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Catalyzed and Uncatalyzed Modifications of Nucleosides, Synthesis of Hippadine, and Deuterated 1,2,3-Triazoles

Hari K. Akula
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CATALYZED AND UNCATALYZED MODIFICATIONS OF NUCLEOSIDES, SYNTHESIS
OF HIPPADINE, AND DEUTERATED 1,2,3-TRIAZoles

by

HARI K. AKULA

A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the
requirements for the degree of Doctor of Philosophy, The City University of New York

2018
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Hari K. Akula

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THE CITY UNIVERSITY OF NEW YORK
ABSTRACT

Catalyzed and Uncatalyzed Modifications of Nucleosides, Synthesis of Hippadine, and Deuterated 1,2,3-Triazoles

by

Hari K. Akula

Advisor: Prof. Mahesh K. Lakshman

The C4 amide carbonyl of O-t-butyldimethylsilyl-protected thymidine, 2’-deoxyuridine, and 3’-azidothymidine (AZT) was activated by reaction with (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in THF as solvent. This led to the formation of corresponding O4-(benzotriazol-1-yl) derivatives, which are reactive intermediates. Substitution at the C4 position was then carried out by reactions with alkyl and aryl amines, and thiols. Typically, reactions were conducted as a two-step, one-pot transformation, and also as a one-step conversion. After examining the reactions, the formation of 1-(4-pyrimidinyl)-1H-benzotriazole-3-oxide derivatives from the pyrimidine nucleosides was identified. However, these too underwent conversion to the desired products. C4 modified pyrimidine nucleosides were desilylated using standard conditions. Desilylated 3’-azido derivatives obtained from AZT were also converted to the 3’-amino derivatives by catalytic reduction. All products were evaluated for their abilities to inhibit cancer cell proliferation and for antiviral activities. Some compounds displayed moderate inhibitory activity against proliferation of murine leukemia (L1210), human cervix carcinoma (HeLa), and human T-lymphocytic (CEM) cell lines. Many were seen to be active against HIV-1 and HIV-2, and
one was active against herpes simplex virus-1 (HSV-1). Evaluations of the structures and activities indicated that the methyl group at the C5 position is important for biological activity.

Chemoselective N-arylation of 8-vinyladenine nucleosides can be carried out with the Pd(OAc)$_2$/Xantphos/Cs$_2$CO$_3$ combination. All the other ligands such as DPEPhos, DPPF, and BIPHEP in combination with Pd(OAc)$_2$, and the complex Pd-118 resulted in Heck arylation, exclusively. Both aryl iodides and bromides can be used under these conditions. Generally, all reactions resulted in N-arylated products in good yields, along with small amounts of Heck-like products and C,N-diarylated products. However, the Heck-like products were observed mostly in the reactions of aryl iodides. The results from the Pd-catalyzed N-arylation reactions of deoxy and ribonucleosides were very similar, but a higher catalyst loading and temperature for reactions of the ribonucleoside was required. A modular, one-pot approach was utilized for the synthesis of diaryl products via sequential C–C reaction and C–N arylation. The generality of chemoselective arylation of simple substrates was tested by exposing p-aminostyrene under N-arylation and Heck-arylation conditions. The results indicated that in this case as well, the Pd/Xantphos/Cs$_2$CO$_3$ combination was effective for chemoselective N-arylation and the Pd/DPEPhos/Cs$_2$CO$_3$ combination was effective for chemoselective Heck-arylation.

In order to synthesize nucleosides adducts produced by a cis ring-opening of benzo[a]pyrene diol epoxide 1, diastereoselective synthesis of (±)-10β-amino-7β,8α,9β-trihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene was carried out in nine steps from (±)-7β,8α-dibenzoyloxy-7,8,9,10-tetrahydrobenzo[a]pyrene. The (±)-7β,8α-dibenzoyloxy-7,8-dihydrobenzo[a]pyrene was converted to the diol epoxide and then reacted with lithium chloride and acetic anhydride to give a peracetyl trans chloro triol with a chloride at the benzylic position. Displacement of chloride by azide, followed by deacylation, and reduction of the azide afforded the requisite amino triol.
B[a]P-deoxyadenosine adducts were synthesized by the reaction of this amino triol with a 6-fluoropurine 2’-deoxyribose derivative. The two adducts obtained from this reaction were separated by preparative TLC and the chirality in each was assigned by comparing their circular dichroism data with the literature. The 2-fluoro-2’-deoxyinosine derivative required for the synthesis of B[a]P-deoxyguanosine adducts, was synthesized by a modified approach utilizing a C6 modification protocol for guanosine nucleosides via the amide activation by BOP. However, the reaction of 2-fluoro-2’-deoxyinosine derivative with the amino triol was unsuccessful. Hence, the hydrochloride salt of amino triol was prepared and then reacted with 2-fluoro-2’-deoxyinosine derivative. This reaction yielded two B[a]P-deoxyguanosine adducts, which were separated by preparative TLC and the chirality in each was assigned by comparing their circular dichroism data with literature. However, careful NMR analysis of B[a]P-dA and dG adducts indicated that the products were not the anticipated cis ring-opened nucleoside adducts as previously reported, but the data were more consistent with trans ring-opened B[a]P DE1-nucleoside adducts. This information suggested that the amino triol synthesized had the undesired trans stereochemistry at the C9 and C10 positions. This was further confirmed by careful evaluation of chemical shift and coupling constant data of synthesized azido triol with known data of trans and cis ring-opened azido triols.

By utilizing PPh3/I2 mediated amidation reaction as a key step, a simple approach was developed for the synthesis of hippadine via anhydrolycorinone. N-(Piperonyl)indoline was synthesized by reacting piperonylic acid and indoline in the presence of PPh3/I2 and iPr2NEt. The combination of polymer-supported PPh3/I2 in place of PPh3/I2 was also very effective under these conditions, and both combinations gave comparable yields of N-(piperonyl)indoline. However, in CDCl3, the 1H NMR data of the amide obtained was missing one aromatic resonance. The
structure of amide obtained via these amidation reactions was further confirmed by obtaining $^1$H NMR in C$_6$D$_6$ at 70 °C and COSY data. The amide was then cyclized using PhI(OTFA)$_2$ and BF$_3$•Et$_2$O to give anhydrolycorinone that was finally oxidized by DDQ to give hippadine in an overall yield of 13%, over three steps.

Deuteration at the C-5 position of the 1,2,3-triazole structure was carried out efficiently during the triazole-forming step by using a copper-catalyzed azide–alkyne cycloaddition (CuAAC) reaction. Reactions of alkynes and azides were conducted in a biphasic medium of CH$_2$Cl$_2$/D$_2$O, using the CuSO$_4$ and Na ascorbate. The mild reaction conditions allow the applicability of this method to relatively high complex substrates, such as nucleosides. Generally, good yields and high levels of deuterium incorporation were observed in all cases. Using appropriately deuterated precursors, partially to fully deuterated triazoles were also assembled under the same conditions. The competition of deuteration vs protonation in the CuAAC reaction was evaluated by conducting a reaction of phenyl azide with 4-ethynyltoluene with equimolar H$_2$O and D$_2$O. Higher hydrogen atom incorporation in the triazole products was observed as compared to deuterium (protonated vs deuterated triazoles were obtained in a 2.7:1 ratio).
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<tr>
<td>AA</td>
<td>Asymmetric Aminohydroxylation</td>
</tr>
<tr>
<td>Ac</td>
<td>Acetyl</td>
</tr>
<tr>
<td>AKR</td>
<td>Aldo-Keto Reductase</td>
</tr>
<tr>
<td>AZT</td>
<td>3'-Azido-2',3'-dideoxythymidine</td>
</tr>
<tr>
<td>BINAP</td>
<td>2,2'-Bis(diphenylphosphino)-1,1'-binaphthalene</td>
</tr>
<tr>
<td>BIPHEP</td>
<td>2,2'-Bis(diphenylphosphino)-1,1'-biphenyl</td>
</tr>
<tr>
<td>BOP</td>
<td>Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium Hexafluorophosphate</td>
</tr>
<tr>
<td>B[a]P</td>
<td>Benzo[a]pyrene</td>
</tr>
<tr>
<td>B[c]Ph</td>
<td>Benzo[c]phenanthrene</td>
</tr>
<tr>
<td>br</td>
<td>Broad</td>
</tr>
<tr>
<td>CD</td>
<td>Circular Dichroism</td>
</tr>
<tr>
<td>CMV</td>
<td>Cytomegalo Virus</td>
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<td>COSY</td>
<td>Correlation Spectroscopy</td>
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<td>CuAAC</td>
<td>Copper(I)-Catalyzed Azide-Alkyne Cycloaddition</td>
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<tr>
<td>Cy</td>
<td>Cyclohexyl</td>
</tr>
<tr>
<td>d</td>
<td>Doublet</td>
</tr>
<tr>
<td>DB[a,h]A</td>
<td>Dibenz[a,h]anthracene</td>
</tr>
<tr>
<td>DB[a,j]A</td>
<td>Dibenz[a,j]anthracene</td>
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<td>DBU</td>
<td>1,8-Diazabicyclo[5.4.0]undec-7-ene</td>
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<td>DDQ</td>
<td>2,3-Dichloro-5,6-dicyano-1,4-benzoquinone</td>
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<td>DE</td>
<td>Diol Epoxide</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Name</td>
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<tr>
<td>DFT</td>
<td>Density Functional Theory</td>
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<td>DHQD</td>
<td>Dihydroquinidine</td>
</tr>
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<td>DIBAL</td>
<td>Diisobutyaluminium Hydride</td>
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<td>DIPEA</td>
<td>N,N-Diisopropylethylamine</td>
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<td>DMAP</td>
<td>4-(Dimethylamino)pyridine</td>
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<td>DMDO</td>
<td>Dimethyldioxirane</td>
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<td>DME</td>
<td>1,2-Dimethoxyethane</td>
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<td>DMF</td>
<td>N,N-Dimethylformamide</td>
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<td>DMSO</td>
<td>Dimethyl Sulfoxide</td>
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<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
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<td>DavePhos</td>
<td>2-Dicyclohexylphosphino-2'-(N,N-dimethylamino)biphenyl</td>
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<td>DPEPhos</td>
<td>Bis[(2-diphenylphosphino)phenyl] Ether</td>
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<td>DPPB</td>
<td>1,4-Bis(diphenylphosphino)butane</td>
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<td>DPPE</td>
<td>1,2-Bis(diphenylphosphino)ethane</td>
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<td>DPPF</td>
<td>1,1''-Ferrocenediyl-bis(diphenylphosphine)</td>
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<td>1,3-Bis(diphenylphosphino)propane</td>
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<td>Ethyl Acetate</td>
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<td>Et₂O</td>
<td>Diethyl Ether</td>
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<td>ESI</td>
<td>Electronspray Ionization</td>
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<td>FDA</td>
<td>Food and Drug Administration</td>
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<td>HEL</td>
<td>Human Embryonic Lung</td>
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<tr>
<td>HF</td>
<td>Hydrogen Fluoride</td>
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<td>Acronym</td>
<td>Full Form</td>
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<td>HFIP</td>
<td>Hexafluoroisopropanol</td>
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<td>HMDS</td>
<td>Hexamethyl Disiloxane</td>
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<td>HMPA</td>
<td>Hexamethylphosphoramide</td>
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<td>HPLC</td>
<td>High-Performance Liquid Chromatography</td>
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<td>High Resolution Mass Spectroscopy</td>
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<td>HSV</td>
<td>Herpes Simplex Virus</td>
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<td>J</td>
<td>Coupling Constant</td>
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<td>Potassium Hydroxide</td>
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<td>LG</td>
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<td>Phenylodine(III) bis(trifluoroacetate)</td>
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PMDTA \(N,N',N'',N'''\)-pentamethyldiethylenetriamine

PS Polystyrene Supported

q Quartet

quint Quintet

\(R_f\) Retention Factor

RNA Ribonucleic Acid

RTA Ricin Toxin A-chain

s Singlet

SARS Severe Acute Respiratory Syndrome

Ts Tosyl

t Triplet

TASF Tris(dimethylamino)sulfonium Difluorotrimethylsilicate

TBDMS \(t\)-Butyldimethylsilyl

TFE Trifluoroethanol

THF Tetrahydrofuran

TIPS Triisopropylsilyl

TLC Thin Layer Chromatography

TMEDA Tetramethylethylenediamine

TMS Trimethylsilyl

TOF Time-of-flight

VSV Vesicular Stomatitis Virus
CHAPTER 1

FACILE MODIFICATION AT THE C-4

POSITION OF PYRIMIDINE NUCLEOSIDES
FACILE MODIFICATION AT THE C-4 POSITION OF PYRIMIDINE NUCLEOSIDES

[1.1] INTRODUCTION

Pyrimidine-based modified nucleosides are prominent because of their applications in biochemistry, biotechnology, and also in medicine.\(^1\) A large group of FDA approved antiviral agents are in fact pyrimidine-based modified nucleosides. Some examples are shown in Figure 1.

![Figure 1. Some clinically used modified pyrimidine nucleoside antiviral agents](image)

Among these unnatural nucleosides, C-4 modified pyrimidine nucleosides are notable due to their antiviral and anticancer activities.\(^2\) For example, 5-methoxymethyl-2'-deoxycytidine (MMdCyd, 1) is highly active against HSV-1 (Figure 2). However, the activity is lost in the presence of cytidine or deoxycytidine deaminases, due to the loss of C-4 amino group. Tetrahydrodeoxyuridine (H\(_4\)dU, 2), a potent inhibitor of deoxycytidine deaminase is often used in combination with MMdCyd (1) in order to retain activity of compound 1. To avoid the usage of H\(_4\)dU (2), Zoghaib et al. developed C-4 modified analogues of MMdCyd (3a–d, Figure 2). They found these analogues to be resistant to deamination by cytidine and deoxycytidine deaminases and were also active against HSV-1, more potently than the parent compound MMdCyd (1) itself.\(^3\)
Apart from their medicinal properties, various C-4 modified pyrimidine nucleosides are also very useful in investigating processes such as mutagenesis and carcinogenesis.\textsuperscript{5,6} Hence, it is important to develop strategies for simple syntheses of C-4 modified nucleosides. One commonly used approach for modification at the C-4 position of pyrimidine nucleosides entails an addition-elimination process on a stable electrophilic nucleoside derivative. In this approach, the amide functionality in the pyrimidine nucleoside is activated by an appropriate reagent and a leaving group (LG) is installed at the C-4 position (Scheme 1). The presence of a leaving group at the C-4 position makes the nucleoside electrophilic and upon exposure to various nucleophiles, produces C-4 modified pyrimidine nucleoside derivatives.

**Figure 2.** 5-Methoxymethyl-2′-deoxycytidine (1), tetrahydro-2′deoxyuridine (2), and C-4 modified analogues of 5-methoxymethyl-2′-deoxycytidine (3a–d)

**Scheme 1.** C-4 Modification via an electrophilic pyrimidine nucleoside derivative

Thus far, a number of electrophilic pyrimidine nucleoside derivatives have been developed and one of the first is a 4-thiopyrimidine nucleoside derivative. The 4-thiopyrimidine nucleoside derivatives can be prepared by the thionation at the 4 position of benzoyl protected pyrimidine
nucleosides 4a–c with P₂S₅ in a pyridine-water mixture, under reflux conditions (Scheme 2). A recent report by Felczak et al. showed that Lawesson’s reagent can also be used for this thionation.

**Scheme 2. Synthesis of 4-thiopyrimidine nucleosides 5a–c from pyrimidine nucleoside derivatives 4a–c**

Typically, benzoyl protection of the free hydroxyl groups on the pyrimidine nucleosides is preferred prior to the thionation with P₂S₅, as it was observed that acetyl groups tend to hydrolyze under the conditions. However, in some cases benzoyl esters were also observed to hydrolyze upon extended reaction times. C-4 modification by using 4-thio pyrimidine nucleosides often requires harsh conditions and special equipment. For example, compound 6 was prepared by heating 5a with 45% methylamine in ethanol solution in a sealed tube at 100 °C (Scheme 3). Similarly, compound 7 was prepared by heating a solution of 5a in ethanol with β-phenylethylamine in a sealed tube at 100 °C (Scheme 3).

**Scheme 3. Synthesis of C-4 modified pyrimidine nucleosides 6 and 7 from 4-thiopyrimidine nucleoside derivative 5a**
Based on the aforementioned drawbacks, Fox et al. modified 4-thiopyrimidine nucleoside derivatives 5a–b to their corresponding 4-methythio derivatives 8a–b.4,9,10 Compounds 8a–b were prepared by initial deacylation of compounds 5a–b, followed by treatment of the resultant product with MeI in 1N NaOH solution (Scheme 4).9 They observed that 4-methythio derivatives 8a, 8b were superior to their precursors 5a, 5b, and underwent displacement reactions with different nucleophiles under milder conditions.4 They also observed that 4-methylthiouridine (8b) was more reactive than 4-methylthiothymidine (8a) towards displacement reactions, when amino acids were used as nucleophiles. However, the stability of 4-methythio derivatives is highly pH dependent and they are prone to hydrolysis even under mildly acidic conditions.4

**Scheme 4. Synthesis of 4-methythio derivatives 8a–b from their corresponding 4-thio derivatives 5a–b**

Saladino et al. developed a different strategy for the C-4 modification of pyrimidine nucleosides by oxidizing 4-thiopyrimidine nucleosides 9a–b with dimethyldioxirane (DMDO) in presence of various nucleophiles (Scheme 5).11-13 Various aliphatic and aromatic amines, and alcohols were used to test the scope of this reaction and good yields were observed in all the reactions.

**Scheme 5. C-4 modification of pyrimidine nucleosides by oxidation of 4-thiopyrimidine nucleosides with DMDO in presence of various amines and alcohols**
Žemlička et al. hypothesized that 4-chloropyrimidine nucleosides could function as excellent electrophilic derivatives for C-4 modification. 4-Chloropyrimidine nucleoside derivatives were synthesized by treating various thymidine and uridine nucleoside derivatives with SOCl₂ and a catalytic amount of DMF (Scheme 6).\(^{14,15}\) The combination of SOCl₂ and DMF generates a reactive species dimethylchloromethyleneammonium chloride. This reagent reacts with the amido group of the pyrimidine nucleoside and generates the corresponding 4-chloro derivative. On the basis of the Appel reaction, Santacroce et al. developed milder reaction conditions for the synthesis of 4-chloropyrimidine nucleosides by using the PPh₃/CCl₄ combination (Scheme 6).\(^{16,17}\) However, 4-chloropyrimidine nucleosides were found to be very unstable and are swiftly used for further transformation upon synthesis.\(^{18}\)

**Scheme 6. Synthesis of a 4-chloropyrimidine nucleoside derivative and subsequent transformation to O⁴-ethylpyrimidine nucleoside derivative**

In one such approach reported by Matsuda et al. the 4-chloropyrimidine nucleoside derivative was generated *in situ* and immediately treated with NaOEt. This resulted in the formation of the corresponding O⁴-ethylpyrimidine nucleoside derivative and this compound was utilized for further transformations at C-4 position (Scheme 6).\(^{19}\) This approach was based on
the observation that \( O^4 \)-alkylpyrimidine nucleoside derivatives are relatively stable but are susceptible to addition-elimination reactions with various nucleophiles.\textsuperscript{20}

**Scheme 7. Synthesis of 4-(3-nitro-1,2,4-triazol-1-yl)pyrimidine nucleoside derivative**

4-(1,2,4-Triazol-1-yl)pyrimidine nucleoside derivatives are an important class of compounds, which are widely used for C-4 modification due to a good balance between their stability and reactivity.\textsuperscript{21,22} For example, 4-(3-nitro-1,2,4-triazol-1-yl)pyrimidine nucleoside \( 14b \) was synthesized by reacting uridine \( 13b \) with 1-(mesitylene-2-sulphonyl)-3-nitro-1,2,4-triazole in pyridine, where diphenyl phosphate \( ((\text{PhO})_2\text{P(O)}\text{OH}) \) was used as a catalyst (Scheme 7).\textsuperscript{21} Intermediate \( 14b \) upon exposure to various nucleophiles led to successful modification at C-4 position. However, thymidine equivalent \( 14a \) cannot be prepared under the conditions used for uridine derivative \( 13b \).\textsuperscript{23,24} An expedient synthesis for the compound \( 14b \) was reported, where uridine derivative \( 13b \) was reacted with readily available diphenyl phosphoryl chloride \( ((\text{PhO})_2\text{POCl}) \) and 3-nitro-1,2,4-triazole.\textsuperscript{22}

Similarly to the synthesis of 3-nitro-1,2,4-triazolyl pyrimidine derivatives, the 1,2,4-triazolyl derivatives also shown remarkable stability and reactivity towards various nucleophiles.\textsuperscript{22} C-4 triazole derivative \( 15b \) can be prepared from the uridine \( 13b \) by either reacting with \( O \)-chlorophenylid(1\( H \)-1,2,4-triazol-1-yl)phosphine oxide or tri(1\( H \)-1,2,4-triazol-1-yl)phosphine.
oxide (Scheme 8).\textsuperscript{22} The reagent tri(1H-1,2,4-triazol-1-yl)phosphine oxide was generated \textit{in situ} by reacting POCl\textsubscript{3} with 1,2,4-triazole in presence of Et\textsubscript{3}N. On the contrary, compound 15b can also be prepared from 14b by reacting it with 1,2,4-triazole and Et\textsubscript{3}N in CH\textsubscript{3}CN (Scheme 8).\textsuperscript{22}

\textbf{Scheme 8. Synthesis of 4-(1,2,4-triazol-1-yl) pyrimidine nucleoside derivative from uridine derivative 13b or 3-nitro-1,2,4-triazolyl derivative 14b}

![Scheme 8](image)

Although the 3-nitro-1,2,4-triazol-1-yl nucleoside derivative 14b is more reactive than the 1,2,4-triazolyl derivative 15b, the latter analogues are more commonly used because of their ease in synthesis and relative stability.\textsuperscript{22} For example, compounds 16a and 16b are easily transformed to their corresponding 1,2,4-triazolyl derivatives 17a and 17b by reaction with \textit{p}-chlorophenyl phosphoryl dichloride and 1,2,4-triazole, in pyridine (Scheme 9).\textsuperscript{25,26} Without any further

\textbf{Scheme 9. Convenient synthesis of 4-(1,2,4-triazol-1-yl)pyrimidine nucleotide derivatives 17a and 17b for the site–specific modification of oligomers}

![Scheme 9](image)
purification, the products thus obtained were treated with 2-cyanoethanol to give their corresponding nucleotides 17a and 17b. Here, the 3'-OH group of the pyrimidine nucleosides 16a and 16b were concurrently phosphorylated while the amide functionality was activated to give the 1,2,4-triazolyl derivatives. This approach enables the easy access for the site–specific modification of pyrimidine bases in oligoribonucleotides.25,26

In their studies, Reese et al. observed that 4-tetrazolyl pyrimidine nucleoside derivatives can also act as excellent electrophilic nucleoside derivatives for accomplishing C-4 modification.23,24 C-4 tetrazole derivative 18 was prepared by reacting uridine derivative 13b with tetrazole, diphenyl phosphate ((PhO)_2P(O)OH) and p-toluenesulfonyl chloride, in pyridine at room temperature for 1.5 days (Scheme 10). C-4 tetrazole derivative 18 was stable, isolable, and underwent nucleophillic displacement with various nucleophiles.

**Scheme 10. Synthesis of 4-tetrazol-1-ylpyrimidine nucleoside derivative 18**

In an independent work, Sung et al. observed that phosphorylation of the 3'-OH group of uridine derivative 16b with p-chlorophenyl phosphoryl ditetrazolide (19) in pyridine followed by treatment with 2-cyanoethanol, yielded compound 20, where a concomitant installation of a tetrazole unit took place at the C-4 position of 16b (Scheme 11).27

**Scheme 11. Concomitant introduction of a tetrazole group at C-4 position during the phosphorylation of compound 16b**
Although, the 4-triazolyl and 4-tetrazolyl pyrimidine nucleoside derivatives are excellent electrophilic nucleosides that undergo nucleophilic displacement with various nucleophiles under mild conditions, their synthesis is inconvenient. The reagents are often generated in situ, are highly moisture sensitive, and in some cases, long reaction times are required.\(^{22-27}\) Focusing on this issue, Matsuda et al. designed an alternate electrophilic nucleoside, 3-Methyl-1-imidazolium intermediates 21a and 21b, which were synthesized by reacting 1-methylimidazole with POCl\(_3\) in CH\(_3\)CN at 0 °C, followed by the addition of pyrimidine nucleoside 4a or 4b (Scheme 12).\(^{28}\) These intermediates 21a and 21b when exposed to diverse nucleophiles underwent displacement readily and resulted in the corresponding C-4 modified pyrimidine nucleosides in good yields.

Unlike the cases of 4-triazolyl and 4-tetrazolyl pyrimidine nucleoside derivatives, this approach

**Scheme 12. Synthesis of 3-methyl-1-imidazolium intermediates 21a and 21b from the reaction of 1-methylimidazole and POCl\(_3\) with pyrimidine nucleosides 4a and 4b**
succeeds only as a one-pot reaction, since the intermediates 21a and 21b are highly labile, and therefore, cannot be isolated.28

Bischofberger has developed a different class of electrophilic nucleoside derivatives with the synthesis of pyrimidine 4-aryl sulfonates.29 Typically, 2,4,6-triisopropylphenyl and 2-mesitylenyl groups were used as the aryl substituents. The O4-triisopropylphenyl sulfonate derivatives 23a and 23b were synthesized by reacting silyl protected pyrimidine nucleosides 22a and 22b with NaH in THF, followed by the addition of 2,4,6-triisopropylphenylsulfonfyl chloride (Scheme 13). Compounds 23a and 23b could also be prepared by treating the nucleosides 22a and 22b with Et3N and DMAP in CH2Cl2, followed by the addition of 2,4,6-triisopropylphenylsulfonfyl chloride. However in the later case, the yields of the products 23a and 23b are low. In a similar manner, O4-mesitylene sulfonate derivatives 24a and 24b were also prepared under the same conditions utilized for the synthesis of compounds 23a and 23b (Scheme 13). Nevertheless, the products 24a and 24b degraded upon storage and have to be utilized quickly upon their synthesis.29

Scheme 13. Synthesis of O4-aryl sulfonate derivatives of pyrimidine nucleosides 22a and 22b
Owing to their greater stability, $O^4$-triisopropylphenyl sulfonate derivatives are most commonly used for the C-4 modification of pyrimidine nucleosides. In addition to the utilities of $O^4$-aryl sulfonate derivatives in nucleophilic displacement reactions, we\textsuperscript{30} and others\textsuperscript{31} showed that these substrates can also be successfully applied to the C–C bond-forming reactions by palladium-catalyzed cross-coupling chemistry (Scheme 14).

**Scheme 14.** *Pd-catalyzed C–C bond forming reactions with $O^4$-aryl sulfonate derivatives of pyrimidine nucleosides*

\[
\begin{align*}
\text{Scheme 14.} & \quad \text{Pd-catalyzed C–C bond forming reactions with } O^4\text{-aryl sulfonate derivatives of pyrimidine nucleosides}
\end{align*}
\]

Similarly to $O^4$-triisopropylphenyl and $O^4$-mesitylene sulfonate pyrimidine nucleoside derivatives, $O^4$-toluene sulfonate derivatives can also be synthesized under milder conditions. At

**Scheme 15.** *One-pot C-4 modification of pyrimidine nucleoside 25 via pyrimidine $O^4$-toluene sulfonate derivative 26*

\[
\begin{align*}
\text{Scheme 15.} & \quad \text{One-pot C-4 modification of pyrimidine nucleoside 25 via pyrimidine $O^4$-toluene sulfonate derivative 26}
\end{align*}
\]
least in one case, we observed that the rate of degradation of \( O^4 \)-toluene sulfonate derivative is much slower than the corresponding \( O^4 \)-mesitylene sulfonate derivative.\(^{30}\) To improve the yield of substitution reactions, C-4 modification reactions with \( O^4 \)-toluene sulfonate derivatives are often carried out in one-pot, by subjecting pyrimidine \( O^4 \)-toluene sulfonate derivatives to nucleophilic displacement reactions with nucleophiles immediately, upon their formation (Scheme 15).\(^{32}\)

In one case, pyrimidine \( O^4 \)-toluene sulfonate derivatives \( 29a \) and \( 29b \) were converted to their corresponding quaternary ammonium salts \( 30a \) and \( 30b \) as reactive electrophilic pyrimidine nucleoside derivatives (Scheme 16).\(^{33}\) However, these salts were also not isolated but were subjected to nucleophilic displacement reaction with aq. \( \text{NH}_3 \) to give the corresponding cytidine derivatives.

**Scheme 16.** One-pot conversion of pyrimidine \( O^4 \)-toluene sulfonate derivatives \( 29a \) and \( 29b \) to their corresponding quaternary ammonium salts \( 30a \) and \( 30b \)

![Scheme 16](image)

Apart from the aforementioned electrophilic pyrimidine nucleoside derivatives, \( O^4 \)-trimethylsilyl pyrimidine nucleoside derivatives are also used for C-4 modification.\(^{34}\) These derivatives can be easily prepared by the silylation of acyl-protected or free pyrimidine nucleosides. However, their usage is limited because of their inability to react with a wide variety of nucleophiles under mild conditions.
Direct alkylation or arylation of pyrimidine nucleosides is an auxiliary strategy to the addition-elimination approach for the C-4 modification of pyrimidine nucleosides. For example, acyl protected thymidine derivatives 4a and 13a were converted to their O4'-alkyl derivatives 31a and 31b, by Ag2CO3 catalyzed reaction of 4a and 13a with various alkyl chlorides (R’Cl) in toluene (Equation 1 in Scheme 17). Similarly, 2’-deoxycytidine (32) could be N-arylated to give the compound 33, by copper catalyzed reaction of the compound 32 with 4-tolylboronic acid and TMEDA in DMSO at 60 °C over 16 h (Equation 2 in Scheme 17). However, only one example was shown and the yield was modest. Kane et al. developed a method for the N4'-monoalkylation of 2’-deoxycytidine (32). This approach involves the formation of the amidine intermediate 34, by reacting 32 with N,N-dimethylformamide dimethyl acetal in DMF at 40 °C for 1 h. The crude mixture containing the compound 34 was then treated with NaBH4 for 2 h, to give the product 35 (Equation 3 in Scheme 17). However, this approach is not highly appreciated since it requires the use of a strong reducing agent and also due to commercial unavailability of N,N-dialkylformamide dimethyl acetal derivatives.

Although C-4 modification of pyrimidine nucleosides via addition-elimination is straightforward as compared to direct alkylation or arylation approaches, there are some drawbacks in terms of the stability and reactivity of electrophilic nucleoside derivatives that are reported so far and the harsh conditions required for the nucleophilic displacement reactions. Hence, we decided to develop a one-pot strategy for the C-4 modification of pyrimidine nucleosides via a new class of electrophilic pyrimidine nucleoside derivatives, which would be readily reactive towards nucleophilic displacement reactions with various nucleophiles.

**Scheme 17. C-4 Modification of pyrimidine nucleosides by direct alkylation and arylation**
4a, \( R = Bz \)  
13a, \( R = Ac \)

\[ \text{Ag}_2\text{CO}_3 \rightrightarrows \]

\[ \text{R}^\prime \text{Cl, PhMe} \]

\[ \text{R}^\prime = \text{alkyl} \]

31a, \( R = Bz \)  
31b, \( R = Ac \)

\[ \text{H}_2\text{C} - \text{B(OH)}_2 \]

\[ \text{Cu(OAc)}_2, \text{TMEDA} \]

\[ \text{DMSO, 60 °C, 16 h} \]

\[ 46\% \]

32

33

32

34

\[ \text{NaBH}_4 \]

\[ 2\, \text{h}, 71\% \]

35
[1.2] RESULTS AND DISCUSSION

[1.2.1] Optimization and Substrate Scope

The premise of developing a new electrophilic pyrimidine nucleoside depends upon our recent success in developing the methodology for C-6 modification of purine nucleosides.\(^{37-45}\) In this strategy, the amide group of protected or unprotected purine nucleoside derivatives \(36a–f\) was activated by (benzotriazol-1-yl)tris(dimethylamino)phosphonium hexafluorophosphate (BOP), a known peptide coupling agent in the presence of a suitable base (Scheme 18). This activation leads to the formation of a \(O^6\)-(benzotriazol-1-yl) purine nucleoside derivatives \(37a–f\), which are stable and reactive. These derivatives upon exposure to various nucleophiles lead to the C-6 modified purine nucleosides under mild conditions.

**Scheme 18.** \(C-6\) Modification of purine nucleosides through the formation of \(O^6\)-(benzotriazol-1-yl) purine nucleoside derivatives \(37a–f\)

During the course of our initial report on the synthesis and reactivity of \(O^6\)-(benzotriazol-1-yl) inosine derivatives \((37a–d)\),\(^{37}\) a similar methodology was also reported for the direct amination of various amide derivatives using BOP, DBU, and an amine in a suitable solvent.\(^{46}\)
Ever since our initial reports on the C-6 modified purine nucleosides, the amide activation with BOP has seen light in various applications in the recent past.\textsuperscript{47-52} Since several pyrimidine nucleosides like thymidine and uridine possess an amide linkage. Thus, we postulated that the corresponding $O^4$-(benzotriazol-1-yl) derivatives can be synthesized by the activation of amide linkage with BOP and a suitable base. By exposing the $O^4$-(benzotriazol-1-yl) derivative to displacement reactions with various nucleophiles, C-4 modification can be achieved.

To test our hypothesis, we started our initial experiments by reacting thymidine (38) with BOP and DBU in THF (entry 1 in Table 1). After 3 h, complete conversion of 38 to the $O^4$-(benzotriazol-1-yl) derivative 39 was observed. However, during isolation by silica gel chromatography, compound 39 decomposed. Hence, we decided to expose the $O^4$-(benzotriazol-1-yl) derivative 39, upon its formation, to morpholine prior to isolation (entry 2). The C-4 modified product 41 was observed to form in 12 h in \textit{ca.} 58\% yield. However, purification by silica gel chromatography was not very successful due to the high polarity of 41, it co-eluted along with several other unknown polar impurities.

\begin{table}[h]
\centering
\caption{Optimization of conditions for the C-4 modification via activation of thymidine with BOP and base\textsuperscript{a}}
\begin{tabular}{llll}
\hline
Entry & Substrate & Conditions & Product: yield\textsuperscript{b} \\
\hline
1 & 38 & BOP, DBU, THF, 3 h & 39: decomp\textsuperscript{c} \\
2$^d$ & 38 & BOP, DBU, THF, 3 h, then morpholine, 12 h & 41: \textit{ca.} 58\%\textsuperscript{e} \\
\hline
\end{tabular}
\end{table}
<table>
<thead>
<tr>
<th>Step</th>
<th>Compound</th>
<th>Conditions</th>
<th>Yield</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>22a</td>
<td>BOP, DBU, THF, 0.5 h</td>
<td>40: decomp$^d$</td>
<td></td>
</tr>
<tr>
<td>4$^f$</td>
<td>22a</td>
<td>BOP, DBU, THF, 0.5 h, then morpholine, 2 h</td>
<td>42: 76%</td>
<td></td>
</tr>
<tr>
<td>5$^g$</td>
<td>22a</td>
<td>BOP, Cs$_2$CO$_3$, THF, 24 h</td>
<td>40: inc$^h$</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>22a</td>
<td>BOP, DBU, DME, 2 h</td>
<td>40: inc$^h$</td>
<td></td>
</tr>
<tr>
<td>7$^f$</td>
<td>22a</td>
<td>BOP, DBU, MeCN, 0.5 h, then morpholine, 2 h</td>
<td>42: 73%</td>
<td></td>
</tr>
<tr>
<td>8$^g,f$</td>
<td>22a</td>
<td>BOP, Cs$_2$CO$_3$, MeCN, 0.5 h, then morpholine, 2 h</td>
<td>42: 94%</td>
<td></td>
</tr>
<tr>
<td>9$^i$</td>
<td>22a</td>
<td>BOP, DBU, morpholine, THF, 20 min</td>
<td>42: 90%</td>
<td></td>
</tr>
<tr>
<td>10$^{g,i}$</td>
<td>22a</td>
<td>BOP, Cs$_2$CO$_3$, morpholine, MeCN, 24 h</td>
<td>42: inc$^j$</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Reactions were conducted at room temperature with 0.424 M solutions of 38 or 22a (except when noted otherwise), BOP (2 equiv.), base (2 equiv.) in anhydrous solvent. $^b$ Where reported, yield of isolated and purified product. $^c$ Product was not isolated due to decomposition over silica. $^d$ Morpholine (4 equiv.) was added to the reaction mixture, after complete conversion of 38 to 39. $^e$ Product isolation was difficult as it co-elutes with morpholine, HMPA and other impurities. $^f$ Morpholine (4 equiv.) was added to the reaction mixture, after complete conversion of 22a to 40. $^g$ Reactions were conducted with 0.1325 M solution of 22a. $^h$ Product was not isolated due to incomplete reaction (As assessed by TLC, ca. 50% conversion of the starting material was observed). $^i$ After stirring a mixture of 22a, BOP (2 equiv.), and base (2 equiv.) in the appropriate solvent for 5 min, morpholine (4 equiv.) was added to the reaction mixture. $^j$ Product was not isolated due to incomplete reaction (As assessed by TLC, ca. 10% conversion of the starting material was observed).

To decrease polarity of the final product and increase ease of purification, we decided to test silyl protected-thymidine derivative 22a under the conditions used in entry 1 (entry 3). The O$^4$-(benzotriazol-1-yl) derivative 40 was formed within a short reaction time of 0.5 h. However, an attempt to isolate the product by silica gel chromatography lead to decomposition of majority of product 40 to its parent compound 22a, and 40 was isolated as a mixture along with 22a. Several other attempts to isolate 40 were made using neutral and basic alumina, but hydrolysis was observed in all cases. Silyl protection of the free hydroxyl groups in 38 did not provide any additional stability to the O$^4$-(benzotriazol-1-yl) derivative 40. As a result, we decided to not isolate O$^4$-(benzotriazol-1-yl) derivative 40, but proceed towards C-4 modification by subjecting 40 to nucleophilic displacement with morpholine upon its formation (entry 4). After the formation of compound 40, the desired product 42 was formed within 2 h. Product 42 was isolated in a good 76% yield, without any problems in the purification. Surprisingly, changing
the base from DBU to Cs$_2$CO$_3$ did not yield 40. To improve the solubility of the base, the same reaction was conducted at higher dilution (entry 5). This reaction seemed to progress really fast within the first 0.5 h and about 70% conversion observed at that stage. To our surprise, upon further extending the reaction time to 24 h, only 50% conversion of 22a was observed. It is possible that product 40 hydrolyzed to the starting material (22a) over a prolonged reaction time, under these conditions. Substituting THF with DME as solvent to the conditions in entry 4, did not provide any improvement, and only 50% conversion of the starting material 22a was observed after 2 h (entry 6). However, switching from THF to acetonitrile did in fact yield the $O^1$-(benzotriazol-1-yl) derivative 40 within 0.5 h (entry 7). Subsequent addition of morpholine to the reaction mixture lead to the formation of the product 42 in 2 h, in a good 73% yield, comparable to that in entry 4. Interchanging DBU with Cs$_2$CO$_3$ to the conditions in entry 7 increased the yield of 42 to 94% (entry 8). However, this reaction proceeds only at higher dilution and requires vigorous stirring. To further improve the conditions of the reaction, we decided to perform a one-step reaction for the formation of product 42 i.e., addition of the nucleophile to the reaction mixture along with BOP and base, rather than adding nucleophile after the formation of intermediate 40. We believe that the availability of the nucleophile within the reaction medium allows the $O^1$-(benzotriazol-1-yl) derivative 40 to react immediately with the nucleophile upon its formation, without any competing processes. As expected, the reaction proceeded to form product 42 within a short 20 min, in an excellent 90% yield (entry 9). Surprisingly, the one-step reaction with Cs$_2$CO$_3$ as a base and acetonitrile as solvent, did not even proceed to the formation of intermediate 40, and only 10% conversion of the starting material 22a was observed even after 24 h (entry 10).
Having optimized conditions for C-4 modification, the scope was investigated with substrates and nucleophiles. Silyl-protected 2’-deoxyuridine 43 and 3’-azido-2’,3’-dideoxythymidine (AZT) 47 were chosen along with the thymidine derivative 22a for this purpose, and various amines and thiols were used as nucleophiles (Table 2). The one-pot, two-step method and the one-step method with BOP, DBU in THF were chosen as standard conditions. Typically, in a one-pot, two-step method, the nucleoside was reacted with 2 equiv. each of BOP and DBU in THF to form the corresponding benzotriazol-1-yl derivative. Then the nucleophile was added to the reaction mixture from step 1 and the reaction was allowed to proceed until the benzotriazol-1-yl derivative completely consumed to the product. Conditions used in step 2 were subject to the nucleophile used and will be discussed later in more detail. In the one-step method, the nucleoside was reacted with BOP (2 equiv.) and DBU, along with the nucleophile in a suitable solvent.

Table 2. Generality of the two-step, one-pot and one-step reactions for the C-4 modification of pyrimidine nucleosides

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Nucleophile</th>
<th>Two-step, one-pot&lt;sup&gt;b&lt;/sup&gt;</th>
<th>One-step&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Reaction conditions</td>
<td>Product: yield&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>22a</td>
<td>MeNH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>rt, 0.5 h</td>
<td>45a: 84%&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>22a</td>
<td>PhCH&lt;sub&gt;2&lt;/sub&gt;NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>rt, 1.5 h</td>
<td>45b: 72%</td>
</tr>
<tr>
<td>Reaction</td>
<td>Reactants</td>
<td>Conditions</td>
<td>Yield</td>
<td>Notes</td>
</tr>
<tr>
<td>----------</td>
<td>-----------</td>
<td>------------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>3</td>
<td>43, PhCH₂NH₂</td>
<td>rt, 2 h</td>
<td>46a: 78%</td>
<td>THF, rt, 1 h</td>
</tr>
<tr>
<td>4</td>
<td>22a, Me₂NH</td>
<td>rt, 2 h</td>
<td>45c: 76%</td>
<td>THF, rt, 1 h</td>
</tr>
<tr>
<td>5</td>
<td>22a, Et₂NH</td>
<td>rt, 2 h</td>
<td>45d: 78%</td>
<td>THF, rt, 1 h</td>
</tr>
<tr>
<td>6</td>
<td>47, Et₂NH</td>
<td>rt, 1 h</td>
<td>49a: 57%</td>
<td>THF, rt, 1 h</td>
</tr>
<tr>
<td>7</td>
<td>22a, NH₂</td>
<td>rt, 2 h</td>
<td>45e: 75%</td>
<td>THF, rt, 20 min</td>
</tr>
<tr>
<td>8</td>
<td>43, NH₂</td>
<td>rt, 2 h</td>
<td>46b: 71%</td>
<td>THF, rt, 30 min</td>
</tr>
<tr>
<td>9</td>
<td>47, NH₂</td>
<td>rt, 2 h</td>
<td>49b: 53%</td>
<td>rt, 1 h</td>
</tr>
<tr>
<td>10</td>
<td>22a, NH₂</td>
<td>rt, 2 h</td>
<td>45f: 75%</td>
<td>THF, rt, 1 h</td>
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<tr>
<td>11</td>
<td>43, NH₂</td>
<td>rt, 2 h</td>
<td>46c: 75%</td>
<td>THF, rt, 30 min</td>
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<tr>
<td>12</td>
<td>47, NH₂</td>
<td>rt, 1.5 h</td>
<td>49c: 63%</td>
<td>rt, 40 min</td>
</tr>
<tr>
<td>13</td>
<td>43, O₂</td>
<td>rt, 2 h</td>
<td>46d: 76%</td>
<td>THF, rt, 30 min</td>
</tr>
<tr>
<td>14</td>
<td>47, O₂</td>
<td>rt, 2 h</td>
<td>49d: 57%</td>
<td>rt, 1 h</td>
</tr>
<tr>
<td>15</td>
<td>22a, iPr₂NEt</td>
<td>EtOH, 50 °C, 16 h</td>
<td>45g: 62%</td>
<td>BOP, DBU, MeCN, reflux, 12 h</td>
</tr>
<tr>
<td>16</td>
<td>43, iPr₂NEt</td>
<td>EtOH, 50 °C, 16 h</td>
<td>46e: 64%</td>
<td>BOP, DBU, MeCN, reflux, 12 h</td>
</tr>
<tr>
<td>17</td>
<td>22a, EtSH</td>
<td>Cs₂CO₃, DME, 3.5 h</td>
<td>45h: 68%</td>
<td>BOP, DBU, DME, rt, 3 h</td>
</tr>
<tr>
<td>18</td>
<td>22a, PhCH₂SH</td>
<td>Cs₂CO₃, DME, 4 h</td>
<td>45i: 70%</td>
<td>BOP, DBU, DME, rt, 3 h</td>
</tr>
<tr>
<td>19</td>
<td>22a, MeSNa</td>
<td>DMSO, 50 °C, 2 h</td>
<td>45j: 49%</td>
<td>not done</td>
</tr>
</tbody>
</table>

Reactions were conducted at 0.424 M concentration using 0.212 mmol of 22a, 0.219 mmol of 43, and 0.262 mmol of 47. BOP (2 equiv.), DBU (2 equiv.) in anhydrous THF were used to form the corresponding O₂(benzotriazol-1-yl) derivatives and then nucleophile (4 equiv.) was added to the reaction mixture. The nucleoside, BOP (2 equiv.), DBU (2 equiv.), and
nucleophile (4 equiv.) were reacted in an appropriate solvent. d Yields are of isolated and purified products. e The following amine solutions were used: 2 M MeNH₂ in THF and 40 wt% Me₂NH in H₂O. f Yield was based on recovery of the compound 22a, the reaction was incomplete (ca. 7% of 22a was reisolated). g After the formation of benzotriazolyl derivative, solvent was evaporated. Then nucleophile (2 equiv.), and base (2 equiv.) in an appropriate solvent (2 mL) were used. h DBU (4 equiv.), nucleophile (2 equiv.), and appropriate solvent (2 mL) were used. i After the formation of Ŭ6-(benzotriazol-1-yl) derivative, solvent was evaporated. Then NaSMe (2 equiv.) was used in dry DMSO. j A one-step approach was not attempted in this case.

Various 1° and 2° aliphatic amines were used as nucleophiles in both the one-pot, two-step and the one-step methods. In the case of one-pot, two-step method, after formation of benzotriazol-1-yl derivative in step 1, 4 equiv. of the nucleophile was added and reaction was conducted until the completion. All the reactions were completed within 0.5–2 h range and the yields were in 71–84% range with substrates 22a and 43(Table 2). Yields were moderate (53–63%) in the case of the AZT derivative 47 (Table 2). The one-step reactions with aliphatic amines were conducted by reacting the nucleoside with 2 equiv. each of BOP, DBU, and 4 equiv. of the amine. These reactions were very rapid and excellent yields (89–98%) were observed in all reactions. Surprisingly, the reaction of MeNH₂ with thymidine derivative 22a did not go to completion after 45 min, and 7% of 22a was reisolated (entry 1 in Table 2). Prolonging the reaction to 16 h, did not improve the outcome. We then queried if aromatic amines can also be used as nucleophiles under these conditions. We chose p-toluidine and initially tested the one-pot, two-step reaction conditions. This was performed by adding p-toluidine (2 equiv.) and iPr₂NEt (2 equiv.) as the base, to the reaction mixture after formation of Ŭ4-(benzotriazol-1-yl) derivative 40 in step 1 and heating the reaction mixture at 50 °C. However, this did not lead to formation of the desired product 45g. Hence, we decided to modify the conditions in the step 2. On the basis of our previous observations, nucleophilic displacement reactions of Ŭ6-(benzotriazol-1-yl) purine nucleosides with p-toluidine and iPr₂NEt, requires a polar solvent like
Hence, we conducted a reaction under similar conditions; by evaporating the solvent after step 1, addition of 2 mL of EtOH, followed by the addition of \( p \)-toluidine (2 equiv.) and iPr\(_2\)NEt (2 equiv.). Heating the resultant reaction mixture at 50 °C for 16 h gave the desired product 45g in 62% yield (entry 15). The one-pot reaction was conducted by refluxing thymidine derivative 22a with BOP (2 equiv.), DBU (4 equiv.) and \( p \)-toluidine (2 equiv) in CH\(_3\)CN for 12 h (entry 15). Desired product 45g was isolated in 67% yield. Similarly, product 46e was synthesized from 2’-deoxyuridine derivative 43 in both the one-pot, two-step, and the one-step methods in yields of 64% and 69% (entry 16). The one-step method seems to be superior to the one-pot, two-step method when aliphatic amines are used as nucleophiles, in terms of both reaction times and yield, especially in the case of AZT derivative 47. Only a slight advantage was observed in the one-step method with \( p \)-toluidine was used as nucleophile.

Next, we wanted to test if thiols would be as good nucleophiles as amines under the standard conditions. However, reaction of \( O^4 \)-(benzotriazol-1-yl) derivative 40 with 2 equiv. each of EtSH and Cs\(_2\)CO\(_3\) in THF was slow and produced the desired product 45h along with multiple byproducts. Reactions of thiols with \( O^6 \)-(benzotriazol-1-yl) purine nucleosides are typically conducted by using Cs\(_2\)CO\(_3\) as base and DME as solvent.\(^{37,42}\) Hence, after the formation of \( O^4 \)-(benzotriazol-1-yl) derivative 40 in step 1 from thymidine derivative 22a, the solvent was evaporated, 2 mL of DME was added, followed by the addition of 2 equiv. each of EtSH and Cs\(_2\)CO\(_3\) (entry 17). After 3.5 h, the product 45h was isolated in 68% yield. For the one-step method, the reaction conditions were slightly modified from the standard conditions. The reaction of 22a was conducted with BOP (2 equiv.), DBU (4 equiv.) and EtSH (2 equiv.) in DME as solvent at room temperature (entry 17). This reaction yielded the product 45h in 3 h in 76% yield. The \( N^4 \)-thiobenzyl product 45i was also synthesized in the above manner via the one-
pot, two-step and the one-step methods in 70% and 82% yield, respectively (entry 18). However, the reaction with MeSNa under the one-pot, two-step reaction conditions used in entry 13, didn’t yield product 45j, due to insolubility of MeSNa in DME. A similar result was also observed when the reaction was conducted in THF as solvent. Hence, to improve the solubility of MeSNa, we decided to conduct step 2 reactions in various polar solvents like CH$_3$CN, DMF, DMSO, and EtOH. Reactions of O$^4$-(benzotriazol-1-yl) derivative 40 with 2 equiv. of MeSNa in CH$_3$CN or in DMF at 50 °C, did not reach completion. When the same reaction was conducted in DMSO at 50 °C over 2 h, the product 45j was isolated in a moderate 49% yield along with two other minor byproducts (entry 19). After comparing the polarity of the byproducts with 40 by TLC and careful evaluation by $^1$H NMR and mass spectrometry, the two byproducts were identified as compounds 50 and 51 (Figure 3). Trace amounts of the compounds 50 and 51 were also observed to form in the reactions conducted in CH$_3$CN and DMF as solvents. However, no attempt was made to isolate the byproducts in these cases. The yield of the product 45j did not improve even after increasing the amount of MeSNa from 2 equiv. to 4 equiv. in a reaction conducted with DMSO as solvent. In the reaction conducted at room temperature with EtOH as solvent, a new product 52 (Figure 3) was formed exclusively in 12 h in 73% yield. It is possible that 52 can form either by displacing BtO$^-$ from the O$^4$-(benzotriazol-1-yl) intermediate 40 or MeS$^-$ from 45j by EtOH. It is well known that 4-methylthiopyrimidine nucleoside derivatives like 45j are good electrophilic nucleosides and easily undergo addition-elimination reactions with nucleophiles.$^{4,9,10}$ A one-step approach for the synthesis of the compound 45j was not attempted. One other important observation that was made during the isolation of the N$^4$-alkylthio derivatives 45h–j is that they decompose on silica. Particularly, compound 45j
decomposes faster than the compounds 45h and 45i. This issue was resolved by neutralizing the silica gel with 5% Et₃N in hexanes prior to loading the crude material.

**Figure 3.** Unusual products furnished during the reaction of O⁴-(benzotriazol-1-yl) derivative 40 with NaSMe

Intrigued with the formation of the byproducts 50 and 51, we then focused on investigating the source of their formation. The N-oxide 50 could arise by the rearrangement of the isomeric O⁴-(benzotriazol-1-yl) derivative 40. Reese *et al.* observed a similar case previously during their studies on activating the amide linkage in 3’,5’-di-O-acetylthymidine (13a) with 1-hydroxy-1H-benzotriazole, 2-chlorophenyl phosphorodichloridate, and Et₃N.⁵³ The corresponding O⁴-(benzotriazol-1-yl) derivative that resulted from this reaction was isolated. This compound upon heating with 1-methylimidazole in dry pyridine at 50 °C underwent rearrangement to give the corresponding N-oxide derivative. Compound 51 could have originated by either deoxygenation of the N-oxide 50 or the O⁴-(benzotriazol-1-yl) derivative 40. In our recent studies, we have shown that diboron reagents B₂(OH)₄ and (pinB)₂ can easily deoxygenate 1-hydroxy-1H-benzotriazoles and O⁶-(benzotriazol-1-yl)purine nucleosides.⁵⁴,⁵⁵ It is possible that the peptide coupling agent BOP may contain P(III) reagents as contaminants. These species could result the formation of the benzotriazolide derivative 51 by deoxygenating either of 50 or 40. Hence, to assess the role of BOP reagent, we conducted a series of reactions with thymidine derivative 22a,
DBU and BOP from two different sources (Chem-Impex and Aldrich), and the products formed in those reactions were examined (Table 3).

**Table 3. Analysis of the products formed with BOP from two different sources**

<table>
<thead>
<tr>
<th>BOP Supplier</th>
<th>DBU</th>
<th>Time</th>
<th>%Yield</th>
<th>40</th>
<th>50</th>
<th>51</th>
<th>Recovered 22a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chem-Impex</td>
<td>2 equiv.</td>
<td>0.5 h</td>
<td>33.3 %</td>
<td>17.6 %</td>
<td>0.8 %</td>
<td>14.1 %</td>
<td></td>
</tr>
<tr>
<td>(99.7% pure)</td>
<td>2 equiv.</td>
<td>24 h</td>
<td>14.5%</td>
<td>31.6%</td>
<td>9.9%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Aldrich</td>
<td>2 equiv.</td>
<td>0.5 h</td>
<td>58.6%</td>
<td>23.5%</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(97% pure)</td>
<td>2 equiv.</td>
<td>24 h</td>
<td>21.7%</td>
<td>46.1%</td>
<td>3.5%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>4 equiv.</td>
<td>24 h</td>
<td>–</td>
<td>8.4%</td>
<td>25.9%</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Reactions were conducted with 0.212 mmol of 22a, BOP (2 equiv.), DBU in THF (0.5 mL). Purities are as stated by the suppliers. Yields are of isolated and purified products. Compounds 40 and 51 were isolated as a mixture to avoid decomposition of 40 to 22a. The yields were calculated based on relative ratios of H-1’ of 40 and 51 in the 1H NMR.

In the reactions conducted, within 0.5 h, the reaction with BOP from Aldrich showed better results, producing *ca.* 60% of the $O^4$-(benzotriazol-1-yl) derivative 40. Although, the N-oxide derivative 50 was also formed in both reactions in *ca.* 20% yields, the reduced product 51 was observed only in the reaction with BOP from Chem-Impex. When the same reactions were conducted over 24 h, a significant increase in the amounts of the N-oxide derivative 50 and reduced product 51 was observed. However, the amount of the reduced product 51 formed in the reaction with BOP from Chem-Impex was relatively high (*ca.* 10% compared to 3.5% with the Aldrich sample). In order to study the role of DBU in these reactions, we conducted a reaction over 24 h with 4 equiv. of DBU and BOP from Aldrich. Surprisingly, the $O^4$-(benzotriazol-1-yl)
derivative 40 was completely consumed, the amount of the reduced product 51 increased to ca. 26%, and only 8% of the N-oxide product 50 was isolated. Several studies have shown that DBU can act as a good nucleophile, and this usually leads to a caprolactam by ring opening.\textsuperscript{56} Since it is known that the N-oxide type derivative of uridine can also undergo displacement reactions with nucleophiles,\textsuperscript{53} it is plausible that DBU could displace the BtO\textsuperscript{−} by reacting with the compounds 40 and/or 50. The BtO\textsuperscript{−} group thus displaced could undergo deoxygenation with the P(III) contaminants from the BOP reagent to form the benzotriazolide (Bt\textsuperscript{−}). Bt\textsuperscript{−} could then react with any of the electrophilic nucleosides generated in the reaction (40 and/or 50 or even the compounds generated from DBU reacting with 40 and 50) to form compound 51. This hypothesis is in congruence with our recent proposal for the mechanism of the deoxygenation of $O^6$-(benzotriazol-1-yl)purine nucleosides.\textsuperscript{55} Despite the presence of P(III) contaminants in BOP, compounds with azide functionality were unaffected during the course of the reaction.

After analysis of the formation of side products 50 and 51, we conducted another reaction for the structural confirmation of these two compounds by using the conditions used for the deoxygenation of 1-hydroxy-1H-benzotriazoles.\textsuperscript{54} This was done by reacting N-oxide 50 with B\textsubscript{2}(OH)\textsubscript{4} in CH\textsubscript{3}CN at 60 °C for 2 h (Figure 4). This resulted in compound 51 as the product with a high 97% yield.
**Figure 4.** Deoxygenation of the N-oxide derivative 50 to compound 51 with $B_2(OH)_4$. Partial $^1H$ NMR of the compounds 50 (in black) and 51 (in blue). Benzotriazolyl resonances are indicated by an asterisk.

### [1.2.2] Mechanistic Studies

We next focused on investigating the mechanistic pathways for the formation of the $O^4$-(benzotriazol-1-yl) derivative 40 and the N-oxide derivative 50. There are 3 plausible pathways as shown in the Scheme 19. These pathways differ in the mode of attack of amide oxygen of 22a.

**Scheme 19.** Plausible pathways for the formation of $O^4$-(benzotriazol-1-yl) derivative 40 and the N-oxide derivative 50.
on to BOP, after undergoing deprotonation with DBU. Pathway a involves the formation of a phosphonium intermediate 53, where as in pathways b and c, the $O^4$-(benzotriazol-1-yl) derivative 40 is directly produced by displacing HMPA from BOP with attack of the amide oxygen either at the N1 atom of the benzotriazolyl moiety via a $S_N2$ process or at the N3 atom of benzotriazolyl moiety via a $S_N2'$-like process. Phosphonium intermediate 53 can react either with BtO$^-$ to form the compounds 40 and 50 or it can directly react with the nucleophile to give the C-4 modified product.

Unlike in the case of pyrimidine nucleosides, formation of $N$-oxide derivatives was not observed with purine nucleosides. However, formation of phosphonium intermediate was clearly evident by $^{31}P\{^1H\}$ NMR experiments.\textsuperscript{37,42} Although, the $N$-oxide derivative 50 was formed as a minor byproduct in the case of pyrimidine nucleosides, it is observed that the $O^4$-(benzotriazol-1-yl) derivative 40 eventually transforms to the compound 50 over a period of time (Table 3). However, the direct formation of the compound 50 cannot be ruled out. Hence, to probe these mechanistic pathways, we conducted several $^{31}P\{^1H\}$ experiments. In the first experiment, the $^{31}P\{^1H\}$ NMR spectrum of BOP ($d = 43.8$ ppm, PF$_6$\textsuperscript{−} $d = -144.5$ ppm) was recorded in CD$_3$CN at room temperature (Figure 5). No new resonances were observed upon exposing this BOP solution to the compound 22a. However, immediately upon addition of DBU to the mixture, a new resonance corresponding to HMPA ($d = 23.5$ ppm) was observed. Over a period of time, the amount of BOP decreased, while the amount of HMPA formation increased. Despite the support from this experiment for pathways b and c, the formation of a short-lived phosphonium species 53 cannot be excluded. To evaluate if a short-lived species could be detected at a lower temperature, we conducted the same $^{31}P\{^1H\}$ NMR experiment at $-25$ °C. Again, no new resonances could correspond to phosphonium species 53 were observed.
Figure 5. $^{31}P({}^1H)$ NMR studies recorded in CD$_3$CN to observe the reaction between BOP, 22a, and DBU

The rapid formation of HMPA upon the addition of DBU to a mixture of compound 22a and BOP in CD$_3$CN could also result via the direct nucleophilic attack by DBU on BOP. Similarly, in the one-step approach, the presence of nucleophile in the reaction medium could also lead to the formation of HMPA by the reaction of the nucleophile with BOP. To further investigate these, we performed three more experiments in CD$_3$CN at room temperature (i) with BOP and DBU (Panel A, Figure 6), (ii) with BOP and pyrrolidine (Panel B, Figure 6), (iii) with BOP, DBU and pyrrolidine (Panel C, Figure 6).
Figure 6. $^{31}P\{^1H\}$ NMR studies: Panel A shows the time course of the reaction between BOP and DBU, Panel B shows the time course of the reaction between BOP and pyrrolidine, and Panel C shows the time course of the reaction between BOP, DBU, and pyrrolidine.

In the reaction of BOP with DBU, a trace amount of HMPA was observed after 1 h, and it slowly increased over a period of 24 h (Panel A, Figure 6). In the reactions of BOP with pyrrolidine and the combination of DBU and pyrrolidine, relatively more HMPA was observed after 1 h, compared to the reaction of BOP with DBU (Panels B and C, Figure 6). The amount of HMPA increased over a 24 h time period in the latter cases as well. However, the amount of HMPA generated in these three conditions was relatively low when compared to the reaction of BOP and DBU in the presence of the nucleoside 22a. The reaction of BOP, DBU, and the nucleoside 22a in CH$_3$CN is typically faster (0.5 h). On the basis of all these experiments, it appears that the formation of the compounds 40 and 50 can indeed proceed via pathways b and/or c. But results are in contrast to those from the reactions of purine nucleosides, where clear evidence for the formation of phosphonium intermediates was observed.$^{37,42}$

[1.2.3] Biological Studies

The C-4 modified pyrimidine nucleosides were desilylated using standard reaction conditions i.e., n-Bu$_4$NF in THF at room temperature, KF in MeOH at 80 °C, and tris(dimethylamino)sulfonium difluorotrimethylsilicate (TASF) in MeCN from 0 °C to room temperature (Figure 7). Desilylation of compound 45h with KF in MeOH at 80 °C in 12 h,
resulted in \(O^4\)-methylthymidine (54i) in a low 28% yield. This further demonstrates the reactivity of \(N^4\)-alkylthiopyrimidine nucleosides. Hence, compound 45h was desilylated with \(n\)-Bu\(_4\)NF in THF at room temperature and the desired compound 54g was isolated in a good 70% yield. Similarly, the labile 45j was desilylated under mild conditions using TASF in MeCN, to give product 8a in a reasonable 68% yield.

\[\text{Method A} \quad \text{(Using } n\text{-Bu}_4\text{NF/THF)}\]
Figure 7. Compounds synthesized with reaction times, yields using desilylation conditions. Method A. \( n\)-Bu4NF, THF, rt; Method B: KF, MeOH, 80 °C; Method C: TASF, MeCN, 0 °C to rt.

Studies have shown that the presence of an amino group at the 3'-position of several pyrimidine nucleosides led to compounds with anticancer properties.\(^{57-60}\) We wondered, if reduction of the 3’-azido group of the C-4 modified AZT analogues to their corresponding 3’-amino derivatives, would lead to potential anti-cancer agents. Hence, 3’-azido pyrimidine nucleoside analogues 56a–d were reduced with 5% palladium on charcoal and H\(_2\) gas, in MeOH as a solvent, at room temperature, to the corresponding 3’-amino pyrimidine nucleoside analogues 57a–d in excellent yields (Scheme 20).

Scheme 20. Azide reduction of C-4 modified AZT analogues to their corresponding amines

Once all the desired compounds were obtained, they were sent to our collaborators at Rega institute for medical research in Belgium in order to evaluate the biological activities. All the desilylated compounds and the 3’-amino pyrimidine nucleoside analogues were tested for
inhibition of proliferation of murine leukemia (L1210), human cervix carcinoma (HeLa), and human T-lymphocytic (CEM) cell lines (Figure 8). The C-4 modified thymidine derivatives 41, 54a, c, d, f–h showed moderate activity in these assays. Interestingly, the corresponding C-4 modified 2'-deoxyuridine derivatives 33, 55a, b, d did not show any activity. This implies that the methyl group at the 5-position is important to the inhibitory properties of these compounds.
IC\textsubscript{50} = 50% Inhibitory concentration or compound concentration required to inhibit tumor cell proliferation by 50%. \textsuperscript{b} EC\textsubscript{50} = 50% effective concentration or compound concentration required to inhibit virus replication by 50%.

**Figure 8. Compounds that displayed biological activities**

The antiviral activity of the compounds was also studied by testing against various viruses: (a) cytomegalo (CMV), varicella-zoster (VZV), herpes simplex-1 (HSV-1), herpes simplex-2 (HSV-2), vaccinia, and vesicular stomatitis (VSV) in human embryonic lung (HEL) cells, (b) VSV, coxsackie, and respiratory syncytial viruses in human cervix carcinoma (HeLa) cells, (c) parainfluenza-3, reo, sindbis, coxsackie B-4, and punta toro viruses in green monkey kidney (VERO) cells, (d) feline corona and feline herpes in feline kidney (CRFK) cells, and (e) influenza A H1N1, influenza B H3N2, and influenza B in canine kidney (MDCK) cells. Except for the 3’-azido pyrimidine analogues 56a–d, none of the other compounds showed any antiviral activity. The 3’-azido group is necessary for the compounds to exhibit antiviral properties. The diethylamino derivative 56a and the piperidinyl derivative 56c showed comparable activity against HIV-1 and HIV-2. Surprisingly, decreasing ring size from piperidinyl to pyrrolidinyl in compound 56b decreased the activity (Figure 8). Most notably, incorporating an oxygen atom in the six-membered ring as in the morpholinyl derivative 56d, increased activity by ten fold. Surprisingly, the byproduct O\textsuperscript{4}-methylthymidine (54i) obtained from the desilylation of 45h, showed inhibition against HSV-1 Kos strain (EC\textsubscript{50} = 5.4 ± 2.0 µM), while the minimum cytotoxic concentration required to cause a microscopically detectable alteration of normal cell morphology was >100 µM (Figure 8). Interestingly, 54i did not inhibit HSV-2 and an acyclovir-resistant (thymidine-kinase deficient) HSV-1 virus.
CONCLUSION

We established a simple and easy method for the C-4 modification of pyrimidine nucleosides using BOP and DBU. Various amines and thiols were used as nucleophiles. This method involves the formation of $O^4$-(benzotriazol-1-yl)pyrimidine nucleosides derivative as intermediates by the activation of the amide linkage with BOP and DBU. A one-pot, two-step and a one-step approach were studied with various amines and thiols. In most of the cases, the one-step approach was superior to the one-pot, two-step method. Our approach is tolerant of a 3’-azido group in the nucleoside. The $O^4$-(benzotriazol-1-yl)pyrimidine nucleoside transforms into an isomeric N-oxide derivative over extended reaction times, and the 4-(benzotriazol-1-yl)pyrimidine nucleoside derivatives are generated possibly by reduction of the N-oxide with P^{III} species in BOP. Deoxygenation of the N-oxide derivative with B$_2$(OH)$_4$ lead to the corresponding (benzotriazol-1-yl)pyrimidine nucleoside confirming the product structure. Spectroscopic studies further confirmed that these compounds were indeed as anticipated. Mechanism of the reaction was investigated by $^{31}$P{$_1^1$H} NMR experiments. All products were desilylated and the 3’-azido compounds were reduced to the corresponding 3’-amines. Anticancer activity of the resultant compounds was assessed using L1210, HeLa, and CEM cell lines, where some C-4 thymidine derivatives showed moderate activity. Out of all the compounds, 3’-azido pyrimidine nucleosides displayed activity against HIV-1 and HIV-2 and $O^4$-methylthymidine (54i) showed anti-HSV-1 activity. In conclusion, this method provides a simple and broadly applicable approach for accessing diverse C-4 modified pyrimidine nucleosides.
[1.4] EXPERIMENTAL SECTION

General Experimental Considerations. Reactions were conducted in screw-cap glass vials with Teflon-lined caps. Thin-layer chromatography was performed on 200 µm aluminum-foil-backed silica gel plates. Column chromatography was performed using 200–300 mesh silica gel. In some cases the silica gel column had to be flushed twice with 5% Et₃N in hexanes before loading the crude material in order to deactivate silica gel (see details under the compound headings). THF was distilled from LiAlH₄ and then from Na prior to use. CH₃CN and 1,2-dimethoxyethane (DME) were distilled from CaH₂. All other reagents were used as received from commercial suppliers. ¹H NMR spectra were obtained at 500 MHz and are referenced to the residual protonated solvent resonance. ¹³C NMR spectra were obtained at 125 MHz and are referenced to the solvent resonance. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (J) are in hertz (Hz). Standard abbreviations are used to designate resonance multiplicities.

General procedure for the C-4 modification of pyrimidine nucleosides 22a, 43, and 47 (One-pot, two-step Method)

\[
\text{Step 1 of two-step procedure (synthesis of 40, 44, and 48)}
\]
To a 0.424 M solution of the nucleoside derivative (22a: 0.212 mmol, 43: 0.219 mmol, and 47: 0.262 mmol) in THF, BOP (2 equiv.) and DBU (2 equiv.) were added, and the mixture was stirred at room temperature for 30 min.

*Step 2 for aliphatic amines (synthesis of 42, 45a–f, 46a–d, 49a–d)*

To the reaction mixture from step 1, the appropriate amine (4 equiv.) was added and the reaction mixture was stirred at room temperature until consumption of the corresponding benzotriazolyl intermediate (40, 44 or 48) occurred. The mixture was diluted with EtOAc (25 mL) and washed with deionized water (3 x 50 mL) followed by brine (15 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered, and evaporated under reduced pressure. The crude material was purified by chromatography on a silica gel column using a suitable eluting solvent (see individual compound headings below).

\[ 1-(3,5\text{-Di-O-(t-butyldimethylsilyl)}\text{-2-deoxy-β-D-ribofuranosyl)}\text{-4-(morpholin-4-yl)}\text{-5-methyl-2(1H)}\text{-pyrimidinone (42)} \]

Chromatography on a silica gel column by sequential elution with 50% EtOAc in hexanes and EtOAc gave compound 42 (86.9 mg, 76%) as a sticky, white solid. \( R_f (\text{SiO}_2/\text{EtOAc}) = 0.32 \). \(^1\)H NMR (500 MHz, acetone-\( d_6 \)):

\[ \delta 7.66 (s, 1\text{H, H-6}), 6.29 (t, J = 6.7 \text{ Hz, 1H, H-1'}), 4.50 (dt, J = 2.8, 5.8 \text{ Hz, 1H, H-3'}), 3.96 (\text{app q, } J_{\text{app}} \sim 3.2 \text{ Hz, 1H, H-4'}), 3.90 (\text{dd, } J = 3.7, 11.3 \text{ Hz, 1H, H-5'}), 3.86 (\text{dd, } J = 3.6, 11.4 \text{ Hz, 1H, H-5'}), 3.70–3.66 (\text{m, 4H, morpholiny1-CH}_2), 3.65–3.61 (\text{m, 4H, morpholiny1-CH}_2), 2.30 (\text{ddd, } J = 2.9, 5.9, 13.3 \text{ Hz, 1H, H-2'}), 2.15 (\text{m, 4H, Me, and H-2'}), 0.92 and 0.91 (2s, 18H, t-Bu), 0.13 and 0.12 (2s, 6H, SiMe), 0.11 and 0.10 (2s, 6H, SiMe). \(^1\)C NMR (125 MHz, acetone-\( d_6 \)):

\[ \delta 166.9, 156.5, 142.2, 104.8, 88.7, 86.4, 73.3, 67.1, 63.8, 48.3, 42.0, 26.3, \]
26.1, 18.9, 18.5, 18.0, −4.5, −4.7, −5.3. HRMS (ESI/TOF) m/z calculated for C_{26}H_{49}N_{3}O_{5}Si_{2}Na [M + Na]^+ : 562.3103, found 562.3107.

3',5'-Di-O-(t-butyldimethylsilyl)-N,5-dimethyl-2'-deoxycytidine (45a)

Chromatography on a silica gel column by sequential elution with EtOAc and 1% MeOH in EtOAc, gave compound 45a (85.8 mg, 84%) as a white solid. R_f (SiO_2/EtOAc) = 0.17. ^1H NMR (500 MHz, CDCl_3): δ 7.49 (s, 1H, H-6), 6.38 (t, J = 6.5 Hz, 1H, H-1’), 5.25 (br s, 1H, NH), 4.36 (dt, J = 3.3, 6.4 Hz, 3H, NMe), 2.39 (ddd, J = 3.7, 6.1, 13.3 Hz, 1H, H-2’), 1.97 (dt, J = 6.6, 13.3 Hz, 1H, H-2’), 1.88 (s, 3H, Me), 0.92 and 0.88 (2s, 18H, t-Bu), 0.10 and 0.09 (2s, 6H, SiMe), 0.06 and 0.05 (2s, 6H, SiMe). ^13C NMR (125 MHz, CDCl_3): δ 163.8, 156.4, 136.5, 102.1, 87.6, 85.7, 71.9, 62.9, 42.1, 28.4, 26.1, 25.9, 18.5, 18.1, 13.3, −4.5, −4.7, −5.2, −5.3. HRMS (ESI/TOF) m/z calculated for C_{26}H_{49}N_{3}O_{5}Si_{2}Na [M + Na]^+ : 562.3103, found 562.3107.

N-Benzyl-3',5'-di-O-(t-butyldimethylsilyl)-5-methyl-2'-deoxycytidine (45b)

Chromatography on a silica gel column by elution with 50% EtOAc in hexanes gave compound 45b (85.7 mg, 72%) as a white solid. R_f (SiO_2/50% EtOAc in hexanes) = 0.24. ^1H NMR (500 MHz, acetone-d_6): δ 7.54 (s, 1H, H-6), 7.36 (d, J = 7.5 Hz, 2H, Ar-H), 7.28 (t, J = 7.5 Hz, 2H, Ar-H), 7.21 (t, J = 7.3 Hz, 1H, Ar-H), 7.06–6.98 (m, 1H, NH), 6.35 (dd, J = 6.0, 7.7 Hz, 1H, H-1’), 4.70–4.68 (m, 2H, NCH_2), 4.50 (dt, J = 2.8, 5.7 Hz, 1H, H-3’), 3.92 (app q, J_{app} ~ 3.1 Hz, 1H, H-4’), 3.90–3.84 (m, 2H, H-5’), 2.24 (ddd, J = 2.7, 5.8, 13.1 Hz, 1H, H-2’), 2.08–2.04 (m, 1H, H-2’), 1.99 (s, 3H, Me), 0.95 and 0.92 (2s, 18H, t-Bu), 0.15 and 0.14 (2s, 6H, SiMe), 0.13 (s, 6H, SiMe).
Chromatography on a silica gel column by sequential elution with 50% EtOAc in hexanes and EtOAc gave compound 45c (79.9 mg, 76%) as a viscous, colorless liquid. R_f (SiO_2/EtOAc) = 0.35. ^1H NMR (500 MHz, CDCl_3): δ 7.48 (s, 1H, H-6), 6.34 (t, J = 6.4 Hz, 1H, H-1’), 4.35 (dt, J = 3.3, 6.4 Hz, 1H, H-3’), 3.90-3.86 (m, 2H, H-4’ and H-5’), 3.75 (dd, J = 2.5, 11.1 Hz, 1H, H-5’), 3.13 (s, 6H, NMe_2), 2.37 (ddd, J = 3.8, 5.9, 13.3 Hz, 1H, H-2’), 2.13 (s, 3H, Me), 1.95 (dt, J = 6.6, 13.3 Hz, 1H, H-2’), 0.91 and 0.87 (2s, 18H, t-Bu), 0.10 and 0.09 (2s, 6H, SiMe), 0.05 and 0.04 (2s, 6H, SiMe). HRMS (ESI/TOF) m/z calculated for C_{24}H_{47}N_3O_4Si_2Na [M + Na]^+: 520.2997, found 520.2974. The ^1H NMR spectrum matches to that of the authentic material.61

3’,5’-Di-O-(t-butyldimethylsilyl)-N,N,5-trimethyl-2’-deoxycytidine (45d)

Chromatography on a silica gel column by sequential elution with 25% EtOAc in hexanes and 50% EtOAc in hexanes gave compound 45d (86.9 mg, 78%) as a viscous, colorless liquid. R_f (SiO_2/50% EtOAc in hexanes) = 0.24. ^1H NMR (500 MHz, CDCl_3): δ 7.43 (s, 1H, H-6), 6.35 (t, J = 6.5 Hz, 1H, H-1’), 4.37 (dt, J = 3.2, 6.3 Hz, 1H, H-3’), 3.90-3.86 (m, 2H, H-4’ and H-5’), 3.76 (dd, J = 2.0, 11.2 Hz, 1H, H-5’), 3.60–3.52 (m, 4H, NCH_2), 2.38 (ddd, J = 3.8, 5.7, 13.2 Hz, 1H, H-2’), 2.13 (s, 3H, Me), 1.99 (dt, J = 6.7, 13.3 Hz, 1H, H-2’), 1.20 (t, J = 7.0 Hz, 6H, Me), 0.92 and 0.88 (2s, 18H, t-Bu), 0.10 (s, 6H, SiMe), 0.06 and 0.05 (2s, 6H, SiMe). ^13C NMR (125 MHz,
CDCl₃: δ 164.5, 155.4, 140.7, 102.2, 87.7, 85.8, 71.9, 62.9, 43.8, 42.2, 26.2, 26.0, 19.0, 18.6, 18.2, 14.0, –4.3, –4.7, –5.1, –5.2. HRMS (ESI/TOF) m/z calculated for C₂₆H₅₂N₅O₄Si₂ [M + H]⁺: 526.3491, found 526.3499.

1-(3,5-Di-O-(t-butyldimethylsilyl)-2-deoxy-ß-D-ribofuranosyl)-5-methyl-4-(pyrrolidin-1-yl)-2(IH)-pyrimidinone (45e)

Chromatography on a silica gel column by sequential elution with 40% EtOAc in hexanes and 75% EtOAc in hexanes gave compound 45e (83.5 mg, 75%) as a viscous, colorless liquid. Rf (SiO₂/EtOAc) = 0.48. ¹H NMR (500 MHz, CDCl₃): δ 7.38 (s, 1H, H-6), 6.26 (t, J = 6.5 Hz, 1H, H-1’), 4.33 (dt, J = 3.1, 6.1 Hz, 1H, H-3’), 3.86 (app q, Jₚₚptic ~ 2.9 Hz, 1H, H-4’), 3.82 (dd, J = 2.8, 11.3 Hz, 1H, H-5’), 3.73–3.71 (m, 5H, H-5’ and N(CH₂)₂), 2.27 (ddd, J = 3.5, 6.0, 13.3 Hz, 1H, H-2’), 2.16 (s, 3H, Me), 1.95 (dt, J = 6.7, 13.4 Hz, 1H, H-2’), 1.92–1.84 (m, 4H, CH₂), 0.89 and 0.84 (2s, 18H, t-Bu), 0.07 and 0.06 (2s, 6H, SiMe), 0.03 (s, 6H, SiMe). ¹³C NMR (125 MHz, acetone-d₆): δ 163.4, 155.1, 140.1, 102.6, 88.2, 85.9, 73.6, 64.0, 49.7, 41.9, 26.3, 26.2, 25.9 (br), 18.9, 18.6, 18.2, –4.5, –4.6, –5.2, –5.3. HRMS (ESI/TOF) m/z calculated for C₂₆H₄₉N₃O₄Si₂Na [M + Na]⁺: 546.3154, found 546.3159.

1-(3,5-Di-O-(t-butyldimethylsilyl)-2-deoxy-ß-D-ribofuranosyl)-5-methyl-4-(piperidin-1-yl)-2(IH)-pyrimidinone (45f)

Chromatography on a silica gel column by sequential elution with 25% EtOAc in hexanes and 50% EtOAc in hexanes gave compound 45f (85.8 mg, 75%) as a viscous, colorless liquid. Rf (SiO₂/50% EtOAc in hexanes) = 0.28. ¹H NMR (500 MHz, CDCl₃): δ 7.53 (s, 1H, H-6), 6.35 (t, J = 6.5 Hz, 1H, H-1’), 4.36 (dt, J = 3.3, 6.4 Hz, 1H, H-3’), 3.92–3.86 (m, 2H, H-4’ and H-5’),...
3.75 (dd, $J = 1.8$, 10.7 Hz, 1H, H-5’), 3.55–3.50 (br m, 4H, NCH$_2$), 2.39 (ddd, $J = 3.9$, 6.1, 13.3 Hz, 1H, H-2’), 2.06 (s, 3H, Me), 1.99 (dt, $J = 6.6$, 13.3 Hz, 1H, H-2’), 1.69–1.58 (br m, 6H, (CH$_2$)$_3$), 0.92 and 0.88 (2s, 18H, t-Bu), 0.10 and 0.09 (2s, 6H, SiMe), 0.06 and 0.05 (2s, 6H, SiMe).

$^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 166.8, 155.6, 140.8, 103.4, 87.6, 85.8, 71.7, 62.8, 48.4, 42.1, 26.2, 26.1, 25.9, 24.6, 18.5, 18.2, 18.1, –4.4, –4.8, –5.2, –5.3. HRMS (ESI/TOF) $m/z$ calculated for C$_{27}$H$_{51}$N$_3$O$_4$Si$_2$Na [M + Na]$^+$: 560.3310, found 560.3315.

$N$-benzyl-3’,5’-Di-$O$-($t$-butyldimethylsilyl)-2’-deoxycytidine (46a)

Chromatography on a silica gel column by sequential elution with 25% EtOAc in hexanes and 60% EtOAc in hexanes gave compound 46a (93.6 mg, 78%) as a viscous, colorless liquid. $R_f$ (SiO$_2$/50% EtOAc in hexanes) = 0.20. $^1$H NMR (500 MHz, acetone-$_d_6$): $\delta$ 7.83 (d, $J = 7.5$ Hz, 1H, H-6), 7.36 (d, $J = 7.2$ Hz, 2H, Ar-H), 7.31 (t, $J = 7.5$ Hz, 2H, Ar-H), 7.24 (t, $J = 7.2$ Hz, 1H, Ar-H), 7.18–7.17 (br t, $J = 6.0$ Hz, 1H, NH), 6.30 (t, $J = 6.3$ Hz, 1H, H-1’), 5.83 (d, $J = 7.5$ Hz, 1H, H-5), 4.63–4.60 (m, 2H, NCH$_2$), 4.52 (dt, $J = 3.7$, 6.0 Hz, 1H, H-3’), 3.92–3.89 (m, 2H, H-4’ and H-5’), 3.87–3.84 (m, 1H, H-2’), 2.30 (ddd, $J = 4.2$, 6.1, 13.2 Hz, 1H, H-2’), 2.11 (dt, $J = 6.5$, 13.1 Hz, 1H, H-2’), 0.94 and 0.92 (2s, 18H, t-Bu), 0.13 (s, 12H, SiMe). $^{13}$C NMR (125 MHz, acetone-$_d_6$): $\delta$ 164.7, 156.4, 140.5, 140.0, 129.1, 128.6, 127.7, 95.5, 88.0, 86.2, 72.5, 63.4, 44.6, 42.2, 26.3, 26.2, 18.9, 18.5, –4.4, –4.6, –5.2, –5.3. HRMS (ESI/TOF) $m/z$ calculated for C$_{28}$H$_{47}$N$_3$O$_4$Si$_2$Na [M + Na]$^+$: 568.2997, found 568.2996.

$1$-(3,5-Di-$O$-($t$-butyldimethylsilyl)-2-deoxy-$\beta$-d-ribofuranosyl)-$4$-(pyrrolidin-1-yl)-$2$(1$H$)-pyrimidinone (46b)
Chromatography on a silica gel column by sequential elution with 50% EtOAc in hexanes and EtOAc, gave compound 46b (79.7 mg, 71%) as a white solid. \( R_f \) (SiO\(_2\)/EtOAc) = 0.23. \(^1\)H NMR (500 MHz, CDCl\(_3\)): \( \delta \) 7.94 (d, \( J = 7.6 \) Hz, 1H, H-6), 6.30 (t, \( J = 5.7 \) Hz, 1H, H-1’), 5.60 (d, \( J = 7.6 \) Hz, 1H, H-5), 4.36 (app q, \( J_{\text{app}} \approx 5.7 \) Hz, 1H, H-3’), 3.91 (dd, \( J = 2.4, 11.3 \) Hz, 1H, H-5’), 3.66 (t, \( J = 6.8 \) Hz, 2H, NCH\(_2\)), 3.38 (t, \( J = 6.8 \) Hz, 2H, NCH\(_2\)), 2.40–2.06 (m, 1H, H-2’), 2.04–1.97 (m, 2H, CH\(_2\)), 1.96–1.87 (m, 2H, CH\(_2\)), 0.92 and 0.86 (2s, 18H, t-Bu), 0.10 and 0.09 (2s, 6H, SiMe), 0.04 and 0.03 (2s, 6H, SiMe). \(^{13}\)C NMR (125 MHz, acetone-d\(_6\)): \( \delta \) 162.3, 155.7, 141.2, 93.0, 88.2, 86.3, 72.8, 63.6, 47.0 (br), HRMS (ESI/TOF) \( m/z \) calculated for C\(_{25}\)H\(_{47}\)N\(_3\)O\(_4\)Si\(_2\)Na [M + Na]\(^+\): 532.2997, found 532.2994.

1-(3,5-Di-O-(t-butyldimethylsilyl)-2-deoxy-β-D-ribofuranosyl)-4-(piperidin-1-yl)-2(1H)-pyrimidinone (46c)

Chromatography on a silica gel column by sequential elution with 30% EtOAc in hexanes and 60% EtOAc in hexanes, gave compound 46c (86.4 mg, 75%) as a white solid. \( R_f \) (SiO\(_2\)/100% EtOAc) = 0.57. \(^1\)H NMR (500 MHz, acetone-d\(_6\)): \( \delta \) 7.91 (d, \( J = 7.8 \) Hz, 1H, H-6), 6.30 (t, \( J = 6.4 \) Hz, 1H, H-1’), 6.28 (d, \( J = 7.8 \) Hz, 1H, H-5), 4.52 (dt, \( J = 3.2, 6.1 \) Hz, 1H, H-3’), 3.94–3.88 (m, 3H, H-4’ and H-5’), 3.85 (dd, \( J = 2.9, 11.2 \) Hz, 1H, H-5’), 3.81–3.49 (br m, 4H, NCH\(_2\)), 2.30 (ddd, \( J = 3.9, 6.0, 13.2 \) Hz, 1H, H-2’), 2.21 (dt, \( J = 6.5, 13.2 \) Hz, 1H, H-2’), 1.71–1.64 (m, 2H, CH\(_2\)), 1.59–1.51 (br m, 4H, CH\(_2\)), 0.93 and 0.91 (2s, 18H, t-Bu), 0.13 (s, 6H, SiMe), 0.12 and 0.11 (2s, 6H, SiMe). \(^{13}\)C NMR (125 MHz, acetone-d\(_6\)): \( \delta \) 163.1, 156.7, 141.7, 92.4, 88.3, 86.3, 72.8, 63.6, 47.0 (br), 43
45.2 (br), 42.2, 26.6 (br), 26.3, 26.1, 25.1, 18.9, 18.5, –4.5, –4.6, –5.2, –5.3. HRMS (ESI/TOF) m/z calculated for C_{26}H_{49}N_{3}O_{4}Si_{2}Na [M + Na]^+: 546.3154, found 546.3159.

1-(3,5-Di-O-(t-butyldimethylsilyl)-2-deoxy-β-D-ribofuranosyl)-4-(morpholin-4-yl)-2(IH)-pyrimidinone (46d)

Chromatography on a silica gel column by sequential elution with 50% EtOAc in hexanes followed by EtOAc, gave compound 46d (87.5 mg, 76%) as a colorless liquid. R_f (SiO_2/EtOAc) = 0.48. ^1H NMR (500 MHz, acetone-d_6): δ 7.93 (d, J = 7.8 Hz, 1H, H-6), 6.27 (t, J = 6.3 Hz, 1H, H-1'), 6.01 (d, J = 7.8 Hz, 1H, H-5), 4.51 (dt, J = 3.5, 5.7 Hz, 1H, H-3'), 3.93–3.89 (m, 2H, H-4' and H-5'), 3.85 (dd, J = 2.4, 10.8 Hz, 1H, H-5'), 3.66 (br s, 8H, O(CH_2)_2 and N(CH_2)_2), 2.31 (ddd, J = 4.1, 6.0, 13.2 Hz, 1H, H-2'), 2.18 (dt, J = 6.5, 13.1 Hz, 1H, H-2'), 0.94 and 0.92 (2s, 18H, t-Bu), 0.13 and 0.12 (2s, 12H, SiMe). ^13C NMR (125 MHz, CDCl_3): δ 164.0, 155.3, 142.1, 91.2, 88.3, 86.4, 72.8, 67.1, 63.6, 45.2 (br), 42.4, 26.4, 26.2, 18.9, 18.6, –4.4, –4.6, –5.3. HRMS (ESI/TOF) m/z calculated for C_{25}H_{47}N_{3}O_{5}Si_{2}Na [M + Na]^+: 548.2946, found 548.2950.

3'-Azido-5'-O-(t-butyldimethylsilyl)-N,N-diethyl-5-methyl-2',3'-dideoxycytidine (49a)

Chromatography on a silica gel column by sequential elution with 25% EtOAc in hexanes and 75% EtOAc in hexanes gave compound 49a (65.4 mg, 57%) as colorless, viscous liquid. R_f (SiO_2/EtOAc) = 0.35. ^1H NMR (500 MHz, acetone-d_6): δ 7.52 (s, 1H, H-6), 6.18 (t, J = 6.5 Hz, 1H, H-1'), 4.43 (dt, J = 3.8, 7.3 Hz, 1H, H-3'), 4.00–3.94 (m, 2H, H-4' and H-5'), 3.92 (dd, J = 3.4, 11.1 Hz, 1H, H-5'), 3.58 (q, J = 7.0 Hz, 4H, NCH_2), 2.43 (ddd, J = 4.6, 6.1, 13.6 Hz, 1H, H-2'), 2.34 (dt, J = 6.9, 13.7 Hz, 1H, H-2'), 2.19 (s, 3H, Me), 1.19 (t, J = 7.0 Hz, 6H, Me),
0.94 (s, 9H, t-Bu), 0.15 (s, 6H, SiMe). $^{13}$C NMR (125 MHz, acetone-$d_6$): δ 165.0, 155.2, 141.6, 102.7, 86.1, 85.2, 64.0, 62.1, 44.2, 38.6, 26.3, 18.9, 18.8, 14.0, -5.2, -5.3. HRMS (ESI/TOF) $m/z$ calculated for C$_{20}$H$_{38}$N$_6$O$_3$SiNa [M + Na]$^+$: 459.2510, found 459.2519.

1-(3-Azido-5-O-($t$-butyldimethylsilyl)-2,3-dideoxy-$\beta$-D-ribofuranosyl)-5-methyl-4-\(1H\)-pyrimidinone (49b)

Chromatography on a silica gel column using EtOAc gave compound 49b (60.4 mg, 53%) as a viscous, colorless liquid. R$_f$ (SiO$_2$/EtOAc) = 0.27. $^1$H NMR (500 MHz, acetone-$d_6$): δ 7.43 (s, 1H, H-6), 6.19 (t, $J = 6.6$ Hz, 1H, H-1’), 4.42 (dt, $J = 3.8$, 7.4 Hz, 1H, H-3’), 3.97–3.90 (m, 3H, H-4’, H-5’, and H-5’), 3.73–3.63 (br m, 4H, NCH$_2$), 2.39 (ddd, $J = 4.4$, 6.3, 13.6 Hz, 1H, H-2’), 2.30 (dt, $J = 6.9$, 13.7 Hz, 1H, H-2’), 2.20 (d, $J = 0.9$ Hz, 3H, Me), 1.93-1.85 (m, 4H, CH$_2$), 0.94 (s, 9H, t-Bu), 0.15 (s, 6H, SiMe). $^{13}$C NMR (125 MHz, acetone-$d_6$): δ 163.6, 154.9, 140.2, 102.7, 85.9, 85.0, 64.1, 62.1, 49.8, 38.5, 26.3, 25.9 (br), 18.9, 18.2, -5.2, -5.3. HRMS (ESI/TOF) $m/z$ calculated for C$_{20}$H$_{34}$N$_6$O$_3$SiNa [M + Na]$^+$: 457.2354, found 457.2365.

1-(3-Azido-5-O-($t$-butyldimethylsilyl)-2,3-dideoxy-$\beta$-D-ribofuranosyl)-5-methyl-4-(piperidin-1-yl)-2\(1H\)-pyrimidinone (49c)

Chromatography on a silica gel column by sequential elution with 50% EtOAc in hexanes and EtOAc gave compound 49c (74.1 mg, 63%) as a colorless, viscous liquid. R$_f$ (SiO$_2$/EtOAc) = 0.55. $^1$H NMR (500 MHz, acetone-$d_6$): δ 7.57 (d, $J = 0.7$ Hz, 1H, H-6), 6.16 (t, $J = 6.5$ Hz, 1H, H-1’), 4.43 (dt, $J = 4.1$, 7.2 Hz, 1H, H-3’), 4.00–3.95 (m, 2H, H-4’ and H-5’), 3.92 (dd, $J = 3.3$, 11.2 Hz, 1H, H-5), 3.51 (t, $J = 5.2$ Hz, 4H, NCH$_2$), 2.44 (ddd, $J = 4.5$, 6.3, 13.7 Hz, 1H, H-2’), 2.33 (dt, $J
\[ \text{CH}_2 \text{CH}_2 \text{CH}_2 \text{SiMe}_3 \] 0.15 (s, 6H, SiMe). \[ ^{13}C \text{NMR (125 MHz, acetone-}d_6\text{)}: \delta 167.4, 155.1, 141.9, 103.6, 86.2, 85.2, 64.0, 62.0, 48.8, 38.7, 26.7, 26.3, 25.3, 18.9, 18.0, –5.2, –5.3. \] HRMS (ESI/TOF) \textit{m/z} calculated for C_{21}H_{36}O_3N_6SiNa [M + Na]^+: 471.2510, found 471.2506.

\[ \text{1-(3-Azido-5-O-(t-butyldimethylsilyl)-2,3-dideoxy-\beta-D-ribofuranosyl)-5-methyl-4-}
\text{(morpholin-4-yl)-2(1H)-pyrimidinone (49d)} \]

Chromatography on a silica gel column by sequential elution with 50% EtOAc in hexanes and EtOAc, gave compound 49d (67.3 mg, 57%) as a colorless liquid. \[ R_f (\text{SiO}_2/\text{EtOAc}) = 0.27. \] \[ ^{1}H \text{NMR (500 MHz, acetone-}d_6\text{): } \delta 7.62 \text{ (s, 1H, H-6), 6.15 (t, } J = 6.4 \text{ Hz, 1H, H-1’), 4.43 (dt, } J = 4.3, 7.1 \text{ Hz, 1H, H-3’), 4.01 (app q, } J_{\text{app}} = 3.7 \text{ Hz, 1H, H-4’), 3.97 (dd, } J = 3.5, 11.3 \text{ Hz, 1H, H-5’), 3.93 (dd, } J = 3.4, 11.3 \text{ Hz, 1H, H-5), 3.73–3.65 (m, 4H, morpholinyl–CH}_2\text{), 3.62–3.52 (m, 4H, morpholinyl–CH}_2\text{), 2.47 (ddd, } J = 4.5, 6.3, 13.7 \text{ Hz, 1H, H-2’), 2.34 (dt, } J = 6.9, 13.7 \text{ Hz, 1H, H-2’), 2.11 (s, 3H, Me), 0.94 (s, 9H, t-Bu), 0.15 (s, 6H, SiMe). \] \[ ^{13}C \text{NMR (125 MHz, acetone-}d_6\text{): } \delta 167.5, 155.0, 142.3, 103.5, 86.4, 85.4, 67.3, 64.0, 62.0, 48.4, 38.7, 26.3, 18.9, 17.8, –5.2. \] HRMS (ESI/TOF) \textit{m/z} calculated for C_{20}H_{34}O_4N_6SiNa [M + Na]^+: 473.2303, found 473.2302.

\textbf{Step 2 for \textit{p}-toluidine (synthesis of 45g and 46e)}

The reaction mixture from step 1 was evaporated on a rotary evaporator and dried under high vacuum for 10 min. The residual material was dissolved in EtOH (2 mL), \textit{p}-toluidine (2 equiv.) and iPr\textsubscript{2}NEt (2 equiv.) were added, and the mixture was stirred at 50 °C for 16 h. The reaction mixture was cooled then diluted with EtOAc (25 mL), washed with deionized water (3 x 50 mL), and brine (15 mL). The organic layer was dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}, filtered, and evaporated.
under reduced pressure. The crude material was purified by chromatography on a silica gel column using a suitable eluting solvent (see individual compound headings below).

3',5'-Di-O-(t-butyldimethylsilyl)-5-methyl-N-(p-tolyl)-2'-deoxycytidine (45g)

Chromatography on a silica gel column by sequential elution with 15% EtOAc in hexanes and 40% EtOAc in hexanes, gave compound 45g (73.6 mg, 62%) as a light-brown solid. R_f (SiO_2/25% EtOAc in hexanes) = 0.14. ^1H NMR (500 MHz, CDCl_3): δ 7.54 (br s, 3H, H-6, Ar-H), 7.36 (d, J = 8.1 Hz, 2H, Ar-H), 6.67–6.40 (m, 1H, NH), 6.34 (t, J = 6.4 Hz, 1H, H-1'), 4.37 (dt, J = 3.3, 6.3 Hz, 1H, H-3'), 3.92–3.89 (m, 2H, H-4' and H-5'), 3.77 (dd, J = 2.3, 11.2 Hz, 1H, H-5'), 2.42 (br s, 1H, H-1'), 2.33 (s, 3H, ArMe), 2.03–1.98 (m, 4H, H-2', Me), 0.93 and 0.89 (2s, 18H, t-Bu), 0.12 and 0.11 (2s, 6H, SiMe), 0.07 and 0.06 (2s, 6H, SiMe). ^13C NMR (125 MHz, CDCl_3): δ 161.2, 155.6, 137.9, 135.8, 134.1, 129.6, 121.9, 102.2, 87.7, 86.0, 71.8, 62.9, 42.1, 26.1, 25.9, 21.0, 18.6, 18.2, 13.8, −4.4, −4.7, −5.2. HRMS (ESI/TOF) m/z calculated for C_{29}H_{50}N_{3}O_{4}Si_{2} [M + H]^+: 560.3334, found 560.3311.

3',5'-Di-O-(t-butyldimethylsilyl)-N-(p-tolyl)-2'-deoxycytidine (46e)

Chromatography on a silica gel column by sequential elution with 20% EtOAc in hexanes and 40% EtOAc in hexanes gave compound 46e (74.1 mg, 64%), as a light-brown solid. R_f (SiO_2/50% EtOAc in hexanes) = 0.24. ^1H NMR (500 MHz, CDCl_3): δ 8.46–8.08 (br, 1H, NH), 8.19 (d, J = 7.4 Hz, 1H, H-6), 7.52–7.22 (br m, 2H, Ar-H), 7.22–7.11 (m, 2H, Ar-H), 6.32–6.27 (m, 1H, H-1'), 5.88 (d, J = 7.4 Hz, 1H, H-5), 4.41–4.34 (m, 1H, H-3'), 3.94–3.87 (m, 2H, H-4' and H-5'), 3.77 (d, J = 11.5 Hz, 1H, H-5'), 2.49–2.40 (m, 1H, H-
2’), 2.34 (s, 3H, ArMe), 2.16-2.08 (m, H-2), 0.90 and 0.88 (2s, 18H, t-Bu), 0.09 (s, 6H, SiMe), 0.06 (s, 6H, SiMe). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 163.9 (br), 155.8 (br), 141.7 (br), 135.3 (br), 130.0, 124.1 (br), 92.1 (br), 87.5, 86.2, 70.4, 62.1, 42.3, 26.1, 25.9, 21.1, 18.5, 18.1, –4.4, –4.8, –5.3, –5.4. HRMS (ESI/TOF) m/z calculated for C$_{28}$H$_{48}$N$_3$O$_4$Si$_2$ [M + H]$^+$: 546.3178, found 546.3173.

**Step 2 for thiols (synthesis of 45h and 45i)**

The reaction mixture from step 1 was evaporated on a rotary evaporator and dried under high vacuum for 10 min. The residual material was dissolved in dry DME (2 mL), the appropriate thiol (0.425 mmol, 2 equiv.) and Cs$_2$CO$_3$ (138.1 mg, 0.425 mmol, 2 equiv.) were added, and the mixture was stirred at room temperature until consumption of 40 occurred. The reaction mixture was then diluted with EtOAc (25 mL), washed with deionized water (3 x 50 mL), and brine (15 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and evaporated under reduced pressure. The crude material was purified by chromatography on a neutralized silica gel column using a suitable eluting solvent (see individual compound headings below and the General Experimental Considerations).

3’,5’-Di-O-(t-butyldimethylsilyl)-5’-ethyl-4-thiothymidine (45h)

Chromatography on a neutralized silica gel column by sequential elution with 5% EtOAc in hexanes and 10% EtOAc in hexanes gave compound 45h (74.4 mg, 68%) as a white solid. R$_f$ (SiO$_2$/50% EtOAc in hexanes) = 0.75. $^1$H NMR (500 MHz, acetone-$d_6$): $\delta$ 7.73 (d, $J$ = 0.4 Hz, 1H, H-6), 6.20 (t, $J$ = 6.6 Hz, 1H, H-1’), 4.52 (dt, $J$ = 2.9, 5.8 Hz, 1H, H-3’), 4.02 (app q, $J_{app}$ ~ 3.1 Hz, 1H, H-4’), 3.93 (dd, $J$ = 3.6, 11.4 Hz, 1H, H-5’), 3.89 (dd, $J$ = 3.3, 11.4 Hz, 1H, H-5’), 3.15 (q, $J$ =
7.4 Hz, 2H, SCH₂) 2.43 (ddd, J = 3.0, 5.9, 13.3 Hz, 1H, H-2’), 2.15 (ddd, J = 6.1, 7.2, 13.3 Hz, 1H, H-2’), 1.99 (d, J = 0.6 Hz, 3H, Me), 1.31 (t, J = 7.3 Hz, 3H, Me), 0.93 and 0.92 (2s, 18H, t-Bu), 0.14 (2s, 6H, SiMe), 0.13 (2s, 6H, SiMe). ¹³C NMR (125 MHz, acetone-d₆): δ 177.6, 153.5, 138.4, 111.2, 88.9, 87.3, 73.3, 63.7, 42.5, 26.3, 26.2, 24.3, 18.9, 18.5, 14.5, 14.3, –4.5, –4.7, –5.3. HRMS (ESI/TOF) m/z calculated for C₂₄H₄₆N₂O₄SSi₂Na [M + Na]^+: 537.2609, found 537.2611.

3’,5’-Di-O-(t-butyldimethylsilyl)-S⁴-benzyl-4-thiothymidine (45i)

Chromatography on a neutralized silica gel column by sequential elution with 5% EtOAc in hexanes, 7% EtOAc in hexanes, and 10% EtOAc in hexanes gave compound 45i (85.6 mg, 70%) as a white solid. Rf (SiO₂/25% EtOAc in hexanes) = 0.57. ¹H NMR (500 MHz, acetone-d₆): δ 7.77 (s, 1H, H-6), 7.46 (d, J = 7.2 Hz, 2H, Ar-H), 7.31 (t, J = 7.4 Hz, 2H, Ar-H), 7.25 (t, J = 7.3 Hz, 1H, Ar-H), 6.23 (t, J = 6.6 Hz, 1H, H-1’), 4.53 (dt, J = 2.9, 5.8 Hz, 1H, H-3’), 4.45 (s, 2H, -SCH₂), 4.03 (app q, Japp ~ 3.2 Hz, 1H, H-4’), 3.94 (dd, J = 3.6, 11.4 Hz, 1H, H-5’), 3.89 (dd, J = 3.3, 11.4 Hz, 1H, H-5’), 2.45 (ddd, J = 3.1, 5.9, 13.3 Hz, 1H, H-2’), 2.17 (ddd, J = 6.2, 7.1, 13.3 Hz, 1H, H-2’), 1.99 (s, 3H, Me), 0.93 (2s, 18H, t-Bu), 0.14 (2s, 12H, SiMe). ¹³C NMR (125 MHz, acetone-d₆): δ 177.1, 153.5, 138.9, 138.3, 130.1, 129.3, 128.0, 111.1, 89.1, 87.5, 73.4, 63.8, 42.5, 34.1, 26.3, 26.2, 18.9, 18.5, 14.3, –4.4, –4.6, –5.2. HRMS (ESI/TOF) m/z calculated for C₂₉H₄₈N₂O₄SSi₂Na [M + Na]^+: 599.2766, found 599.2764.

Step 2 for NaSMe (synthesis of 45j)

The reaction mixture from step 1 was evaporated on a rotary evaporator and dried under high vacuum for 10 min. The residual material was dissolved in dry DMSO (1 mL) and NaSMe (29.7 mg, 2 equiv.) was added, and the mixture and stirred at 50 °C for 2 h. The reaction mixture was
then diluted with EtOAc (25 mL), washed with deionized water (3 x 50 mL), and brine (15 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and evaporated under reduced pressure. The crude material was purified on a neutralized silica gel column by sequential elution with 10% EtOAc in hexanes, 15% EtOAc in hexanes, 20% EtOAc in hexanes, and 50% EtOAc in hexanes to give: 51 (6.5 mg, 5%) as an off-white solid, 45j (52.4 mg, 49%) as a colorless, viscous oil, and 50 (15.4 mg, 12%) as an off-white solid.

$3',5'$-Di-O-(t-butyldimethylsilyl)-5'$-$methyl-4-thiothymidine (45j)

\[ \text{Rf} (\text{SiO}_2/50\% \text{EtOAc in hexanes}) = 0.67. \]

$^1$H NMR (500 MHz, CDCl$_3$): δ 7.67 (s, 1H, H-6), 6.27 (t, $J = 6.3$ Hz, 1H, H-1$'$), 4.36 (dt, $J = 3.6, 6.4$ Hz, 1H, H-3$'$), 3.96 (app q, $J_{app} \sim 3.0$ Hz, 1H, H-4$'$), 3.91 (dd, $J = 2.6, 11.4$ Hz, 1H, H-5$'$), 3.77 (dd, $J = 2.5, 11.4$ Hz, 1H, H-5$'$), 2.56 (s, 3H, SMe), 2.51 (ddd, $J = 4.0, 6.2, 13.4$ Hz, 1H, H-2$'$), 2.04-1.99 (m, 4H, H-2$'$, Me), 0.91 and 0.89 (2s, 18H, t-Bu), 0.11 and 0.10 (2s, 6H, SiMe), 0.07 and 0.06 (2s, 6H, SiMe).

$^{13}$C NMR (125 MHz, CDCl$_3$): δ 178.4, 154.0, 136.9, 111.7, 88.2, 86.8, 71.6, 62.7, 42.5, 26.1, 26.0, 18.6, 18.2, 14.5, 13.3, –4.3, –4.7, –5.2. HRMS (ESI/TOF) $m/z$ calculated for C$_{23}$H$_{45}$N$_2$O$_4$SSi$_2$ [M + H]$^+$: 501.2633, found 501.2631.

1-(3,5-Di-O-(t-butyldimethylsilyl)-2-deoxy-$\beta$-D-ribofuranosyl)-5-methyl-4-(3-oxido-1H-benzotriazol-1-yl)-2(1H)-pyrimidinone (50)

$\text{Rf} (\text{SiO}_2/50\% \text{EtOAc in hexanes}) = 0.32. \]

$^1$H NMR (500 MHz, CDCl$_3$): δ 8.88 (d, $J = 8.6$ Hz, 1H, Ar-H), 8.19 (s, 1H, H-6), 8.02 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.75 (t, $J = 7.8$ Hz, 1H, Ar-H), 7.55 (t, $J = 7.7$ Hz, 1H, Ar-H), 6.30 (t, $J = 6.3$ Hz, 1H, H-1$'$), 4.40 (dt, $J = 3.1, 6.1$ Hz, 1H, H-3$'$), 4.07 (app q, $J_{app} \sim 2.8$ Hz, 1H, H-4$'$), 3.95 (dd, $J = 2.5, 11.5$ Hz, 1H, H-5$'$), 3.80 (dd, $J = 2.4, 11.5$ Hz,
1H, H-5’), 2.64 (ddd, J = 3.6, 6.1, 13.5 Hz, 1H, H-2’), 2.50 (s, 3H, Me), 2.09 (dt, J = 6.5, 13.3 Hz, 1H, H-2’), 0.91 and 0.90 (2s, 18H, t-Bu), 0.12 and 0.11 (2s, 6H, SiMe), 0.09 and 0.08 (2s, 6H, SiMe). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 158.5, 154.0, 146.0, 133.9, 132.5, 131.9, 126.8, 118.6, 115.3, 105.3, 89.0, 87.9, 72.0, 62.9, 42.8, 26.1, 25.9, 18.6, 18.4, 18.2, –4.3, –4.7, –5.2. HRMS (ESI/TOF) m/z calculated for C$_{28}$H$_{46}$N$_5$O$_5$Si$_2$ [M + H]$^+$: 588.3032, found 588.3032.

4-(1H-Benzotriazol-1-yl)-1-(3,5-di-O-(t-butyldimethylsilyl)-2-deoxy-β-D-ribofuranosyl)-5-methyl-2(1H)-pyrimidinone (51)

$R_f$ (SiO$_2$/20% EtOAc in hexanes) = 0.50. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.16 (s, 1H, H-6), 8.07 (d, J = 8.4 Hz, 1H, Ar-H), 7.52 (t, J = 7.5 Hz, 1H, Ar-H), 7.44–7.40 (m, 2H, Ar-H), 6.19 (t, J = 6.2 Hz, 1H, H-1’), 4.38 (dt, J = 3.4, 6.3 Hz, 1H, H-3’), 4.00 (app q, $J_{app}$ ~ 2.9 Hz, 1H, H-4’), 3.96 (dd, J = 2.2, 11.5 Hz, 1H, H-5’), 3.80 (dd, J = 2.0, 11.5 Hz, 1H, H-5’), 2.51 (ddd, J = 4.1, 6.0, 13.4 Hz, 1H, H-2’), 2.28 (s, 3H, Me), 2.02 (dt, J = 6.4, 13.2 Hz, 1H, H-2’), 0.96 and 0.88 (2s, 18H, t-Bu), 0.16 and 0.14 (2s, 6H, SiMe), 0.07 (s, 6H, SiMe). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 169.1, 154.0, 144.1, 143.8, 129.1, 128.9, 125.0, 120.8, 108.9, 100.7, 88.6, 87.6, 71.6, 62.7, 42.7, 26.2, 26.0, 18.7, 18.2, 12.2, –4.3, –4.7, –5.1. HRMS (ESI/TOF) m/z calculated for C$_{28}$H$_{46}$N$_5$O$_5$Si$_2$ [M + H]$^+$: 588.3032, found 588.3018.

General procedure for the C-4 modification of pyrimidine nucleosides 22a, 43, and 47
(One-step method)
Procedure for aliphatic amines (synthesis of 42, 45a–f, 46a–d, 49a–d)

To a 0.424 M solution of the nucleoside derivative (22a: 0.212 mmol, 43: 0.219 mmol, 47: 0.262 mmol) in THF, BOP (2 equiv.) and DBU (2 equiv.) were added, and the mixture was stirred at room temperature for 5 min. Then an appropriate amine (4 equiv.) was added to the mixture and the stirring was continued at room temperature until the consumption of the starting material was observed. The mixture was then diluted with EtOAc (25 mL), washed with deionized water (3 x 50 mL), and brine (15 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered, and evaporated under reduced pressure. The crude material was purified by chromatography on a silica gel column using a suitable eluting solvent (see individual compound headings above).

3',5'-Di-O-(t-butyldimethylsilyl)-N,5-dimethyl-2’-deoxycytidine (45a)

Chromatography on a silica gel column by sequential elution with EtOAc and 1% MeOH in EtOAc gave compound 45a (92.6 mg, 90%) as a white solid, and the starting material 22a (7 mg, 7%).

Procedure for reaction with p-toluidine (synthesis of 45g and 46e)

To a solution of the nucleoside derivative (22a: 0.212 mmol, 43: 0.219 mmol) in dry CH₃CN (2 mL), BOP (2 equiv.) and DBU (4 equiv.) were added, and the mixture was stirred at room temperature for 5 min. Then p-toluidine (2 equiv.) was added and the mixture was stirred at
50 °C for 16 h. The mixture was then diluted with EtOAc (25 mL), washed with deionized water (3 x 50 mL), and brine (15 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered, and evaporated under reduced pressure. The crude material was purified by chromatography on a silica gel column using a suitable eluting solvent (see individual compound headings above).

*Procedure for reactions with thiols (synthesis of 45i–j)*

To a solution of nucleoside derivative 22a (100 mg, 0.212 mmol, 1 equiv.) in dry DME (2 mL), BOP (188 mg, 0.425 mmol, 2 mol equiv) and DBU (127 µL, 0.850 mmol, 4 mol equiv) were added, and the mixture was stirred at room temperature for 5 min. Then an appropriate thiol (0.425 mmol, 2 equiv.) was added to the mixture and the stirring was continued at room temperature for 3 h. The reaction mixture was then diluted with EtOAc (25 mL), washed with deionized water (3 x 50 mL), and brine (15 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered, and evaporated under reduced pressure. The crude material was purified by chromatography on a neutralized silica gel column using a suitable eluting solvent (see individual compound headings above).

*Undesired 3’,5’-di-O-(t-butyldimethylsilyl)-O⁴-ethylthymidine (52) obtained in a reaction with NaSMe*

To a solution of the nucleoside derivative 22a (100 mg, 0.212 mmol, 1 equiv.) in THF (0.5 mL), BOP (187.5 mg, 0.424 mmol, 2 mol equiv) and DBU (64 µL, 0.424 mmol, 2 mol equiv) were
added, and the mixture was stirred at room temperature for 30 min. The mixture was evaporated on a rotary evaporator and dried under high vacuum for 10 min. The residual material was then dissolved in dry EtOH (1 mL), NaSMe (59.4 mg, 0.848 mmol, 4 equiv.) was added, and the mixture was stirred at room temperature for 12 h. The mixture was evaporated and the crude material was loaded onto a silica gel column. Sequential elution with 15% hexanes in EtOAc and 30% hexanes in EtOAc gave compound 52 (77.5 mg, 73%) as a colorless, viscous liquid. \( R_f \) (SiO\(_2\)/50% EtOAc in hexanes) = 0.68. \(^1\)H NMR (500 MHz, CDCl\(_3\)): \( \delta \) 7.69 (s, 1H, H-6), 6.32 (t, \( J = 6.4 \) Hz, 1H, H-1’), 4.42 (t, \( J = 7.1 \) Hz, 2H, OCH\(_2\)), 4.35 (dt, \( J = 3.3, 6.3 \) Hz, 1H, H-3’), 3.91 (app q, \( J_{app} \approx 2.8 \) Hz, 1H, H-4’), 3.88 (dd, \( J = 2.6, 11.3 \) Hz, 1H, H-5’), 3.74 (dd, \( J = 2.5, 11.3 \) Hz, 1H, H-5’), 2.42 (ddd, \( J = 3.7, 6.1, 13.3 \) Hz, 1H, H-2’), 1.97 (dt, \( J = 6.6, 13.3 \) Hz, 1H, H-2’), 1.90 (s, 3H, Me), 1.33 (q, \( J = 7.1 \) Hz, 3H, Me), 0.89 and 0.86 (2s, 18H, t-Bu), 0.09 and 0.08 (2s, 6H, SiMe), 0.04 and 0.03 (2s, 6H, SiMe). \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \( \delta \) 170.5, 156.1, 139.4, 104.5, 88.0, 86.4, 71.8, 63.3, 62.8, 42.4, 26.0, 25.9, 18.5, 18.2, 14.4, 12.5, –4.4, –4.7, –5.2. HRMS (ESI/TOF) m/z calculated for C\(_{24}\)H\(_{46}\)N\(_2\)O\(_5\)Si\(_2\)Na [M + Na]\(^+\): 521.2837, found 521.2858.

**General procedure for the evaluation of various products formed with BOP from two commercial suppliers**

![Chemical structures](image-url)
To a solution of 22a (100.0 mg, 0.212 mmol, 1 equiv.) in dry THF (0.5 mL), BOP (188.0 mg, 0.424 mmol, 2 equiv.) and DBU were added, and the mixture was stirred at room temperature for a certain period of time.

Reactions with BOP from Chem-Impex

Conditions 1. DBU (64 µL, 0.424 mmol, 2 equiv.) was used. The reaction mixture was stirred for 0.5 h and then evaporated. Chromatography through a plug of neutralized silica gel by initial elution with 15% EtOAc in hexanes gave compound 51 (1.0 mg, 1%) as a white solid. Subsequent elution with 25% EtOAc in hexanes gave a mixture of compounds 40 (39.1 mg, 31%) and 22a (14.1 mg, 14%) as a white, sticky solid. Further elution with 50% EtOAc in hexanes gave compound 50 (21.9 mg, 18%) as a white solid. Yields of 40 and 22a were calculated on the basis of the integration in the 1H NMR spectrum of the mixture.

Conditions 2. DBU (64 µL, 0.424 mmol, 2 equiv.) was used. The reaction mixture was stirred for 24 h and then evaporated. Chromatography through a plug of neutralized silica gel by initial elution with 30% EtOAc in hexanes gave compound 40 (73.0 mg, 59%) as a white solid.

Reactions with BOP from Sigma-Aldrich

Conditions 1. DBU (64 µL, 0.424 mmol, 2 equiv.) was used. The reaction mixture was stirred for 0.5 h and then evaporated. Chromatography through a plug of neutralized silica gel by initial elution with 30% EtOAc in hexanes gave compound 40 (73.0 mg, 59%) as a white solid.
Subsequent elution with 50% EtOAc in hexanes gave compound 50 (29.3 mg, 23%) as a white solid.

\[ O^1(1H-\text{Benzotriazol}-1-yl)-3',5'-\text{di-O-(t-butyldimethylsilyl)}\text{thymidine} (40) \]

![Chemical Structure](image)

\[ R_f (\text{SiO}_2/20\% \text{EtOAc in hexanes}) = 0.50. \]

\[
^1\text{H NMR (500 MHz, CDCl}_3): \delta \\
8.16 (s, 1H, H-6), 8.07 (d, J = 8.4 Hz, 1H, Ar-H), 7.52 (t, J = 7.5 Hz, 1H, Ar-H), 7.44–7.40 (m, 2H, Ar-H), 6.19 (t, J = 6.2 Hz, 1H, H-1’), 4.38 (dt, J = 3.4, 6.3 Hz, 1H, H-3’), 4.00 (app q, \text{J}_{app} \sim 2.9 Hz, 1H, H-4’), 3.96 (dd, J = 2.2, 11.5 Hz, 1H, H-5’), 3.80 (dd, J = 2.0, 11.5 Hz, 1H, H-5’), 2.51 (ddd, J = 4.1, 6.0, 13.4 Hz, 1H, H-2’), 2.28 (s, 3H, Me), 2.02 (dt, J = 6.4, 13.2 Hz, 1H, H-2’), 0.96 and 0.88 (2s, 18H, t-Bu), 0.16 and 0.14 (2s, 6H, SiMe), 0.07 (s, 6H, SiMe).
\]

\[
^{13}\text{C NMR (125 MHz, CDCl}_3): \delta 169.1, 154.0, 144.1, 143.8, 129.1, 128.9, 125.0, 120.8, 108.9, 100.7, 88.6, 87.6, 71.6, 62.7, 42.7, 26.2, 26.0, 18.7, 18.2, 12.2, –4.3, –4.7, –5.1. \]

HRMS (ESI/TOF) \text{m/z} calculated for C\text{_{28}}H\text{_{46}}N\text{_{5}}O\text{_{5}}Si\text{_{2}} [M + H]^+: 588.3032, found 588.3018.

**Conditions 2.** DBU (64 \mu L, 0.424 mmol, 2 equiv.) was used. The reaction mixture was stirred for 24 h and then evaporated. Chromatography through a plug of neutralized silica gel by initial elution with 30% EtOAc in hexanes gave a mixture of compounds 51 (4.2 mg, 3%) and 40 (27.0 mg, 22%) as a white solid. Subsequent elution with 50% EtOAc in hexanes gave compound 50 (57.4 mg, 46%) as a white solid. Yields of 51 and 40 were calculated on the basis of the integration in \(^1\text{H NMR spectrum of the mixture.}\)

**Conditions 3.** DBU (127 \mu L, 0.424 mmol, 4 equiv.) was used. The reaction was stirred for 24 h and then evaporated. Chromatography through a plug of neutralized silica gel by initial elution with 30% EtOAc in hexanes gave a mixture of compounds 51 (31.4 mg, 26%) as a white solid.
Subsequent elution with 50% EtOAc in hexanes gave compound 50 (10.5 mg, 8%) as a white solid.

**Procedure for the deoxygenation of 50 to 51 with B$_2$(OH)$_4$**

To a solution of N-oxide 50 (20 mg, 0.034 mmol, 1 mol equiv) in dry MeCN (0.2 mL) B$_2$(OH)$_4$ (3.7 mg, 0.041 mmol, 1.2 equiv.) was added, and the mixture was stirred at 60 °C for 2 h. The mixture was then evaporated and the crude material was purified on a silica gel column eluted with 30% EtOAc in hexanes to give compound 51 (18.8 mg, 97%) as a white solid.

**Procedure for desilylation reactions**

**Method A** *(with nBu$_4$NF/THF for synthesis of 41, 54a–h, 55a–d, 56a–d)*

To a 0.115 M solution of the appropriate nucleoside (1 equiv.) in dry THF, nBu$_4$NF (1 M solution in THF, 2.4 equiv.) was added, and the mixture was stirred at room temperature for 20 min. The mixture was evaporated and the crude material was purified by chromatography on a
short silica gel column using a suitable eluting solvent (see individual compound headings below)

**1-(2-Deoxy-β-D-ribofuranosyl)-5-methyl-4-(morpholin-4-yl)-2(1H)-pyrimidinone (41)**

Chromatography on a silica gel column by sequential elution with EtOAc and 10% MeOH in EtOAc gave compound 41 (83.4 mg, 96%) as a white solid. R_f (SiO_2/10% MeOH in EtOAc) = 0.18. 1H NMR (500 MHz, CD_3OD): δ 7.98 (s, 1H, H-6), 6.26 (t, J = 6.4 Hz, 1H, H-1’), 4.41 (dt, J = 3.7, 6.4 Hz, 1H, H-3’), 3.96 (app q, J_app ~ 3.5 Hz, 1H, H-4’), 3.85 (dd, J = 3.1, 12.1 Hz, 1H, H-5’), 3.78–3.75 (m, 5H, morpholinyl–CH_2 and H-5’), 3.72–3.70 (m, 4H, morpholinyl–CH_2), 2.39 (ddd, J = 4.0, 6.1, 13.5 Hz, 1H, H-2’), 2.21–2.14 (m, 4H, Me and H-2’). 13C NMR (125 MHz, CD_3OD): δ 167.4, 157.5, 143.6, 105.8, 88.9, 87.6, 71.8, 67.8, 62.6, 48.8, 42.1, 17.9. HRMS (ESI/TOF) m/z calculated for C_{14}H_{21}N_{3}O_{5}Na [M + Na]^+: 334.1373, found 334.1366.

**N-Benzyl-5-methyl-2’-deoxycytidine (54a)**

Chromatography on a silica gel column by sequential elution with EtOAc and 10% MeOH in EtOAc gave compound 54a (54.2 mg, 92%) as a white solid. R_f (SiO_2/10% MeOH in EtOAc) = 0.24. 1H NMR (500 MHz, CD_3OD): δ 7.82 (s, 1H, H-6), 7.35 (d, J = 7.1 Hz, 2H, Ar-H), 7.31 (t, J = 7.5 Hz, 2H, Ar-H), 7.23 (t, J = 7.2 Hz, 1H, Ar-H), 6.30 (t, J = 6.5 Hz, 1H, H-1’), 4.71 (s, 2H, CH_2), 4.40 (dt, J = 3.3, 6.4 Hz, 1H, H-3’), 3.94 (app q, J_app ~ 3.4 Hz, 1H, H-4’), 3.84 (dd, J = 3.1, 12.1 Hz, 1H, H-5’), 3.76 (dd, J = 3.8, 12.1 Hz, 1H, H-5’), 2.34 (ddd, J = 3.8, 5.9, 13.5 Hz, 1H, H-2’), 2.17 (dt, J = 6.7, 13.5 Hz, 1H, H-2’), 2.00 (s, 3H, Me). 13C NMR (125 MHz, CD_3OD): δ 164.9, 158.6, 140.3, 138.9, 129.3,
128.6, 128.0, 104.9, 88.7, 87.3, 72.0, 62.8, 45.1, 41.9, 13.2. HRMS (ESI/TOF) m/z calculated for C₁₇H₂₁N₃O₄Na [M + Na]^+: 354.1424, found 354.1440.

\( \text{N,N,5-Trimethyl-2'-deoxycytidine (54b)} \)

Chromatography on a silica gel column by sequential elution with EtOAc and 20% MeOH in EtOAc gave compound 54b (51.5 mg, 96%) as an off-white solid. \( R_f \) (SiO₂/10% MeOH in EtOAc) = 0.10. \(^1\)H NMR (500 MHz, CD₃OD): \( \delta \) 7.81 (s, 1H, H-6), 6.23 (t, \( J = 6.5 \) Hz, 1H, H-1’), 4.38 (dt, \( J = 3.4, 6.5 \) Hz, 1H, H-3’), 3.92 (app q, \( J_{app} \sim 3.5 \) Hz, 1H, H-4’), 3.81 (dd, \( J = 3.2, 12.0 \) Hz, 1H, H-5’), 3.73 (dd, \( J = 3.7, 12.0 \) Hz, 1H, H-5’), 3.21 (s, 6H, NMe₂), 2.32 (ddd, \( J = 3.8, 6.1, 13.5 \) Hz, 1H, H-2’), 2.25 (s, 3H, Me), 2.15 (dt, \( J = 6.7, 13.5 \) Hz, 1H, H-2’). \(^{13}\)C NMR (125 MHz, CD₃OD): \( \delta \) 166.9, 157.4, 142.3, 105.2, 88.8, 87.3, 71.9, 62.7, 41.9, 40.6, 18.7. HRMS (ESI/TOF) m/z calculated for C₁₂H₁₉N₃O₄Na [M + Na]^+: 292.1268, found 292.1272.

\( \text{N,N-Diethyl-5-methyl-2'-deoxycytidine (54c)} \)

Chromatography on a silica gel column by sequential elution with EtOAc and 10% MeOH in EtOAc gave compound 54c (81.1 mg, 96%) as a white solid. \( R_f \) (SiO₂/10% MeOH in EtOAc) = 0.20. \(^1\)H NMR (500 MHz, CD₃OD): \( \delta \) 7.83 (s, 1H, H-6), 6.26 (t, \( J = 6.5 \) Hz, 1H, H-1’), 4.41 (dt, \( J = 3.4, 6.5 \) Hz, 1H, H-3’), 3.95 (app q, \( J_{app} \sim 3.5 \) Hz, 1H, H-4’), 3.84 (dd, \( J = 3.1, 12.0 \) Hz, 1H, H-5’), 3.76 (dd, \( J = 3.7, 12.0 \) Hz, 1H, H-5’), 3.66 (q, \( J = 7.0 \) Hz, 4H, NCH₂), 2.35 (ddd, \( J = 3.8, 6.1, 13.5 \) Hz, 1H, H-2’), 2.25 (s, 3H, Me), 2.19 (dt, \( J = 6.7, 13.5 \) Hz, 1H, H-2’), 1.26 (t, \( J = 7.0 \) Hz, 6H, Me). \(^{13}\)C NMR (125 MHz, CD₃OD): \( \delta \) 165.1, 157.4, 142.6, 104.8, 88.8, 87.3, 71.9, 62.7, 45.0, 41.9, 18.7, 14.1. HRMS (ESI/TOF) m/z calculated for C₁₄H₂₃N₃O₄Na [M + Na]^+: 320.1581, found 320.1568.
1-(2-Deoxy-β-D-ribofuranosyl)-5-methyl-4-(pyrrolidin-1-yl)-2(1H)-pyrimidinone (54d)

Chromatography on a silica gel column by sequential elution with EtOAc and 20% MeOH in EtOAc gave compound 54d (54.6 mg, 97%) as an off-white solid. Rf (SiO2/10% MeOH in EtOAc) = 0.12. 1H NMR (500 MHz, CD3OD): δ 7.75 (s, 1H, H-6), 6.24 (t, J = 6.6 Hz, 1H, H-1’), 4.38 (dt, J = 3.4, 6.5 Hz, 1H, H-3’), 3.91 (app q, Japp ~ 3.4 Hz, 1H, H-4’), 3.81 (dd, J = 3.1, 12.0 Hz, 1H, H-5’), 3.78-3.72 (m, 5H, H-5’ and NCH2), 2.30 (ddd, J = 3.8, 6.1, 13.5 Hz, 1H, H-2’), 2.25 (s, 3H, Me), 2.17–2.12 (m, 1H, H-2’), 1.93 (br s, 4H, (CH2)2). 13C NMR (125 MHz, CD3OD): δ 163.9, 157.6, 141.2, 105.2, 88.7, 87.2, 72.0, 62.8, 50.6, 41.9, 26.1, 18.1. HRMS (ESI/TOF) m/z calculated for C14H21N3O4Na [M + Na]+: 318.1424, found 318.1403.

1-(2-Deoxy-β-D-ribofuranosyl)-5-methyl-4-(piperidin-1-yl)-2(1H)-pyrimidinone (54e)

Chromatography on a silica gel column by sequential elution with EtOAc and 10% MeOH in EtOAc, gave compound 54e (53.9 mg, 94%) as a white solid. Rf (SiO2/10% MeOH in EtOAc) = 0.20. 1H NMR (500 MHz, CD3OD): δ 7.90 (s, 1H, H-6), 6.26 (t, J = 6.5 Hz, 1H, H-1’), 4.41 (dt, J = 3.5, 6.6 Hz, 1H, H-3’), 3.95 (app q, Japp ~ 3.5 Hz, 1H, H-4’), 3.85 (dd, J = 3.2, 12.1 Hz, 1H, H-5’), 3.76 (dd, J = 3.7, 12.1 Hz, 1H, H-5’), 3.68–3.64 (m, 4H, NCH2), 2.37 (ddd, J = 3.9, 6.1, 13.5 Hz, 1H, H-2’), 2.21–2.15 (m, 4H, Me and H-2’), 1.77–1.72 (m, 2H, CH2), 1.71–1.66 (m, 4H, CH2). 13C NMR (125 MHz, CD3OD): δ 167.2, 153.8, 143.1, 105.9, 88.9, 87.4, 71.9, 62.7, 49.4, 42.0, 27.3, 25.5, 18.2. HRMS (ESI/TOF) m/z calculated for C15H23N3O4Na [M + Na]+: 332.1581, found 332.1584.

5-Methyl-N-(p-tolyl)-2’-deoxycytidine (54f)
Chromatography on a silica gel column by sequential elution with EtOAc and 10% MeOH in EtOAc gave compound **54f** (83.2 mg, 94%) as an off-white solid. Rf (SiO2/10% MeOH in EtOAc) = 0.30. $^1$H NMR (500 MHz, CD$_3$OD): $\delta$ 7.90 (s, 1H, H-6), 7.52 (d, J = 8.3 Hz, 2H, Ar-H), 7.12 (d, J = 8.2 Hz, 2H, Ar-H), 6.26 (t, J = 6.5 Hz, 1H, H-1’), 4.38 (dt, J = 3.4, 6.6 Hz, 1H, H-3’), 3.93 (app q, $J_{app}$ ~ 3.5 Hz, 1H, H-4’), 3.83 (dd, J = 3.2, 12.1 Hz, 1H, H-5’), 3.74 (dd, J = 3.8, 12.1 Hz, 1H, H-5’), 2.34 (ddd, J = 3.9, 6.1, 13.5 Hz, 1H, H-2’), 2.30 (s, 3H, Me), 2.19 (dt, J = 6.7, 13.5 Hz, 1H, H-2’), 2.08 (s, 3H, Me). $^{13}$C NMR (125 MHz, CD$_3$OD): $\delta$ 163.5, 158.3, 139.8, 136.9, 135.6, 130.0, 124.5, 105.5, 88.8, 87.5, 71.9, 62.7, 42.0, 21.0, 13.6. HRMS (ESI/TOF) m/z calculated for C$_{17}$H$_{22}$N$_3$O$_4$ [M + H]$^+$: 332.1605, found 332.1604.

**S'4-Ethyl-4-thiothymidine (54g)**

Chromatography on a neutralized silica gel column by sequential elution with EtOAc and 5% MeOH in EtOAc gave compound **54g** (58.7 mg, 70%) as a white solid. Rf (SiO2/10% MeOH in EtOAc) = 0.36. $^1$H NMR (500 MHz, CD$_3$OD): $\delta$ 8.09 (s, 1H, H-6), 6.20 (t, J = 6.2 Hz, 1H, H-1’), 4.38 (dt, J = 3.6, 6.6 Hz, 1H, H-3’), 3.98 (app q, $J_{app}$ ~ 3.5 Hz, 1H, H-4’), 3.85 (dd, J = 2.9, 12.2 Hz, 1H, H-5’), 3.75 (dd, J = 3.6, 12.2 Hz, 1H, H-5’), 3.20 (q, J = 7.4 Hz, 2H, SCH$_2$), 2.46 (ddd, J = 4.5, 6.0, 13.6 Hz, 1H, H-2’), 2.16 (dt, J = 6.6, 13.4 Hz, 1H, H-2’), 2.04 (s, 3H, Me), 1.35 (t, J = 7.4 Hz, 3H, Me). $^{13}$C NMR (125 MHz, CD$_3$OD): $\delta$ 179.9, 156.0, 139.4, 114.2, 89.3, 88.3, 71.5, 62.4, 42.4, 25.1, 14.5, 14.2. HRMS (ESI/TOF) m/z calculated for C$_{12}$H$_{18}$N$_2$O$_4$SNa [M + Na]$^+$: 309.0879, found 309.0866.

**S'4-Benzyl-4-thiothymidine (54h)**
Chromatography on neutralized silica gel column by sequential elution with EtOAc and 5% MeOH in EtOAc, gave compound 54h (95.7 mg, 79%) as a white solid. R$_f$ (SiO$_2$/10% MeOH in EtOAc) = 0.37. $^1$H NMR (500 MHz, CD$_3$OD): δ 8.13 (s, 1H, H-6), 7.42 (d, J = 7.9 Hz, 2H, Ar-H), 7.30 (t, J = 7.3 Hz, 2H, Ar-H), 7.24 (t, J = 7.1 Hz, 1H, Ar-H), 7.24 (t, J = 7.1 Hz, 1H, Ar-H), 6.22 (t, J = 6.2 Hz, 1H, H-1'), 4.47 (s, 2H, SCH$_2$), 4.38 (dt, J = 4.0, 5.7 Hz, 1H, H-3'), 3.98 (app q, $J_{app}$ ~ 3.5 Hz, 1H, H-4'), 3.85 (dd, J = 12.2 Hz, 1H, H-5'), 3.75 (dd, J = 3.7, 12.2 Hz, 1H, H-5'), 2.48 (ddd, J = 4.4, 6.2, 13.6 Hz, 1H, H-2'), 2.18 (dt, J = 6.6, 13.4 Hz, 1H, H-2'), 2.04 (s, 3H, Me). $^{13}$C NMR (125 MHz, CD$_3$OD): δ 179.1, 155.9, 139.8, 138.3, 129.6, 128.4, 113.9, 89.2, 88.3, 71.5, 62.3, 42.4, 34.7, 14.1. HRMS (ESI/TOF) m/z calculated for C$_{17}$H$_{20}$N$_2$O$_4$SNa [M + Na]$^+$: 371.1036, found 371.1035.

$\text{N-Benzyl-2'\textquotesingle-deoxycytidine (55a)}$

Chromatography on a silica gel column by sequential elution with EtOAc and 10% MeOH in EtOAc gave compound 55a (53.6 mg, 92%) as a white solid. R$_f$ (SiO$_2$/10% MeOH in EtOAc) = 0.24. $^1$H NMR (500 MHz, CD$_3$OD): δ 7.91 (d, J = 7.5 Hz, 1H, H-6), 7.34–7.28 (m, 4H, Ar-H), 7.25–7.22 (m, 1H, Ar-H), 6.27 (t, J = 6.5 Hz, 1H, H-1'), 5.90 (d, J = 7.5 Hz, 1H, H-5), 4.59–4.56 (m, 2H, NCH$_2$), 4.36 (dt, J = 3.3, 6.4 Hz, 1H, H-3'), 3.93 (app q, $J_{app}$ ~ 3.6 Hz, 1H, H-4'), 3.78 (dd, J = 3.3, 12.0 Hz, 1H, H-5'), 3.72 (dd, J = 3.9, 12.0 Hz, 1H, H-5'), 2.34 (ddd, J = 3.7, 6.1, 13.5 Hz, 1H, H-2'), 2.13 (dt, J = 6.7, 13.5 Hz, 1H, H-2'). $^{13}$C NMR (125 MHz, CD$_3$OD): δ 165.4, 158.7, 141.3, 139.7, 129.5, 128.8, 128.3, 96.9, 88.8, 87.5, 72.1, 62.8, 45.2, 41.9. HRMS (ESI/TOF) m/z calculated for C$_{16}$H$_{19}$N$_3$O$_4$Na [M + Na]$^+$: 340.1268, found 340.1265.

1-(2-Deoxy-$\beta$-D-ribofuranosyl)-4-(pyrrolidin-1-yl)-2(1H)-pyrimidinone (55b)
Chromatography on a silica gel column by sequential elution with EtOAc and 10% MeOH in EtOAc gave compound 55b (72.1 mg, 87%) as a white solid. R_f (SiO_2/20% MeOH in EtOAc) = 0.22. \(^1\)H NMR (500 MHz, CD_3OD): \(\delta\) 8.01 (d, \(J = 7.6\) Hz, 1H, H-6), 6.26 (t, \(J = 6.5\) Hz, 1H, H-1’), 5.96 (d, \(J = 7.7\) Hz, 1H, H-5), 4.36 (dt, \(J = 3.3, 6.4\) Hz, 1H, H-3’), 3.93 (app q, \(J_{app} \sim 3.5\) Hz, 1H, H-4’), 3.79 (dd, \(J = 3.2, 12.0\) Hz, 1H, H-5’), 3.72 (dd, \(J = 3.8, 12.0\) Hz, 1H, H-5’), 3.56 (t, \(J = 6.8\) Hz, 2H, NCHH), 3.46 (t, \(J = 6.8\) Hz, 2H, NCHH), 3.23 (ddd, \(J = 4.0, 5.9, 13.5\) Hz, 1H, H-2’), 2.03 (dt, \(J = 6.6, 13.4\) Hz, 1H, H-2’), 1.95 (quint, \(J = 6.7\) Hz, 2H, CH_2). \(^{13}\)C NMR (125 MHz, CD_3OD): \(\delta\) 162.8, 158.0, 142.0, 95.0, 88.8, 87.5, 72.0, 62.8, 48.2, 48.1, 42.1, 26.5, 25.7. HRMS (ESI/TOF) \(m/z\) calculated for C_{13}H_{19}N_3O_4Na [M + Na]^+: 304.1268, found 304.1272.

1-(2-Deoxy-\(\beta\)-D-ribofuranosyl)-4-(piperidin-1-yl)-2(1H)-pyrimidinone (55c)

Chromatography on a silica gel column by sequential elution with EtOAc and 10% MeOH in EtOAc gave compound 55c (52.1 mg, 90%) as a white solid. R_f (SiO_2/20% MeOH in EtOAc) = 0.17. \(^1\)H NMR (500 MHz, CD_3OD): \(\delta\) 8.00 (d, \(J = 7.9\) Hz, 1H, H-6), 6.24 (t, \(J = 6.5\) Hz, 1H, H-1’), 6.21 (d, \(J = 7.9\) Hz, 1H, H-5), 4.36 (dt, \(J = 3.4, 6.5\) Hz, 1H, H-3’), 3.93 (app q, \(J_{app} \sim 3.5\) Hz, 1H, H-4’), 3.89–3.69 (m, 2H, NCHH), 3.79 (dd, \(J = 3.3, 12.0\) Hz, 1H, H-5’), 3.72 (dd, \(J = 3.7, 12.1\) Hz, 1H, H-5’), 3.67–3.55 (m, 2H, NCHH), 2.35 (ddd, \(J = 3.8, 6.1, 13.5\) Hz, 1H, H-2’), 2.35 (dt, \(J = 6.7, 13.5\) Hz, 1H, H-2’), 1.76–1.69 (m, 2H, CH_2), 1.65–1.57 (m, 4H, CH_2). \(^{13}\)C NMR (125 MHz, CD_3OD): \(\delta\) 163.8, 158.3 (br), 142.5, 93.6, 88.8, 87.5, 72.1, 62.8, 47.8 (br), 47.8 (br), 42.0, 27.0 (br), 25.5. HRMS (ESI/TOF) \(m/z\) calculated for C_{14}H_{21}N_3O_4Na [M + Na]^+: 318.1424, found 318.1449.

1-(2-Deoxy-\(\beta\)-D-ribofuranosyl)-4-(morpholin-4-yl)-2(1H)-pyrimidinone (55d)
Chromatography on a silica gel column by sequential elution with EtOAc followed by 15% MeOH in EtOAc gave compound 55d (54.2 mg, 93%) as a white solid. \( R_f \) (SiO\(_2\)/10% MeOH in EtOAc) = 0.12. \(^1\)H NMR (500 MHz, CD\(_3\)OD): \( \delta \) 8.08 (d, \( J = 7.8 \) Hz, 1H, H-6), 6.24 (t, \( J = 6.4 \) Hz, 1H, H-1’), 6.20 (d, \( J = 7.8 \) Hz, 1H, H-5), 4.36 (dt, \( J = 3.4 \), 6.4 Hz, 1H, H-3’), 3.94 (app q, \( J_{app} \approx 3.5 \) Hz, 1H, H-4’), 3.79 (dd, \( J = 3.3 \), 12.0 Hz, 1H, H-5’), 3.74–3.55 (m, 9H, H-5’ and morpholinyl-CH\(_2\)), 2.37 (ddd, \( J = 3.9 \), 6.1, 13.5 Hz, 1H, H-2’), 2.14 (dt, \( J = 6.7 \), 13.4 Hz, 1H, H-2’). \(^{13}\)C NMR (125 MHz, CD\(_3\)OD): \( \delta \) 164.5, 158.1, 143.0, 93.3, 88.9, 87.7, 72.0, 67.5, 62.7, 46.6 (br), 45.1 (br), 42.1. HRMS (ESI/TOF) \( m/z \) calculated for C\(_{13}\)H\(_{19}\)N\(_3\)O\(_5\)Na [M + Na]\(^+\): 320.1217, found 320.1213.

3’-Azido-N,N-diethyl-5-methyl-2’,3’-dideoxycytidine (56a)

Chromatography on a silica gel column by sequential elution with EtOAc and 10% MeOH in EtOAc gave compound 56a (71.0 mg, 96%) as an off-white solid. \( R_f \) (SiO\(_2\)/10% MeOH in EtOAc) = 0.59. \(^1\)H NMR (500 MHz, CD\(_3\)OD): \( \delta \) 7.80 (s, 1H, H-6), 6.13 (t, \( J = 6.2 \) Hz, 1H, H-1’), 4.32 (app q, \( J_{app} \approx 6.1 \) Hz, 1H, H-3’), 3.92 (dt, \( J = 3.2 \), 4.9 Hz, 1H, H-4’), 3.86 (dd, \( J = 3.1 \), 12.2 Hz, 1H, H-5’), 3.75 (dd, \( J = 3.2 \), 12.2 Hz, 1H, H-5’), 3.63 (q, \( J = 7.0 \) Hz, 4H, NCH\(_2\)), 2.44 (dt, \( J = 6.4 \), 13.2 Hz, 1H, H-2’), 2.36 (dt, \( J = 6.7 \), 13.5 Hz, 1H, H-2’), 2.22 (s, 3H, Me), 1.23 (t, \( J = 7.0 \) Hz, 6H, Me). \(^{13}\)C NMR (125 MHz, CD\(_3\)OD): \( \delta \) 165.2, 157.3, 142.5, 104.8, 87.0, 86.2, 62.3, 61.4, 45.0, 39.0, 18.7, 14.1. HRMS (ESI/TOF) \( m/z \) calculated for C\(_{14}\)H\(_{22}\)N\(_6\)O\(_3\)Na [M + Na]\(^+\): 345.1646, found 345.1646.

1-(3-Azido-2,3-dideoxy-\( \beta \)-d-ribofuranosyl)-5-methyl-4-(pyrrolidin-1-yl)-2(\(IH\))pyrimidinone (56b)
Chromatography on a silica gel column by sequential elution with EtOAc and 10% MeOH in EtOAc gave compound 56b (71.5 mg, 97%) as an off-white solid. $R_f$ (SiO$_2$/10% MeOH in EtOAc) = 0.20. $^1$H NMR (500 MHz, CD$_3$OD): $\delta$ 7.75 (s, 1H, H-6), 6.13 (t, $J = 6.2$ Hz, 1H, H-1’), 4.32 (app q, $J_{app} \sim 6.0$ Hz, 1H, H-3’), 3.91 (app q, $J_{app} \sim 3.9$ Hz, 1H, H-4’), 3.85 (dd, $J = 3.1$, 12.2 Hz, 1H, H-5’), 3.76–3.66 (m, 5H, H-5’ and NCH$_2$), 2.41 (dt, $J = 6.3$, 13.1 Hz, 1H, H-2’), 2.36 (dt, $J = 6.7$, 13.5 Hz, 1H, H-2’), 2.24 (s, 3H, Me), 1.92 (br s, 4H, CH$_2$). $^{13}$C NMR (125 MHz, CD$_3$OD): $\delta$ 163.2, 157.4, 141.0, 105.2, 86.9, 86.1, 62.4, 61.5, 50.6 (br), 38.9, 26.2 (br), 18.1. HRMS (ESI/TOF) m/z calculated for C$_{14}$H$_{21}$N$_6$O$_3$ [M + H]$^+$: 321.1670, found 321.1663.

1-(3-Azido-2,3-dideoxy-β-D-ribofuranosyl)-5-methyl-4-(piperidin-1-yl)-2(IH)-pyrimidinone (56c)

Chromatography on a silica gel column by sequential elution with EtOAc and 10% MeOH in EtOAc gave compound 56c (122.1 mg, 99%) as an off-white solid. $R_f$ (SiO$_2$/10% MeOH in EtOAc) = 0.60. $^1$H NMR (500 MHz, CD$_3$OD): $\delta$ 7.87 (s, 1H, H-6), 6.13 (t, $J = 6.1$ Hz, 1H, H-1’), 4.32 (app q, $J_{app} \sim 6.1$ Hz, 1H, H-3’), 3.93 (dt, $J = 2.8$, 5.3 Hz, 1H, H-4’), 3.86 (dd, $J = 3.0$, 12.2 Hz, 1H, H-5’), 3.75 (dd, $J = 3.2$, 12.2 Hz, 1H, H-5’), 3.66–3.60 (m, 4H, NCH$_2$), 2.45 (dt, $J = 6.4$, 13.2 Hz, 1H, H-2’), 2.35 (dt, $J = 6.7$, 13.5 Hz, 1H, H-2’), 2.15 (s, 3H, Me), 1.75–1.68 (m, 2H, CH$_2$), 1.68–1.61 (m, 4H, CH$_2$). $^{13}$C NMR (125 MHz, CD$_3$OD): $\delta$ 167.2, 157.5, 142.9, 105.9, 87.1, 86.3, 62.3, 61.3, 49.4, 39.1, 27.3, 25.5, 18.2. HRMS (ESI/TOF) m/z calculated for C$_{15}$H$_{22}$N$_6$O$_3$Na [M + Na]$^+$: 357.1646, found 357.1670.
1-(3-Azido-2,3-dideoxy-β-D-ribofuranosyl)-5-methyl-4-(morpholin-4-yl)-2(1H)-pyrimidinone (56d)

Chromatography on a silica gel column by sequential elution with EtOAc and 10% MeOH in EtOAc gave compound 56d (73.2 mg, 98%) as a white solid. $R_f$ (SiO$_2$/10% MeOH in EtOAc) = 0.23. $^1$H NMR (500 MHz, CD$_3$OD): $\delta$ 7.96 (s, 1H, H-6), 6.13 (t, $J = 6.0$ Hz, 1H, H-1’), 4.32 (app q, $J_{app} \approx 6.2$ Hz, 1H, H-3’), 3.94 (dt, $J = 2.9$, 5.4 Hz, 1H, H-4’), 3.87 (dd, $J = 3.1$, 12.2 Hz, 1H, H-5’), 3.77-3.73 (m, 5H, H-5’, and morpholinyl–CH$_2$), 3.71-3.68 (m, 4H, morpholinyl–CH$_2$), 2.47 (dt, $J = 6.5$, 13.3 Hz, 1H, H-2’), 2.37 (dt, $J = 6.6$, 13.5 Hz, 1H, H-2’), 2.15 (s, 3H, Me). $^{13}$C NMR (125 MHz, CD$_3$OD): $\delta$ 167.0, 156.9, 143.6, 105.8, 87.3, 86.4, 67.8, 62.1, 61.1, 48.9, 39.1, 17.9. HRMS (ESI/TOF) m/z calculated for C$_{14}$H$_{20}$N$_6$O$_4$Na [M + Na]$^+$: 359.1438, found 359.1434.

Method B (with KF for synthesis of 6 and 33)

To a 0.096 M solution of the appropriate nucleoside (1 equiv.) in dry MeOH, KF (4 equiv.) was added, and the mixture was stirred at 80 °C for 12 h. The mixture was evaporated and the crude material was purified through a short silica gel column by elution with suitable solvent system (see individual compound headings below).

N,5-Dimethyl-2’-deoxycytidine (6)

Chromatography on a silica gel column by sequential elution with EtOAc and 20% MeOH in EtOAc gave compound 6 (35.4 mg, 89%) as a white solid. $R_f$ (SiO$_2$/20% MeOH in EtOAc) = 0.16. $^1$H NMR (500 MHz, CD$_3$OD): $\delta$ 7.73 (s, 1H, H-6), 6.28 (t, $J = 6.6$ Hz, 1H, H-1’), 4.37 (dt, $J = 3.4$, 6.5 Hz, 1H, H-3’), 3.91 (app q, $J_{app} \approx 3.5$ Hz, 1H, H-4’), 3.80 (dd, $J = 3.2$, 12.0 Hz, 1H, H-5’), 3.73 (dd, $J = 3.8$,
12.1 Hz, 1H, H-5’), 2.94 (s, 3H, NMe), 2.30 (ddd, J = 3.7, 6.1, 13.5 Hz, 1H, H-2’), 2.13 (dt, J = 6.7, 13.5 Hz, 1H, H-2’), 1.93 (s, 3H, Me). 13C NMR (125 MHz, CD3OD): δ 165.4, 158.6, 138.3, 105.0, 88.7, 87.2, 72.0, 62.8, 41.9, 28.3, 13.1. HRMS (ESI/TOF) m/z calculated for C11H17N3O4Na [M + Na]+: 278.1111, found 278.1123.

N-(p-Toly)-2’-deoxycytidine (33)

Chromatography on a silica gel column by sequential elution with EtOAc and 10% MeOH in EtOAc gave compound 33 (42.9 mg, 87%) as a white solid. Rf (SiO2/10% MeOH in EtOAc) = 0.40. 1H NMR (500 MHz, CD3OD): δ 8.05 (d, J = 7.5 Hz, 1H, H-6), 7.60 (br s, 2H, Ar-H), 7.14 (d, J = 7.3 Hz, 2H, Ar-H), 6.27 (t, J = 6.5 Hz, 1H, H-1’), 6.03 (d, J = 7.5 Hz, 1H, H-5), 4.37 (dt, J = 3.4, 6.5 Hz, 1H, H-3’), 3.95 (app q, Japp ~ 3.6 Hz, 1H, H-4’), 3.80 (dd, J = 3.3, 12.0 Hz, 1H, H-5’), 3.73 (dd, J = 3.9, 12.1 Hz, 1H, H-5’), 2.39 (ddd, J = 3.8, 6.1, 13.6 Hz, 1H, H-2’), 2.31 (s, 3H, Me), 2.16 (dt, J = 6.7, 13.5 Hz, 1H, H-2’). 1H NMR (500 MHz, DMSO-d6): δ 9.62 (br s, 1H, NH), 7.93 (d, J = 7.5 Hz, 1H, H-6), 7.63 (br s, 2H, Ar–H), 7.13 (d, J = 8.2 Hz, 2H, Ar–H), 6.17 (t, J = 6.6 Hz, 1H, H-1’), 5.97 (d, J = 7.4 Hz, 1H, H-5), 5.22 (d, J = 4.2 Hz, 1H, OH), 4.99 (t, J = 5.2 Hz, 1H, OH), 4.24–4.18 (m, 1H, H-3’), 3.79 (app q, Japp ~ 3.5 Hz, 1H, H-4’), 3.62–3.51 (m, 2H, H-5’ and H-5’), 2.26 (s, 3H, Me), 2.16 (ddd, J = 3.3, 5.8, 13.2 Hz, 1H, H-2’), 1.97 (dt, J = 6.6, 13.3 Hz, 1H, H-2’). HRMS (ESI/TOF) m/z calculated for C16H20N3O4 [M + H]+: 318.1448, found 318.1443. The 1H NMR spectrum matches to that of the authentic material.35

O4'-Methylthymidine (54i)

Chromatography on a silica gel column by sequential elution with EtOAc and 10% MeOH in EtOAc gave compound 54i (20.7 mg, 28%) as a white solid. Rf (SiO2/10% MeOH in EtOAc) =
0.37. \(^1\)H NMR (500 MHz, CD\(_3\)OD): \(\delta\) 8.13 (s, 1H, H-6), 6.24 (t, \(J = 6.4\) Hz, 1H, H-1’), 4.38 (dt, \(J = 3.8, 6.3\) Hz, 1H, H-3’), 3.97–3.94 (m, 4H, H-4’ and OCH\(_3\)), 3.84 (dd, \(J = 3.1, 12.1\) Hz, 1H, H-5’), 3.75 (dd, \(J = 3.7, 12.1\) Hz, 1H, H-5’), 2.41 (ddd, \(J = 4.1, 6.2, 13.6\) Hz, 1H, H-2’), 2.15 (dt, \(J = 6.6, 13.4\) Hz, 1H, H-2’), 1.97 (s, 3H, Me). \(^{13}\)C NMR (125 MHz, CD\(_3\)OD): \(\delta\) 172.5, 158.2, 142.0, 106.6, 89.1, 87.9, 71.7, 62.5, 55.1, 42.2, 12.1. HRMS (ESI/TOF) \(m/z\) calculated for C\(_{11}\)H\(_{16}\)N\(_2\)O\(_5\)Na [M + Na]\(^+\): 279.0951, found 279.0948.

**Method C (with TASF for synthesis of 8a)**

\(\text{S}^4\)-Methyl-4-thiothymidine (8a)

To a solution of the compound 45j (50 mg, 0.099 mmol, 1 equiv.) in 1 mL dry MeCN at 0 °C, a solution of TASF (412.5 mg, 0.499 mmol, 5 equiv.) in 0.33 mL dry MeCN was added dropwise and stirred at 0 °C for 1 h. Then the reaction mixture was allowed to stir at room temperature for 16 h. The crude material after desilylation was quickly filtered through a short silica plug plug using 10% MeOH in EtOAc. The filtrate was evaporated and washed with EtOAc followed by 2% MeOH in EtOAc to obtain compound 8a (18.3 mg, 68%) as a white solid. \(R_f\) (SiO\(_2\)/10% MeOH in EtOAc) = 0.49. \(^1\)H NMR (500 MHz, CD\(_3\)OD): \(\delta\) 8.10 (s, 1H, H-6), 6.21 (t, \(J = 6.2\) Hz, 1H, H-1’), 4.39 (dt, \(J = 4.1, 6.1\) Hz, 1H, H-3’), 3.99 (app q, \(J_{app} \sim 3.5\) Hz, 1H, H-4’), 3.85 (dd, \(J = 3.0, 12.1\) Hz, 1H, H-5’), 3.76 (dd, \(J = 3.7, 12.1\) Hz, 1H, H-5’), 2.54 (s, 3H, SCH\(_3\)), 2.46 (ddd, \(J = 4.4, 6.1, 13.6\) Hz, 1H, H-2’), 2.17 (dt, \(J = 6.6, 13.4\) Hz, 1H, H-2’), 2.10 (s, 3H, Me). \(^{13}\)C NMR (125 MHz, CD\(_3\)OD): \(\delta\) 180.3, 156.0, 139.2, 114.1, 89.3, 88.2, 71.6, 62.4, 42.4, 14.1, 13.0. HRMS (ESI/TOF) \(m/z\) calculated for C\(_{11}\)H\(_{16}\)N\(_2\)O\(_4\)SNa [M + Na]\(^+\): 295.0723, found 295.0716.
General procedure for reduction of 3’-azido nucleosides To amines (synthesis of 57a–d)

To a solution of the AZT derivative (0.131 mmol, 1 equiv.) in dry MeOH (0.6 mL) in a 4 mL vial, 5% Pd/C (27.5 mg) was added. The reaction vial was placed in a two-necked flask to which a hydrogen balloon was attached via a gas inlet adapter with a Teflon stopcock at one neck and the other neck was stoppered with a rubber septum. The flask was connected to a vacuum line via a needle inserted through the septum. The flask was degassed and filled with H₂ gas via the balloon, and this process was repeated three times. Finally, the reaction was allowed to proceed for 1 h at room temperature under a balloon filled with hydrogen gas. The reaction mixture was filtered through a short plug of Celite and the residue was washed with methanol. The filtrate was evaporated under reduced pressure and washed with EtOAc followed by 5% MeOH in EtOAc. The resulting solid was dried under high vacuum. All the products are very polar materials.

3’-Amino-N,N-diethyl-5-methyl-2’,3’-dideoxycytidine (57a)

Compound 57a (38.4 mg, 99%) was obtained as a light-yellow solid. R_f (SiO₂/MeOH) = 0.34. ¹H NMR (500 MHz, CD₃OD): δ 7.90 (s, 1H, H-6), 6.12 (t, J = 5.4 Hz, 1H, H-1’), 3.89 (dd, J = 2.4, 12.2 Hz, 1H, H-5’), 3.79 (dd, J = 3.2, 12.2 Hz, 1H, H-5’), 3.72 (dt, J = 3.2, 6.7 Hz, 1H, H-4’), 3.62 (q, J = 7.0 Hz, 4H, NCH₂), 3.50 (app q, J_app ~ 7.4 Hz, 1H, H-3’), 2.26–2.22 (m, 5H, H-2’, H-2’, and Me), 1.22 (t, J =
7.0 Hz, 6H, Me). $^{13}$C NMR (125 MHz, CD$_3$OD): δ 165.2, 157.4, 142.7, 104.4, 88.8, 86.7, 61.8, 51.0, 45.0, 42.7, 18.2, 14.1. HRMS (ESI/TOF) m/z calculated for C$_{14}$H$_{25}$N$_4$O$_3$ [M + H]$^+$: 297.1921, found 297.1916.

1-(3-Amino-2,3-dideoxy-β-D-ribofuranosyl)-5-methyl-4-(piperidin-1-yl)-2(1H)-pyrimidinone (57b)

Compound 57b (37.5 mg, 97%) was obtained as a light-yellow solid. R$_f$ (SiO$_2$/MeOH) = 0.23. $^1$H NMR (500 MHz, CD$_3$OD): δ 7.84 (s, 1H, H-6), 6.12 (t, $J = 5.5$ Hz, 1H, H-1’), 3.89 (dd, $J = 2.6$, 12.2 Hz, 1H, H-5’), 3.80–3.69 (m, 6H, H-4’, H-5’, and NCH$_2$), 3.49 (app q, $J_{app} \sim 7.4$ Hz, 1H, H-3’), 2.28–2.20 (m, 5H, H-2’, H-2’, and Me), 1.96–1.90 (m, 4H, CH$_2$). $^{13}$C NMR (125 MHz, CD$_3$OD): δ 164.0, 157.5, 141.2, 104.8, 88.8, 86.6, 61.9, 51.1, 50.5, 42.6, 26.2, 18.1. HRMS (ESI/TOF) m/z calculated for C$_{14}$H$_{22}$N$_4$O$_3$Na [M + Na]$^+$: 317.1584, found 317.1598.

1-(3-Amino-2,3-dideoxy-β-D-ribofuranosyl)-5-methyl-4-(pyrrolidin-1-yl)-2(1H)-pyrimidinone (57c)

Compound 57c (39.9 mg, 99%) was obtained as a white solid. R$_f$ (SiO$_2$/MeOH) = 0.31. $^1$H NMR (500 MHz, CD$_3$OD): δ 7.98 (s, 1H, H-6), 6.12 (t, $J = 5.3$ Hz, 1H, H-1’), 3.90 (dd, $J = 2.6$, 12.3 Hz, 1H, H-5’), 3.80 (dd, $J = 3.3$, 12.3 Hz, 1H, H-5’), 3.72 (dt, $J = 3.2$, 6.7 Hz, 1H, H-4’), 3.65–3.58 (m, 4H, NCH$_2$), 3.49 (app q, $J_{app} \sim 7.5$ Hz, 1H, H-3’), 2.27–2.22 (m, 2H, H-2’ and H-2’), 2.14 (s, 3H, Me), 1.74–1.68 (m, 2H, CH$_2$), 1.68–1.61 (m, 4H, CH$_2$). $^{13}$C NMR (125 MHz, CD$_3$OD): δ 167.3, 157.6, 143.1, 105.5, 88.9, 86.8, 61.7, 50.9, 49.4, 42.7, 27.3, 25.5, 18.2. HRMS (ESI/TOF) m/z calculated for C$_{15}$H$_{24}$N$_4$O$_3$Na [M + Na]$^+$: 331.1741, found 331.1744.
1-(3-Amino-2,3-dideoxy-β-D-ribofuranosyl)-5-methyl-4-(morpholin-4-yl)-2(1H)-pyrimidinone (57d)

Compound 57d (39 mg, 96%) was obtained as a light-yellow solid. Rf (SiO2/MeOH) = 0.17. 1H NMR (500 MHz, CD3OD): δ 8.07 (s, 1H, H-6), 6.11 (t, J = 5.2 Hz, 1H, H-1’), 3.91 (dd, J = 2.3, 12.3 Hz, 1H, H-5’), 3.80 (dd, J = 3.2, 12.3 Hz, 1H, H-5’), 3.76–3.71 (m, 5H, H-4’, morpholinyl-CH2), 3.70–3.65 (m, 4H, morpholinyl-CH2), 3.49 (app q, Japp ~ 7.5 Hz, 1H, H-3’), 2.31-2.21 (m, 2H, H-2’ and H-2’), 2.14 (s, 3H, Me). 13C NMR (125 MHz, CD3OD): δ 167.4, 157.4, 143.6, 105.4, 89.0, 87.0, 67.8, 61.6, 50.8, 48.7, 42.7, 17.9. HRMS (ESI/TOF) m/z calculated for C14H23N4O4 [M + H]+: 311.1714, found 311.1712.
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CHAPTER 2

PALLADIUM CATALYZED

CHEMOSELECTIVE ARYLATION OF 8-VINYLPURINE NUCLEOSIDES
[2.1] INTRODUCTION

Palladium-catalyzed carbon–nitrogen (C–N) bond-forming reactions have become an important area in the field of organic synthesis. This is because, palladium-catalyzed cross-coupling reactions allow access not only to small molecules but also to larger dendrimers, oligoanilines, and biologically important nucleoside analogues. Typically, a palladium-catalyzed C–N bond-forming reaction occurs between an aryl halide or aryl sulfonate and an amine, in the presence of palladium catalyst, ligand, base and a suitable solvent (Scheme 1).

Scheme 1. C–N cross-coupling reaction catalyzed by palladium-ligand complex

Before the exploration of palladium catalysis, the first C–N cross-coupling reaction was reported with copper. In this method, anthranilic acid was reacted with bromobenzene or p-bromonitrobenzene in the presence of K₂CO₃ and a catalytic amount of copper, at an elevated

Scheme 2. Copper catalyzed C–N cross-coupling reactions
temperature in nitrobenzene (Scheme 2, eq. 1). A similar reaction was also reported between benzamide and bromobenzene under the same conditions (Scheme 2, eq. 2). Typically, these reactions require high temperatures, long reaction times and, in some cases, stoichiometric amounts of copper.

The limitations of copper-mediated C–N bond-forming reactions invoked exploration of new methods and that resulted in palladium catalysis. Migita et al. reported the first Pd-catalyzed C–N bond-forming reaction between aryl bromides and a N-stannyl amide. These reactions were catalyzed by bis(tri-O-toly1phosphine)palladium(II)chloride ([(o-tolyl)3P]2PdCl2) in toluene at 100 °C over 3 h (Scheme 3). Modest to good yields (16–81%) were obtained with a wide variety of substituted aryl bromides. However, this strategy is not devoid of limitations, such as i) unreactivity of aryl chlorides and iodides under the reaction conditions, and ii) necessity for toxic tin substrates and production of new tin derivatives in the reaction.

Scheme 3. Palladium-catalyzed cross-coupling reactions between aryl bromides and a N-stannyl amide

Intrigued with the successful aryl amination protocol of Migita et al., Hartwig et al. investigated the mechanistic processes involved in the Pd-catalyzed C–N bond-forming reactions. In their studies, they found that when tin amides 1a and 1b were individually reacted with [(o-tolyl)3P]2PdCl2 in toluene at 90–110 °C, new phosphine and amine bound complexes 2a and 2b were formed in 50–60% yields (Scheme 4). The complexes 2a and 2b showed catalytic activity comparable to that of [(o-tolyl)3P]2PdCl2. On the other hand, the Pd(0) species ([(o-tolyl)3P]2Pd) under identical conditions displayed superior catalytic activity than the Pd(II)
complexes 2a and 2b. This led to the belief that a Pd(0) species is generated in the reaction and is responsible for the C–N bond formation. On the basis of their findings, Hartwig et al. proposed a plausible mechanism for Pd-catalyzed C–N bond-forming reactions between aryl bromides and tin amides (Scheme 4). In this mechanism, Pd(0) species 3, generated from a Pd(II) complex, undergoes oxidative addition with the aryl bromide to give either monomeric intermediate 4a or dimeric form 4b. X-ray and solution molecular weight analysis clearly supported the formation of dimeric form 4b. However, stoichiometric and kinetic studies indicated that the ligand dissociates from the dimeric aryl halide complex 4b to form the three coordinated monomeric species 4a, in order to undergo transmetalation with tin amide to give complex 5. Reductive elimination of intermediate 5 then yields the aryl amine and Pd(0) species 3.

Scheme 4. Reaction intermediates generated in the palladium-catalyzed C–N bond-forming reactions between aryl bromides and N-stannyl amides and a plausible mechanism
While Hartwig et al. were investigating the mechanistic aspects of Pd-catalyzed C–N bond-forming reactions, Buchwald et al. focused on improving the reaction conditions for the Pd-catalyzed C–N reactions. They were able to conduct reactions with lowered catalyst loadings and expanded the scope of the reaction by generating various tin amides in situ from (N,N-diethylamino)tributyltin via transamination reactions. Moderate to high yields (55–88%) were obtained with various electron-rich, -poor and -neutral aryl bromides. Nevertheless, there were certain aspects that still need to be addressed, such as, toxicity issues related to tin derivatives, poor reactivity of aryl iodides and reactivity confined to only secondary amines and primary anilines.

Addressing the issue related to the necessity of tin amide derivatives, Buchwald et al. and Hartwig et al. independently reported tin-free Pd-catalyzed C–N bond-forming reactions by using free amine and a strong base. In Buchwald’s protocol, reactions between aryl bromides and amines were conducted with either [(o-tolyl)₃P]₂PdCl₂ or Pd(dba)₂/(o-tolyl)₃P in combination with sodium tert-butoxide (NaO⁻Bu) in toluene, at elevated temperature (Scheme 5). Similar conditions were employed in Hartwig’s protocol except that [(o-tolyl)₃P]₂Pd or [(o-tolyl)₃P]₂PdCl₂ were used as the catalyst and lithium hexamethyldisilazide (LiHMDS) was used as the base. On the basis of their observations, a plausible mechanism was proposed (Scheme 5).

In this mechanism, Pd(0) species (L-Pd, 3) undergoes oxidative addition with the aryl bromide to give the intermediate 4, in a similar fashion to that shown in Scheme 4. The amine then coordinates to the intermediate 4 to give the Pd-amine complex 6. The base (either NaO⁻Bu or LiHMDS) then abstracts a proton from the complex 6, leading to the intermediate 7. Reductive elimination from intermediate 7 results in the aryl amine product (Scheme 5).
Scheme 5. Plausible mechanism for tin-free, palladium-catalyzed aryl amination reactions

Although the tin-free protocols do produce good yields with various aryl bromides and amines, reactions with primary amines such as butylamine and hexylamine produced lower yields. Significant amounts of reduction products of aryl bromides were also observed in these reactions. This is because of the competing β-hydride elimination from the Pd-amine complex 7 leading to imines and arenes (Scheme 5). Reactions conducted with aryl iodides and primary amines in the presence of the Pd(dba)$_2$/(o-tolyl)$_3$P combination and NaOrBu in dioxane at 100 °C also produced similar results. Even though ortho substitution on the aryl iodides did suppress β-hydride elimination to some extent and produced decent yields of aryl amine products, this strategy still warrants an alternate solution to suppress β-hydride elimination. Typically, bidentate phosphine ligands were utilized in transition metal complexes to inhibit β-hydride elimination. However, Hartwig’s results indicated that Pd-catalyzed C–N bond-forming reactions requires the three coordinate monophosphine species (4a, Scheme 5) and thus, the
sterically bulky monophosphine ligand such as (o-tolyl)$_3$P was commonly used in these reactions. Since, bidentate phosphine ligands under Pd-catalyzed C–N bond-forming reaction conditions do not result in three-coordinate monophosphine species, their use was discouraged. Despite the negative feedback from the initial experiments on the C–N coupling reactions with bidentate phosphine ligands, Buchwald et al., experimented with various bis(phosphine) ligands. Eventually, promising results were obtained when a combination of Pd$_2$(dba)$_3$/(-)-2,2'-bis(diphenylphosphino)-1,1'-binaphthalene (BINAP) and NaOtBu were utilized for the reactions of aryl bromides with amines in toluene at 80 °C. The Pd$_2$(dba)$_3$/BINAP combination showed high efficiency with various aryl bromides and amines. Comparative studies on reaction rates, yields, and suppression of reduction products with various ligands in a reaction of n-hexylamine with 1-bromo-3,5-dimethylbenzene (8), clearly indicated that BINAP was superior to (o-tolyl)$_3$P, and other bidentate phosphine ligands (Scheme 6). Although, 1,1'-ferrocenediyli-

**Scheme 6. Effects of ligands in palladium-catalyzed C–N bond-forming reaction of n-hexylamine with 5-bromo-3,5-dimethylbenzene (8)**

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Time</th>
<th>Conversion</th>
<th>Yield</th>
<th>Ratio of 9/10</th>
</tr>
</thead>
<tbody>
<tr>
<td>BINAP</td>
<td>2 h</td>
<td>100%</td>
<td>88%</td>
<td>39/1</td>
</tr>
<tr>
<td>(o-tolyl)$_3$P</td>
<td>22 h</td>
<td>88%</td>
<td>35%</td>
<td>1.5/1</td>
</tr>
<tr>
<td>DPPF</td>
<td>3 h</td>
<td>100%</td>
<td>–</td>
<td>13.2/1</td>
</tr>
</tbody>
</table>


bis(diphenylphosphine) (DPPF) showed faster conversion rate compared to (o-tolyl)$_3$P, significant amount of reduction product 10 was also observed. Other bidentate phosphine ligands such as 1,2-bis(diphenylphosphino)ethane (DPPE), 1,3-bis(diphenylphosphino)propane (DPPP), 1,4-bis(diphenylphosphino)butane (DPPB) were less reactive and showed 2–18% conversion of 8, over a period of 3–6 h (data not shown).

Later Hartwig et al. also showed that using a chelating ligand based catalyst such as (DPPF)PdCl$_2$ in the place of Pd(dba)$_2$(o-tolyl)$_3$P in aryl amination reactions restricted the $\beta$-hydride elimination process (Scheme 7). Reactions of both aryl iodides and aryl bromides with primary alkyl and aryl amines resulted in high yields (80–96%). These results are clearly superior to those reactions conducted with similar substrates using (o-tolyl)$_3$P based Pd catalysts. The suppression of $\beta$-hydride elimination with DPPF, which is smaller in size as compared to (o-tolyl)$_3$P, indicates that the chelation is more important than the steric bulk of the ligands.

**Scheme 7.** (DPPF)PdCl$_2$ catalyzed C–N bond-forming reactions of aryl halides with primary alkyl and aryl amines

From the time success with Pd-catalyzed aryl amination reactions of aryl halides with alkyl and aryl amines has been realized, $N$-arylation of imines, azoles, imides, amides, carbamates, sulfonamides, sulfoximines have all been achieved with some modifications to the reaction conditions. These modifications involve i) use of milder bases such as Cs$_2$CO$_3$ and K$_3$PO$_4$, ii) reactions with aryl triflates and unreactive aryl chlorides. One important feature of the reactions with aryl chlorides is that some of the reactions could be carried out at room temperature. Although, the combination of Pd catalysts and chelating
ligands such as BINAP or DPPF successfully led to aryl amination reactions, the overall mechanism was unknown. Hatwig et al. conducted various experiments with aryl bromides and amines using the catalysts Pd(BINAP)$_2$ (11a) and Pd(DPPF)$_2$ (11b)$^{30}$ Their results were consistent with the previously proposed mechanism for aryl amination reactions, which involves oxidative addition of an aryl bromide, generation of an amine ligated Pd complex, followed by reductive elimination from the palladium amido complex to give the product (Schemes 4 and 5). However, kinetic studies indicated that ligand dissociation from 11a to Pd(BINAP) (12a) was the rate-limiting step and the resultant complex 12a undergoes oxidative addition with the aryl bromide. This led to the proposal of a new catalytic cycle for the Pd-catalyzed C–N bond forming reactions where catalysts 11a and 11b are part of the catalytic cycle (Scheme 8).

**Scheme 8.** Catalytic cycle for the C–N bond forming reactions catalyzed by Pd(BINAP)$_2$ or Pd(DPPF)$_2$, as proposed by Hartwig et al.
Dissociation of catalyst 11 is followed by the oxidative addition of aryl bromide to the Pd complex 12, the resultant aryl halide complex 13 reacts with the amine (HNR₁R₂) and the base (NaOR) to give Pd-amido complex 14. Intermediate 14 then undergoes C–N bond formation to give the three-coordinated amine-ligated Pd complex 15. Dissociation of the intermediate 15 then results the aryl amine.

However, results from the kinetic studies by Buchwald et al. in collaboration with Blackmond et al. on aryl amination catalyzed by Pd₂(dba)₃/BINAP combination or 11a, were in contradiction with the results of Hartwig et al. In their studies, they observed that Pd complex 12a generated from either Pd₂(dba)₃/BINAP combination or pure 12a, initially coordinates to the amine forming three-coordinated Pd complex 16 rather than undergoing oxidative addition (Scheme 9). The rate-limiting oxidative addition is observed to occur much faster with the intermediate 16 rather than 12a. Thus the resultant aryl amine Pd complex 17 reacts with the base and undergoes reductive elimination to give the aryl amine product. They also observed

**Scheme 9. Catalytic cycle for the aryl amination reactions catalyzed by Pd(BINAP)₂ (12a), as proposed by Buchwald et al.**
that the when Pd$_2$(dba)$_3$/(±)-BINAP combination was used in the reaction, the amine activates Pd$_2$(dba)$_3$ by forming [Pd(BINAP)(amine)].

The contradictions in the mechanisms of (±)-BINAP ligated Pd-catalyzed aryl amination reactions proposed by Hartwig et al. and Buchwald et al. led to reassessment of the mechanism. This was carried out by a collaborative effort of Hartwig, Buchwald, and Blackmond. After careful evaluation of the identity of the Pd(0) species and the rates of reactions of aryl halides with amines in the aryl amination reactions, a new mechanism was proposed (Scheme 10).$^{31}$ In this mechanism, active catalyst 12a is initially generated from 11a, with 11a being out of the catalytic cycle. Unlike in the case of the catalytic cycle proposed by Buchwald et al., 12a undergoes oxidative addition with the aryl bromide to give aryl palladium complex 13a. Then, 13a reacts with amine and the base to give aryl amido palladium complex 14a. Reductive

**Scheme 10. Revised mechanistic cycle for the Pd-catalyzed aryl amination reactions with BINAP as ligand**
elimination from 14a results in the aryl amine product. Due to the extensive mechanistic studies and the improvements to aryl amination reactions by Buchwald and Hartwig, Pd-catalyzed C–N bond forming reactions have been named as *Buchwald-Hartwig amination reactions*.

On this premise, we were interested in investigating the applicability of Pd-catalyzed C–N bond-forming reactions to nucleosides. This chemistry is of importance because several C-6, C-8, and C-2 modified purine nucleosides has therapeutic value, and have also been used in structural, biochemical, and biological studies. For example, nucleoside analogues 19a–d act as modulators of adenosine receptors, 20 exhibited anti-malarial properties, and 21 inhibits DNA polymerase-α (Figure 1). Carcinogenic amines such as 2-aminofluorene and 2-amino-1-

![Figure 1. Biologically important C-2, C-6 and C-8 modified purine nucleosides](image-url)
methyl-6-phenylimidazolo[4,5-b]pyridine covalently bind to DNA and cause mutations, which may lead to cancer. Thus, nucleoside adducts 22 and 23 have been used to study the structural and biochemical processes that lead to carcinogenesis (Figure 1).\textsuperscript{36,37}

Typically, the amine modified purine nucleosides were synthesized by displacing halogens from the C-2, C-6 or C-8 position of the purine nucleosides \textit{via} an SN\textsubscript{Ar} pathway or by conjugate addition-elimination pathway. However, these reactions are typically limited to aliphatic amines and electron-rich aryl amines.\textsuperscript{38} This limits availability of compounds such as 19d, 21–23, where the nucleoside is bonded to an aromatic amine. Hence, it was important to investigate Pd-catalyzed aryl amination reactions of nucleosides in order to access aryl amine modified purine nucleosides.

Buchwald \textit{et al.} had reported a protocol for the synthesis of aminopyridines using Pd-catalyzed C–N bond-forming reactions.\textsuperscript{39} In this approach, halopyridines were coupled with alkyl and aryl amines by using a combination of Pd catalysts (Pd\textsubscript{2}(dba)\textsubscript{3} or Pd(OAc)\textsubscript{2}) and chelating phosphine ligands (DPPP or (±)-BINAP), with NaOtBu as the base (Scheme 11). It was known from previous studies that pyridine inhibits Pd/(o-tolyl)\textsubscript{3}P catalyzed arylamination reactions \textit{via} the formation of bis-pyridyl complexes by displacing phosphine ligands from critical reaction intermediates.\textsuperscript{39,40} This new protocol showcased that the chelating ligands DPPP or (±)-BINAP enable Pd-catalyzed synthesis of aminopyridines by preventing the formation bis-

\textbf{Scheme 11.} Pd-catalyzed synthesis of aminopyridines from halopyridines and amines in the presence of chelating ligands
pyridyl complexes that are detrimental to reactivity.

On the basis of Buchwald’s synthesis of aminopyridines, and due to structural features of 4-bromo and 2-bromopyridines within 6-bromopurine nucleoside 18, Lakshman et al. were interested in studying reaction of 6-bromopurine nucleoside 18 with aryl amines, under Pd-catalyzed C–N bond-forming conditions (Figure 2). This strategy would allow facile access to C-6 modified adenine nucleoside derivatives. However, there are some important considerations: i) nucleoside 18 contains multiple heteroatoms and can potentially lead to ligand exchange with metal complexes and inhibit the reaction, despite the use of chelating ligands, ii) unlike the bromopyridines, nucleosides are more labile in nature and can easily undergo deglycosylation.

Initially, the conditions for Pd-catalyzed aryl amination reaction of 18 with p-toluidine were carefully evaluated. The combination of Pd$_2$(dba)$_3$ and 2-(dicyclohexylphosphino)-2′-(N,N-dimethylamino)-1,1′-biphenyl (DavePhos) with K$_3$PO$_4$ as the base, in 1,2-DME as solvent, at 80 °C, was found to be optimal (Table 1). Moderate to good yields (52–72%) were obtained for the C-6 amine-modified purine nucleosides 24a–f. Use of strong bases such as NaOtBu resulted in low yields of the products due to the formation of byproducts. Notably, the reaction between 18 and p-toluidine conducted in the absence of the catalyst and ligand, resulted in no product. This result ruled out the possibility for formation of products 24a–f via a $S_N$Ar pathway.
Table 1. Palladium-catalyzed C–N bond-forming reactions between 6-bromopurine nucleoside (18) with various aryl amines

<table>
<thead>
<tr>
<th>Entry</th>
<th>Aryl Amine</th>
<th>Product: Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H₂C[\text{NH}_2]</td>
<td>24a: 69</td>
</tr>
<tr>
<td>2</td>
<td>NC[\text{NH}_2]</td>
<td>24b: 61</td>
</tr>
<tr>
<td>3</td>
<td>OCH₃[\text{NH}_2]</td>
<td>24c: 72</td>
</tr>
<tr>
<td>4</td>
<td>H₃C[\text{NH}_2}</td>
<td>24d: 52</td>
</tr>
<tr>
<td>5</td>
<td>[\text{NH}_2}</td>
<td>24e: 64</td>
</tr>
<tr>
<td>6</td>
<td>[\text{NH}_2}</td>
<td>24f: 68</td>
</tr>
</tbody>
</table>

Later, Koomen et al. reported Pd-catalyzed amination of 6-chloropurine ribonucleoside 25 with alkyl and aryl amines using Pd₂(dba)₃/(±)-BINAP combination with either Cs₂CO₃ or KOtBu.⁴³ However, only reaction with simple aliphatic amines resulted in good yields and the reactions with sterically hindered aliphatic amines and aromatic amines resulted in either low yields or no products.⁴³ This led Lakshman and co-workers to evaluate the reactivity of 6-chloro and 6-bromo purine nucleosides with sterically hindered aliphatic amines of polycyclic aromatic hydrocarbons (PAHs). This strategy, if effective, would allow easy access to several PAH-nucleoside adducts. PAHs are health hazards and are common pollutants present in air, water and soil.⁴⁴ PAHs undergo metabolic activation and form diol epoxides. These diol epoxides are highly electrophilic and react at the exocyclic amino groups of purine bases in DNA resulting in
covalent DNA modification. These DNA lesions can lead to mutations and tumorigenesis. Hence, it is important to have access to PAH-nucleoside adducts for studies directed towards understanding the structural, functional, and biological factors influenced by nucleoside modification within DNA. Hence, the hypothesis was tested by initially reacting 6-bromo and 6-chloropurine nucleosides 18 and 25 respectively, with a relatively simpler benzo[a]pyrene derivative (±)-26. Use of Pd(OAc)$_2$/(-)-BINAP/Cs$_2$CO$_3$ in toluene at 80 °C, resulted a diastereomeric pair of B[a]P-dA adducts 27a, b (eq. 1, Scheme 12). Surprisingly, the reaction times of 6-chloropurine nucleoside 25 and 6-bromopurine nucleoside 18 were comparable (5.5 and 5 h respectively). However, the yield with 6-chloropurine nucleoside 25 was higher as compared to that from 6-bromopurine nucleoside 18 (90% vs 80%). This clearly shows that 6-chloropurine nucleoside 25 is at least as effective as 6-bromopurine nucleoside 18 in Pd-catalyzed aryl amination reactions. Next, the conditions were applied to the synthesis of B[a]P-dA adducts 29a, b by reacting 6-chloropurine nucleoside 25 with benzoyl-protected B[a]P

**Scheme 12. Synthesis of B[a]P-nucleoside adducts 27a,b and 29a,b via Pd-catalysis**
amino triol (±)-28. This reaction was complete within 4 h and yielded products 29a, b in 71% yield (eq. 2, Scheme 12). One important feature of this strategy is that, despite the possibility of β-hydride elimination, no attrition of chirality was observed in either of the products. This may be attributable to the bis coordinating (±)-BINAP ligand used in these reactions.

With this success, we and others reported synthesis of several other PAH-nucleoside adducts by coupling PAH amine donors with electrophilic halo nucleosides using Pd-catalysis. Some of the examples of PAH–nucleoside adducts with PAHs bonded at C-2 (in the case of 2’-deoxyguanosine), and C-6 (in the case of 2’-deoxyadenosine) positions of purine nucleosides are shown in Figure 3.

Figure 3. PAH-nucleoside adducts accessed by palladium catalysis

Next, Lakshman et al. decided to evaluate the reactivity of aromatic amines with 6-chloro- and bromo ribo- and 2’-deoxyribonucleosides. Hence, ribose and 2’-deoxyribonucleosides, 18, and 25, as well as 30 and 31 were considered as model substrates. After evaluating several conditions, Pd(OAc)$_2$/Xantphos with Cs$_2$CO$_3$ as the base in toluene, at 100 °C, proved to be the best conditions for aryl amination reactions of 18, 25, 30, and 31. These reactions were generally very fast and were complete within 1–1.5 h, in very high yields (86–99%). An example is shown in Scheme 13, where 6-bromo and 6-chloro purine nucleosides 18, 25, 30, and 31 were reacted with silylated 2’-deoxyadenosine (dA) and adenosine (A) analogues 32a, b under the aforementioned optimal conditions to give the dimers 33a (dA-dA) and 33b (A-A),
respectively. These dimers were known to form in vitro by DNA cross-linking processes mediated by nitrous acid. Since these processes can lead to deleterious biological changes, synthetic access to dimers 33a and 33b will enable to study the physiological transformations.

**Scheme 13. Pd-catalyzed aryl amination reactions of 6-halonucleosides 18, 25, 30, and 31 with 2'-deoxyadenosine and adenosine derivatives 32a,b**

Lakshman et al. also reported the synthesis of C6-azolyl purine nucleosides by use of Pd-catalyzed C–N bond-forming reactions. In this strategy, 6-chloropurine nucleoside 25 and 6-bromopurine nucleosides 18, 30 were coupled with various heteroaryl amines by use of Pd(OAc)₂/Xantphos as the catalyst combination, Cs₂CO₃ as the base in toluene or 1,4-dioxane, at 100 °C (Scheme 14). Interestingly, the ligands that were commonly used for Pd-catalyzed aryl amination reactions such as Davephos and (+)-BINAP were not effective under these conditions and only xantphos produced high yields. This could be due to a combination of factors, such as i) the large bite-angle of Xantphos allowing the formation of stable complexes and also enhancing reductive elimination, ii) the apical oxygen atom in Xantphos stabilizing intermediates in the cis-trans isomerization of oxidative-addition complexes. Both 6-chloropurine nucleoside 25 and 6-bromopurine nucleosides 18 and 30 were effective substrates under these conditions. In general, the reactions conducted in toluene were much faster as compared to 1,4-dioxane and
resulted in higher yields. However, reactions of benzimidazole, carbazole, and 1,2,4-triazole in 1,4-dioxane were clearly superior to those in toluene.⁵³

**Scheme 14. Synthesis of C6-azolylpurine nucleoside analogues via Pd-catalyzed coupling of halonucleosides 18, 25, and 30 with heteroaryl amines**

![Scheme 14](image)

From the previous reports, it was known that arene aryl sulfonates could also be used for Pd-catalyzed aryl amination reactions.⁵⁴,⁵⁵ However, there were no reports on the utility of O₆-aryl sulfonate derivatives of nucleosides in Pd-catalyzed C–N coupling reactions. Lakshman et al. investigated the reactivity of nucleosides with a sulfonate group at the C6 position of purine nucleosides.⁵⁶ However, the stability of O₆-aryl sulfonate nucleoside derivatives under Pd-catalyzed aryl amination reaction conditions is a matter of concern. Therefore, several O₆-aryl sulfonate derivatives of 2'-deoxyguanosine were tested in reactions with p-toluidine, in the presence of different Pd catalyst and ligand combinations. It was observed that the O₆-tosyl derivative of 2'-deoxyguanosine 36 was most stable and produced better yields under these conditions compared to the sterically bulky mesylate or 2,4,6-trisopropylphenyl analogues of 36. The reactions required a combination of Pd(OAc)₂/Davephos/K₃PO₄ with 1,4-dioxane/t-BuOH mixture as solvent, at 110 °C (Scheme 15). The reactions were, in general, fast (1–1.5 h) and gave products in moderate to high yields (55–98%).⁵⁶ In the search of designing new ligands, Lakshman et al. recently reported the synthesis of ligand 38, which is isomeric to DavePhos.⁵⁷ Ligand 38 in combination with Pd(OAc)₂ was successfully used to couple aryl amines with the
$O^6$-tosyl derivative of 2'-deoxyguanosine 36 (Scheme 15).\textsuperscript{57} Studies on reaction times and product yields showed that ligand 38 was comparable to DavePhos. However, in some cases, ligand 38 was clearly superior to DavePhos. Pd(OAc)$_2$/ligand 38 combination was also very effective in C–C bond-forming reactions and were much superior to the Pd(OAc)$_2$/DavePhos combination. Notably, some of the reactions were also carried out at room temperature by using the Pd(OAc)$_2$/ligand 38 combination.\textsuperscript{57}

**Scheme 15.** C–N coupling reactions of aryl amines with a $O^6$-tosyl nucleoside derivative catalyzed by a Pd(OAc)$_2$/biarylphosphine ligand combination

![Scheme 15](image)

Although Pd-catalyzed aryl amination reaction of electrophilic halonucleosides with aryl amines is an effective method for the synthesis of $N^6$-aryl purine nucleosides, an easy and alternative route would involve Pd-catalyzed coupling of aryl halides with nucleosides containing an amine functionality (Figure 4). Typically, synthesis of 6-bromo purine nucleoside 18 and its chloro analogue 25 was carried out from 2'-deoxyadenosine in 2 and 3 steps, respectively.\textsuperscript{41,58} Since, a vast number of aryl halides are commercially available, coupling of
aryl halides with amino group of nucleosides would be cost-effective and a less labor-intensive approach to access \( N^6 \)-aryl purine nucleosides.

![Figure 4. Alternative route to the synthesis of \( N^6 \)-aryl purine nucleosides](image)

In this regard, Sigurdsson and Hopkins et al. reported the first Pd-catalyzed C–N bond-forming reaction on nucleosides.\(^{59}\) They successfully synthesized a dG-dG dimer by coupling protected 2’-deoxyguanosine and protected 2-bromo-2’-deoxyinosine derivatives through the use of the \( \text{Pd(OAc)}_2/R-(-)\text{-BINAP/NaOtBu} \) combination in toluene, at 80 °C. Later, De Riccardis and Johnson et al. reported the synthesis of \( N^6 \)-aryl purine nucleosides by Pd-catalyzed aryl amination reaction of 2’-deoxyadenosine derivative 32a with various aryl bromides and triflates, using the \( \text{Pd(OAc)}_2/(\pm)-\text{BINAP/Cs}_2\text{CO}_3 \) combination in toluene, at 80 °C (Scheme 16).\(^{60}\) Although, high yields (84–88%) were obtained in these reactions, this method is applicable to only aryl bromides and triflates that contain a nitro group at the ortho position. The presence of electron-withdrawing groups such as nitro group activates aryl bromides and triflates increases the rate of oxidative addition in Pd-catalyzed coupling reactions. Interestingly, at least in one case, a \( N^6, N^6 \)-diarylated nucleoside (40) was formed as the major product, when equivalent amounts of 1-(2-nitronaphthyl)triflate and 32a were coupled. Hence, to suppress the diarylation, these reaction were conducted with excess 32a as compared to the aryl bromides and triflates.\(^{60}\)

**Scheme 16.** \( N^6 \)-arylation of 32a with aryl bromides and triflates under Pd-catalyzed C–N coupling reaction conditions, and the structure of a \( N^6, N^6 \)-diarylated product (40)
In an extension of their work, De Riccardis and Johnson et al. utilized Pd-catalyzed arylamination reactions of 32a to synthesize cross-linked purine nucleosides 33a and 43. This was achieved by reacting bromo nucleosides 18 and 42 with 32a under the conditions used for N^6-arylation of 32a (Scheme 17). However, the yields for the products 33a and 43 were very low (21% and 24% respectively). Modifying reaction conditions did not improve the yields. Replacing the 6-bromo purine nucleoside 18 with its iodo analogue 41, increased the yield of the product 33a to 53% (Scheme 17). In order to improve the yield of 43, the iodo analogue of 42 was needed. However, attempts towards the synthesis of iodo analogue of 42 failed. Later, Hopkins and Sigurdsson et al. reported an improved synthesis of 43 by using the same substrates as De Riccardis and Johnson, i.e., 32a and 42. However, the Pd_2(dba)_3/R-(+)-BINAP/tBuONa combination was used in toluene, at 80 °C. These conditions lead to the product 43 in relatively better yield (44%), despite use of a stronger base tBuONa, which was known to be detrimental to nucleosides.
Scheme 17. Synthesis of cross-linked purine nucleosides 33a and 43 by Pd-catalyzed C–N coupling reactions

The limitations of using only activated aryl bromides and triflates in N6-arylation of 32a, and the low product yields in the case of cross-linked purine nucleoside synthesis, caused Lakshman et al. to reevaluate the Pd-catalyzed transformation of 2-deoxyadenosine derivative 32a. Initial attempts at the arylation of 32a with bromo PAHs using the Pd(OAc)2/(±)-BINAP/Cs2CO3 combination in toluene, at 100 °C, had been shown to be inadequate in applicability. In our previous reports on Pd-catalyzed aryl amination reactions of 6-halonucleosides with aryl and heteroaryl amines, it was observed that a Pd catalyst/Xantphos combination was very effective as compared to other Pd catalyst /ligand combinations. In this context, we wanted to investigate how effective Pd catalyst/Xantphos combinations would be as compared to other catalytic systems. Hence, we evaluated the coupling of aryl bromides with 32a by screening different combinations of Pd catalysts with Xantphos and other ligands. In our evaluations, optimal conditions were established as Pd2(dba)3/Xantphos/Cs2CO3 combination
in toluene, at 90 °C (Scheme 18). Then, the scope of the reaction was tested by reacting 32a with several aryl bromides under the optimized conditions. Both electron-deficient and electron-rich aryl bromides reacted well under the conditions. However, product yields in the latter case were slightly lower as compared to the former. Bromonaphthalenes reacted very slowly with 32a (27–48 h). Surprisingly, the reaction times for electron-deficient aryl bromides especially in the case of 4-formyl bromobenzene and 4-acetyl bromobenzene were also longer (24 h). However, the yields, in general, were very high in most cases. Later, we turned our focus to improve the yield of the cross-linked purine nucleoside 33a. For this, we conducted the reaction of 32a with 6-bromopurine nucleoside 18 and its chloro analogue 25 in the presence of the Pd(OAc)$_2$/Xantphos/Cs$_2$CO$_3$ combination in toluene, at 100 °C. This resulted in the dA-dA dimer 32a in relatively high 65–77% yields compared to the previous reports (Scheme 13).$^{64}$

Scheme 18. Pd-Xantphos catalyzed N$^\alpha$-arylation of 32a

Recently, Lakshman *et al.* reported a study on Heck arylation chemistry of 8-vinyladenine nucleosides 45 and 46.$^{65}$ For this coupling, Pd(OAc)$_2$/P(o-tol)$_3$/Et$_3$N combination in DMF at 100 °C proved to be the most effective among all conditions that were tested. A wide array of aryl iodides and aryl bromides were successfully reacted at the $\beta$-position of 8-vinyladenine nucleosides 45 and 46 (Scheme 19). These reactions lead to the trans-alkenes as the major products. However, in some cases, where electron-rich aryl halides were used, the product isomerization to the corresponding cis-alkene was observed. Overall, moderate to high yields
(57–92%) were obtained in all cases. Attempts at utilizing aryl chlorides for the Heck arylation were successful with minor modifications to the optimized conditions. However, it was limited to only activated aryl halides.\textsuperscript{65}

**Scheme 19. Chemoselective Heck-arylation of 8-vinlyadenine nucleoside derivatives 45 and 46**

![Chemoselective Heck-arylation of 8-vinlyadenine nucleoside derivatives 45 and 46](image_url)

Typically, in Heck arylation reactions, a new C–C bond is formed by reacting aryl/vinyl halides or triflates with alkenes that contain an electron-withdrawing group in the presence of Pd catalyst and a base, in a suitable solvent.\textsuperscript{66,67} Pd(OAc)$_2$ is the most commonly used Pd source, where as P(PPh$_3$)$_3$ and P(o-tol)$_3$ are the commonly used ligands for Heck arylation reactions. The mechanism of Heck coupling reactions involves 4 main steps in the catalytic cycle. They are *oxidative addition*, *syn-insertion*, *syn β-hydride elimination*, and *reductive elimination*. The general mechanistic pathway proposed by Heck for reactions catalyzed by Pd(OAc)$_2$ and monophosphine ligand (L) combination is shown in Scheme 20.\textsuperscript{66,68} In this mechanism, initially Pd(0) species 50 was generated by the reduction of Pd(OAc)$_2$ by the phosphine ligand L. Then, 50 undergo *oxidative addition* with aryl/vinyl halide or triflate to give trans-aryl palladium complex 51. This is followed by the dissociation of a phosphine ligand L from 51 to give three co-ordinated palladium species 52, that then co-ordinates to the alkene. The resultant species 53 undergoes *syn insertion* to the alkene to give alkyl palladium species 54. Internal C–C bond rotation in 54 allows the β-hydrogen to align *syn* to the palladium atom, and this facilitates a *syn β-hydride elimination*, resulting in the alkene ligated-palladium species 56. Subsequently, the
alkene dissociates from 56 to give the free alkene 49 and a hydridopalladium complex 57. 

*Reductive elimination* from 57 via action of a base then regenerates the catalytic species 50.

**Scheme 20. Proposed mechanistic pathway for Heck-arylation reactions catalyzed by Pd(OAc)$_2$ and monophosphine ligand (L) combination**

On the basis of the mechanisms proposed for Pd-catalyzed C–N bond-forming reactions and Heck C–C arylations, it is evident that both processes are catalyzed by a Pd(0) species, generated *in situ*, and involve a phosphine ligand, a base and an aryl halide. This implies that under Pd-catalyzed Heck C–C arylation conditions, substrates that contain both amino and alkene
functionalities such as 45 and 46, C–N arylation could potentially take place. However, in our analysis, Heck arylation reactions of 45 and 46 with aryl halides resulted only in C–C products 47 and 48. Practically, no C–N arylated products were observed. As mentioned earlier, we have reported that Pd-Xantphos combinations are very effective for $N^6$-arylation of the exocyclic amino group of 2'-deoxyadenosine derivative 32a (Scheme 18). All these results intrigued us and led us to evaluate the chemoselective $N^6$-arylation of 8-vinyladenine nucleosides 45 and 46 by Pd-catalysis (Scheme 21). In particular, we wanted to investigate how important ligand structure was for these reactions.

**Scheme 21.** Chemoselective $N^6$-arylation of 8-vinlyadenine nucleoside derivatives 45 and 46 to be evaluated

Recently, it was reported that 8-vinyl-2'-deoxyadenosine (8vdA, 60) exhibited fluorescent properties and its quantum yield is comparable to that of 2-aminopurine nucleoside 61, one of the most commonly used fluorophores (Figure 5). Similarly, 8vdA analogue 62 also showed strong fluorescence in non-polar solvents (Figure 5). On the other hand, 8vdA-10, a 10-mer stem–tetraloop RNA in which 60 was incorporated, inhibits ricin toxin A-chain (RTA). On the basis of the biological and fluorescent properties of 60 and its analogues, $N^6$-modification of 60, in principle, can lead to potential fluorophores and biologically active compounds. Pd-catalyzed chemoselective $N^6$-arylation of 8-vinyladenine nucleosides 45 and 46 provides an excellent route to access such modified 8-vinyladenine nucleosides (58 and 59).
Figure 5. Fluorescent purine nucleosides
[2.2] RESULTS AND DISCUSSION

In order to evaluate conditions for chemoselective $N^6$-arylation, we needed access to $3',5'$-silyl protected 8-vinyl-2'-deoxyadenosine 45. The synthesis of 45 has previously been reported starting from $3',5'$-silyl-protected 2'-deoxyadenosine 32a, in two steps.\textsuperscript{65,72} Hence, following the reported procedure, compound 45 was synthesized by lithiation at the C-8 position of nucleoside 32a with lithium diisopropyl amide (LDA) followed by iodination with iodine in anhydrous THF (Scheme 22). This gave the 8-iodo-2'-deoxyadenosine nucleoside 63 in 80% yield. Stille coupling of 8-iodo nucleoside 63 with tributyl vinyltin in the presence of Pd(PPh$_3$)$_4$ in anhydrous THF, at 90 °C, gave the desired product 45 in 80% yield (Scheme 22).

\textbf{Scheme 22.} Synthesis of 8-vinyl-2'-deoxyadenosineadenosine derivative 45 from $3',5'$-disilyl 2'-deoxyadenosine 32a

With compound 45 in hand, we began to evaluate conditions for selective $N^6$-arylation of 45. For this, we choose six ligands i.e., Xantphos, bis[(2-diphenylphosphino)phenyl] ether (DPEPhos), (±)-BINAP, 2,2'-Bis(diphenylphosphino)-1,1'-biphenyl (BIPHEP), and DPPF (Figure 6). Xantphos, (±)-BINAP and DPPF were chosen because it was already proven that they are effective in aryl amination reactions of alkyl, and aromatic amines with aryl halides and also in reactions involving nucleosides. DPEPhos and BIPHEP were chosen due to their structural similarities to Xantphos and (±)-BINAP, respectively. The reactions were conducted in toluene and Pd(OAc)$_2$, Pd$_2$(dba)$_3$ and Pd-118 (Figure 6) were chosen as the source of catalytic Pd.
Figure 6. Ligands and one Pd catalyst tested for the reactions

Due to our success with the Pd/Xantphos combination for aryl amination reactions of nucleosides, we initially conducted a reaction of 45 with 1.5 equiv. of PhI in the presence of 10 mol% Pd(OAc)$_2$/20 mol% Xantphos/1.5 equiv. of Cs$_2$CO$_3$ combination in toluene, at 100 °C. This reaction was complete within 5 h and the desired $N^6$-arylated product 58a was isolated in a 65% yield (entry 1, Table 2). However, some C,N-diarylated product 64a (18%) and Heck-like product 47a (7%) were also observed in this reaction. Reducing the Xantphos stoichiometry to 10 mol% did not influence the outcome of the reaction (entry 2, Table 2). However, replacing the base Cs$_2$CO$_3$ with K$_3$PO$_4$ effected the chemoselectivity of the reaction, resulting in the Heck-like product 47a as the major isomer (75%) and the $N^6$-arylated product 58a (10%) and C,N-diarylated product 64a (5%), as the minor byproducts (entry 3, Table 2). Use of the combination of 10 mol% Pd(OAc)$_2$/20 mol% DPEphos/1.5 molar equiv. of Cs$_2$CO$_3$ resulted exclusively in the Heck-like product 47a in a 75% yield (entry 4, Table 2). This is quite interesting, since DPEPhos is structural very similar to Xantphos, except that DPEPhos is more flexible as compared to Xantphos. Reducing the ligand stoichiometry to 10 mol% or changing the base to K$_3$PO$_4$, from the conditions in entry 4 did not effect the product formed or the yield (entries 5 and 6, Table 2). Poorer results were observed when the ligand in entry 1 (Xantphos) was
replaced with the bidentate (±)-BINAP, with a slight selectivity for Heck-like reaction (entry 7, Table 2). Surprisingly, use of Pd(OAc)₂/BIPHEP/1.5 molar equiv. of Cs₂CO₃ resulted exclusively in the Heck-like product 47a (entries 8 and 9, Table 2). Similarly, DPPF also gave the Heck-like product 47a, exclusively (entries 10 and 11, Table 2). In general, 1:1 and 1:2 Pd/ligand combinations resulted in closely similar outcome and the ligands DPEPhos, BIPHEP and DPPF showed selectivity for Heck-like reactions, resulting in product 47a, in comparable yields (74–80%). Remarkably, substituting Pd(OAc)₂ with Pd₂(dba)₃, from the conditions in entry 1, resulted in the N⁶-arylated product 58a (30%), C,N-diarylated product 64a (15%), and Heck-like product 47a (40%), with complete loss of selectivity (entry 12, Table 2). Next, in an attempt to reduce the amount of diarylated product 64a formation, we conducted the reactions with 1.3 equiv. of PhI at 75 °C (entry 13, Table 2). This significantly reduced the amount of the diarylation product and also increased the yield of the N⁶-arylation product. Under conditions similar to entry 13, 1.3 equiv. of PhBr was reacted with 45, resulting in products 58a (78%) and 64a (5%) (entry 14, Table 2). However, a higher catalyst loading and temperature were required for this reaction, but no formation of the Heck-like product 47a was observed. Lowering the temperature and reducing the ligand stoichiometry in entry 3, successfully led to the formation of Heck-like product 47a, with little effect on the yield (compare entry 3 to entries 15 and 16 in Table 2). Lastly, with preformed complex Pd-118, Heck-like product 47a (82%) was isolated exclusively (entry 17, Table 2). This result was comparable to the results observed in the reactions conducted with Pd(OAc)₂/DPPF combination (entries 11 vs 17, Table 2).

Table 2. Conditions evaluated for C–N bond-forming reactions of protected 8-vinyl-2'-deoxyadenosine derivative 45⁶
<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst (mol%)</th>
<th>Ligand (mol%)</th>
<th>Base</th>
<th>Reaction Time, Temp</th>
<th>58a Yield (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>47a Yield (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>64a Yield (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pd(OAc)&lt;sub&gt;2&lt;/sub&gt; (10)</td>
<td>Xantphos (20)</td>
<td>Cs&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>5 h, 100 °C</td>
<td>65%</td>
<td>7%</td>
<td>18%</td>
</tr>
<tr>
<td>2</td>
<td>Pd(OAc)&lt;sub&gt;2&lt;/sub&gt; (10)</td>
<td>Xantphos (10)</td>
<td>Cs&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>5 h, 100 °C</td>
<td>67%</td>
<td>7%</td>
<td>17%</td>
</tr>
<tr>
<td>3</td>
<td>Pd(OAc)&lt;sub&gt;2&lt;/sub&gt; (10)</td>
<td>Xantphos (20)</td>
<td>K&lt;sub&gt;3&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>18 h, 100 °C</td>
<td>10%</td>
<td>75%</td>
<td>5%</td>
</tr>
<tr>
<td>4</td>
<td>Pd(OAc)&lt;sub&gt;2&lt;/sub&gt; (10)</td>
<td>DPEPhos (20)</td>
<td>Cs&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>18 h, 100 °C</td>
<td>75%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Pd(OAc)&lt;sub&gt;2&lt;/sub&gt; (10)</td>
<td>DPEPhos (10)</td>
<td>Cs&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>18 h, 100 °C</td>
<td>74%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Pd(OAc)&lt;sub&gt;2&lt;/sub&gt; (10)</td>
<td>DPEPhos (20)</td>
<td>K&lt;sub&gt;3&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>18 h, 100 °C</td>
<td>76%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Pd(OAc)&lt;sub&gt;2&lt;/sub&gt; (10)</td>
<td>BINAP (20)</td>
<td>Cs&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>18 h, 100 °C</td>
<td>15%</td>
<td>25%</td>
<td>5%</td>
</tr>
<tr>
<td>8</td>
<td>Pd(OAc)&lt;sub&gt;2&lt;/sub&gt; (10)</td>
<td>BIPHEP (20)</td>
<td>Cs&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>18 h, 100 °C</td>
<td>74%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Pd(OAc)&lt;sub&gt;2&lt;/sub&gt; (10)</td>
<td>BIPHEP (10)</td>
<td>Cs&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>18 h, 100 °C</td>
<td>79%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Pd(OAc)&lt;sub&gt;2&lt;/sub&gt; (10)</td>
<td>DPPF (20)</td>
<td>K&lt;sub&gt;3&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>18 h, 100 °C</td>
<td>78%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Pd(OAc)&lt;sub&gt;2&lt;/sub&gt; (10)</td>
<td>DPPF (10)</td>
<td>Cs&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>18 h, 100 °C</td>
<td>80%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Pd&lt;sub&gt;2&lt;/sub&gt;(dba)&lt;sub&gt;3&lt;/sub&gt; (10)</td>
<td>Xantphos (20)</td>
<td>Cs&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>18 h, 100 °C</td>
<td>30%</td>
<td>40%</td>
<td>15%</td>
</tr>
<tr>
<td>13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Pd(OAc)&lt;sub&gt;2&lt;/sub&gt; (10)</td>
<td>Xantphos (10)</td>
<td>Cs&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>5 h, 75 °C</td>
<td>75%</td>
<td>5%</td>
<td>5%</td>
</tr>
<tr>
<td>14&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Pd(OAc)&lt;sub&gt;2&lt;/sub&gt; (15)</td>
<td>Xantphos (15)</td>
<td>Cs&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>12 h, 85 °C</td>
<td>78%</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Pd(OAc)&lt;sub&gt;2&lt;/sub&gt; (10)</td>
<td>Xantphos (20)</td>
<td>K&lt;sub&gt;3&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>18 h, 75 °C</td>
<td>5%</td>
<td>77%</td>
<td>10%</td>
</tr>
<tr>
<td>16</td>
<td>Pd(OAc)&lt;sub&gt;2&lt;/sub&gt; (10)</td>
<td>Xantphos (10)</td>
<td>K&lt;sub&gt;3&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>18 h, 75 °C</td>
<td>5%</td>
<td>76%</td>
<td>9%</td>
</tr>
<tr>
<td>17</td>
<td>Pd-118 (10)</td>
<td>None</td>
<td>Cs&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>18 h, 75 °C</td>
<td>82%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Reactions were conducted at a 0.1 M concentration of the nucleoside in PhMe (1 mL).
Next, we wanted to evaluate the scope of the Pd-catalyzed chemoselective \( N^6 \)-arylation of 8-vinyl-2'-deoxyadenosine derivative 45 by using the combination of Pd(OAc)\(_2\)/Xantphos/Cs\(_2\)CO\(_3\) with various aryl bromides and aryl iodides. The results are shown in Table 3. All reactions were selective towards \( N^6 \)-arylation of 45 and good yields (70–80%) were observed for the \( N \)-aryl products 58b–e in all cases. Small amounts of diaryl products 64b–e (3–7%) were also observed in all cases. However, the Heck-like products 47b–e were observed mostly with aryl iodides. Reactions with aryl bromides required higher temperature. As compared to aryl bromides, aryl iodides are more reactive in Pd-catalysis. Hence, this could be the reason why the reactions with aryl bromides require higher temperature. Both electron-rich and electron-deficient aryl halides were reactive under these conditions. However, aryl halides that are activated for cross-coupling i.e., 4-iodo and 4-bromoacetophenone resulted in relatively higher yields (entries 3 and 4, Table 3). Sterically hindered 2-iodotoluene also effectively reacted under these conditions (entry 5, Table 3). Reactions with 4-idoanisole and 4-bromoanisole were also successful, however, product isolation was not easy in these cases.

Table 3. Pd-catalyzed aryl amination reactions of 8-vinyl-2'-deoxyadenosine derivative 45 with aryl iodides and bromides

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ar-X</th>
<th>Conditions</th>
<th>N-Aryl Product 58b–e</th>
<th>Heck-like Product 47b–e</th>
<th>C,N-Diaryl Product 64b–e</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( ^a \) Yields are of isolated and purified products. \( ^b \) Reactions was conducted 1.3 equiv. of PhI, and 1.5 equiv. of base. \( ^c \) Reactions was conducted 1.3 equiv. of PhBr, and 1.5 equiv. of base.
Reactions were conducted at a 0.1 M concentration of the nucleoside in PhMe (1 mL), using 0.1 mmol of 45. Conditions A: Reaction was conducted with 10 mol% of Pd(OAc)$_2$, 10 mol% of Xantphos, 1.3 equiv. of aryl iodide, 1.5 equiv. of Cs$_2$CO$_3$, in PhMe, 85 °C, 12 h. Conditions B: Reaction was conducted with 15 mol% of Pd(OAc)$_2$, 15 mol% of Xantphos, 1.3 equiv. of aryl bromide, 1.5 equiv. of Cs$_2$CO$_3$, in PhMe, 85 °C, 12 h. Yields are of isolated and purified products.

<table>
<thead>
<tr>
<th></th>
<th>![Structure]</th>
<th>Product$^c$</th>
<th>Product$^c$</th>
<th>Product$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>![Structure]</td>
<td>A</td>
<td>58b: 71%</td>
<td>47b: 7%</td>
</tr>
<tr>
<td>2</td>
<td>![Structure]</td>
<td>B</td>
<td>58b: 70%</td>
<td>47b: trace</td>
</tr>
<tr>
<td>3</td>
<td>![Structure]</td>
<td>A</td>
<td>58c: 77%</td>
<td>47c: 6%</td>
</tr>
<tr>
<td>4</td>
<td>![Structure]</td>
<td>B</td>
<td>58c: 80%</td>
<td>47c: trace</td>
</tr>
<tr>
<td>5</td>
<td>![Structure]</td>
<td>A</td>
<td>58d: 72%</td>
<td>47d: 6%</td>
</tr>
</tbody>
</table>

* Reactions were conducted at a 0.1 M concentration of the nucleoside in PhMe (1 mL), using 0.1 mmol of 45.  
* Conditions A: Reaction was conducted with 10 mol% of Pd(OAc)$_2$, 10 mol% of Xantphos, 1.3 equiv. of aryl iodide, 1.5 equiv. of Cs$_2$CO$_3$, in PhMe, 85 °C, 12 h. Conditions B: Reaction was conducted with 15 mol% of Pd(OAc)$_2$, 15 mol% of Xantphos, 1.3 equiv. of aryl bromide, 1.5 equiv. of Cs$_2$CO$_3$, in PhMe, 85 °C, 12 h.  

From Table 3, it is apparent that the reactions of 8-vinyl-2'-deoxyadenosine 45 with aryl iodides and bromides were, in fact, chemoselective giving primarily N-arylation. We then queried if the ribose analogue of 45 would also undergo comparable chemoselective N-arylation under the same conditions used for 45. For this, we needed 8-vinyladenosine 46. Hence, the synthesis of 46 was carried out as shown in Scheme 23, following previous reports. 8-Iodo purine riboside 65 was synthesized by lithiation-iodonation of 32b, following the same procedure used for the synthesis of 8-ido-2'-deoxyriboside 63. In this reaction, compound 65 was isolated in a good 69% yield. Suzuki coupling of 65 with potassium vinyl tetrafluoroborate in the presence of the Pd(PPh$_3$)$_4$/Cs$_2$CO$_3$ combination in toluene, at 90 °C gave the required product 46 in 74% yield (Scheme 23).

**Scheme 23. Synthesis of 8-vinlyadenosine derivative 46 from 2',3',5'-trisilyladenosine 32b**
With the availability of 8-vinlyadenosine derivative 46, we tested the conditions for the chemoselectivity \( N \)-arylation of 46, by using the combination of 10 mol% \( \text{Pd(OAc)}_2 \)/10 mol% Xantphos/1.5 molar equiv. of \( \text{Cs}_2\text{CO}_3 \)/1.3 molar equiv. of PhI, at 85 °C. However, these reactions were less successful under these conditions and required higher catalyst load and temperature. The reactions with aryl iodides were conducted using 15 mol% \( \text{Pd(OAc)}_2 \)/15 mol% Xantphos at 100 °C, and the reactions with aryl bromides were conducted with 20 mol% \( \text{Pd(OAc)}_2 \)/20 mol% Xantphos at 100 °C. In both cases, 1.5 equiv. of \( \text{Cs}_2\text{CO}_3 \) and 1.3 equiv. of the aryl halide were used. The results from these reactions are shown in Table 4. In general, good yields (68–79%) yields were obtained for the \( N \)-arylated products 59a–e. Diaryl products 66a–e were formed in all cases, but only in small amounts (1–7%). As observed in the case of 8-vinyl-2’-deoxyadenosine 45, the Heck-like products 48a–e were more likely to form with aryl iodides. Also, 4-iodo and 4-bromoacetophenone resulted in relatively high yields of the \( N \)-arylated product 59c. Overall, the results from the reactions of 8-vinlyadenosine derivative 46 under the optimal conditions were comparable to that of 8-vinyl-2’-deoxyadenosine 45.

**Table 4.** \( \text{Pd-catalyzed aryl amination reactions of 8-vinlyadenosine derivative 46 with aryl iodides and bromides}^{a} \)
<table>
<thead>
<tr>
<th>Entry</th>
<th>Ar-X</th>
<th>Conditions</th>
<th>N-Aryl Product</th>
<th>Heck-like Product</th>
<th>C,N-Diaryl Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>![Iodobenzene]</td>
<td>A</td>
<td>59a: 71%</td>
<td>48a: 3%</td>
<td>66a: 7%</td>
</tr>
<tr>
<td>2</td>
<td>![Bromoiodobenzene]</td>
<td>B</td>
<td>59a: 74%</td>
<td>48a: trace</td>
<td>66a: 6%</td>
</tr>
<tr>
<td>3</td>
<td>![Iodobenzene]</td>
<td>A</td>
<td>59b: 70%</td>
<td>48b: 7%</td>
<td>66b: 5%</td>
</tr>
<tr>
<td>4</td>
<td>![Bromoiodobenzene]</td>
<td>B</td>
<td>59b: 69%</td>
<td>48b: 2%</td>
<td>66b: 2%</td>
</tr>
<tr>
<td>5</td>
<td>![O-bromobenzyl phenyl]</td>
<td>A</td>
<td>59c: 78%</td>
<td>48c: 5%</td>
<td>66c: –d</td>
</tr>
<tr>
<td>6</td>
<td>![O-bromobenzyl phenyl]</td>
<td>B</td>
<td>59c: 79%</td>
<td>48c: trace</td>
<td>66c: –d</td>
</tr>
<tr>
<td>7</td>
<td>![Iodo-o-bromobenzyl phenyl]</td>
<td>A</td>
<td>59d: 71%</td>
<td>48d: 7%</td>
<td>66d: 4%</td>
</tr>
<tr>
<td>8c</td>
<td>![Iodo-o-bromobenzyl phenyl]</td>
<td>A</td>
<td>59e: 68%</td>
<td>48e: 4%</td>
<td>66e: 3%</td>
</tr>
</tbody>
</table>

a Reactions were conducted at a 0.1 M concentration of the nucleoside in PhMe (0.8 mL), using 0.08 mmol of 46. b Conditions A: Reaction was conducted with 15 mol% of Pd(OAc)$_2$, 15 mol% of Xantphos, 1.3 equiv. of aryl iodide, 1.5 equiv. of Cs$_2$CO$_3$, in PhMe, 100 °C, 12 h. Conditions B: Reaction was conducted with 20 mol% of Pd(OAc)$_2$, 20 mol% of Xantphos, 1.3 equiv. of aryl bromide, 1.5 equiv. of Cs$_2$CO$_3$, in PhMe, 100 °C, 12 h. c Yields are of isolated and purified products. d Product formation was not observed. e Reaction time was 24 h.

We next intended to query a sequential diarylation of 8-vinyl-2'-deoxyadenosine 45 and its ribose analogue 46 in a one-pot operation, by modulating the catalytic conditions. In principle, this can be achieved in two ways. In one approach N-arylation is carried out first, then followed by a C–C reaction. Complimentary to this approach, C–C reaction followed by N-arylation could also result diarylated products (Scheme 24).

**Scheme 24.** Possible approaches for the sequential diarylation of 8-vinyladenine nucleosides 45 and 46 via Pd-catalysis
We initially attempted sequential diarylation of 45 by Pd-catalyzed C–N bond-formation with 4-iodotoluene using Pd(OAc)$_2$/Xantphos/1.5 equiv. of Cs$_2$CO$_3$ combination followed by C–C bond-forming reaction with PhI using Pd(OAc)$_2$/DPEphos/1.5 equiv. of Cs$_2$CO$_3$ combination. The diaryl product formation was clearly observed. However, due to formation of byproducts in the N-arylation step, isolation of the final product was very difficult. Hence, the complimentary approach, i.e., C–C bond-formation followed by C–N bond-formation was used to carry out the diarylation of nucleosides 45 and 46 (Table 5). The combination of Pd(OAc)$_2$/DPEPhos/1.5 molar equiv. of Cs$_2$CO$_3$ was used for the C–C bond-forming reactions and Pd(OAc)$_2$/Xantphos/1.5 equiv. of Cs$_2$CO$_3$ was used for C–N bond-forming reactions. For both steps 1.3 equiv. of aryl iodides were used and the reactions were conducted sequentially in a single pot. Three C,N-diarylated compounds 64f, g and 66f were synthesized via this approach. Moderate yields (35–42%) were obtained over two steps but a proof of principle was clearly obtained. However, when we tried to synthesize C,N-diarylated compound 64h via this approach, it was unsuccessful. Although the C–C bond-forming reaction of 45 with 4-iodoacetophenone was successful, the resulting Heck-like product 47c did not undergo N-arylation with PhI. Increasing the catalyst loading, temperature or the amount of PhI did not yield the product 64h.
Since, the acetyl group is in direct conjugation with the amino group in 47c (Figure 7), this could potentially deactivate the amino group and, thus, inhibit the C–N bond-forming reaction. In principle, 64h can be synthesized by C–N bond-forming reaction with PhI followed by a C–C bond-forming reaction with 4-iodoacetophenone.

**Table 5. One-pot sequential C–C followed by C–N arylation of 8-vinlyadenine nucleoside derivatives 45 and 46 via Pd-catalysis**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ar₁-I</th>
<th>C–C Coupling Conditions</th>
<th>Ar₂-I</th>
<th>C–N Coupling Conditions</th>
<th>C,N-Diaryl Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1" alt="Image" /></td>
<td>A</td>
<td><img src="image2" alt="Image" /></td>
<td>B</td>
<td>64f: 38%</td>
</tr>
<tr>
<td>2</td>
<td><img src="image3" alt="Image" /></td>
<td>A</td>
<td><img src="image4" alt="Image" /></td>
<td>B</td>
<td>64g: 42%</td>
</tr>
<tr>
<td>3</td>
<td><img src="image5" alt="Image" /></td>
<td>A</td>
<td><img src="image6" alt="Image" /></td>
<td>B or C</td>
<td>64h: d</td>
</tr>
<tr>
<td>4</td>
<td><img src="image7" alt="Image" /></td>
<td>A</td>
<td><img src="image8" alt="Image" /></td>
<td>C</td>
<td>66f: 35%</td>
</tr>
</tbody>
</table>

*Reactions were conducted at a 0.1 M concentration of the nucleoside in PhMe, using 0.1 mmol of 45 and 0.08 mmol of 46. a Conditions A: Reaction was conducted with 10 mol% of Pd(OAc)$_2$, 10 mol% of DPEPhos, 1.3 equiv. of aryl iodide, 1.5 equiv. of Cs$_2$CO$_3$, in PhMe, 100 °C, 12 h. Conditions B: Reaction was conducted with 10 mol% of Pd(OAc)$_2$, 10 mol% of Xantphos, 1.3 equiv. of aryl iodide, 1.5 equiv. of Cs$_2$CO$_3$, in PhMe, 100 °C, 12 h. Conditions C: Reaction was conducted with 20 mol% of Pd(OAc)$_2$, 20 mol% of Xantphos, 1.3 equiv. of aryl iodide, 1.5 equiv. of Cs$_2$CO$_3$, in PhMe, 100 °C, 12 h. c Yields are of isolated and purified products. d Product formation was not observed.*
Finally, we wanted to investigate if simple substrates that contain both amino and vinyl groups, such as $p$-aminostyrene can be chemoselectively arylated under the conditions developed. Hence, we conducted a reaction of $p$-aminostyrene and 1.3 equiv. of PhI by using the combination of Pd(OAc)$_2$/Xantphos/Cs$_2$CO$_3$ in toluene, at 80 °C. Although, $N$-aryl product 67 was observed to form in this reaction, the $C,N$-diaryl product 68 was isolated as the major product, and the reaction was incomplete. Conducting a reaction with 1.3 molar equiv. of $p$-aminostyrene and 1.0 equiv. of PhI under the same conditions went to completion and reduced the diarylation. From this reaction, the $N$-aryl product 67 was isolated in 62% yield as the major product, along with $C,N$-diaryl product 68 in 17% yield (Scheme 25). Similarly, when a reaction was conducted with 1.3 equiv. of $p$-aminostyrene and 1.0 equiv. of PhI by using Pd(OAc)$_2$/DPEPhos/Cs$_2$CO$_3$ combination in toluene, at 80 °C, primarily the Heck-like product 69 was obtained in a high 88% yield (Scheme 25). A small amount of $C,N$-diaryl product 68

**Scheme 25. Pd-catalyzed chemoselective arylation of $p$-aminostyrene**

![Scheme 25. Pd-catalyzed chemoselective arylation of $p$-aminostyrene](image-url)
(1%) was also isolated in this reaction. The results from Scheme 25 clearly indicates that Pd(OAc)$_2$/Xantphos and Pd(OAc)$_2$/DPEPhos combinations are highly selective for $N$-arylation and Heck arylation, respectively even in simple substrates. Further, these combinations can be used for sequential arylation reactions.
CONCLUSION

In summary, we have demonstrated that the Pd(OAc)$_2$/Xantphos combination with Cs$_2$CO$_3$ as the base is an effective catalytic system for chemoselective $N$-arylation of 8-vinyladenine nucleosides 45 and 46. All the other catalytic systems with Pd(OAc)$_2$ in combination of ligands such as DPEPhos, DPPF, BIPHEP, as well as Pd-118 resulted in Heck arylation, exclusively. Various aryl iodides and bromides were tested under these conditions. In general, all reactions resulted $N$-arylated products in good yields. Small amounts of Heck-like products and C,N-diarylated products were also isolated in these reactions. However, the Heck-like products were observed mostly in the reactions of aryl iodides. This could be due to higher reactivity of aryl iodides in Pd-catalysis. The results from the Pd-catalyzed $N$-arylation reactions of deoxy and ribonucleosides, 45 and 46, respectively, were very similar. However, ribonucleoside 46 required a higher catalyst loading and temperature. Further, by modulating the chemoselective catalytic conditions, diaryl products 64g,h and 66f were also synthesized in a one-pot, via sequential C–C reaction and C–N arylation. To test the generality of chemoselective arylation of simple substrates, $p$-aminostyrene was exposed to $N$-arylation and Heck-arylation conditions identified here. The results indicated that in this case as well, the Pd/Xantphos combination was effective for chemoselective $N$-arylation, whereas the Pd/DPEPhos combination was effective for chemoselective Heck-arylation of this simple substrate as well. As observed in the case of nucleoside derivatives, small amount of C,N-diaryl product was also isolated in these two reactions.
[2.4] EXPERIMENTAL SECTION

General Experimental Considerations. Reactions were conducted in screw-cap glass vials with Teflon-lined caps. Thin-layer chromatography was performed on 200 µm aluminum-foil-backed silica gel plates. Column chromatography was performed using 200–300 mesh silica gel. THF and PhMe were distilled from LiAlH₄ and then from Na prior to use. All other reagents were used as received from commercial suppliers. ¹H NMR spectra were obtained at 500 MHz and are referenced to the residual protonated solvent resonance. ¹³C NMR spectra were obtained at 125 MHz and are referenced to the solvent resonance. Chemical shifts (d) are reported in parts per million (ppm) and coupling constants (J) are in hertz (Hz). Standard abbreviations are used to designate resonance multiplicities.

3',5'-Bis-O-(tert-butyldimethylsilyl)-8-iodo-2'-deoxyadenosine (63)²²,²³

![Chemical structure of 3',5'-Bis-O-(tert-butyldimethylsilyl)-8-iodo-2'-deoxyadenosine (63)]

Into a clean, dry, 250 mL three-neck round-bottomed flask equipped with a stirring bar was placed 32a (3.79 g, 7.90 mmol, 1 equiv.) in dry THF (76 mL). The solution was cooled down to −70 °C and LDA (19.8 mL, 39.5 mmol, 5 equiv.) was added dropwise to the stirring mixture. The mixture was stirred at −70 °C for 1 h. Then a solution of I₂ (4.00 g, 15.8 mmol, 2 equiv.) in THF (38 mL) was added to the reaction mixture dropwise, maintaining the temperature at −70 °C. The reaction mixture was brought to 0 °C and stirred for 1 more hour. The mixture was diluted with Et₂O (50 mL) and washed with water (50 mL). The aqueous layer was back extracted with
Et₂O (3 x 50 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous Na₂SO₄, filtered, and evaporated to dryness under reduced pressure. The crude product was chromatographed on a short silica gel column using CH₂Cl₂ followed by 5% MeOH in CH₂Cl₂. The product 63 (3.83 g, 80% yield) was obtained as a light-brown solid. ¹H NMR (500 MHz, CDCl₃): δ 8.21 (s, 1H, Ar-H), 6.24 (t, J = 6.7 Hz, 1H, H-1’), 5.46 (br s, 2H, NH₂), 4.87 (dt, J = 3.7, 5.9 Hz, 1H, H-3’), 3.98–3.90 (m, 2H, H-4’, H-5’), 3.73–3.66 (m, 2H, H-5’, H-2’), 2.23 (ddd, J = 4.1, 6.8, 13.1 Hz, 1H, H-2’’), 0.94 and 0.83 (2s, 18H, t-Bu), 0.15 (s, 6H, SiMe), 0.01 and –0.05 (2s, 6H, SiMe).

8-Ethenyl-3’,5’-bis-O-(tert-butyldimethylsilyl)-2’-deoxyadenosine (45)

Into a clean, dry, 100 mL round-bottomed flask containing a stirring bar and with an attached condenser, was placed 63 (3.20 g, 5.28 mmol, 1 equiv.) in dry THF (54 mL). Then tri(n-buty)tin (3.10 mL, 10.6 mmol, 2 equiv.) and Pd(PPh₃)₄ (0.610 g, 0.528 mmol, 0.1 equiv.) were added to the solution, and the resulting mixture was stirred at 90 °C for 24 h. After cooling to room temperature, the reaction mixture was diluted with EtOAc (30 mL) and washed with water (15 mL). The aqueous layer was back extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous Na₂SO₄, filtered, and evaporated to dryness under reduced pressure. The crude product was chromatographed on a silica gel column using CH₂Cl₂ followed by 5% acetone in CH₂Cl₂. The product 45 (2.14 g, 80% yield) was obtained as a white solid. ¹H NMR (500 MHz, CDCl₃): δ 8.28 (s, 1H, Ar-H), 7.03 (dd, J = 11.1, 17.2 Hz, 1H, =CH), 6.46 (d, J = 17.2 Hz, 1H, =CHtrans), 6.41 (t, J = 6.7 Hz, 1H, H-1’),
5.67 (d, J = 11.1 Hz, 1H, =CH\textsubscript{cis}), 5.56 (s, 2H, NH\textsubscript{2}), 4.77 (dt, J = 3.3, 6.4 Hz, 1H, H-3’), 3.91–3.88 (m, 2H, H-4’, H-5’), 3.72 (dd, J = 3.6, 10.7 Hz, 1H, H-5’), 3.23 (dt, J = 3.7, 6.5, 13.1 Hz, 1H, H-2’), 2.22 (ddd, J = 3.7, 6.5, 13.1 Hz, 1H, H-2’), 2.93 (dd, J = 6.5, 11.1 Hz, 1H, H-3’), 2.86 (d, J = 11.1 Hz, 1H, H-3’), 2.52 (dd, J = 3.3, 6.5 Hz, 1H, H-3’), 2.24 (ddd, J = 6.5, 13.1 Hz, 1H, H-2’), 1.10 (t, J = 7.0 Hz, 18H, t-Bu), 0.86 (s, 6H, SiMe), 0.13 (s, 6H, SiMe). 0.03 and 0.00 (2s, 6H, SiMe).

**General procedure for the N-arylation of 8-ethenyl-3’,5’-bis-O-(tert-butyldimethylsilyl)-2’-deoxyadenosine (45)**

With aryl iodides

In a clean, dry, 8 mL vial equipped with a stirring bar were placed Pd(OAc)$_2$ (2.2 mg, 1.0 µmol) and Xantphos (5.7 mg, 1.0 µmol) in dry PhMe (1 mL), and the mixture was stirred for 2 min. Then 45 (50 mg, 0.10 mmol), Cs$_2$CO$_3$ (48.4 mg, 0.15 mmol) and the aryl iodide (1.3 equiv.) were added to the mixture. The reaction mixture was flushed with nitrogen gas, sealed with a Teflon-lined cap, and placed in a sand bath that was maintained at 85 °C. Reactions were monitored by TLC and upon consumption of the starting material, the reaction mixtures were diluted with EtOAc (20 mL) and washed with water (3 x 10 mL), brine (10 mL), dried over anhydrous Na$_2$SO$_4$, filtered, and evaporated to dryness under reduced pressure. The crude reaction products were purified by column chromatography and then by preparative TLC using appropriate solvents (see the individual compound headings below).

With aryl bromides
In a clean, dry, 8 mL vial equipped with a stirring bar were placed Pd(OAc)$_2$ (3.3 mg, 1.5 µmol) and Xantphos (8.6 mg, 1.5 µmol) in dry PhMe (1 mL), and the mixture was stirred for 2 min. Then 45 (50 mg, 0.10 mmol), Cs$_2$CO$_3$ (48.4 mg, 0.15 mmol) and the aryl bromide (1.3 equiv.) were added to the mixture. The reaction mixture was flushed with nitrogen gas, sealed with a Teflon-lined cap, and placed in a sand bath that was maintained at 85 °C. Reactions were monitored by TLC and upon consumption of the starting material, the reaction mixtures were diluted with EtOAc (20 mL) and washed with water (3 x 10 mL), brine (10 mL), dried over anhydrous Na$_2$SO$_4$, filtered, and evaporated to dryness under reduced pressure. The crude reaction products were purified by column chromatography and then by preparative TLC using appropriate solvents (see the individual reactions below).

**N-Arylation of 8-ethenyl-3’,5’-bis-O-(tert-butyldimethylsilyl)-2’-deoxyadenosine (45) with PhI**

The reaction was carried out with Pd(OAc)$_2$ (2.2 mg, 1.0 µmol, 0.1 equiv.), Xantphos (5.7 mg, 1.0 µmol, 0.1 equiv.), 45 (50 mg, 0.10 mmol, 1 equiv.), Cs$_2$CO$_3$ (48.4 mg, 0.15 mmol, 1.5 equiv.) and PhI (15.0 µL, 0.13 mmol, 1.3 equiv.) in dry PhMe (1 mL) at 75 °C for 6 h. Purification of the crude material by chromatography on a silica gel column (200–300 mesh) with 5–10% EtOAc in hexanes gave compound 64a (3.2 mg, 5% yield) as a brown, viscous liquid and compound 58a (43.1 mg, 75% yield) as a pale-yellow, viscous liquid. Subsequent elution with 30% EtOAc in hexanes gave compound 47a (2.9 mg, 5% yield) as a pale-yellow solid.

**3’,5’-Bis-O-(tert-butyldimethylsilyl)-N$^\theta$-phenyl-8-ethenyl-2’deoxyadenosine (58a)**
R_{f} (SiO_{2}/10\%\ EtOAc\ in\ hexanes) = 0.13. ¹H NMR (500 MHz, CDCl₃):
δ 8.45 (s, 1H, Ar-H), 7.81 (d, J = 7.8 Hz, 2H, Ar-H), 7.64 (br s, 1H, NH), 7.38 (t, J = 7.7 Hz, 2H, Ar-H), 7.11–7.03 (m, 2H, Ar-H, =CH), 6.51 (d, J = 17.2 Hz, 1H, =CH_{trans}), 6.44 (t, J = 6.8 Hz, 1H, H-1’), 5.71 (d, J = 11.1 Hz, 1H, =CH_{cis}), 4.79 (dt, J = 3.3, 6.4 Hz, 1H, H-3’), 3.97–3.90 (m, 2H, H-4’, H-5’), 3.73 (dd, J = 10.8 Hz, 1H, H-5’’), 3.38 (dt, J = 13.4 Hz, 1H, H-2’), 2.25 (ddd, J = 3.6, 6.5, 13.1 Hz, 1H, H-2’’), 0.94, and 0.86 (2s, 18H, t-Bu), 0.14 (s, 6H, SiMe), 0.03 and 0.00 (2s, 6H, SiMe). ¹³C NMR (125 MHz, CDCl₃): δ 152.4, 151.9, 150.5, 149.7, 139.0, 129.3, 124.5, 124.1, 123.6, 120.4, 120.3, 87.7, 83.9, 72.1, 62.6, 38.9, 26.1, 18.6, 18.3, –4.4, –4.5, –5.3. HRMS (ESI/TOF) m/z calculated for C₃₀H₄₈N₅O₃Si₂ [M + H]⁺: 582.3290, found 582.3281.

3’,5’-Bis-O-(tert-butyldimethylsilyl)-8-[(E)-2-phenylethenyl]-2’-deoxyadenosine (47a)⁶⁵

¹H NMR (500 MHz, CDCl₃): δ 8.27 (s, 1H, Ar-H), 7.82 (d, J = 15.9 Hz, 1H, =CH_{trans}), 7.60 (d, J = 7.5 Hz, 2H, Ar-H), 7.41 (t, J = 7.4 Hz, 2H, Ar-H), 7.36 (t, J = 7.2 Hz, 1H, Ar-H), 7.23 (d, J = 17.2 Hz, 1H, =CH_{trans}), 6.43 (t, J = 6.6 Hz, 1H, H-1’), 5.53 (br s, 2H, NH₂), 4.84 (dt, J = 3.5, 6.3 Hz, 1H, H-3’), 3.98 (app q, J_{app} ~ 4.9 Hz, 1H, H-4’), 3.88 (dd, J = 6.2, 10.8 Hz, 1H, H-5’), 3.72 (dd, J = 4.8, 10.8 Hz, 1H, H-5’’), 3.60 (dt, J = 6.5, 13.1 Hz, 1H, H-2’), 2.26 (ddd, J = 4.5, 6.8, 13.0 Hz, 1H, H-2’’), 0.94 and 0.82 (2s, 18H, t-Bu), 0.15 (2s, 6H, SiMe), 0.00 and –0.05 (2s, 6H, SiMe).

3’,5’-Bis-O-(tert-butyldimethylsilyl)-N⁶-phenyl-8-[(E)-2-phenylethenyl]-2’-deoxyadenosine (64a)
R_f(SiO_2/10% EtOAc in hexanes) = 0.33. ^1^H NMR (500 MHz, CDCl_3):
δ 8.44 (s, 1H, Ar-H), 7.88 (d, J = 15.9 Hz, 1H, =CH_{trans}), 7.84 (d, J = 7.7 Hz, 2H, Ar-H), 7.76 (br s, 1H, NH), 7.62 (d, J = 7.3 Hz, 2H, Ar-H), 7.45–7.34 (m, 5H, Ar-H), 7.26 (d, J = 15.9 Hz, 1H, =CH_{trans}), 7.11 (t, J = 7.4 Hz, 1H, Ar-H), 6.46 (t, J = 6.7 Hz, 1H, H-1’), 4.85 (dt, J = 3.9, 5.9 Hz, 1H, H-3’), 4.00 (app q, J_{app} ~ 4.9 Hz, 1H, H-4’), 3.90 (dd, J = 6.2, 10.8 Hz, 1H, H-5’), 3.74 (dd, J = 4.7, 10.8 Hz, 1H, H-5’’), 3.66 (dt, J = 6.5, 13.1 Hz, 1H, H-2’), 2.29 (ddd, J = 4.3, 6.7, 13.1 Hz, 1H, H-2’’), 0.95 and 0.82 (2s, 18H, t-Bu), 0.16, 0.00, and −0.04 (4s, 12H, SiMe). ^13^C NMR (125 MHz, CDCl_3): δ 152.0, 151.7, 150.5, 150.2, 139.1, 138.3, 136.0, 129.5, 129.2, 129.1, 127.7, 123.4, 120.9, 120.2, 114.2, 87.7, 84.3, 72.6, 63.0, 37.9, 26.1, 26.0, 18.6, 18.3, −4.4, −4.5, −5.2, −5.3. HRMS (ESI/TOF) m/z calculated for C_{36}H_{52}N_{5}O_{3}Si_{2}[M + H]^+: 658.3603, found 658.3607.

^N^-Arylation of 8-ethenyl-3’,5’-bis-O-( tert-butyldimethylsilyl)-2’-deoxyadenosine (45) with PhBr

The reaction was carried out with Pd(OAc)_2 (3.3 mg, 0.15 µmol, 0.15 equiv.), Xantphos (8.6 mg, 0.15 µmol, 0.15 equiv.), 45 (50 mg, 0.10 mmol, 1 equiv.), Cs_2CO_3 (48.4 mg, 0.15 mmol, 1.5 equiv.), and PhBr (13.5 µL, 0.13 mmol, 1.3 equiv.) in dry PhMe (1 mL), at 85 °C for 12 h. Purification of the crude material by chromatography on a silica gel column (200–300 mesh) with 5–10% EtOAc in hexanes gave compound 64a (3.5 mg, 5% yield) as a brown, viscous liquid and compound 58a (45.0 mg, 78% yield) as a pale-yellow, viscous liquid.

^N^-Arylation of 8-ethenyl-3’,5’-bis-O-( tert-butyldimethylsilyl)-2’-deoxyadenosine (45) with 4-iodotoluene
The reaction was carried out with Pd(OAc)$_2$ (2.2 mg, 1.0 µmol, 0.1 equiv.), Xantphos (5.7 mg, 1.0 µmol, 0.1 equiv.), 45 (50 mg, 0.10 mmol, 1 equiv.), Cs$_2$CO$_3$ (48.4 mg, 0.15 mmol, 1.5 equiv.), and 4-iodotoluene (28.1 mg, 0.13 mmol, 1.3 equiv.) in dry PhMe (1 mL), at 85 °C for 12 h. Purification of the crude material by chromatography on a silica gel column (200–300 mesh) with 7–15% EtOAc in hexanes gave compounds 64b and 58b as a mixture. Subsequent elution with 30% EtOAc in hexanes gave compound 47b (4.1 mg, 7% yield) as a pale-yellow solid. Purification of the mixture of 64b and 58b by preparative TLC (1 mm, 20 x 20 cm SiO$_2$ plate eluted with 15% EtOAc in hexanes) gave compound 64b (2.6 mg, 4% yield) as a pale-yellow, viscous liquid and compound 58b (41.7 mg, 71% yield) as a pale-yellow, viscous liquid.

3’,5’-Bis-O-(tert-butyldimethylsilyl)-N6-(4-tolyl)-2’-deoxyadenosine (58b)

R$_f$(SiO$_2$/10% EtOAc in hexanes) = 0.13. $^1$H NMR (500 MHz, CDCl$_3$): δ 8.42 (s, 1H, Ar-H), 7.71–7.60 (m, 3H, Ar-H, NH), 7.19 (d, $J$ = 7.8 Hz, 2H, Ar-H), 7.06 (dd, $J$ = 11.1, 17.2 Hz, 1H, =CH), 6.49 (d, $J$ = 17.2 Hz, 1H, =CH$_{trans}$), 6.44 (t, $J$ = 6.9 Hz, 1H, H-1’), 5.70 (d, $J$ = 11.1 Hz, 1H, =CH$_{cis}$), 4.80–4.76 (m, 1H, H-3’), 3.99-3.89 (m, 2H, H-4’, H-5’), 3.73 (dd, $J$ = 3.3, 10.6 Hz, 1H, H-5’’), 3.38–3.34 (m, 1H, H-2’), 2.40–2.20 (m, 4H, Me, H-2’’), 0.93 and 0.86 (2s, 18H, t-Bu), 0.13 (s, 6H, SiMe), 0.03 and 0.00 (2s, 6H, SiMe). HRMS (ESI/TOF) 

m/z calculated for C$_{31}$H$_{50}$N$_5$O$_3$Si$_2$ [M + H]$^+$: 596.3447, found 596.3445.

3’,5’-Bis-O-(tert-butyldimethylsilyl)-8-[(E)-2-(4-tolyl)ethenyl]-2’-deoxyadenosine (47b)\textsuperscript{65}

$^1$H NMR (500 MHz, CDCl$_3$): δ 8.26 (s, 1H, Ar-H), 7.80 (d, $J$ = 15.8 Hz, 1H, =CH$_{trans}$), 7.50 (d, $J$ = 7.9 Hz, 2H, Ar-H), 7.21 (d, $J$ = 7.9 Hz, 2H, Ar-H), 7.17 (d, $J$ = 15.8 Hz, 1H, =CH$_{trans}$), 6.42 (t, $J$ = 6.6 Hz, 1H, H-1’), 5.50 (s, 2H, NH$_2$), 4.84 (app q, $J_{app}$ ~ 4.7 Hz, 1H, H-3’), 3.98 (app q, $J_{app}$...
\[ \text{~4.8 Hz, 1H, H-4'}, 3.89 \text{ (dd, } J = 6.2, 10.8 \text{ Hz, 1H, H-5'}, 3.72 \text{ (dd, } J = 4.8, 10.8 \text{ Hz, 1H, H-5''), 3.61 \text{ (dt, } J = 4.8, 10.8 \text{ Hz, 1H, H-5''),} \]

\[ \text{2.39 (s, 3H, Me), 2.25 \text{ (ddd, } J = 4.7, 6.8, 12.8 \text{ Hz, 1H, H-2''), 0.94 and 0.82 (2s, 18H, t-Bu), 0.15, 0.14, 0.00, and } \]

\[ \text{–0.05 (4s, 12H, SiMe).} \]

3’,5’-Bis-O-(tert-butyldimethylsilyl)-N⁶-(4-tolyl)-8-[(E)-2-(4-tolyl)ethenyl]-2’-deoxyadenosine (64b)

\[ \text{R}_f \text{(SiO}_2/20\% \text{ EtOAc in hexanes) = 0.55.} \]

\[ ^1\text{H NMR (500 MHz, CDCl}_3\): } \delta \text{ 8.41 (s, 1H, Ar-H), 7.83 (d, } J = 15.6 \text{ Hz, 1H, =CH}_\text{trans}), \]

\[ 7.70–7.64 \text{ (m, 2H, Ar-H, NH), 7.51 (d, } J = 8.0 \text{ Hz, 2H, Ar-H), 7.24–7.17 \text{ (m, 5H, Ar-H, =CH}_\text{trans}), 6.45 \text{ (t, } J = 6.6 \text{ Hz, 1H, H-1’), 4.84 \text{ (dt, } J = 3.2, 5.7 \text{ Hz, 1H, H-3’), 4.00 (app q, } J_{app} \approx 4.6 \text{ Hz, 1H, H-4’), 3.90 (dd, } J = 6.3, 10.7 \text{ Hz, 1H, H-5'}, 3.73 \text{ (dd, } J = 4.7, 10.8 \text{ Hz, 1H, H-5’’), 3.65 \text{ (dt, } J = 6.5, 13.1 \text{ Hz, 1H, H-2’), 2.40 (s, 3H, Me), 2.35 (s, 3H, Me), 2.27 (ddd, } J = 4.3, 6.5, 12.8 \text{ Hz, 1H, H-2’’), 0.95 and 0.82 (2s, 18H, t-Bu), 0.16 (s, 6H, SiMe), 0.00 and } \]

\[ –0.04 \text{ (2s, 6H, SiMe).} \]

\[ \text{HRMS (ESI/TOF) } m/z \text{ calculated for C}_{38}H_{56}N_5O_3Si_2 [M + H]^+: 686.3916, \text{ found 686.3911.} \]

N-Arylation of 8-ethenyl-3’,5’-bis-O-(tert-butyldimethylsilyl)-2’-deoxyadenosine (45) with 4-bromotoluene

The reaction was carried out with Pd(OAc)_2 (3.3 mg, 1.5 µmol, 0.15 equiv.), Xantphos (8.6 mg, 1.5 µmol, 0.15 equiv.), 45 (50 mg, 0.10 mmol, 1 equiv.), Cs_2CO_3 (48.4 mg, 0.15 mmol, 1.5 equiv.), and 4-bromotoluene (15.8 µL, 0.13 mmol, 1.3 equiv.) in dry PhMe (1 mL), at 85 °C for 12 h. Purification of the crude material by chromatography on a silica gel column (200–300 mesh) with 7–15% EtOAc in hexanes gave the compounds 64b and 58b as a mixture. Further
purification of the mixture of 64b and 58b by preparative TLC (1 mm, 20 x 20 cm SiO₂ plate eluted with 10% EtOAc in hexanes) gave compound 64b (1.9 mg, 3% yield) as a pale-yellow, viscous liquid and compound 58b (41.3 mg, 70% yield) as a pale-yellow, viscous liquid.

**N-Arylation of 8-ethenyl-3’,5’-bis-O-(tert-butyldimethylsilyl)-2’-deoxyadenosine (45) with 4-iodoacetophenone**

The reaction was carried out with Pd(OAc)₂ (2.2 mg, 1.0 µmol, 0.1 equiv.), Xantphos (5.7 mg, 1.0 µmol, 0.1 equiv.), 45 (50 mg, 0.10 mmol, 1 equiv.), Cs₂CO₃ (48.4 mg, 0.15 mmol, 1.5 equiv.) and 4-iodoacetophenone (31.7 mg, 0.13 mmol, 1.3 equiv.) in dry PhMe (1 mL) at 85 °C for 12 h. Purification of the crude material by chromatography on a silica gel column (200–300 mesh) with 30% EtOAc in hexanes gave compounds 58c and 64c as a mixture. Subsequent elution with 60% EtOAc in hexanes gave compound 47c (3.9 mg, 6% yield) as a pale-yellow solid. Purification of the mixture of 64c and 58c by preparative TLC (1 mm, 20 x 20 cm SiO₂ plate eluted with 30% EtOAc in hexanes) gave compound 58c (47.6 mg, 77% yield) as a pale-yellow solid and compound 64c (5.2 mg, 7% yield) as a pale-yellow solid.

**3’,5’-Bis-O-(tert-butyldimethylsilyl)-N⁶-(4-acetylphenyl)-8-ethenyl-2’-deoxyadenosine (58c)**

![Image of 58c](image.png)

Rₛ(SiO₂/50% EtOAc in hexanes) = 0.61. ¹H NMR (500 MHz, CDCl₃):

δ 8.51 (s, 1H, Ar-H), 8.15 (br s, 1H, NH), 8.01–7.97 (m, 4H, Ar-H), 7.07 (dd, J = 11.1, 17.2 Hz, 1H, =CH), 6.51 (d, J = 17.1 Hz, 1H, =CH<sub>trans</sub>), 6.44 (t, J = 6.9 Hz, 1H, H-1’), 5.74 (d, J = 11.0 Hz, 1H, =CH<sub>trans</sub>), 4.80 (dt, J = 3.3, 6.4 Hz, 1H, H-3’), 3.96 (app q, J<sub>app</sub> ~ 4.1 Hz, 1H, H-4’), 3.91 (dd, J = 4.9, 11.0 Hz, 1H, H-5’), 3.73 (dd, J = 3.8, 11.0 Hz, 1H, H-5’), 3.36 (dt, J = 6.7, 13.3 Hz, 1H, H-2’), 2.59 (s, 3H, Me), 2.26 (ddd, J = 3.8, 6.5, 13.1 Hz, 1H, H-2’), 0.93
and 0.85 (2s, 18H, t-Bu), 0.14 (s, 6H, SiMe), 0.03 and 0.01 (2s, 6H, SiMe). $^{13}$C NMR (125 MHz, CDCl$_3$): δ 197.1, 152.1, 151.2, 150.8, 150.2, 143.8, 131.9, 130.1, 124.7, 124.3, 120.7, 119.0, 87.7, 84.0, 72.0, 62.6, 38.9, 26.6, 26.1, 26.0, 18.6, 18.3, –4.4, –4.5, –5.3. HRMS (ESI/TOF) m/z calculated for C$_{32}$H$_{49}$N$_5$O$_4$Si$_2$Na [M + Na]$^+$: 646.3215, found 646.3226.

3′,5′-Bis-O-(tert-butyldimethylsilyl)-8-[(E)-2-(4-acetylphenyl)ethenyl]-2′-deoxyadenosine (47c)

1H NMR (500 MHz, CDCl$_3$): δ 8.29 (s, 1H, Ar-H), 7.99 (d, J = 8.1 Hz, 2H, Ar-H), 7.86 (d, J = 15.8 Hz, 1H, =CH$_{trans}$), 7.68 (d, J = 8.1 Hz, 2H, Ar-H), 7.34 (d, J = 15.8 Hz, 1H, =CH$_{trans}$), 6.43 (t, J = 6.5 Hz, 1H, H-1’), 5.52 (s, 2H, NH$_2$), 4.85 (app q, $J_{app}$ ~ 4.6 Hz, 1H, H-3’), 3.99 (app q, $J_{app}$ ~ 4.7 Hz, 1H, H-4’), 3.87 (dd, J = 6.0, 10.9 Hz, 1H, H-5’), 3.71 (dd, J = 4.7, 10.9 Hz, 1H, H-5’’), 3.64 (dt, J = 6.4, 13.1 Hz, 1H, H-2’), 2.63 (s, 3H, Me), 2.29 (ddd, J = 4.7, 6.7, 13.0 Hz, 1H, H-2’’), 0.95 and 0.81 (2s, 18H, t-Bu), 0.16, –0.01, and –0.06 (3s, 12H, SiMe).

3′,5′-Bis-O-(tert-butyldimethylsilyl)-N$^6$-(4-acetylphenyl)-8-[(E)-2-(4-acetylphenyl)ethenyl]-2′-deoxyadenosine (64c)

$R_f$ (SiO$_2$/50% EtOAc in hexanes) = 0.46. 1H NMR (500 MHz, CDCl$_3$): δ 8.52 (s, 1H, Ar-H), 8.02–7.99 (m, 7H, Ar-H, NH), 7.92 (d, J = 15.9 Hz, 1H, =CH$_{trans}$), 7.70 (d, J = 8.2 Hz, 2H, Ar-H), 7.38 (d, J = 15.9 Hz, 1H, =CH$_{trans}$), 6.45 (t, J = 6.5 Hz, 1H, H-1’), 4.88 (app q, $J_{app}$ ~ 5.0 Hz, 1H, H-3’), 4.01 (app q, $J_{app}$ ~ 4.8 Hz, 1H, H-4’), 3.89 (dd, J = 5.8, 10.9 Hz, 1H, H-5’), 3.73–3.67 (m, 2H, H-5’’’, H-2’’), 2.64 (s, 3H, Me), 2.60 (s, 3H, Me), 2.33 (ddd, J = 4.7, 6.7, 13.0 Hz, 1H, H-2’’’), 0.95 and 0.80 (2s, 18H, t-Bu), 0.17 (s, 6H, SiMe), –
0.02 and –0.07 (2s, 6H, SiMe). \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \(\delta\) 197.5, 197.1, 152.1, 151.2, 150.8, 150.2, 143.7, 140.2, 137.6, 137.2, 131.9, 130.1, 129.2, 127.8, 121.4, 118.9, 116.4, 87.8, 84.5, 72.4, 62.9, 37.9, 26.9, 26.6, 26.1, 26.0, 18.6, 18.3, –4.4, –4.5, –5.2, –5.3. HRMS (ESI/TOF) \(m/z\) calculated for \(C_{40}H_{56}N_5O_5Si_2\) \([M + H]^+\): 742.3814, found 742.3834.

**N-Arylation of 8-ethenyl-3',5'-bis-O-(tert-butyldimethylsilyl)-2'-deoxyadenosine (45) with 4-bromoacetophenone**

The reaction was carried out with Pd(OAc)\(_2\) (3.3 mg, 1.5 \(\mu\)mol, 0.15 equiv.), Xantphos (8.6 mg, 1.5 \(\mu\)mol, 0.15 equiv.), 45 (50 mg, 0.10 mmol, 1 equiv.), Cs\(_2\)CO\(_3\) (48.4 mg, 0.15 mmol, 1.5 equiv.) and 4-bromotoluene (15.8 \(\mu\)L, 0.13 mmol, 1.3 equiv.) in dry PhMe (1 mL) at 85 °C for 12 h. Purification of the crude material by chromatography on a silica gel column (200–300 mesh) with 30% EtOAc in hexanes gave compounds 58c and 64c as a mixture. Further purification of the mixture of 64c and 58c by preparative TLC (1 mm, 20 x 20 cm SiO\(_2\) plate eluted with 30% EtOAc in hexanes) gave compound 58c (49.2 mg, 80% yield) as a yellow solid and compound 64c (2.8 mg, 4% yield) as a pale-yellow solid.

**N-Arylation of 8-ethenyl-3',5'-bis-O-(tert-butyldimethylsilyl)-2'-deoxyadenosine (45) with 2-iodotoluene**

The reaction was carried out with Pd(OAc)\(_2\) (2.2 mg, 1.0 \(\mu\)mol, 0.1 equiv.), Xantphos (5.7 mg, 1.0 \(\mu\)mol, 0.1 equiv.), 45 (50 mg, 0.10 mmol, 1 equiv.), Cs\(_2\)CO\(_3\) (48.4 mg, 0.15 mmol, 1.5 equiv.), and 2-iodotoluene (16.4 \(\mu\)L, 0.13 mmol, 1.3 equiv.) in dry PhMe (1 mL), at 85 °C for 12 h. Purification of the crude material by chromatography on a silica gel column (200–300 mesh) with 10–20% EtOAc in hexanes gave compounds 64d and 58d as a mixture. Subsequent elution with 40% EtOAc in hexanes gave the compound 47d (3.6 mg, 6% yield) as a white solid.
Purification of the mixture of 64d and 58d by preparative TLC (1 mm, 20 x 20 cm SiO₂ plate eluted with 20% EtOAc in hexanes) gave compound 64d (3.3 mg, 5% yield) as a yellow viscous liquid and compound 58d (42.6 mg, 72% yield) as a yellow viscous liquid.

3’,5’-Bis-O-(tert-butyldimethylsilyl)-N⁶-(2-tolyl)-8-ethenyl-2’-deoxyadenosine (58d)

\[
\text{R}_f(\text{SiO}_2/20\% \text{ EtOAc in hexanes}) = 0.34.
\]

\[\begin{align*}
\delta & 8.38 (s, 1\text{H, Ar-H}), 7.94 (d, J = 7.9 \text{ Hz, 1H, Ar-H}), 7.51 (\text{br s, 1H, NH}), 7.29–7.25 (m, 2\text{H, Ar-H}), 7.18 (t, J = 7.9 \text{ Hz, 1H, Ar-H}), 7.06 (d, J = 11.1, 17.2 \text{ Hz, 1H, =CH}), 6.52 (d, J = 17.2 \text{ Hz, 1H, =CH}_\text{trans}), 6.44 (t, J = 6.9 \text{ Hz, 1H, H-1’}), 5.71 (d, J = 11.2 \text{ Hz, 1H, =CH}_\text{cis}), 4.79 (d, J = 3.2, 6.4 \text{ Hz, 1H, H-3’}), 3.96-3.89 (m, 2\text{H, H-4’, H-5’}), 3.73 (d, J = 3.6, 10.7 \text{ Hz, 1H, H-5”}), 3.37 (d, J = 6.8, 13.4 \text{ Hz, 1H, H-2’}), 2.37 (s, 3\text{H, Me}), 2.25 (d, J = 3.6, 6.5, 13.1 \text{ Hz, 1H, H-2”}), 0.93 and 0.86 (2\text{s, 18H, t-Bu}), 0.13 (s, 6\text{H, SiMe}), 0.03 and 0.00 (2\text{s, 6H, SiMe}).
\end{align*}\]

\[\begin{align*}
\text{^13C NMR (125 MHz, CDCl}_3\text{): }\delta & 152.6, 152.5, 150.5, 149.5, 136.6, 130.9 (2\text{s}), 126.9, 125.2, 124.5, 124.0, 123.9, 120.4, 87.6, 83.9, 72.0, 62.6, 38.8, 26.1, 26.0, 18.6, 18.3, 18.2, -4.4, -4.5, -5.3.
\end{align*}\]

HRMS (ESI/TOF) \(m/z\) calculated for C₃₁H₅₀N₅O₃Si₂ [M + H]⁺: 596.3447, found 596.3461.

3’,5’-Bis-O-(tert-butyldimethylsilyl)-8-[(E)-2-(2-tolyl)ethenyl]-2’-deoxyadenosine (47d)

\[\begin{align*}
\delta & 8.27 (s, 1\text{H, Ar-H}), 8.08 (d, J = 15.7 \text{ Hz, 1H, =CH}_\text{trans}), 7.65 (d, J = 7.1 \text{ Hz, 1H, Ar-H}), 7.29–7.24 (m, 2\text{H, Ar-H}), 7.23–7.20 (m, 1\text{H, Ar-H}), 7.13 (d, J = 15.7 \text{ Hz, 1H, =CH}_\text{trans}), 6.42 (t, J = 6.6 \text{ Hz, 1H, H-1’}), 5.49 (s, 2\text{H, NH}_2), 4.84–4.81 (m, 1\text{H, H-3’}), 3.97
\end{align*}\]
(app q, $J_{app} \sim 4.8$ Hz, 1H, H-4’), 3.87 (dd, $J = 6.2, 10.8$ Hz, 1H, H-5’), 3.71 (dd, $J = 4.8, 10.8$ Hz, 1H, H-5’), 3.62 (dt, $J = 6.5, 13.1$ Hz, 1H, H-2’), 2.50 (s, 3H, Me), 2.25 (ddd, $J = 4.4, 6.6, 13.0$ Hz, 1H, H-2’), 0.93 and 0.82 (2s, 18H, t-Bu), 0.14, –0.01, and –0.06 (3s, 12H, SiMe).

$3',5'$-Bis-O-(tert-butyldimethylsilyl)-N$^6$-(2-tolyl)-8-[(E)-2-(2-tolyl)ethenyl]-2'-deoxyadenosine (64d)

\[
\text{R}_{f} (\text{SiO}_{2}/20\% \text{ EtOAc in hexanes}) = 0.54. \hspace{1cm} \text{^1H NMR (500 MHz, CDCl}_3) : \hspace{1cm} \delta 8.37 (s, 1H, Ar-H), 8.12 (d, } J = 15.8 \text{ Hz, 1H, =CH}_{trans}, 7.96 (d, } J = 7.9 \text{ Hz, 1H, Ar-H}), 7.67 (d, } J = 6.6 \text{ Hz, 1H, Ar-H}), 7.45 (br s, 1H, NH), 7.30–7.23 (m, 5H, Ar-H), 7.16 (d, } J = 15.8 \text{ Hz, 1H, =CH}_{trans}, 7.12 (t, } J = 7.6 \text{ Hz, 1H, Ar-H}), 6.45 (t, } J = 6.6 \text{ Hz, 1H, H-1’}), 4.84 (dt, } J = 3.7, 5.9 \text{ Hz, 1H, H-3’}), 3.99 (app q, } J_{app} \sim 4.9 \text{ Hz, 1H, H-4’}), 3.89 (dd, } J = 6.3, 10.9 \text{ Hz, 1H, H-5’}), 3.72 (dd, } J = 4.8, 10.8 \text{ Hz, 1H, H-5’}), 3.67 (dt, } J = 6.5, 13.2 \text{ Hz, 1H, H-2’}), 2.52 (s, 3H, Me), 2.41 (s, 3H, Me), 2.27 (ddd, } J = 4.1, 6.7, 13.2 \text{ Hz, 1H, H-2’}), 0.94 and 0.82 (2s, 18H, t-Bu), 0.15 (s, 6H, SiMe), 0.00 and –0.05 (2s, 6H, SiMe). \text{^13C NMR (125 MHz, CDCl}_3) : \delta 152.5, 152.2, 150.7, 150.3, 137.3, 136.6, 136.1, 135.1, 131.0, 129.3, 129.1, 127.7, 126.9, 126.5, 126.0, 125.2, 124.0, 120.9, 115.3, 87.7, 84.3, 72.6, 63.0, 37.8, 26.1, 20.2, 18.6, 18.4, 18.3, –4.4, –4.5, –5.2, –5.3. \text{HRMS (ESI/TOF) } m/z \text{ calculated for C}_{38}H_{56}N_{8}O_{3}Si_{2} [M + H]^+ : 686.3916 found 686.3903.

$2',3',5'$-Tris-O-(tert-butyldimethylsilyl)-8-iodoadenosine (65)
To a clean, dry, 250 mL three-neck round-bottomed flask, a solid addition tube containing I₂ (0.48 g, 1.88 mmol, 2 equiv.) was connected to one-neck. A solution of 32b (0.45 g, 0.94 mmol, 1 equiv.) in dry THF (14 mL) was introduced into the flask, and the solution was cooled to –70 °C. LDA (2.34 mL, 4.69 mmol, 5 equiv.) was added dropwise to the stirring mixture. The mixture was stirred at –70 °C for 1 h, brought to 0 °C, and I₂ was added to the reaction mixture by twisting the addition tube. The mixture was stirred for 1 more hour. The reaction mixture was diluted with Et₂O (30 mL) and washed with water (20 mL). The aqueous layer was back extracted with Et₂O (3 x 20 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na₂SO₄, filtered, and evaporated to dryness under reduced pressure. The crude product was chromatographed on a short silica gel column using CH₂Cl₂ followed by 5% MeOH in CH₂Cl₂. The product 65 (0.392 mg, 69% yield) was obtained as a light-brown solid.

\(^1\)H NMR (500 MHz, CDCl₃): \(\delta 8.21\) (s, 1H, Ar-H), \(5.86\) (d, \(J = 5.9\) Hz, 1H, H-1’), \(5.64\) (br s, 2H, NH₂), \(5.56\) (dd, \(J = 4.4, 5.8\) Hz, 1H, H-2’), \(4.57\) (dd, \(J = 2.1, 4.2\) Hz, 1H, H-3’), \(4.11-4.04\) (m, 2H, H-5’, H-5’’), \(3.71\) (app q, \(J_{app} \approx 7.4\) Hz, 1H, H-4’), \(0.92, 0.84\) and \(0.80\) (3s, 27H, t-Bu), \(0.16\) (2s, 6H, SiMe), \(0.02, -0.02, -0.07\) and \(-0.36\) (4s, 12H, SiMe).

**8-Ethenyl-2’,3’,5’-tris-O-(tert-butyldimethylsilyl)adenosine (46)**

\[\text{65} \rightarrow \text{46}\]

Into a clean, dry, 50 mL round-bottomed flask containing a stirring bar and with an condenser, was placed 65 (1.65 g, 2.24 mmol, 1 equiv.) in a 9:1 THF-H₂O (23 mL). Potassium vinyltrifluoroborate (449 mg, 3.35 mmol, 1.5 equiv.), Pd(PPh₃)₄ (387 mg, 0.335 mmol, 0.15
equiv.), and Cs$_2$CO$_3$ (2.18 g, 6.70 mmol, 3 equiv.) were added, and the resulting mixture was stirred at 90 °C for 20 h. After cooling to room temperature, the reaction mixture was diluted with EtOAc (30 mL) and washed with water (3 x 15 mL), brine (20 mL), dried over anhydrous Na$_2$SO$_4$, filtered, and evaporated to dryness under reduced pressure. The crude product was chromatographed on a silica gel column using CH$_2$Cl$_2$ followed by 2% MeOH in CH$_2$Cl$_2$. Product 46 (1.08 g, 74% yield) was obtained as a white solid. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.28 (s, 1H, Ar-H), 6.96 (dd, $J = 11.1$, 17.2 Hz, 1H, =CH), 6.48 (d, $J = 17.2$ Hz, 1H, =CH$_{\text{trans}}$), 5.98 (d, $J = 6.2$ Hz, 1H, H-1’), 5.70 (d, $J = 11.1$ Hz, 1H, =CH$_{\text{cis}}$), 5.44 (s, 2H, NH$_2$), 5.30 (t, $J = 5.3$ Hz, 1H, H-2’), 4.53–4.48 (m, 1H, H-3’), 4.07–4.04 (m, 2H, H-5’, H-5”), 3.77–3.73 (m, 1H, H-4’), 0.96, 0.86, and 0.74 (3s, 27H, t-Bu), 0.15 (2s, 6H, SiMe), 0.05, 0.01, –0.09, and –0.41 (4s, 12H, SiMe).

**General procedure for the N-arylation of 8-ethenyl-2’,3’,5’-tris-O-(tert-butyldimethylsilyl) adenosine (46)**

![Diagram of the reaction](image)

*With aryl iodides*

In a clean, dry, 8 mL vial equipped with a stirring bar were placed Pd(OAc)$_2$ (2.6 mg, 1.2 µmol), and Xantphos (6.8 mg, 1.2 µmol) in dry PhMe (0.8 mL), and the mixture was stirred for 2 min. Then 46 (50 mg, 0.08 mmol), Cs$_2$CO$_3$ (38.4 mg, 0.12 mmol) and the aryl iodide (1.3 equiv.) were added to the mixture. The reaction mixture was flushed with nitrogen gas, sealed with a Teflon-lined cap, and placed in a sand bath that was maintained at 100 °C. Reactions
were monitored by TLC and upon consumption of the starting material, the reaction mixtures were diluted with EtOAc (20 mL) and washed with water (3 x 10 mL), brine (10 mL), dried over anhydrous Na₂SO₄, filtered, and evaporated to dryness under reduced pressure. The crude reaction products were purified by column chromatography and then by preparative TLC using appropriate solvents (see the individual compound headings below).

*With aryl bromides*

In a clean, dry, 8 mL vial equipped with a stirring bar were placed Pd(OAc)₂ (3.5 mg, 1.6 µmol) and Xantphos (9.1 mg, 1.6 µmol) in dry PhMe (0.8 mL) and the mixture was stirred for 2 min. Then 46 (50 mg, 0.08 mmol), Cs₂CO₃ (38.4 mg, 0.12 mmol) and the aryl bromide (1.3 equiv.) were added to the mixture. The reaction mixture was flushed with nitrogen gas, sealed with a Teflon-lined cap, and placed in a sand bath that was maintained at 100 °C. Reactions were monitored by TLC and upon consumption of the starting material, the reaction mixtures were diluted with EtOAc (20 mL) and washed with water (3 x 10 mL), brine (10 mL), dried over anhydrous Na₂SO₄, filtered, and evaporated to dryness under reduced pressure. The crude reaction products were purified by column chromatography and then by preparative TLC using appropriate solvents (see the individual reactions below).

*N-Arylation of 8-ethenyl-2’,3’,5’-tris-O-(tert-butylidimethylsilyl)adenosine (46) with PhI*

The reaction was carried out with Pd(OAc)₂ (2.6 mg, 1.2 µmol, 0.15 equiv.), Xantphos (6.8 mg, 1.2 µmol, 0.15 equiv.), 46 (50 mg, 0.08 mmol, 1 equiv.), Cs₂CO₃ (38.4 mg, 0.12 mmol, 1.5 equiv.), and PhI (12.0 µL, 0.10 mmol, 1.3 equiv.) in dry PhMe (0.8 mL), at 100 °C for 12 h. Purification of the crude material by chromatography on a silica gel column (200–300 mesh) with 5–10% EtOAc in hexanes gave compounds 66a and 59a as a mixture. Subsequent elution
with 30% EtOAc in hexanes gave compound 48a (1.8 mg, 3% yield) as an off-white solid. Purification of the mixture of 66a and 59a by preparative TLC (1 mm, 20 x 20 cm SiO₂ plate eluted with 5% EtOAc in hexanes) gave compound 66a (4.3 mg, 7% yield) as a light brown, viscous liquid and compound 59a (39.7 mg, 71% yield) as a brown, viscous liquid.

\[ 2',3',5'-\text{Tris}-O-(\text{tert-butyldimethylsilyl})-N^6-\text{phenyl}-8-\text{ethenyladenosine (59a)}^{65} \]

\[
\text{R_f(SiO}_2/10\% \text{ EtOAc in hexanes)} = 0.35. \]

\[ ^1\text{H NMR (500 MHz, CDCl}_3): \]
\[ \delta 8.45 \ (s, 1\text{H, Ar-H}), 7.83 \ (d, J = 7.8 \text{ Hz}, 2\text{H, Ar-H}), 7.66 \ (\text{br s, 1H, NH}), 7.39 \ (t, J = 7.8 \text{ Hz}, 2\text{H, Ar-H}), 7.10 \ (t, J = 7.3 \text{ Hz}, 1\text{H, Ar-H}), 7.00 \ (dd, J = 11.1, 17.1 \text{ Hz}, 1\text{H, }=\text{CH}), 6.52 \ (d, J = 17.1 \text{ Hz}, 1\text{H, }=\text{CH}_{\text{trans}}), 6.02 \ (d, J = 6.3 \text{ Hz}, 1\text{H, H-1'}), 5.74 \ (d, J = 11.2 \text{ Hz}, 1\text{H, }=\text{CH}_{\text{cis}}), 5.32 \ (t, J = 5.3 \text{ Hz}, 1\text{H, H-2'\text{)}}), 4.50 \ (dd, J = 1.8, 4.2 \text{ Hz}, 1\text{H, H-3'\text{)}}), 4.12-4.05 \ (m, 2\text{H, H-4', H-5'\text{)}}), 3.79-3.74 \ (m, 1\text{H, H-5''\text{)}}), 0.97, 0.87, \text{ and 0.74 (3s, 27H, t-Bu)}, 0.16, 0.15, 0.06, 0.02, -0.09, \text{ and -0.41 (6s, 18H, SiMe)}.

\[ 2',3',5'-\text{Tris}-O-(\text{tert-butyldimethylsilyl})-8-\text{[(E)-2-phenylethenyl]adenosine (48a)}^{65} \]

\[ ^1\text{H NMR (500 MHz, CDCl}_3): \]
\[ \delta 8.26 \ (s, 1\text{H, Ar-H}), 7.83 \ (d, J = 15.9 \text{ Hz}, 1\text{H, }=\text{CH}_{\text{trans}}), 7.59 \ (d, J = 7.3 \text{ Hz}, 2\text{H, Ar-H}), 7.59 \ (t, J = 7.3 \text{ Hz}, 2\text{H, Ar-H}), 7.36 \ (t, J = 7.3 \text{ Hz}, 1\text{H, Ar-H}), 7.16 \ (d, J = 15.9 \text{ Hz}, 1\text{H, }=\text{CH}_{\text{trans}}), 6.00 \ (d, J = 5.3 \text{ Hz}, 1\text{H, H-1'}), 5.55 \ (\text{br s, 2H, NH}_2), 5.51 \ (t, J = 4.9 \text{ Hz}, 1\text{H, H-2'\text{)}}), 4.60 \ (t, J = 3.6 \text{ Hz}, 1\text{H, H-3'\text{)}}), 4.12-4.04 \ (m, 2\text{H, H-4', H-5'\text{)}}), 3.76 \ (dd, J = 4.0, 10.6 \text{ Hz}, 1\text{H, H-5''\text{)}}), 0.98, 0.83, \text{ and 0.77 (3s, 27H, t-Bu)}, 0.18, 0.17, 0.02, -0.04, -0.06, \text{ and -0.33 (6s, 18H, SiMe)}.

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2',3',5'-Tris-\(\text{O-(tert-butyldimethylsilyl)-N}^6\)-phenyl-8-[(E)-2-phenylethenyl]adenosine (66a)

\[ \text{R}_f(\text{SiO}_2/10\% \text{ EtOAc in hexanes}) = 0.51. \]

\(^1\text{H NMR (500 MHz, CDCl}_3\):}

\[ \delta 8.45 \ (s, 1\text{H, Ar-H}), 7.88 \ (d, J = 16.4 \text{ Hz, 1H, } =\text{CH}_{\text{trans}}), 7.85 \ (d, J = 8.2 \text{ Hz, 2H, Ar-H}), 7.72 \ (\text{br s, 1H, NH}), 7.61 \ (d, J = 7.4 \text{ Hz, 2H, Ar-H}), 7.45-7.36 \ (m, 5\text{H, Ar-H}), 7.19 \ (d, J = 15.9 \text{ Hz, 1H, } =\text{CH}_{\text{trans}}), 7.11 \ (t, J = 7.3 \text{ Hz, 1H, Ar-H}), 6.03 \ (d, J = 5.4 \text{ Hz, 1H, H-1’}), 5.57 \ (t, J = 4.8 \text{ Hz, 1H, H-2’}), 4.61 \ (t, J = 3.5 \text{ Hz, 1H, H-3’}), 4.15-4.08 \ (m, 2\text{H, H-4’, H-5’}), 3.78 \ (dd, J = 3.7, 10.3 \text{ Hz, 1H, H-5’’}), 0.99, 0.84, \text{and 0.78 (3s, 27H, t-Bu)}, 0.19, 0.18, 0.03, -0.02, -0.05, \text{and } -0.33 \ (6\text{s, 18H, SiMe}). \]

\(^{13}\text{C NMR (125 MHz, CDCl}_3\):} \[ \delta 152.1, 151.8, 150.5 \ (2\text{s}), 139.1, 138.7, 136.0, 129.6, 129.3, 129.1, 127.7, 123.5, 121.0, 120.3, 113.6, 88.7, 85.6, 72.7, 72.5, 62.7, 26.2, 26.1, 26.0, 18.5, 18.4, 18.1, -4.2, -4.3, -4.9, -5.2, -5.3. \]

HRMS (ESI/TOF) \[ m/z \text{ calculated for C}_{42}\text{H}_{66}\text{N}_5\text{O}_4\text{Si}_3 \ [\text{M + H}]^+: 788.4417, \text{ found 788.4411.} \]

\( \text{N-Arylation of 8-ethenyl-2’,3’,5’-tris-\(\text{O-(tert-butyldimethylsilyl)adenosine (46)} \) with PhBr} \)

The reaction was carried out with \(\text{Pd(OAc)}_2\) (3.5 mg, 1.6 \text{ µmol, 0.2 equiv.}), Xantphos (9.1 mg, 1.6 \text{ µmol, 0.2 equiv.}), 46 (50 mg, 0.08 mmol, 1 equiv.), \(\text{Cs}_2\text{CO}_3\) (38.4 mg, 0.12 mmol, 1.5 equiv.), and PhBr (11.0 \text{ µL, 0.10 mmol, 1.3 equiv.}) in dry PhMe (0.8 mL), at 100 °C for 12 h. Purification of the crude material by chromatography on a silica gel column (200–300 mesh) with 5–10\% EtOAc in hexanes gave compound 66a (3.8 mg, 6\% yield) as a light brown, viscous liquid and compound 59a (41.3 mg, 74\% yield) as a brown, viscous liquid.

\( \text{N-Arylation of 8-ethenyl-2’,3’,5’-tris-\(\text{O-(tert-butyldimethylsilyl)adenosine (46)} \) with 4-iodotoluene} \)
The reaction was carried out with Pd(OAc)$_2$ (2.6 mg, 1.2 µmol, 0.15 equiv.), Xantphos (6.8 mg, 1.2 µmol, 0.15 equiv.), 46 (50 mg, 0.08 mmol, 1 equiv.), Cs$_2$CO$_3$ (38.4 mg, 0.12 mmol, 1.5 equiv.), and 4-iodotoluene (22.3 mg, 0.10 mmol, 1.3 equiv.) in dry PhMe (0.8 mL), at 100 °C for 12 h. Purification of the crude material by chromatography on a silica gel column (200–300 mesh) with 5–15% EtOAc in hexanes gave compounds 66b and 59b as a mixture. Subsequent elution with 30% EtOAc in hexanes gave compound 48b (4.1 mg, 7% yield) as a pale-yellow solid. Purification of the mixture of 66b and 59b by preparative TLC (1 mm, 20 x 20 cm SiO$_2$ plate eluted with 10% EtOAc in hexanes) gave compound 66b (3.1 mg, 5% yield) as a yellow solid and compound 59b (39.8 mg, 70% yield) as a yellow solid.

2’,3’,5’-Tris-O-(tert-butyldimethylsilyl)-N$^6$-(4-tolyl)-8-ethenyladenosine (59b)

\[ \text{R}_{5}(\text{SiO}_2/20\% \text{EtOAc in hexanes}) = 0.36. \]

$^1$H NMR (500 MHz, CDCl$_3$): \( \delta \) 8.43 (s, 1H, Ar-H), 7.68 (d, \( J = 8.2 \) Hz, 2H, Ar-H), 7.58 (br s, 1H, NH), 7.19 (d, \( J = 8.0 \) Hz, 2H, Ar-H), 7.00 (dd, \( J = 11.1, 17.0 \) Hz, 1H, Ar-H, =CH), 6.52 (d, \( J = 17.1 \) Hz, 1H, =CH$_{\text{trans}}$), 6.01 (d, \( J = 6.1 \) Hz, 1H, H-1’), 5.73 (d, \( J = 11.0 \) Hz, 1H, =CH$_{\text{cis}}$), 5.34 (t, \( J = 5.0 \) Hz, 1H, H-2’), 4.53–4.48 (m, 1H, H-3’), 4.12-4.07 (m, 2H, H-5’, H-5’’), 3.77 (app q, \( J_{\text{app}} \sim 6.9 \) Hz, 1H, H-4’), 2.34 (s, 3H, Me), 0.97, 0.87, and 0.75 (3s, 27H, t-Bu), 0.16 (2s, 6H, SiMe), 0.06, 0.02, –0.08, and –0.40 (4s, 12H, SiMe). HRMS (ESI/TOF) m/z calculated for C$_{37}$H$_{64}$N$_5$O$_4$Si$_3$ [M + H]$^+$: 726.4261, found 726.4249.

2’,3’,5’-Tris-O-(tert-butyldimethylsilyl)-8-[(E)-2-(4-tolyl)ethenyl]adenosine (48b)$^{65}$

$^1$H NMR (500 MHz, CDCl$_3$): \( \delta \) 8.26 (s, 1H, Ar-H), 7.80 (d, \( J = 15.8 \) Hz, 1H, =CH$_{\text{trans}}$), 7.48 (d, \( J = 7.9 \) Hz, 2H, Ar-H), 7.22 (d, \( J = 7.9 \) Hz, 2H, Ar-H), 7.11 (d, \( J = 15.9 \) Hz, 1H, =CH$_{\text{trans}}$), 5.99 (d,
$J = 5.4 \text{ Hz, 1H, H-1'}$, 5.55 (br s, 2H, NH$_2$), 5.51 (t, $J= 4.9 \text{ Hz, 1H, H-2'}$), 4.60 (t, $J= 3.6 \text{ Hz, 1H, H-3'}$), 4.12–4.05 (m, 2H, H-4’, H-5”), 3.76 (dd, $J= 3.9, 10.5 \text{ Hz, 1H, H-5'}$), 2.39 (s, 3H, Me), 0.98, 0.83, and 0.77 (3s, 27H, t-Bu), 0.18, 0.02, –0.03, –0.06, and –0.34 (5s, 18H, SiMe)

$2',3',5'$-Tris-O-(tert-butyldimethylsilyl)-N$^6$-(4-tolyl)-8-[(E)-2-(4-tolyl)ethenyl]adenosine (66b)

R$_f$ (SiO$_2$/10% EtOAc in hexanes) = 0.28. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.41 (s, 1H, Ar-H), 7.85 (d, $J= 15.6 \text{ Hz, 1H, =CH}_\text{trans}$), 7.70 (d, $J= 8.1 \text{ Hz, 2H, Ar-H}$), 7.56 (br s, 1H, NH), 7.50 (d, $J= 7.7 \text{ Hz, 2H, Ar-H}$), 7.23 (d, $J= 7.7 \text{ Hz, 2H, Ar-H}$), 7.20 (d, $J= 8.0 \text{ Hz, 2H, Ar-H}$), 7.13 (d, $J= 15.8 \text{ Hz, 1H, =CH}_\text{trans}$), 6.01 (d, $J= 5.3 \text{ Hz, 1H, H-1'}$), 5.56 (t, $J= 4.3 \text{ Hz, 1H, H-2'}$), 4.61 (t, $J= 3.3 \text{ Hz, 1H, H-3'}$), 4.14–4.07 (m, 2H, H-5’, H-5”), 3.80–3.74 (m, 1H, H-4’), 2.40 (s, 3H, Me), 2.35 (s, 3H, Me), 0.98, 0.84, and 0.77 (3s, 27H, t-Bu), 0.18 (2s, 6H, SiMe), 0.27, –0.02, –0.06, and –0.34 (4s, 12H, SiMe). HRMS (ESI/TOF) m/z calculated for C$_{44}$H$_{69}$N$_5$O$_4$Si$_3$Na [M + Na]$^+$: 838.4550, found 838.4542.

$N$-Arylation of 8-ethenyl-2’,3’,5’-tris-O-(tert-butyldimethylsilyl)adenosine (46) with 4-bromotoluene

The reaction was carried out with Pd(OAc)$_2$ (3.5 mg, 1.6 µmol, 0.2 equiv.), Xantphos (9.1 mg, 1.6 µmol, 0.2 equiv.), 46 (50 mg, 0.08 mmol, 1 equiv.), Cs$_2$CO$_3$ (38.4 mg, 0.12 mmol, 1.5 equiv.), and PhBr (13.0 µL, 0.10 mmol, 1.3 equiv.) in dry PhMe (0.8 mL), at 100 °C for 12 h. Subsequent elution with 30% EtOAc in hexanes gave the compound 48b (1.1 mg, 2% yield) as a
pale-yellow solid. Purification of the crude material by chromatography on a silica gel column (200–300 mesh) with 5–10% EtOAc in hexanes gave compounds \(66b\) and \(59b\) as a mixture. Subsequent elution with 30% EtOAc in hexanes gave compound \(48b\) (1.1 mg, 2% yield) as a white solid. Purification of the mixture of \(66b\) and \(59b\) by preparative TLC (1 mm, 20 x 20 cm SiO\(_2\) plate eluted with 10% EtOAc in hexanes) gave compound \(66b\) (1.3 mg, 2% yield) as a yellow solid and compound \(59b\) (39.5 mg, 69% yield) as a yellow solid.

\textbf{N-Arylation of 8-ethenyl-2',3',5'-tris-(tert-butyldimethylsilyl)adenosine (46) with 4-iodoacetophenone}

The reaction was carried out with Pd(OAc)\(_2\) (2.6 mg, 1.2 µmol, 0.15 equiv.), Xantphos (6.8 mg, 1.2 µmol, 0.15 equiv.), \(46\) (50 mg, 0.08 mmol, 1 equiv.), Cs\(_2\)CO\(_3\) (38.4 mg, 0.12 mmol, 1.5 equiv.), and 4-iodoacetophenone (25.1 mg, 0.10 mmol, 1.3 equiv.) in dry PhMe (0.8 mL), at 100 °C for 12 h. Purification of the crude material by chromatography on a silica gel column (200–300 mesh) with 30% EtOAc in hexanes gave compound \(59c\) with minor impurities. Subsequent elution with 50% EtOAc in hexanes gave compound \(48c\) (3.1 mg, 5% yield) as a pale-yellow solid. Purification of the impure \(59c\) using preparative TLC (1 mm, 20 x 20 cm SiO\(_2\) plate eluted with 30% EtOAc in hexanes) gave compound \(59c\) (46.2 mg, 78% yield) as a yellow solid.

\textbf{2',3',5'-Tris-(tert-butyldimethylsilyl)-N\(^6\)-(4-acetylphenyl)-8-ethenyladenosine (59c)}

\[
\text{R}_f(\text{SiO}_2/20\% \text{ EtOAc in hexanes}) = 0.27. \quad ^1\text{H NMR (500 MHz, CDCl}_3\text{):}
\]
\[
\delta 8.52 (s, 1H, Ar-H), 8.01 (d, \(J = 8.9 \text{ Hz}, 2\text{H}, \text{Ar-H}), 7.99 (d, \(J = 8.9 \text{ Hz, 2H, Ar-H}), 7.91 (\text{br s, 1H, NH}), 7.02 (dd, \(J = 11.0, 17.1 \text{ Hz, 1H, =CH}), 6.55 (d, \(J = 17.1 \text{ Hz, 1H, =CH}_{\text{trans}})), 6.03 (d, \(J = 6.3 \text{ Hz, 1H, H-1'})), 5.77 (d, \(J = 11.4 \text{ Hz, 1H, =CH}_{\text{cis}})), 5.31–5.27 (m, 1H, H-2'), 4.50 (dd, \(J = 2.3, \]
4.4 Hz, 1H, H-3’), 4.12–4.04 (m, 2H, H-4’, H-5’), 3.77 (dd, J = 3.5, 10.7 Hz, 1H, H-5’’), 2.60 (s, 3H, –COCH₃), 0.97, 0.87, and 0.74 (3s, 27H, t-Bu), 0.17, 0.16, 0.06, 0.03, –0.09, and –0.42 (6s, 18H, SiMe). ¹³C NMR (125 MHz, CDCl₃): δ 197.0, 152.1, 151.2, 151.0, 150.5, 143.7, 131.9, 130.1, 124.9, 123.9, 120.9, 118.9, 88.0, 86.2, 73.1, 72.4, 62.8, 26.6, 26.1 (2s), 25.8, 18.6, 18.3, 18.0, –4.2, –4.4, –5.1, –5.2, –5.3. HRMS (ESI/TOF) m/z calculated for C₃₈H₆₃N₅O₄Si₃Na [M + Na]⁺: 776.4029, found 776.4031.

2’,3’,5’-Tris-O-(tert-butyldimethylsilyl)-8-[(E)-2-(4-acetylphenyl)ethenyl]adenosine (48c) ⁶⁵

![Structure of 48c](image)

¹H NMR (500 MHz, CDCl₃): δ 8.28 (s, 1H, Ar-H), 8.00 (d, J = 8.3 Hz, 2H, Ar-H), 7.86 (d, J = 15.9 Hz, 1H, =CH₉₆trans), 7.66 (d, J = 8.2 Hz, 2H, Ar-H), 7.26 (d, J = 15.9 Hz, 1H, =CH₉₆trans), 5.99 (d, J = 5.4 Hz, 1H, H-1’), 5.54 (t, J = 4.8 Hz, 1H, H-2’), 5.49 (br s, 2H, NH₂), 4.60 (t, J = 3.7 Hz, 1H, H-3’), 4.12 (dt, 1H, J = 3.5, 6.8 Hz, H-4’), 4.07 (dd, J = 7.0, 10.7 Hz, 1H, H-5’’), 3.76 (dd, J = 4.2, 10.8 Hz, 1H, H-5’’’), 2.63 (s, 3H, –COCH₃), 0.98, 0.82, and 0.77 (3s, 27H, t-Bu), 0.18, 0.17, 0.02, –0.04, –0.05, and –0.33 (6s, 18H, SiMe).

N-Arylation of 8-ethenyl-2’,3’,5’-tris-O-(tert-butyldimethylsilyl)adenosine (46) with 4-bromoacetophenone

The reaction was carried out with Pd(OAc)₂ (3.5 mg, 1.6 µmol, 0.2 equiv.), Xantphos (9.1 mg, 1.6 µmol, 0.2 equiv.), 46 (50 mg, 0.08 mmol, 1 equiv.), Cs₂CO₃ (38.4 mg, 0.12 mmol, 1.5 equiv.), and 4-bromoacetophenone (20.3 mg, 0.10 mmol, 1.3 equiv.) in dry PhMe (0.8 mL), at 100 °C for 12 h. Purification of the crude material by chromatography on a silica gel column (200–300 mesh) with 30% EtOAc in hexanes gave compound 59c with minor impurities.
Further purification of impure 59c using preparative TLC (1 mm, 20 x 20 cm SiO2 plate eluted with 30% EtOAc in hexanes) gave compound 59c (46.9 mg, 79% yield) as a yellow solid.

*N-Arylation of 8-ethenyl-2’,3’,5’-tris-O-(tert-butyldimethylsilyl)adenosine (46) with 2-iodotoluene*

The reaction was carried out with Pd(OAc)$_2$ (2.6 mg, 1.2 µmol, 0.15 equiv.), Xantphos (6.8 mg, 1.2 µmol, 0.15 equiv.), 46 (50 mg, 0.08 mmol, 1 equiv.), Cs$_2$CO$_3$ (38.4 mg, 0.12 mmol, 1.5 equiv.), and 2-iodotoluene (13.0 µL, 0.10 mmol, 1.3 equiv.) in dry PhMe (0.8 mL), at 100 ºC for 12 h. Purification of the crude material by chromatography on a silica gel column (200–300 mesh) with 7–15% EtOAc in hexanes gave compounds 66d and 59d as a mixture. Subsequent elution with 30% EtOAc in hexanes gave compound 48d (4.2 mg, 7% yield) as a white solid. Purification of the mixture of 66a and 59a by preparative TLC (1 mm, 20 x 20 cm SiO2 plate eluted with 10% EtOAc in hexanes) gave compound 66d (2.7 mg, 4% yield) as a yellow, viscous liquid and compound 59d (40.8 mg, 71% yield) as a yellow, viscous liquid.

2’,3’,5’-Tris-O-(tert-butyldimethylsilyl)-N$^6$-(2-tolyl)-8-ethenyladenosine (59d)

![Structure of 59d](structure.png)

R$_f$(SiO$_2$/10% EtOAc in hexanes) = 014. $^1$H NMR (500 MHz, CDCl$_3$): δ 8.38 (s, 1H, Ar-H), 7.98 (d, J = 7.9 Hz, 1H, Ar-H), 7.50 (s, 1H, NH), 7.29–7.26 (m, 2H, Ar-H), 7.12 (d, J = 7.4 Hz, 1H, Ar-H), 7.00 (dd, J = 11.1, 17.1 Hz, 1H, Ar-H, =CH), 6.53 (dd, J = 1.3, 17.1 Hz, 1H, Ar-H, =CH$_{trans}$), 6.01 (d, J = 6.1 Hz, 1H, H-1’), 5.73 (dd, J = 1.3, 11.1 Hz, 1H, Ar-H, =CH$_{cis}$), 5.34 (t, J = 5.3 Hz, 1H, H-2’), 4.54–4.50 (m, 1H, H-3’), 4.12–4.04 (m, 2H, H-4’, H-5’), 3.76–3.75 (m, 1H, H-5’’), 2.38 (s, 3H, Me), 0.97, 0.86, and 0.75 (3s, 27H, t-Bu), 0.16, 0.15, 0.05, 0.01, −0.07, and −0.38 (6s, 18H, SiMe). $^{13}$C NMR (125 MHz, CDCl$_3$): δ 152.7, 152.5, 140
150.6, 149.8, 136.6, 130.9, 129.2, 126.9, 124.4, 123.9, 120.6, 120.3, 88.1, 86.0, 73.0, 72.4, 62.7, 26.2, 26.1, 25.9, 18.6, 18.4, 18.3, 18.0, –4.2, –4.3, –4.4, –5.1, –5.2, –5.3. HRMS (ESI/TOF) m/z calculated for C_{37}H_{64}N_{5}O_{4}Si_{3} [M + H]^+: 726.4261, found 726.4240.

2',3',5'-Tris-O-(tert-butyldimethylsilyl)-8-[(E)-2-(2-tolyl)ethenyl]adenosine (48d)

\[
\text{\includegraphics[width=0.5\textwidth]{48d}}
\]

\[^1\text{H NMR (500 MHz, CDCl}_3\text{): }\delta 8.27 (s, 1\text{H, Ar-H}), 8.08 (d, } J = 15.8 \text{ Hz, } 1\text{H, } =\text{CH}_\text{trans}, 7.62 (d, } J = 7.6 \text{ Hz, } 1\text{H, Ar-H}), 7.28–7.24 (m, 2\text{H, Ar-H}), 7.24–7.21 (m, 1\text{H, Ar-H}), 7.06 (d, } J = 15.7 \text{ Hz, } 1\text{H, } =\text{CH}_\text{trans}, 5.98 (d, } J = 5.4 \text{ Hz, } 1\text{H, H-1'}), 5.58 (\text{br s, } 2\text{H, NH}_2\text{), } 5.52 (t, } J = 4.8 \text{ Hz, } 1\text{H, H-2'}), 4.59 (t, } J = 3.5 \text{ Hz, } 1\text{H, H-3'}), 4.11–4.04 (m, } 2\text{H, H-4', H-5'}), 3.75 (dd, } J = 4.0, 10.4 \text{ Hz, } 1\text{H, H-5''}), 2.49 (s, } 3\text{H, Me), 0.97, 0.82, and 0.77 (3s, 27\text{H, } t-\text{Bu), } 0.17, 0.16, 0.01, –0.04, –0.06, and –0.34 (6s, 18\text{H, SiMe).}

2',3',5'-Tris-O-(tert-butyldimethylsilyl)-N^6-(2-tolyl)-8-[(E)-2-(2-tolyl)ethenyl]adenosine (66d)

\[
\text{\includegraphics[width=0.5\textwidth]{66d}}
\]

\[R_f(\text{SiO}_2/10\% \text{EtOAc in hexanes}) = 0.18. \] \[^1\text{H NMR (500 MHz, CDCl}_3\text{): }\delta 8.37 (s, 1\text{H, Ar-H}), 8.12 (d, } J = 15.7 \text{ Hz, } 1\text{H, } =\text{CH}_\text{trans}, 7.98 (d, } J = 7.9 \text{ Hz, } 1\text{H, Ar-H}), 7.66–7.62 (m, 1\text{H, Ar-H}), 7.47 (s, 1\text{H, NH}), 7.31–7.22 (m, 5\text{H, Ar-H}), 7.15–7.06 (m, } 2\text{H, Ar-H, } =\text{CH}_\text{trans}, 6.01 (d, } J = 5.4 \text{ Hz, } 1\text{H, H-1'}), 5.58 (t, } J = 4.8 \text{ Hz, } 1\text{H, H-2'}), 4.61 (t, } J = 3.6 \text{ Hz, } 1\text{H, H-3'}), 4.14–4.05 (m, } 2\text{H, H-4', H-5'}), 3.75 (dd, } J = 3.6, 10.3 \text{ Hz, } 1\text{H, H-5''}), 2.51 (s, } 3\text{H, Me), 2.40 (s, } 3\text{H, Me), 0.97, 0.82, and 0.78 (3s, 27\text{H, } t-\text{Bu), } 0.18, 0.17, and 0.01 (3s, 9\text{H, SiMe), } –0.04 (s, } 6\text{H, SiMe), } –0.31 (s, } 6\text{H, SiMe). \] \[^1\text{C NMR (125 MHz, CDCl}_3\text{): }\delta 152.5, 155.3, 150.6, 150.5, 137.3, 136.7, 136.5, 135.2, 131.0, 130.9, 129.4, 126.9, 126.6, 126.2, 125.2, 123.9, 121.0, 114.9,
88.8, 85.5, 72.6, 72.5, 62.6, 26.2, 26.1, 25.9, 20.2, 18.5, 18.4, 18.1, −4.2, −4.3, −4.4, −4.9, −5.2, −5.3. HRMS (ESI/TOF) m/z calculated for C_{44}H_{70}N_{5}O_{4}Si_{3} [M + H]^{+}: 816.4730, found 816.4718.

**N-Arylation of 8-ethenyl-2’,3’,5’-tris-(tert-butyldimethylsilyl)adenosine (46) with 1-iodonaphthalene**

The reaction was carried out with Pd(OAc)$_2$ (2.6 mg, 1.2 µmol, 0.15 equiv.), Xantphos (6.8 mg, 1.2 µmol, 0.15 equiv.), 46 (50 mg, 0.08 mmol, 1 equiv.), Cs$_2$CO$_3$ (38.4 mg, 0.12 mmol, 1.5 equiv.), and 1-iodonaphthalene (15.0 µL, 0.10 mmol, 1.3 equiv.) in dry PhMe (0.8 mL), at 100°C for 12 h. Purification of the crude material by chromatography on a silica gel column (200–300 mesh) with 7–15% EtOAc in hexanes gave compounds 66e and 59e as a mixture. Subsequent elution with 30% EtOAc in hexanes gave compound 48e (2.5 mg, 4% yield) as a white solid. Purification of the mixture of 66e and 59e by preparative TLC (1 mm, 20 x 20 cm SiO$_2$ plate eluted with 10% EtOAc in hexanes) gave compound 66e (2.1 mg, 3% yield) as a yellow solid and compound 59e (40.6 mg, 68% yield) as a pale-yellow solid.

**2’,3’,5’-Tris-(tert-butyldimethylsilyl)-N^6-(naphthalene-1-yl)-8-ethenyladenosine (59e)**

![Structure of 59e](image)

R$_f$(SiO$_2$/5% EtOAc in hexanes) = 0.14. $^1$H NMR (500 MHz, CDCl$_3$): δ 8.39 (s, 1H, Ar-H), 8.16 (d, $J = 7.4$ Hz, 1H, Ar-H), 8.09–8.08 (m, 1H, Ar-H), 7.93–7.88 (m, 2H, Ar-H, NH), 7.74 (d, $J = 8.2$ Hz, 1H, Ar-H), 7.57–7.51 (m, 3H, Ar-H), 7.03 (dd, $J = 11.1, 17.1$ Hz, 1H, =CH), 6.58 (d, $J = 17.1$ Hz, 1H, =CH$_{trans}$), 6.03 (d, $J = 6.1$ Hz, 1H, H-1’), 5.77 (d, $J = 11.1$ Hz, 1H, =CH$_{cis}$), 5.38 (t, $J = 5.2$ Hz, 1H, H-2’), 4.53 (dd, $J = 2.2, 4.3$ Hz, 1H, H-3’), 4.14–4.06 (m, 2H, H-4’, H-5’), 3.80–3.74 (m, 1H, H-5’’), 0.97, 0.87, and 0.77 (3s, 27H, t-Bu), 0.17, 0.16, 0.05, 0.02, −0.06, and −0.35 (6s, 18H, SiMe). $^{13}$C NMR (125 MHz, CDCl$_3$): δ 153.2, 152.6,
150.8, 150.1, 134.6, 133.5, 128.9, 126.4, 126.3, 126.0, 125.7, 124.5, 124.0, 121.7, 120.9 (2s), 88.2, 86.0, 73.0, 72.4, 62.7, 26.2, 26.1, 25.9, 18.6, 18.4, 18.1, –4.2, –4.3, –4.4, –5.0, –5.2, –5.3. HRMS (ESI/TOF) m/z calculated for C_{40}H_{64}N_{5}O_{4}Si_{3} [M + H]^+ : 762.4261, found 762.4260.

2',3',5'-Tris-O-(tert-butyldimethylsilyl)-8-[(E)-2-(naphthalene-1-yl)ethenyl]adenosine (48e)

\[
\text{1H NMR (500 MHz, CDCl}_3\text{): } \delta 8.65 (d, J = 15.7 \text{ Hz, 1H, } =CH_{trans}), 8.30–8.28 (m, 2H, Ar-H), 7.89 (d, J = 7.9 \text{ Hz, 2H, Ar-H}), 7.81 (d, J = 7.2 \text{ Hz, 1H, Ar-H}), 7.61–7.51 (m, 3H, Ar-H), 7.24 (d, J = 15.9 \text{ Hz, 1H, } =CH_{trans}), 6.03 (d, J = 5.4 \text{ Hz, 1H, H-1'}), 5.55–5.50 (m, 3H, NH\text{2}, H-2'), 4.61 (t, J = 3.7 \text{ Hz, 1H, H-3'}), 4.12–4.05 (m, 2H, H-4', H-5'), 3.76 (dd, J = 4.0, 10.7 \text{ Hz, 1H, H-5'''}, 0.96, 0.82, and 0.77 (3s, 27H, t-Bu), 0.17, 0.16, 0.01, –0.05, and –0.31 (5s, 18H, SiMe).
\]

R_f(SiO_2/10% EtOAc in hexanes) = 0.18. 1H NMR (500 MHz, CDCl_3):

\[
\delta 8.75 (d, J = 15.6 \text{ Hz, 1H, } =CH_{trans}), 8.40 (s, 1H, Ar-H), 8.35 (d, J = 8.4 \text{ Hz, 1H, Ar-H}), 8.19 (d, J = 7.5 \text{ Hz, 1H, Ar-H}), 8.16 (dd, J = 0.7, 8.1 \text{ Hz, 1H, Ar-H}), 8.00 (br s, 1H, NH), 7.94–7.89 (m, 3H, Ar-H), 7.86 (d, J = 7.1 \text{ Hz, 1H, Ar-H}), 7.76 (d, J = 8.1 \text{ Hz, 1H, Ar-H}), 7.64–7.51 (m, 6H, Ar-H), 7.30 (d, J = 15.6 \text{ Hz, 1H, } =CH_{trans}), 6.01 (d, J = 5.2 \text{ Hz, 1H, H-1'}), 5.62 (t, J = 4.8 \text{ Hz, 1H, H-2'}), 4.65 (t, J = 3.8 \text{ Hz, 1H, H-3'}), 4.16–4.09 (m, 2H, H-4', H-5'), 3.78 (dd, J = 3.9, 10.6 \text{ Hz, 1H, H-5'''}, 0.97, 0.83, and 0.81 (3s, 27H, t-Bu), 0.19, 0.18, 0.02, –0.01, –0.03, and –0.26 (6s, 18H, SiMe). 13C NMR (125 MHz, CDCl_3): \delta 153.1, 152.4, 150.8, 150.6, 135.8, 134.7, 134.0,
133.7, 133.6, 131.7, 129.9, 128.9, 128.4, 126.9, 126.4 (2s), 126.3, 126.1, 125.9, 125.7, 124.6, 124.0, 121.8, 121.3, 121.1, 116.4, 88.9, 85.6, 72.8, 72.5, 62.6, 26.2, 26.0 (2s), 18.5, 18.4, 18.1, –4.2, –4.3, –4.4, –4.8, –5.2, –5.3. HRMS (ESI/TOF) m/z calculated for C_{50}H_{70}N_{5}O_{4}Si_{3} [M + H]^+: 888.4730, found 888.4737.

**General procedure for the one-pot sequential diarylation of 8-ethenyl-3’,5’-bis-O-(tert-butyl dimethylsilyl)-2’-deoxyadenosine (45)**

![Diagram of the reaction](image)

Into a clean, dry, 8 mL vial equipped with a stirring bar were placed Pd(OAc)$_2$ (2.2 mg, 1.0 µmol), and DPEPhos (5.3 mg, 1.0 µmol) in dry PhMe (1 mL), and the mixture was stirred for 2 min. Then 45 (50 mg, 0.10 mmol), Cs$_2$CO$_3$ (48.4 mg, 0.15 mmol), and aryl iodide (1.3 equiv.) were added to the mixture. The reaction mixture was flushed with nitrogen gas, sealed with a Teflon-lined cap, and placed in a sand bath that was maintained at 100 °C. Reactions were monitored by TLC and upon consumption of the starting material, Pd(OAc)$_2$ (2.2 mg, 1.0 µmol), Xantphos (5.7 mg, 1.0 µmol), Cs$_2$CO$_3$ (48.4 mg, 0.15 mmol), and aryl iodide (1.3 equiv.) were added to the mixture and stirred at 100 °C. Upon completion, the reaction mixtures were diluted with EtOAc (20 mL) and washed with water (3 x 10 mL), brine (10 mL), dried over anhydrous Na$_2$SO$_4$, filtered, and evaporated to dryness under reduced pressure. The crude reaction products were purified by column chromatography and then by preparative TLC using appropriate solvents (see the individual compound headings below).
3',5'-Bis-\(O\)-(tert-butyldimethylsilyl)-\(N^6\)-(4-acetylphenyl)-8-[(\(E\))-2-phenylethenyl]-2'-deoxy-adenosine (64f)

In step 1, the reaction was carried out with \(\text{Pd(OAc)}_2\) (2.2 mg, 1.0 µmol, 0.1 equiv.), DPEPhos (5.3 mg, 1.0 µmol, 0.1 equiv.), \(\text{Cs}_2\text{CO}_3\) (48.4 mg, 0.15 mmol, 1.5 equiv.), and PhI (15.0 µL, 0.13 mmol, 1.3 equiv.) in dry PhMe (1 mL), at 100 °C for 18 h. After completion of step 1, \(\text{Pd(OAc)}_2\) (2.2 mg, 1.0 µmol, 0.1 equiv.), Xantphos (5.7 mg, 1.0 µmol, 0.1 equiv.), \(\text{Cs}_2\text{CO}_3\) (48.4 mg, 0.15 mmol, 1.5 equiv.), and 4-iodoacetophenone (31.7 mg, 0.13 mmol, 1.3 equiv.) were added to the vial containing the reaction mixture and stirred at 100 °C for 12 h. Purification of the crude material by chromatography on a silica gel column (200–300 mesh) with 20% EtOAc in hexanes gave the compound 64f along with some impurities. Further purification using preparative TLC (1 mm, 20 x 20 cm SiO\(_2\) plate eluted with 20% EtOAc in hexanes) gave compound 64f (26.3 mg, 38% yield) as a light-brown solid. \(R_f(\text{SiO}_2/20\% \text{ EtOAc in hexanes}) = 0.34\). \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 8.51 (s, 1H, Ar-H), 8.01 (d, \(J = 9.0\) Hz, 2H, Ar-H), 8.00 (d, \(J = 9.0\) Hz, 2H, Ar-H), 7.94 (br s, 1H, NH), 7.90 (d, \(J = 15.8\) Hz, 1H, =CH\(_{trans}\)), 7.63 (d, \(J = 7.5\) Hz, 2H, Ar-H), 7.43 (t, \(J = 7.3\) Hz, 2H, Ar-H), 7.38 (t, \(J = 7.2\) Hz, 1H, Ar-H), 7.27 (d, \(J = 15.8\) Hz, 1H, =CH\(_{trans}\)), 6.46 (t, \(J = 6.5\) Hz, 1H, H-1’), 4.87 (app q, \(J_{app} \sim 4.7\) Hz, 1H, H-3’), 4.00 (app q, \(J_{app} = 4.8\) Hz, 1H, H-4’), 3.89 (dd, \(J = 6.0, 10.8\) Hz, 1H, H-5’), 3.73 (dd, \(J = 4.6, 10.9\) Hz, 1H, H-5’’), 3.66 (dt, \(J = 6.5, 13.1\) Hz, 1H, H-2’), 2.60 (s, 3H, CH\(_3\)), 2.31 (ddd, \(J = 4.4, 6.7, 13.1\) Hz, 1H, H-2’’), 0.95 and 0.81 (2s, 18H, t-Bu), 0.16 (s, 6H, SiMe), 0.00 and –0.06 (2s, 6H, SiMe). \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \(\delta\) 197.0, 151.8, 151.0 (2s), 143.8, 138.9, 135.9, 131.9, 130.1, 129.8, 129.2, 127.8, 121.3,
118.9 (2s), 114.0, 87.8, 84.4, 75.5, 63.0, 37.9, 29.9, 26.1 (2s), 18.6, 18.3, –4.4, –4.5, –5.2, –5.3.

HRMS (ESI/TOF) m/z calculated for C_{38}H_{54}N_{5}O_{4}Si_{2} [M + H]^+: 700.3709, found 700.3724.

3',5'-Bis-O-(tert-butyldimethylsilyl)-N'-phenyl-8-[(E)-2-(4-tolyl)-ethenyl]-2'-deoxyadenosine (64g)

In step 1, The reaction was carried out with Pd(OAc)$_2$ (2.2 mg, 1.0 µmol, 0.1 equiv.), DPEPhos (5.3 mg, 1.0 µmol, 0.1 equiv.), 45 (50 mg, 0.10 mmol, 1 equiv.), Cs$_2$CO$_3$ (48.4 mg, 0.15 mmol, 1.5 equiv.), and 4-iodotoluene (28.1 mg, 0.13 mmol, 1.3 equiv.) in dry PhMe (1 mL), at 100 °C for 18 h. After completion of step 1, Pd(OAc)$_2$ (2.2 mg, 1.0 µmol, 0.1 equiv.), Xantphos (5.7 mg, 1.0 µmol, 0.1 equiv.), Cs$_2$CO$_3$ (48.4 mg, 0.15 mmol, 1.5 equiv.), and PhI (15.0 µL, 0.13 mmol, 1.3 equiv.) were added to the vial containing the reaction mixture and stirred at 100 °C for 12 h. Purification of the crude material by chromatography on a silica gel column (200–300 mesh) with 5–10% EtOAc in hexanes gave compound 64g along with some impurities. Further purification using preparative TLC (1 mm, 20 x 20 cm SiO$_2$ plate eluted with 5% EtOAc in hexanes) gave compound 64g (28.1 mg, 42% yield) as a pale-yellow solid. $R_f$(SiO$_2$/10% EtOAc in hexanes) = 0.43. $^1$H NMR (500 MHz, CDCl$_3$): δ 8.44 (s, 1H, Ar-H), 7.85 (d, $J$ = 15.9 Hz, 1H, =CH$_{trans}$), 7.84 (d, $J$ = 8.1 Hz, 2H, Ar-H), 7.69 (br s, 1H, NH), 7.52 (d, $J$ = 8.0 Hz, 2H, Ar-H), 7.39 (t, $J$ = 7.8 Hz, 2H, Ar-H), 7.25–7.17 (m, 3H, Ar-H, =CH$_{trans}$), 7.10 (t, $J$ = 7.4 Hz, 1H, Ar-H), 6.45 (t, $J$ = 6.6 Hz, 1H, H-1’), 4.85 (dt, $J$ = 4.0, 5.2 Hz, 1H, H-3’), 4.00–4.05 (app q, $J_{app}$ ~ 4.7 Hz, 1H, H-4’), 3.91 (dd, $J$ = 6.3, 10.8 Hz, 1H, H-5’), 3.73 (dd, $J$ = 4.7, 10.8 Hz, 1H, H-5’), 3.67 (dt, $J$ = 6.5, 13.1 Hz, 1H, H-2’), 2.40–2.60 (s, 3H, Me), 2.28 (ddd, $J$ = 4.2, 6.7, 13.1 Hz, 1H, H-2’), 0.95 and 0.83 (2s, 18H, t-Bu), 0.17, 0.16, 0.00, and –0.04 (4s, 12H, SiMe). $^{13}$C NMR (125 MHz, CDCl$_3$): δ 151.9, 151.6, 150.6, 150.5, 139.8, 139.1, 138.4, 133.2,
129.8, 129.2, 127.7, 123.4, 120.9, 120.3, 113.1, 87.8, 84.3, 72.7, 63.1, 37.9, 26.1 (2s), 21.7, 18.6, 18.3, –4.4, –4.5, –5.2, –5.3. HRMS (ESI/TOF) m/z calculated for C_{37}H_{54}N_{5}O_{3}Si_{2} [M + H]^{+}: 672.3760, found 672.3734.

**N-Arylation of p-aminostyrene**

![N-Arylation of p-aminostyrene](image)

In a clean, dry, 16 mL vial equipped with a stirring bar were placed Pd(OAc)$_2$ (18.9 mg, 0.084 mmol, 0.2 equiv.), and Xantphos (48.6 mg, 0.084 mmol, 0.2 equiv.) in dry PhMe (4.3 mL) and the mixture was stirred for 2 min. Then p-aminostyrene (65 mg, 0.55 mmol, 1 equiv.), Cs$_2$CO$_3$ (205 mg, 0.63 mmol, 1.5 equiv.), and PhI (47.0 µL, 0.42 mmol, 1.3 equiv.) were added to the mixture. The reaction mixture was flushed with nitrogen gas, sealed with a Teflon-lined cap, and stirred at 100 °C for 6 h. Upon consumption of the starting material, the reaction mixtures were diluted with EtOAc (20 mL) and washed with water (3 x 10 mL), brine (10 mL), dried over anhydrous Na$_2$SO$_4$, filtered, and evaporated to dryness under reduced pressure. Purification of the crude material by chromatography on a silica gel column (200–300 mesh) with 1–5% EtOAc in hexanes gave compound 67 (50.8 mg, 62% yield) as a light-brown solid and compound 68 (6.8 mg, 17% yield) as an off-white solid. $^1$H NMR of 67$^{74}$ (500 MHz, CDCl$_3$): δ 7.32 (d, J = 8.5 Hz, 2H, Ar-H), 7.27 (d, J = 7.7 Hz, 2H, Ar-H), 7.08 (d, J = 7.8 Hz, 2H, Ar-H), 7.02 (d, J = 8.5 Hz, 2H, Ar-H), 6.94 (t, J = 7.2 Hz, 1H, Ar-H), 6.66 (dd, J = 17.6, 10.8 Hz, 1H, =CH), 5.75 (br s, 1H, NH), 5.61 (d, J = 17.6 Hz, 1H, =CH$_{trans}$), 5.11 (d, J = 10.9 Hz, 1H, =CH$_{cis}$). $^1$H NMR of 68$^{75}$ (500 MHz, CDCl$_3$): δ 7.52 (d, J = 7.7 Hz, 2H, Ar-H), 7.45 (d, J = 8.3 Hz, 2H, Ar-H), 7.37 (t, J = 7.6 Hz, 2H, Ar-H), 7.31 (t, J = 7.8 Hz, 2H, Ar-H), 7.25 (t, J = 7.8 Hz, 1H, Ar-H), 7.13 (d,
$J = 7.9$ Hz, 2H, Ar-H), 7.11–7.06 (m, 3H, Ar-H, =CH), 6.99 (m, 2H, Ar-H, =CH), 5.81 (br s, 1H, NH). HRMS (ESI/TOF) $m/z$ calculated for C$_{20}$H$_{18}$N [M + H]$^+$: 272.1434, found 272.1415.

**Heck-arylation of $p$-aminostyrene**

In a clean, dry, 16 mL vial equipped with a stirring bar were placed Pd(OAc)$_2$ (18.9 mg, 0.084 mmol, 0.2 equiv.), and DPEPhos (45.3 mg, 0.084 mmol, 0.2 equiv.) in dry PhMe (4.3 mL) and the mixture was stirred for 2 min. Then $p$-aminostyrene (65 mg, 0.55 mmol, 1 equiv.), Cs$_2$CO$_3$ (205 mg, 0.63 mmol, 1.5 equiv.), and PhI (47.0 µL, 0.42 mmol, 1.3 equiv.) were added to the mixture. The reaction mixture was flushed with nitrogen gas, sealed with a Teflon-lined cap, and stirred at 100 °C for 24 h. Upon consumption of the starting material, the reaction mixtures were diluted with EtOAc (20 mL) and washed with water (3 x 10 mL), brine (10 mL), dried over anhydrous Na$_2$SO$_4$, filtered, and evaporated to dryness under reduced pressure. Purification of the crude material by chromatography on a silica gel column (200–300 mesh) with 5% EtOAc in hexanes gave compound 68 (0.8 mg, 1% yield) as an off-white solid. Subsequent elution with 20% EtOAc in hexanes gave compound 69 (72.2 mg, 88% yield) as a brown solid. $^1$H NMR of 69$^{76}$ (500 MHz, CDCl$_3$): $\delta$ 7.47 (d, $J = 7.6$ Hz, 2H, Ar-H), 7.36–7.30 (m, 4H, Ar-H), 7.21 (t, $J = 7.3$ Hz, 1H, Ar-H), 7.02 (d, $J = 16.3$ Hz, 1H, =CH$_{trans}$), 6.92 (d, $J = 16.3$ Hz, 1H, =CH$_{trans}$), 6.6d (d, $J = 8.3$ Hz, 2H, Ar-H), 3.74 (br s, 2H, NH$_2$).
REFERENCES


CHAPTER 3

STEREOSELECTIVE SYNTHESIS OF (±)-10β-AMINO-7β,8α,9β-
TRIHYDROXY-7,8,9,10-TETRAHYDROBENZO[a]PYRENE AND
THE SYNTHESIS OF 2’-DEOXYADENOSINE AND 2’-
DEOXYGUANOSINE ADDUCT
STEREOSELECTIVE SYNTHESIS OF (±)-10β-AMINO-7β,8a,9β-
TRIHYDROXY-7,8,9,10-TETRAHYDROBENZO[a]PYRENE AND
THE SYNTHESIS OF 2’-DEOXYADENOSINE AND 2’-
DEOXYGUANOSINE ADDUCTS

[3.1] INTRODUCTION

In 1775, Percivall Pott, a British surgeon observed the prevalence of scrotal cancer among chimney sweeps and reasoned that it was due to their exposure to soot particles. This phenomenon was again observed 100 years later, among many people working in paraffin industry. Eventually, the causation of scrotal cancer and ulceration of skin were officially attributed to pitch, tar, bitumen, mineral oil and paraffin. These observations led to the identification of carcinogenic substance present in all the above mentioned materials. Hence, as an initial step towards finding the carcinogenic substance, several attempts were made to reproduce the same cancerous outcome in laboratory animals. After a string of failed attempts, Yamagiwa and Ichikawa successfully developed a system, where repetitive application of coal tar to the skin of rabbits led to invasive skin tumors. Further investigation by Passey et al., showed that the active substance responsible for cancer can be extracted from soot with ether. In a separate study, Bloch and Dreifuss identified a high boiling, nitrogen and sulfur-free complex hydrocarbon as the active carcinogenic substance from coal tar. This observation was further supported by Kennaway’s investigation, where he observed that the pyrolysis products of acetylene or isoprene at 700–920 °C under a hydrogen atmosphere were carcinogenic. Later studies on products obtained by reaction of tetralin with AlCl₃ at 30–40 °C also showed
carcinogenic activity. These observations led to the conclusion that the active substance in all the carcinogenic materials is a polycyclic aromatic hydrocarbon (PAH).

![Diagram of polycyclic aromatic hydrocarbons]

**Figure 1. Some carcinogenic polycyclic aromatic hydrocarbons (PAHs)**

Later, Mayneord obtained crucial information during his investigation on the fluorescence of carcinogenic substances. He observed that the carcinogenic substances showed distinct bands at 400, 418 and 440 nm in their fluorescence spectrum. Although benz[a]anthracene (B[a]A, Figure 1) showed a fluorescence spectrum similar to that of the carcinogenic substances, none of the other compounds available at that period of time showed similar fluorescence. Eventually, after the initial reports on the synthesis of dibenz[a,h]anthracene (DB[a,h]A, Figure 1) and dibenz[a,j]anthracene (DB[a,j]A, Figure 1) by Clar in 1930, Kennaway and Hieger conducted studies on these two compounds for their carcinogenic activity. They observed that DB[a,h]A and DB[a,j]A not only showed strong carcinogenic activity but also showed fluorescence bands similar to that of carcinogenic tars. The next breakthrough was made by Hieger et al. by the isolation about 7 g of active carcinogenic crystalline material, from two tons of coal tar utilizing extensive separation techniques. The material thus obtained showed carcinogenic activity and displayed identical fluorescence bands like the carcinogenic tar. Further purification of this
material yielded perylene and two unknown isomeric compounds. After several evaluations, the major isomer with high carcinogenic activity was identified as benzo[a]pyrene (B[a]P, Figure 1) and the minor isomer with no carcinogenic activity was identified as benzo[e]pyrene (B[e]P, Figure 1). Based on these studies, it was confirmed that B[a]P is the active species responsible for carcinogenicity of coal tar and the distinct fluorescence bands.\(^5\)

Apart from coal tar, B[a]P and other PAHs can be the source of any “carcinogenic materials” as they can be practically formed by thermal decomposition of any organic compound. For example, 1 g of glucose, tobacco and paraffin wax decomposes at 700 °C under a nitrogen atmosphere, to yield 886 ng, 752 ng, and 66.6 µg, respectively, of B[a]P apart from other PAHs.\(^15\) Small portions of PAHs in the environment are produced due to natural events such as forest fires and volcanic activities, while human activities are the major contributing factors. Industries, which operate processes like coke production, aluminum smelting, iron and steel sintering, foundry operations, petrochemical processing, petroleum catalytic cracking, coal gasification, shale oil and asphalt production contribute in a major to the production of PAHs in the environment.\(^2\) Combustion for residential heating, power and heat generation, incineration, open fires, and combustion of gasoline or diesel fuels in motor vehicles also releases PAHs into the environment. Due to all these reasons, PAHs have become common pollutants in air, soil, water, and even in food. A study by the Agency for Toxic Substances and Disease Registry in 1995 showed that an average human consumes up to 0.207 µg, 0.027 µg, and 0.16–1.6 µg, of PAHs on a daily basis from air, water, and food.\(^2\) Apart from these common sources, tobacco smoke also plays a key role in the generation of PAHs in indoor air. Cigarette smoke produces around 150 different PAHs, among which many are carcinogenic. About 10–50 ng of B[a]P is produced
from one cigarette alone. Although modern cigarettes produce less than 10 ng of B[a]P, the production of other potential carcinogenic PAHs cannot be underestimated.

After recognizing the carcinogenic activity of B[a]P and other PAHs, the focus then shifted to understanding the metabolism of PAHs. Initial investigation by various research groups indicated that PAHs bind to proteins and DNA.\textsuperscript{2,16,17} PAHs have to be electrophilic in nature, in order to bind with the amino groups of the nucleobase in DNA. However, PAHs are highly lipophilic and unreactive towards such reactions. During that period of study, Boyland and Levi isolated different stereomers of 1,2-dihydroxy-1,2-dihydroanthracene from the urine of anthracene-fed rabbits and rats.\textsuperscript{18} This finding supported the hypothesis that PAHs undergo enzymatic transformations to electrophilic PAH derivatives that can easily bind to DNA. Recent studies also support the theory that carcinogenesis occurs only from the metabolites of PAHs but not from the unmetabolized PAHs.\textsuperscript{2} Early studies on the metabolites showed that metabolism of anthracene and DB[a,h]A generated the corresponding arene oxides,\textsuperscript{18,19} which bound to DNA.\textsuperscript{20,21} Further investigation by Borgen \textit{et al.} showed that the 7,8-dihydrodiol of B[a]P binds to DNA 10 times stronger than B[a]P itself.\textsuperscript{22} Later, Sims \textit{et al.} observed that the 7,8-dihydrodiol-9,10-epoxides of B[a]P are generated as the secondary metabolites from B[a]P. They also observed that the diol epoxide binds directly to the DNA and was relatively more carcinogenic and mutagenic than the arene oxides.\textsuperscript{23}

The metabolic activation of PAHs can take place in several steps and via different pathways. However, in order for a PAH to show carcinogenic activity, it has to possess certain structural features. On the basis of observations made on the structural features of various carcinogenic and non-carcinogenic PAHs, it was initially hypothesized that PAHs that contain regions with notable olefinic character, termed as a K-region (shown in Figure 2), are carcinogenic. However,
this hypothesis was dismissed as the $K$-region epoxides showed little carcinogenic activity compared to non $K$-region metabolites.$^{2,5}$ Eventually, Jerina et al. proposed a new theory, where PAHs containing bay or fjord regions are more likely to be carcinogenic.$^{24,25}$ A bay region is defined as the concave shape that arises due to the angular arrangement of benzo rings, whereas the fjord region is described as the narrow U-shaped region arising by fusing benzo ring to bay region PAH (Figure 2). According to this hypothesis, PAHs undergo epoxidation adjacent to bay or fjord regions and the ensuing diol epoxide metabolites$^{22}$ react with the amino moieties of nucleobases in DNA.

Figure 2. PAHs containing $K$, bay and fjord regions

Consistent with the proposal of Borgen et al., Jerina et al. developed the diol epoxide theory (explained later). Apart from the diol epoxides formed from bay and fjord-region containing PAHs, several other metabolites can damage DNA. The three commonly considered pathways that lead to all metabolites are discussed in detail below.

1. One-Electron Oxidation Pathway

In the one-electron oxidation pathway proposed by Cavalieri et al., the PAH undergoes enzymatic metabolism to generate radical cations via removal of an electron.$^{26,27}$ This removal
of electron usually takes place at the site of highest electron density. For example, position 6 of B[a]P and the benzylic position of 6-methyl B[a]P are more likely oxidized than other positions of B[a]P and 6-methyl B[a]P (as shown in Figure 3).

After the removal of an electron from the PAH, the radical cation thus generated reacts with the DNA, specifically at the N7, N3 or C8 positions of the nucleobases. The resulting DNA adducts possess a weakened glycosidic bond, and are susceptible to easy depurination. This results in the formation of apurinic sites, which may induce mutations leading to tumor formation. A representative one electron oxidation reaction of B[a]P and leading to a DNA adduct by reaction at the N7 position of guanine in DNA, is shown in Scheme 1.

Scheme 1. DNA adduct formation and leading to tumorigenesis via one electron oxidation pathway
2. Formation of Ortho-Quinones and Reactive Oxygen Species Pathway

In this pathway, the initial activation of a PAH involves a non-\(K\)-region mono-oxygenation by cytochrome P450 (CYP450), followed by hydrolysis of the formed arene oxide with microsomal epoxide hydrolase (mEH).\(^2\) This results in the formation of non-\(K\)-region trans dihydrodiols that are further oxidized to ortho-quinones by dihydrodiol dehydrogenase. Dihydrodiol dehydrogenase belongs to the nicotinamide adenine dinucleotide phosphate-dependent (NADPH-dependent) aldo-keto reductase (AKR) family and is most commonly found in rat liver as AKR1C9 and in human liver as AKR1C1–1C4.\(^2\) The enzymatic oxidation of non-\(K\)-region dihydrodiols by NADPH-dependent aldo-keto reductase proceeds by initial generation of a ketol derivative that immediately tautomerises to a catechol (Scheme 2).\(^{28-30}\) Autoxidation of the catechol in the presence of superoxide anion radical generates unstable semiquinone anion radical along with hydrogen peroxide, via a single-electron-transfer mechanism.\(^{28-30}\) Further oxidation of semiquinone anion radical leads to the formation of ortho-quinone along with superoxide anion radical.\(^{28-30}\) Although the PAH ortho-quinones are good Michael acceptors,
they are not very reactive for covalent modification of DNA. The PAH *ortho*-quinones once generated can again undergo reduction to give semiquinone anion radical and catechol.

The *ortho*-quinones cause DNA damage by forming stable and depurinating adducts with exocyclic amino groups of the DNA. However, the redox cycle involving catechol and *ortho*-quinone generates reactive oxygen species such as superoxide anion radical and hydrogen peroxide, which are known to cause major DNA damage. The levels of the reactive oxygen species do not depend upon the amount of *ortho*-quinone produced, as the *ortho*-quinone redox cycle generates the reactive oxygen species continuously. After their generation, the super oxide radical anion and \( \text{H}_2\text{O}_2 \) generate hydroxyl radical (\( \text{HO}' \)) either by reaction with each other or by

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**Scheme 3. DNA damage caused by reactive oxygen species**

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decomposing in presence of transition metal atoms such as Cu(I), Cu(II), Fe (II) or Fe (III). The hydroxyl radical oxidizes guanine residues of DNA to 8-hydroxy-2′-deoxyguanosine (8-OH-dG DNA). The 8-OH-dG DNA tautomerizes to the corresponding amido form (Scheme 3). This leads to base mispairing with deoxyadenosine (dA) during DNA replication. *In vitro* studies showed that these lesions cause deoxyguanosine (dG) to deoxythymidine (dT) transversions, making them mutagenic.29

3. Diol Epoxide Pathway

The diol epoxide pathway is considered to be the predominant pathway through which tumorigenesis and carcinogenesis are mostly likely to occur. In this pathway, PAHs initially undergo monoxygenation, catalyzed by CYP450 enzymes such as CYP450 1A1, CYP450 1B1 (expressed in the extrahepatic tissue), and CYP450 1A2, CYP450 3A4 (expressed in the liver).2 This oxidation process is highly stereoselective and produce an enantiomeric pair of arene oxides. For example, monoxygenation of B[a]P by CYP450 produces the (7R, 8S)-oxide isomer predominantly and a minor amount of (7S, 8R)-oxide is also formed (Scheme 4). These oxides undergo hydrolysis mediated by mEH (expressed highly in the liver and to a lesser extent in other organs) to give trans dihydrodiols.31-33 These trans dihydrodiols can produce reactive oxygen species and ortho quinone derivatives, as discussed above, or they can undergo detoxification either by glucorinic acid conjugation and/or sulfation.34,35 Concurrently, the trans dihydrodiols undergo secondary epoxidation with CYP450 enzymes to give diol epoxides.36,37 Hence, the two enantiomeric dihydrodiols produce two enantiomeric pairs of diastereomers *via* a face-selective epoxidation. The diol epoxides with epoxide ring *cis* to the benzylic hydroxyl group are labeled as series 1 diol epoxides (DE1 or syn), where as epoxide ring *trans* to the benzylic hydroxyl group are labeled as series 2 diol epoxides (DE2 or trans). In the secondary
oxidation of the \((-\text{-}\text{trans-B}[a]P-(7R, 8R)\text{-dihydrodiol, (+)-trans-B}[a]P\text{-DE2 is formed predominantly and in the case of (+)-trans-B}[a]P-(7S,8S)\text{-dihydrodiol, (+)-cis-B}[a]P\text{-DE1 is the preferentially produced isomer.}^{38,39}\) In the overall three-step process, (+)-\text{trans-B}[a]P\text{-DE2 is produced in higher quantities as compared to other three diol epoxides.}

**Scheme 4. Metabolism of B[a]P via diol epoxide pathway**

Out of all the four diol epoxides of B[a]P, series 2 diol epoxides are highly tumorigenic, whereas the series 1 diol epoxides lack tumorigenicity.\(^2\) Among the 4 diol epoxides, (+)-\text{trans-B}[a]P\text{-DE2 exhibited the highest mutagenicity in mammalian cells and highest carcinogenic potency in mouse tumor models.}^{40,41}\) All four diol epoxides of B[a]P are good electrophiles and
are DNA alkylating agents.\textsuperscript{2,5} However, the extent of covalent binding to DNA by these diol epoxides is low.\textsuperscript{42} On the other hand, B[c]Ph is a weak carcinogen, but its diol epoxides covalently bind to DNA at significantly higher levels.\textsuperscript{43} Hence, the carcinogenic activity of a PAH is likely not dependent upon the ability of its diol epoxides to alkylate DNA.\textsuperscript{44} It was observed that the B[a]P diol epoxides alkylate predominantly the $N^2$-amino group of 2’-deoxyguanosine residues in the minor groove of DNA.\textsuperscript{42,45} By comparison, with the diol epoxides of B[c]Ph and DB[a,l]P, alkylation takes place predominantly at the $N^6$-amino group of 2’-deoxyadenosine residues in the major groove of the DNA.\textsuperscript{46,47} Extensive adduct-forming studies of various PAH diol epoxides with DNA indicate that the diol epoxides of planar PAHs are prone to producing a higher proportion of guanine adducts as compared to adenine adducts, whereas an opposite trend is observed with the non planar PAHs.\textsuperscript{44}

**Scheme 5. DNA alkylation pathway by a diol epoxide**

DNA alkylation by the diol epoxides takes place in multiple steps. After their metabolic formation, diol epoxides initially undergo DNA intercalation. Then, the epoxide ring is protonated. This facilitates epoxide ring-opening and formation of a stable, benzylic carbocation (Scheme 5).\textsuperscript{48,49} Nucleophilic capture of the benzylic carbocation by the exocyclic amino group of 2’-deoxyadenosine and 2’-deoxyguanine residues in DNA then leads to the formation of DNA adducts.
Because, the overall process takes place via an S_{N}1-like mechanism, nucleophilic attack by DNA produces two DNA adducts each 2’-deoxyadenosine and 2’-deoxyguanine, with a single diol epoxide. Hence, the 4 diol epoxides of any PAH produce 16 nucleoside adducts (Figure 4).\textsuperscript{2,50} The formation of these adducts then leads to perturbation of the local DNA structures, which can eventually influence replication and/or repair mechanisms. These events can then lead to mutations and then to tumorigenesis.\textsuperscript{42,51-57} Hence, it is important to have access to stereospecifically defined nucleoside and DNA adducts of diol epoxides in order to understand

\textbf{Figure 4}. Nucleoside adducts formed by DNA alkylation at the adenine and guanine residues by the four diol epoxides.
how covalent interactions of PAH metabolites with DNA can lead to adverse or innocuous biological effects depending upon the diol epoxide stereochemistry.

To date three approaches have been developed for the site-specific modification of DNA with stereochemically defined diol epoxide adducts, and these are discussed below.

1. Direct Reaction of Diol Epoxides with DNA Oligomers

This approach developed by Loechler et al.\textsuperscript{58} and Geacintov et al.\textsuperscript{59,60} involves direct reaction of a specific PAH diol epoxide with short oligonucleotides (Scheme 6). However, the kind of adducts produced and the yields of the adducts completely depend up on the specific PAH diol epoxide used. For example, in the case of B[α]P DE2, higher quantities of guanine adducts were

\textbf{Scheme 6. An example of site-specific DNA modification by the direct reaction of a diol epoxide with a DNA oligomer}
obtained compared to adenine adducts.\textsuperscript{61,62} Whereas, in the case of DB[\(a,\)]P DE2, adenine adducts were obtained in excess compared to guanine adducts.\textsuperscript{63} Since the minor adducts may play an important role in events leading to mutagenesis and carcinogenesis, and obtaining minor adducts is difficult \textit{via} this approach, it is not broadly applicable. Another factor that limits this approach is the low yields of the adducts with many diol epoxides, due to competing hydrolysis of the diol epoxides.\textsuperscript{58-62} Besides, the presence of multiple exocyclic amino groups may lead to alkylation at multiple sites or result in multiple products, and cis and trans ring-opened products can result. These pose separation and characterization problems.

2. \textit{Post-Oligomerization Modification Approach}

In this approach, the DNA adducts were obtained by reaction of stereochemically defined PAH amino triols with DNA oligonucleotides containing a specifically placed electrophilic nucleoside (Scheme 7).\textsuperscript{64-66} The desired PAH amino triols are synthesized by diastereoselective chemical approaches and the reactive nucleoside in oligonucleotides typically contain either a halo or triflate group at the desired reaction site. This approach is complementary to the direct

\textbf{Scheme 7. An example of the reaction of a PAH amino triol with a DNA oligomer containing an electrophilic nucleoside residue}
reaction approach, as the PAH intermediate acts as a *nucleophile* and the DNA fragment acts as the *electrophile*. As a consequence, this approach is more advantageous over the direct reaction approach. However, there are some limitations to this approach such as low yields of the products and difficulties with DNA synthesis.\(^{64-67}\)

3. Total Synthesis Approach

In this approach, nucleosides adducts are initially synthesized by reacting stereochemically defined PAH amino triols with electrophilic nucleosides (See similarity to the post-oligomerization modification approach). The PAH-adducted nucleosides so obtained are then utilized in the solid-phase DNA synthesis to prepare site-specifically modified DNA (Scheme 8).\(^{68-74}\) Typically fluorinated purine nucleosides such as 6-fluoro-9-(2-deoxy-β-D-erythropentafuranosyl)purine (6-FP) and 2-fluoro-2’-deoxyinosine (2-FdI) are used as the electrophilic nucleosides for preparing dA and dG adducts, respectively (Figure 5).\(^{68-75}\) However, the C-2-triflate derivative of 2’-deoxyxanthosine (2-TdX) can also be applied for the synthesis of dG adducts (Figure 5).\(^{66}\) Even though this approach is labor intensive, it avoids the problems associated with direct reaction and post-oligomerization techniques. This approach, in principle, allows access to all the nucleoside adducts and in turn, DNA adducts, and in substantial amounts. Hence this approach is more versatile than the other approaches.
Figure 5. Commonly used electrophilic nucleosides for the synthesis of nucleoside adducts

In order to gain a greater understanding of the roles of specific B[a]P DE-DNA adducts in mutational events, the ensuing biological effects, and structure-activity relationships, the total synthesis approach seems to be a more reliable approach. However, this requires the development of robust synthetic routes to various B[a]P metabolites and their nucleoside adducts. We\textsuperscript{68,76-79} and others\textsuperscript{73,74,80-86} have been extensively involved in synthetic studies leading to facile routes to PAH metabolites, stereochemically defined PAH-nucleoside adducts, and PAH-DNA adducts.

Access to the B[a]P amino triols 1, 5 arising by a trans ring-opening of B[a]P series 1 and 2 diol epoxides can be achieved fairly easily by either direct aminolysis\textsuperscript{68,77,80} or nucleophilic displacement with azide anion on B[a]P series 1 and 2 diol epoxides (Scheme 9).\textsuperscript{81,87,88} The azido triol derivatives 2 and 4 upon catalytic reduction produce amino triols 1 and 2, respectively.\textsuperscript{81,87,88} Typically, the free hydroxyl groups of the B[a]P azido triols 2 and 4 were benzyolated to give 3 and 7, prior to the catalytic hydrogenation (Scheme 9). The resultant benzoyl-protected amino triols 4 and 8 were then reacted with fluorinated nulceosides (2-FdI or 6-FdP) to give the nucleoside adducts.\textsuperscript{77,78,81,87,88} We\textsuperscript{78,79} and others\textsuperscript{89} recently developed a new strategy for the synthesis of B[a]P-nucleoside adducts using palladium-catalyzed C–N bond-forming reactions. The N\textsuperscript{6}-deoxyadenosine adducts were synthesized by the reaction of B[a]P amino tribenzoates with either 6-chloro purine nucleoside 9 or 6-bromo purine nucleoside 10.
Scheme 9. Amino triol synthesis by the trans ring-opening displacement on B[a]P series 1 and 2 DEs

On the other hand the $N^2$-deoxyguanosine adducts were synthesized by the reaction of B[a]P amino tribenzoates with 2-bromo purine nucleoside 12. Representative reactions by the

Scheme 10. Synthesis of nucleoside adducts 11a,b and 13a,b by palladium-catalyzed C–N bond-forming reactions
palladium-catalyzed synthesis of the $N^6$-deoxyadenosine adducts 11a,b and $N^2$-deoxyguanosine adducts 13a,b are shown in Scheme 10. Typically, synthesis of 6-FdP and 2-FdI (commonly used for displacement reactions with PAH amines) requires several steps. On the other hand, halo nucleoside derivatives 9 and 10, which do not undergo S$_{N}$Ar reactions with PAH amino triols effectively, are more easily accessible. However, the Pd-catalyzed strategy allows the use of chloro and bromo nucleosides 9, 10, and 12 for the nucleoside adduct synthesis in relatively high yields, thus, providing a simple alternative route to the nucleoside-PAH adduct synthesis.

Unlike the trans ring-opened B[a]P amino triol derivatives, the synthesis of cis ring-opened B[a]P amino triol derivatives is more complex. Due to the difficulties in their synthesis, the DNA adducts derived from cis ring-opening of B[a]P DEs, they are less studied as compared to DNA adducts derived from trans ring-opening of B[a]P DEs. The first diastereoselective synthesis of a cis ring-opened B[a]P amino triol from (±)-B[a]P DE2 was reported by Meehan et al. In this, the trimethylsilyl ether derivative of (±)-B[a]P DE2 14 was reacted with trimethylsilyl azide (TMS-N$_3$) and titanium tetraisopropoxide (Ti(OiPr)$_4$) in THF to give the corresponding B[a]P azido derivative (±)-15 (Scheme 11). Desilylation followed by catalytic hydrogenation led to the formation of the cis ring-opened B[a]P amino triol ((±)-16). However, the reasons for this stereochemical outcome are unknown. This approach, however, when

**Scheme 11. Diastereoselective synthesis of cis ring-opened B[a]P amino triol ((±)-16) from B[a]P DE2**
applied to trimethylsilyl ether derivative of (±)-B[a]P DE1 resulted in 1.5:1 ratio of cis/trans B[a]P azido triol derivatives.  

An alternate approach for the synthesis of cis ring-opened B[a]P DE2-2′-deoxyadenosine adducts was developed by Jerina et al. This approach, which is based on Sharpless asymmetric aminohydroxylation (AA) protocol, involves the reaction of silyl protected 2′-deoxyadenosine 17 with (±)-trans-7,8-dihydroxy-7,8-dihydro B[a]P (18) in presence tert-butyl hypochlorite, cat. (DHQD)2PHAL, and K2OsO2(OH)4 in n-propanol to give the desired diastereomeric pair of adducts 19a and 19b with 85% yield (Scheme 12). Although this method circumvents the use of B[a]P amino triol derivatives, the dG adducts cannot be synthesized by this approach.

**Scheme 12. Synthesis of cis ring-opened B[a]P DE2-2′-deoxyadenosine adducts by using asymmetric aminohydroxylation approach**

Jerina et al. reported another approach for the synthesis of cis ring-opened B[a]P-dG adducts. In this approach, direct ring-opening of B[a]P DE1 and B[a]P DE2 was conducted in trifluoroethanol (TFE) in the presence of disilyl O6-allyl protected 2′-deoxyguanosine 20 (dGally), at room temperature (Scheme 13). However, these reactions were not stereoselective and resulted in both cis and trans ring-opened B[a]P-dG adducts. In the reaction with B[a]P DE2 two diastereomeric pairs of adducts 21a,b and 22a,b were obtained, whereas with B[a]P DE1 two diastereomeric pairs of adducts 23a,b and 24a,b were isolated. Notably, when reactions of silyl protected 2′-deoxyadenosine 17 were conducted with B[a]P DE2 and B[a]P
DE1 in TFE, only cis ring-opened B[a]P-dA adducts 19a,b and 25a,b respectively, were obtained (Scheme 13). The major drawback of this approach is that TFE reacts at the C-10 position of the B[a]P DE1 and DE2 and forms trans ring-opened B[a]P tetraol derivatives in

Scheme 13. TFE mediated synthesis of cis ring-opened B[a]P-dG adducts 21a,b and 23a,b and cis ring-opened B[a]P-dA adducts 19a,b and 25a,b
significant amounts (~50% yield). As a result of competing reaction with TFE and lack of stereoselectivity, the yields of the products in these reactions were very low. Use of other fluorinated solvents such as hexafluoroisopropanol (HFIP) and perfluoro-\(\text{t}\)-butanol instead of TFE, improved the yields to some extent in the case of B[a]P-dG adducts. However, no improvement was observed in the case of B[a]P-dA adducts. The mixtures obtained in these reactions were separated by HPLC. Because of all these complications, large scale synthesis of dG adducts by this approach is difficult.

This led Lakshman et al. to develop a novel diastereoselective approach for the synthesis of cis ring-opened B[a]P DE2 adducts of dG and dA.\(^{92}\) In this approach, the synthesis of cis ring-opened B[a]P amino triol (±)-16 starts with 7,8-bis-benzyloxy-7,8-dihydro B[a]P derivative (±)-27 (Scheme 14), which can be easily synthesized in three steps from 7,8,9,10-tetrahydrobenzo[\(\text{a}\)]pyrene-7-ol (B[a]P-7-ol, 26).\(^{93}\) Diastereoselective dihydroxylation of dihydrodibenzoate B[a]P derivative (±)-27 with catalytic OsO\(_4\), NMO and PhB(OH)\(_2\) in CH\(_2\)Cl\(_2\) resulted in a single boronate ester B[a]P derivative (±)-28. Oxidation of the boronate ester (±)-

with 50% H$_2$O$_2$ in EtOAc-acetone gave the tetraol dibenzoate (±)-29. Evaluation of the relative energies of formation for the boronate diastereomers (±)-28 and the diol diastereomers (±)-29 showed that the cis $\alpha$ isomers of boronate diastereomers and diol diastereomers (±)-28 and (±)-29 are more favored than their cis $\beta$ counterparts. From the plausible transition state structures (shown in Figure 6), the addition of OsO$_4$ is likely to occur from the bottom face of dihydrodibenzoate (±)-27 resulting in the formation of boronate ester (±)-28 via transition state 1 (TS1). The top face addition of OsO$_4$ to dihydrodibenzoate (±)-27 via transition state 2 (TS2) is unlikely, since it contains eclipsing interactions. These studies illustrate that torsion control on the OsO$_4$ addition may be a key factor that influences the diastereoselectivity of the dihydroxylation reaction.

![Transition State 1 (TS1)](image1)

![Transition State 2 (TS2)](image2)

**Figure 6. Plausible transition states TS1 and TS2 for OsO$_4$ addition to dihydrodibenzoate B[a]P derivative (±)-27**

The tetraol B[a]P derivative (±)-29 was treated with 1-chorocarbonyl-1-methylethyl acetate (30) in MeCN to give the trans-chloro acetate intermediate (±)-31 (Scheme 14). Nucleophilic displacement of chloro acetate derivative (±)-31 with NaN$_3$ in DMF at 50 °C gave the desired cis azidotriol B[a]P derivative (±)-32 (Scheme 14). Deacylation followed by catalytic reduction of the azidotriol (±)-32 gave the cis ring-opened B[a]P amino triol (±)-16. The amino triol (±)-16 facilitated the synthesis of dA and dG adducts from a common intermediate, and was also the first report on the synthesis of cis ring-opened dG adducts from B[a]P DE2. The synthetic strategy for the conversion of (±)-29 to (±)-16 relies on earlier reports by Lakshman et al., where
cis aminoalcohols were synthesized from cis diols.\textsuperscript{94,95} In that work, the cis diols 33 when treated with 1-chlorocarbonyl-1-methylethyl acetate (30) in MeCN, gave the trans chloro acetates 34 (Scheme 15). Displacement of chloride with azide ion, followed by deacylation, and reduction of the azido group produced the desired cis aminoalcohols 36 with the amino group at the benzylic position.

**Scheme 15. Synthetic approach to cis aminoalcohols from cis diols**

![Scheme 15 diagram]

Although, attempts towards synthesis of amino triols by cis ring-opening of B[a]P DE2 were successful, the same was not true for the synthesis of cis ring-opened B[a]P DE1 amino triols. As mentioned earlier, Jerina et al. synthesized cis ring-opened B[a]P DE1 amino triol, by utilizing a procedure developed by Jhingan and Meehan for cis ring-opening of B[a]P DE2 by azide. In their synthesis, trimethylsilyl ether derivative of B[a]P DE1 (±)-37 was treated with

**Scheme 16. Synthesis of a cis ring-opened azido triol (±)-35 from a disilyl B[a]P DE1 derivative (±)-34**

![Scheme 16 diagram]
Ti(OiPr)$_4$ and TMSN$_3$ in THF at room temperature. Subsequent desilylation with dil. HCl in MeOH resulted in the desired cis ring-opened B[a]P azido triol (±)-38 along with trans ring-opened B[a]P azido triol (±)-6 in 1.5:1 ratio, in an overall yield of 89% (Scheme 16).

The desired B[a]P azido triol (±)-38 was separated from the mixture by HPLC and was then reduced by catalytic hydrogenation to give the desired cis ring-opened B[a]P amino triol (±)-39 (Scheme 17). The B[a]P amino triol (±)-39 was then reacted with silyl protected 6-fluoro purine nucleoside 40 in the presence of 2,6-lutidine, hexamethyl disiloxane ((Me$_3$Si)$_2$O) in DMSO at 90 °C. This gave the diastereomeric pair of B[a]P DE1-dA adducts 25a and 25b respectively (Scheme 16). The adducts 25a and 25b were separated and confirmed by various spectroscopic techniques. Although, the strategy by Jerina et al. granted access to cis ring-opened B[a]P DE1-dA adducts, the azido triol synthesis was not stereoselective and no attempts were made to synthesize B[a]P DE1-dG adducts arising via a cis ring-opening of B[a]P DE1. Kroth et al. reported the synthesis of B[a]P DE1-dG adducts, however the process was not stereoselective.  

This resulted in a mixture of adducts, in which two of them were cis ring-opened B[a]P DE1-dG adducts and the remaining two were trans ring-opened B[a]P DE1-dG adducts. The adducts in the mixture were separated using HPLC.\textsuperscript{85} Hence, a highly diastereoselective synthetic route to B[a]P amino triol (±)-39 is still essential in order to synthesize substantial amounts of B[a]P DE1 adducts.

In order to address the issue, we designed a new approach for the synthesis of B[a]P amino triol (±)-39.\textsuperscript{96} The synthesis in this approach starts with 7,8-dibenzyloxy-7,8-dihydro B[a]P derivative (±)-27, which is also a common precursor for the synthesis of cis ring-opened B[a]P amino triol (±)-16. This can be synthesized from B[a]P-7-ol (26) in three steps.\textsuperscript{93} The B[a]P DE1 dibenzoate (±)-27 upon reaction with N-bromo succinimide (NBS) and NaOAc in THF-H$_2$O at room temperature for 18 h gave bromohydrin (±)-41 in a yield of 62\% (Scheme 18). Epoxide (±)-42 was synthesized from reacting the bromohydrin (±)-41 with NaH in THF at 0 °C for 1.5 h. The epoxide ring in the compound (±)-42 was opened by reacting it with dried LiCl, Ac$_2$O, and a catalytic amount of DMAP in DMF at room temperature over 18 h. The resultant mixture containing benzylic chlorohydrin derivative (±)-43 was then subjected to nucleophilic displacement with NaN$_3$ in DMF at 50 °C for 18 h. This resulted in the B[a]P azido triol derivative (±)-44 in 53\% yield over two steps. The stereochemistry of (±)-40 was confirmed by correlating the spatial interactions between H-9 and H-10 in its NOESY spectrum. Deacylation of (±)-44 with NH$_3$ saturated MeOH, at 50 °C over 24 h gave the B[a]P azido triol (±)-38 in 91\% yield. Reduction of azido triol by catalytic hydrogenation with Lindlar catalyst (Pd/CaCO$_3$) in EtOH under an atmosphere of H$_2$ at room temperature over 2.5 h gave the B[a]P amino triol (±)-39 in a 96\% yield.
Scheme 18. Stereoselective approach towards the synthesis of cis ring-opened B[a]P amino triol (±)-39

Via this approach a diastereoselective synthesis of 10β-amino-7β,8α,9β-trihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene (±)-39 is possible. However, it requires certain improvements. One such essential improvement is improving the yield for the transformation of (±)-42 to (±)-44. Although, a moderate 53% yield was obtained previously, a higher yielding transformation would be optimal for carrying out large-scale reactions. The deacylation reaction of (±)-44 and the reduction reaction of (±)-38 were carried out on a relatively small scale (0.05 mmol of (±)-44 and 0.087 mmol of (±)-38). High-yielding, large-scale experiments have to be performed in order to show feasibility of this approach. Hence, we intended focus on these two issues: i) improving the yield of the (±)-42 to (±)-44 conversion, and ii) carrying out deacylation and reduction reactions on larger scales. Once the B[a]P amino triol (±)-39 was obtained, we intended to synthesize the 2 cis ring-opened B[a]P DE1-dA adducts 25a, b and the 2 cis ring-opened B[a]P DE1-dG adducts 46a, b (Scheme 19). With the synthesis of dA and dG adducts, the total synthesis of all sixteen nucleoside adducts from the B[a]P diol epoxides will be completed.
Scheme 19. The dA and dG adducts to be synthesized by reacting amino triol (±)-39 with fluorinated nucleosides 40 and 45

Once the PAH-nucleoside adducts are acquired, PAH-DNA adducts becomes accessible. This allows studies on the structure and function of the PAH-DNA adducts. Typically, the structures of modified DNAs were resolved by solution NMR studies.\textsuperscript{97} Several reports on the structure of \textit{cis} and \textit{trans} ring-opened B[\textit{a}]P-nucleoside adducts in DNA duplexes were made possible by solution NMR studies.\textsuperscript{98-112} Since formation of B[\textit{a}]P DE2-dG adduct is highly preferred, studies were mainly focused on these.\textsuperscript{98-102} In the case of deoxyguanosine adducts with 10\textit{S} configuration, NMR studies showed that the PAH is oriented towards the 5’ end of the modified strand, not intercalated, and residing in the minor groove of the DNA.\textsuperscript{98,101,102} Whereas in the case of deoxyguanosine adducts with 10\textit{R} configuration, the PAH may or may not intercalate but resides in the minor groove of the DNA, oriented towards the 3’ end of the modified strand.\textsuperscript{98,100} So far, no structural data on \textit{cis} ring-opened B[\textit{a}]P DE1-deoxyguanosine adducts were available. However, unlike the case of deoxyguanosine adducts, solution structures of B[\textit{a}]P DE2 and DE1 adducts of deoxyadenosine have been well studied. In the adducts with a
10S configuration, NMR studies indicated that the PAH is oriented towards the 3’ end of the modified strand, intercalated, and located in the major groove of the DNA. Similar results were also observed in the case of adducts with 10R configuration, where the PAH is oriented towards the 3’ end of the modified strand, intercalated, and located in the major groove of the DNA.

Biochemical and biological experiments are employed to study the function of PAH-DNA adducts. These experiments enable an understanding of the types of mutations produced, and effects on DNA replication and repair caused by specific DNA adducts. For instance, in one of the studies, it was observed that subtle differences in adduct conformation can greatly influence the UvrABC repair system and these also affect the excision repair process. Studies on different DNA adducts showed that efficiency of UvrABC incision also depends upon the size of the aromatic system bound to the DNA. DNA bound with smaller aromatic groups such as C8-guanine adducts of 2-aminoflourene displayed the best incision efficiency in a bubble of three mismatched nucleotides. However, in the case of DNA attached to larger aromatic groups like in the case of cis ring-opened B[a]P DE2-guanine adduct, the best incision efficiency observed in a bubble of six mismatched nucleotides. Studies were also conducted on the activity of DNA polymerases (specifically Y family of DNA polymerases) with B[a]P diol epoxide adducts. It was observed that DNA polymerase ζ bypasses B[a]P DE-dG adducts in yeast cells. On the other hand, DNA polymerase κ successfully cleaves the undesired lesions and protects the mammalian cells against the mutagenic effects of B[a]P.

Hence from all the information discussed so far, it is evident that in order to have a better understanding on structure-activity relations of the B[a]P-DNA adducts, collaborative efforts that entails facile synthetic strategies, structural studies, biochemical and biological experiments
are necessary. The synthesis of the stereochemically defined cis ring-opened B[a]P DE1-nucleoside adducts 36a,b and 42a,b will help achieve one of these goals.
[3.2] RESULTS AND DISCUSSION

In our efforts to improve the diastereoselective synthesis of 10β-amino-7β,8α,9β-trihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene (±)-39 discussed in the introduction, we initially focused on improving the yield of B[a]P azido triol (±)-44. We needed the B[a]P diol epoxide (±)-42 for this purpose and this was synthesized from 7,8-bis-benzylxylo-7,8-dihydro B[a]P (±)-27 in two steps as shown in Scheme 18. The synthesis of (±)-27 was previously reported in three steps from B[a]P 7-ol (26).93 Hence, by following the reported procedure, we started the synthesis by a p-toluenesulfonic acid (p-TsOH) catalyzed dehydration of B[a]P 7-ol (26) in refluxing toluene over 0.5 h (Scheme 20). This gave the B[a]P alkene 47 in a 90% yield. Alkene 47 was then subjected to Prévost reaction using iodine and silver benzoate in dry toluene at reflux over 16 h. This resulted in the B[a]P dibenzoate (±)-48 in 88% yield (Scheme 20). Oxidation of the dibenzoate derivative (±)-48 with DDQ in refluxing dry toluene over 17 h gave the 7,8-bis-benzyloxy-7,8-dihydro B[a]P derivative (±)-27 in a 75% yield. Reaction of compound (±)-27 with N-bromosuccinimide (NBS) and sodium acetate in a THF-H2O mixture at room temperature over 16 h gave the B[a]P bromohydrin (±)-41 in a 75% yield (Scheme 20).

Scheme 20. Synthesis of B[a]P bromohydrin derivative (±)-41 from B[a]P 7-ol (26)
As shown in Scheme 18, the required B[a]P dibenzoyloxy epoxide (±)-42 can be synthesized by reaction of B[a]P bromohydrin (±)-41 with NaH in THF. The epoxide (±)-42 from this reaction was obtained as a pink solid. However, reports show that B[a]P diol epoxide derivatives are usually obtained as white solids or colorless crystals. This led us to believe that the pink color of (±)-42 is due to the presence of unknown impurities. The impurities produced in this reaction could affect the yield in the next epoxide ring-opening reaction. Because, epoxide (±)-42 is reactive and sensitive to robust purification techniques, we decided to consider a different protocol to access epoxide (±)-42, involving mild conditions and easy purification. In this regard, Jerina et al. have developed an efficient synthesis of (±)-42 from (±)-41 under mild conditions. Following that protocol, epoxide (±)-42 was synthesized by reacting bromohydrin (±)-41 with dry Amberlite IRA-400 HO⁻ form resin under subdued light for 3 days (Scheme 21). The epoxide (±)-41 was obtained as a creamy-white solid in a 98% yield.

Scheme 21. B[a]P epoxide (±)-41 synthesis by the reaction of B[a]P bromohydrin (±)-42 with Amberlite resin

Ring opening of epoxide (±)-42 was carried out by reaction with dried LiCl, anhydrous Ac₂O, and DMAP (catalytic amount), in DMF at room temperature (Scheme 22). After 18 h, the crude mixture containing product (±)-43 was worked-up to remove any byproducts and unreacted reagents. The resultant crude material was dissolved in anhydrous DMF and subjected to nucleophilic displacement with NaN₃ at 50 °C over 18 h. This resulted in the B[a]P azido derivative (±)-44 in a yield of 74% over two steps (Scheme 22). The increase in the yield of (±)-
44 from 53% (as in Scheme 18) to 74% (as in Scheme 22) showed that the epoxide synthesized from (±)-41 using Amberlite IRA-400 resin (Scheme 21) was obtained in a pure form and was responsible for the high yield in this reaction. Subsequent deacylation of (±)-44 with 7 N NH₃ in MeOH at 50 °C over 6 h resulted the azido triol (±)-38 in a quantitative yield (Scheme 22).

Scheme 22. Synthesis of B[a]P azido triol (±)-38

Next, reduction of the azido triol (±)-38 using Lindlar catalyst, in EtOH under a H₂ atmosphere, gave the desired B[a]P amino triol (±)-39 in a 93% yield (Scheme 23). To confirm the stereochemistry in amino triol (±)-39, the amino triol tetraacetate (±)-49 was synthesized directly from azido triol (±)-38 in two steps (Scheme 23). Initially, azido triol (±)-38 was reduced by using Lindlar catalyst in EtOH, under H₂ gas. This was followed by treatment with

Ac₂O, DMAP (catalytic amount), and pyridine, in N,N-dimethyl formamide (DMF) to give the peracetate (±)-49 in a 71% yield over two steps (Scheme 23).

The structure and stereochemistry of the final product (±)-39 was assigned by comparative NMR analysis of various stereochemically defined B[a]P derivatives. As a first step, the NMR of azido triol (±)-38 was compared to that of reported by Jerina et al (Table 1). The key chemical shifts H-7 (4.98 ppm), H-8 (3.68 ppm), H-9 (4.23 ppm), H-10 (5.44 ppm) and coupling constants J<sub>7,8</sub> = 8.6, J<sub>8,9</sub> = 8.5, J<sub>9,10</sub> = 5.7 recorded in CD<sub>3</sub>OD at 500 MHz (entry 1, Table 1) correlate well with the reported chemical shifts H-7 (5.03 ppm), H-8 (3.79 ppm), H-9 (4.33 ppm), H-10 (5.52 ppm) and coupling constants J<sub>7,8</sub> = 8.2, J<sub>8,9</sub> = 8.2, J<sub>9,10</sub> = 5.4 recorded in CDCl<sub>3</sub>-CD<sub>3</sub>OD at 300 MHz by Jerina et al (entry 1, Table 1). On the other hand, the chemical shifts and coupling constants of (±)-38 were not in congruence with the chemical shifts H-7 (4.85 ppm), H-8 (4.13 ppm), H-9 (4.07 ppm), H-10 (5.78 ppm) and coupling constants J<sub>7,8</sub> = 8.3, J<sub>8,9</sub> = 10.7, J<sub>9,10</sub> = 4.2 that were reported for the <i>trans</i> ring-opened B[a]P azido triol (±)-6 (entry 2, Table 1). The coupling constant J<sub>7,8</sub> ~ 8.2 in both cis and <i>trans</i> ring-opened B[a]P azido triols (±)-38 and (±)-6 respectively (entries 1 and 2, Table 1), indicates a quasi diequatorial orientation of the hydroxyl groups. Similar trend was also observed in the case of B[a]P amino triol (±)-39, where the coupling constant J<sub>7,8</sub> = 7.4 also indicating a quasi diequatorial orientation of the hydroxyl groups (entry 4, Table 1). However, upon acylation of compound (±)-39, compound (±)-49 displayed a small coupling constant J<sub>7,8</sub> = 4.5 (entry 5, Table 1), implying a quasi diaxial orientation of the substituents at the 7 and 8 positions. A similar trend was also observed in other <i>cis</i> ring-opened peracylated derivatives derived from B[a]P DE2 (±)-32, (±)-52–(±)-54 (entries 8–11, Table 1) with the coupling constant range J<sub>7,8</sub> = 3.4–4.0. The differences in the orientation of substituents at 7 and 8 positions in unprotected and acyl protected B[a]P
derivatives can be rationalized as follows. In the case of azido and amino triols, the quasi diequatorial orientation of the hydroxyl groups is preferred possibly due to internal hydrogen bonding, resulting in a quasi diaxial arrangement for protons H-7 and H-8. Upon peracylation of aminotriols, the substituents adopt a quasi diaxial arrangement possibly to avoid steric interactions between the substituents, loss of H-bonding, and dipole-dipole repulsions. However, a large coupling constant $J_{7,8} = 8.0$ was observed in the case of cis ring-opened tetraol tetraacetate derivative (±)-50 (entry 6, Table 1), which is an exception to the trend observed in other compounds.

**Table 1. Comparison of chemical shifts and coupling constants of tetrahydro ring protons in B[a]P derivatives**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>H-7</th>
<th>H-8</th>
<th>H-9</th>
<th>H-10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>d 4.98</td>
<td>t 3.68</td>
<td>dd 4.23</td>
<td>d 5.44</td>
</tr>
<tr>
<td>1</td>
<td><img src="image1" alt="Schema 1" /></td>
<td>(d 5.03)&lt;sup&gt;83&lt;/sup&gt;</td>
<td>(t 3.79)&lt;sup&gt;83&lt;/sup&gt;</td>
<td>(dd 4.33)&lt;sup&gt;83&lt;/sup&gt;</td>
<td>(d 5.52)&lt;sup&gt;83&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>500 MHz (CD&lt;sub&gt;3&lt;/sub&gt;OD): $J_{7,8} = 8.0$; $J_{8,9} = 8.0$; $J_{9,10} = 5.5$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[300 MHz (CDCl&lt;sub&gt;3&lt;/sub&gt;-CD&lt;sub&gt;3&lt;/sub&gt;OD): $J_{7,8} = 8.0$; $J_{8,9} = 8.0$; $J_{9,10} = 5.5$]&lt;sup&gt;83&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2&lt;sup&gt;83&lt;/sup&gt;</td>
<td><img src="image2" alt="Schema 2" /></td>
<td>d 4.85</td>
<td>dd 4.13</td>
<td>dd 4.07</td>
<td>d 5.78</td>
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<td></td>
<td>500 MHz (CDCl&lt;sub&gt;3&lt;/sub&gt;-CD&lt;sub&gt;3&lt;/sub&gt;OD): $J_{7,8} = 8.3$; $J_{8,9} = 10.7$; $J_{9,10} = 4.2$</td>
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<td>3</td>
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<td>d 7.14</td>
<td>t 5.84</td>
<td>dd 5.91</td>
<td>d 5.78</td>
</tr>
<tr>
<td></td>
<td>500 MHz (CDCl&lt;sub&gt;3&lt;/sub&gt;): $J_{7,8} = 7.4$; $J_{8,9} = 5.9$; $J_{9,10} = 3.8$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><img src="image4" alt="Schema 4" /></td>
<td>m 5.09–5.03</td>
<td>t 3.92</td>
<td>dd 4.19</td>
<td>m 5.09–5.03</td>
</tr>
<tr>
<td></td>
<td>500 MHz (CD&lt;sub&gt;3&lt;/sub&gt;OD): $J_{7,8} = 7.4$; $J_{8,9} = 7.0$; $J_{9,10} = 4.4$</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5</td>
<td><img src="image5" alt="Schema 5" /></td>
<td>d 6.61</td>
<td>t 5.63</td>
<td>dd 5.52</td>
<td>dd 6.34</td>
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<tr>
<td></td>
<td>500 MHz (CDCl&lt;sub&gt;3&lt;/sub&gt;): $J_{7,8} = 4.5$; $J_{8,9} = 5.5$; $J_{9,10} = 3.2$; $J_{10,NH} = 9.0$</td>
<td></td>
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</tr>
</tbody>
</table>
Stereochemistry of B[a]P amino triol (±)-39 was further verified by evaluating the NOESY data of compound (±)-49 (Figure 7). The NOESY data showed through-space interaction between H-9 and H-10. A clear through-space interaction was also observed between H-9 and H-7, implying that H-7 and H-9 are located on the same face. Similar interactions were also observed in the NOESY spectrum of B[a]P derivatives (±)-38 and (±)-44. Strong through-space interactions for H-7 and H-9, H-9 and H-10 were observed in the case of B[a]P azido triol.
(±)-38, whereas only weak through-space interactions for H-7 and H-9 were observed in the case of azido triacyl derivative (±)-44.

**Figure 7. NOESY spectrum showing interactions between H-7 and H-9, as well as H-9 and H-10**

After verifying the supporting data for the stereochemistry of B[a]P amino triol (±)-39, we then focused on B[a]P-dA adduct synthesis. Following our previous protocol on dA adduct synthesis,92 B[a]P amino triol (±)-39 was subjected to S_N_Ar displacement reaction with 6-fluoropurine nucleoside 40 in the presence hexamethyl disiloxane (HMDS) and diisopropylethylamine (DIPEA) in DMSO, at 80 °C for 5 h (Scheme 24). This resulted in a diastereomeric pair of B[a]P-dA adducts 25a and 25b. Several attempts were made to separate
adducts 25a and 25b by using preparative TLC. However, these attempts were unsuccessful due to the polar nature of the adducts. Hence, the free hydroxyl groups of adducts were acetylated in the hope that a solvent system could be found for the adduct separation on preparative TLC. Thus, adducts 25a and 25b were then treated with acetic anhydride and pyridine at room temperature for 14 h to give the acetylated B[a]P-dA adducts 55a and 55b. The two adducts 55a and 55b could be carefully separated by chromatography on a preparative TLC plate (SiO$_2$, 500 µ, 20 x 20 cm), by eluting with 10% EtOAc in CH$_2$Cl$_2$. This gave compounds 55a and 55b in a combined yield of 42% over two steps. On the basis of the Circular Dichroism (CD) data of dA adducts 55a and 55b, the faster-eluting diastereomer was assigned as the 10S isomer (55a), as it displayed a positive band at 281 nm in its CD spectrum (Figure 8). The slower-eluting diastereomer was assigned as the 10R isomer (55b) as it displayed a negative band at 281 nm in

**Scheme 24. Synthesis of B[a]P DE1-dA adducts 55a and 55b from B[a]P amino triol (±)-39 and 6-fluoropurine nucleoside 40**
its CD spectrum (Figure 8). The CD spectra of the B[a]P-dA adducts 55a and 55b were consistent with the data reported by Jerina et al.\textsuperscript{83}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{cd_spectra.png}
\caption{Normalized CD spectra (in MeOH) of (±)-B[a]P DE1-dA adducts 55a (red = 10S trans) and 55b (blue = 10R cis)}
\end{figure}

Following the successful synthesis of B[a]P-dA adducts, we moved to synthesis of the B[a]P-dG adducts by utilizing an addition-elimination reaction. For this purpose, 2-fluoro-2’-deoxyinosine derivative 45 was needed. Typically, 45 was synthesized from a protected 2’-deoxyguanosine derivative via its O6-benzyl derivative 59.\textsuperscript{71} The introduction of a benzyl group at the O-6 position of 2’-deoxyguanosine is usually carried out under Mitsunobu conditions.\textsuperscript{71} However, these reaction conditions require high temperature, involve tedious purification process, and produce moderate yields.\textsuperscript{71} Hence, we decided to modify the strategy by using our previous report on a guanosine etherification protocol.\textsuperscript{126} The synthesis starts by reacting silyl-protected 2’-deoxyguanosine derivative 56 with benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) and 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU) in dry MeCN, at room temperature for 1 h (Scheme 25), following the reported procedure.\textsuperscript{126} This resulted in the formation of O6-benzotriazol-1-yl-2’-deoxyguanosine derivative 57 in a 88% yield. Then 57 was subjected to S\textsubscript{N}Ar displacement reaction with benzyl alcohol and Cs\textsubscript{2}CO\textsubscript{3} in dry DME at room temperature for 36 h to give O6-
benzyl-2’-deoxyguanosine derivative 58 in a 92% yield. Subsequent desilylation of 58 with KF in MeOH at 80 °C for 12 h resulted O6-benzyl-2’-deoxyguanosine 59 in a 95% yield (Scheme 25). 2-Fluoro-2’-deoxyinosine derivative 47 was synthesized from 59 in three steps, with minor modifications to the reported procedure.71 Diazotization and fluorination of 59, with t-butylnitrite (t-BuONO) and 60% HF in pyridine at –60 °C for 25 min gave the 2-fluoropurine nucleoside 60 in 85% yield. Silyl protection of the free hydroxyl groups of 60 with TBDMSOTf and pyridine in DME at 0 °C over 45 min afforded the silyl derivative 61 in a 92% yield.

**Scheme 25. Modified synthesis of 2-fluoro-2’-deoxyinosine derivative 41**
Debenzylation of 61 under catalytic hydrogenation conditions i.e., 5% Pd/C, H₂ (1 atm) in THF-CH₃OH mixture, at room temperature for 1 h, furnished the desired product 47 in a 96% yield.

With 2-fluoro-2'-deoxyinosine derivative 45 in hand, we then pursued the B[a]P-dG adduct synthesis. In this context, B[a]P amino triol (±)-39 was reacted with fluorinated nucleoside 45 under the same conditions used for the synthesis of dA adducts 25a and 25b. However, these attempts were unsuccessful and no product formation was observed. We wondered whether the presence of any minor impurities in B[a]P amino triol (±)-39 could be preventing the reaction. Since, B[a]P amino triol (±)-39 is a sensitive substrate, we decided to synthesize the hydrochloride salt of amino triol, (±)-62, as it could be relatively easy to purify and can also be directly used for adduct synthesis. The amino triol salt (±)-62 was obtained in quantitative yield by treating B[a]P azido triol (±)-38 with Lindlar catalyst and 6 M HCl in EtOH under a hydrogen atmosphere, at room temperature over 2 h (Scheme 26). Treatment of B[a]P amino triol (±)-39 with 6 M HCl in EtOH at room temperature for 2 h also afforded the amino triol hydrochloride salt (±)-62 in a quantitative yield. Since the synthesis of amino triol hydrochloride.

salt (±)-62 involves acidic conditions, it is important to make sure no stereochemical changes occurred during the process. Hence, we synthesized B[a]P amino triol tetraacetate (±)-49 by treating hydrochloride salt (±)-62 with Ac₂O, pyridine and catalytic amount of DMAP in DMF at room temperature for 14 h (Scheme 26). The ¹H NMR data of (±)-49 was consistent with the same compound synthesized previously, shown in Scheme 23, indicating no stereochemical changes to (±)-62.

Next, we attempted the B[a]P-dG adduct synthesis, by coupling the amino triol hydrochloride salt (±)-62 with 2-fluoro-2'-deoxyinosine derivative 45 in the presence of (Me₃Si)₂O and DIPEA in DMSO at 80 °C for 6 h (Scheme 27). This afforded the diastereomeric pair of B[a]P-dG adducts 46a and 46b. The diastereomeric mixture 46a and 46b were then reacted with acetic anhydride and pyridine at room temperature for 12 h to give the desired acetylated B[a]P-dG adducts 63a and 63b. The two adducts 63a and 63b were separated by chromatography on a preparative TLC plate (SiO₂, 1 mm, 20 cm × 20 cm), by eluting with 22% over two steps.

Scheme 27. B[a]P DE1-dG adduct synthesis via nucleophilic displacement by amino triol hydrochloride (±)-62 on 2-fluoro-2'-deoxyinosine derivative 45
1:19:80 MeOH-EtOAc-CH$_2$Cl$_2$. Compounds 63a and 63b were obtained in a combined yield of 22% over two steps. On the basis of the CD data of the dG adducts 63a and 63b, the faster-eluting diastereomer was assigned as the 10S isomer (63a), as it displayed a positive band at 250.6 nm (Figure 9). On the other hand, the slower-eluting diastereomer was assigned as the 10R isomer (63b) as it displayed a negative band at 250.8 nm in its CD spectrum (Figure 9). The CD spectra of the B[a]P-dG adducts 63a and 63b were consistent with the data reported by Jerina et al.$^{85}$

![Figure 9. Normalized CD spectra (in MeOH) of (±)-B[a]P DE1-dG adducts 63a (red = 10S trans) and 63b (blue = 10R cis)](image)

To further confirm the structure and stereochemistry of the adducts 55a (10S), 55b (10R), 63a (10S), and 63b (10R) key coupling constants were compared to that of reported cis and trans ring-opened B[a]P DE1-nucleoside adducts (Table 2). To our surprise, no correlation was observed with the reported coupling constant of the cis ring-opened B[a]P DE1-dA adducts and 55a ($J_{7,8} = 7.9$ vs 6.2, $J_{8,9} = 11.5$ vs 6.9, entry 1, Table 2) and 55b ($J_{7,8} = 8.0$ vs 5.5, $J_{8,9} = 11.5$ vs 6.5, entry 2, Table 2). On the other hand, the coupling constants of 55a and 55b matched those of the trans ring-opened B[a]P DE1-dA adducts 64a and 64b (entries 1–4, Table 2). Similarly, the coupling constants of B[a]P DE1-dG adducts 63a and 63b correlated with the reported
coupling constant of the *trans* ring-opened B[α]P DE1-dA adducts 65a and 65b rather than *cis* ring-opened B[α]P DE1-dA adducts (entries 5–8, Table 2).

**Table 2.** *Comparison of coupling constants of the tetrahydro ring protons in B[α]P-nucleoside adducts*<sup>a</sup>

<table>
<thead>
<tr>
<th>Entry</th>
<th>Adduct</th>
<th>(J_{7,8})</th>
<th>(J_{8,9})</th>
<th>(J_{9,10})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2'-deoxyadenosine adducts <em>via cis</em> ring-opening of B[α]P DE1</td>
<td>6.2&lt;sup&gt;b&lt;/sup&gt; (3.4)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.9&lt;sup&gt;b&lt;/sup&gt; (4.1)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.2&lt;sup&gt;b&lt;/sup&gt; (2.3)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td><img src="image1.png" alt="Diagram" /></td>
<td>7.9&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>11.5&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>4.5&lt;sup&gt;b,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>2'-deoxyadenosine adducts <em>via trans</em> ring-opening of B[α]P DE1</td>
<td>5.5&lt;sup&gt;b&lt;/sup&gt; (3.0)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.5&lt;sup&gt;b&lt;/sup&gt; (3.7)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.4&lt;sup&gt;b&lt;/sup&gt; (2.9)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td><img src="image2.png" alt="Diagram" /></td>
<td>8.0&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>11.5&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>4.5&lt;sup&gt;b,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>3&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2'-deoxyguanosine adducts <em>via cis</em> ring-opening of B[α]P DE1</td>
<td>6.0&lt;sup&gt;b&lt;/sup&gt; (3.1)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.9&lt;sup&gt;b&lt;/sup&gt; (3.3)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.2&lt;sup&gt;b&lt;/sup&gt; (2.9)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2'-deoxyguanosine adducts <em>via cis</em> ring-opening of B[α]P DE1</td>
<td>5.8&lt;sup&gt;b&lt;/sup&gt; (3.1)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.6&lt;sup&gt;b&lt;/sup&gt; (3.6)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.9&lt;sup&gt;b&lt;/sup&gt; (2.9)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>2'-deoxyguanosine adducts <em>via cis</em> ring-opening of B[α]P DE1</td>
<td>7.0&lt;sup&gt;f&lt;/sup&gt;</td>
<td>7.2&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4.5&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td><img src="image3.png" alt="Diagram" /></td>
<td>8.2&lt;sup&gt;b,g&lt;/sup&gt;</td>
<td>11.8&lt;sup&gt;b,g&lt;/sup&gt;</td>
<td>2.2&lt;sup&gt;b,g&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>2'-deoxyguanosine adducts <em>via trans</em> ring-opening of B[α]P DE1</td>
<td>6.3&lt;sup&gt;f&lt;/sup&gt;</td>
<td>6.5&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3.6&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td><img src="image4.png" alt="Diagram" /></td>
<td>8.2&lt;sup&gt;b,g&lt;/sup&gt;</td>
<td>11.8&lt;sup&gt;b,g&lt;/sup&gt;</td>
<td>NA&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>7&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2'-deoxyguanosine adducts <em>via trans</em> ring-opening of B[α]P DE1</td>
<td>6.9&lt;sup&gt;f&lt;/sup&gt;</td>
<td>7.0&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4.4&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
These observations suggest that the substituents at the C-9 and C-10 positions in the tetrahydro ring of dA and dG adducts $55\text{a, b}$ and $63\text{a, b}$ possess a trans stereochemistry rather than the desired cis orientation. In comparing the \textsuperscript{1}H NMR data of the adducts $55\text{a, b}$ and $63\text{a, b}$ to the trans ring-opened B[a]P DE1-nucleoside adducts reported by Lakshman \textit{et al.},{\textsuperscript{78}} it is clearly evident that the adducts generated herein possess an all trans stereochemistry and the corrected structures of the adducts are shown in Figure 10.

\textit{Figure 10. Corrected structures of the B[a]P DE1-nucleoside adducts $55\text{a, b}$ and $63\text{a, b}$}
Since the adduct synthesis involves $S_{N}Ar$ and addition-elimination mechanisms, it is highly unlikely for change in chirality at the C-10 position to occur in the adduct-forming step. Thus, any determination of chirality can occur only during the synthesis of azido triacyl derivative $\pm$-44, since the process of installing the azido group at the C-10 position involves a double displacement reaction. Hence, we re-evaluated the $^1$H NMR data of azido triol $\pm$-38, recorded in CD$_3$OD and CDCl$_3$-CD$_3$OD at 500 MHz by comparing to the $^1$H NMR data of cis and trans ring-opened azido triols $\pm$-38 and $\pm$-6 (Table 3). The chemical shift data for the H-7, H-8, H-9, and H-10 protons observed for azido triol $\pm$-38 synthesized herein, in CD$_3$OD and CDCl$_3$-CD$_3$OD were comparable to those reported (entries 1–3, Table 3) and was different from trans ring-opened azido triol $\pm$-6 (entries 2 and 4, Table 3). However, comparing the coupling constants for the presently synthesized $\pm$-38 obtained in CDCl$_3$-CD$_3$OD to those of the cis or trans ring-opened azido triols reported (entries 2–4, Table 3) did not provide adequate confidence for unequivocal structure determination. The data from entries 1–4 in Table 3 were not adequately clear-cut to indicate that the double displacement reaction on epoxide $\pm$-42 had successfully occurred. Hence, we compared the data for the azido triol $\pm$-38 we synthesized to the trans ring-opened azido triol $\pm$-6 previously synthesized by Lakshman.$^{127}$ In acetone-$d_6$ at 500 MHz, the chemical shift and coupling constant data were exactly comparable to those previously obtained at 300 MHz in the same solvent (entries 5 and 6, Table 3). This indicates that the double-displacement reaction on epoxide $\pm$-42, followed by deacylation of triacyl azidotriol $\pm$-44, resulted in the trans ring-opened B[a]P azido triol $\pm$-6 rather than the desired cis isomer $\pm$-38. The reasons behind the failure of proposed double displacement reaction and new avenues for the synthesis of cis ring-opened B[a]P amino triol are currently being investigated.
Table 3. Comparison of coupling constants of the tetrahydro ring protons in B[a]P-azidotriols (±)-38 and (±)-6

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>H-7</th>
<th>H-8</th>
<th>H-9</th>
<th>H-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>![38 (anticipated)]</td>
<td>d 4.98</td>
<td>t 3.68</td>
<td>dd 4.23</td>
<td>d 5.44</td>
</tr>
<tr>
<td>2</td>
<td>![38 (anticipated)]</td>
<td>d 4.98</td>
<td>t 3.66</td>
<td>dd 4.22</td>
<td>d 5.30</td>
</tr>
<tr>
<td>3&lt;sup&gt;b, c&lt;/sup&gt;</td>
<td>![38 (authentic)]</td>
<td>d 5.03</td>
<td>t 3.79</td>
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<td>![6 (authentic)]</td>
<td>d 5.04</td>
<td>t 3.79</td>
<td>dd 4.33</td>
<td>d 5.52</td>
</tr>
<tr>
<td>5</td>
<td>![38 (anticipated)]</td>
<td>d 5.03</td>
<td>t 3.78</td>
<td>dd 4.32</td>
<td>d 5.51</td>
</tr>
<tr>
<td>6&lt;sup&gt;c, d&lt;/sup&gt;</td>
<td>![6 (authentic)]</td>
<td>d 5.04</td>
<td>t 3.79</td>
<td>dd 4.33</td>
<td>d 5.52</td>
</tr>
</tbody>
</table>

CD<sub>3</sub>OD: \(J_{7,8} = 8.0; J_{8,9} = 8.0; J_{9,10} = 5.5\)

CDCl<sub>3</sub>-CD<sub>3</sub>OD: \(J_{7,8} = 8.5; J_{8,9} = 8.9; J_{9,10} = 6.3\)

CDCl<sub>3</sub>-CD<sub>3</sub>OD: \(J_{7,8} = 8.0; J_{8,9} = 8.0; J_{9,10} = 5.5\)

CDCl<sub>3</sub>-CD<sub>3</sub>OD: \(J_{7,8} = 8.3; J_{8,9} = 10.7; J_{9,10} = 4.2\)

acetone-\(d_6\): \(J_{7,8} = 8.1; J_{8,9} = 7.9; J_{9,10} = 5.4\)

acetone-\(d_6\): \(J_{7,8} = 8.3; J_{8,9} = 8.0; J_{9,10} = 5.5\)

<sup>a</sup> Data obtained at 500 MHz under ambient conditions.  
<sup>b</sup> As reported by Jerina et al. (ref. 83).  
<sup>c</sup> Data obtained at 300 MHz under ambient conditions.  
<sup>d</sup> Unpublished results by Lakshman (ref. 127).
[3.3] CONCLUSION

In conclusion, we prepared B[a]P epoxide (±)-42 from bromohydrin (±)-41 under mild conditions using hydroxide form resin (Amberlite IRA-400-OH) as previously reported. Epoxide (±)-42 was obtained in high yield and with high purity. Using this epoxide in a double displacement reaction with LiCl, Ac₂O, followed by NaN₃, an improved the yield of B[a]P azido triacyl derivative (±)-44 was attained. This modification along with minor changes in purification techniques improved the overall yield of (±)-10β-amino-7β,8a,9β-trihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene. The structure and stereochemistry of this amino triol was analyzed and by comparison of the NMR data with various B[a]P derivatives. The amino triol was then used for the synthesis of dA adducts. Moderate yields were obtained in this process and the diastereomeric pair of dA adducts 55a and 55b were separated using preparative TLC. The absolute configuration in the adducts were determined by the sign of the CD band at 281 nm and by comparing the CD data with the literature. By utilizing a C-6 modification protocol for guanosine nucleosides via the amide activation by BOP, we developed a modified synthesis of 2-fluoro-2’-deoxyinosine derivative 45. This compound is necessary for the B[a]P-dG adduct synthesis. However, the reaction of amino triol with 45 was unsuccessful. Hence, the hydrochloride salt of the amino triol (±)-62 was prepared and was used in the dG adduct synthesis. This reaction afforded the desired B[a]P-dG adducts 63a and 63b. Adducts 63a and 63b were separated using preparative TLC and the adducts were determined by comparing the CD data with the literature. Surprisingly, the key coupling constant data of the adducts 55a,b and 63a,b did not correlate with the data of previously reported cis ring-opened B[a]P DE1-nucleoside adducts and matched well with trans ring-opened B[a]P DE1-nucleoside adducts 64a,b and 65a,b respectively. This indicated that the double displacement reaction had failed.
and the following steps yielded *trans* ring-opened B[a]P azido triol (±)-6 rather than *cis* ring-opened isomer (±)-38. Since, the *trans* ring-opened B[a]P azido triol was carried on for further steps, the nucleoside adducts prepared were not of the desired stereochemistry and, hence, structures of adducts were corrected. Analysis of the double displacement reaction will be performed in the future to evaluate the failure of this reaction, because all indications were that successful reaction had occurred.
[3.4] EXPERIMENTAL SECTION

General Experimental Considerations. Thin layer chromatography was performed on 200 µm aluminum-foil-backed silica gel plates. Column chromatography was performed using 200–300 mesh silica gel. Toluene was distilled over Na. THF was distilled over LiAlH₄ and then over Na. CH₃CN and 1,2-dimethoxyethane (DME) were distilled from CaH₂. NaN₃ was washed with acetone and dried under vacuum overnight before use. Amberlite was stored in dry THF for a week under subdued light and the THF was replaced daily with freshly distilled THF. The THF was filtered off before the Amberlite was used in the cyclization reaction. All other reagents were used as received from commercial suppliers. ¹H NMR spectra were obtained at 500 MHz and are referenced to the residual protonated solvent resonance. ¹³C NMR spectra were obtained at 125 MHz and are referenced to the solvent resonance. Freshly deacidified CDCl₃ was used as the solvent for acquiring NMR data of some samples (deacidification is done by percolating CDCl₃ through a bed of basic alumina and NaHCO₃ and discarding the first few drops). Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (J) are in hertz (Hz). Standard abbreviations are used to designate resonance multiplicities.

9,10-Dihydrobenzo[a]pyrene (47)₉³

In a clean, dry, 500 mL round bottom flask equipped with a stir bar was placed 7,8,9,10-tetrahydrobenzo[a]pyrene-7-ol (7.0 g, 25.7 mmol, 1 equiv.) in dry toluene (266 mL). Then p-toluenesulfonic acid (0.489 g, 2.57 mmol, 0.1 equiv.) was added to the mixture and the resulting
mixture was heated at reflux for 0.5 h. The mixture was cooled, evaporated to half of its volume, and filtered through a plug of cotton. The filtrate was then washed with saturated aq. NaHCO₃ (2 x 100 mL) and brine (50 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered, and evaporated to dryness under reduced pressure. Chromatography of the crude material on a silica gel column by sequential elution with hexanes, 5% CH₂Cl₂ in hexanes, and 10% CH₂Cl₂ in hexanes gave compound 47 (5.88 g, 90%) as a pale yellow solid. ¹H NMR (500 MHz, CDCl₃): δ 8.28 (d, 1H, J = 9.3 Hz, Ar-H), 8.12 (d, 2H, J = 7.5 Hz, Ar-H), 8.07 (d, 1H, J = 9.3 Hz, Ar-H), 7.99 (app s, 2H, Ar-H), 7.95 (t, 1H, J = 7.6 Hz, Ar-H), 7.87 (s, 1H, Ar-H), 6.87 (d, 1H, J = 9.5 Hz, Ar-H), 6.27 (dt, 1H, J = 4.5, 9.3 Hz, Ar-H), 3.52 (t, 2H, J = 8.2 Hz, CH₂), 2.62–2.56 (m, 2H, CH₂).

(±)-7β,8α-Dibenzoyloxy-7,8,9,10-tetrahydrobenzo[a]pyrene ((±)-48)

To a suspension of BzOAg (7.63 g, 33.30 mmol, 2.2 equiv.) in dry toluene (170 mL), was added iodine (4.23 g, 16.65 mmol, 1.1 equiv.) with dry toluene (30 mL). The mixture was stirred for 0.5 h, at which point a yellow suspension had formed. To this mixture compound 47 (3.85 g, 15.13 mmol, 1 equiv.) was added with dry toluene (77 mL) and the mixture was stirred for 2.5 h at which point TLC showed consumption of compound 47. The resulting mixture was then heated at reflux for 16 h, and filtered hot through Celite. The filtrate was concentrated and suspended in acetone. The suspension was sonicated and filtered. The solid was recovered and the filtrate was concentrated. The solid obtained from the filtrate was again sonicated with
acetone and filtered. This process was repeated several times until complete recovery of the product. Compound (±)-48 (6.63 g, 88% yield) was obtained as a light-brown solid. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.33 (d, 1H, $J = 9.3$ Hz, Ar-H), 8.24–8.16 (m, 4H, Ar-H), 8.11 (d, 2H, $J = 7.7$ Hz, Ar-H), 8.04–7.94 (m, 5H, Ar-H), 7.55 (t, 1H, $J = 7.4$ Hz, Ar-H), 7.49 (t, 1H, $J = 7.4$ Hz, Ar-H), 7.42 (t, 2H, $J = 7.7$ Hz, Ar-H), 7.35 (t, 2H, $J = 7.7$ Hz, Ar-H), 6.99 (d, 1H, $J = 6.0$ Hz, H-7), 5.82–5.76 (m, 1H, H-8), 3.81–3.68 (m, 2H, H-9, H-9’), 2.74 (dq, 1H, $J = 3.3$, 10.0 Hz, H-10), 2.54 (dq, 1H, $J = 6.9$, 13.9 Hz, H-10’).

(±)-7β,8α-Dibenzoyloxy-7,8-dihydrobenzo[a]pyrene ((±)-27)$^{93}$

In a clean, dry, 500 mL round bottom flask equipped with a stir bar, DDQ (4.74 g, 20.88 mmol, 1.3 equiv.) was stirred in dry toluene (100 mL) to give a dark reddish-brown solution. Then compound (±)-48 (7.95 g, 16.02 mmol, 1 equiv.) was added to the mixture, with dry toluene (219 mL), and the resulting mixture was heated at reflux for 17 h. The reaction mixture was filtered hot through Celite. Evaporation of the filtrate resulted in a greenish solid. The solid was suspended in acetone, sonicated and filtered. The solid was recovered and the filtrate was concentrated. The solid obtained from the filtrate was sonicated again with acetone and filtered. This process was repeated several times until complete recovery of the product. Compound (±)-27 (4.37 g, 75% yield) was obtained as a green solid. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.42 (d, 1H, $J = 9.3$ Hz, Ar-H), 8.22–8.15 (m, 4H, Ar-H), 8.13 (d, 2H, $J = 7.6$ Hz, Ar-H), 8.08 (d, 1H, $J = 8.9$ Hz, Ar-H), 8.03-7.98 (m, 4H, Ar-H), 7.79 (d, 1H, $J = 10.0$ Hz, H-10), 7.57 (t, 1H, $J = 7.4$ Hz, Ar-
H), 7.51 (t, 1H, J = 7.4 Hz, Ar-H), 7.44 (t, 2H, J = 7.8 Hz, Ar-H), 7.37 (t, 2H, J = 7.8 Hz, Ar-H), 7.07 (d, 1H, J = 7.5 Hz, H-7), 6.47 (dd, 1H, J = 3.6, 10.1 Hz, H-9), 6.22 (ddd, 1H, J = 1.1, 3.6, 7.4 Hz, H-8).

(±)-9α-Bromo-7β,8α-dibenzoyloxy-10β-hydroxy-7,8,9,10-tetrahydrobenzo[α]pyrene ((±)-41)<sup>125</sup>

\[ \begin{array}{c}
\text{(±)-27} \\
\text{NBS, NaOAc} \\
\text{THF, H}_{2}\text{O, 16 h} \\
\text{75\%} \\
\text{(±)-41}
\end{array} \]

To a pale yellow solution of dibenzoate (±)-27 (1.0 g, 2.022 mmol, 1 equiv.) in 250 mL of THF, NBS (464.8 mg, 2.63 mmol, 1.3 equiv.), NaOAc (497.6 mg, 6.066 mmol, 3 equiv.) and 100 mL of deionized water were added. The mixture was stirred, at room temperature, under subdued light for 16 h. The reaction mixture was evaporated to a third of its volume and was extracted with CH₂Cl₂ (3 x 100 mL). The organic layers were combined, washed with brine (50 mL), dried over anhydrous Na₂SO₄, filtered, and evaporated to dryness under reduced pressure. The crude mixture was chromatographed on a silica gel column using 50% CH₂Cl₂ in hexanes as the eluent. The resulting solid was washed with 1:1 hexanes-Et₂O and 1:1 hexanes-CH₂Cl₂. Compound (±)-41 (896.9 mg, 75% yield) was obtained as a creamy, white solid.

(±)-7β,8α-Dibenzoyloxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[α]pyrene ((±)-42)<sup>125</sup>

\[ \begin{array}{c}
\text{(±)-41} \\
\text{Amberlite-IRA-400-OH} \\
\text{THF, r.t., 72 h, 98\%} \\
\text{(±)-42}
\end{array} \]
In a clean, dry, 500 mL round bottom flask equipped with a stir bar, was placed dry Amberlite IRA 400 (OH\textsuperscript{−}) (36.0 g) in dry THF (250 mL). To this, bromohydrin (±)-41 (1.0 g, 1.69 mmol) was added and the mixture was stirred at room temperature under subdued light for 3 days. The mixture was filtered and the Amberlite was washed with dry THF. The filtrate was concentrated under reduced pressure. The resulting solid was suspended in dry Et\textsubscript{2}O, sonicated and filtered to give product (±)-42 (845.0 mg, 98% yield) as a white solid. \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}): δ 8.62 (d, 1H, J = 9.3 Hz, Ar-H), 8.33 (s, 1H, Ar-H), 8.29–8.21 (m, 3H, Ar-H), 8.13 (br d, 3H, J = 8.6 Hz, Ar-H), 8.09–8.03 (m, 2H, Ar-H), 7.92 (br d, 2H, J = 7.8 Hz, Ar-H), 7.54 (t, 1H, J = 7.3 Hz, Ar-H), 7.50 (t, 1H, J = 7.4 Hz, Ar-H), 7.42 (t, 2H, J = 7.6 Hz, Ar-H), 7.34 (t, 2H, J = 7.6 Hz, Ar-H), 6.96 (d, 1H, J = 3.9 Hz, H-7), 6.04–5.99 (m, 1H, H-9), 5.12 (d, 1H, J = 3.6 Hz, H-10), 4.26–4.22 (m, 1H, H-8).

(±)-9β-Acetoxy-10β-azido-7β,8α-dibenzoyloxy-7,8,9,10-tetrahydrobenzo[a]pyrene ((±)-44)

To a solution of the BaP diol epoxide (±)-42 (200 mg, 0.392 mmol, 1 equiv.) in dry DMF (4 mL), prepared in a clean, dry, 8 mL reaction vial equipped with a stirring bar, DMAP (a few crystals) and Ac\textsubscript{2}O (741 µL, 7.834 mmol, 20 equiv.) were added under dry N\textsubscript{2} gas, in a glove bag. In a separate 4 mL reaction vial, LiCl (249.0 mg, 5.88 mmol, 15 equiv.) was dried overnight at 150 °C in an oven and then heated at 150 °C in a sand bath under vacuum for 2 h. After cooling to room temperature under vacuum, the vial was transferred to the glove bag and the LiCl was added to the reaction mixture. The reaction vial was stoppered, covered with aluminum foil, and
the mixture was stirred at room temperature for 18 h. Four other reactions on the same scale were setup simultaneously. After completion of the reactions as assessed by TLC, the reaction mixtures were combined, and diluted with EtOAc (60 mL). The organic layer was washed with saturated aq. NaHCO$_3$ (2 x 20 mL), 1:1 deionized water-brine (3 x 50 mL), and brine (10 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and evaporated to dryness under reduced pressure. To the concentrated and dried crude reaction mixture from step 1, dry DMF (20 mL) and NaN$_3$ (1.27 g, 19.6 mmol) were added, the mixture was flushed with N$_2$ gas, stoppered, and stirred at 50 °C for 18 h. The reaction mixture was diluted with EtOAc (50 mL), washed with 1:1 deionized water-brine (3 x 30 mL), and brine (10 mL). The aqueous layers were combined and back extracted with EtOAc (2 x 30 mL). The organic layers were combined, washed with brine (10 mL), dried over anhydrous Na$_2$SO$_4$, filtered, and evaporated to dryness under reduced pressure. The crude product was chromatographed on a silica gel column using CH$_2$Cl$_2$ as eluent. The product obtained was then washed with cold EtOAc to give the compound (±)-44 (752.0 mg, 78% yield) as a pinkish-red solid. $^1$H NMR (500 MHz, CDCl$_3$): δ 8.42 (d, 1H, $J = 9.3$ Hz, Ar-H), 8.30 (d, 2H, $J = 8.5$ Hz, Ar-H), 8.26 (d, 1H, $J = 7.6$ Hz, Ar-H), 8.17 (s, 1H, Ar-H), 8.16–8.11 (m, 3H, Ar-H), 8.09 (t, 1H, $J = 7.6$ Hz, Ar-H), 8.06–8.02 (m, 3H, Ar-H), 7.59 (t, 1H, $J = 7.4$ Hz, Ar-H), 7.54 (t, 1H, $J = 7.3$ Hz, Ar-H), 7.46 (t, 2H, $J = 7.5$ Hz, Ar-H), 7.41 (t, 2H, $J = 7.5$ Hz, Ar-H), 7.14 (d, 1H, $J = 7.4$ Hz, H-7), 5.91 (dd, 1H, $J = 4.1, 5.9$ Hz, H-9), 5.84 (t, 1H, $J = 6.7$ Hz, H-8), 5.78 (d, 1H, $J = 3.8$ Hz, H-10), 2.03 (s, 3H, Me).

(±)-10β-Azido-7β,8α,9β-trihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene ((±)-38)$^{96}$

![Chemical Reaction Diagram]
In a clean, dry, 15 mL pressure vial equipped with a stir bar were placed azido acetoxy dibenzoate (±)-44 (300 mg, 0.504 mmol, 1 equiv.) and 7 N NH₃ in MeOH (10.5 mL). The vial was tightly sealed, and the reaction mixture was stirred at 50 °C for 6 h, at which point the reaction mixture turned into a clear brownish-yellow solution. The reaction mixture was diluted with EtOAc (60 mL) and washed with deionized water (2 x 15 mL). The aqueous layers were combined and back extracted with EtOAc (2 x 20 mL). The combined organic layer was washed with brine (15 mL), dried over anhydrous Na₂SO₄, filtered, and evaporated to dryness under reduced pressure. The resulting yellow solid was washed with 1:1 Et₂O-hexanes to give compound (±)-38 (173.7 mg, quantitative yield) as a light brown solid. ¹H NMR (500 MHz, acetone-\textit{d₆}): δ 8.55 (s, 1H, Ar-H), 8.52 (d, 2H, \textit{J} = 9.7 Hz, Ar-H), 8.35–8.28 (m, 3H, Ar-H), 8.18 (s, 2H, Ar-H), 8.08 (t, 1H, \textit{J} = 7.6 Hz, Ar-H), 5.52 (d, 1H, \textit{J} = 5.4 Hz, H-10), 5.05 (d, 1H, \textit{J} = 8.1 Hz, H-7), 4.96 (br s, 2H, OH-7, OH-8), 4.90–4.79 (br, 1H, OH-9), 4.34 (dd, 1H, \textit{J} = 5.7, 7.9 Hz, H-9), 3.80 (t, 1H, \textit{J} = 8.2 Hz, H-8). ¹H NMR (500 MHz, CD₃OD): δ 8.49 (s, 1H, Ar-H), 8.46 (d, 2H, \textit{J} = 9.3 Hz, Ar-H), 8.27 (d, 1H, \textit{J} = 7.8 Hz, Ar-H), 8.24 (d, 2H, \textit{J} = 8.6 Hz, Ar-H), 8.14–8.10 (m, 2H, Ar-H), 8.04 (t, 1H, \textit{J} = 7.6 Hz, Ar-H), 5.44 (d, 1H, \textit{J} = 5.6 Hz, H-10), 4.98 (d, 1H, \textit{J} = 8.7 Hz, H-7), 4.23 (dd, 1H, \textit{J} = 5.7, 8.5 Hz, H-9), 3.68 (t, 1H, \textit{J} = 8.6 Hz, H-8).

(±)-10β-amino-7β,8α,9β-trihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene ((±)-38)\textsuperscript{96}

\[ \text{HO} \hspace{1cm} \text{HO} \hspace{1cm} \text{HO} \hspace{1cm} \text{HO} \hspace{1cm} \text{HO} \hspace{1cm} \text{HO} \]

(±)-38

\[ \text{Pd-CaCO}_3 \hspace{1cm} \text{EtOH, r.t.} \hspace{1cm} 2 \text{ h, 93%} \]

(±)-39

In a clean, dry, 100 mL two-neck round bottom flask, equipped with a stir bar, was placed azido triol (±)-38 (115 mg, 0.333 mmol, 1 equiv.) in EtOH (25 mL). To the mixture was added
Lindlar catalyst (575 mg). One neck of the flask was stoppered with a rubber septum and to the other neck, a balloon filled with H₂ gas was attached via a gas inlet adapter with a Teflon stopcock. The mixture was stirred and the entire unit was evacuated via a needle inserted through the rubber septum and connected to a vacuum line. The flask was then filled with H₂ gas from the balloon, and this evacuation-refill process was repeated three times. Finally, the reaction was allowed to proceed for 2 h at room temperature under the H₂ gas-filled balloon. Then the reaction mixture was filtered through Celite and washed with EtOH (50 mL). The solid material obtained by concentrating the filtrate under reduced pressure was suspended in Et₂O, sonicated, and filtered to obtain compound (±)-39 (98.6 mg, 93% yield) as a light-brown solid. 

R_f (10% MeOH in CH₂Cl₂): 0.05. ¹H NMR (500 MHz, CD₃OD): δ 8.47 (d, 1H, J = 9.4 Hz, Ar-H), 8.44 (s, 1H, Ar-H), 8.27–8.22 (m, 3H, Ar-H), 8.13–8.11 (m, 2H, Ar-H), 8.04 (t, 1H, J = 7.6 Hz, Ar-H), 5.09–5.03 (m, 2H, H-7, H-10), 4.19 (dd, 1H, J = 4.4, 7.2 Hz, H-9), 3.92 (t, 1H, J = 7.0 Hz, H-8). ¹³C NMR (125 MHz, CD₃OD): δ 137.0, 132.8, 132.6, 132.0, 130.3, 129.6, 128.9, 128.5, 127.3, 126.7, 126.5, 125.9 (2s), 125.8, 123.8, 75.6, 75.4, 73.5, 54.7. HRMS (ESI/TOF) m/z calculated for C₂₀H₁₈NO₃ [M + H]⁺: 320.1281, found 320.1282.

**Hydrochloride salt of (±)-10β-amino-7β,8α,9β-trihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene (±)-62**

*From (±)-10β-Azido-7β,8α,9β-trihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene (±)-38⁹²*
In a clean, dry, 25 mL two-neck round bottom flask, equipped with a stir bar, was placed azidotriol (±)-38 (50.0 mg, 0.145 mmol, 1 equiv.) in EtOH (10.8 mL). To the solution were added Lindlar catalyst (250 mg) and 6 M aq. HCl (48 µL, 0.290 mmol, 2 equiv.). One neck of the flask was stoppered with a rubber septum and to the other neck a balloon filled with H₂ gas was attached via a gas inlet adapter with a Teflon stopcock. The mixture was stirred and the entire unit was evacuated via a needle inserted through the rubber septum and connected to a vacuum line. The flask was then filled with H₂ gas from the balloon, and this evacuation-refill process was repeated three times. Finally, the reaction was allowed to proceed for 2 h at room temperature under the H₂ gas-filled balloon. Then the reaction mixture was filtered through Celite, washed with EtOH (20 mL), and the filtrate was concentrated under reduced pressure. The resulting solid material was suspended in Et₂O, sonicated and filtered. The product was then washed with EtOAc, followed by 1:1 Et₂O-MeOH to obtain compound (±)-62 (46.2 mg, quantitative yield) as a light-brown solid. Rₓ (SiO₂/MeOH) = 0.10. ¹H NMR (500 MHz, CD₃OD): δ 8.49 (s, 1H, Ar-H), 8.40–8.29 (m, 4H, Ar-H), 8.22–8.14 (m, 2H, Ar-H), 8.11 (t, 1H, J = 7.6 Hz, Ar-H), 5.52 (d, 1H, J = 4.0 Hz, H-10), 5.08 (d, 1H, J = 5.2 Hz, H-7), 4.47 (dd, 1H, J = 4.0, 6.3 Hz, H-9), 4.17 (t, 1H, J = 5.8 Hz, H-8). ¹³C NMR (125 MHz, CD₃OD): δ 137.5, 133.7, 132.6, 131.9, 130.5, 130.4, 129.7, 128.3, 127.7, 127.2, 127.1, 126.8, 125.9, 125.6, 123.2, 122.8, 74.1, 73.1, 72.6, 53.4. HRMS (ESI/TOF) m/z calculated for C₂₀H₁₉ClNO₃ [M + H]⁺: 356.1048, found 356.1048.

From (±)-10β-amino-7β,8α,9β-trihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene ((±)-39)
In a clean, dry, 8 mL reaction vial equipped with a stir bar, was placed BaP amino triol (±)-39 (40.0 mg, 0.125 mmol, 1 equiv.) in EtOH (1 mL). To the stirring solution 6 M aq. HCl (42 µL, 0.250 mmol, 2 equiv.) was added. The vial was sealed, the mixture was stirred at room temperature for 2 h. The mixture was evaporated under reduced pressure. The resulting brown solid was suspended in Et₂O, sonicated, and filtered. The product was washed with EtOAc, followed by 1:1 Et₂O-MeOH to obtain compound (±)-62 (44.5 mg, quantitative yield) as a light-brown solid.

(±)-10β-Acetylamino-7β,8α,9β-trisacetoxy-7,8,9,10-tetrahydrobenzo[α]pyrene ((±)-49)

From (±)-10β-azido-7β,8α,9β-trihydroxy-7,8,9,10-tetrahydrobenzo[α]pyrene ((±)-38)

In a clean, dry, 25 mL two-neck round bottom flask, equipped with a stir bar, was placed azidotriol (±)-32 (30.0 mg, 0.0869 mmol, 1 equiv.) in EtOH (6.5 mL). To the mixture Lindlar catalyst (150 mg) was added. One neck of the flask was stoppered with a rubber septum and to the other neck a balloon filled with H₂ gas was attached via a gas inlet adapter with a Teflon stopcock. The mixture was stirred and the entire unit was evacuated via a needle inserted through the rubber septum and connected to a vacuum line. The flask was then filled with H₂ gas from the balloon, and this evacuation-refill process was repeated three times. Finally, the reaction was allowed to proceed for 2 h at room temperature under the H₂ gas-filled balloon. Then the reaction mixture was filtered through Celite and washed with EtOH (20 mL). The pale-yellow-colored filtrate was concentrated under reduced pressure and dried under high vacuum.
for 2 h. The resulting crude material was dissolved in dry DMF (1 mL), Ac₂O (164 µL, 1.737 mmol, 20 equiv.), pyridine (140 µL, 1.737 mmol, 1 equiv.), and DMAP (a few crystals) were added. The reaction mixture was stirred at room temperature for 18 h. The mixture was then diluted with EtOAc (30 mL) and washed with 1 N aq. HCl (10 mL), deionized water (10 mL), saturated aq. NaHCO₃ (2 x 10 mL), deionized water (10 mL), and brine (10 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure, to give an off-white solid. This material was chromatographed on a silica gel column by sequential elution with 20% EtOAc in CH₂Cl₂ and 50% EtOAc in CH₂Cl₂ to give product (±)-45 (30.0 mg, 71% yield) as an off-white solid. ¹H NMR (500 MHz, CDCl₃): δ 8.27–8.19 (m, 4H, Ar-H), 8.11–8.00 (m, 4H, Ar-H), 6.61 (d, 1H, J = 4.5 Hz, H-7), 6.34 (dd, 1H, J = 3.2, 9.0 Hz, H-10), 5.87 (d, 1H, J = 9.0 Hz, NH), 5.63 (t, 1H, J = 5.2 Hz, H-8), 5.52 (dd, 1H, J = 3.5, 5.5 Hz, H-9), 2.21 (s, 3H, Me), 2.11 (s, 3H, Me), 2.06 (s, 3H, Me), 2.00 (s, 3H, NHCOMe).

From the salt of (±)-10β-amino-7β,8α,9β-trihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene (±)-62

To a solution of BaP aminotriol hydrochloride (±)-62 (40.0 mg, 0.112 mmol, 1 equiv.) in dry DMF (1 mL), Ac₂O (212 µL, 2.25 mmol, 20 equiv.), pyridine (227 µL, 2.81 mmol, 25 equiv.), and DMAP (a few crystals) were added. The reaction mixture was stirred at room temperature for 14 h. The reaction mixture was then diluted with EtOAc (30 mL) and washed with 1 N aq. HCl (2 x 10 mL), saturated aq. NaHCO₃ (2 x 10 mL), deionized water (10 mL), and brine (10 mL).
mL). The organic layer was dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure to give an off-white solid. This material was chromatographed on a silica gel column by sequential elution with 20% EtOAc in CH₂Cl₂ and 50% EtOAc in CH₂Cl₂ to give product (±)-49 (47.1 mg, 86% yield) as an off-white solid.

\[ N^6-[10-(7,8,9-Triacetoxy-7,8,9,10-tetrahydrobenzo[a]pyrenyl)-3',5'-bis-O-(t-butyldimethylsilyl)2'-deoxyadenosine (63a,b) \]

To a solution of fluoro nucleoside 40 (50.4 mg, 0.104 mmol, 1 equiv.) in DMSO (0.54 mL) were added BaP aminetriol (±)-39 (40.0 mg, 0.125 mmol, 1.2 equiv.), iPr₂NEt (182 µL, 1.04 mmol, 10 equiv.) and (Me₃Si)₂O (0.89 mL, 4.18 mmol, 40 equiv.). The biphasic mixture was flushed with N₂ gas, sealed, and vigorously stirred at 85 °C for 5 h. The mixture was cooled to room temperature, diluted with EtOAc (30 mL), and washed with deionized water (15 mL). The aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layer was dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The crude mixture was carefully chromatographed on a silica gel column using CH₂Cl₂ followed by 3% MeOH-CH₂Cl₂. To the resulting off-white product were added Ac₂O (441 µL, 4.67 mmol, 45 equiv.), pyridine (441 µL, 5.46 mmol, 52.5 equiv.), and DMAP (a few crystals). The mixture was stirred at room temperature, overnight. The mixture was diluted with EtOAc (30 mL) and washed with deionized water (15 mL). The aqueous layer was extracted with EtOAc (2 x 20 mL). The
combined organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The two diastereomeric products 55a and 55b were carefully separated by chromatography on a preparative TLC plate (SiO$_2$, 500 µ, 20 x 20 cm), eluted with 10% EtOAc in CH$_2$Cl$_2$. This separation yielded 9.7 mg of the less-polar adduct 55a as a pale-yellow solid, 10.0 mg of the more-polar adduct 55b as an off-white solid and 20.0 mg of the mixture of diastereomers (combined yield of 42% over the two steps).

The faster-eluting diastereomer was the 10S isomer (55a) as determined by the presence of a positive band at 281 nm in its CD spectrum. $R_f$ (5% EtOAc in CH$_2$Cl$_2$): 0.33. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.63 (br s, 1H, Ar-H), 8.30 (d, $J$ = 9.3 Hz, 1H, Ar-H), 8.22–8.15 (m, 3H, Ar-H), 8.12–7.99 (m, 5H, Ar-H), 6.78–6.76 (br m, 1H, H-10), 6.67 (d, $J$ = 3.4 Hz, 1H, H-7), 6.46 (t, $J$ = 6.2 Hz, 1H, H-1’), 6.19–6.17 (br m, 1H, –NH), 5.70 (t, $J$ = 3.7 Hz, 1H, H-8), 5.64 (dd, $J$ = 2.3, 4.1 Hz, 1H, H-9), 4.60 (app q, $J_{app}$ ~ 4.2 Hz, 1H, H-3’), 4.01 (app q, $J_{app}$ ~ 3.4 Hz, 1H, H-4’), 3.87 (dd, $J$ = 3.9, 11.3 Hz, 1H, H-5’), 3.76 (dd, $J$ = 2.6, 11.2 Hz, 1H, H-5’’), 2.65 (dt, $J$ = 6.0, 12.3 Hz, 1H, H-2’), 2.46 (dt, $J$ = 2.6, 12.5 Hz, 1H, H-2’’), 2.18, 2.11, and 2.05 (3s, 9H, OCOCH$_3$), 0.92 and 0.86 (2s, 18H, $t$-Bu), 0.11, 0.10, 0.05, and 0.03 (4s, 12H, SiMe). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 170.2, 169.8, 169.1, 163.4, 153.4, 153.0, 139.0, 132.1, 131.4, 131.0, 130.4, 129.7, 129.2, 128.7, 127.3, 126.7, 126.3, 126.1, 126.0, 125.8, 125.5, 124.5, 123.3, 120.5, 88.0, 84.7, 71.8, 69.2, 69.1, 68.5, 62.8, 41.6, 29.9, 26.1, 26.0, 21.4, 21.2, 21.1, 18.6, 18.2, −4.4, −4.6, −5.2, −5.3. HRMS (ESI/TOF) $m/z$ calculated for C$_{48}$H$_{62}$N$_5$O$_9$Si$_2$ [M + H]$^+$: 908.4081, found 908.4076.

The slower-eluting diastereomer was the 10R isomer (55b) as determined by the presence of a negative band at 281 nm in its CD spectrum. $R_f$ (5% EtOAc in CH$_2$Cl$_2$): 0.16. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.62 (br s, 1H, Ar-H), 8.32 (d, $J$ = 9.3 Hz, 1H, Ar-H), 8.22–8.15 (m, 3H, Ar-H),
8.12–7.99 (m, 5H, Ar-H), 6.80–6.76 (br m, 1H, H-10), 6.67 (d, \( J = 2.9 \) Hz, 1H, H-7), 6.46 (t, \( J = 6.0 \) Hz, 1H, H-1’), 6.21–6.13 (br m, 1H, –NH), 5.69 (t, \( J = 3.1 \) Hz, 1H, H-8), 5.67–5.63 (m, 1H, H-9), 4.62 (app q, \( J_{app} \sim 4.5 \) Hz, 1H, H-3’), 4.00 (app q, \( J_{app} \sim 3.4 \) Hz, 1H, H-4’), 3.89 (dd, \( J = 4.1, 11.2 \) Hz, 1H, H-5’), 3.77 (dd, \( J = 2.8, 11.2 \) Hz, 1H, H-5’’), 2.64 (dt, \( J = 6.3, 12.9 \) Hz, 1H, H-2’), 2.44 (dt, \( J = 5.8, 12.1 \) Hz, 1H, H-2’’), 2.18, 2.10, and, 2.04 (3s, 9H, OCOCH\(_3\)), 0.91 and 0.87 (2s, 18H, t-Bu), 0.10 (s, 6H, SiMe), 0.05 and 0.03 (2s, 6H, SiMe). \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \( \delta \) 170.2, 169.8, 169.1, 163.4, 153.4, 153.0, 139.1, 132.1, 131.4, 131.0, 130.4, 129.7, 129.2, 128.7, 127.4, 126.7, 126.4, 126.0 (2c), 125.8, 125.5, 124.5, 123.3, 120.4, 88.0, 84.5, 71.9, 69.1 (2c), 68.5, 62.8, 41.5, 29.9, 26.1, 26.0, 21.4, 21.2, 21.1, 18.6, 18.2, –4.4, –4.6, –5.2, –5.3. HRMS (ESI/TOF) \( m/z \) calculated for C\(_{48}\)H\(_{61}\)N\(_5\)O\(_9\)Si\(_2\)Na [M + Na]\(^+\): 930.3900, found 930.3904.

\( O^6\)-(Benzotriazol-1-yl)-3’,5’-di-O-(t-butyldimethylsilyl)-2’-deoxyguanosine (57)\(^{126}\)

![Chemical Structure](image)

To a suspension of 3’,5’-di-O-(t-butyldimethylsilyl)-2’-deoxyguanosine (56, 2.5 g, 5.04 mmol, 1 equiv.) in dry CH\(_3\)CN (20 mL), BOP (3.34 g, 7.56 mmol, 1.5 equiv.) and DBU (1.39 mL, 10.09 mmol, 2 equiv.) were added. The mixture was stirred at room temperature for 1 h. The mixture was then diluted with EtOAc (50 mL) and washed with deionized water (2 x 30 mL), and brine (10 mL). The organic layer was dried over anhydrous Na\(_2\)SO\(_4\), filtered, and concentrated under reduced pressure. The crude product was chromatographed on a silica gel
column by eluting with 10% EtOAc in hexanes, followed by 25% EtOAc in hexanes, to yield the product 57 (2.71 g, 88% yield) as a white solid. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.17–8.09 (m, 2H, H-8, Ar-H), 7.58–7.42 (m, 3H, Ar-H), 6.35 (t, 1H, $J = 6.3$ Hz, H-1’), 4.74 (s, 2H, NH$_2$), 4.61 (dt, 1H, $J = 3.2$, 5.9 Hz, H-3’), 4.00 (app q, 1H, $J_{app}$ ~ 3.2 Hz, H-4’), 3.85 (dd, 1H, $J = 3.8$, 11.3 Hz, H-5’), 3.77 (dd, 1H, $J = 2.9$, 11.3 Hz, H-5’), 2.58 (dt, 1H, $J = 6.3$, 12.9 Hz, H-2’), 2.45–2.34 (m, 1H, H-2’), 0.92 (s, 18H, $t$-Bu), 0.11 and 0.10 (2s, 12H, SiMe).

$O^6$-Benzyl-3’,5’-di-O-($t$-butyldimethylsilyl)-2’-deoxyguanosine (58)$^{128}$

![Chemical structure image](image_url)

To a suspension of $O^6$-(benzotriazol-1-yl)-2’-deoxyguanosine derivative 57 (742.0 mg, 1.212 mmol, 1 equiv.) in DME (7.4 mL), BnOH (314.0 µL, 3.03 mmol, 2.5 equiv.) and Cs$_2$CO$_3$ (987.0 mg, 3.030 mmol, 2.5 equiv.) were added, and the mixture was stirred at room temperature. After 24 h, the reaction was incomplete, hence, additional BnOH (157 µL, 1.515 mmol, 1.25 equiv.) and Cs$_2$CO$_3$ (494.0 mg, 1.515 mmol, 1.25 equiv.) were added to the mixture, and was stirred at room temperature for 12 h. The mixture was then diluted with EtOAc (30 mL), washed with deionized water (2 x 20 mL), and brine (10 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The resulting material was purified over a long silica gel column by sequential elution initially with 5% EtOAc in hexanes, 10% EtOAc in hexanes, and 20% EtOAc in hexanes, gave the product 58 (650 mg, 92% yield) as a
white solid. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.91 (s, 1H, H-8), 7.50 (d, 2H, $J = 7.3$ Hz, Ar-H), 7.35 (t, 2H, $J = 7.3$ Hz, Ar-H), 7.30 (t, 1H, $J = 7.4$ Hz, Ar-H), 6.32 (t, 1H, $J = 6.5$ Hz, H-1’), 5.60–5.52 (m, 2H, OCH$_2$), 4.82 (s, 2H, NH$_2$), 4.58 (dt, 1H, $J = 3.1$, 5.8 Hz, H-3’), 3.97 (app q, 1H, $J_{app} \sim 3.5$ Hz, H-4’), 3.81 (dd, 1H, $J = 4.3$, 11.2 Hz, H-5’), 3.75 (dd, 1H, $J = 3.2$, 11.1 Hz, H-5’), 2.57 (dt, 1H, $J = 6.5$, 13.1 Hz, H-2’), 2.34 (ddd, 1H, $J = 3.7$, 6.0, 13.0 Hz, H-2’), 0.91 and 0.90 (2s, 18H, t-Bu), 0.10 (s, 6H, SiMe), 0.07 (s, 6H, SiMe).

$O^6$-Benzyl-2’-deoxyguanosine (59)

To a solution of $O^6$-benzyl-2’-deoxyguanosine derivative 58 (600 mg, 1.024 mmol, 1 equiv.) in MeOH (10.5 mL), KF (238.0 mg, 4.096 mmol, 4 equiv.) was added, and the mixture was stirred at 80 °C for 12 h. After completion of the reaction, the mixture was concentrated under reduced pressure, and purified over a short silica gel column by sequential elution with 2% MeOH in EtOAc and 5% MeOH in EtOAc, to yield product 59 (0.35 g, 95% yield) as a pale-yellow solid. $^1$H NMR (500 MHz, CD$_3$OD): $\delta$ 8.04 (s, 1H, H-8), 7.51 (d, 2H, $J = 7.4$ Hz, Ar-H), 7.37 (t, 2H, $J = 7.4$ Hz, Ar-H), 7.31 (t, 1H, $J = 7.2$ Hz, Ar-H), 6.32 (dd, 1H, $J = 6.2$, 8.0 Hz, H-1’), 5.55 (s, 2H, OCH$_2$), 4.56 (dt, 1H, $J = 2.5$, 5.4 Hz, H-3’), 4.04 (app q, 1H, $J_{app} \sim 2.8$ Hz, H-4’), 3.84 (dd, 1H, $J = 3.0$, 12.2 Hz, H-5’), 3.74 (dd, 1H, $J = 3.4$, 12.2 Hz, H-5’), 2.79 (ddd, 1H, $J = 5.8$, 7.9, 13.6 Hz, H-2’), 2.34 (ddd, 1H, $J = 2.5$, 6.0, 13.4 Hz, H-2’).

$O^6$-Benzyl-2-fluoro-2’-deoxyinosine (60)$^{71}$
To a 60% HF in pyridine solution (13.47 mL) at −60 °C in a 25 mL polypropylene vial, was added \( \text{O}^6\)-benzyl-2’-deoxyguanosine (59, 432.0 mg, 1.209 mmol) and the mixture was stirred until a clear solution was observed. Then \( t\)-BuONO (971.0 µL, 8.160 mmol, 6.75 equiv.) precooled to −50 °C was slowly added to the reaction mixture and the mixture was stirred at −60 °C for 25 min. The dark-yellow reaction mixture was then poured into cold water (75 mL), and was extracted with CHCl₃ (5 x 75 mL). The organic layer was passed through a column of solid NaHCO₃ and washed with saturated aq. NaHCO₃ (2 x 20 mL), dried over a mixture of anhydrous Na₂SO₄ and solid NaHCO₃, filtered, and concentrated under reduced pressure. The resulting material was washed with 1:1 CH₂Cl₂-hexanes, to obtain product 60 (0.37 g, 85% yield) as a pale-yellow solid. \(^1\)H NMR (500 MHz, CDCl₃): \( \delta \) 8.04 (s, 1H, H-8), 7.51 (d, 2H, \( J = 7.2 \) Hz, Ar-H), 7.41–7.29 (m, 3H, Ar-H), 6.32 (dd, 1H, \( J = 5.9, 8.3 \) Hz, H-1’), 5.63 (s, 2H, OCH₂), 4.75 (dt, 1H, \( J = 2.4, 5.1 \) Hz, H-3’), 4.2 (app q, 1H, \( J_{\text{app}} \sim 2.0 \) Hz, H-4’), 3.94 (dd, 1H, \( J = 2.1, 12.7 \) Hz, H-5’), 3.78 (dd, 1H, \( J = 2.2, 12.7 \) Hz, H-5’), 2.86 (ddd, 1H, \( J = 5.3, 8.5, 13.6 \) Hz, H-2’), 2.36 (ddd, 1H, \( J = 1.9, 5.7, 13.4 \) Hz, H-2’).

\( \text{O}^6\)-Benzyl-3’,5’-di-O-(\( t\)-butyldimethylsilyl)-2-fluoro-2’-deoxyinosine (61) \(^71\)
To a solution of 60 (100.0 mg, 0.277 mmol, 1 equiv.) in dry DME (1.8 mL), pyridine (112.0 μL, 1.387 mmol, 5 equiv.) was added and the stirring mixture was cooled down to 0 °C. TBDMSOTf (160.0 μL, 0.694 mmol, 2.5 equiv.) was added to the solution and the mixture was stirred at 0 °C for 45 min. The resulting yellow solution was diluted with EtOAc (20 mL), washed with brine (2 x 10 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The crude material was chromatographed on a silica gel column by sequential elution with hexanes and CH₂Cl₂ to yield product 61 (154.6 mg, 95% yield) as a pale-yellow oil.

^H NMR (500 MHz, CDCl₃): δ 8.23 (s, 1H, H-8), 7.54 (d, 2H, J = 7.6 Hz, Ar-H), 7.40–7.31 (m, 3H, Ar-H), 6.39 (t, 1H, J = 6.3 Hz, H-1’), 5.69–5.60 (m, 2H, OCH₂), 4.61–4.59 (m, 1H, H-3’), 4.00 (app q, 1H, J_app ~ 3.4 Hz, H-4’), 3.88 (dd, 1H, J = 4.0, 11.3 Hz, H-5’), 3.77 (dd, 1H, J = 2.9, 11.2 Hz, H-5’), 2.58 (dt, 1H, J = 6.4, 12.9 Hz, H-2’), 2.42 (ddd, 1H, J = 4.1, 6.0, 13.2 Hz, H-2’), 0.91 and 0.90 (2s, 18H, t-Bu), 0.10 and 0.08 (2s, 12H, SiMe).

3’,5’-Di-O-(t-butyldimethylsilyl)-2-fluoro-2’-deoxyinosine (45)^71

To a suspension of 5% Pd/C (30.0 mg) in 1:1 THF-MeOH (5.6 mL) in an 8 mL reaction vial, 61 (135.0 mg, 0.279 mmol, 1 equiv.) was added. The vial was placed into a two-necked round bottom flask. To one neck was attached a hydrogen balloon via a gas inlet adapter with a Teflon stopcock and the other neck was stoppered with a rubber septum. The flask was connected to a vacuum line via a needle inserted through the septum. The flask was evacuated and filled with H₂ gas via the balloon. The evacuation-refill process was repeated three times. Finally, the
reaction was allowed to proceed for 1 h at room temperature under a balloon filled with H₂ gas. The mixture was filtered through Celite and the residue was washed with THF (50 mL). The filtrate was concentrated under reduced pressure and dried under high vacuum to give the compound 45 (109.6 mg, 96%) as a white solid. \(^1\)H NMR (500 MHz, CD\(_3\)OD): \(\delta\) 8.16 (s, 1H, H-8), 6.28 (t, 1H, \(J = 6.3\) Hz, H-1’), 4.73 (app q, 1H, \(J_{app} \sim 4.6\) Hz, H-3’), 3.96 (app q, 1H, \(J_{app} \sim 3.8\) Hz, H-4’), 3.88 (dd, 1H, \(J = 4.6, 11.3\) Hz, H-5’), 3.78 (dd, 1H, \(J = 3.5, 11.2\) Hz, H-5’), 2.77 (dt, 1H, \(J = 6.3, 13.0\) Hz, H-2’), 2.43 (dd, 1H, \(J = 4.7, 6.4, 13.2\) Hz, H-2’), 0.95 and 0.89 (2s, 18H, t-Bu), 0.15 (s, 6H, SiMe), 0.08 and 0.05 (2s, 6H, SiMe).

\(N^2\)-[10-(7,8,9-Trisacetoxy-7,8,9,10-tetrahydrobenzo[a]pyrenyl)-3’,5’-bis-O-(t-butyldimethylsilyle)2’-deoxyguanosine (63a,b)

The aminotriol hydrochloride (±)-62 (30.0 mg, 0.0843 mmol, 1 equiv.) was suspended in DIPEA (147.0 \(\mu\)L, 0.843 mmol, 10 equiv.) and the mixture was stirred for 15 min. After this dry DMSO (0.42 mL), (Me\(_3\)Si)\(_2\)O (723.0 \(\mu\)L, 3.384 mmol, 40 equiv.), and 2-fluoro-2’-deoxyinosine derivative 45 (63.0 mg, 0.126 mmol, 1.5 equiv.) were added. The biphasic mixture was flushed with \(N_2\) gas, capped, and vigorously stirred at 80 \(^\circ\)C for 6 h. The mixture was cooled to room
temperature, diluted with EtOAc (30 mL) and washed with deionized water (2 x 15 mL), brine (15 mL), dried over anhydrous Na$_2$SO$_4$, filtered, and evaporated under reduced pressure. To the crude material (81.3 mg), pyridine (954.0 µL, 11.80 mmol, 140 equiv.), DMAP (a few crystals), and Ac$_2$O (956.0 µL, 10.12 mmol, 120 equiv.) were added and the mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with EtOAc (40 mL) and washed with deionized water (20 mL). The aqueous layer was back extracted with EtOAc (2 x 30 mL). The combined organic layers was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The crude material was chromatographed on a short silica gel column by sequential elution with 50% EtOAc in hexanes, EtOAc, and 1% MeOH in EtOAc to yield peracylated amino triol derivative (±)-49 (10.8 mg) as a dark-brown solid and the mixture of two diastereomers 63a and 63b (31.2 mg) also as a dark-brown solid. The two diastereomers 63a and 63b were carefully separated by preparative TLC (SiO$_2$, 1 mm, 20 cm × 20 cm) by elution with 1:19:80 MeOH-EtOAc-CH$_2$Cl$_2$ to yield 9.5 mg of the less-polar adduct 63a, and 7.9 mg of the more-polar adduct 63b (combined yield of 22% over the two steps and 30% based on the recovered (±)-49).

The faster-eluting diastereomer was the 10S isomer (63a) as determined by the presence of a positive band at 250.6 nm in its CD spectrum. $R_f$(5% MeOH in EtOAc): 0.44. $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 10.68 (br s, 1H, guanine ring NH), 8.36 (d, $J = 7.5$ Hz, 1H, Ar-H), 8.33–8.27 (m, 2H, Ar-H), 8.26–8.20 (m, 4H, Ar-H), 8.12 (t, $J = 7.6$ Hz, 1H, Ar-H), 7.98 (s, 1H, Ar-H), 7.21 (br d, $J = 7.7$ Hz, 1H, exocyclic NH), 6.68 (d, $J = 7.0$ Hz, 1H, H-7), 6.26 (t, $J = 6.4$ Hz, 1H, H-1’), 6.20 (dd, $J = 4.5$, 7.6 Hz, 1H, H-10), 5.86 (dd, $J = 4.5$, 7.2 Hz, 1H, H-9), 5.44 (t, $J = 7.2$ Hz, 1H, H-8), 4.50 (app q, $J_{app} \sim 4.7$ Hz, 1H, H-3’), 3.89 (app q, $J_{app} \sim 4.2$ Hz, 1H, H-4’), 3.79 (dd, $J = 5.0$, 11.2 Hz, 1H, H-5’), 3.74 (dd, $J = 4.5$, 11.2 Hz, 1H, H-5’’), 2.59–2.56 (m, 1H, H-2’), 2.32–
2.28 (m, 4H, H-2′′, OCOCH₃), 2.08, and 1.99 (2s, 6H, OCOCH₃), 0.88 and 0.85 (2s, 18H, r-Bu), 0.07 (s, 3H, SiMe), 0.05 (s, 6H, SiMe), 0.04 (s, 3H, SiMe). ¹³C NMR (125 MHz, DMSO-d₆): δ 170.3, 169.3 (2c), 156.7, 151.3, 149.9, 135.5, 131.3, 130.9, 130.7, 130.1, 128.8 (2c), 128.3, 127.2, 126.9, 126.7, 126.1, 125.9, 124.0, 123.4, 122.9, 122.8, 117.2, 86.9, 82.4, 76.6, 71.7, 71.1, 70.1, 69.8, 62.7, 50.8, 25.8, 25.7, 20.9, 20.6, 20.5, 18.0, 17.8, –4.7, –5.0, –5.4. HRMS (ESI/TOF) m/z calculated for C₄₈H₆₂N₅O₁₀Si₂ [M + H]⁺: 924.4030, found 924.4039.

The slower-eluting diastereomer was the 10R isomer (63b) as determined by the presence of a negative band at 250.8 nm in its CD spectrum. Rf (5% MeOH in EtOAc): 0.36. ¹H NMR (500 MHz, DMSO-d₆): δ 10.60 (br s, 1H, guanine ring NH), 8.35 (d, J = 7.6 Hz, 1H, Ar-H), 8.33–8.28 (m, 2H, Ar-H), 8.26–8.22 (m, 3H, Ar-H), 8.16–8.01 (m, 2H, Ar-H), 7.97 (s, 1H, Ar-H), 7.14 (br d, J = 8.0 Hz, 1H, exocyclic NH), 6.63 (d, J = 6.3 Hz, 1H, H-7), 6.24 (dd, J = 6.1, 7.7 Hz, 1H, H-1′), 6.19 (dd, J = 3.6, 8.0 Hz, 1H, H-10), 5.81 (dd, J = 4.0, 6.5 Hz, 1H, H-9), 5.43 (t, J = 6.5 Hz, 1H, H-1), 4.49 (dt, J = 2.5, 5.0 Hz, 1H, H-3′), 3.86–3.81 (m, 1H, H-4′), 3.75 (dd, J = 7.4, 10.5 Hz, 1H, H-8), 3.71 (dd, J = 4.5, 10.8 Hz, 1H, H-5′′), 3.08 (ddd, J = 5.6, 7.7, 13.1 Hz, 1H, H-2″), 2.28 (ddd, J = 2.7, 5.4, 13.0 Hz, 1H, H-2′″), 2.25, 2.07, and 1.99 (3s, 9H, OCOCH₃), 0.87, and 0.64 (2s, 18H, r-Bu), 0.01, 0.08. –0.23, and –0.31 (4s, 12H, SiMe). HRMS (ESI/TOF) m/z calculated for C₄₈H₆₂N₅O₁₀Si₂ [M + H]⁺: 924.4030, found 924.4039.
[3.5] REFERENCES

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CHAPTER 4

SYNTHESIS OF HIPPADINE
SYNTHESIS OF HIPPADINE

[4.1] INTRODUCTION

Amaryllidaceae alkaloids represent a vast and important group of natural products and plants belonging to this family have been used in medicine for years. These alkaloids are classified into nine main subgroups based on their structural features. Lycorine alkaloids, a main subgroup of the amaryllidaceae alkaloid family, consist of a pyrrolophenanthridine skeleton, and possess interesting pharmacological properties. As examples, hippadine (1) inhibits fertility in male rats reversibly, kalbretorine (3) has shown antitumor activity, and anhydrolycorinium chloride (5) is active against P-338 murine leukemic cell line, ungeremine (4) shows antitumor as well as antileukemic properties. Oxoassoanine (6) is the synthetic precursor to pratosine (2), anhydrolycorinone (7) is the synthetic precursor for hippadine (1), and anhydrolycorinium chloride (5), respectively (Figure 1). Because of the biological properties and common structural features exhibited by these compounds, we wanted to develop a modular approach towards the synthesis of hippadine (1) via anhydrolycorinone (7).

![Molecule Images]

1. Hippadine (\(R_1 = -\text{CH}_2\), \(R_2 = \text{H}\))
2. Pratosine (\(R_1 = -\text{CH}_3\), \(R_2 = \text{H}\))
3. Kalbretorine (\(R_1 = -\text{CH}_2\), \(R_2 = \text{OH}\))
4. Ungeremine (\(R_1 = \text{OH}\))
5. Anhydrolycorinium chloride (\(R_1 = \text{H}\))
6. Oxoassoanine (\(R_1 = \text{CH}_3\))
7. Anhydrolycorinone (\(R_1 = -\text{CH}_2\))

Figure 1. Some important molecules belonging to the lycorine alkaloid family

To date, different research groups have developed various strategies for the synthesis of hippadine (1) and anhydrolycorinone (7). Some of these strategies include oxazoline mediated biarylation reaction, cycloaddition reaction, radical cyclization, heteroaryl alkyne
cyclization,\textsuperscript{11} metal-mediated coupling reaction,\textsuperscript{12-15} and C–H functionalization\textsuperscript{16-18} as one of their key steps. More detailed descriptions of some of the important strategies are discussed below.

\textbf{[4.1.1] Oxazoline-Mediated Synthesis}\textsuperscript{5}

Meyers \textit{et al.} developed a synthetic route where a Grignard reagent of 7-bromo indole derivative 8 was reacted with oxazoline derivative 9 to give the biaryl compound 10 (Scheme 1). In this reaction, the oxazoline group of 9 serves both as a protecting group for the carboxylic acid and directs the Grignard reagent to couple at the ortho position to oxazoline ring of compound 9. Biaryl compound 10 was then subjected to hydrolysis and reduction, leading to lactamization and anhydrolycorinone (7). Subsequent oxidation of 7 with DDQ resulted in the formation of hippadine (1) in an overall yield of 32.7% over 4 steps (Scheme 1).

\textit{Scheme 1. Hippadine synthesis mediated by aryl oxazoline 9}
[4.1.2] Via Intramolecular Diels-Alder Reaction

Castedo et al. have developed a method to construct the pyrrolophenanthridine ring system by an intramolecular Diels-Alder reaction of appropriately substituted pyrones with alkynes.\(^7\) On the basis of this strategy, they synthesized anhydrolycorinone (7) from the pyrone derivative 14 (Scheme 2).\(^6\) Reaction of the homophthalic anhydride derivative 11 with pent-4-yn-1-amine gave the corresponding imide 12, which upon heating with malonyl derivative 13, resulted in the formation of 14. Heating a solution of 14 in nitrobenzene at 210 °C, promoted the intramolecular cycloaddition reaction, with the loss of CO\(_2\) via retro Diels-Alder reaction, gave 15. Subsequent hydrolysis followed by decarboxylation of 15 resulted in the formation of anhydrolycorinone (7), in an overall yield of 31.9% over four steps.

**Scheme 2. Synthesis of anhydrolycorinone (7) via intramolecular an cycloaddition reaction of alkynyl pyrone derivative 14**

A similar strategy was developed by Padwa et al.,\(^8\) where they initially synthesized substituted indolines and tetrahydroquinolines from the intramolecular cycloaddition reaction of 2-amido furans. They then extended this to the synthesis of anhydrolycorinone (7, Scheme 3).\(^8\)
The key compound, amido furan derivative 18 was synthesized in two steps. The amidation of 6-iodopiperonylic acid with the amino oxazole derivative 16, gave the amide 17. The resultant amide 17 along with trimethylsilylacetylene, under Sonogoshira cross-coupling conditions, underwent a subsequent intramolecular cycloaddition reaction to give the desired amido furan derivative 18. Heating 18 at 320 °C in 1,2,4-trichlorobenzene caused the intramolecular cycloaddition reaction to give anhydrolycorinone (7), in an overall yield of 11.3 % over four steps (Scheme 3).  

**Scheme 3. Synthesis of anhydrolycorinone (7) via an intramolecular cycloaddition reaction of amido furan derivative 18**

Boger et al. synthesized hippadine (1) in 7 steps with an overall yield of 21%, by using a sequential intramolecular azadiene cycloaddition reaction (Scheme 4). The synthesis starts with the displacement of one of the thiomethyl groups of tetrazine 19 with but-3-yne-1-amine hydrochloride to give the key intermediate 20. Protection of the free amine in 20 with (Boc)₂O/DMAP, and intramolecular inverse-electron-demand Diels-Alder reaction gave the diazine 21. An amidation reaction with piperonylic acid derivative 23, was carried out after the Boc-deprotection of 21, to give amide 24. The second intramolecular cycloaddition and
subsequent aromatization reaction was achieved by heating the amide 24 at 265 °C, which gave 25. From 25, hippadine (1) was prepared by reduction of the thiomethyl group with Raney Ni, followed oxidation with DDQ.

**Scheme 4.** *Sequential intramolecular cycloaddition reaction of tetrazine 19 leading to the synthesis of hippadine (1)*

[4.1.3] Through Radical Cyclization

The first attempt to synthesize pyrrolophenanthridine based compounds *via* radical cyclization of 1-o-iodobenzoxyindole was made by Carruthers *et al.* but instead they obtained 1,2-fused aryl indole as the major product.\(^{19}\) Later, Tsuge *et al.* successfully synthesized hippadine (1) under the radical cyclization conditions from the benzoyl indole derivative 26,\(^ {10}\) which was prepared from 7-bromoindole and piperonylchloride (Scheme 5). Even though this method complements previous attempts, the regioisomeric product 27 was also formed along with 1 under these radical cyclization conditions.
Scheme 5. Intramolecular radical cyclization of 26 leading to hippadine (1) and regioisomer 27

[4.1.4] Via Iron-Mediated Oxidative Cyclization

Knölker et al. have developed a unique strategy for the synthesis of hippadine (1) by using iron-mediated oxidative-cyclization as the key step (Scheme 6). In this strategy, ester 30 was prepared by treating lithium ester enolate 29 with cyclohexadienyl iron salt 28. Ester 30 was then reduced to aldehyde 31 with DIBAL. Reductive amination of 31 with amine 32 resulted in the formation of alkylamine substituted iron complex 33. Complex 33 upon oxidative cyclization with Cp2FePF6/Na2CO3 yielded the tetrahydroindolo complex 34. Demetalation of 34 led to tetrahydroindole 35, which upon treatment with stoichiometric Pd(PPh3)4 underwent a Heck-type cyclization reaction, concurrent aromatization of the cyclohexadiene ring, and oxidation at the benzylic position, to give anhydrolycorinone (7). Hippadine (1) was synthesized by the oxidation of 7 with DDQ, in an overall yield of 7.8% over 7 steps.

Scheme 6. Synthesis of hippadine (1) via iron-mediated oxidative-cyclization of 17
[4.1.5] Via Copper-Mediated Alkyne Cyclization

Recently, an interesting method was developed by Hiroya et al. for the synthesis of indoles by a copper-catalyzed intramolecular cyclization of 2-alkynylanilines. On the basis of this

Scheme 7. Copper-mediated intramolecular cyclization of 36 en route to hippadine (1)
methodology, they have developed an approach for the synthesis of hippadine (1) (Scheme 7). Biaryl alkyne derivative 36, upon refluxing with a stoichiometric amount of Cu(OAc)$_2$, underwent cyclization to give the indole derivative 37, which upon deprotection followed by formylation led to the formamide 39. Under Bischler-Napieralski reaction conditions, compound 39 gave hippadine (1), in an overall yield of 6.2% over four steps.

[4.1.6] Using Palladium-Mediated Coupling Reactions

It has been reported in the literature that during the synthesis of aryltrialkyl stannanes, often homocoupled products were obtained as undesired compounds when aryl iodides or bromides were treated with hexamethyl- or hexabutylditin and a Pd(0) source. On the basis of these results, Grigg et al. investigated the use of Pd(0)/R$_3$Sn-SnR$_3$ combination for the intramolecular coupling of aryl halides, and have successfully developed a route to the synthesis of hippadine (1). In their strategy, anhydrolycorinone (7) was prepared by the intramolecular coupling of the appropriately substituted diiodide derivative 40. Then compound 7 was oxidized with DDQ to give hippadine (1), in an overall yield of 54% over two steps (Scheme 8).

**Scheme 8.** Palladium-mediated intramolecular cyclization approach to hippadine (1)

![Scheme 8](image)

Sneickus et al. attempted the synthesis of hippadine (1), utilizing the Suzuki cross-coupling chemistry. One of the Suzuki coupling partners 41 was synthesized by the thallium-mediated iodination and deacylation of N-acetyl indoline. Then boronic acid 42 was cross-coupled with
and after a subsequent lactamization gave anhydrolycorinone (7). Oxidation of 7, with DDQ
gave hippadine (1) in an overall yield of 34.2% over five steps (Scheme 9).

**Scheme 9. Hippadine (1) synthesis via Suzuki cross-coupling**

Having observed that the Suzuki coupling conditions developed by Snieckus et al. resulted in
low yields (41 + 42, Scheme 9), Tønder et al. extensively studied on this particular coupling
reaction by modifying and interchanging the coupling partners.\textsuperscript{14} They also tested the other
cross-coupling reactions such as the Kumada, the Negishi and the Stille reactions, but they were
unsuccessful in preparing the requisite metalated coupling partners. However, they developed a
one-pot synthesis of hippadine (1), by borylation of 7-bromoindole followed by cross-coupling
with 6-iodopiperonyl ester 44 and subsequent lactamization (Scheme 10).

**Scheme 10. One-pot synthesis of hippadine (1) from 7-bromoindole**

Kerr et al. developed a strategy that involves a palladium-mediated amidation as a key
transformation in the synthesis of hippadine (1).\textsuperscript{15} In their synthesis of 1, N-\(\text{N}-(\text{piperonyl})\)indoline
46 was prepared by a palladium-mediated amidation of carbonate 45 with piperonylamide, and a subsequent intramolecular cyclization reaction. Amide 46 upon oxidative-cyclization with PIFA/\(\text{BF}_3 \cdot \text{Et}_2\text{O}\) gave anhydrolycorinone (7), which upon oxidation with DDQ resulted in the formation of hippadine (1), in an overall yield of 56.4% over three steps (Scheme 11).

**Scheme 11. Synthesis of hippadine (1) via a palladium-mediated amidation reaction**

![Scheme 11](image)

**[4.1.7] By C–H Functionalization**

Knowing the importance of 7-substituted indoles and the difficulties involved in their synthesis, Snieckus et al. studied the synthesis of 7-substituted indoles by C–H functionalization. They were able to successfully install various electrophiles at position C-7 of 1-(N,N-diethylcarbomyl)-2-trimethylsilyl indole under strongly basic conditions, with the amide group acting as a metalation director, and the trimethylsilyl group acts as a blocking agent at the C-2 position. On the basis of these results they have synthesized 7-borylated indole derivative 48 by lithiation followed by borylation of 1-(N,N-diethylcarbomyl)-2-trimethylsilyl indole 47 (Scheme 12). The borylated indole derivative 48 was then coupled with bromo ester
49 under Suzuki conditions, to give biaryl compound 50 as well as hippadine (1) in 9:1 ratio, respectively. The biaryl compound 50 was cyclized with either NaOH or LiOH to give hippadine (1).

**Scheme 12. Lithium mediated C–H functionalization reaction leading to 1**

Hartwig et al. further explored the synthesis of 7-borylated indoles, by an using iridium-catalyzed C–H functionalization, precluding the necessity of blocking groups at the C-2 of the indole.17 On the basis of this methodology, they have developed a one-pot synthesis of hippadine (1, Scheme 13). Initially, the nitrogen atom of the indole was silylated using Et3N, Me2SiCl. The silyl group attached to the nitrogen directs the iridium catalyst to selectively activate C–H bond at the C-7 of the indole, forming the plausible intermediate 52. This intermediate undergoes borylation to give the 7-borylated indole derivative. Once the borylated species is formed, it was subjected to Suzuki cross-coupling conditions with the bromo ester 51. Under these conditions, the silyl group on the nitrogen of the indole underwent desilylation, and this was followed by intramolecular lactamization to give hippadine (1) in a 48% overall yield.
Scheme 13. One-pot synthesis of I by C–H functionalization of indole

Garden et al. developed a method for C–H functionalization at the C-7 position of indole but by a completely different approach. In this approach, benzyl isatin 54 was synthesized from isatin and benzyl chloride derivative 53 (Scheme 14). The keto group of benzyl isatin 54, was then converted to its corresponding spiro-dioxolane 55. Palladium catalyzed C–H functionalization at the C-7 position of isatin part of spiro-dioxolane 55 gave the cyclized product 56. Once the cyclized product 56 was obtained, it was hydrolyzed to isatin derivative 57, which upon carbonyl reduction followed by oxidation with MnO2-SiO2 gave hippadine (1).

Scheme 14. Synthesis of I via C–H functionalization of spiro-dioxolane 55
Many of the strategies developed so far for the synthesis of hippadine (1), require multiple steps, harsh reaction conditions, specialized starting materials that are not easily accessible, and use of stoichiometric amounts of expensive catalysts. Hence we decided to evaluate a relatively inexpensive and easy amidation route to hippadine (1), via anhydrolycorinone (7).
[4.2] RESULTS AND DISCUSSION

Recently, Lakshman *et al.* developed a simple method for the synthesis of amides from various carboxylic acids and amines by using the inexpensive combination of PPh$_3$ and I$_2$ or polymer-supported PPh$_3$ and I$_2$ (Scheme 15).$^{25}$ Reactions of wide variety of aromatic, alkyl, and hetero-aromatic carboxylic acids with aromatic, alkyl ($1^\circ$, $2^\circ$) amines were tested under the conditions developed, and these gave fruitful results. In those cases where the yields were moderate, an additional equivalent of phosphine and I$_2$ or the base led to improve yields.

The scope, generality, involvement of cheap reagents such as PPh$_3$, I$_2$, as well as the ease of purification made this an attractive route to hippadine (1), which possess an amide functionality. According to our strategy, illustrated in Scheme 16, hippadine (1) can be easily obtained by the oxidation$^{2,13}$ of anhydrolycorinone (7), which can be obtained by

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**Scheme 15.** Synthesis of amides from carboxylic acids and amines via use of PPh$_3$/I$_2$ or Pol–PPh$_3$/I$_2$ combinations

| R = Aryl, alkyl, heteroaryl, |
| R' = aryl, alkyl, |
| R'' = H, alkyl |

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**Scheme 16.** Retrosynthetic analysis for the synthesis of hippadine (1)
the oxidative-cyclization of amide 46. By employing the PPh$_3$/I$_2$ amidation reaction conditions to piperonylic acid and indoline, the amide 46 can be easily attained.

On the basis of our strategy discussed above, we initially started the synthesis of N-(piperonoyl)indoline (46), by reaction of piperonylic acid and indoline using 1 molar equivalent each of PPh$_3$ and I$_2$, and 1.5 molar equivalents of iPr$_2$NEt in CH$_2$Cl$_2$ as solvent (Scheme 17). Under these conditions, amide 46 was obtained in 70% yield. When the reaction was performed under similar conditions except that PPh$_3$ was replaced by Pol-PPh$_3$, amide 46 was obtained in a comparable 74% yield (Scheme 17).

**Scheme 17. Synthesis of N-(piperonoyl)indoline (46) using PPh$_3$/I$_2$ and Pol–PPh$_3$/I$_2$**

In the course of examining the spectral data of product 46, we observed some unusual features. Even though the $^1$H NMR data of our product matched that reported,$^{15}$ it is quite surprising that in CDCl$_3$, compound 46 showed only six aromatic resonances instead of seven aromatic resonances (Panel A, Figure 2). To be certain that no other side reactions occurred on one of the aromatic rings during the course of the reaction, we sought additional data. Hence, the $^1$H NMR spectrum of compound 46 was recorded in C$_6$D$_6$. Here we observed an additional broad signal at 8.1 ppm (Panel B, Figure 2), which increased in intensity upon heating the $^1$H NMR sample. Finally, a clearly discernible resonance was observed when the spectrum was obtained at 70 °C (Panel C, Figure 2). A COSY spectrum of the same sample recorded at 70 °C
in C₆D₆, further confirmed that the new downfielded resonance that appeared in the spectrum indeed belonged to the amide 46. Also, a long-range coupling of a benzylic CH₂ with an aromatic proton of the indoline ring was observed (Figure 3).

Figure 2. NMR studies on amide 46. Panel A: ′H NMR spectrum of compound 46 in CDCl₃ at 25 °C. Panel B: ′H NMR spectrum of compound 46 in C₆D₆ at 25 °C. Panel C: ′H NMR spectrum of compound 46 in C₆D₆ at 70 °C.
Figure 3. COSY spectrum of 46 in C₆D₆ at 70 °C showing a correlation between the aromatic proton and the new resonance and a long range coupling between the benzylic CH₂ and an aromatic proton.

Oxidative cyclization of amide 46 with PIFA and BF₃•Et₂O in CH₂Cl₂ at –25 °C, resulted anhydrolycorinone (7) in 23% yield (Scheme 18). This result is much lower than what is reported in literature (83% reported yield). However, the 23% yield was consistently reproducible in our hands. One possible reason for the low yield could be the subambient temperature of the reaction. At low temperature there could be restricted rotation around the amide linkage. Thus if in the rotamers the two aryl rings do not come in proximity for the reaction to occur, this could produce a low yield. This argument is supported by the fact that, the ¹H NMR spectrum of an amide similar to 46 (N-(3,4-dimethoxybenzoyl)indoline) in CDCl₃ at room temperature, showed four distinct methoxy resonances at δ = 3.31, 3.30, 3.28, and 3.27 ppm. These slowly merged into two signals upon heating the sample. This amide under the same oxidation conditions did not cyclize. These results clearly support the possibility of rotamers in the case of N-(piperonoyl)indoline (46) at sub-ambient temperature, and thus the low yielding reaction. Oxidation of anhydrolycorinone (7) with DDQ, proceeded uneventfully to yield hippadine (1) in 78% yield (Scheme 18).

Scheme 18. Synthesis of hippadine (1) from anhydrolycorinone (7) by oxidative cyclization of 46 using PIFA and BF₃•Et₂O and DDQ olefination.
CONCLUSION

We have developed a straightforward approach for the synthesis of hippadine (1) via anhydrolycorinone (7) using a PPh₃/I₂ mediated amidation reaction as a key step. Both PPh₃/I₂ and Pol-PPh₃/I₂ combinations gave comparable amidation yields, when piperonylic acid and indoline were reacted, to give N-(piperonoyl)indoline 46. The amide obtained via this amidation conditions was confirmed as the desired amide 46, with its ¹H NMR and COSY data. Amide 46 was cyclized by PIFA and BF₃•Et₂O to give anhydrolycorinone (7), which was finally oxidized by DDQ to give hippadine (1) in an overall yield of 13.3% over three steps.
[4.4] EXPERIMENTAL SECTION

**General Experimental Considerations.** Thin-layer chromatography was performed on 200 µm aluminum-back silica gel plates and column chromatographic purifications were performed on 200–300 mesh silica gel. CH₂Cl₂ and iPr₂NEt were distilled from CaH₂, and THF was distilled from LiAlH₄ and redistilled from Na prior to use. All other reagents were obtained from commercial sources and were used without further purification. Pol–PPh₃ (PS-triphenylphosphine, 2.28 mmol/g) was obtained from Biotage. ¹H NMR spectra were recorded at 500 MHz and are referenced to the residual protonated solvent. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (J) are in hertz (Hz).

**N-(Piperonoyl)indoline (46)⁵**

Using Pol–PPh₃: To a stirring solution of I₂ (253.8 mg, 1.0 mmol) in dry CH₂Cl₂ (5 mL) at 0 °C was added Pol–PPh₃ (438.0 mg, 1.0 mmol). The reaction mixture was flushed with nitrogen gas and allowed to stir at 0 °C for 5 min. At this temperature, piperonylic acid (166.1 mg, 1.0 mmol) was added, followed by the dropwise addition of iPr₂NEt (260.0 µL, 1.5 mmol) and indoline (112 µL, 1.0 mmol). The reaction mixture was slowly brought to room temperature and allowed to stir for 2 h. The mixture was filtered and evaporated to dryness. Chromatographic purification on a silica gel column (30% EtOAc in hexanes) afforded 199.2 mg (74% yield) of N-(piperonoyl)indoline as a colorless solid.
Using PPh₃: To a stirring solution of I₂ (253.8 mg, 1.0 mmol) in dry CH₂Cl₂ (5 mL) at 0 °C was added PPh₃ (438.0 mg, 1.0 mmol). The reaction mixture was flushed with nitrogen gas and allowed to stir at 0 °C for 5 min. At this temperature, piperonylic acid (166.1 mg, 1.0 mmol) was added, followed by the dropwise addition of iPr₂NEt (260.0 µL, 1.5 mmol) and indoline (112 µL, 1.0 mmol). The reaction mixture was slowly brought to room temperature and allowed to stir for 2 h. The reaction mixture was diluted with water and extracted with CH₂Cl₂. The organic layer was dried with anhydrous Na₂SO₄ and evaporated under reduced pressure. Chromatographic purification on a silica gel column (30% EtOAc in hexanes) afforded 188.4 mg (70% yield) of N-(piperonyl)indoline as a colorless solid. Rf (SiO₂/40% EtOAc in hexanes) = 0.60. ¹H NMR (500 MHz, CDCl₃, ambient temperature): δ 7.21 (d, 1H, Ar-H, J = 7.4 Hz), 7.18–7.09 (br s, 1H, Ar-H), 7.09 (dd, 1H, Ar-H, J = 1.3, 8.0 Hz), 7.05 (s, 1H, Ar-H), 7.01 (t, 1H, Ar-H, J = 7.4 Hz), 6.85 (d, 1H, Ar-H, J = 8.0 Hz), 6.03 (s, 2H, OCH₂), 4.10 (t, 2H, NCH₂, J = 7.9 Hz), 3.11 (t, 2H, indolinyl-CH₂, J = 7.9 Hz). ¹H NMR (500 MHz, C₆D₆, 70 °C): δ 7.96 (br s, 1H, Ar-H), 7.01 (t, 1H, Ar-H, J = 8.0 Hz), 6.98 (s, 1H, Ar-H), 6.91 (d, 2H, Ar-H, J = 7.8 Hz), 6.84 (t, 1H, Ar-H, J = 7.3 Hz), 6.53 (d, 1H, Ar-H, J = 7.8 Hz), 5.31 (s, 2H, OCH₂), 3.54 (t, 2H, NCH₂, J = 8.0 Hz), 2.47 (t, 2H, indolinyl-CH₂, J = 8.0 Hz).

Anhydrolycorinone (7)²,²⁶

In a clean and dry 50 mL round-bottom flask equipped with a stir bar, phenyl iodonium trifluoroacetate (450 mg, 1.047 mmol) was dissolved in dry CH₂Cl₂ (14 mL) and stirred for 10
min at –25 °C under an argon balloon. To this stirring reaction mixture, BF₃•Et₂O (228 µL, 1.8 mmol) was added dropwise followed by the addition of a solution of N-(piperonoyl)indoline (200 mg, 0.748 mmol) in dry CH₂Cl₂ (6 mL) maintaining the temperature at –25 °C. This reaction mixture was stirred at the same temperature for 1.5 h at which point TLC showed the consumption of the starting material. The mixture was then neutralized with saturated aq NaHCO₃ (25 mL) and washed with deionized water (3 x 20 mL). The aqueous layer was back extracted with EtOAc (3 x 15 mL). The organic layers were combined, washed with brine (20 mL), dried over anhydrous Na₂SO₄, and carefully evaporated. Purification of the crude material on a short silica gel column using 50% EtOAc in hexanes yielded 45.6 mg (23% yield) of anhydrolycorinone (7) as a dark-brown solid. Rₐ (SiO₂/100% EtOAc) = 0.32. ¹H NMR (500 MHz, CDCl₃): δ 7.93 (s, 1H), 7.76 (d, 1H, J = 7.7 Hz), 7.56 (s, 1H), 7.30 (dd, 1H, J = 0.9, 7.2 Hz), 7.20 (t, 1H, J = 7.6 Hz), 6.13 (s, 2H), 4.47-4.50 (m, 2H), 3.44 (t, 2H, J = 8.2 Hz).

Hippadine (1)

In a clean, dry 100 mL round-bottom flask equipped with a stir bar, anhydrolycorinone (7) (40 mg, 0.15 mmol) dissolved in dry benzene (50 ml). DDQ (136.2 mg, 0.60 mmol) was added and the mixture was refluxed for 18 h, and then benzene was evaporated. The resulting crude solid was dissolved in EtOAc (15 mL) and washed with saturated aq NaHCO₃ (3 x 15 mL), deionized water (10 mL), and then brine (10 mL). The organic layer was dried over Na₂SO₄ and evaporated. Purification of the crude material over a short silica gel plug using 20% EtOAc in hexanes gave 31.1 mg (78% yield) of hippadine (1) as a white solid. ¹H NMR (500 MHz, CDCl₃): δ 8.05 (d,
1H, $J = 3.6$ Hz), 7.99 (s, 1H), 7.93 (d, 1H, $J = 7.7$ Hz), 7.76 (d, 1H, $J = 7.7$ Hz), 7.67 (s, 1H), 7.48 (t, 1H, $J = 7.7$ Hz), 6.90 (d, 1H, $J = 3.6$ Hz), 6.17 (s, 2H).
[4.5] REFERENCES


CHAPTER 5

SYNTHESIS OF DEUTERATED 1,2,3-TRIAZOLES
SYNTHESIS OF DEUTERATED 1,2,3-TRIAZoles

[5.1] INTRODUCTION

Among the heterocyclic compounds, 1,2,3-triazoles are of great importance because of their applications in various fields. They are found to be active against HIV,\(^1\) Cantagalo,\(^2\) SARS, and orthopox viruses.\(^3\) 1,2,3-Triazole derivatives are also used as insecticides,\(^4\) fungicides,\(^5\) plant growth regulators,\(^6,7\) optical brighteners,\(^8\) corrosion inhibitors,\(^9\) and photo stabilizers.\(^10\) The Huisgen 1,3-dipolar cycloaddition of azides and alkynes is one of the most atom-economical synthesis of 1,2,3-triazoles.\(^11,12\) However, under the thermal conditions two isomers, 1,4- and 1,5-disubstituted 1,2,3-triazoles are often observed, as this process lacks regioselectivity (Scheme 1).

**Scheme 1.** 1,2,3-Triazole synthesis by Huisgen azide–alkyne ligation

Independent research by Sharpless and coworkers\(^13\) and Meldal and coworkers\(^14\) led to the discovery of a copper-catalyzed azide–alkyne cycloaddition (CuAAC) reaction, and 1,4-disubstituted 1,2,3-triazoles became more accessible. These reactions are mild and highly regioselective (Scheme 2). The CuAAC reactions are catalyzed by Cu(I) salts in presence of protic solvents. Some of the Cu(I) sources typically used in the CuAAC reactions are CuI\(^14\) and a combination of CuSO\(_4\)/Na ascorbate.\(^13\)

**Scheme 2.** Regioselective synthesis of 1,4-disubstituted 1,2,3-triazoles by the CuAAC reaction
Sharpless et al. first proposed the mechanism for the CuAAC reaction (Figure 1), where the Cu(I) catalyst initially reacts with the alkyne to form a copper (I) acetylide 1A. This species 1A, coordinates to the azide to form intermediate 1B, which then cyclizes to give a six-membered metallocycle 1C. This intermediate rearranges to give the cuprated triazole 1D. Upon abstraction of a proton from the solvent, the cuprated triazole 1D is converted to the 1,4-disubstituted 1,2,3-triazole (1) and the Cu(I) catalyst. There are assumptions that once copper (I) acetylide 1A reacts with the azide, it directly forms the cuprated triazole intermediate 1D in a concerted pathway. However, the DFT calculations showed that a step-wise mechanistic pathway is favored over the concerted pathway by about 12-15 kcal.

**Figure 1. Proposed mechanism for the CuAAC reaction**
A number of important features were recognized during recent mechanistic investigations of CuAAC reaction. It was found that CuAAC reactions are second order in Cu(I) under catalytic conditions. One of the two possible explanations is that the azide and alkyne are activated by different metal centers like in the intermediate 1E (Figure 2a). The second explanation was on the basis of the observation that the electron density of C-1 in the intermediate 1C (Figure 1) is localized. Hence it was postulated that the second copper atom may provide extra stability to the intermediates and the transition state to the path of 1I, by coordinating with either the triple bond of the alkyne (1F, 1G) or the C-1 carbon of the intermediates 1H and 1I (Figure 2b).

Figure 2. (a) Azide-alkyne interaction by a dinuclear metal complex. (b) Mechanistic outline of the intermediates with σ- and π-coordinated metal centers to the alkyne leading to 1I.

Although the involvement of Cu-acetylides in CuAAC reaction is highly anticipated, the involvement of π-coordinating copper with the alkyne is questionable. However, recent studies by Cantillo et al. proved the intermediacy of the Cu-acetylides formed from the terminal alkynes. They observed that the copper-catalyzed reaction of benzyl azide with (ethynyl-\textit{D})benzene in \textit{t}-BuOH/H\textsubscript{2}O gave only the C-5 protio triazole \textit{3a}, rather than the C-5 deuterated triazole \textit{3b} (Scheme 3). If the transformation took place via a copper π-complex with the alkyne,
3b would be the product. Since 3a is exclusively formed under these conditions, it is clearly evident that Cu-acetylides are formed and may be responsible for the CuAAC reaction.

**Scheme 3. Regioselective synthesis of 1,4-disubstituted 1,2,3-triazoles by CuAAC**

In order to prove the intermediacy of a $\pi$-complex between copper with the alkyne in CuAAC reactions, Fokin et al. conducted experiments with benzyl azide and a stoichiometric amount of Cu-acetylide 4 in the presence and absence of Cu(PPh$_3$)$_4$NO$_3$ catalyst (Scheme 4). These reactions were monitored by real-time heat-flow reaction calorimetry and showed that the catalyzed reaction was complete within 20 min to form the copper triazolide 5. On the other hand, the uncatalyzed reaction showed no conversion to the copper triazolide 5. Similar results were observed when other azides and soluble copper catalysts were used. These results clearly indicated that a second copper atom is necessary for the CuAAC reactions. To further establish

**Scheme 4. Experiments conducted to study the role of copper $\pi$-complex and isotopic enrichment of copper triazolide 5**
the role of two copper atoms, another experiment was conducted with benzyl azide and copper acetylide $4$ ($^{63}$Cu-69% and $^{65}$Cu-31%) with isotopically pure copper-63 catalyst ($^{63}$Cu(MeCN)$_4$PF$_6$) in THF at room temperature (Scheme 4). This reaction was monitored by time-of-flight mass spectrometry (TOF-MS) and showed the formation of copper triazolide $5$. Surprisingly, the triazolide $5$ was 50% isotopically enriched ($^{63}$Cu-85% and $^{65}$Cu-15%). The isotopic enrichment of copper triazolide $5$ could have taken place via the copper acetylide $4$ and/or via triazolide intermediate $5$ and/or within an intermediate during cycloaddition reaction. However, when copper acetylide $4$ and triazolide $5$ were individually reacted with isotopically pure catalyst $^{63}$Cu(MeCN)$_4$PF$_6$ neither showed any isotopic enrichment. This clearly indicated that the enrichment event took place during the cycloaddition reaction.

On the basis of all the observations discussed above, Fokin et al. proposed a plausible mechanism for the CuAAC reaction (Figure 3). In this mechanism, the alkyne initially forms a copper $\pi$-complex $1J$, which then undergoes deprotonation to form $\pi$-coordinated copper acetylide species $1K$ with another copper atom. Then the azide coordinates to the $\pi$-bound copper atom to form intermediate $1L$. Next, the $\beta$-carbon atom of the alkyne attacks at the N-3 atom of the azide to give the intermediate $1M$. The ligand exchange is more likely to take place within intermediate $1M$, since from the previous discussion, it is known that ligand exchange did not occur from the copper acetylide $4$ and the triazolide $5$ to the $^{63}$Cu catalyst. Intermediate $1M$, containing two chemically equivalent copper atoms, also accounts for the isotopic enrichment observed in the case of triazolide $5$. Intermediate $1M$ then results in the formation of cuprated triazole $1N$ via the mononuclear metallacycle stabilized by the second copper atom (as shown in Figure 2b). By abstracting a proton from solvent, cuprated triazole $1N$ is converted to the 1,4-disubstituted 1,2,3-triazole (1) and the Cu(I) catalyst.
As discussed earlier, C-5 deuterated triazoles from terminal deuterated alkynes is highly improbable to form, under aqueous conditions (Scheme 3). However, aqueous conditions are essential as they have been shown to increase the rate of CuAAC reactions.\(^{17}\) Thus, it was quite surprising that very few transformations were reported that lead to the deuterated triazoles and none involving CuAAC. Some of the previously reported methodologies are shown in Scheme 5.

Recently, Micouin \textit{et al.} reported a methodology for the synthesis of C-5 deuterated triazoles (Scheme 5, eq. 1).\(^{19}\) In this transformation, ethynylbenzene is aluminated by treating it with AlMe\(_3\) and MeN(SiMe\(_3\))\(_2\) to give dimethylphenylalkynylaluminum 6. Then compound 6 was reacted with benzyl azide in the presence of CuI and \(N,N,N',N'',N'''-\)pentamethyldiethylenetriamine (PMDTA) to give aluminated triazole intermediate 7, which upon exposure to DCl/D\(_2\)O resulted in the formation of C-5 deuterated triazole 8. Hu \textit{et al.} reported another transformation for the synthesis of C-5 deuterated triazoles by AcOD promoted copper catalysis (Scheme 5, eq. 2).\(^{20}\) Dinuclear copper acetylide 9 was initially prepared from a Cu(I) complex polymer [(MeCO\(_2\)Cu)\(_2\)]\(_n\) and ethynylbenzene in cyclohexane. Ligation of compound 7

\[\text{Figure 3. Proposed mechanistic model for the CuAAC reaction showing the involvement of two copper atoms in the mechanism}\]
with benzyl azide to give the C-5 deuterated triazole 8. However, unlike other processes, this process is stoichiometric in Cu-acetylide and does not lead to 8 from the corresponding cuprated triazole in the absence of AcOD.

**Scheme 5. Previously reported syntheses of deuterated triazoles**

One of the oldest strategies utilized for the synthesis of C-5 deuterated triazole synthesis was developed by Ohta et al., where the triazole 10 is initially lithiated at the C-5 position by treatment with n-BuLi in THF (Scheme 5, eq. 3).\(^1\) Quenching this lithiated triazole 11 with D\(_2\)O gave the C-5 deuterated triazole 12. A complementary method was reported by Krasiński et al. for the synthesis of C-4 deuterated triazole from magnesium acetylide (Scheme 5, eq. 4).\(^2\) Ethynylbenzene was reacted with 1 M solution of EtMgBr in THF at \(-40^\circ\)C, to give magnesium acetylide 13, which upon treatment with phenyl azide gave the corresponding magnesiated...
Triazole 14. Triazole 14 upon reaction with DCl/D$_2$O gave the C-4 deuterated triazole 15. Dideuterated triazole 16 was also reported by the reaction of CaC$_2$ with an azide in the presence of CuI, Et$_3$N and D$_2$O, at 55 °C (Scheme 5, eq. 5).$^{23}$ Sharpless et al. reported that C-5 deuteration of triazoles by CuAAC reaction, but the details are unavailable.$^{17}$

The role of deuterated molecules in studying reaction mechanisms, biosynthetic pathways, and mass spectrometry is well known. Also, deuterium substituted drug candidates, called as “heavy drugs,” have shown potential advantages over their unsubstituted analogues. The effectiveness of a drug may decrease due to its metabolism in the body by the cleavage of C-H bonds. This sometimes may lead to side effects. Hence, by switching those weak C–H bonds with the relatively stronger C–D bonds can slow down this unwanted metabolic activation.$^{24-28}$ Examples of deuterated analogues of some of the drug candidates are shown in Figure 4.

The deuterated analogues of several drugs have shown superiority over their protio analogues by circumventing the undesired metabolic activation and longer retention in the biological system. For example, the deuterated analogue of the HIV protease inhibitor atazanavir, CTP-518 (Figure 4), has proven to show slower hepatic metabolism.$^{24}$ It also eliminated the necessity of “anti-HIV boosters”, which are very expensive.$^{27}$ In the undeuterated version of CTP-347 (paroxetine), the methylene dioxy group generates a carbene during the metabolism which irreversibly inactivates hepatic CYP2D6.$^{24}$ This process inhibits the uptake of other drugs co-administered with it.$^{24-26}$ CTP-347 successfully circumvents the formation of carbene and thereby prevents the inactivation of CYP2D6. Other deuterated analogues of commercial drugs C-20081 (deuterated analogue of the antibiotic, linezolid), SD-254 (deuterated analogue of the antidepressant, venlafaxine), and tamoxifen-$d_5$ (dueterated analogue of tamoxifen, used to treat breast cancer), exhibited superior activity over their fully protio analogues.$^{24-29}$
Figure 4. Some deuterated pharmaceutical agents.

On the basis of all these considerations of isotopic labeling, as well as the medicinal importance of 1,2,3-triazoles, we decided to develop a simple and mild synthesis of C-5 deuterated triazoles via the CuAAC reaction.
RESULTS AND DISCUSSION

Our hypothesis for the one-pot triazole-forming deuteration strategy mainly relies on the following mechanistic considerations of CuAAC reaction. The acidity of alkynyl proton is increased by the π-coordination of Cu(I) to the terminal alkyne. This facilitates the formation of Cu-acetyldides in aqueous media.\textsuperscript{17} Since the azide coordination to the Cu center occurs after the formation of Cu-acetylide, terminal alkynes can be directly utilized. However, our hypothesis mainly relies on the cuprated triazole intermediate 1D, (Figure 1) formation step. In a typical CuAAC reaction, 1D is protonated from a proton source, to give 1,4-disubstituted C-5 protio triazole.\textsuperscript{13} Hence, by deuteration of 1D, from a cheap deuterium source like D\textsubscript{2}O, 1,4-disubstituted C-5 deuterated triazole can be obtained.

Typical solvent system used in CuAAC reactions is a mixture of t-BuOH and H\textsubscript{2}O.\textsuperscript{13} Hence, the mixture of t-BuOD and D\textsubscript{2}O can be used for our methodology, but that would be cost prohibitive. Recently, Lakshman \textit{et al.} observed the competing azide reduction in their CuAAC reactions of 6-azidopurine nucleosides.\textsuperscript{30} This is not a typical case in CuAAC reactions, and they have overcome it by using biphasic reaction medium i.e., 1:1 CH\textsubscript{2}Cl\textsubscript{2} and H\textsubscript{2}O. This guided us to use the biphasic solvent system with D\textsubscript{2}O as one of the phases. This should allow the C-5 cuprated triazole 1D formed in the CuAAC reaction, to get deuterated from D\textsubscript{2}O.

On the basis of the aforementioned considerations, the initial optimization conditions for the formation of 1,4-disubstituted, C-5 deuterated triazoles were tested (Table 1). CuSO\textsubscript{4}$\cdot$5H\textsubscript{2}O was dried and desiccated prior to the reaction, to reduce the risk of competing protonation of cuprated triazole intermediate. The extent of %D incorporation increased with the usage of rigorously dried CuSO\textsubscript{4} and 99.8% D\textsubscript{2}O (entry 2). However, the reaction was incomplete and 8% of phenyl
azide (14) was recovered. By increasing the catalyst loading, a complete reaction was achieved with 75% yield and 96% D incorporation (entry 3). In this case %D incorporation was assessed in two ways. 96% D incorporation was observed when CH2Cl2 was used as internal NMR standard, whereas 94% D incorporation was observed when residual H-5 resonance was compared with the other resonances of 16a. Additional attempts were made to enhance the %D incorporation by performing a reaction under the same conditions as in entry 3, except that Na ascorbate was evaporated from D2O under vacuum. However, this did not improve the %D incorporation (entry 4). To reduce the proton source, a reaction was carried out with 1 equivalent of alkyne, but this resulted in an incomplete reaction (entry 5). In order to evaluate whether a water miscible solvent would ameliorate the reaction, a 1:1 mixture of CH3CN/D2O was used as the solvent system. To our surprise, only 5% of the product was observed after 24 h (entry 6). In order to confirm this result, another reaction was carried out with 1:1 CH3CN/H2O, again 5% product was observed even after 5 d (entry 7). Prior investigations on CuAAC reaction reported the primacy of CH2Cl2/H2O over CH3CN/H2O, DMSO/H2O, EtOH/H2O, t-BuOH/H2O and other solvent combinations.31 Other Cu(I) source, CuCl was also evaluated, but it did not turn out to be advantageous over CuSO4·5H2O (entry 8).

Table 1. Optimization for Cu(I) catalyzed synthesis of C-5 deuterated 1,2,3-triazoles

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent, Catalytic System, Reaction Time</th>
<th>Product: Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1d,e</td>
<td>1:1 CH2Cl2/D2O, 5 mol% CuSO4, 10 mol% Na ascorbate, 12 h</td>
<td>16a: 60% yield, 80% D incorporation, reaction was incomplete</td>
</tr>
<tr>
<td>2g,h</td>
<td>1:1 CH2Cl2/D2O, 7 mol% CuSO4, 15 mol% Na ascorbate, 12 h</td>
<td>16a: 65% yield, 95% D incorporation, reaction was incomplete</td>
</tr>
</tbody>
</table>
Once the initial optimization conditions were evaluated, we next focused on assessing the generality of C-5 deuteration strategy. A wide spectrum of various azides and alkynes were tested, and good to excellent yields were obtained with high %D incorporation (Table 2). $^2$H NMR chemical shifts of C-5 deuterium of all the triazole are shown in parenthesis. Compounds 17-23 are structurally similar to resveratrol triazole analogues that have shown potent cytotoxic and antiproliferative activity higher than resveratrol. Complex nucleoside triazoles 25 and 26 were also synthesized with high yields and excellent %D incorporation. The yield of the AZT based triazole 25 is comparable to the microwave assisted CuAAC of AZT.$^{32}$ These AZT based protio triazoles, have proven to be excellent *Ureaplasma parvum* thymidine kinase inhibitors.$^{32}$ The C-6 azidopurine nucleoside, which is the precursor of 26 was known to show significant azide-tetrazole equilibrium, and readily reduced under CuAAC conditions.$^{30}$ However, we did not encounter any issues under our CuAAC conditions. Pyridine-based triazole derivative 24, a
deuterated analogue of a highly active nicotinic acetylcholine receptor modulator 29, was also synthesized in good yield.

Table 2. Scope of the one-pot triazole-forming deuteration strategy^ae

| R'   | N=N+D_R^+ | R'   | N=N+D_R^+ | Reactions were conducted at room temperature with 0.2 M azide and 2 mol equiv of alkyne. ^b Yields are of isolated and purified products. ^c %D incorporation was assessed from the residual H-5 resonance in the ^1H NMR. Deuterium resonances are referenced to the ^2H resonance of CDCl3 (δ = 7.24 ppm). ^d Syntheses of 15 to 23 were performed with 10 mol% CuSO4, 20 mol% Na ascorbate (0.048 M solutions of CuSO4 and 0.096 M Na ascorbate were prepared in 99.8% D2O and used). ^e Synthesis of 24 was performed with 5 mol% CuSO4, 10 mol% Na ascorbate (0.048 M solutions of CuSO4 and 0.096 M Na ascorbate were prepared in 99.8% D2O and used). | 17: 72%, (rxn time 24 h) 94% D (δ = 8.22 ppm) | 18: 87%, (rxn time 24 h) 96% D (δ = 8.10 ppm) | 19: 70%, (rxn time 24 h) 95% D (δ = 8.09 ppm) | 20: 91%, (rxn time 12 h) 97% D (δ = 8.16 ppm) | 21: 85%, (rxn time 48 h) 96% D (δ = 8.20 ppm) | 22: 72%, (rxn time 48 h) 92% D (δ = 8.17 ppm) | 23: 71%, (rxn time 24 h) 98% D (δ = 7.97 ppm) | 24: 70%, (rxn time 48 h) 94% D (δ = 8.38 ppm) | 25: 96%, (rxn time 7 h) 96% D (δ = 7.65 ppm) | 26: 88%, (rxn time 4 h) 95% D (δ = 9.35 ppm) |
Compound 29 was also synthesized under the same CuAAC reaction conditions except that D₂O was replaced with H₂O (Scheme 6). 29 was obtained in 70% yield, while the literature reported yield was 54%.³³

**Scheme 6. Synthesis of 29 under CuAAC reaction conditions**

These results clearly display the efficiency and generality of this methodology. This made us curious to build poly- and fully deuterated molecules by using our deuteration strategy, where the final deuteration takes place in the CuAAC step. Hence by utilizing the optimized conditions,
three compounds (31, 34 and 35) were synthesized (Scheme 7). Compound 31 was synthesized from phenyl azide-$D_5$ (30) and 4-ethynyltoluene (15), where as compound 34 was synthesized from ethynylbenzene-$D_5$ (32) and TBDMS-protected AZT 33. Fully deuterated 1,2,3-triazole 35 was synthesized from phenyl azide-$D_5$ (30) and ethynylbenzene-$D_5$ (32). The %D incorporation of the triazolyl deuterium in compounds 31 and 33 was assessed from the residual H-5 resonance in the $^1$H NMR spectra, where as in the case of 35, it was assessed by using CH$_2$Cl$_2$ as an internal standard.

We next focused on evaluating the extent of protonation versus deuteration, when both processes compete in the CuAAC reaction. Since the O–D bond is more stronger than O–H bond, deuteration should be less favorable than protonation. In order to assess that, we have conducted two parallel experiments with phenyl azide (14) and 4-ethynyltoluene (15). The co-solvent in one reaction was D$_2$O (99.8%), and in the other reaction was equimolar H$_2$O/ D$_2$O (99.8%) (Scheme 8). The amount of 16b formed in the reaction with D$_2$O (99.8%) basing on the $^1$H NMR, using CH$_2$Cl$_2$ as internal standard, was calculated to be 4%. Where as in the case of the other reaction it was 73% (77% observed–4% observed in the control experiment). The yields from the reactions with D$_2$O and H$_2$O/ D$_2$O are 74% and 76% respectively. The ratio of 16b/16a

**Scheme 8. Competition experiment to assess the extent of deuteration**

\[ \text{14} + \text{15} \xrightarrow{10 \text{ mol\% CuSO}_4, 20 \text{ mol\% Na ascorbate}} \text{16a}, \text{16b} \]

- **16a**: 19.7%  
  **16b**: 56.3%

- **16a**: 70.9%  
  **16b**: 3%
in the reaction with H₂O/ D₂O is 2.7. The higher percentage of \textbf{16b} as compared to \textbf{16a} could be due to the rapid protonation of cuprated triazole. This could be one of the major reasons for the formation of protio products in these reactions.
In summary, a simple and mild method has been developed for the regioselective C-5 deuteration of 1,2,3-triazoles by the CuAAC reaction, using dehydrated CuSO$_4$ and Na ascorbate. For these reactions, the biphasic CH$_2$Cl$_2$/D$_2$O system was clearly superior to CH$_3$CN/D$_2$O system. Under these conditions, no reduction of the azide in the azido purine nucleosides occurs. Poly- and fully deuterated 1,2,3-triazoles were also synthesized by using this methodology. On the basis of our strategy, multiply labeled compounds can be synthesized by combining deuteration strategy with the incorporation of other isotopes such as $^{13}$C and $^{15}$N. This one-pot deuteration strategy is likely to be of importance to medicinal chemistry applications, as it avoids the chemical manipulations of triazoles, which may not be easy in the case of complex molecules.
[5.4] EXPERIMENTAL SECTION

General Experimental Considerations. Thin layer chromatography was performed on aluminum foil-backed TLC plates of 200 µm thickness and column chromatographic purifications were performed on 200–300 mesh silica gel. CH₂Cl₂ and MeCN were distilled over CaH₂, Et₂O was distilled over LiAlH₄ and then over Na. All other reagents were obtained from commercial sources and were used without further purification. Glassware used for reactions was dried at 150 °C in an oven and cooled in a desiccator. Melting points reported are of chromatographically purified materials and are uncorrected. ¹H NMR spectra were recorded at 500 MHz and are referenced to the residual solvent resonance. ¹³C NMR spectra were recorded at 125 MHz in CDCl₃ and are referenced to the solvent resonance. ²H NMR spectra were recorded at 77 MHz using CDCl₃ as an internal standard. For this, first the ¹H NMR spectrum of CDCl₃ was recorded at 500 MHz and the residual protonated solvent resonance was set to δ 7.26 ppm. Next, the ²H NMR of the same sample was recorded at 77 MHz, and this showed a resonance at δ 7.24 ppm. From this analysis, CDCl₃ was referenced to δ 7.24 ppm for all ²H NMR experiments. ¹⁹F NMR spectra was recorded at 282 MHz using CFCl₃ as internal standard. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (J) are in hertz (Hz). HRMS analyses were performed using a TOF analyzer, the ionization methods are provided in the compound characterization.

Synthesis of 3-Ethynylpyridine (28)
Step 1. Synthesis of 3-((triisopropylsilyl)ethynyl)pyridine (36)

Following a literature procedure,\textsuperscript{35} in a clean, dry three-neck round-bottom flask equipped with a stirring bar, 3-bromopyridine (122 µL, 1.26 mmol) was dissolved in (iso-Pr)\textsubscript{2}NH (5 mL). TIPS-acetylene (336 µL, 1.512 mmol), (Ph\textsubscript{3}P)\textsubscript{2}PdCl\textsubscript{2} (44.0 mg, 0.063 mmol), and CuI (12.0 mg, 0.063 mmol) were added and the reaction mixture was heated with stirring under a reflux condenser at 100 °C for 1 h, in a nitrogen atmosphere. The reaction mixture was then diluted with EtOAc (20 mL) and filtered through Celite. The filtrate was washed with deionized H\textsubscript{2}O (3 x 20 mL), of brine (15 mL). The organic layer was separated, dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}, and concentrated under reduced pressure. Purification of the crude material, by passing it through a short silica gel plug with the initial elution using hexanes followed by 5% EtOAc in hexanes, gave 294 mg (90% yield) of TIPS-protected ethynylpyridine (36) as a pale-yellow, viscous liquid. \(R_f\) (SiO\textsubscript{2}/10% EtOAc in hexanes) = 0.40. \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}): \(\delta\) 8.69 (s, 1H, H-2), 7.51 (d, 1H, H-6, \(J = 4.1\) Hz), 7.73 (dt, 1H, H-4, \(J = 1.9, 7.8\) Hz), 7.22 (dd, 1H, H-5, \(J = 4.9, 7.8\) Hz), 1.13 (s, 21H, Si(iso-Pr)\textsubscript{3}). \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}): \(\delta\) 153.0, 148.8, 139.0, 123.0, 120.9, 103.8, 95.1, 18.7, 11.6. HRMS (ESI) calculated for C\textsubscript{16}H\textsubscript{26}NSi [M + H]\textsuperscript{+} 260.1829, found 260.1835.

Step 2. Synthesis of 3-ethynlpyridine (28)\textsuperscript{36}

Following a literature procedure,\textsuperscript{37} in a clean, dry round-bottom flask equipped with a stirring bar, TIPS-protected ethynlpyridine (36, 286 mg, 1.102 mmol) was dissolved in dry MeOH (15 mL). Powdered KOH (433 mg, 7.72 mmol) was added and the reaction mixture was stirred at 45 °C for 24 h. To the reaction mixture was added a saturated aqueous NH\textsubscript{4}Cl solution, and the mixture was extracted with Et\textsubscript{2}O (3 x 15 mL). The organic layer was separated, washed with
brine (10 mL), dried over anhydrous Na₂SO₄, and carefully evaporated. The reaction mixture was carefully evaporated. Purification of the crude material on a short silica gel column using 10% Et₂O in hexanes yielded 51.0 mg (45% yield) of 3-ethynylpyridine (28) as a white solid. \( R_f \) (SiO₂/20% Et₂O in hexanes) = 0.39. \(^1\)H NMR (500 MHz, CDCl₃): \( \delta \) 8.73 (s, 1H, H-2), 8.57 (d, 1H, H-6, \( J = 4.6 \) Hz), 7.77 (d, 1H, H-4, \( J = 7.8 \) Hz), 7.37 (t, 1H, H-5, \( J = 6.4 \) Hz), 3.22 (s, 1H, \( \equiv \text{CCH} \)).

**Synthesis of Ethynylbenzene-\( D_5 \) (29)**

![Reaction Scheme](image)

**Step 1. Synthesis of triisopropyl(phenylethynyl)silane-\( D_5 \) (37)**

Following a literature procedure, in a clean, dry 3-neck round-bottom flask equipped with a stirring bar, bromobenzene-\( D_5 \) (500 mg, 3.085 mmol) was dissolved in \( (\text{iso-Pr})_2\text{NH} \) (12.5 mL). TIPS-acetylene (823 \( \mu \)L, 3.702 mmol), \( (\text{Ph}_3\text{P})_2\text{PdCl}_2 \) (108.3 mg, 0.154 mmol), and CuI (29.4 mg, 0.154 mmol) were added and the reaction mixture was heated with stirring at 100 °C in a nitrogen atmosphere, under a reflux condenser for 1.5 h. The reaction mixture was then diluted with EtOAc (20 mL) and filtered through Celite. The filtrate was washed with deionized H₂O (3 x 20 mL), of brine (15 mL). The organic layer was separated, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. Purification of the crude material, by passing it through a short silica gel plug using hexanes as the eluting solvent gave 500 mg (61% yield) of TIPS-protected ethynylbenzene-\( D_5 \) (37) as a colorless viscous liquid. \( R_f \) (hexanes/SiO₂) = 0.70. \(^1\)H NMR (500 MHz, CDCl₃): \( \delta \) 1.16 (s, 21H, Si(\text{iso-Pr})₃). \(^{13}\)C NMR (125 MHz, CDCl₃): \( \delta \) 131.8 (t,
Step 2. Synthesis of ethynylbenzene-\(\text{D}_5\) (29)

By modifying a literature procedure,\(^{39}\) in a clean, dry 25 mL round-bottom flask equipped with a stirring bar, TIPS-protected ethynylbenzene-\(\text{D}_5\) (35, 700 mg, 2.656 mmol) was dissolved in dry Et\(_2\)O (11 mL) with stirring. A 1 M solution of \(n\)-Bu\(_4\)N\(^+\)F\(^-\) in THF (3.2 mL, 3.2 mmol) was added and the mixture was stirred for 5 min. The mixture was concentrated under a stream of nitrogen gas and loaded onto a short silica gel column. Elution with hexanes, followed by careful rotary evaporation of the fractions containing the product using a water bath at \(\leq 65^\circ\text{C}\), gave 171 mg (61% yield) of ethynylbenzene-\(\text{D}_5\) (29) as a pale-yellow liquid. \(R_f\) (SiO\(_2\)/hexanes) = 0.42. \(^1\text{H}\) NMR (500 MHz, CDCl\(_3\)): \(\delta\ 3.08\) (s, 1H, \(\equiv\text{CCH}\)). \(^2\text{H}\) NMR (77 MHz, CHCl\(_3\)): \(\delta\ 7.52, 7.38, 7.35\).

Synthesis of azides

Unless mentioned otherwise, all azides were synthesized from the corresponding boronic acids.\(^{40}\) 6-Azido-9-[2,3,5-tri-O-(tert-butyldimethylsilyl)]-\(\beta\)-D-ribofuranosyl]purine (38) was previously synthesized in the lab.\(^{30}\)

General procedure for the synthesis of azides (14, 39-41)

Following the literature procedure,\(^{40}\) in a clean 10 mL round-bottom flask equipped with a stirring bar, appropriate boronic acid (2 mmol) was dissolved in MeOH (6 mL). NaN\(_3\) (156 mg,
2.4 mmol) and CuSO₄•5H₂O (50 mg, 0.2 mmol) were added to the reaction mixture, and the mixture was stirred in an open flask at room temperature until the starting material was consumed. The solvent was evaporated under reduced pressure and EtOAc (10 mL) was added. The mixture was extracted with H₂O (10 mL) and the aqueous layer was back extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was purified by chromatography on a silica gel column. See compound headings below for details.

**Phenyl azide (14)**

\[
\text{N}_3 \quad ^1H \text{ NMR (500 MHz, CDCl}_3\text{): } \delta \text{ 7.38-7.34 (m, 2H), 7.15 (t, 1H, } J = 7.4 \text{ Hz), 7.04 (d, 2H, } J = 7.6 \text{ Hz).}
\]

**1-Azido-4-methoxybenzene (39)**

\[
\begin{array}{c}
\text{MeO} \\
\text{N}_3
\end{array} \\
^1H \text{ NMR (500 MHz, CDCl}_3\text{): } \delta \text{ 6.96 (d, 2H, } J = 8.9 \text{ Hz), 6.90 (d, 2H, Ar-H, } J = 8.9 \text{ Hz), 3.80 (s, 4H, OMe).}
\]

**1-azidonaphthalene (40)**

\[
\begin{array}{c}
\text{N}_3
\end{array} \\
^1H \text{ NMR (500 MHz, CDCl}_3\text{): } \delta \text{ 8.13 (d, 1H, } J = 7.7 \text{ Hz), 7.79 (d, 1H, } J = 7.4 \text{ Hz), 7.66 (d, 1H, } J = 8.3 \text{ Hz), 7.57-7.52 (m, 1H), 7.47 (t, 1H, } J = 7.8 \text{ Hz), 7.29 (d, 1H, } J = 7.6 \text{ Hz).}
\]

**4-((Tert-butyl)phenyl azide (41)**

Chromatography using hexanes yielded 311.5 mg (89% yield) of 39 as an orange-red liquid. \( R_f \text{ (SiO}_2/\text{hexanes) = 0.60.} \)

\[
^1H \text{ NMR (500 MHz, CDCl}_3\text{): } \delta \text{ 7.38 (d, 2H, Ar-H, } J = 8.6 \text{ Hz), 6.98 (d, 2H, Ar-H, } J = 8.6 \text{ Hz), 1.32 (s, 9H, } \text{tert-Bu).}
\]

4-(Tert-butyl)phenyl azide has previously been synthesized by diazotization of 4-tert-butylaniline.\(^{41}\)
**m-Azidobenzonitrile (27)**

Following a literature procedure, in a clean, dry round-bottom flask equipped with a stirring bar, 3-aminobenzonitrile (200 mg, 1.69 mmol) was dissolved in dry MeCN (4 mL) and cooled to 0 °C. To the stirring solution tert-butyl nitrite (300 µL, 2.54 mmol) was added dropwise followed by dropwise addition of Me₃SiN₃ (270 µL, 2.03 mmol). The mixture was warmed to room temperature and stirred for 2 h. This reaction mixture was diluted with EtOAc (15 mL) and washed with deionized H₂O (3 x 15 mL), and brine (10 mL). The organic layer was separated, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. Chromatography of the crude material on a silica gel column using 5% EtOAc in hexanes yielded 190 mg (78% yield) of the title compound as a brown solid. $R_f$ (SiO₂/10% EtOAc in hexanes) = 0.30. $^1$H NMR (500 MHz, CDCl₃): δ 7.49-7.42 (m, 2H), 7.29-7.26 (m, 2H). *m*-Azidobenzonitrile (27) has previously been synthesized by diazotization of *m*-cyanoaniline.

**Phenyl azide-D₅ (30)**

Following a literature procedure, in a clean, dry 2-neck round-bottom flask equipped with a stirring bar, bromobenzene-D₅ (400 mg, 2.468 mmol) was dissolved in 7:3 EtOH/H₂O (4.8 mL). $N,N'$-Dimethylethylenediamine (40 µL, 0.37 mmol), NaN₃ (321 mg, 4.936 mmol), Na ascorbate (24.4 mg, 0.123 mmol), and CuI (47 mg, 0.247 mmol) were added and the mixture was heated
with stirring at 100 °C, in an argon atmosphere, under a reflux condenser for 2 h. The mixture was cooled to room temperature and diluted with Et₂O (20 mL) and deionized H₂O (10 mL). The aqueous layer was separated and extracted with Et₂O (3 x 20 mL). The organic layers were combined and washed with brine (10 mL), dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. Chromatography of the crude material on a short silica gel column using hexanes yielded 261 mg (85% yield) of 30 as a yellow liquid. \( R_f \) (SiO₂/hexanes) = 0.50. \(^{13}\)C NMR (125 MHz, CDCl₃): \( \delta \) 139.8, 129.2 (t, \( J = 24.2 \) Hz), 124.3 (t, \( J = 24.2 \) Hz), 118.6 (t, \( J = 24.2 \) Hz). \(^2\)H NMR (77 MHz, CHCl₃): \( \delta \) 7.29, 7.08, 6.98. Phenyl azide-\( D_5 \) (28) has previously been synthesized by diazotization of aniline-\( D_5 \).\(^{44}\)

3’-azido-3’-deoxy-5’-O-(tert-butyldimethylsilyl)thymidine (33)\(^{46}\)

Following the literature procedure,\(^{46}\) in a clean, dry, RB flask equipped with a stirring bar were placed AZT (400 mg, 1.496 mmol) and dissolved in 8 mL of Dry DMF. Then TBDMSCl (338 mg, 2.245 mmol) and imidazole (305 mg, 4.49 mmol) were added to the stirring mixture, the flask was flushed with nitrogen gas and stoppered. After being stirred at room temperature for overnight, the reaction mixture was diluted with water (50 mL) and then washed with ethyl acetate (3 x 50 mL). The collected organic layers were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. Chromatography of the crude material on a short silica gel column using 40% EtOAc in hexanes yielded 502 mg (88% yield) of the 33 as a white solid. \(^1\)H NMR (500 MHz, CDCl₃): \( \delta \) 8.37 (br s, 1H, N-H), 7.44 (s, 1H, Ar-H), 6.22 (t,
1H, H-1’, $J = 6.5$ Hz), 4.24 (dt, 1H, H-3’, $J = 3.7$, 7.3 Hz), 3.98-3.93 (m, 2H, H-4’, H-5’), 3.80 (dd, 1H, H-5’, $J = 2.2$, 11.4 Hz), 2.43 (ddd, 1H, H-2’, $J = 4.1$, 6.1, 13.6 Hz), 2.22 (dt, 1H, H-2’, $J = 7.0$, 13.8 Hz), 1.92 (s, 3H, CH$_3$), 0.93 (s, 9H, tert-Bu), 0.13 (s, 6H, SiCH$_3$).

**General procedure for the synthesis of deuterated triazoles (16-26, 31, 34 and 35)**

In a clean, dry, 10 mL round-bottom flask equipped with a stirring bar, was placed the appropriate azide (0.335 mmol) in 1.6 mL of dry CH$_2$Cl$_2$. To this stirred solution the appropriate alkyne (0.67 mmol) was added. In a separate vial CuSO$_4$•5H$_2$O (8.4 mg, 0.0335 mmol, 10 mol %) was heated at 120 °C under vacuum until the color changed from blue to grey. Using this desiccated CuSO$_4$ a 0.048 M solution was prepared in a glove bag by the addition of D$_2$O (99.8% D, 0.7 mL), and this solution was transferred to the reaction mixture. To the reaction mixture a 0.098 M solution of Na ascorbate (13.3 mg in 0.7 mL of 99.8% D$_2$O, 20 mol %) was added. The reaction mixture was sealed and stirred under nitrogen gas at room temperature until TLC showed consumption of azide. Then the organic layer of the reaction mixture was separated and the aqueous layer was back extracted with EtOAc (3 x 5 mL). The combined organic layers were washed with brine (5 mL), dried over anhydrous Na$_2$SO$_4$, and concentrated under reduced pressure. The crude material was purified by chromatography on a silica gel column. See compound headings below for details.

**5-Deutero-1-phenyl-4-(p-tolyl)-1H-1,2,3-triazole (16a)**

Chromatography using hexanes followed by 20% EtOAc in hexanes gave an off-white solid. $R_f$ (SiO$_2$/25% EtOAc in hexanes) = 0.34. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.82-7.79 (m, 4H, Ar-H),
Assessment of %D incorporation using an external standard. CH₂Cl₂ (16 µL, 0.25 mmol) was added to the solution of the product (16a, 11.8 mg) in CDCl₃ (0.5 mL). The ¹H NMR of this solution was acquired and the resonances were integrated. The signal at δ 5.30 ppm (CH₂Cl₂) was set to 10 whereby the signal at δ 8.16 ppm (residual triazolyl C5-H) integrated to 0.04. Thus, the %D incorporation in the reaction was estimated to be 4%.

5-Deutero-1,4-diphenyl-1H-1,2,3-triazole (17)

Chromatography using hexanes followed by 20% EtOAc in hexanes gave a white solid. Rₛ (SiO₂/25% EtOAc in hexanes) = 0.34. ¹H NMR (500 MHz, CDCl₃): δ 7.92 (d, 2H, Ar-H, J = 8.1 Hz), 7.80 (d, 2H, Ar-H, J = 7.8 Hz), 7.55 (t, 2H, Ar-H, J = 7.8 Hz), 7.48-7.45 (m, 3H, Ar-H), 7.37 (t, 1H, Ar-H, J = 7.4 Hz). ¹³C NMR (125 MHz, CDCl₃): δ 148.6, 137.4, 130.6, 130.0, 129.1, 128.9, 128.6, 126.2, 120.8, 117.6 (s, J = 28.1 Hz). ²H NMR (77 MHz, CHCl₃): δ 8.22. HRMS (ESI) calculated for C₁₄H₁₁DN₃ [M + H]⁺ 223.1089, found 223.1086.

5-Deutero-1-(4-methoxyphenyl)-4-(p-tolyl)-1H-1,2,3-triazole (18)

Chromatography using hexanes followed by 20% EtOAc in hexanes gave a white solid. Rₛ (SiO₂/25% EtOAc in hexanes) = 0.26. ¹H NMR (500 MHz, CDCl₃): δ 7.80 (d, 2H, Ar-H, J = 7.6 Hz), 7.67 (d, 2H, Ar-H, J = 8.6 Hz), 7.26
(d, 2H, Ar-H, $J = 7.6$ Hz), 7.02 (d, 2H, Ar-H, $J = 8.6$ Hz), 3.87 (s, 3H, OCH$_3$), 2.40 (s, 3H, CH$_3$).

$^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 159.9, 148.3, 138.4, 130.7, 129.7, 127.7, 125.9, 122.3, 117.5 (t, $J = 28.9$ Hz), 114.9, 55.8, 21.5.

$^2$H NMR (77 MHz, CHCl$_3$): $\delta$ 8.10. HRMS (ESI) calculated for C$_{16}$H$_{15}$DN$_3$O [M + H]$^+$ 267.1351, found 267.1356.

5-Deutero-4-(4-fluorophenyl)-1-(4-methoxyphenyl)-1H-1,2,3-triazole (19)

Chromatography using hexanes followed by 20% EtOAc in hexanes gave an off-white solid, $R_f$ (SiO$_2$/25% EtOAc in hexanes) = 0.22. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.89-7.86 (m, 2H, Ar-H), 7.67 (d, 2H, Ar-H, $J = 8.9$ Hz), 7.14 (t, 2H, Ar-H, $J = 8.6$ Hz), 7.04 (d, 2H, Ar-H, $J = 8.9$ Hz), 3.88 (s, 3H, OCH$_3$). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 164.1, 162.1, 160.3, 147.5, 130.8, 127.8 (d, $J = 8.1$ Hz), 127.0 (d, $J = 3.2$ Hz), 122.4, 117.6 (t, $J = 28.9$ Hz), 116.1 (d, $J = 21.8$ Hz), 115.1, 55.9. $^2$H NMR (77 MHz, CHCl$_3$): $\delta$ 8.09. $^{19}$F NMR (282 MHz, CDCl$_3$): $\delta$ –113.8 (relative to CFCl$_3$). HRMS (ESI) calculated for C$_{15}$H$_{12}$DFN$_3$O [M + H]$^+$ 271.1100, found 271.1105.

5-Deutero-1-(4-methoxyphenyl)-4-phenyl-1H-1,2,3-triazole (20)

Chromatography using hexanes followed by 20% EtOAc in hexanes gave a white solid. $R_f$ (SiO$_2$/25% EtOAc in hexanes) = 0.23. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.90 (d, 2H, Ar-H, $J = 7.8$ Hz), 7.69 (d, 2H, Ar-H, $J = 8.9$ Hz), 7.46 (t, 2H, Ar-H, $J = 7.6$ Hz), 7.37 (t, 1H, Ar-H, $J = 7.4$ Hz), 7.04 (d, 2H, Ar-H, $J = 8.9$ Hz), 3.88 (s, 3H, OCH$_3$). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 160.2, 148.4, 130.9, 130.8, 129.1, 128.5, 126.1, 122.4, 117.8 (t, $J = 28.3$ Hz), 115.1, 55.9. $^2$H NMR (77 MHz, CHCl$_3$): $\delta$ 8.16. HRMS (ESI) calculated for C$_{15}$H$_{13}$DN$_3$O [M + H]$^+$ 253.1194, found 253.1199.

5-Deutero-1-(4-(tert-butyl)phenyl)-4-phenyl-1H-1,2,3-triazole (21)
Chromatography using hexanes followed by 20% EtOAc in hexanes gave an off-white solid. $R_f$ (SiO$_2$/25% EtOAc in hexanes) = 0.46. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.92 (d, 2H, Ar-H, $J = 8.1$ Hz), 7.70 (d, 2H, Ar-H, $J = 8.5$ Hz), 7.53 (d, 2H, Ar-H, $J = 8.5$ Hz), 7.44 (t, 2H, Ar-H, $J = 7.6$ Hz), 7.35 (dt, 1H, Ar-H, $J = 1.0$, 7.4 Hz), 1.38 (s, 9H, tert-Bu). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 152.3, 148.3, 134.8, 130.5, 129.1, 128.5, 126.8, 126.0, 120.4, 117.8 (t, $J = 28.9$ Hz), 35.0, 31.5. $^2$H NMR (77 MHz, CHCl$_3$): $\delta$ 8.20. HRMS (ESI) calculated for C$_{18}$H$_{19}$D$_3$N$_3$ [M + H]$^+$ 279.1715, found 279.1709.

**5-Deutero-1-(naphthalen-1-yl)-4-phenyl-1H-1,2,3-triazole (22)**

Chromatography using hexanes followed by 20% EtOAc in hexanes gave a brown solid. $R_f$ (SiO$_2$/25% EtOAc in hexanes) = 0.34. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.00-7.92 (m, 4H, Ar-H), 7.69 (d, 1H, Ar-H, $J = 8.3$ Hz), 7.59-7.49 (m, 4H, Ar-H), 7.46 (t, 2H, Ar-H, $J = 7.7$ Hz), 7.04 (dt, 1H, Ar-H, $J = 1.0$, 7.4 Hz). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 147.9, 134.5, 134.0, 130.6, 130.5, 129.1, 128.9, 128.6, 128.5, 128.1, 127.3, 126.1, 125.2, 123.7, 122.6, 122.2 (t, $J = 28.6$ Hz). $^2$H NMR (77 MHz, CHCl$_3$): $\delta$ 8.17. HRMS (ESI) calculated for C$_{18}$H$_{13}$D$_3$N$_3$ [M + H]$^+$ 273.1245, found 273.1247.

**5-Deutero-1-(4-methoxyphenyl)-4-(N-phthalimidomethyl)-1H-1,2,3-triazole (23)**

Chromatography using hexanes followed by 40% EtOAc in hexanes gave an off-white solid. $R_f$ (SiO$_2$/50% EtOAc in hexanes) = 0.40. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.85 (dd, 2H, Ar-H, $J = 3.1$, 5.3 Hz), 7.72 (dd, 2H, Ar-H, $J = 3.1$, 5.3 Hz), 7.57 (d, 2H, Ar-H, $J = 8.9$ Hz), 6.97 (d, 2H, Ar-H, $J = 8.9$ Hz), 5.06 (s, 2H, CH$_2$), 3.83 (s, 3H, OCH$_3$). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 167.8, 159.9, 143.3,
134.3, 132.2, 130.5, 123.6, 122.4, 121.1 (t, \( J = 28.8 \) Hz), 114.8, 55.8, 33.2. \(^2\)H NMR (77 MHz, CHCl\(_3\)): \( \delta \) 7.97. HRMS (ESI) calculated for C\(_{18}\)H\(_{14}\)DN\(_3\)O\(_3\) [M + H]\(^{+}\) 336.1201, found 336.1204.

**5-Deutero-1-(3-cyanophenyl)-4-(pyridin-3-yl)-1H-1,2,3-triazole (24)**

Chromatography using 10% EtOAc in hexanes followed by EtOAc gave an off-white solid. \( R_f (\text{SiO}_2/\text{EtOAc}) = 0.34 \). \(^1\)H NMR (500 MHz, CDCl\(_3\)): \( \delta \) 9.17 (br s, 1H, Ar-H), 8.73 (br s, 1H, Ar-H), 8.31 (d, 1H, Ar-H, \( J = 7.9 \) Hz), 8.15 (t, 1H, Ar-H, \( J = 1.6 \) Hz), 8.11 (ddd, 1H, Ar-H, \( J = 1.1, 2.2, 8.1 \) Hz), 7.79 (dt, 1H, Ar-H, \( J = 1.3, 7.7 \) Hz), 7.74 (t, 1H, Ar-H, \( J = 7.9 \) Hz), 7.47 (br s, 1H, Ar-H). \(^{13}\)C NMR (125 MHz, CD\(_3\)OD, 55 °C): \( \delta \) 150.2, 147.8, 146.8, 139.1, 135.2, 132.5, 128.4, 126.1, 125.7, 125.1, 121.2 (t, \( J = 29.6 \) Hz), 118.7, 115.4. \(^2\)H NMR (77 MHz, CHCl\(_3\)): \( \delta \) 8.38. HRMS (ESI) calculated for C\(_{14}\)H\(_9\)DN\(_5\) [M + H]\(^+\) 249.0993, found 249.0997.

**5’-O-(Tert-butyldimethylsilyl)-3’-deoxy-3’-(5-deutero-4-(4-methylphenyl)-1H-1,2,3-triazole-1-yl)thymidine (25)**

For this reaction 0.131 mmol of azide, 0.262 mmol of alkyne, 10 mol % of anhydrous CuSO\(_4\) in 273 µL of D\(_2\)O, 20 mol % of Na ascorbate in 273 µl of D\(_2\)O, and 0.65 mL of dry CH\(_2\)Cl\(_2\) were used. Chromatography using hexanes followed by 45% EtOAc in hexanes gave a white solid. \( R_f (\text{SiO}_2/75\% \text{ EtOAc in hexanes}) = 0.48 \). \(^1\)H NMR (500 MHz, CDCl\(_3\)): \( \delta \) 9.41 (br s, 1H, NH), 7.70 (d, 2H, Ar-H, \( J = 7.9 \) Hz), 7.49 (s, 1H, Ar-H), 7.22 (d, 2H, Ar-H, \( J = 7.9 \) Hz), 6.44 (t, 1H, H-1’, \( J = 6.5 \) Hz), 5.33 (app dt, 1H, H-3’, \( J_{\text{app}} = 4.6, 9.1 \) Hz), 4.49-4.47 (m, 1H, H-4’), 4.02 (dd, 1H, H-5’, \( J = 2.4, 11.6 \) Hz), 3.88 (dd, 1H, \( J = 2.4, 11.6 \) Hz), 3.00 (app dt, 1H, H-2’, \( J_{\text{app}} = 5.9, 13.1 \) Hz), 2.63 (ddd, 1H, H-2’, \( J = 6.8, 8.5, 14.6 \) Hz), 2.37 (s, 3H, CH\(_3\)), 1.94, (s,
3H, CH₃), 0.93 (s, 9H, *tert*-Bu), 0.13 and 0.12 (2s, 6H, SiCH₃). ^13^C NMR (125 MHz, CDCl₃): δ 163.8, 150.5, 148.6, 138.5, 135.5, 129.8, 127.7, 125.9, 118.5 (t, *J* = 28.7 Hz), 111.3, 85.8, 85.0, 62.9, 59.8, 38.8, 26.1, 21.4, 18.6, 12.7, –5.1, –5.2. ^2^H NMR (77 MHz, CHCl₃): δ 7.85. HRMS (ESI) calculated for C₂₅H₃₅DN₅O₄Si [M + H]⁺ 499.2594, found 499.2600.

**6-[5-Deutero-4-(4-methylphenyl)-1,2,3-triazol-1-yl]-9-[2,3,5-tri-O-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]purine (26)**

For this reaction 0.198 mmol of azide, 0.395 mmol of alkyne, 5 mol% of anhydrous CuSO₄ in 0.2 mL of D₂O, 10 mol % of Na ascorbate in 0.2 ml of D₂O, and 0.95 mL of CH₂Cl₂ were used. Chromatography using hexanes followed by 15% EtOAc in hexanes gave a pale-yellow, viscous liquid. *R*₇ (SiO₂/20% EtOAc in hexanes) = 0.28. ^1^H NMR (500 MHz, CDCl₃): δ 8.97 (s, 1H, Ar-H, *J* = 7.8 Hz), 8.65 (s, 1H, Ar-H), 7.91 (d, 1H, Ar-H, *J* = 8.0 Hz), 7.29 (d, 2H, Ar-H, *J* = 8.0 Hz), 6.24 (d, 1H, H-1', *J* = 5.1 Hz), 4.68 (t, 1H, H-2', *J* = 4.7 Hz), 4.35 (t, 1H, H-3', *J* = 3.9 Hz), 4.20 (app q, 1H, H-4', *J* app = 3.0 Hz), 4.06 (dd, 1H, H-5', *J* = 3.5, 11.4 Hz), 3.85 (dd, 1H, H-5', *J* = 2.4, 11.4 Hz), 2.41 (s, 3H, CH₃), 0.99, 0.96, and 0.81 (3s, 27H, *tert*-Bu), 0.19, 0.18, 0.13, 0.12, –0.02, and –0.21 (6s, 18H, Si-CH₃). ^13^C NMR (125 MHz, CDCl₃): δ 154.5, 152.5, 148.5, 145.2, 145.0, 138.8, 129.8, 127.5, 126.4, 123.5, 119.5 (t, *J* = 26 Hz), 88.9, 86.1, 76.5, 72.3, 62.8, 26.4, 26.1, 25.9, 21.5, 18.8, 18.3, 18.1, –4.1, –4.3, –4.4, –4.6, –4.7, –5.1. ^2^H NMR (77 MHz, CHCl₃): δ 9.35. HRMS (ESI) calculated for C₃₈H₆₀DN₇O₄Si₃Na [M + Na]⁺ 775.4048, found 775.4046.

**5-Deutero-1-phenyl-D₅-4-(4-methylphenyl)-1H-1,2,3-triazole (31)**

Chromatography using hexanes followed by 20% EtOAc in hexanes gave an off-white solid. *R*₇
(SiO$_2$/50% EtOAc in hexanes) = 0.60. $^1$H NMR (500 MHz, CDCl$_3$):  
\[ \delta 7.83 \text{ (d, 2H, Ar-H, } J = 7.8 \text{ Hz)}, \delta 7.29 \text{ (d, 2H, Ar-H, } J = 7.8 \text{ Hz), 2.42} \text{ (s, 3H, -CH$_3$).} \]  
$^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 148.6, 138.5, 137.2, 129.8, 129.5 (t, $J = 25.0$ Hz), 128.4 (t, $J = 25.0$ Hz), 127.6, 126, 120.3 (t, $J = 24.9$ Hz), 117.2 (t, $J = 29.4$ Hz), 21.5. $^2$H NMR (77 MHz, CHCl$_3$): $\delta$ 8.19, 7.82, 7.58, 7.49. HRMS (ESI) calculated for C$_{13}$H$_9$D$_6$N$_3$ [M + H]$^+$ 242.1559, found 242.1553.

5'-O-(Tert-butyldimethylsilyl)-3'-deoxy-3'-(5-deutero-4-(phenyl-D$_5$)-1H-1,2,3-triazol-1-yl)thymidine (34)

For this reaction 0.131 mmol of azide, 0.262 mmol of alkyne, 10 mol % of anhydrous CuSO$_4$ in 0.273 mL of D$_2$O, 20 mol % of Na ascorbate in 0.273 ml of D$_2$O, and 0.65 mL of dry CH$_2$Cl$_2$ were used in this reaction. Chromatography using hexanes followed by 50% EtOAc in hexanes gave a white solid. $R_f$ (SiO$_2$/50% EtOAc in hexanes) = 0.21. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 9.20 (br s, 1H, NH), 7.50 (s, 1H, Ar-H), 6.45 (t, 1H, H-1', $J = 6.5$ Hz), 5.34 (dt, 1H, H-3', $J = 4.6, 8.8$ Hz), 4.49-4.51 (m, 1H, H-4'), 4.03 (dd, 1H, H-5', $J = 2.1, 11.5$ Hz), 3.88 (dd, 1H, H-5', $J = 1.8, 11.5$ Hz), 3.01 (dd, 1H, H-2', $J = 5.2, 6.3, 14.0$ Hz), 2.64 (ddd, 1H, H-2', $J = 6.5, 8.1, 14.3$ Hz), 1.95 (s, 3H, CH$_3$), 0.94 (s, 9H, tert-Bu), 0.14 and 0.13 (2s, 6H, SiCH$_3$). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 163.8, 150.5, 148.4, 135.5, 130.1, 128.6 (t, $J = 23.8$ Hz), 128.1 (t, $J = 23.0$ Hz), 125.6 (t, $J = 24.1$ Hz), 118.7 (t, $J = 28.8$ Hz), 111.4, 85.6, 85.0, 62.8, 59.8, 38.9, 29.9, 26.1, 18.6, 12.8, -5.1, -5.2. $^2$H NMR (77 MHz, CHCl$_3$): $\delta$ 7.87, 7.45. HRMS (ESI) calculated for C$_{24}$H$_{28}$D$_8$N$_5$O$_4$Si [M + H]$^+$ 490.2751, found 490.2766.

5-Deutero-1,4-diphenyl-D$_{10}$-1H-1,2,3-triazole (35)
Chromatography using hexanes followed by 20% EtOAc in hexanes gave an off-white solid. \( R_f \) (SiO\textsubscript{2}/50% EtOAc in hexanes) = 0.60. 

\[ ^{13}\text{C} \text{NMR (125 MHz, CDCl}_3): \delta 148.5, 137.2, 130.3, 129.5 \text{ (t, } J = 24.3 \text{ Hz)}, 128.6 \text{ (t, } J = 23.3 \text{ Hz)}, 128.5\text{ (t, } J = 23.1 \text{ Hz)}, 128.1 \text{ (t, } J = 21.8 \text{ Hz)}, 125.7 \text{ (t, } J = 24.2 \text{ Hz)}, 120.3 \text{ (t, } J = 25.2 \text{ Hz)}, 117.6 \text{ (t, } J = 26.9 \text{ Hz)}. \]

\[ ^2\text{H} \text{NMR (77 MHz, CHCl}_3): \delta 8.23, 7.94, 7.83, 7.58, 7.49. \]

HRMS (ESI) calculated for C\textsubscript{14}H\textsubscript{11}D\textsubscript{11}N\textsubscript{3} [M + H]\textsuperscript{+} 233.1716, found 233.1717.

Assessment of %D in 33. CH\textsubscript{2}Cl\textsubscript{2} (16 \text{ µL, 0.25 mmol}) was added to the solution of 33 (11.7 mg, 0.05 mmol) in CDCl\textsubscript{3} (0.5 mL). The \(^1\text{H} \text{NMR of this solution was acquired and the resonances were integrated. The signal at } \delta 5.30 \text{ ppm (CH}_2\text{Cl}_2) \text{ was set to 10 whereby the signal at } \delta 8.19 \text{ ppm (residual triazolyl C5-H) integrated to 0.06. Thus, the %D incorporation in the reaction was estimated to be 94%}. \]

1-(3-Cyanophenyl)-4-(pyridin-3-yl)-1H-1,2,3-triazole (29)\textsuperscript{33}

In a clean, extremely dry, 10 mL round-bottom flask equipped with a stirring bar, m-azidobenzonitrile (25, 57 mg, 0.335 mmol) was dissolved in CH\textsubscript{2}Cl\textsubscript{2} (1.6 mL). 3-Ethynylpyridine (26, 69 mg, 0.67 mmol) was added to the flask, followed by CuSO\textsubscript{4}·5H\textsubscript{2}O (8.4 mg, 0.0335 mmol), Na ascorbate (13.3 mg, 0.067 mmol), and H\textsubscript{2}O (1.4 mL). The mixture was stirred at room temperature for 48 h and then diluted with CH\textsubscript{2}Cl\textsubscript{2} (5 mL). The aqueous layer was separated and back extracted with CH\textsubscript{2}Cl\textsubscript{2} (3 x 5 mL). The combined organic layers were washed with brine (5 mL), dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}, and concentrated under reduced pressure. Chromatography of the crude material on a short silica gel plug, by sequential elution
with 10% EtOAc in hexanes followed by EtOAc, yielded 58 mg (70% yield) of 29 as an off-white solid. $R_f$ (SiO$_2$/EtOAc) = 0.34. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 9.13 (br s, 1H, Ar-H), 8.68 (br s, 1H, Ar-H), 8.33 (s, 1H, Ar-H), 8.31 (d, 1H, Ar-H, $J$ = 7.7 Hz), 8.15 (t, 1H, Ar-H, $J$ = 1.5 Hz), 8.11 (ddd, 1H, Ar-H, $J$ = 1.1, 2.1, 8.1 Hz), 7.79 (dt, 1H, Ar-H, $J$ = 1.2, 7.8 Hz), 7.73 (t, 1H, Ar-H, $J$ = 7.9 Hz), 7.47 (br s, 1H, Ar-H). $^{13}$C NMR (125 MHz, CD$_3$OD, 55 °C): $\delta$ 150.2, 147.8, 146.8, 139.1, 135.2, 133.7, 132.5, 128.4, 126.1, 125.7, 125.1, 121.4, 118.7, 115.4.

**Competitive Experiment using H$_2$O and D$_2$O**

In a clean, extremely dry, 10 mL round-bottom flask equipped with a stirring bar, was placed the phenyl azide (14, 40.0 mg, 0.335 mmol) in dry CH$_2$Cl$_2$ (1.6 mL). To this stirring solution $p$-ethynyl toluene (15, 85 µL, 0.67 mmol) was added. In a separate vial CuSO$_4$•5H$_2$O (8.4 mg, 0.0335 mmol, 10 mol %) was heated at 120 °C under vacuum until the color changed from blue to grey. Using this dehydrated CuSO$_4$, a 0.048 M solution was prepared in a glove bag by the addition of D$_2$O (99.8% D, 0.7 mL) and this solution was transferred to the reaction mixture. To the reaction mixture a 0.098 M solution of Na ascorbate (13.3 mg in 0.7 mL of 99.8% H$_2$O, 20 mol %) was then added. The reaction mixture was sealed and stirred under nitrogen gas at room temperature until TLC showed consumption of azide. Then the organic layer of the reaction mixture was separated and the aqueous layer was back extracted with EtOAc (3 x 5 mL). The organic layers were combined and washed with brine (5 mL), dried over anhydrous Na$_2$SO$_4$, and concentrated under reduced pressure. The crude material was purified by chromatography on a silica gel column to give 60 mg of an off-white solid. On the basis of the $^1$H NMR, this mixture
contained 15.6 mg (19.7 %) of 16a and 44.4 mg (56.3 %) of 16b. \( R_f \) (25% EtOAc in hexanes/SiO\(_2\)) = 0.34.

*Assessment of the amounts of 14a and 14b in the product mixture.* CH\(_2\)Cl\(_2\) (16 \(\mu\)L, 0.25 mmol) was added to the solution of the product mixture (11.8 mg) in CDCl\(_3\) (0.5 mL). The \(^1\)H NMR of this solution was acquired and the resonances were integrated. The signal at \(\delta\) 5.30 ppm (CH\(_2\)Cl\(_2\)) was set to 10 whereby the signal at \(\delta\) 8.16 ppm (residual triazolyl C5-H) integrated to 0.77. On the basis of these values the amount of 16b was estimated at 9.058 mg (77%) and that of 16a was estimated as 2.741 mg (23%).
REFERENCES


APPENDIX

CHAPTER 1 NMR DATA
gCOSY spectrum of compound 45b in acetone-$d_6$
ONON
Me
Me
TBDMSO
TBDMSO
45d
Me
Me
Pd

0 ppm

140
160
120
100
80
60
40
20
0
Me
TBDMSO
TBDMSO
45f
gCOSY spectrum of compound 45f in CDCl₃
gCOSY spectrum of compound 45g in CDCl₃
Expansion of gCOSY spectrum of compound 11g in CDCl₃
TBDMSO

Ph

HN

N

O

TBDMSO

TBDMSO

46a
gCOSY spectrum of compound 46c in acetone-$d_6$
gHMOC spectrum of compound 46c in acetone-$d_6$
gCOSY spectrum of compound 46e in CDCl$_3$
gCOSY spectrum of compound 49c in acetone-$d_6$
gCOSY spectrum of compound 54d in CD$_3$OD
gHMOC spectrum of compound 54d in CD$_3$OD
gHMOC spectrum of compound 54d in CD$_3$OD
gCOSY spectrum of compound 55d in CD$_3$OD
gCOSY spectrum of compound 56b in CD$_3$OD
gHMQC spectrum of compound 56b in CD$_3$OD
gCOSY spectrum of compound 57c in CD$_3$OD
CHAPTER 2 NMR DATA

![NMR Spectrogram]

- Signals at 6 ppm
- Peak at 2.1 ppm
- Additional peaks at 3.0, 4.0, and 5.0 ppm

Chemical structure with labels:
- NH<sub>2</sub>
- OTBDMS
- TBDMSO
- 63
TBDMSO
TBDMSO
NH
Me
Me
64d
OBz

BzO

(±) 27
gCOSY spectrum of compound ((±)-34) in CD$_3$OD
Cl^+NH_3^-\text{OH}\_\text{OH}\_\text{OH}\_\text{OH}\_\text{OH}^{-}\_\text{Cl}^{-} \text{ (476 ppm)}
gCOSY spectrum of compound ((§)-53) in CD$_3$OD

(§)-62
NOESY spectrum of compound ((±)-49) in CDCl₃
Expansion of NOESY spectrum of compound ((±)-45) in CDCl₃
gCOSY spectrum of compound (55a) in CDCl$_3$
Expansion of gCOSY spectrum of compound (55a) in CDCl₃
gCOSY spectrum of compound (55b) in CDCl$_3$
Expansion of gCOSY spectrum of compound (55b) in CDCl₃
gCOSY spectrum of compound (63a) in DMSO-$d_6$
Expansion of gCOSY spectrum of compound (63a) in DMSO-$d_6$

![gCOSY spectrum of compound (63a)](image-url)
gCOSY spectrum of compound (63b) in DMSO-$_{d_6}$
Expansion of gCOSY spectrum of compound \((63b)\) in DMSO-\(d_6\)
CHAPTER 4 NMR DATA

$^1H$ NMR of compound in CDCl$_3$ at 25°C
46

$C_6D_6$ at 70 °C
Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 °C / 298.1 K
Operator: mkl
File: 1203-HK-03-22
INOVA-500 "riga"

Pulse 38.6 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
132 repetitions

OBSERVE H1, 499.7707095 MHz
DATA PROCESSING
FT size 32768
Total time 6 min, 20 sec
1203-HX-03-23-13C

Archive directory: /export/home/mkl/vnmrsys/data
Sample directory:

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 24.0 C / 297.1 K
Operator: mkl
File: 1203-HX-03-22-13C
INOVA-500 "riga"

Relax. delay 4.000 sec
Pulse 52.1 degrees
Acq. time 1.300 sec
Width 29996.3 Hz
400 repetitions

OBSERVED C13, 125.6674109 MHz
DECOUPLE H1, 649.7743279 MHz
Power 42 dB
on during acquisition
WALTZ-16 modulated

DATA PROCESSING
Line broadening 1.0 Hz
Gauss apodization 0.200 sec
FT size 131072
Total time 117 hr, 59 min, 33 sec
1203-HK-04-21-1H-pure

Pulse Sequence: s2pul

Solvent: CDC13
Temp. 24.0 C / 297.1 K
Operator: mk1
File: 1203-HK-04-21-1H-pure
INOVA-500 "riga"

Pulse 38.6 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
44 repetitions

OBSERVE H1, 499.7707202 MHz

DATA PROCESSING
FT size 32768
Total time 6 min, 20 sec
1203-HK-04-21-13C-pure

Archive directory: /export/home/mkl/vnmrsys/data
Sample directory:

Pulse Sequence: z2pul
Solvent: CDCl3
Temp. 24.0 °C / 297.1 K
Operator: mkl
File: 1203-HK-04-21-13C-pure
INNOVA-500 "riga"

Relax. delay 4.000 sec
Pulse 52.1 degrees
Acq. time 1.300 sec
Width 29996.3 Hz
10242 repetitions

OBSERVE C13, 125.6674191 MHz
DECOUPLE H1, 699.7743279 MHz
Power 42 dB
on during acquisition
WALTZ-16 modulated

DATA PROCESSING
Line broadening 1.0 Hz
Gauss apodization 0.200 sec
FT size 131072
Total time 117 hr, 59 min, 33 sec
Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 °C / 298.1 K
Operator: mk1
File: 1203-HK-04-20-2Deuterium
INOMA-500 "riga"

Relax. delay 1.000 sec
Pulse 516.7 degrees
Acq. time 1.780 sec
Width 1150.8 Hz
100 repetitions
OBSERVE 1k, 76.7178480 kHz
DECOUPLE X1, 499.7732084 kHz
Power 39 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.1 Hz
Gauss apodization 0.400 sec
FT size 8192
Total time 5 min, 56 sec
1203-HK-04-25-1H-pure

Pulse Sequence: s2pul

Solvent: CDCl3
Temp. 24.0°C / 297.1 K
Operator: mkl
File: 1203-HK-04-25-1H-pure
INOVA-500 "riga"

Pulse 38.6 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
18 repetitions

OBSERVE: 1H, 499.7707207 MHz
DATA PROCESSING
FT size 32768
Total time 6 min, 20 sec
1203-RK-04-25-2Deutirium

Pulse Sequence: s2pul
Solvent: CDC13
Temp. 25.0 C / 298.1 K
Operator: mlk
File: 1203-RK-04-25-2Deutirium
INOVA-500 "riga"

Relax. delay 1.000 sec
Pulse 516.7 degrees
Acq. time 1.780 sec
Width 1190.8 Hz
108 repetitions

OBSERVE 1k, 76.7178492 MHz
DECOUPLE 1k, 499.7732084 MHz
Power 39 dB
continuously on
WAITY-16 modulated

DATA PROCESSING
Line broadening 0.1 Hz
Gauss apodization 0.400 sec
FT size 8192
Total time 18 min, 35 sec
1203-MK-02-Phenyl azide

Archive directory: /export/home/mkl/vnmrsys/data
Sample directory:

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Operator: mkl
File: 1203-MK-02-06PhenylAzide
INOVAS-500 "rigo"

Relax. delay 4.000 sec
Pulse 43.4 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
12 repetitions
OBSERVE H1, 499.7707197 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 32768
Total time 19 min, 40 sec
1203-HK-02-31

Pulse Sequence: s2pu1
Solvent: CDCl3
Ambient temperature
Operator: mkl
File: 1203-HK-02-31
INova-500 "riga"

Pulse 48.0 degrees
Acq. time 1.992 sec
Width 8000.0 Hz
8 repetitions
OBSERVE H1, 499.7707207 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 15 sec
N

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 24.0 C / 297.1 K
Operator: mkl
File: 1203-HK-02-45
INOVA-500 "riga"

Pulse 38.6 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
75 repetitions
OBSERVE H1, 499.7707212 MHz
DATA PROCESSING
FT size 32768
Total time 6 min, 20 sec
Pulse Sequence: s2yul
Solvent: cdc13
Temp. 25.0 C / 298.1 K
Operator: mkl
File: 1203-HK-03-15
INVUA-500 "riga"

Relax. delay 2.000 sec
Pulse 45.0 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
63 repetitions

AS95 499.37707095 MHz
DATA PROCESSING
Resol. enhancement 0.5 Hz
Gauss apodization 0.400 sec
FT size 32768
Total time 3 min, 43 sec
1203-EX-04-45-phenylazido-2Deuterium

Pulse sequence: z2pul
Solvent: CDCl3
Temp. 25.0 °C / 298.1 K
Operator: mlk
File: 1203-EX-04-19-2Deuterium-1
INova-500 "sign"

Relax. delay 1.000 sec
Pulse 515.7 degrees
Avg. time 1.780 sec
Width 1150.8 Hz
200 repetitions
OBSERVE 1k, 76.7178937 MHz
DECOUPLE H1, 459.7733084 MHz
Power 39 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING:
Line broadening 0.1 Hz
Gauss apodization 0.400 sec
FT size 8192
Total time 9 min, 17 sec
Pulse Sequence: m2pul
Solvent: CDC13
Ambient temperature
Operator: mkl
File: 1203-HX-02-08
INOVA-500 "riga"

Relax. delay 4.000 sec
Pulse 57.9 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
12 repetitions
OBSERVE H1, 499.7707222 MHz
DATA PROCESSING
FT size 32768
Total time 4 hr, 55 min, 2 sec
1203-HK-04-30-d1-3

Pulse Sequence: s2pul
Solvent: CCl3
Temp: 25.0 C / 298.1 K
Operator: mkl
File: 1203-HK-04-30-d1-3
INOWA-500 "riga"

Relax. delay 3.000 sec
Pulse 90.6 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
16 replications
OBSERVE H1, 499.7707202 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 18 sec
Pulse Sequence: s2pol
Solvent: CDCl3
Temp. 24.0 C / 297.1 K
Operator: mk1
File: 1203-02-63-19c-final
INova-500 "riga"
Relax. delay 4.000 sec
Pulse 52.1 degrees
Acq. time 1.300 sec
Width 29996.3 Hz
1676 repetitions
OBSERVE C13, 125.6674132 MHz
DECOMPLEX H1, 499.7741279 MHz
Power 42 dB
on during acquisition
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
Gauss apodization 0.200 sec
FT size 131072
Total time 117 hr, 59 min, 33 sec
Pulse Sequence: z2pol
Solvent: CDCl3
Ambient temperature
Operator: mk1
File: 1203-EX-02-07-Deutirium
INOWA-500 "rigo"

Relax. delay 4.000 sec
Pulse 1000.0 degrees
Acq. time 2.636 sec
Width 1536.7 Hz
32 repetitions

Observer 1k, 76.7173670 MHz
Decouple H1, 496.7731086 MHz
Power 40 dB
continuously on
WALKE-16 modulated

DATA PROCESSING
FT size 8192
Total time 3 min, 32 sec
1203-EX-02-63-15C-pure

Archive directory: /export/home/mkl/vmanSys/data
Sample directory:

Pulse Sequence: m2pul
Solvent: CDCl3
Temp. 24.0 °C / 297.1 K
Operator: mkl
File: 1203-EX-02-63-15C-pure
INova-500 “riga”

Relax. delay 4.000 sec
Pulse 52.1 degrees
Acq. time 1.300 sec
Width 29996.3 Hz
1620 repetitions

OBSERVE C13, 125.6674122 MHz
DECOUPLE H1, 499.7742792 MHz
Power 42 dB
cc during acquisition
WALTZ-16 modulated

DATA PROCESSING
Line broadening 1.0 Hz
Gauss apodisation 0.200 sec
FT size 131072
Total time 117 hr, 59 min, 33 sec
Pulse Sequence: "NOMA"
Solvent: CDCl3
Temp. 298.15 K
Operator: skl
File: 1203-EX-02-49-1M
INOVA-500 "riga"

Relax. delay 1.000 sec
Pulse 65.0 degrees
Acq. time 1.892 sec
Width 0.0000 Hz
14 repetitions

OBSERVE 60.77/7095 MHz
DATA PROCESSING
Baseline enhancement 0.5 Hz
Gauss apodization 0.600 sec
FT size 32768
Total time 5 min, 43 sec
Pulse Sequence: s2pol
Solvent: CDCl3
Ambient temperature
Operator: mkl
File: 1203-EX-02-49-13C-final
Methylene 500 "riga"

Pulse 70.1 degrees
Acq. time 1.300 sec
Width 25000.0 Hz
40624 repetitions
OBSERVE C13, 125.657625 MHz
DECOUPLE H1, 499.7732086 MHz
Power 60 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 18 hr, 11 min, 9 sec
1203-HK-02-49-2Deuterium

Pulse Sequence: <2pul>
Solvent: CDCl3
Temp. 25.0 °C / 298.1 K
Operator: mkl
File: 1203-HK-02-49-2Deuterium
INOVA-500 "riga"

Relax. delay 1.000 sec
Pulse 516.7 degrees
Acq. time 1.780 sec
Width 1150.8 Hz
200 repetitions

OBSERVE 1k, 76.7178489 MHz
DECOUPLE 1k, 495.7735084 MHz
Power 39 dB
continuously on

WALTZ-16 modulated

DATA PROCESSING
Line broadening 0.1 Hz
Gauss apodization 0.400 sec
FT size 8192
Total time 9 min, 17 sec
1201-HK-02-39

Pulse Sequence: ad2pul

Solvent: cdcl3

Temp. 25.0 C / 298.1 K

Operator: mkl

File: 1201-HK-02-39

IMAGA-500 "riga"

Relax. delay 2.000 sec

Pulse 45.0 degrees

Acq. time 1.692 sec

Width 8000.0 Hz

13 repetitions

OBSERVE 81.4997787197 MHz

DATA PROCESSING

Baseline enhancement 0.5 Hz

Gauss apodisation 0.600 sec

FT size 32768

Total time 5 min, 43 sec

MeO

N=N

D

F

19
N=N
|     |
|     |
|     |
|     |

F

D

MeO

19
1203-HE-02-59-2Deuterium

Pulse Sequence: s2pul
Solvent: CDC13
Temp. 25.0 C / 298.1 K
Operator: mkl
File: 1203-HE-02-59-2Deuterium
INOWA-500 "rigs"

Relax. delay 1.000 sec
Pulse 516.7 degrees
Acq. time 1.780 sec
Width 1150.8 Hz
150 repetitions

OBSERVE 1k, 56.7178480 MHz
DECOPLING 31, 499.7732084 MHz
Power 39 dB
continuously on

DATA PROCESSING
Line broadening 0.1 Hz
Gate suppression 0.400 sec
FT size 8192
Total time 9 min, 17 sec
Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 °C / 298.1 K
File: 1203-EK-02-59-19F
INOUA-50D "zigs"

Relax. delay 4.000 sec
Pulse 25.0 degrees
Acq. time 0.300 sec
Width 100.0 kHz
24 repetitions
OBSERVE F19, 282.1394306 MHz
DATA PROCESSING
Line broadening 2.0 Hz
FT size 83536
Total time 2 hr, 23 min, 36 sec
Pulse Sequence: z2pol
Solvent: cdcl3
Temp. 25.0 C / 298.1 K
Operator: mkl
File: 1203-EX-02-48-pure
INova-500 “riga”

Relax. delay 2.000 sec
Pulse 45.0 degrees
Acq. time 1.092 sec
Width 8000.0 Hz
25 repetitions
OBSERVE H1, 499.7707217 MHz
DATA PROCESSING
Resol. enhancement 0.5 Hz
Gauss apodisation 0.600 sec
FT size 32768
Total time 5 min, 43 sec
Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
Operator: mkl
File: 1203-EN-02-88-2Deuterium
INOMA-500 “riga”

Relax. delay 1.000 sec
Pulse 516.7 degrees
Acq. time 1.780 sec
Width 1150.8 Hz
128 repetitions

DESOSE 1k, 76.7170478 MHz
DECOMPLX 81, 499.77132086 MHz
Power 39 dB
continuously on
WALTE-16 modulated

DATA PROCESSING
Line broadening 0.1 Hz
Gauss apodisation 0.400 sec
FT size 8192
Total time 5 min, 56 sec
1203-EK-02-57

Pulse Sequence: s2pol
Solvent: cdc13
Temp. 25.0 °C / 298.1 K
Operator: mkl
File: 1203-EK-02-57
INova-500 "riga"

Relax. delay 2.000 sec
Pulse 45.0 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
24 repetitions

OBSERVE H1, 499.7707202 MHz
DATA PROCESSING
Baseline enhancement 0.5 Hz
Gauss apodization 0.600 sec
FT size 22768
Total time 5 min, 43 sec
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Operator: mk1
File: 1203-EX-02-57-13C
ISOVA-50D "rigs"

Pulse 70.1 degrees
Acq. time 1.300 sec
Width 25000.0 Hz
39480 repetitions
OBSERVE C13, 125.6634232 MHz
DECOUPLE H1, 499.7712084 MHz
Power 60 dB
continuously on
WALTZ-16 modulated

DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 18 hr, 11 min, 9 sec
Pulse Sequence: m2pu1
Solvent: CDCl3
Temp. 25.0 °C / 298.1 K
Operator: Mxl
File: 1203-MX-02-57-2Deuterium
INova-500 "riga"

Relax. delay 1.600 sec
Pulse 516.7 degrees
Acq. time 1.780 sec
Width 1150.8 Hz
200 repetitions

OBSERVE 1k, 76.7178689 MHz
DECOUPLE H1, 499.7732084 MHz
Power 39 dB
continuously on

DATA PROCESSING
Line broadening 0.1 Hz
Gauss apodization 0.400 sec
FT size 6192
Total time 9 min, 17 sec
1201-EX-02-61-13C-pure

Archive directory: /export/home/skl/vnmrj/nmr/data
Sample directory:

 Pulse Sequence: s2pal
 Solvent: CDCl3
 Temp. 24.0 C / 297.1 K
 Operator: skl
 File: 3200-EX-02-61-13C-pure
 INOVA-500D "rigs"

relax. delay 6.000 sec
Pulse 52.1 degrees
Acq. time 1.300 sec
Width 29996.3 Hz
1429 repetitions

observe C13, 125.6674198 MHz
Decouple H1, 698.7743079 MHz
Power 42 dB
on during acquisition

Wait-16 modulated

DATA PROCESSING
Line broadening 1.0 Hz
Gauss apodization 0.200 sec
FT size 131072
Total time 117 hr, 59 min, 32 sec
1H03-EX-02-61-2Deuterium

Pulse Sequence: a2pol
Solvent: CDCl3
Temp. 25.0 °C / 298.1 K
Operator: mkl
File: 1H03-EX-02-61-2Deuterium

Relax delay 1.000 sec
Pulse 516.7 degrees
Acq. time 1.780 sec
Width 1150.8 Hz
112 repetitions

OBSERVE 1k, 76.1778478 MHz
DECouple: R1, 699.7732084 MHz
Power 39 dB
continuously on

WALD-16 modulated
DATA PROCESSING
Line broadening 0.1 Hz
Gauss apodization 0.400 sec
FT size E192
Total time 9 min, 17 sec
Pulse Sequence: s2pal
Solvent: CDCl3
Ambient temperature
Operator: mkl
File: 1203-NE-02-5B-13C
INova-500 "riga"

Pulse 70.1 degrees
Acq. time 1.300 sec
Width 25000.0 Hz
36140 repetitions
OBSERVE C13, 125.6674271 MHz
DECOUPLE H1, 499.7732086 MHz
Power 40 SB continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broading 0.5 Hz
FT size 66516
Total time 18 hr, 11 min, 9 sec
1H3-EX-02-5E-2Deuterium

Pulse sequence: s2pul
Solvent: CDCl3
Temp. 25.0 °C / 298.1 K
Operator: mki
File: 1H3-EX-02-5E-2Deuterium

Relax. delay 1.000 sec
Pulse 515.7 degrees
Acq. time 1.780 sec
Width 1130.8 Hz
165 repetitions

OBSEVE 1k, 76.7175478 MHz
DECouple H1, 499.7722084 MHz
Power 39 dB
continuously on

DATA PROCESSING
Line broadening 0.1 Hz
Gauss apodization 0.600 sec
FT size 6192
Total time 9 min, 17 sec
1203-EK-03-24D

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 24.0 °C / 297.1 K
Operator: mkl
File: 1203-EK-03-24D
INOVA-500 "riga"

Pulse 38.6 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
123 repetitions
OBSERVE R1, 499.7707228 MHz
DATA PROCESSING
FT size 32768
Total time 6 min, 20 sec
Pulse Sequence: sdpul
Solvent: CDCl3
Temp. 25.0°C / 298.1 K
Operator: mkl
File: 1203-MK-03-24D-2deuterium
INova-50D "ziga"
Relax. delay 1.000 sec
Pulse 516.7 degrees
Acq. time 1.780 sec
Width 1350.8 Hz
400 repetitions
OBSERVE 1K, 76.1108613 MHz
DECOUPLER H1, 499.7733084 MHz
Power 39 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.1 Hz
Gauss apodization 0.400 sec
FT size 512
Total time 18 min, 35 sec
500 MHz gCOSY NMR spectrum of 25

![NMR spectrum image]
1203-EK-02-71-13C-final

Archive directory: /expert/home/ml/vmmrsa/data
Sample directory:

Pulse Sequence: m2pal
Solvent: CDCl3
Temp. 24.0 C / 297.1 K
Operator: mkl
File: 1203-EK-02-71-13C-final
INNOVA-500 "riga"

Relax. delay 4.000 sec
Pulse 53.1 degrees
Acq. time 1.300 sec
Width 29996.3 Hz
10567 repetitions
Observe C13, 125.6674155 MHz
Decouple H1, 499.7743279 MHz
Power 43 dB
cc during acquisition
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
Gauss apodisation 0.200 sec
FT size 131072
Total time 217 hr, 59 min, 33 sec
Pulse Sequence: e2puls
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
Operator: mkl
File: 1203-END-02-71-2Deuterium

Relax. delay 1.000 sec
Pulse 516.7 degrees
Acq. time 1.780 sec
Width 1150.8 Hz
200 repetitions
OBSERVE 1H, 75.717 ppm
DECOUPLE 1H, 499.773 ppm
Power 39 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.1 Hz
Gauss apodization 0.400 sec
FT size 8192
Total time 9 min, 17 sec
Pulse Sequence: s2pul
Solvent: ccdll
Temp. 25.0 °C / 298.1 K
Operator: srl
File: 1203-EX-02-70
LBN-100 "riga"

Relax. delay 2.000 sec
Pulse 45.0 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
26 repetitions
OBSERVE H1, 499.77707655 MHz
DATA PROCESSING
Resol. enhancement 0.5 Hz
Gauss. apodization 0.600 sec
FT size 32768
Total time 5 min, 43 sec

[Graphical representation of a chemical structure with peaks labeled from 0 to 9 ppm]
500 MHz gCOSY NMR spectrum of 26
1203-EX-02-70-13C

Archive directory: /export/home/mkl/vnmrsys/data
Sample directory:

Pulse Sequence: m2pol
Solvent: CDCl3
Temp. 24.0 C / 297.1 K
Operator: mkl
File: 1203-EX-02-70-13C

1H spectrum 500 MHz, "riga"

Relax. delay 4.000 sec
Pulse 51.1 degrees
Acq. time 1.300 sec
Width 2996.1 Hz
11663 repetitions

Spectrum: Cl3, 125.656895 MHz
DECORREL H1, 499.774227 MHz
Power 81 dB

Data Processing
Line broadening 1.0 Hz
Gauss apodisation 0.200 sec
FT size 131072
Total time 117 hr, 59 min, 33 sec
1203-MK-02-76-2Deutetium

Pulse Sequence: z2pol
Solvent: CDCl3
Temp. 25.0 °C / 298.1 K
Operator: mk
File: 1203-MK-02-76-2Deutetium

Relax. delay 1.000 sec
Pulse 516.7 degrees
Acq. time 1.780 sec
Width 1150.8 Hz
200 repetitions
OBSE Hass. 76.5178579 MHz
DECOUPLE I:\, 498.7738084 MHz
Power 39 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.1 Hz
Gauss spedgradation 0.400 sec
FT size 8192
Total time 9 min, 17 sec

[Graph of a molecular structure with labels TBDMSO and 26]
Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 24.0 C / 297.1 K
Operator: skl
File: 1203-HK-06-23-1-pure
INOVA-500 "riga"

Pulse 28.6 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
49 repetitions

OSERVE B1, 499.7707095 MHz
DATA PROCESSING
FT size 32768
Total time 6 min, 20 sec
Pulse Sequence: s2pol
Solvent: CDCl3
Temp. 24.0 °C / 297.1 K
Operator: mk1
File: 1201-EX-04-23-2-13C
INova-300 "riga"

Relax. delay 4.000 sec
Pulse 52.1 degrees
Acq. time 1.300 sec
Width 29996.3 Hz
11833 repetitions
OBSERVE C13, 125.4674205 MHz
DECOUPLE H1, 498.7442379 MHz
Power 62 dB
on during acquisition
WALTZ-16 modulated

DATA PROCESSING
Line broadening 1.0 Hz
Gauss apodisation 0.200 sec
FT size 131072
Total time 117 hr, 59 min, 33 sec
Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
Operator: mkl
File: 1203-EK-00-23-2deutirium-1
INova-500 "rigs"

Relax. delay 1.000 sec
Pulse 516.7 degrees
Acq. time 1.780 sec
Width 1150.8 Hz
200 repetitions
OBSERVE 1k, 76.7178689 MHz
DECOUPLE 1k, 499.7732086 MHz
Power 39 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.1 Hz
Gauss apodization 0.400 sec
FT size 8192
Total time 9 min, 17 sec
1203-HK-04-36-1H-pure

Pulse Sequence: m2pul

Solvent: CDCl3
Temp. 24.0 C / 297.1 K
Operator: mkl
File: 1203-HK-04-36-1H-pure
INOWA-500 "zign"

Pulse 38.6 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
146 repetitions
OBSERVE H1, 499.7707212 MHz
DATA PROCESSING
FT size 32768
Total time 6 min, 20 sec

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TBDMSO
Pulse Sequence: s2pal
Solvent: CDCl3
Temp. 298.0 K
Operator: mkk
File: 1203-EK-04-26-13C-final
INOVA-500 "ziga"

Relax. delay 4.000 sec
Pulse 51.1 degrees
Acq. time 1.301 sec
Width 34995.6 Hz
11759 repetitions
OBSERVE C13, 125.6674218 MHz
DECOUPLE H1, 499.77174379 MHz
Power 41 dB
oc during acquisition
WHITE-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
Gauss apodization 0.200 sec
FT size 131072
Total time 27 hr, 42 min, 14 sec
1201-EN-04-26-2deutrium

Pulse Sequence: x2ps1
Solvent: CDC13
Temp. 25.0°C / 298.1 K
Operator: skl
File: 1201-EN-04-26-2deutrium
INNVA-500 "ziga"

Relax. delay 1.600 sec
Pulse 516.7 degrees
Acq. time 1.780 sec
Width 1150.8 Hz
200 repetitions

OBSERVE 1x, 76.1778500 MHz
DECOMPLEX H1, 499.7732084 MHz
Power 39 dB
continuously on
WALTZ-16 modulated

DATA PROCESSING
Line broadening 0.1 Hz
Gauss apodisation 0.400 sec
FT size 8192
Total time 9 min, 17 sec
Pulse Sequence: z2pul
Solvent: CDCl3
Temp. 25.0 °C / 298.1 K
Operator: mki
File: 1203-ER-06-37-2Deuterium
INVA-500 "zign"

Relax. delay 1.000 sec
Pulse 516.7 degrees
Acc. time 1.780 sec
Width 1150.6 Hz
200 repetitions
OBSERVE 1H, 76.7178528 MHz
DECOUPL E1, 699.7731084 MHz
Power 39 dB
continuously on
WAIT1-16 modulated

DATA PROCESSING
Line broadening 0.1 Hz
Gauss apodization 0.400 sec
FT size 61521
Total time 9 min, 37 sec
Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 24.0 °C / 297.1 K
Operator: mkl
File: 1203-EK-03-24-1E-pure
INova-500 "riga"

Pulse 38.6 degrees
Acq. time 1.892 sec
Width 8000.0 Hz
200 repetitions

Observe E3, 495.3707212 MHz
Data Processing
Resol. enhancement -0.0 Hz
FT size 32768
Total time 6 min, 20 sec
Pulse Sequence: m2pal
Solvent: CD3OD
Temp. 55.0 C / 328.1 K
Operator: mlk
File: 1203-03-24-13C-CD3OD
INOVVA-500 "riga"

Relax. delay 3.000 sec
Pulse 45.0 degrees
Acq. time 1.300 sec
Width 28996.0 Hz
3824 repetitions

OBSERVE C13, 128.6677437 MHz
DECouple H1, 699.7769775 MHz
Power 42 dB
on during acquisition
WALTZ-16 modulated

DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 16 hr, 45 min, 31 sec
10 mol% CuSO₄
20 mol% Na ascorbate
CH₂Cl₂/H₂O/D₂O

16a:16b = 23:77

16a, 19.7%
16b, 56.3%
CHAPTER 1


CHAPTER 2


### CHAPTER 3

1. Pott, P.: *Chirurgical observations relative to the cataract: The polypus of the nose, the cancer of the scrotum, the different kinds of ruptures, and the mortification of the toes and feet*; T.J. Carnegy, L. Hawes, W. Clarke, and R. Collins: London, 1775.


**CHAPTER 4**


**CHAPTER 5**


