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Purine Nucleosides Modified at C8 or C2 Position with (β-Halo)vinylsulfone and β-Ketosulfone Reactive Groups and Their Incorporation into DNA: Synthesis of the Organoarsenical Antibiotic Arsinothricin and Polyaromatic Hydrocarbons

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PURINE NUCLEOSIDES MODIFIED AT C8 OR C2 POSITION WITH (β-HALO)VINYLSULFONE AND β-KETOSULFONE REACTIVE GROUPS AND THEIR INCORPORATION INTO DNA: SYNTHESIS OF THE ORGANOARSENICAL ANTIBIOTIC ARSINOTHRICIN AND POLYAROMATIC HYDROCARBONS

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in

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by

Md Abu Hasan Howlader

2021

To: Dean Michael R. Heithaus
College of Arts, Sciences, and Education

This dissertation, written by Md Abu Hasan Howlader, and entitled Purine Nucleosides Modified at C8 or C2 Position with (β-Halo)vinylsulfone and β-Ketosulfone Reactive Groups and Their Incorporation into DNA: Synthesis of the Organoarsenical Antibiotic Arsinothricin and Polyaromatic Hydrocarbons, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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Date of Defense: June 29, 2021

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Andrés G. Gil
Vice President for Research and Economic Development
and Dean of the University Graduate School

Florida International University, 2021

DEDICATION

I dedicate this work to my wife Merina Pervin, my parents Halima Akter and Md Abu Hanif Howlader and my late mother-in-law Aleya Khatun for their love and support. Without their understanding and encouragement, the completion of this work would not have been possible.

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ABSTRACT OF THE DISSERTATION

PURINE NUCLEOSIDES MODIFIED AT C8 OR C2 POSITION WITH (β-HALO)VINYLSULFONE AND β-KETOSULFONE REACTIVE GROUPS AND THEIR INCORPORATION INTO DNA: SYNTHESIS OF THE ORGANOARSENICAL ANTIBIOTIC ARSINOTHRICIN AND POLYAROMATIC HYDROCARBONS

by

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Florida International University, 2021

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Modified nucleosides gained great attention as potential anticancer and antiviral therapeutics. In this dissertation, synthesis and reactivity of (β-iodovinyl)sulfone and β-ketosulfone groups incorporated into purine nucleosides at C8 or C2 positions and DNA incorporation of their 5' triphosphates have been developed. Moreover, synthesis of novel antibiotic arsinothricin (AST) as well as polycyclic aromatic hydrocarbons (PAHs) have been discussed. The 8-(1-iodo-2-tosylvinyl)-2'-deoxyadenosine and 8-(1-Iodo-2-tosylvinyl)adenosine were synthesized employing iodovinylsulfonation of 8-ethynyl precursors with TsNa/I_2/NaOAc. The 8-(β-iodovinyl)sulfonyl-2'-deoxyguanosine was prepared via radical mediated iodovinylsulfonation of 8-ethynyl-2'-deoxyguanosine with $TsNHNH_2$/KI/$(BzO)_2$. Conformationally different C2 substituted isomeric adenosine analogues were prepared by iodovinylsulfonation of 2-alkynyl precursors with TsI/NaOAc. The (β-iodovinyl)sulfone probes underwent conjugated addition-elimination reaction with

nucleophilic ammonia to give corresponding β-aminovinylsulfones. The β-ketosulfones were prepared from β-iodovinylsulfone via the intermediary β-aminovinylsulfones. The β-ketosulfones underwent efficient reactions with alkyl halides in the presence of aqueous NaOH at ambient temperature providing a diastereotopic mixture of the α-monoalkylated products. The 8-(2-tosylacetyl)-2'-deoxyadenosine was converted to its 5'-triphosphate and incorporated into double-stranded DNA by human DNA polymerase using one nucleotide gap substrates. Our work showed that the 5'-triphosphate bearing bulky β-ketosulfone group at C8 position is a good substrate for polymerase. Interesting and unexpected conversion of 2-(2-tosylacetyl)-dATP/ATP to 2-carboxylate-dATP/ATP has been observed. The 2-carboxylate-dATP efficiently incorporates into double-stranded DNA by human DNA polymerase into substrates of one nucleotide gap.

A chemoenzymatic procedure has been developed for the synthesis of antibiotic arsinothricin (AST). It involves chemical synthesis of the precursor hydroxyarsinothricin (AST-OH) from sodium arsenite via 2-chloroethyarsonic acid which was then enzymatically methylated to AST with CmArsM, the As(III)-SAM- methyltransferase. The chemical synthesis of racemic AST and the enzymatic isolation of natural L enantiomer has also been developed. It involves reduction of the *N*-acetyl protected analogue of AST-OH and subsequent methylation of the resulting trivalent arsenic intermediate with methyl iodide or condensation of the 2-chloroethyl(methyl)arsinic acid with diethylacetamidomalonate followed by deprotection and decarboxylation. In addition, several novel methods to synthesize polycyclic aromatic hydrocarbons and their “enyne” derivatives, as well their application as precursors or calibration compounds for the gas phase synthesis of PAHs will also be discussed.

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LIST OF ABBREVIATIONS

Ar	aryl
$As(GS)_3$	arsenic triglutathione
AS3MT	*S*-adenosylmethionine arsenic (III) methyltransferase
AST	arsinothricin
AST-OH	hydroxyarsinothricin
Bn	benzyl
BPO	benzoyl peroxide
calcd	calculated (HRMS)
CAN	Cerium(IV) ammonium nitrate
CRE	carbapenem-resistant *Enterobacter cloacae*
Cys	Cysteine
d	doublet (NMR)
DAPA	dihydrodipicolinate
dATP	deoxy adenosine triphosphate
DCM	dichloro methane
DMA	dimethylarsinic acid
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	nucleotide triphosphate
DTBP	di-tertiary-butyl peroxide
ESI	electrospray ionization

Et	ethyl
FAD	flavin adenine dinucleotide
FDA	Food and Drug Administration
FdC	2'-deoxy-2'-fluorocytidine
g	gram(s)
h	hour(s)
HACA	Hydrogen Abstraction-acetylene Addition
HAVA	Hydrogen Abstraction-Vinylacetylene Addition
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
Hcy	homocysteine
HIV	Human immunodeficiency virus
HMDS	Hexamethyldisilazane
HPLC	High-performance liquid chromatography
HRMS	High-resolution mass spectrometry
HSD1	hydroxy steroid dehydrogenase type 1
HSV	Herpes Simplex Virus
Hz	hertz
ICP-MS	inductively coupled plasma mass spectrometry
J	coupling constant in Hz (NMR)
L	liter(s)
m	milli; multiplet (NMR)
M	molar

m/z	mass to charge ratio (MS)
MACA	Methylidyne Addition-Cyclization-Aromatization
MAs	methylarsate
MDR TB	multidrug-resistant tuberculosis
MERS-CoV	Middle East respiratory syndrome-coronavirus
Min	minute(s)
MMA	monomethylarsonic acid
mol	mole(s)
MS	mass spectrometry
MTB	*Mycobacterium tuberculosis*
NAD	nicotinamide adenine dinucleotide
NBS	N-Bromosuccinimide
NCS	N-Chlorosuccinimide
nM	nano molar
NMR	Nuclear magnetic resonance
NXS	N-halo succinimide
^{o}C	degrees Celsius
ON	oligonucleotides
p	para
PAC	Phenyl Addition-Dehydro Cyclization
PAH	polyaromatic hydrocarbon
PCR	polymerase chain reaction
pol β	polymerase beta

q	quartet (NMR)
quin	quintet (NMR)
R_f	retention factor
RNA	ribonucleic acid
RRR	Radical-Radical Reactions
rt	room temperature
s	second(s); singlet (NMR)
SAH	S-adenosylhomocysteine
SAM	S-Adenosyl-L-methionine
t	triplet (NMR)
TBA	tributylamine
TBAF	Tetra-n-butylammonium fluoride
TBAPP	tributylammonium pyrophosphate
TBDMS	tert-Butyldimethylsilyl
TBHP	tertiary butyl hydroperoxide
t-Bu	*tert*-butyl
t-BuO$^{\cdot}$	tertiary-butyl peroxide radical
TEAA	triethylammonium acetate
TEAB	triethylammonium bicarbonate buffer
TFA	trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMAO	trimethylarsine oxide

TMS	Trimethylsilyl
Ts	Tosyl
VCAM	vascular cell adhesion molecule
VZV	Varicella Zoster Virus
WHO	World Health Organization
α	alpha
β	beta
δ	delta
μ	mu (micro)

1. INTRODUCTION

1.1. Nucleosides and nucleotides.

Nucleosides are the structural subunit of nucleic acids such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). They are composed of nucleobases covalently bonded with a five carbon sugar either ribose or deoxyribose. The nucleobase is a nitrogen containing heterocyclic aromatic compound either purine (adenine or guanine) or pyrimidine (cytosine, thymine or uracil). In ribonucleosides, the purine or pyrimidine base is linked to ribose, whereas in deoxynucleosides these bases are linked to deoxyribose. The nucleosides, adenosine, guanosine, cytidine, and uridine are formed from adenine, guanine, cytosine, and uracil, respectively. While the deoxynucleosides, deoxyadenosine, deoxyguanosine deoxycytidine, and deoxythymidine are formed from adenine, guanine, cytosine, and thymine, respectively. The nucleotides additionally have one or more phosphate groups at the sugar moiety. In the cell the nucleotides can be produced via phosphorylation of sugar's primary alcohol group at C5' position with specific kinases. They are present in DNA, RNA, and various energy carriers such as nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD). The ribonucleotides are synthesized as monophosphates that must be transformed to diphosphates and then to triphosphates before being incorporated into RNA. Moreover deoxyribonucleotides are produced from ribonucleoside diphosphates by ribonucleotide reductase enzyme before incorporation into DNA.[1] Both nucleosides and nucleotides play vital roles in DNA and RNA replication which is critical for cell proliferation. Nucleosides and nucleoside analogs are utilized in antiviral and anticancer treatment. These drugs, for the most part, are hydrophilic in nature and require specific transport proteins

to help their uptake and/or release from the cell.[2] Nucleoside transporters are thought to play a key role in the transport of nucleoside analogues within the cells, where they are phosphorylated by nucleoside kinases.[3] Certain nucleotide analogs (e.g., 2',3'-dideoxy derivatives) can be incorporated into DNA by DNA polymerases and terminate the chain elongation,[4] induce gene mutation[5] as well as cell death.[6]

1.1.1. Nucleoside analogs as anticancer drugs

Since nucleosides play an important role in human metabolic process, continuous effort has been made to reveal facts about nucleosides and their derivatives. There are numerous anticancer and antiviral drugs have been invented by modifying the different parts of base and sugar moiety of the nucleosides. A number of nucleoside analogues have been synthesized and some of them shown inhibitory activity toward adenosine deaminase.[7] Currently, several nucleosides analogues such as Cytarabine, Fludarabine, Cladribine, Gemcitabine, Clofarabine, Nelarabine, Capecitabine, Floxuridine, Deoxycoformycin are being used as anti-cancer drugs.[8] There are several potent anticancer drugs have been developed by modifying purine or pyrimidine nucleoside. For example, Cytarabine is being used to treat acute myeloid leukemia[9], while Decitabine is used for treatment of myelodysplastic syndromes.[10] Some of the current approved nucleoside/tide analogues by US Food and Drug Administration (FDA) for the treatment of various cancers are listed in Figure 1, in which modified positions are highlighted in blue. Besides several nucleoside analogs such as Sapacetabine[11], 8-chloroadenosine[12], 8-aminoadenosine[13] are waiting for the approval of FDA for clinical use. The approval of numerous nucleoside/tide analogs in the past decade demonstrates that there is still an ample opportunity to develop new candidates for cancer treatment.

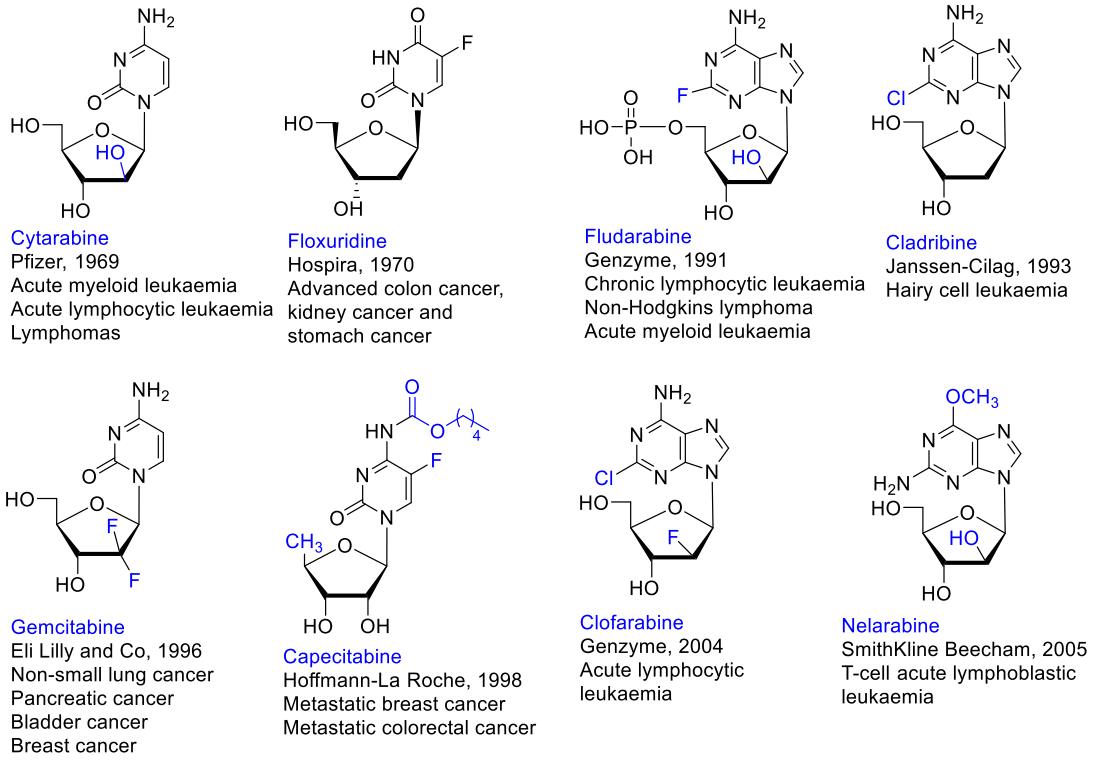


Figure 1. FDA approved anticancer purine and pyrimidine nucleoside analogs

1.1.2. Nucleoside analogs as antiviral drugs

Nucleoside analogues are well-known medications that can cure a host of viral infections, such as Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Influenza Virus, Herpes Simplex Virus (HSV), Human Immunodeficiency Virus, and Varicella Zoster Virus (VZV). For example, Didanosine, Emtricitabine, Zalcitabine, Abacavir, Zidovudine are used to treat HIV virus infection. While, Lamivudine, Entecavir, Telbivudine are used to treat hepatitis B virus and Idoxuridine and Trifluridine are used to treat herpes simplex keratitis. Furthermore, Azacitidine and Decitabine are used as demethylating agents.[8] Several 2'-deoxy-2'-fluorocytidine (FdC) derivatives, such as the methylated version of FdC (PSI-6130), are well-known anti-HCV drugs.[14] Festinavir is a chemically synthesized first-generation anti-HIV nucleoside analog, while stavudine has greater potency and can

lower the mitochondrial toxicity.[15] Mericitabine in combination with pegylated interferon and ribavirin is very effective to inhibit hepatitis C viral replication.[16] Cyclopropavir, a guanosine derivative similar to actyclovir and ganciclovir in structure, is effective against a variety of herpesviruses.[17] Some of the nucleoside/tide analogues approved by US Food and Drug Administration (FDA) for the treatment of viral infection are shown in Figure 2, with modified positions highlighted in blue.[8]

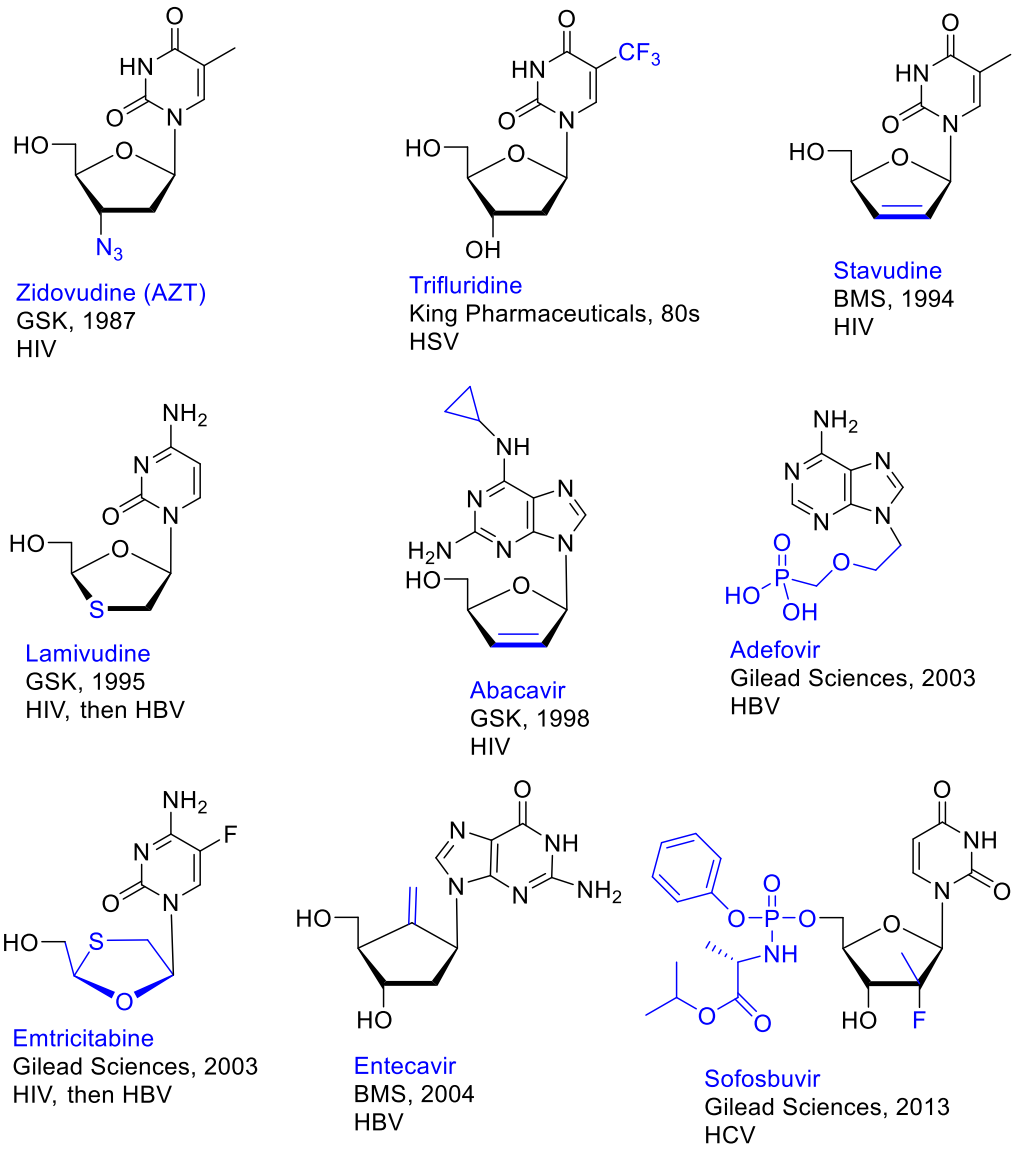


Figure 2. FDA Approved antiviral purine and pyrimidine nucleoside analogs

1.1.3. Base modified nucleosides as reactive probes for bioconjugation

DNA-protein conjugates gain interest because they add DNA programmability with varied functionalities of the protein molecules. Bioconjugation of DNA to protein can be achieved by expressing the interest of protein with chemical means that reacted with functional groups modified DNA. Controlled hybridization of DNA-protein conjugates permit enzyme cascades construction.[18] It also permits detection of proteins by using proximity dependent DNA ligation assays.[19] To construct biochips and biosensors, solid support functionalization of DNA-protein conjugates have also been reported.[20] Combination of proteins function and recognition capacity of DNA is very important to immune-PCR.[21] Bioconjugation of DNA fragments with numerous molecular compounds and various materials has reported to create different nanoscaled controlled functional devices.[22,23] For example, the particular molecular recognition capacity of complementary DNA sequences has been used for designing micrometer and nanometer scale immobilized solid support with DNA-protein conjugates. [24,25] Moreover, DNA-protein conjugates have been used to form fluorescence resonant energy transfer systems in nanobiotechnology.[26,27] Furthermore, DNA-protein hybrid has been used to develop universal adapter for protein detection system which does not necessary secondary antibodies.[28]

DNA-Protein bioconjugates preparation can involve several difficulties, as in protein molecules there are several functional groups in the different amino acid side chains those can form nonspecific conjugation. On the other hand, there are few functional groups in DNA molecules and most of the reactive functional groups (e.g., $-NH_2$, -NH, >C=O) in DNA are necessary for Watson-Crick base pairing. Therefore, for DNA-protein conjugation, it is necessary to incorporate a new reactive functional group in one of the

nucleotides of DNA oligonucleotide, which preferentially would not impede stabilities because of potential interfering with "natural" base pairing. The addition of reactive functionalities such as alkyne, alkene, azide, diene, or aldehyde at the base moiety of nucleosides/nucleotides and their incorporation into DNA offer good programmability to connect the modified DNA with important biomolecules using click chemistry,[29-31] Staudinger ligation,[32] Diels-Alder reactions[33] and other techniques. Hocek's group inserted several reactive groups into the bases of pyrimidime and purine nucleosides and examined their bioconjugation with amino acid, peptides or proteins (Figure 3).[34]

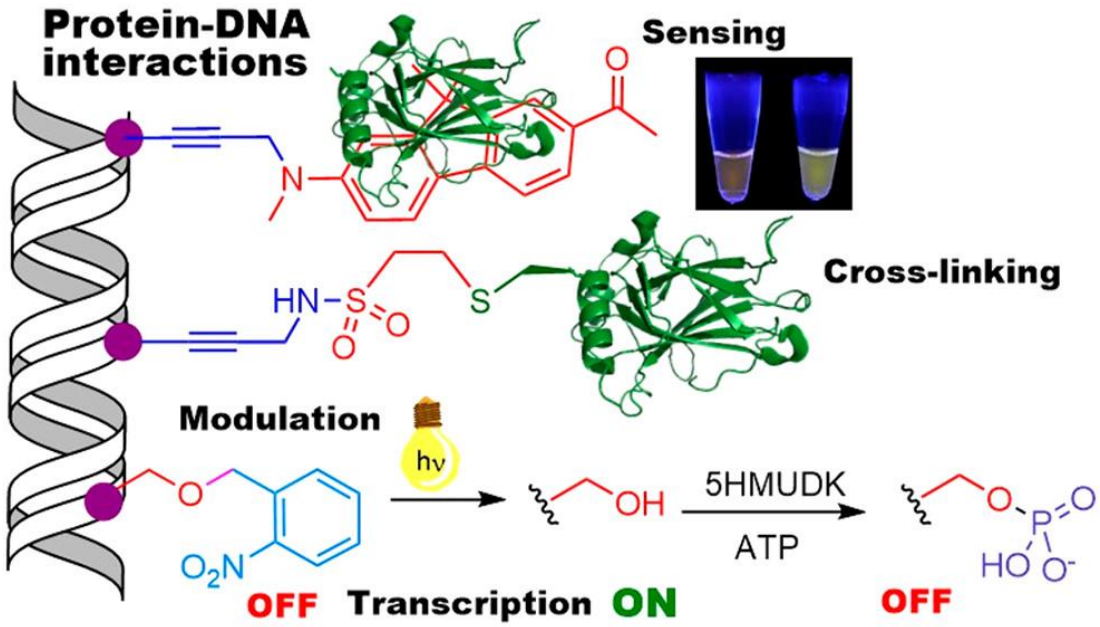


Figure 3. Bioconjugation modified DNA with protein and modulation and switching of protein-DNA interactions (*Copyright © 2020, American Chemical Society)*

For example, modified DNA with a reactive aldehyde functional group at 5 position of 2'-deoxycytidine was bioconjugated with amino acid lysine and peptides.[35] Hocek group reported the synthesis of oligonucleotides (ON) and DNA with reactive acrylamide or vinylsulfonamide groups, as well as their covalent cross-linking reactions with cysteine-

containing peptides and DNA binding domain of protein p53.[36] Previously, our group reported iron-catalyzed or radical-mediated halovinylsulfonation of 5-ethynyluracil nucleosides with TsNa or $TsNHNH_2$ in the presence of halogen sources and the efficient reactions of the resulting 5-(1-halo-2-tosylvinyl) probes with nucleophiles via addition−elimination.[37,38] We also reported uracil and cytosine nucleosides modified at C5 position with β-halovinylsulfones and β-ketosulfones and their reactivity with nucleophiles and electrophiles and polymerase-catalyzed incorporation into DNA.[39]

1.1.3.1. C8-modified purine nucleosides/tides

There are numerous anticancer and antiviral drugs which have been invented by modifying different parts of nucleobase and sugar moiety of the nucleosides[9,40] (see also section 1.1.1 and 1.1.2). Modifications in the base moiety of purine nucleosides at 8-position are easily accessible from the corresponding aryl halides via transition metal mediated cross-coupling reactions.[41,42] Modified nucleosides gained great attention as potential anticancer and antiviral therapeutics.[8,43] Nucleosides with a modified purine bases bearing reactive groups such as azide,[30,44] alkene,[45,46] alkyne,[46] or aldehyde[47] have been explored for imaging cellular DNA, bioconjugation with DNA-bound proteins, and applications in the fluorescent bioanalysis[43,48] of DNA and RNA. The 5′ triphosphates or 3′ phosphoroamidite of these probes were incorporated into oligonucleotides by DNA/RNA polymerases[46,49] or solid-phase synthesis.[45]

Purine nucleosides modified at C8 position[50-53] are easily accessible by transition metal-mediated cross-coupling reactions[42,54] including direct C8-H functionalization[41] and were subject of recent review.[55] The 8-modified purine analogues exhibit biological activity[56] and serve as substrates in vivo and in vitro alterations.[43,57] For example, 8-

chloroadenosine induces apoptosis in myeloma cell lines,[58] while 8-phenyl nicotinamide adenine dinucleotide derivatives show inhibitory activity against the human sirtuin SIRT2 and SIRT1.[59]

1.1.3.2. C2-modified purine nucleosides/tides

Although there are many reports on 2-modified nucleosides, which are not as abundant as 8-modified nucleosides due the complexity in the synthesis of halogenated precursors for further modifications. Namely, Nair's group reported novel protocols to prepare C2-modified purine nucleosides by utilizing palladium-catalyzed cross-coupling reactions.[60,61] Matsuda group then described several C2-substituted adenosine analogs as potent agonists against the adenosine A2 receptor.[62] The minor groove modification was mostly focused on 2'-and 4'-sugar modified derivatives.[63-67] Previously, 2-Chloroadenine[68] and 2,6-diaminopurine[69,70] dNTPs were the only reported minor-groove base-modified nucleotides as substrates for DNA polymerases.

Recently, Hocek's group have reported synthesis of six C2-substituted dATP derivatives bearing Cl, NH_2, CH_3, vinyl, ethynyl, and phenyl substituents to study the effect of substituents on their polymerase-catalyzed DNA incorporation.[46] They found that all of the dATP derivatives were good substrates in primer-extension experiments, except the bulky 2-phenyl-dATP. Among them, the vinyl-modified DNA was reacted with thiol 4-methylmercaptocoumarin, and ethynyl modified DNA was reacted with azide-conjugated Cy3 to label the minor grove. This was the first example of enzymatic synthesis of minor groove modified DNA via nucleobase modification. Later, Hocek's group reported synthesis of a set of 2-alkylamino-2'-deoxyadenosine triphosphates, which incorporated only one modified nucleotide into the primer by Therminator DNA polymerase.[71] The

allylamino-substituted DNA was utilized for the thiol-ene addition, while the propargylamino-DNA was reacted with an azide to label the DNA with a fluorescent dye in the minor groove.

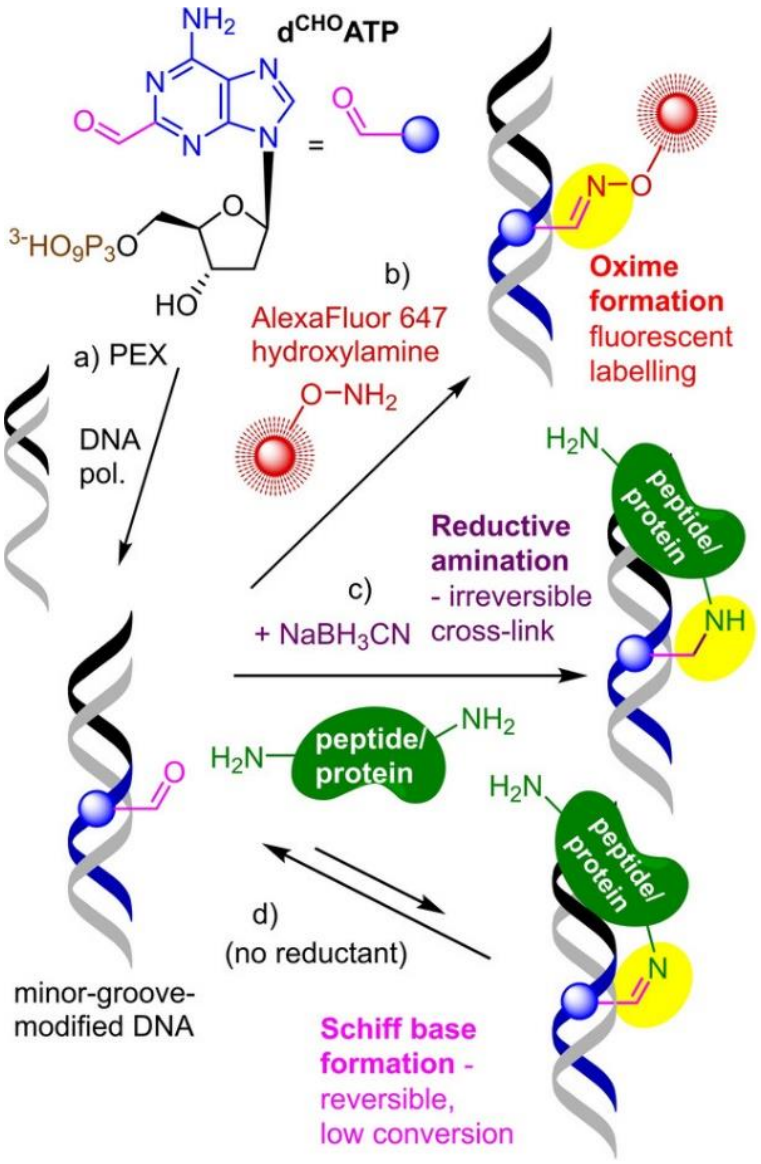


Figure 4. Polymerase synthesis (a) of minor-groove CHO-modified DNA and its conjugation with AlexaFluor647-hydroxylamine (b), cross-linking with peptides or proteins through reductive amination (c) or Schiff-base formation (d) *Copyright © 2020,* John Wiley and Sons

Recently, they also synthesized five 2-substituted 2'-deoxyinosine triphosphates containing Cl, CH_3, vinyl, ethynyl, and phenyl and tested as substrates for the enzymatic synthesis of minor-groove base-modified DNA.[72] They found only 2-methyl and 2-vinyl derivatives proved to be good substrates for Therminator DNA polymerase while the other three are poor substrates for DNA incorporation. Also, 2-formyl-2'-deoxyadenosine triphosphate was tested as a substrate for the enzymatic synthesis of minor-groove modified DNA (Figure 4).[47] The reactive formyl group was used for cross-linking with peptides and proteins as well as for fluorescent labelling through oxime formation.

1.2. Overview of (*β*-halo)vinylsulfones chemistry

A halogen atom at the *β*-position in respect to the sulfone group of the vinyl functional group is defined as a (β-halo)vinylsulfones as shown in Figure 5. Vinylsulfones are generally accepted as useful intermediate for organic synthesis and in medicinal chemistry.[73] They serve efficiently as both Michael acceptors and as 2π partners in cycloaddition reactions[74] as well as substrates in multicomponent reactions.[75] Moreover, vinylsulfone are potent inhibitors of a variety of enzymatic processes including vascular cell adhesion molecule-1 (VCAM-1) expression inhibition and cysteine protease[76]

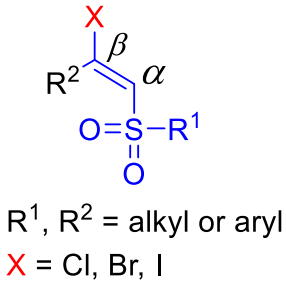
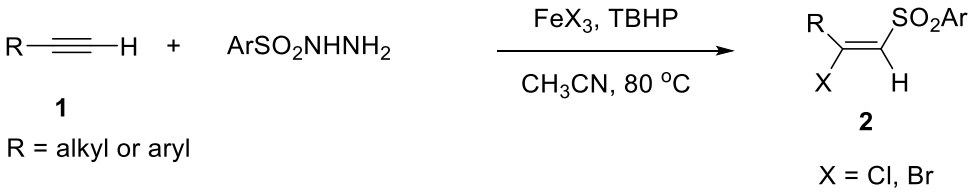


Figure 5. General structure of (*β*-halo)vinylsulfone

1.2.1. Synthesis of (*β*-halo)vinylsulfones

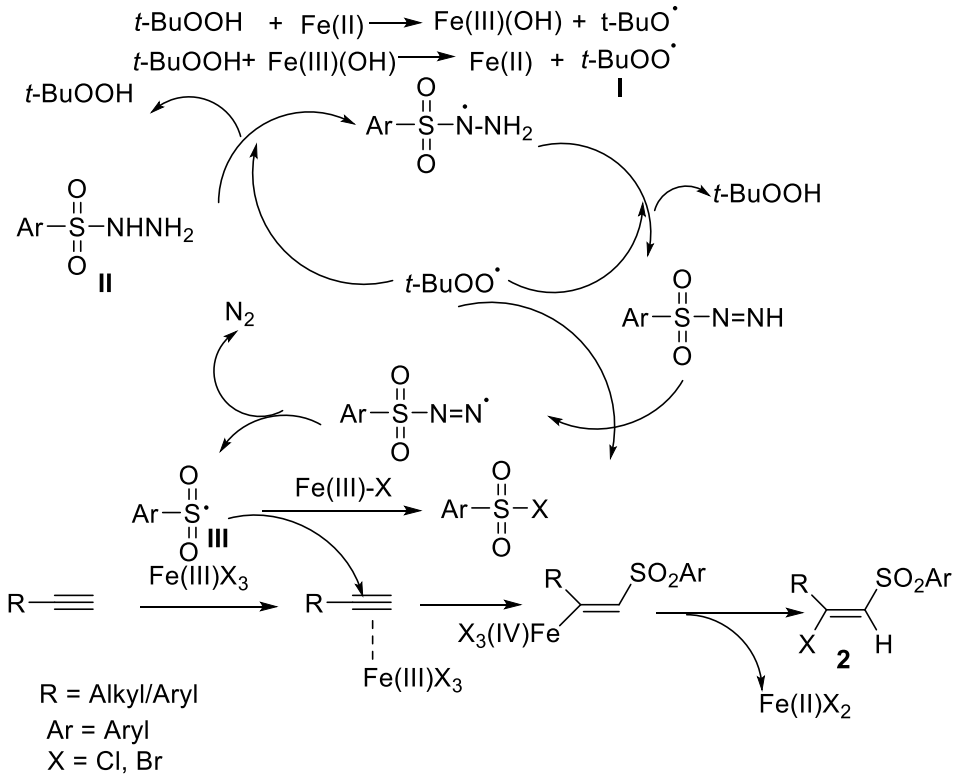
Terminal alkyne functional group provides excellent opening to prepare regio and stereoselective (β-halo)vinylsulfone with various tosyl sources.[77] Nair's group reported Cerium(IV) ammonium nitrate (CAN) mediated reaction of aryl sulfinates and sodium iodide with alkenes[78] or alkynes for the synthesis of vinylsulfones and (β-iodo)vinylsulfones.[79]

Sulfonylhydrazide is a common precursor of sulfonyl group and widely used reagent for the synthesis of halovinylsulfonylation compounds. Xu's group reported iron-mediated regio and stereoselective halosulfonylation reaction to prepare (*E*)-(*β*-chloro/bromo)vinylsulfone **2** by the reaction of alkyl/aryl acetylene **1** with aryl sulfonylhydrazide in the presence of iron(III) halides and tertiary butyl hydroperoxide (TBHP) (Scheme 1).[80]



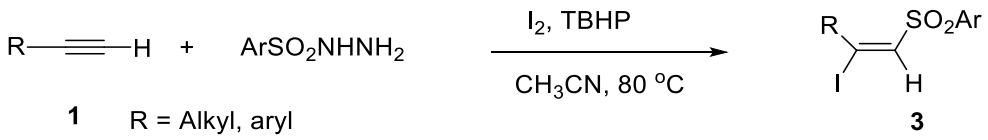
Scheme 1. Synthesis of (*E*)-(*β*-chloro/bromo)vinylsulfone **2** by iron catalyzed halosulfonylation reaction

The probable mechanism of the halovinylation reaction can be explained by the interaction of TBHP with FeX_3 to generate *t*-butyl peroxide radical (*t*-BuO˙) **I** (Scheme 2). This radical makes aryl radical **III** from hydrazide by removal of nitrogen gas. The reactive aryl radical attacks the alkyne group and then interacts with iron (III) halide to generate regio and stereoselective products (*E*)-*β*-chloro/bromovinylsulfone **2**.



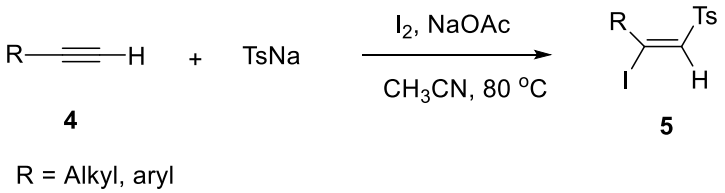
Scheme 2. Proposed mechanism of iron (III) halide-mediated halosulfonylation reactions for the formation of (*E*)-*β*-chloro/bromovinylsulfone

Later, Xu's group also developed a simple methodology to synthesize (*E*)-β-iodovinylsulfones through TBHP mediated reaction of sulfonylhydrazides and iodine with alkyl/aryl acetylenes without using any transition metal[81] (Scheme 3**)**. Here, the interaction of *t*-BuO˙ radical with sulfonyl hydrazide generates sulfonyl radicals. Then, the addition of resulting sulfonyl radicals to aryl acetylenes and subsequent trapping of alkenyl radicals with I_2 afford (*E*)-β-iodovinylsulfones **3**.



Scheme 3. Synthesis of (*E*)-(*β*-iodo)vinylsulfone using $ArSO_2NHNH_2$/ I_2/ TBHP

Kuhakarn's group also reported iodosulfonylation to synthesize (*E*)-*β*-Iodovinylsulfone from aryl or alkyl acetylene **4 (Scheme 4)** with molecular I_2 and Sodium *p*-toluenesulfonate (TsNa) instead of $TsNHNH_2$, in the presence of NaOAc (CH_3CN/80 ^oC) to afford *β*-iodovinylsulfone **5**.[82]



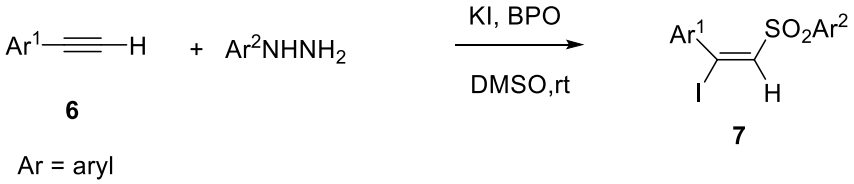
4

R = Alkyl, aryl

5

Scheme 4. Synthesis of (*E*)-(*β*-iodo)vinylsulfone using TsNa/NaOAc

Liu's group reported a new synthetic route for the synthesis of *E*-iodovinylsulfones via the iodosulfonylation of terminal alkynes **6** (**Scheme 5**) employing potassium iodide and sulfonyl hydrazine as reaction partners at room temperature in the presence of only benzoyl peroxide (BPO).[83]



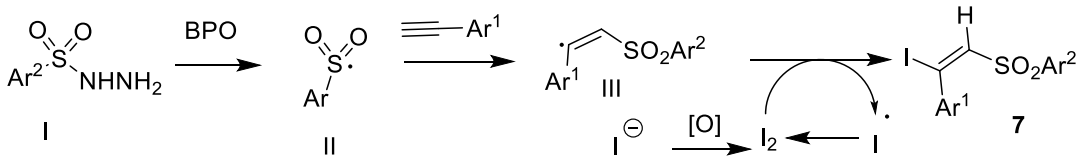
6

Ar = aryl

7

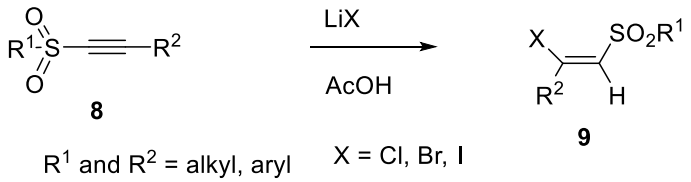
Scheme 5. Synthesis of (*E*)-(*β*-iodo)vinylsulfone using $TsNHNH_2$/BPO

A possible mechanism has been proposed as outlined in **Scheme 6**. In the presence of peroxide species, the sulfonyl hydrazine **I** may be converted to the sulfonyl radical **II**. Then this radical interacts with terminal alkyne to generate the vinylsulfone radical **III** via radical addition to the triple bond. Meanwhile, the oxidation of the iodide produce molecular iodine which capture the radical intermediate **III** to afford product **7**.[83]



Scheme 6. Proposed mechanism of iodosulfonylation using $TsNHNH_2$/BPO.

Chen's group developed a simpler and efficient approach for the synthesis of (*E*)-*β*-iodovinylsulfones via the molecular iodine and di-tert-butyl peroxide (DTBP) promoted difunctionalization of alkynes with sodium benzenesulfinates without transition-metal catalyst.[84] Recently, Xu's group developed a highly efficient synthesis of Z- halovinyl sulfonone **9** via hydrohalogenations of sulfonyl alkynes **8** with LiX ((X = Cl, Br, I) in the presence of acetic acid as hydrogen bonding donor and solvent (**Scheme 7**).[85]

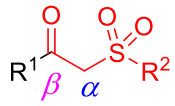


Scheme 7. Hydrohalogenations of sulfonyl alkynes

1.3. Overview of *β*-ketosulfones chemistry

β-Ketosulfones, also known as 2-oxo-sulfones, are an organosulfur compound of alkyl, aryl, or heteroaryl groups at carbonyl or sulfonyl moieties, and the C=O and SO_2 functionalities isolated by the unsubstituted CH_2 group (Figure 6).[86] Due to their encouraging bio-logical activities, such as anti-hepatitis, anti-bacterial, antifungal, and non-nucleoside inhibitors, ketosulfones and their analogues have attracted great attention.[87] Since *β*-ketosulfones contain multifunctional functional groups like sulfonyl, carbonyl, and active methylene (acidic proton) moieties which could be used in a variety of chemical manipulations. *β*-Ketosulfones have emerged as effective building blocks due to their ease

of removing sulfonyl moieties from and constructing new carbocyclic and heterocyclic compounds. As *β*-ketosulfones have an active methylene group, they are often used as model compounds for study of reactivity. The chemistry of *β*-ketosulfones are seeing a surge in interest in recent decades, and it now comprises the full branch of organosulfur chemistry.[88] It is also reported that certain *β*-ketosulfones are selective inhibitors of 11*β*-hydroxy steroid dehydrogenase type 1 (11*β*-HSD1).[89]

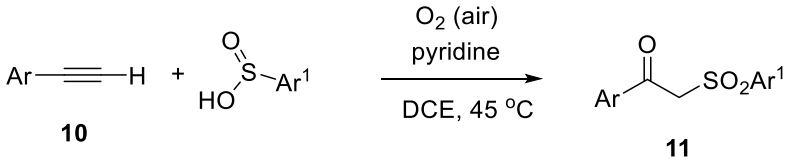


R^1, R^2 = Alkyl, Aryl

Figure 6 General structure of *β*-ketosulfone

1.3.1. Synthesis of *β*-ketosulfones

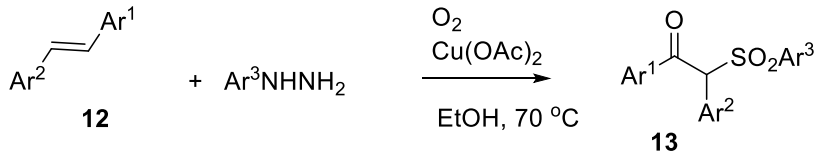
The β-ketosulfones have gained wide range of applications in the organic synthesis over the last decade. Lei's group developed a synthetic methodology to prepare *β*-ketosulfones **11** from terminal alkyne **10** with acid using dioxygen as the only oxidant in presence of pyridine (**Scheme 8**).[90] The authors also claimed that the radical process involved during the reaction and pyridine not only serves as base but also prevents the atom transfer radical addition mechanism, which affords to generate *β*-ketosulfones instead of vinylsulfone.



10 **11**

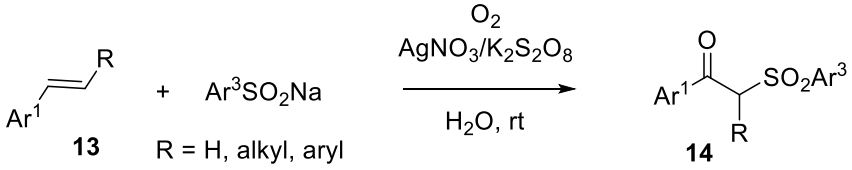
Scheme 8. Synthesis of *β*-ketosulfones via pyridine mediated dioxygen as an oxidant

Wang's group reported the synthesis of *β*-ketosulfones **12** via copper-catalyzed direct oxysulfonylation of alkenes **13** with dioxygen and sulfonylhydrazides (Scheme 9).[91]



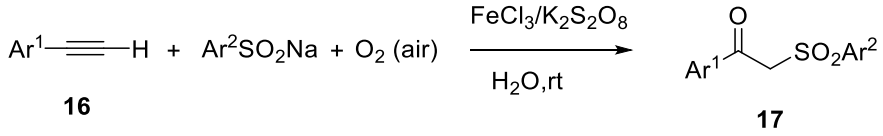
Scheme 9. Synthesis of *β*-ketosulfones via copper catalyzed oxysulfonylation

Later Wang's group reported another method for the synthesis of *β*-ketosulfones via iron-catalyzed oxidative difunctionalization of alkene with sulfinic acids and atmospheric dioxygen instead of copper catalyst.[92] Then, using an $AgNO_3/K_2S_2O_8$ catalyst system in an aqueous medium, Yadav's group developed an efficient methodology for the preparation of *β*-ketosulfones **14** by oxidative oxysulfonylation of alkenes **13** with dioxygen and sodium arenesulfinates at room temperature (Scheme 10).[93]



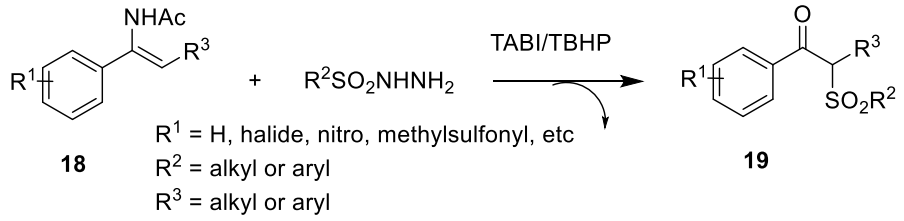
Scheme 10. Synthesis of *β*-ketosulfones via silver catalyzed oxysulfonylation from alkene

Subsequently, the same group also reported conversion of alkyne **16** to *β*-keto sulfones **17** using $FeCl_3/K_2S_2O_8$ catalytic system by aerobic oxysulfonylation with dioxygen and sodium arenesulfinates at room temperature in water (Scheme 11).[94]



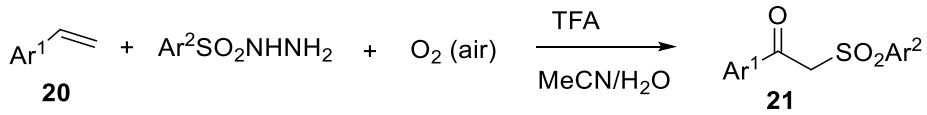
Scheme 11. Synthesis of *β*-ketosulfones via iron catalyzed oxysulfonylation from alkyne

Du's group developed a simple synthetic method for the preparation of *β*-ketosulfone derivatives **19** using TBAI and TBHP-mediated oxidative coupling of readily available enamides **18** with sulfonylhydrazides (Scheme 12).[95] Thus, $TsNHNH_2$ reacted smoothly with a wide variety of vinylacetamides with various benzene ring substituents to give the corresponding *β*-ketosulfones in high yields.



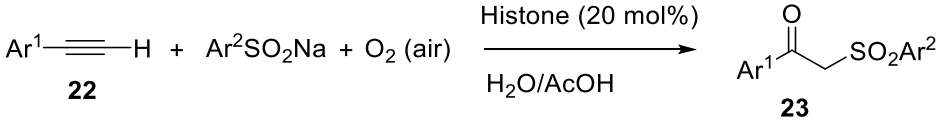
Scheme 12. Oxidative sulfonylation of enamides with sulfonylhydrazides

In 2016, Liu's group developed a trifluoroacetic acid (TFA) mediated oxosulfonation to syntesize *β*-ketosulfone **21** with alkene **20** and sulfonylhydrazides in aerobic condition in acetonitrile and water (Scheme 13).[96] A wide range of aryl-, heteroaryl-, and alkyl sulfonylhydrazides are attached with styrene to produce structurally distinct *β*-ketosulfones in excellent yields.



Scheme 13. TFA catalyzed sulfonylation of alkene with sair and ulfonylhydrazides

Very recently, Kumar's group reported an amino acid catalyzed protocol to synthesize *β*-ketosulfone **23** from terminal alkyne **22** with sodium salt of arysulfinic acid under oxygen in water/acetic acid (Scheme 14).[97] A variety of aryl acetylenes coupled with sodium sulfinates to give moderate to excellent yields of ketosulfones **23**.

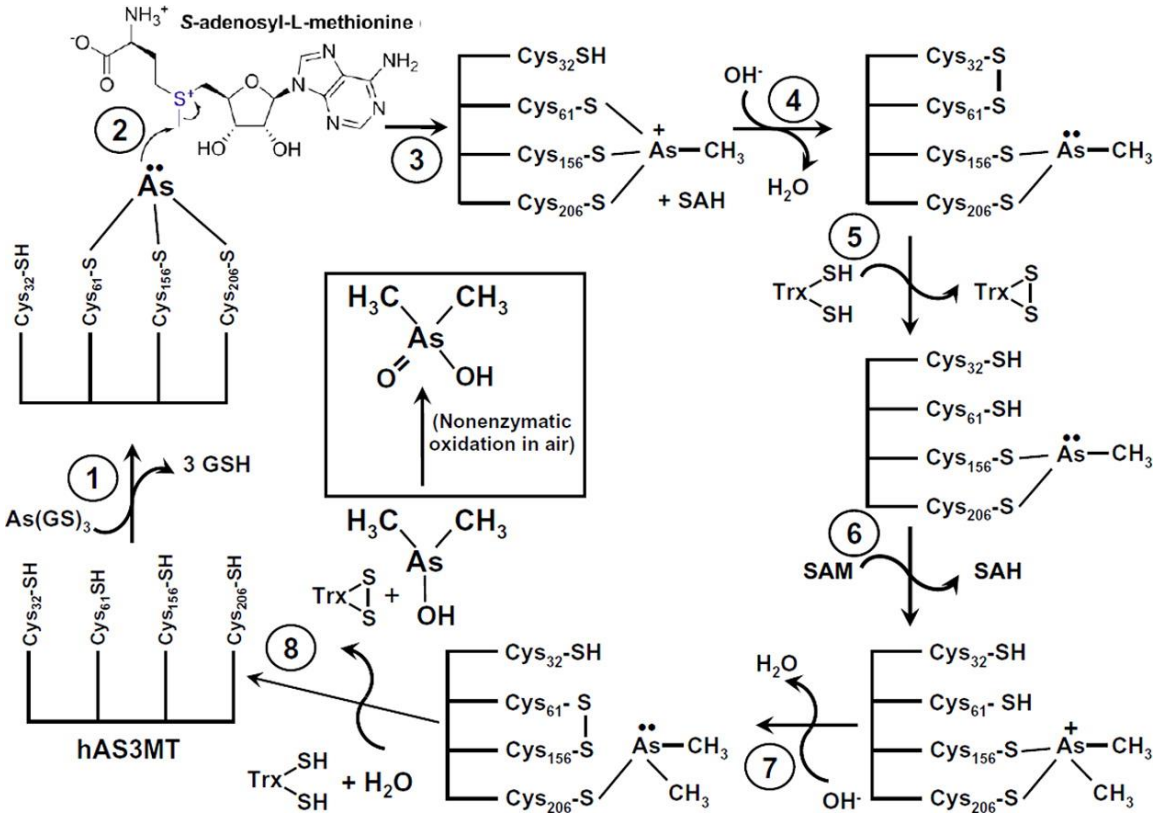


Scheme 14. Histone catalyzed sulfonylation of alkene with air and sulfonylhydrazides

1.4. S-Adenosyl-L-methionine as source for methylation of arsenic species.

Arsenic is a poisonous substance which can be a carcinogen but, when controlled, can be used as a therapeutic agent. Arsenic metabolism mechanisms have been thoroughly researched. From the genomic sequenced results, it is now clear that most of the bacteria and single-celled prokaryotic organisms contain arsenic-resistance (*ars*) operons that regulate the resistance to arsenite [As(III)] and arsenate [As(V)].[98] The widespread presence of *ars* genes validates the fact that arsenic is a prevalent environmental toxic metal. For the detoxification process arsenic(III), methylation has long been considered since pentavalent arsenic species are less toxic than trivalent arsenic.[99] Previously, it was reported that As(III) metabolism and detoxification occurs in many mammals, including humans, through methylation in the liver followed by urinary excretion.[99] Later, it was found that in humans, *S*-adenosylmethionine As(III) methyltransferase (AS3MT), primarily a liver enzyme, catalyzes transfer of methyl groups from S-Adenosyl-L-methionine (SAM) to As(III), producing monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and finally trimethylarsine oxide (TMAO) or the trivalent arsenic species.[99] AS3MT is thought to have two rounds of As (III) methylation, with each round consisting of oxidative methylation accompanied by reduction using SAM as the methyl donor. The first round reaction yields MMA (V), which is then reduced to MMA (III) followed by a second methylation to produce DMA (V).[100,101] In 2014, Rosen's group proposed a new pathway for human AS3MT catalyzes transfer of methyl groups from SAM to As(III)[102] as shown in Scheme 15. (1) first, hAS3MT binds As(III) in a series of three thiol transfer reactions from arsenic triglutathione [As(GS)3]; (2) then CH_3 group of SAM is attacked by the lone pair of arsenic; (3) next, pentavalent methylarsate [MAs(V)] intermediate is

formed and (4) which is reduced to an enzyme-bound MAs(III) intermediate by Cys32 with formation of a Cys32–Cys61 disulfide; (5) the disulfide is reduced with thioredoxin (Trx) and the enzyme undergoes the next round of methylation, (6) forming a pentavalent dimethylarsenate [DMAs(V)] intermediate; which implies that monoalkyl aresenite can be also methylated to form alkyl-methyl pentavalent arsenic motif such as present in AST, (7) DMAs(V) is reduced to dimethylarsenite [DMAs(III)] by Cys61 which make Cys61–Cys156 disulfide, (8) which is reduced by Trx, regenerating the enzyme and liberating the key soluble product, DMAs(III). Finally, air oxidation convert trivalent DMAs(III) to DMAs(V) nonenzymatically.



Scheme 15. Proposed human AS3MT catalyzed methylation reaction pathway *(Copyright, American Chemical Society)*

S-Adenosyl-L-methionine (SAM) is a substrate in various enzyme-catalyzed reactions in which it acts as a universal methyl group donor to nucleic acids, proteins, lipids, and neurotransmitters precursors in the biosynthesis of natural products.[103,104] The by-product of this methylation reaction is S-adenosylhomocysteine (SAH) which is recycled enzymatically to methionine and adenine (Figure 7).[105,106] The hydrolysis of SAH by the enzyme SAH hydrolase (SAHase) is the sole intracellular source of homocysteine (Hcy), which is needed to control accurate cellular level of SAM.[106,107] The amino group of SAM is also transferred in the dihydrodipicolinate (DAPA) synthase-catalyzed biotin metabolic pathways, while ribosyl group serves as a precursor to a transformed tRNA nucleoside in the biosynthesis pathway.[108] The methionine metabolic cycle, which is involved in the synthesis of SAM, is tightly regulated at both the genetic and protein levels, which is consistent with these essential functions.[108] SAM is also a source of catalytic 5'-deoxyadenosyl radical, which is produced as reaction intermediate by the superfamily of radical SAM enzymes[104] and is involved in the arsenosugar derivatives biosynthesis.[109]

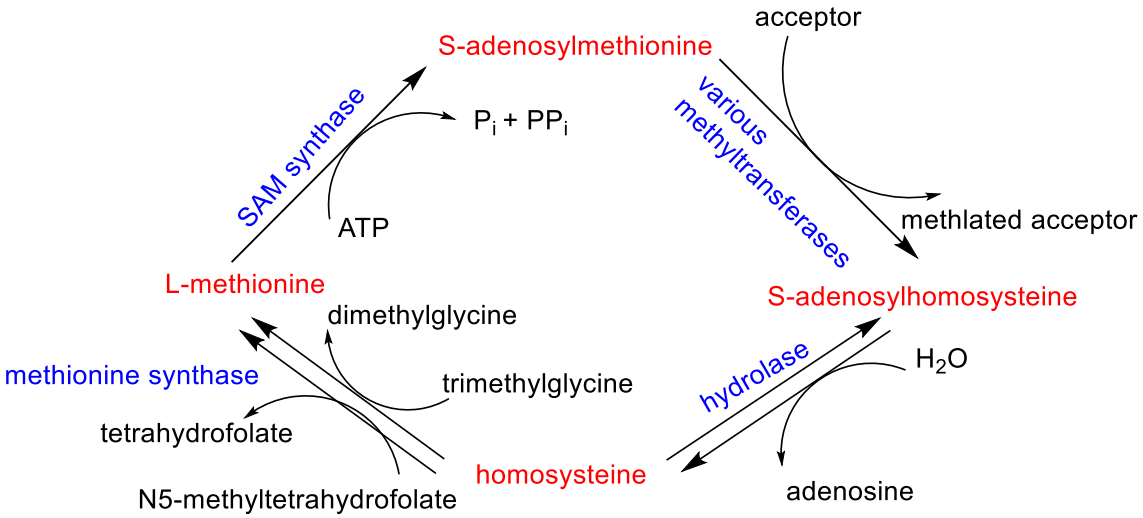


Figure 7. S-Adenosyl-L-methionine biosynthesis pathway

Additionally, SAH analogs have been developed as broad-spectrum antiviral agents[106] and advances in SAHase inhibitors in clinical applications have been covered in details by Borchardt and Robins groups.[110] Aspects of the "hydrolytic" activity of the S-adenosyl-L-homocysteine hydrolase has been also reviewed.[111] Potent inhibitory activity of 6'-(*E* and *Z*)-halohomovinyl, dihalovinyl and acetylene derivatives of adenosine against SAHase and the correlation of enzyme inhibition with their anticancer and antiviral potencies have been reported.[112-114] Jeong's group showed that adenosine and N-6-methyladenosine analogues possess potent inhibition against SAH hydrolase, while only the adenosine derivatives exhibited potent antiviral activity against RNA viruses such as Middle East respiratory syndrome-coronavirus I deleted bold (MERS-CoV).[115]

1.4.1. Organoarsenicals as a therapeutics

Arsenicals have a long history of use in humans as an antimicrobial and anticancer agents or as a venomous for bad intentions.[116] Aminophenyl arsenic acid, is a crystalline powder that was initially used in medicine in the late 19th century as Atoxyl. The sodium salt was used as the first organic arsenical compound by injection in the early 20th century, but it was quickly discovered to be too poisonous for human use. Arsanilic acid has long been used as a veterinary feed supplement to promote growth of poultry and swine, as well as to avoid or cure dysentery.[117] However, its approval as an animal treatment by the US government was voluntarily revoked by its sponsors in 2013. Then, in the early 1910s, Arsphenamine, also known as Salvarsan or compound 606, was introduced as the first arsenic-based modern antimicrobial agent as a therapy for syphilis and African trypanosomiasis.[118] Although, salvarsan is no longer in clinical use, the World Health Organization still recommends the organoarsenical melarsoprol, which was developed in

1949, for the treatment of second-stage *Trypanosoma brucei* sleeping sickness.[119] Antimicrobials such as atoxyl and the relevant synthetic aromatic arsenicals carbarsone [4-(Carbamoylamino)phenyl arsenic] acid, roxarsone (4-hydroxy-3-nitrophenylarsenate), and nitarsone (4-nitrophenylarsenate) are used in poultry to combat Coccidia and Histomonas infections (Figure 8).[120] Finally, in humans with all-trans retinoic acid unresponsive acute promyelocytic anemia, arsenic trioxide is actually the medication of choice.[121]

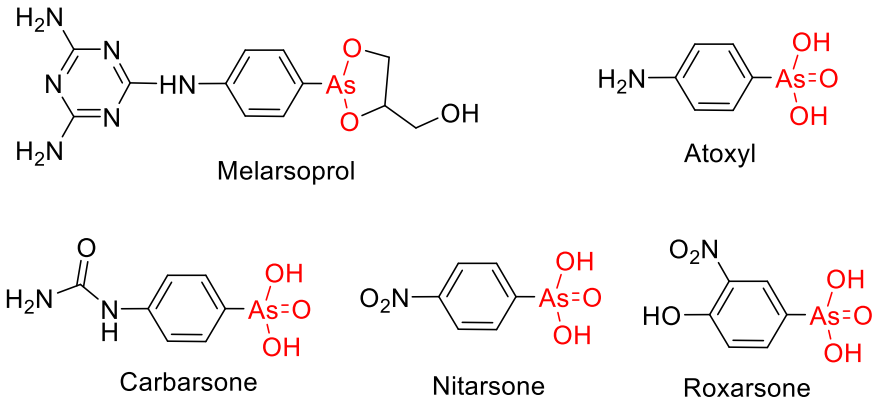


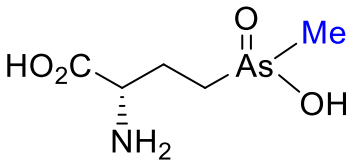
Figure 8. Structure of Arsenic based drugs

1.4.2. A new broad-spectrum organoarsenical antibiotic Arsinothricin

The emergence and spread of bacterial resistance have rendered nearly every clinically used antibiotic ineffective. This emphasizes the urgent need for new antibiotics. Infectious diseases such as tuberculosis, the top global infectious disease killer caused by the bacterium *Mycobacterium tuberculosis* (MTB), has become even more difficult to treat due to drug resistance. The World Health Organization (WHO) has declared multidrug-resistant tuberculosis (MDR TB) a global public health crisis, calling for a pressing need for development of new and innovative antibiotics. In addition to *M. tuberculosis*, the WHO recently issued a global priority pathogen list of antibiotic-resistant bacteria that pose the greatest threat to human health, including the six nosocomial pathogens whose first

initials form the acronym ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp*.) pathogens.[122]

Most organisms merely try to survive life in arsenic. In contrast, some members of microbial communities have found ways to use arsenic as weapons against other bacteria in the continual battle for dominance in microbial warfare. Recently, a new arsenic-containing compound, arsinothricin [2-amino-4-(hydroxymethylarsinoyl)butanoic acid or AST], was shown to be synthesized by the rice rhizosphere bacterium *Burkholderia gladioli* GSRB05 (**Figure 9**).[123] AST has broad-spectrum antibiotic activity and is effective against both Gram-positive and Gram-negative bacteria, including the World Health Organization ESKAPE pathogen carbapenem-resistant *Enterobacter cloacae* (CRE) and *Mycobacterium bovis* BCG, a causative agent of animal tuberculosis that is closely related to the human pathogen MTB.[124] AST is a nonproteinogenic amino acid analog of glutamate that inhibits glutamine synthetase, a crucial enzyme in formation of nitrogen compounds, presumably by mimicking the γ-acylphosphoglutamate intermediate in the glutamine synthetase reaction.



AST

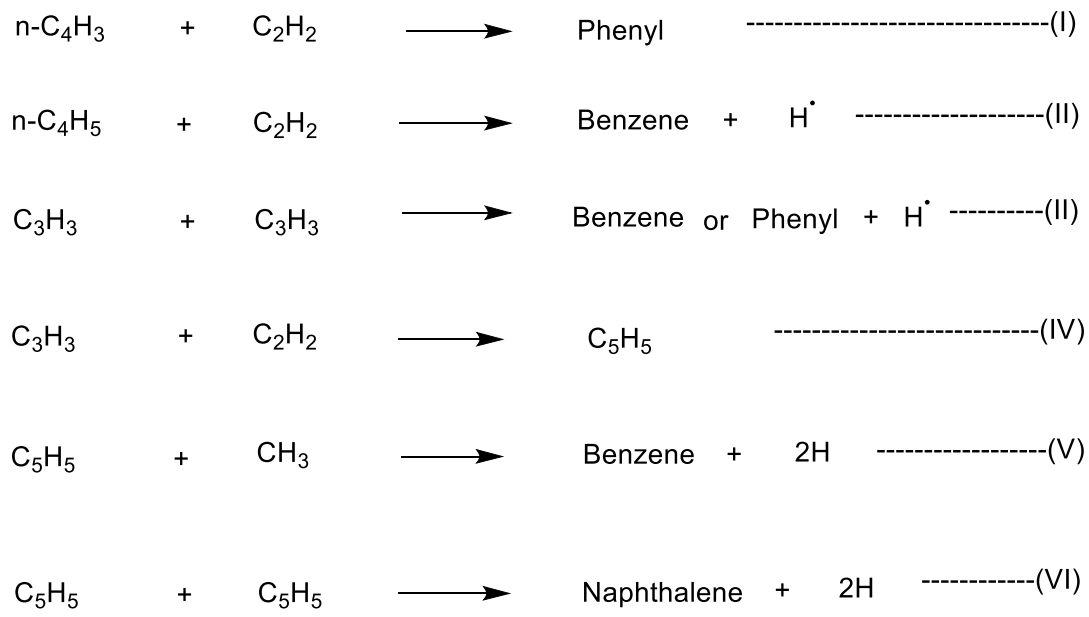
Figure 9. Structure of arsinothricin (AST)

1.5. Polyaromatic hydrocarbon (PAH)

A chemical compound that contains only carbon and hydrogen and composed with multiple aromatic rings is defined as a polyaromatic hydrocarbon (PAH). Naphthalene, which has two aromatic rings, and the three-ring compounds anthracene and phenanthrene are the simplest compound of these classes. The formation of PAH and soot has been one of the major attention of study in the field of hydrocarbon pyrolysis and fuel combustion.[125] PAHs are debated as potential starting materials for abiotic synthesis of necessary ingredients by life's earliest forms. These compounds have been of great interest since the hypothesis of them being carriers of the diffuse interstellar bands in the interstellar medium, and their direct link in the formation of amorphous carbon dust or Soot.[126] It has been proposed that PAHs could account for up to 20% of the galactic carbon budget and serve as a bridge between resonantly stabilized free radicals and carbonaceous nanoparticles in interstellar and circumstellar settings.[127,128]

1.5.1. Formation of simple benzene and naphthalene hydrocarbons

The formation of precursor phenyl radical for growth of PAH from through the very simplest molecules acetylene and n-C_4H_3 and n-C_4H_5. Various possible pathway is shown in Scheme 16 for the formation of simple aromatic hydrocarbon benzene or naphthalene.[125]



Scheme 16. Formation pathway of benzene or naphthalene

1.5.2. Molecular mass growth process of PAH

On Earth, the primary source of PAHs in the environment has been noted as the incomplete combustion of natural and anthropogenic.[129] There are several proposed pathways for PAH mass growth, but Kaiser and Hansen recently outlined the formation and molecular growth processes of PAHs using five elementary mechanisms, which were validated using molecular beam experiments and high-level electronic structure measurements.[130] The five elementary steps are (i) Hydrogen Abstraction-C_2H_2 (acetylene) Addition (HACA), (ii) Hydrogen Abstraction-Vinylacetylene Addition (HAVA), (iii) Phenyl Addition-Dehydro Cyclization (PAC), (iv) Radical-Radical Reactions (RRR), and (v) Methylidyne Addition-Cyclization-Aromatization (MACA). The HACA process entails a repeated reaction sequence involving the abstraction of a hydrogen atom from the reacting aromatic hydrocarbon by a hydrogen atom, accompanied by the addition of an acetylene molecule to the radical site which add two extra carbons.[131,132] However, the ring

annulation and addition of a six-membered ring to an established aromatic moiety are involved in the HAVA reaction mechanisms for the molecular mass growth processes of PAHs.[133-135] While in PAC mechanism, a phenyl radical is first added to an aromatic hydrocarbon, followed by hydrogen loss.[136] The resulting phenyl-substituted aromatic system is dehydrogenated, cyclized, and aromatized, essentially transforming three and four-carbon bays at aromatic hydrocarbons to indene and naphthalene moieties. In RRR mechanism reactions, it is involved between two hydrocarbon radicals to generate a new aromatic system. For example, reaction of reaction of the methyl radical (CH_3) with indenyl (C_9H_7) leading to formation of naphthalene.[137] Finally, the MACA process effectively transforms a PAH's vinyl side chain into a five-membered ring via ring annulation.[138]

1.5.3. Gas phase synthesis of PAH

For the gas phase synthesis of PAH, the reactions mainly involve either reaction of a closed-shell molecule and a radical or between two radicals. The radicals can be a simple atomic H to a complex hydrocarbon radical. The gas phase reaction is very useful to construct multiple aromatic ring from a simple phenyl ring system through the aforementioned pathway. Generally, methyl (Me) radical is used for installation of one extra carbon to the existing ring.[137] Similarly acetylene (C_2H_2) is being used for addition of two carbons,[139] propylene or allene (C_3H_4) for three carbons,[140] vinyl acetylene (C_4H_4) for four carbons,[141] and phenyl (C_6H_5) radical for six carbon[136] to the existing ring.

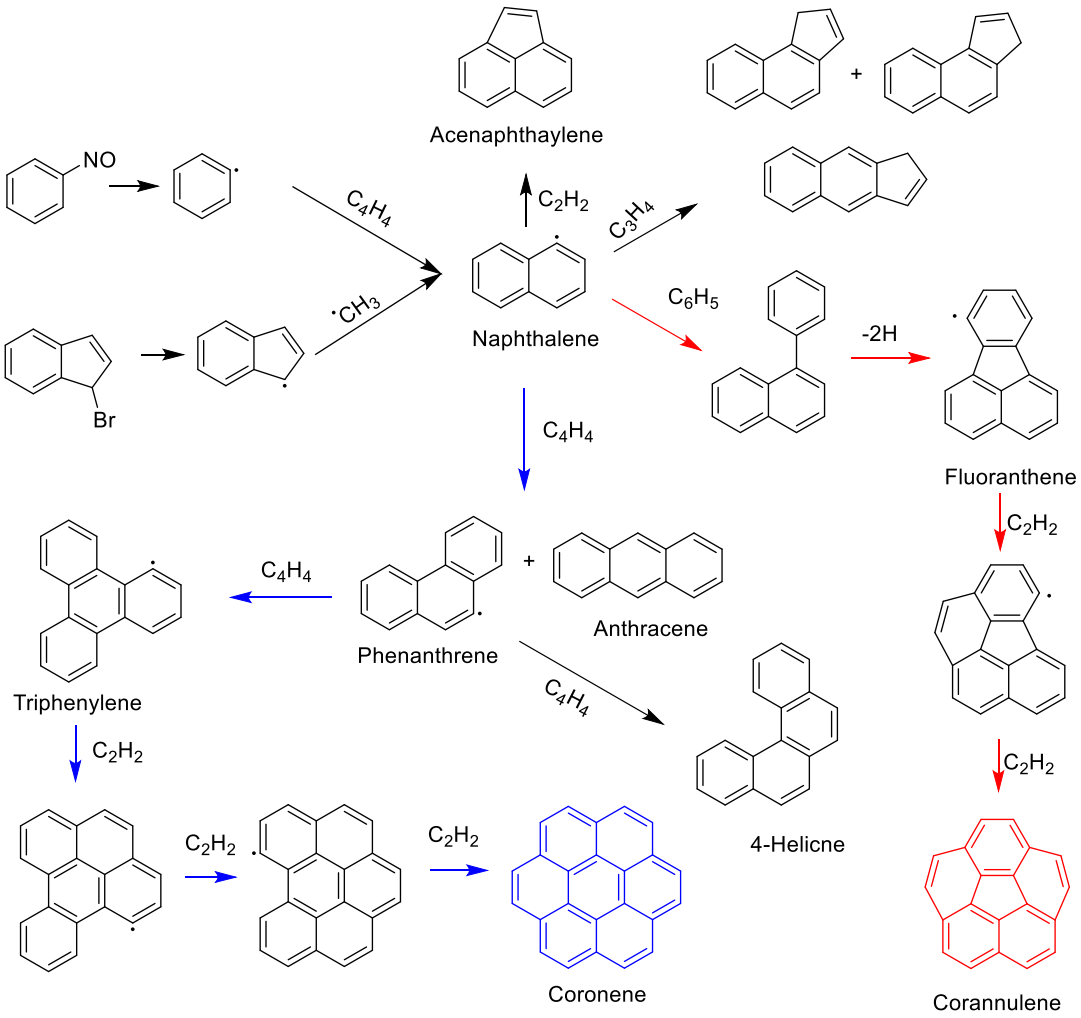
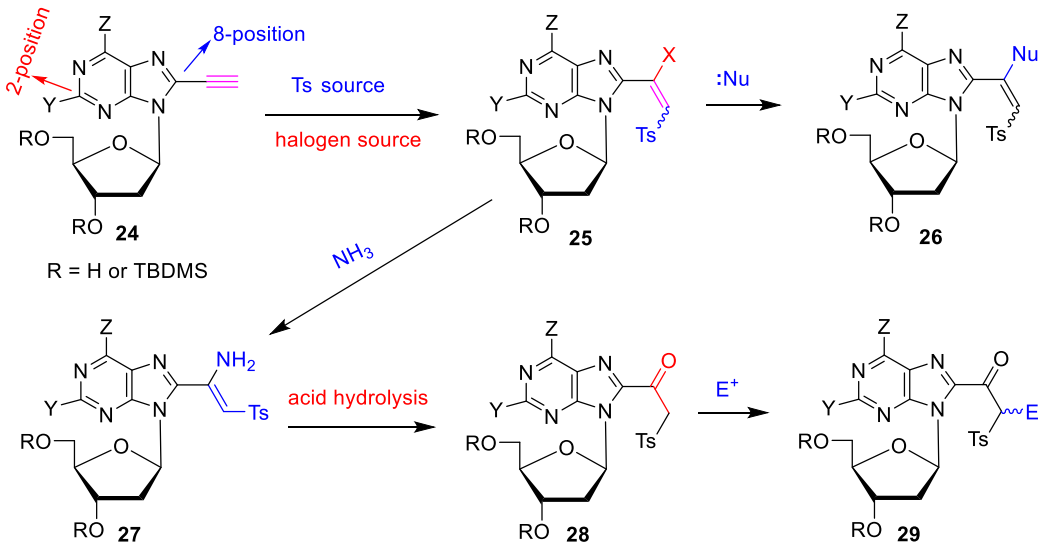


Figure 10. Gas phase formation and mass growth of PAH

While for the aromatic precursor nitroso or halogenated aromatic compounds are used to generate corresponding radical. For example, simple phenyl radical with vinyl acetylene or indene radical with methyl radical can growth simplest PAH naphthalene. Which can grow different three ring (e.g., anthracene or phenanthrene, acenapthylene or benzindene) PAHs with different radicals (Figure 10). Those three ring cyclic compound act as a precursor for further growth of four member PAH (e.g., triphenylene, 4-helicene, fluoranthene). Finally, those four member PAHs radicals in gas phase can grow coronene (blue arrow) or corannulene (red arrow) with acetylene in multiple steps.

2. RESEARCH OBJECTIVE

The first overall goal was to attach the reactive (*β*-halo)vinylsulfone group to the purine bases of the nucleosides/tides as well as to study their reactions with amino acids such as cysteine or lysine in order to further study DNA/RNA–protein interaction. The nucleoside analogues modified with (*β*-halo)vinylsulfone group could be good candidates for the bioconjugation since they are very reactive toward nucleophiles (e.g., amines or thiols). Therefore, my first research objective was the synthesis of C-8 modified purine nucleosides/tides bearing reactive (*β*-halo)vinylsulfone group. To achieve this goal, I plan to utilize transition-metal-catalyzed or radical mediated halovinylsulfonylation of 8-alkynyl precursors **24** with TsCl, TsNa, or $TsNHNH_2$ in the presence of NXS, X_2, or FeX_3 as halogen source to carry out transformation of alkyne to (*β*-halo)vinylsulfone motif **25 (**Scheme 17**)**. Due to the high reactivity of *β*-halovinylsulfone towards addition-elimination reaction with amines (e.g. product **26** or **27**), I expect to study the behavior of these compounds towards nucleophiles present in proteins by modeling the reaction with amino acids such as cysteine (contains SH group) or lysine (contains NH_2 group) which should similarly react with (*β*-halo)vinylsulfones. It is worth mentioning that although Hocek's group demonstrated bionconjugation of pyrimidine nucleoside bearing reactive groups at C5 position via Michael addition pathway,[142] conjugation with (*β*-halo)vinylsulfone probes is different because it should occur via-addition-elimination path.[37,39]

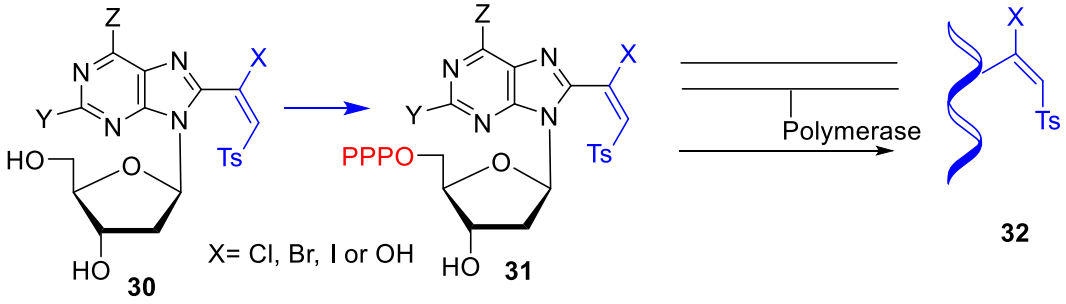


Scheme 17. Purine nucleosides bearing reactive (*β*-halo)vinylsulfone and *β*-ketosulfone groups and their reactivity

My second research objective within this aim was to synthesize nucleoside/tide analogues modified with reactive *β*-ketosulfone group at C8-position of purine bases and to study their reactivity with electrophiles. To achieve this goal, I plan to synthesize *β*-ketosulfone **28 (**Scheme 17**)** from intermediary *β*-aminovinylsulfone **27**, formed when *β*-halovinylsulfone **25** is treated with methanolic ammonia, by the controlled acid hydrolysis. As the *α*-hydrogen of methylene unit of *β*-ketosulfone is acidic (pKa ~ 10-11), I expect this *β*-ketosulfone would deprotonate in physiological condition with sufficiently basic amino acid residues and can be trapped by the electrophile to give α-alkylated product **29**. Model reaction with methyl iodide, allyl bromide and benzyl bromide will be explored for potential utilization of these probes for bioconjugation with proteins.

My third objective within this aim was to study the polymerase catalyzed incorporation of these reactive probes to DNA for potential further post-synthetic modifications, labelling and cross-linking. To accomplish his goal, nucleosides bearing with reactive *β*-

halovinylsulfone and *β*-ketosulfone probes **30** will be phosphorylated at 5'-position and resulting triphosphates **31** will be investigated as possible substrate for polymerase synthesis of reactive DNA fragments **32** and/or chain termination inhibitors (Scheme 18).

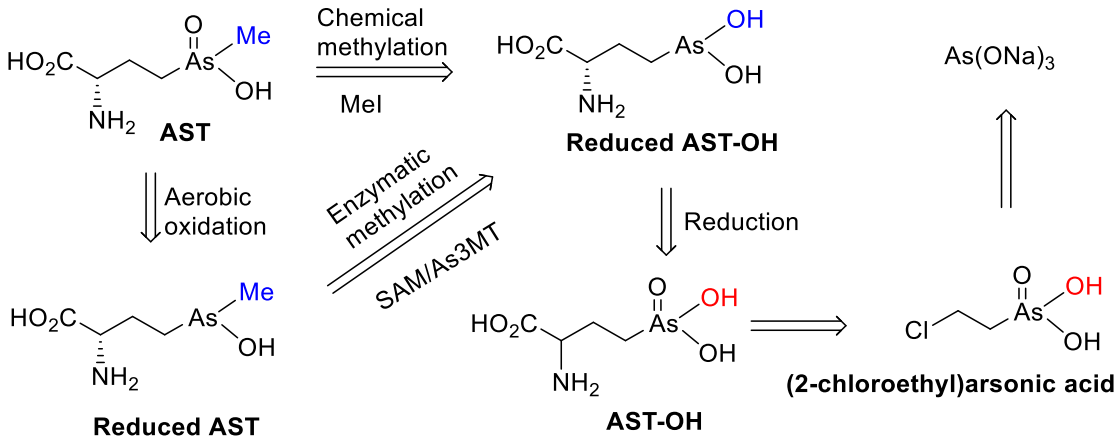


Scheme 18**.** Incorporation *β*-halovinylsulfones and *β*-ketosulfones into DNA

My second aim was the synthesis of C2 modified adenine nucleosides/tides bearing (*β*-halo)vinylsulfone and *β*-ketosulfone reactive groups and their polymerase catalyzed incorporation into DNA. The C2 modified probes will have different conformation as compared to C8 modified counterparts with pronounced effect on Watson-Crick hydrogen bonding and stability of DNA and RNA oligomers. Cell proliferation studies and polymerase-catalyzed DNA incorporation of these nucleosides/tides will provide assessment to what degree position or size of the new modifications effect activity.

Very recently, a new arsenic-containing compound, arsinothricin [2-amino-4-(hydroxymethylarsinoyl)butanoic acid or AST; Scheme 19] has been discovered[123] and show broad-spectrum antibiotic activity being effective against both Gram-positive and Gram-negative bacteria.[124] We predict that AST and related arsenic-containing compounds may be the progenitors of a new class of antibiotics. They may prove to be more effective as drugs than chemically related phosphonates, which include some of the most effective commercially available herbicides, pesticides, and human drugs. Although modest

amounts of AST can be generated using the rhizosphere bacterium culture source the drug development requires a reliable source of the compound. To reduce effort and complexities associated with production of pure AST from bacterial culture medium[123] and to provide reliable source of larger quantities of AST for future drug development, we undertaken efforts to synthesize AST by chemoenzymatic and chemical methods from readily available sodium arsenite [$As(ONa)_3$] (Scheme 19). Since AS3MT can transfer methyl groups from SAM to As(III), producing pentavalent methylarsonic acid after oxidation (as depicted as enzymatic methylation in Scheme 19) , I thought it would be challenging but interesting to find also chemical substitute of SAM for the methylation of 2-amino-4-arsonobutanoic acid (AST-OH) to produce AST. As the synthesis of AST-OH was reported[143] and reduction of pentavalent arsenic to trivalent is well established, I decided to mimic the enzymatic methylation of reduced AST-OH with methyl iodide (as depicted as chemical methylation in Scheme 19) instead of enzymatic methylation with SAM and AS3MT to synthesize AST form AST-OH. So, my third research objective was the chemical synthesis of novel organoarsenical antibiotic arsinothricin (AST).



Scheme 19. Retrosynthesis of hydroxyarsinothricin (AST-OH) and arsinothricin (AST)

In addition, during my dissertation work, I have also investigated synthesis of polyaromatic hydrocarbons (PAH) containing two to six ring system and their derivatives. They would act as precursors or calibration compounds for the gas phase synthesis of PAHs through the exploitation of molecular beam experiments mimicking the conditions of extreme environments from low temperatures settings to high-temperature environments like circumstellar envelopes of carbon-rich asymptotic giant branch stars. The project also would investigate the process of mass growth of polycyclic aromatic hydrocarbon (PAH) products from simple aromatic cyclic aromatic hydrocarbons. So, my final objective is to synthesize different PAHs and their "enyne" derivatives which will be used as precursors or calibration compounds for the gas phase synthesis of PAHs.

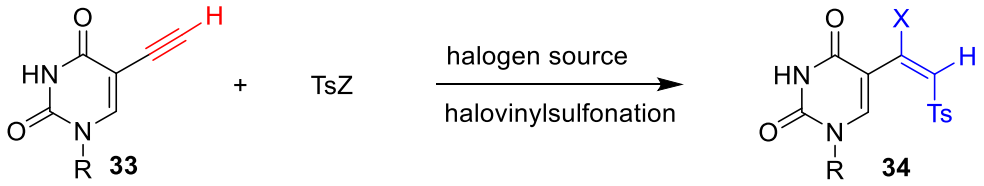
3. RESULT AND DISCUSSION

3.1. Synthesis and reactivity of adenine and guanine nucleosides modified at C8 position with (*β*-halo)vinylsulfone and *β*-ketosulfone reactive groups

3.1.1. Adenine nucleosides modified at C8 position with reactive groups

3.1.1.1. Adenine nucleosides modified at C8 position with (*β*-halo)vinylsulfone group

Recently our group reported a stereoselective synthesis of (*E*)-5-(*β*-halovinyl)sulfone derivatives of uracil nucleosides **34** by transition-metal-catalyzed or radical-mediated halovinylsulfonylation of 5-ethynyluracils **33** with TsNa or $TsNHNH_2$ in the presence of NXS (X = Br, I) or FeX_3 (X = Cl, Br) respectively, as halogen sources (**Scheme 20**).[37]

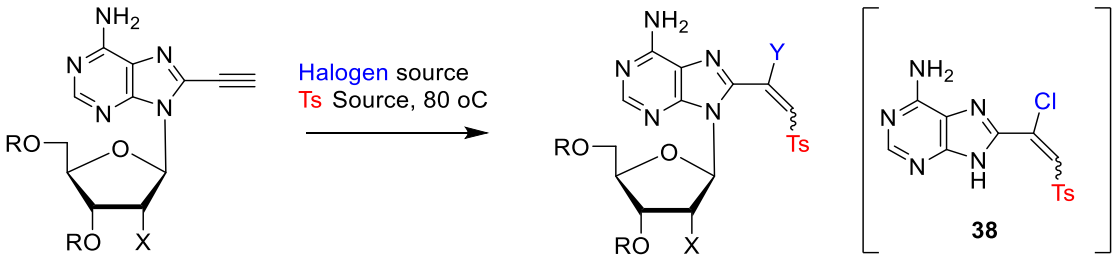


R = Bn, robosyl, arabinosyl, 2'-deoxyribosyl: Z = Cl, $NHNH_2$, Na; X = Cl, Br, I

Scheme 20. Halovinylsulfonation on uracil nucleosides

For the synthesis of C8-modified adenine nucleosides bearing reactive group, we employed the 8-ethynyl-2'-deoxyadenosine substrates **35** and **36** which were prepared as reported.[50] $FeCl_3$-mediated chlorosulfonylation of unprotected **35** with tosyl hydrazide (CH_3CN/80 ^oC) failed to produce the expected *β*-chlorovinyl sulfone **39** (Table 1, entry 1); although this protocol was successful for the pyrimidine nucleosides.[37,39]

Table 1. Synthesis of C8-modified *β*-halovinylsulfones of 2'-deoxyadenosine and adenosine



35 R = X = H
36 R = TBDMS, X = H
37 R = TBDMS, X = TBDMSO

39 R = X = H, Y = Cl
40 R = TBDMS, X = H, Y = Cl
41 R = X = H, Y = I
42 R = TBDMS, X = H, Y = I
43 R = TBDMS, X = TBDMSO, Y = I
44 R = TBDMS, X = H, Y = Br

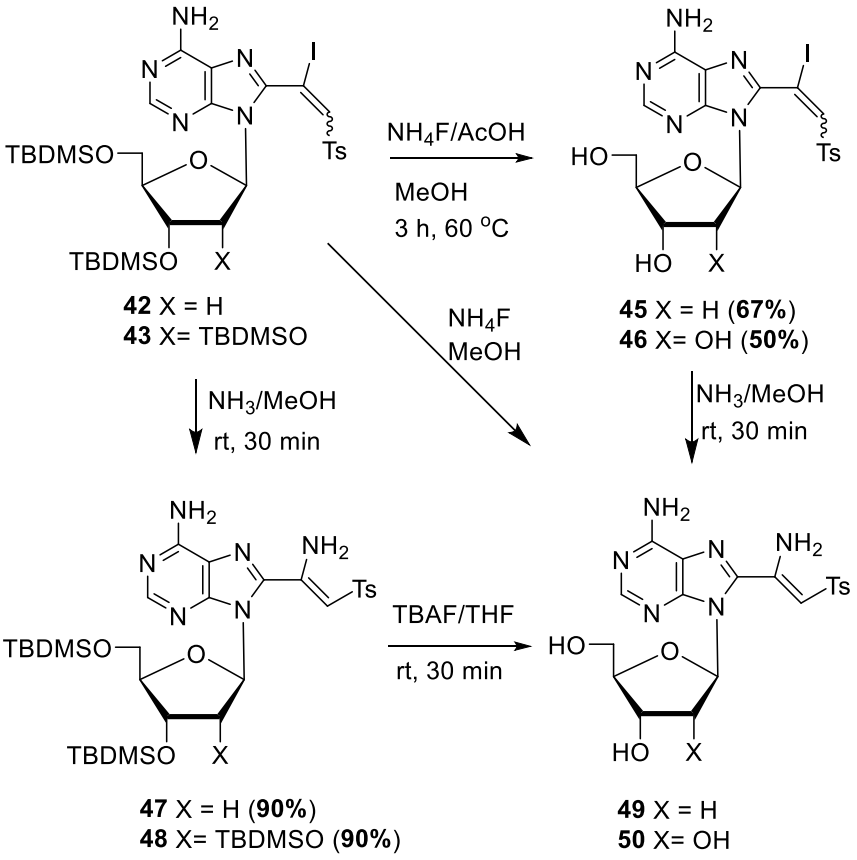
Entry	Substrate	Halogen Source	Ts source	Solvent	Additive	Products	Yields (%)
1	**35**	$FeCl_3$	$TsNHNH_2$	CH_3CN	TBHP	**39**	0
2	**36**	$FeCl_3$	$TsNHNH_2$	CH_3CN	TBHP	**38**	88
3	**35**	$FeCl_3$	$TsNHNH_2$	DMF	TBHP	**39**	0
4	**36**	$FeCl_3$	$TsNHNH_2$	CH_3CN	TBHP/Et_3N	**40**	0
5	**36**	$FeCl_3$	$TsNHNH_2$	CH_3CN	TBHP/NaOAc	**40**	0
6	**36**	I_2	TsNa	CH_3CN	NaOAc	**42**	64
7	**37**	I_2	TsNa	CH_3CN	NaOAc	**43**	50
8	**35**	I_2	TsNa	CH_3CN	NaOAc	**41**	0
9	**35**	I_2	TsNa	DMF	NaOAc	**41**	0
10	**36**	Br_2	TsNa	CH_3CN	NaOAc	**44**	0
11	**36**	NBS	TsNa	CH_3CN	NaOAc	**44**	0
12	**36**	NCS	TsNa	CH_3CN	NaOAc	**40**	0

Interestingly similar chlorosulfonylation of silyl protected **36** led to the isolation of 8-[(*β*-chlorovinyl)sulfone]adenine **38** resulting from cleavage of the glycosidic bond caused presumably by the *in situ* generated HCl from $FeCl_3$. Nonetheless, it also demonstrated high-yield halosulfonylation of alkyne attached to the imidazole ring of purine (entry 2). Use of polar solvent to improve solubility of **35** or adding weak bases to avoid cleavage were also ineffective (entries 3-5). We found however that iodosulfonylation[82] of **36** with

molecular I_2 and TsNa in the presence of NaOAc (CH_3CN/80 ^oC) gave **42** (*E/Z,* 70/30; 64%; entry 6). Similar treatment of protected 8-ethynyladenosine **37** afforded **43** (*E/Z,* 70/30; 50%; entry 7). The use of protected precursors **36** or **37** seems to be necessary for successful iodosulfonvlation since reaction with unprotected **35** even in DMF was unsuccessful (entries 8 and 9). Analogous bromosulfonylation (Br_2 or NBS) or chlorosulfonylation (NCS) of **36** failed to give desired **44** or **40** (entries 10-12).

Deprotection of TBDMS groups and conversion of iodovinylsulfones to aminovinylsulfones

Attempted deprotection of iodovinylsulfone **42** with TBAF gave mixture of byproducts instead of unprotected iodovinylsulfone **45 (**Scheme 21**).** Moreover, treatment of **42** with milder deprotecting conditions[144] (NH_4F/MeOH/ 60 ^oC) led to the formation of the unprotected 8-(*β*-aminovinyl)sulfone **49** (50%) as a single Z isomer instead of 8-(*β*-iodovinyl)sulfone **45**. The *Z* stereochemistry was tentatively assigned in agreement with literature precedence.[37,39,145,146] This suggests that 8-(*β*-iodovinyl)sulfone moiety is very labile toward nucleophilic substitution. Most probably, NH_3 formed during deprotection with NH_4F substituted the iodo group by an addition-elimination mechanism. It was found that addition of AcOH to the reaction mixture to neutralize the *in situ* generated NH_3, gave desired **45** (67%)**.** Similarly 8-(iodovinyl)sulfone of adenosine **46** (50%) was also prepared from **43**.

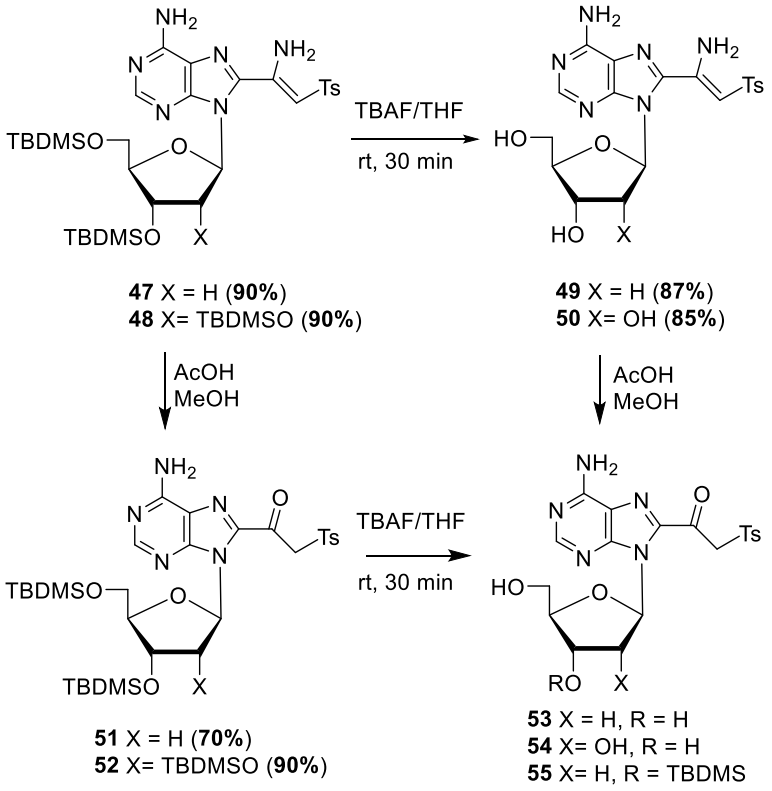


Scheme 21. Deprotection of TBDMS group and conversion of iodovinylsulfones to aminovinylsulfones of 2'-deoxyadenosine and adenosine

Treatment of unprotected iodovinylsulfones **45** or **46 (**Scheme 21**)** with NH_3/MeOH at 0 °C for 30 min gave (*Z*)-*β*-aminovinylsulfones **49** (87%) or **50** (85%). However, removal of TBDMS groups from iodovinylsulfones (e.g., **42** and **43**) with NH_4F or NH_4F/AcOH resulted in lower yields of the expected products (e.g., **45** and **46**). Interestingly, deprotection with TBAF of aminovinylsulfones (e.g., **47** and **48**) gave higher yields without any decomposition. Therefore, at first the TBDMS-protected 8-(iodovinyl)sulfones **42** and **43** were converted to the corresponding aminovinylsulfones **47** (90%) and **48** (90%) upon treatment NH_3/MeOH at 0°C followed by deprotection with TBAF to give 8-(aminovinyl)sulfones **49** (87%) and **50** (85%) in higher yields.

3.1.1.2. Conversion of *β*-aminovinylsulfones to *β*-ketosulfones

Treatment of *β*-aminovinylsulfone **49** with dilute HCl in CH_3CN, by recently reported protocol for pyrimidine nucleosides[39] failed to give the expected *β*-ketosulfone of 2'-deoxyadenosine **53** (Scheme 22) resulting instead in the cleavage of the glycosidic bond.



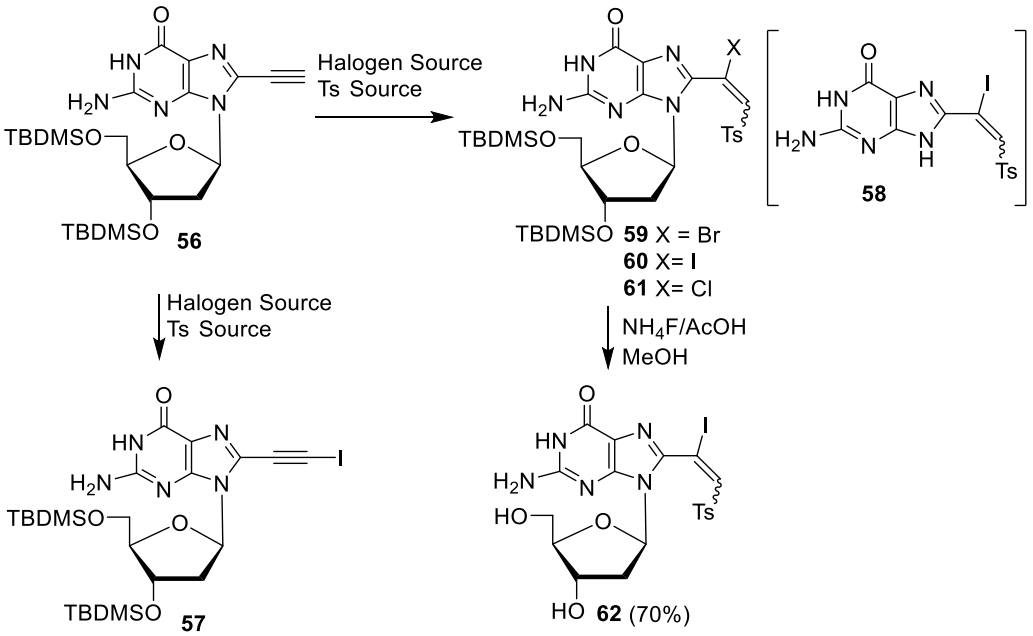
Scheme 22. Synthesis of C8-modified *β*-ketolsulfones of 2'-deoxyadenosine and adenosine

Reaction of **49** under milder condition with AcOH (CH_3CN, 24 h) was also unsuccessful. However, treatment of unprotected **49** with dilute AcOH in MeOH instead of CH_3CN to increase solubility gave desired **53** (90%). Similarly, TBDMS protected *β*-ketosulfones **51** (70%, along with 30% monoprotected **55**) and **52** (90%) as well unprotected **54** (88%) were prepared. The unprotected *β*-ketosulfones **53** (90%) and **54** (90%) were also prepared by TBAF deprotection from **51** and **52**, respectively (Scheme 22).

3.1.2. C8 modified 2'-deoxy guanosine with reactive groups

Guanine nucleosides with reactive (β-halovinyl)sulfone or (β-keto)sulfone group at C8 were prepared from TBDMS protected 8-ethynyl-2'-deoxyguanosine **56.** Substrate **56** was prepared by standard protection/deprotection protocols form 8-[2-(trimethylsilyl)ethynyl]-2'-deoxyguanosine.[52] The iodosulfonylation of **56** with I_2/TsNa/NaOAc (CH_3CN/80 ^oC), gave mixture of products with one being identified as 8-(iodoethynyl)-2'-deoxyguanosine **57** instead of desired iodovinylsulfone **60** (**Table 2**, entry 1); although this protocol was successful on adenine nucleosides (see section 3.1.1; Table 1, entry 6). Similar treatment of **56** with NIS instead of iodine also gave **57** (70%, entry 2). Analogous treatment of **56** with NBS failed to produce either the expected bromovinylsulfone **59** or 8-(bromoethynyl) derivative of type **57** resulting instead starting material back (entry 3).

Table 2. Synthesis of C8-modified *β*-halovinylsulfones of 2'-deoxyguanosine



Entry	Halogen Source	Ts source	Solvent	Additive	Products	Yields (%)
1	I_2	TsNa	CH_3CN	NaOAc	**57**	50
2	NIS	TsNa	CH_3CN	NaOAc	**57**	70
3	NBS	TsNa	CH_3CN	NaOAc	**59**	0
4	I_2	$TsNHNH_2$	CH_3CN	TBHP	**58**	65
5	I_2	$TsNHNH_2$	CH_3CN		**60**	0[a]
6	I_2	TsNa	$EtOH/H_2O$	DTBP	**60**	0
7	I_2	$TsNHNH_2$	CH_3CN	DTBP	**60**	0
8	I_2	TsNa	CH_3CN	DTBP	**60**	0
9	$FeCl_3$	TsNa	EtOH		**61**	0
10	KI	$TsNHNH_2$	DMSO	$(BzO)_2$	**60**	60

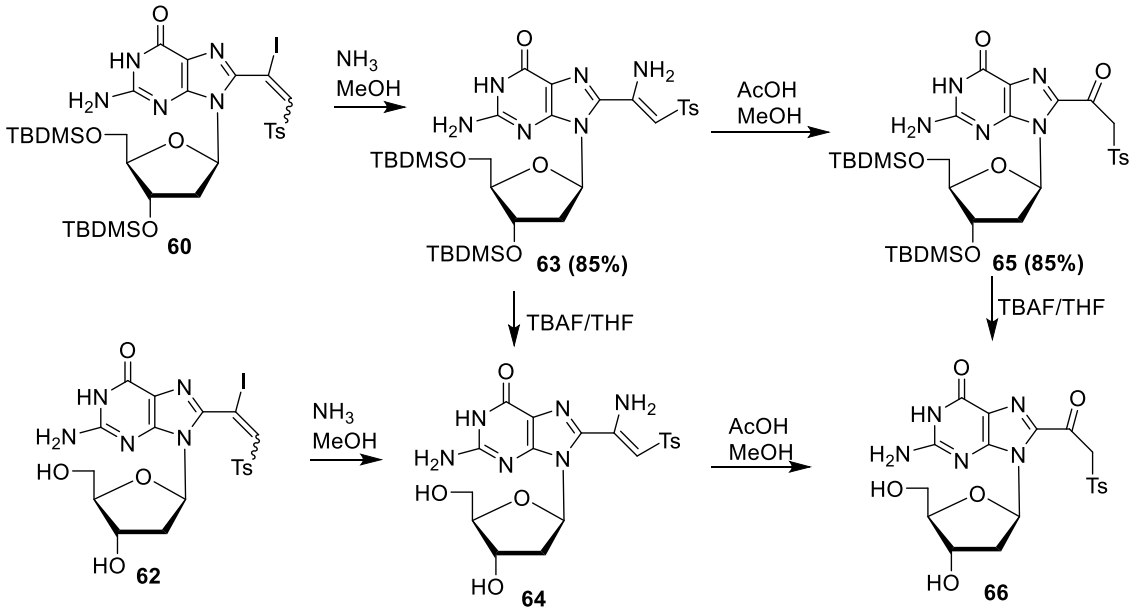
[a]Instead of **60**, monoprotected substrate **56** was isolated.

Subjection of **56** to radical conditions with $I_2/TsNHNH_2/TBHP$ (CH_3CN/80 ^oC) as reported for pyrimidine nucleosides[39] effected iodosulfonylation resulting in the glycosidic cleavage, which gave 8-(*β*-iodovinyl)sulfone guanine **58** (65%, entry 4). Similar treatment of **56** without TBHP also failed to produce **60,** instead yielding 8-ethynylguanine (entry 5). Iodosulfonylation with I_2/TsNa/DTBP ($EtOH/H_2O$, 60 ^oC; entry 6)[84] and its modification

fail to produce **60** (entries 7 and 8). $FeCl_3$ mediated chlorosulfonylation with TsNa in EtOH also failed to give chlorovinyl sulfone **61** (entry 9**)**. Subjection of **56** to the recently reported iodosulfonylation of aryl alkynes[83] with $TsNHNH_2$/KI/benzoyl peroxide in DMSO at rt produced expected *β*-iodovinylsulfone of 2'-deoxyguanosine **60** (*E/Z* = 70/30, 60%; entry 10). Subsequent treatment of **60** with NH_4F/AcOH in MeOH at 60 ^oC for 3 h provided unprotected iodovinylsulfone **62** (70%).

Conversion of β-iodovinylsulfones to β-ketosulfones via β-aminovinylsulfones

The 2'-deoxyguanosine with iodovinylsulfone group **60** at C8 was converted to (*Z*)-amnovinylsulfone **63** (85%) upon treatment with methanolic ammonia at rt for 1 h (Scheme 23). Subsequent hydrolysis of **63** with AcOH/MeOH (rt, 8 h) provided *β*-ketosulfone probe **65** (85%). Then aminovinylsulfone **64** was prepared either treatment of **62** with methanolic ammonia or deprotection of **63** with TABF. Unprotected sulfone **66** was prepared analogously either from **64** with AcOH/MeOH or from **65** with TBAF.



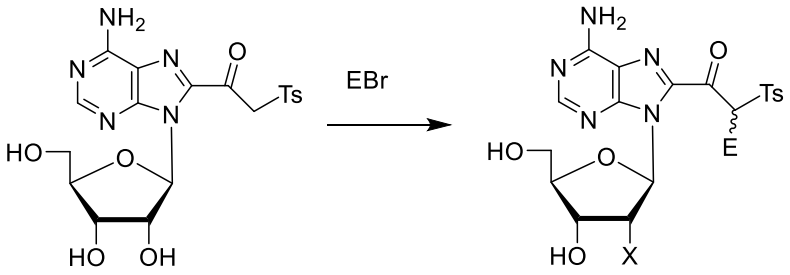
Scheme 23. Conversion of *β*-iodovinylsulfones to *β*-ketosulfones via *β*-aminovinylsulfones of 2'-deoxyguanosine

3.1.3. Reactivity of 8-*β*-iodovinylsulfone probes

The 8-modified (*β*-iodo)vinylsulfones react fast with methanolic ammonia at rt resulting in quantitative conversion to the corresponding enamines (*vide supra*). Reactions with other nucleophiles such propanethiol, glutathione, butyl amine, cysteine etc. are still being optimized

3.1.4. Reactivity of 8-(*β*-keto)sulfone probes

The C8 modified purine nucleosides with reactive *β*-ketosulfones can be envisioned as mechanistically different probes than 8-(*β*-iodoovinyl)sulfones since they can capture electrophiles rather than nucleophiles as they have a methylene acidic proton. (pKa = 10−11). To test the reactivity, *β*-ketosulfone **54** was treated with BnBr in the presence of aqueous NaOH at room temperature resulted in a diastereotopic mixture of α-monobenzylated substance **67** with no α-dialkylated product (Scheme 24). The alkylation of **54** with allyl bromide also provided α-alkylated products **68** (50%).



54 X= OH

67 E = Bn
68 E = Allyl

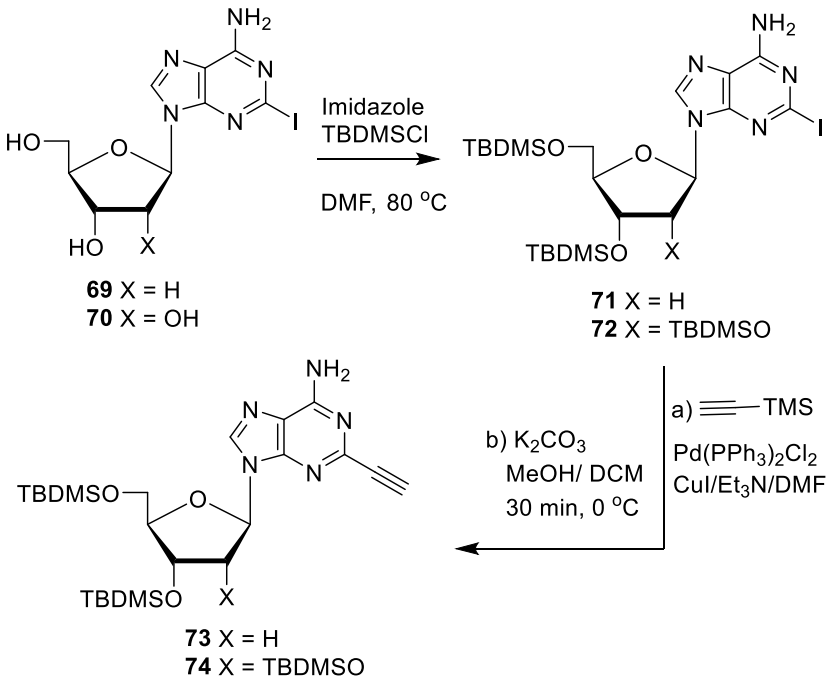
Scheme 24. α-Alkylation of the 8-(*β*-keto)sulfones adenine nucleosides

3.2. Synthesis and reactivity of adenine nucleosides modified at C2 position with (*β*-halo)vinylsulfone and *β*-ketosulfone with reactive groups

3.2.1. Adenine nucleosides modified at C2 position with reactive groups

3.2.1.1. Adenine nucleosides modified at C2 position with (*β*-halo)vinylsulfone

For the synthesis of C2 modified *β*-Iodovinylsulfone for adenine nucleosides, the requisite precursors 2-iodo-2'-deoxyadenosine **69**[147] or 2-iodoadenosine **70**[148] (Scheme 25) was synthesized in multistep procedures following previously reported methods. Then the critical substrates 3', 5'-di-*O*-TBDMS-2-ethynyl-2'-deoxyadenosine **73** and 2', 3', 5'-di-*O*-TBDMS-2-ethynyladenosine **74** were prepared by conventional TBDMS protection of **69** and **70** and Sonogashira coupling and TMS deprotection of iodo-precursors **71**[61] and **72**[60] respectively.

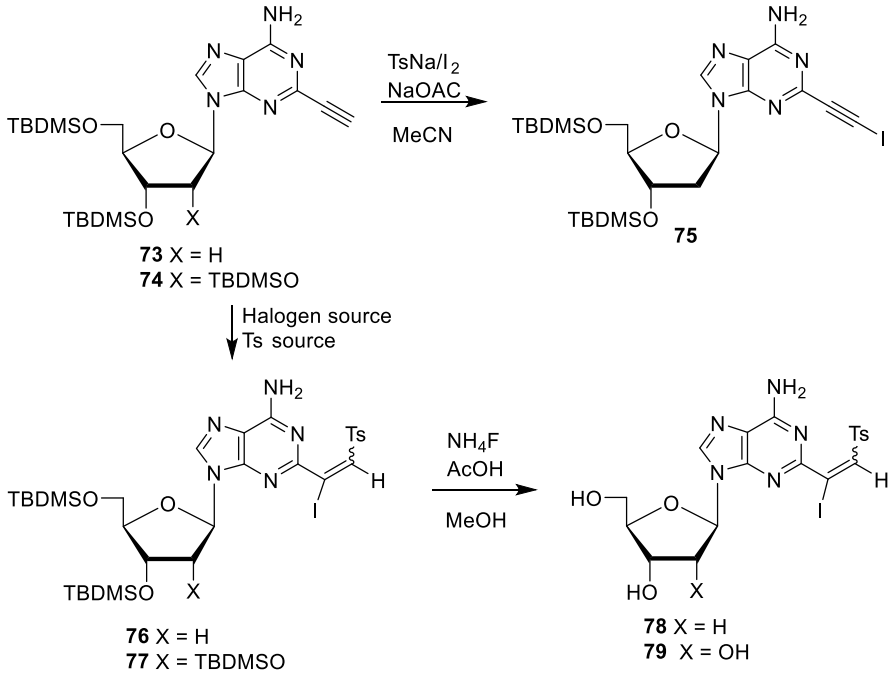


Scheme 25. Synthesis of TBDMS protection 2-ethynyl adenine substrate

The iodosulfonylation condition which we developed for 8-position of adenine nucleosides (see section 3.1.1; Table 1, entry 6) was tested for iodosulfonylation of 2-position of adenine nucleosides **73** with I_2/TsNa/NaOAc (CH_3CN/80 ^oC). This gave byproducts, identified as 8-(iodoethynyl)-2'-deoxyadenosine **75** in 80% yield (Table 3, entry 1). Analogous treatment in absence of NaOAc in H_2O or mixture of H_2O/MeCN gave the same byproduct **75** in lower yields (entries 2 and 3).[149] On the other hand, iodosulfonylation condition, which we developed for 8-position of guanine nucleosides (see section 3.2.1; Table 2, entry 10) with $TsNHNH_2$/KI/$(BzO)_2$ in DMSO at rt, also failed to give any expected product at 2-position, and instead it gave mono-protected starting material (entry 4). Altering the source of iodide to CuI also gave similar result (entry 5). Next, several other reported methods[79,150,151,152] were also applied to synthesize C2 position modified iodovinylsulfone, however, none of them were successful (entries 6 and 7). Another reported iodosulfonylation was tested to iodosulfonylation of **73** with freshly prepared TsI[153] in THF, which failed to give expected *β*-iodovinylsulfone **76**, rather instead gave cleavage of gylcosidic bond (entry 8). Excitingly, similar iodosulfonylation with TsI (2 equiv.) in presence of NaOAc (3 equiv.) afforded expected iodovinylsulfone **76** in 80% yield (*E/Z* = 70/30; entry 9). Similar treatment of ribose analogue **74** gave **77** even in higher yield (92%, entry 10). We also tested this condition for iodosulfonylation of 8-ethynyl adenine nucleosides and 8-ethynyl-2'-deoxyguanosine afforded corresponding iodovinylsulfones in higher yield as compared to protocol described in section 3.1.1; Table 1, entry 6 and section 3.2.1; Table 2, entry 10 illustrating its general character for iodosulfonylation of purine nucleosides. Subsequent treatment of TBDMS protected *β*-

iodovinylsulfones **76** or **77** with NH_4F/AcOH in MeOH at 60 ^oC for 6 h provided unprotected 8-(*β*-iodovinyl)sulfone **78** (78%) or **79** (80%) tentatively assigned as *E* isomer.

Table 3. Synthesis of C2-modified *β*-iodovinylsulfones of 2'-deoxyadenosine and adenosine

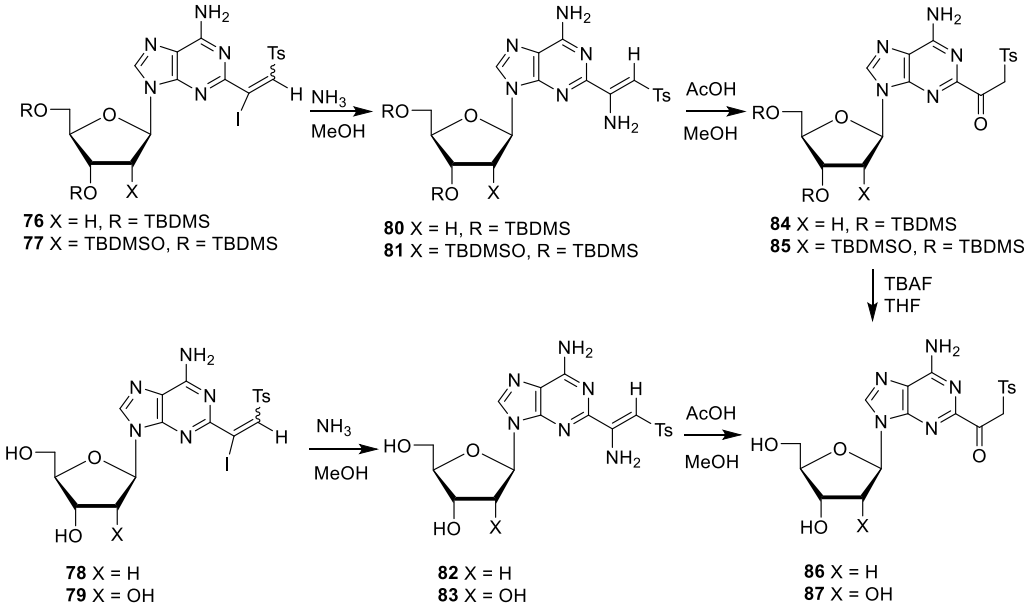


Entry	Halogen Source	Ts source	Solvent	Additive	Products	Yields (%)
1	I_2	TsNa	CH_3CN	NaOAc	**75**	80
2	I_2	TsNa	H_2O		**75**	40
3	I_2	TsNa	H_2O/CH_3CN		**75**	60
4	KI	$TsNHNH_2$	DMSO	$(BzO)_2$	**76**	0
5	CuI	$TsNHNH_2$	DMSO	$(BzO)_2$	**76**	0
6	NaI	TsNa	CH_3CN	CAN	**76**	0
7	KI	TsNa	CH_3CN	$PhI(OAc)_2$	**76**	0
8		TsI	THF		**76**	0
9		TsI	THF	NaOAc	**76**	80
10		TsI	THF	NaOAc	**77**	92

3.2.1.2. Conversion of (*β*-iodo)vinylsulfone to *β*-ketosulfone

The *β*-iodovinylsulfones were then converted to the corresponding *β*-ketosulfones via intermediary *β*-aminovinylsulfones. Thus, treatment of unprotected iodovinylsulfones **78**

or **79** (Scheme 26) with NH_3/MeOH at rt for 1 h gave corresponding (Z)-*β*-aminovinylsulfones **82** or **83**. Protected (*Z*)-*β*-aminovinylsulfones **80** or **81** was prepared analogously from **76** or **77**. Next, treatment of unprotected *β*-aminovinylsulfone **82** or **83** with AcOH (aq) in MeOH at rt for 24 h afforded **86** (84%) or **87** (82%). The TBDMS protected *β*-ketosulfones **84** (90%) and **85** (95%) were prepared using a similar protocol. The unprotected *β*-ketosulfones **86** and **87** were also prepared by TBAF deprotection from **84** and **85** respectively.



Scheme 26. Synthesis of C2 modified *β*-ketosulfones from (*β*-iodo)vinylsulfones

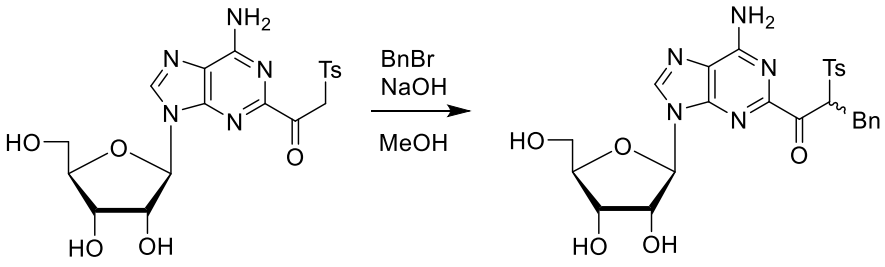
Comparison between 8-modified and 2-modified adenine nucleosides

It is worth mentioning that the C2-modified adenine nucleosides behave differently compare to C8-modified adenine nucleosides. For example, C2-modified aminovinylsulfones **80**, **81**, **82** and **83** are unstable in silica column, which partially convert to corresponding *β*-ketosulfones (**84**, **85**, **86** and **87**) while C8-modified aminovinylsulfones are stable in silica column. The R_f value of the C2-modified protected

iodovinylsulfone **76** was higher than the starting material **73** in all the solvent systems. On the other hand, R_f value of the C8-modified of the protected iodovinylsulfones were lower than the corresponding starting material. Similarly, R_f value of the C2-modified protected aminovinylsulfone **80** was higher than the iodovinylsulfone **76**. In contrast, R_f value of the C8-modified protected aminovinylsulfone was lower than the corresponding iodovinylsulfone. Specially, the C2-modified *β*-ketosulfones (e.g., **84**, **85**, **86** and **87**) are much polar than the corresponding C8 modified analogues. For example, R_f values of the C2-modified *β*-ketosulfones **86** and **87** were much lower than the corresponding aminovinylsulfones. On the other hand, R_f values of the C8-modified *β*-ketosulfones were higher than the corresponding aminovinylsulfones**.**

3.2.2. Reactivity of 2-(*β*-keto)sulfone probes

To test the reactivity, *β*-ketosulfone **87** was treated with benzyl bromide (BnBr) in the presence of NaOH (aq) at room temperature afforded in a diastereotopic mixture of α-monobenzylated substance **88** with the yield of 72% with no α-dialkylated product (Scheme 27).



87 **88**

Scheme 27. α-Alkylation of the 2-(*β*-betoketo)sulfones of adenine nucleosides

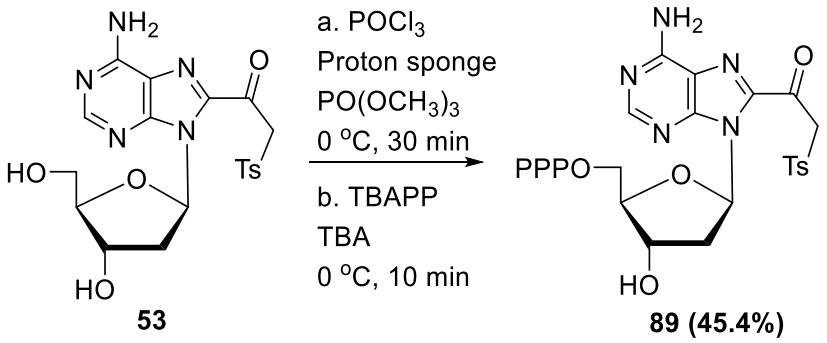
Compound **88** shows two peaks of H8 proton at 8.46 and 8.48 ppm with the integration ratio of 1:1 which proof the presence of diastereomeric product. Similarly, methyl protons of the tosyl (Ts) group also shows two singlet at 2.24 and 2.25 ppm, which again proof the

presence of diastereomeric product. The methylene proton of the Bn group was observed at 3.47-3.51 ppm as a multiplet. Additionally, aromatic protons of Bn group was detected in the region of 7.14-7.25 ppm. In ^{13}C NMR the peak for α-carbon was detected at 70.4 ppm whereas the methylene carbon peak of Bn group was observed at 32.4 and 32.7 ppm. The aromatic peaks of the Bnl group were showed in the range 127.0-130.0 ppm which merge with the Ts peaks.

3.3. Phosphorylation and polymerase catalyzed DNA incorporation

3.3.1. Phosphorylation of 8-(*β*-keto)sulfone probes of 2'-deoxyadenosine

Phosphorylation of *β*-ketosulfone **53** with $POCl_3$ in the presence of proton sponge[154] followed by treatment with mixture of tributylammonium pyrophosphate (TBAPP) and tributylamine (TBA) at 0 °C afforded after Sephadex purification 2'-deoxyadenosine 5'-triphosphate derivative **89** with yield of 45.4% (Scheme 28).



Scheme 28. Phosphorylation 8-*β*-ketosulfone probes of 2'-deoxyadenosine

3.3.2. DNA incorporation of 8-*β*-ketosulfone-dATP by pol *β*

The incorporation of the nucleotide **89** modified with *β*-ketosulfone probe was examined in an oligonucleotide DNA substrate containing a nucleotide gap by pol β. The pol β efficiently incorporated **89** into the one-nucleotide gap substrate containing a phosphate group on the downstream strand (Figure 11). Incorporation of **89** increased with

increasing concentrations of pol β (1−100 nM; lanes 2−7). One nM pol β was sufficient to insert **89** into the DNA to generate ~35% of the DNA synthesis product (lane 2). The 25 nM concentration of pol β was enough to give maximum 80% incorporate (lane 5) as increasing concentration of pol β does not change the incorporation percentage (lane 6 and 7). Polymerase-catalyzed incorporation of the 8-substituted purine nucleotides was known to depend on the size of the substituent and preference for *syn/anti* conformation of the base. Although it was reported that 8-substitued purine nucleotides with bulky group are poor substrates for polymerases, our result suggest that purine nucleotide with bulky *β*-ketosulfone group at C8 position is a good substrate for polymerase.

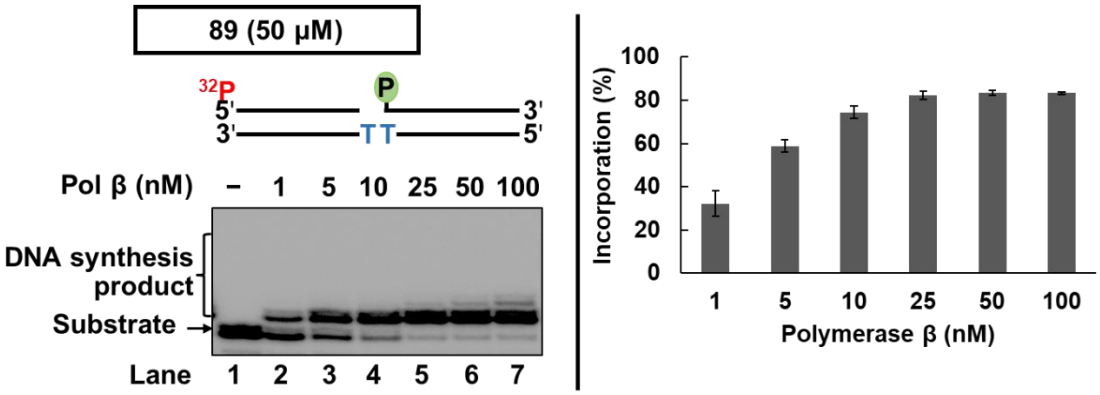
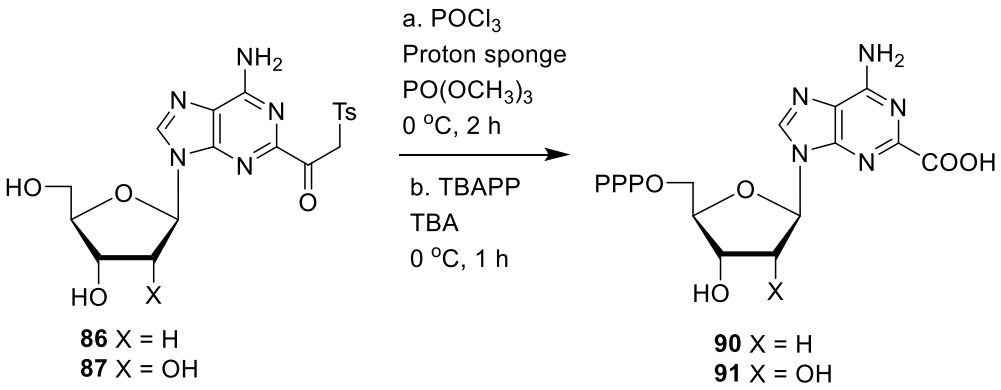


Figure 11. Incorporation of **89** into duplex DNA containing a one-nucleotide gap by pol β. Substrates were ^{32}P-labeled at the 5′-end of the upstream strand of the substrate. Lane 1 indicates substrate only. Lanes 2−7 indicate the DNA synthesis product at increasing concentrations of pol β (1−100 nM). The percentage of **89** insertion product by pol β was quantified and illustrated in the bar chart

3.3.3. Unexpected results of phosphorylation 2-(*β*-keto)sulfone probes of adenine nucleosides

Phosphorylation of **86** (Scheme 29) with $POCl_3$ in the presence of proton sponge followed by treatment with mixture tributylammonium pyrophosphate (TBAPP, 5.0 equiv.) and tributylamine (TBA, 4.0 equiv.) at 0 ^{o}C initially formed expected 5'-triphosphate of *β*-ketosulfone (observed in NMR). However, during purification on

Sephadex with triethylammonium bicarbonate buffer (TEAB), it gradually converted to 2-carboxylic acid-2'-deoxyadenosine-5'-triphosphate **90** (25%). Analogous phosphorylation of **87** also afforded 2-carboxylic acid-adenosine-5'-triphosphate **91** (22%). In order to verify the instability of 5'-triphosphate, **86** and **87** were treated with the same sephadex in 0.5 M TEAB solution at rt for overnight. The TLC and ^{1}H NMR analysis showed no changes of the starting materials, which indicated the triphosphate group was responsible for the instability of the *β*-ketosulfones.

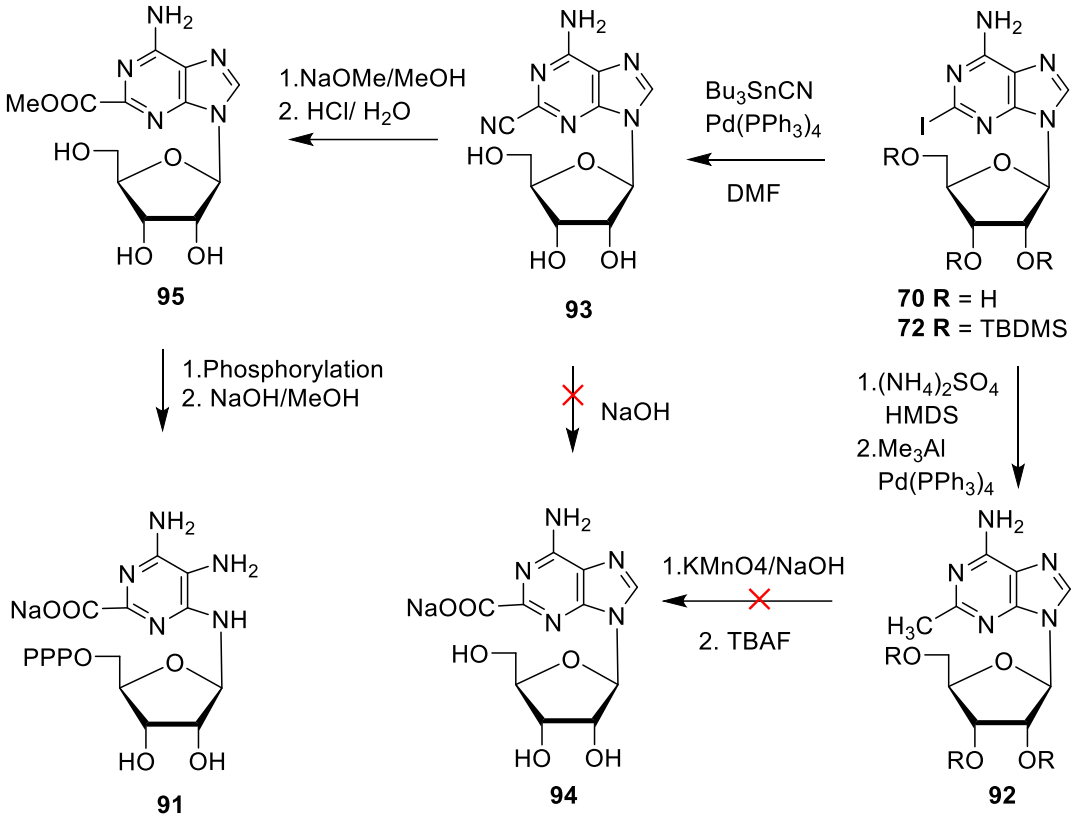


Scheme 29. Phosphorylation of 2-*β*-ketosulfone of adenine nucleosides

3.3.4. Direct synthesis of 2-carboxylate-ATP

To verify the formation of 2-carboxylic acid-5'-triphosphate, we designed alternative synthesis of 2-carboxylic acid-adenosine-5'-triphosphate **91**. At first, we planned methylation of **72** followed by oxidation and de-protection to synthesize **94,** which upon phosphorylation, will produce our target **91** (Scheme 30). Although treatment of **72** with $Me_3Al/Pd(PPh_3)_4$/HMDS in presence of catalytic $(NH_4)_2SO_4$ afforded **92** in 78% yield. However, reflux of **92** with basic $KMnO_4$ failed to oxidize methyl group to produce carboxylic acid **94**. Next, we converted unprotected iodo compound **70** to cyano compound

93 with Bu_3SnCN in presence of catalytic $Pd(PPh_3)_4$ in 80% yield. Which upon treatment with NaOH failed to produce corresponding carboxylic acid **94**.



Scheme 30. Alternative synthesis of 2-carboxylic acid-adenosine-5'-triphosphate

However, we found treatment of cyano compound **93** with NaOMe followed by hydrolysis with HCl (aq) afforded corresponding methyl ester **95** in 80% yield. Which upon phosphorylation by aforementioned condition and hydrolysis with aqueous NaOH, afforded **91** in 25% yield after Sephadex purification. ^{1}H NMR and ^{31}P NMR analysis of compound **91** from both methods show similar spectra as well as HRMS also confirmed the presence of **91**.

3.3.5. DNA incorporation of C2-carboxylate-dATP by pol *β*

The incorporation triphosphate **90** into duplex DNA by pol β was examined by incubating 50 nM substrate containing a 1 nt gap and 5'-phosphate with increasing concentrations of pol β (1 nM-100 nM) in the presence of 50 μM (Figure 12). The results

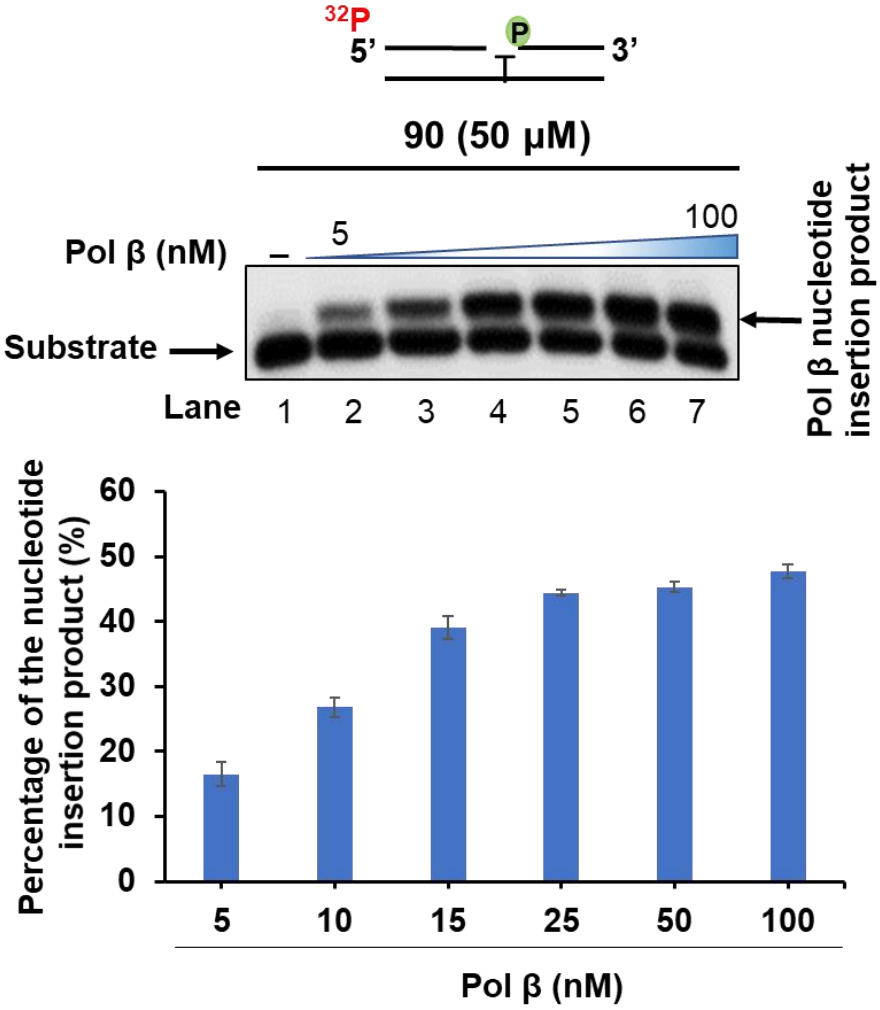


Figure 12. Incorporation of **90** by pol β. The incorporation of **90** into a 1 nt gap by pol β was examined in the presence of 50 nM of 1 nt-gap substrate and increasing concentration of pol β (5 nM-100 nM). Lane 1 indicates substrate only. Lanes 2−7 show **90** insertion product by pol β at 5 nM-100 nM. The percentage of **90** insertion product by pol β was quantified and illustrated in the bar chart below the gel. The substrate was ^{32}P-labeled at the 5′-end of the upstream primer

showed that pol β readily incorporated **90** to fill in the 1 nt gap at a low concentration of 5 nM (Figure 12, lane 2). Increasing concentrations of pol β from 5 nM to 25 nM led to significantly elevated incorporation of the **90** nucleotide analog (lanes 2-5). However, pol β at 50 nM and 100 nM exhibited a similar percentage of nucleotide insertion products to 25 nM (compare lanes 6-7 with lane 5). The results indicate that the **90** nucleotide analog can be readily incorporated into the DNA by a repair DNA polymerase. A previous study has shown the incorporation of C2-modified purines and the potential application of their fluorescence conjugates in detecting DNA and RNA in cells[155]. It is possible that **90** can also be conjugated with a fluorescent tag to be developed as a new probe for staining of DNA in cells. Besides, the modification at C2 can be used to investigate the effect of the functional groups at the position on their insertion by DNA polymerases[156]. On the other hand, since nucleotide analogs are extensively used for the treatment of diseases, such as cancer and viral infection[157,158] **90** may also be developed as a new analog for disease treatment.

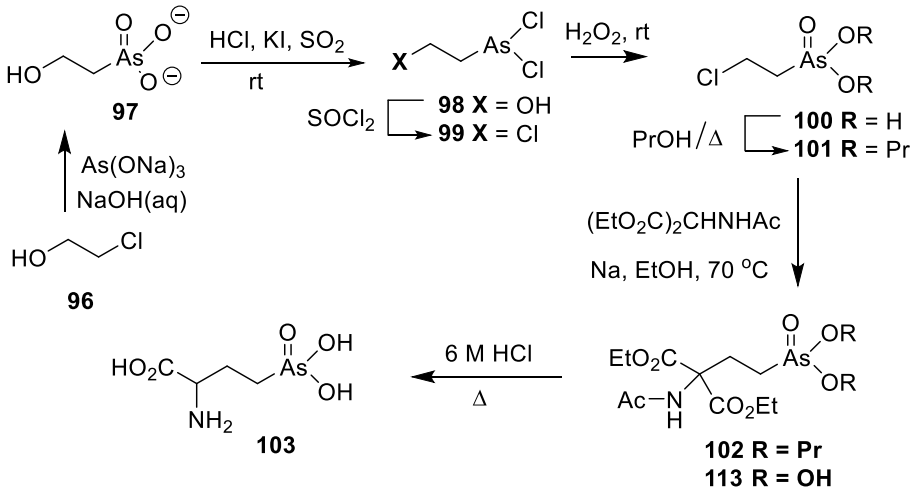
3.4. Synthesis of the organoarsenical antibiotic arsinothricin (AST).

3.4.1. Chemoenzymatic synthesis of the organoarsenical antibiotic arsinothricin

The work in section 3.4.1 and part of experimental section 4.3 was published as an original research paper in the *Journal Natural Products* for which I was one of the co-first authors. Adapted with permission from Suzol, S. H.; Hasan Howlader, A.; Galván, A. E.; Radhakrishnan, M.; Wnuk, S. F.; Rosen, B. P.; Yoshinaga, M. Semi-synthesis of the organoarsenical antibiotic arsinothricin, *J. Nat. Prod.* **2020**, *83*, 2809. *Copyright © 2020, American Chemical Society.*

3.4.1.1. Synthesis of AST-OH from 2-chloroethanol.

In 1983, the synthesis of 2-amino-4-arsonobutanoic acid (AST-OH; **103**) was reported.[143] AST-OH is an immediate precursor of 4-arsono-2-hydroxybutanoic acid, which was identified from the crystal structure as a racemic mixture of the D/L-enantiomers.[159] AST-OH **103** was prepared from arsonic acid **97** by modification of the reported method (Scheme 31).[143] Thus, treatment of 2-chloroethanol **96** with Na_3AsO_3 generated *in situ* from arsenic trioxide (0.5 equiv) and aqueous NaOH (3 equiv), afforded crude (2-hydroxyethyl)arsonic acid **97** with unidentified impurities (based on ^{1}H NMR). Arsenite acts as a nucleophile that displaces the chlorine atom to give pentavalent arsonic acid **97**. To substitute the ethylene hydroxyl group in **97** with a chloride atom to get **100,** polar pentavalent arsonic acid **97** was reduced to the less polar trivalent arsine derivative **98**. Treatment of crude **97** with SO_2 gas (3 equiv) in the presence of catalytic amounts of KI and excess HCl afforded dichloro-(2-hydroxyethyl)arsine **98**. An excess of hydrochloric acid was required to minimize hydrolysis of **98**. Subsequent reaction of crude **98** with thionyl chloride (2.5 equiv) yielded pure dichloro-(2-chloroethyl)arsine **99** in a 52% yield (overall from **96**) after vacuum distillation. The arsine **99** was oxidized into (2-chloroethyl)arsonic acid **100** with excess H_2O_2. To prevent decomposition of **100**, the reaction mixture was carefully evaporated under reduced pressure, providing pure **100** (65%) after recrystallization from acetone/ethyl ether. The structure of **100** was confirmed from HRMS and NMR data.



Scheme 31**.** Synthesis of AST-OH from 2-chloroethanol

Reflux of **100** with anhydrous propanol afforded the propyl ester **101** (73%), which contained ~30% of the vinyl arsonic byproduct(s) probably formed by elimination of HCl. Treatment of crude **101** with diethyl acetamidomalonate (3 equiv) in the presence of freshly prepared sodium ethoxide (4 equiv) at 70 ^{o}C yielded the crude malonate product **102**. Heating the reaction mixture is necessary since this reaction at ambient temperature failed to produce **102**. Reflux of crude **102** in 6 M HCl effected global deprotection and decarboxylation to yield crude **103**. Purification by cation exchange chromatography on a Dowex$^{(R)}$ 50WX8 (H^+ form) column with triethylammonium acetate (TEAA)/AcOH buffer afforded 2-amino-4-arsonobutanoic acid **103** (18%, from **100**).

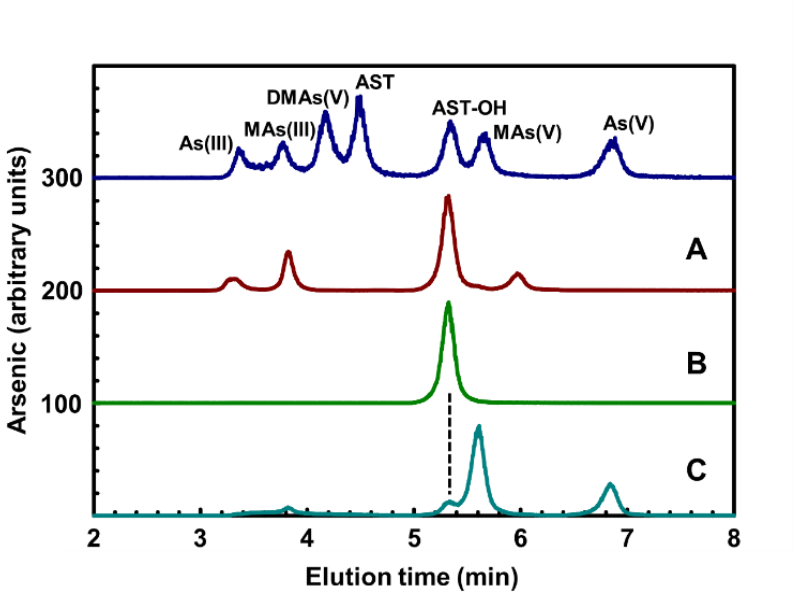


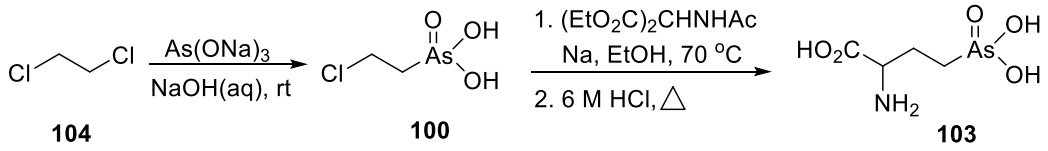
Figure 13. HPLC-ICP-MS analysis of chemically synthesized AST-OH. Line A, crude AST-OH synthesized from **96**; Line B, purified AST-OH; Line C, crude AST-OH synthesized from **104**

Esterification of (2-chloroethyl)arsonic **100** was found not to be necessary since subjection of **100** directly to coupling with diethyl acetamidomalonate produced **113** followed by deprotection and decarboxylation also provided AST-OH **103** (56%) even in higher yield. HPLC coupled with ICP-MS (inductively coupled plasma mass spectrometry) analysis suggests that the purity of the crude **103** and the purified **103** is approximately 60% and nearly 100%, respectively (Figure 13, lines A and B).

3.4.1.2. Synthesis of AST-OH from 1,2-dichloroethane

A shorter 3-steps synthesis of AST-OH from 1,2-dichloroethane **104** was also developed (Scheme 32). Condensation of 1,2-dichloroethane **104** with basic sodium arsenite afforded (2-chloroethyl)arsonic acid **100** (13%) in a single step. Product **100** contained ~10% of vinyl arsonic byproduct(s) as judged by the appearance of the characteristic vinylic peaks from CH_2=CH group in the ^{1}H NMR spectrum in addition to

the 1,2-diarsonoethane adduct detected in HRMS. This approach eliminates (a) the necessity of conversion of the pentavalent (2-hydroxyethyl)arsonic acid **97** to trivalent dichloro(2-hydroxyethyl)arsine **99** with toxic SO_2 gas and (b) challenging displacement of hydroxyl group with chloride. Coupling of crude **100** with diethyl acetamidomalonate followed by deprotection and decarboxylation also yielded AST-OH **103** but in lower yield (8%). HPLC-ICP-MS analysis suggests that the purity is roughly 8% with respect to arsenic (Figure 13, line C).

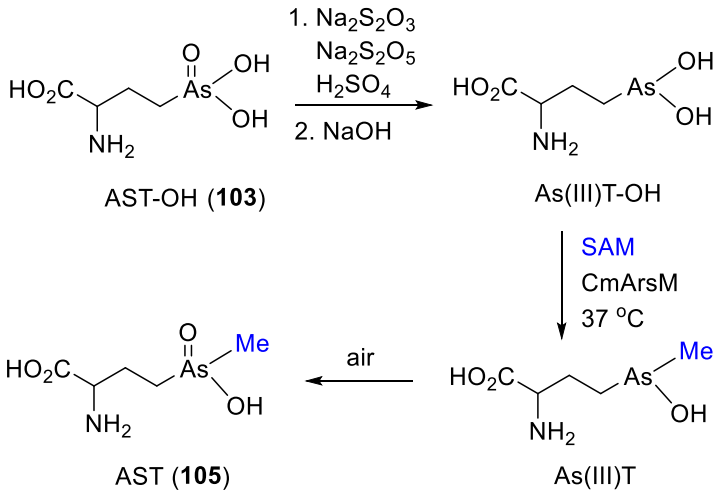


104 **100** **103**

Scheme 32. Synthesis of AST-OH from 1, 2-dichloroethane

3.4.1.3. Enzymatic methylation of AST-OH to produce AST

When exposed to trivalent inorganic arsenite, *B. gladioli* GSRB05 initially produces AST-OH, followed by gradual biotransformation to AST, indicating that the final step of AST biosynthesis is methylation of AST-OH to AST.[123] Microbial methylation of trivalent arsenicals is catalyzed by the enzyme ArsM, an As(III) *S*-adenosylmethionine (SAM) methyltransferase.[160] AST-OH is structurally similar to methylarsenate [MAs(V)], which suggested that it could be a substrate for enzymatic methylation by ArsM to produce AST. To examine this possibility, AST-OH was first chemically reduce to trivalent AST-OH (As(III)T-OH) with an acidic mixture of $Na_2S_2O_3$ and $Na_2S_2O_5$[161] and then incubated with the purified CmArsM enzyme from *Cyanidioschyzon merolae*[162] (Scheme 33).



Scheme 33. Enzymatic methylation of AST-OH to AST

CmArsM catalyzed transfer of the *S*-methyl group of SAM to As(III)T-OH, nearly complete converting it into the trivalent form of AST (As(III)T), presumably as a mixture of the D/L-enantiomers, which then spontaneously oxidized in air to the final product, AST **105** (Figure 14). In the absence of CmArsM, most of the As(III)T-OH re-oxidized to AST-OH. Thus, AST can be quantitatively produced from chemically synthesized AST-OH by enzymatic methylation.

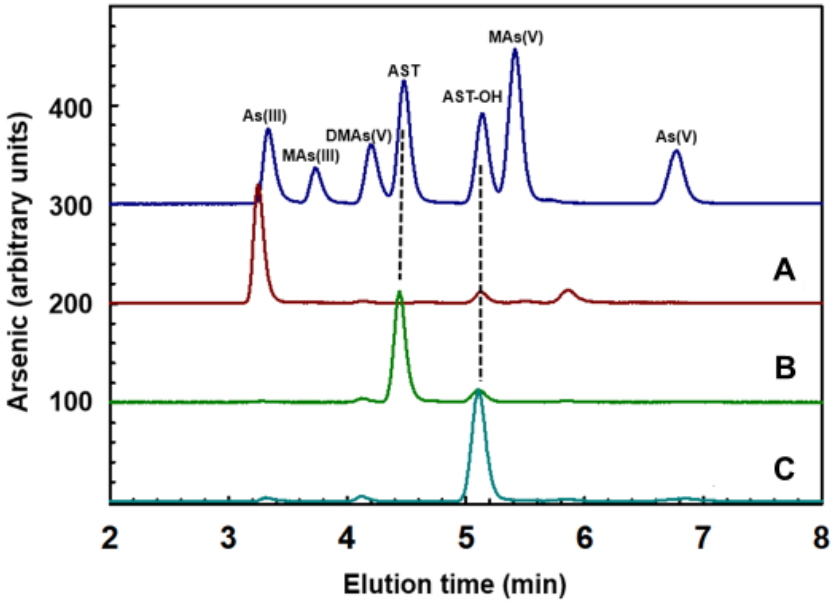


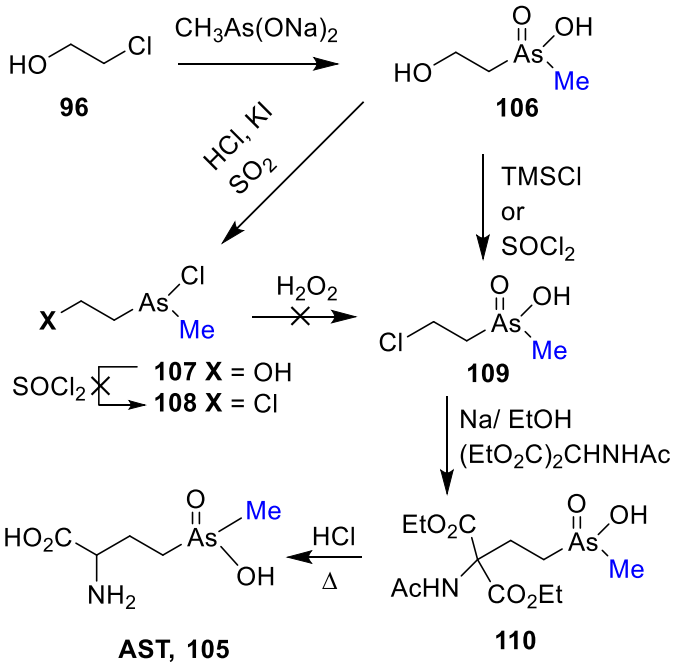
Figure 14. Enzymatic methylation of AST-OH to produce AST. Reduced As(III)T-OH (line A) was incubated in the presence (line B) or absence (line C) of CmArsM, and the arsenic species in the reaction solutions were analyzed by HPLC-ICP-MS

3.4.2. Chemical Synthesis of the Organoarsenical Antibiotic Arsinothricin

3.4.2.1. Synthesis of 2-chloroethyl(methyl)arsinic acid and its conversion to AST

In our first approach for the chemical synthesis of AST **105**, pentavalent 2-hydroxyethyl(methyl)arsinic acid **106** and 2-chloroethyl(methyl)arsinic acid **109** were designed as crucial precursors. Thus, nucleophilic displacement of chloride in 2-chloroethanol **96** with sodium methylarsonite [$CH_3As(ONa)_2$] provided **106** in approximately 86% yield (Scheme 34). The sodium methylarsonite was prepared in high yield by *in situ* reduction of the sodium salt of methyl arsonate [$CH_3As(O)(OH)ONa$] with SO_2 gas in the presence of HCl and catalytic amount of KI[163] followed by hydrolysis of the resulting diiodo(methyl)arsine (CH_3AsI_2) with aqueous NaOH. Reported synthesis of sodium methylarsonite [$CH_3As(ONa)_2$] involves the multistep preparation of chloro(2-chloroethyl)(methlyl)arsane from methyl(oxo)arsane which upon treatment with aq. NaOH

yields $CH_3As(ONa)_2$.[164] Reduction of **106** with SO_2/HCl/KI yielded less polar trivalent chloro(2-hydroxyethyl)(methyl)arsine **107**, which appears to be susceptible to hydrolysis as it was observed for dichloro(2-hydroxyethyl)arsine.[165] Treatment of crude **107** with $SOCl_2$ resulted in vigorous reaction and failed to give **108**, instead producing dichloro(2-hydroxyethyl)arsine **98** (as confirmed by the loss of methyl group signal in NMR). Treatment of the latter with H_2O_2, afforded (2-hydroxyethyl)arsonic acid **97** instead of **109.**



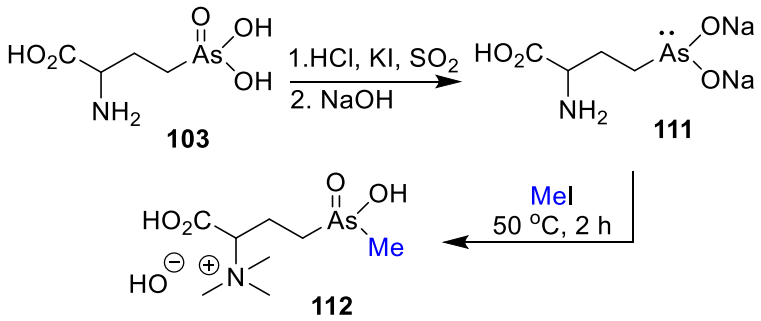
Scheme 34. Synthesis of 2-chloroethyl(methyl)arsinic acid and conversion to AST

Treatment of **106** with TMSCl in DMSO afforded **109** [8%, based on ^{1}H NMR and HPLC-ICP-MS in addition to unchanged **106**. Subsequent reaction of this mixture with acetamidomalonate in the presence of sodium ethoxide at 70 °C yielded malonate **110.** Reflux of crude **110** in 6 M HCl effected global deprotection and decarboxylation providing AST **105** (5% overall from **106**) as estimated by ICP-MS. However, chlorination of the purified and iodide-free sodium salt of **106** with $SOCl_2$ provided **109** (85%, based

on ^{1}H NMR) containing also acidic form of substrate **106** (15%). Treatment of crude **109** with acetamidomalonate followed by deprotection and decarboxylation of the resulting **110** yielded AST **105** (17%) after purification by Dowex and Sephadex column chromatography.

3.4.2.2. Attempted synthesis of AST via direct methylation of AST-OH.

Building on our enzymatic methylation of AST-OH to AST[165] (see section 3.4.1.3), we chemically methylated reduced As(III)T-OH **111** with MeI as a source of an electrophilic methyl group. Reduction of **103** with SO_2/HCl/KI (rt/15 min) followed by treatment with 6 M NaOH gave the reduced arsenic salt **111** (Scheme 35). Treatment of the alkaline solution of crude **111** with excess MeI effected methylation at arsenic atom. However, the reaction also resulted in methylation of the amino group yielding, after purification on cation exchange resin (Dowex$^{(R)}$ H^+ form) with NH_4OH, the trimethylammonium salt **112** (70%, from **103**).

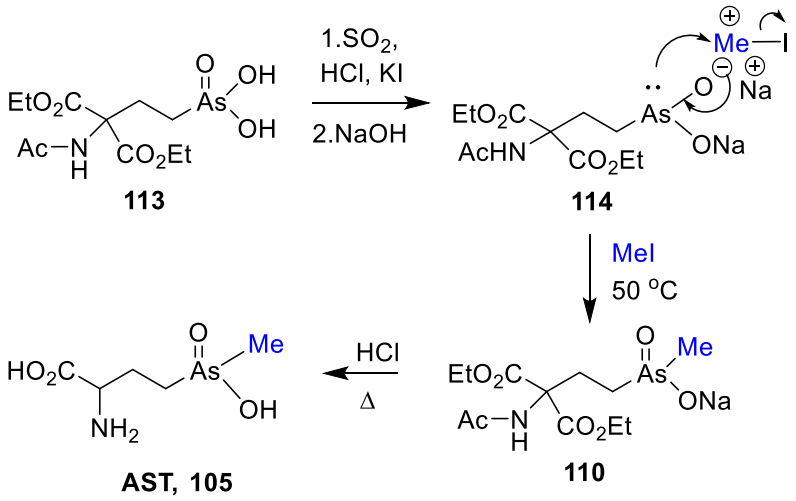


Scheme 35. Attempted synthesis of AST via direct methylation of AST-OH

3.4.2.3. Synthesis of AST from *N*-acetyl protected AST-OH derivative via reduction and methylation

Following encouraging methylation of **111** with MeI, we envisioned that reduction/methylation sequence of the AST-OH derivative bearing the protected amino group would result in straightforward synthesis of AST. We selected *N*-acyl protection for

the amino group in AST-OH **113**, especially since the original synthesis of AST-OH[143] and our improved protocol required synthesis of the *N*-acetyl protected derivative of type **113**[165] (Scheme 36). Thus, treatment of (2-chloroethyl)arsonic acid **100** with acetamidomalonate in the presence of freshly prepared sodium ethoxide (4 equiv) at 70 ºC following purification from the excess of malonate afforded **113**. Reduction of pure **113** with SO_2/HCl and catalytic KI followed by pH adjustment to ~11 with 6 M NaOH gave sodium salt of the trivalent arsenic compound **114.** Subsequent treatment of **114** with MeI (50 ºC/4 h) resulted in exclusive methylation at the arsenic atom, providing protected pentavalent AST derivative **110**. Excess MeI and elevated temperature were crucial for the optimal yield. The progress of the methylation reaction was monitored by HPLC-ICP-MS. Reflux of sodium salt of **110** in 6 M HCl effected global deprotection and decarboxylation providing crude AST. Purification on Dowex (H^+ form) column with 0.25 M NH_4OH followed by size-exclusion chromatography on Sephadex LH-20 with 70% (v/v) of EtOH/H_2O afforded AST **105** (60%, from **113**), presumably as a mixture of the D/L-enantiomers. The reduced As(III)T-OH byproduct (30%) was also isolated, whereas formation of dimethylated product was not observed.



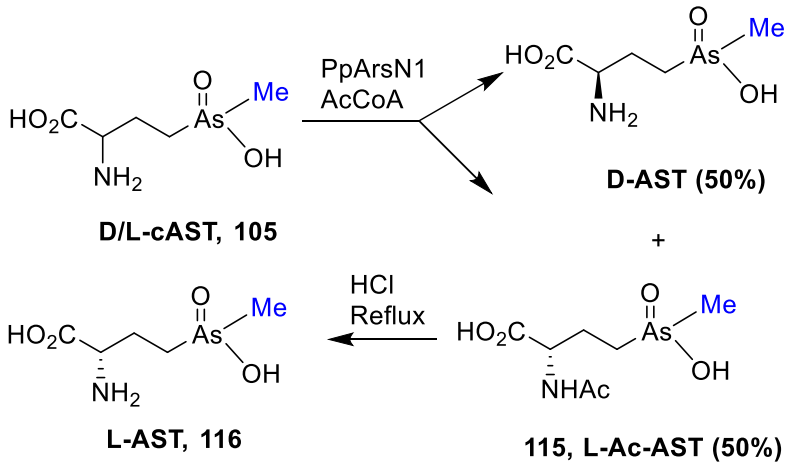
Scheme 36. Synthesis of AST from *N*-acetyl protected AST-OH derivative **113** via reduction and methylation

We propose that the methylation of **114** with MeI in basic solution involves S_N2 attack of the nucleophilic arsenic species on the electrophilic methyl iodide with concurrent formation of the arsenic-oxygen double bond, which also oxidized trivalent arsenic to the pentavalent species **110**. The reaction resembles a Michaelis–Arbuzov reaction of trivalent phosphorus esters with alkyl halides to form pentavalent phosphonate esters. Analogous conversion of trivalent to pentavalent organoarsenicals with alkyl halides has been noted.[166]

3.4.2.4. Enantiomeric separation of racemic AST

Once we completed the chemical synthesis of racemic arsinotricin (cAST), PpArsN1 was utilized to purify L-AST from D/L-cAST. 7 mg of cAST was incubated with purified PpArsN1 and AcCoA overnight, resulting in a mixture of D-AST and L-AcAST **115** (Scheme 37). Purification by size-exclusion chromatography on Sephadex LH-20 afforded **115** (3.0 mg, 36%) and D-AST (2.1 mg, 30%). Reflux of **115** in 2 M HCl effected acetyl deprotection providing L-AST **116** after purification on Sephadex LH-20 column with 70%

(v/v) of EtOH/H_2O. This product (L-AST, **116**), when treated PpArsN1/AcCoA, was acetylated quantitatively to **115**, proving its enantiomeric purity (Figure 15).



Scheme 37. Enzymatic acetylation of cAST to L-AcAST and chemical deacetylation to L-AST

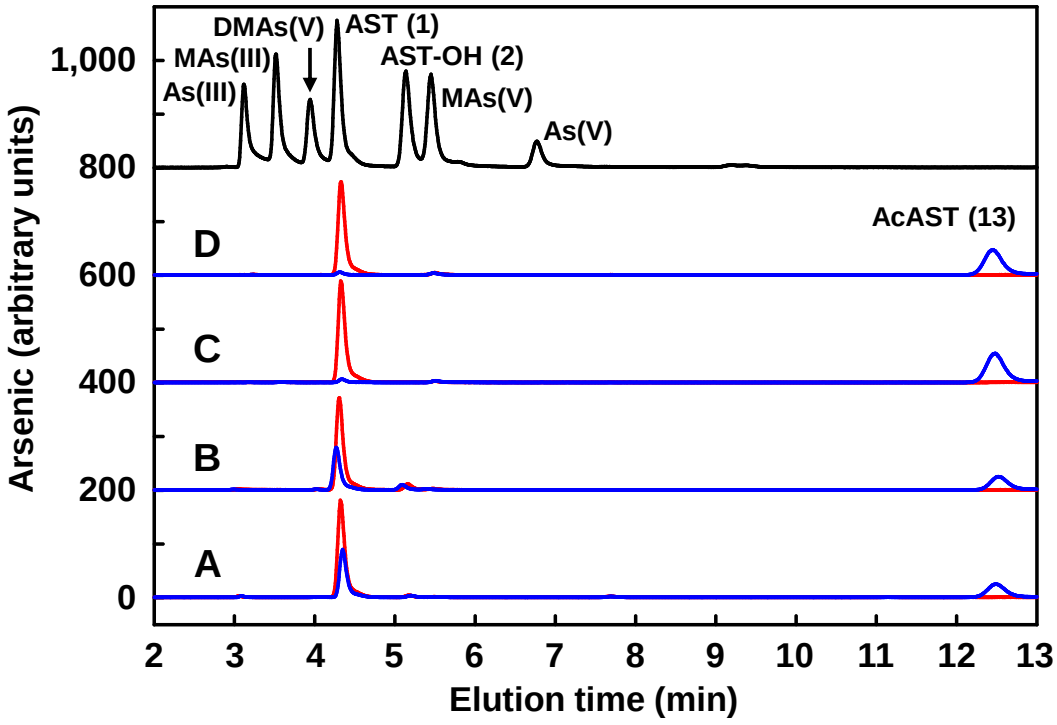


Figure 15. Enzymatic acetylation of AST to produce L-Ac-AST. Chemically synthesized D/L-cAST (A), semisynthetic D/L-sAST (B), biogenic L-bAST (C) or L-enantiomeric (D) **116** was incubated in the absence (red lines) or presence (blue lines) of PpArsN1

3.4.3. The antibiotic properties of AST

The antibiotic properties of the chemically synthesized AST **105** (cAST, presumably a mixture of the D/L-enantiomers) were characterized and compared with those of biogenic AST[123,124] (bAST, the L-enantiomer) and semi-synthesized AST (sAST, a mixture of the D/L-enantiomers). Approximately twice as much cAST or sAST was required to inhibit growth (Figure 16) and GS activity (Table 4) of *Escherichia coli* as bAST, consistent with the L-enantiomer of b-AST as the active species. ArsN1, the bacterial enzyme that confers AST resistance, catalyzes transfer of the acetyl group of acetyl coenzyme A (AcCoA) to the amine group of **105**, generating acetyl-AST (AcAST, **115**; Scheme 37).

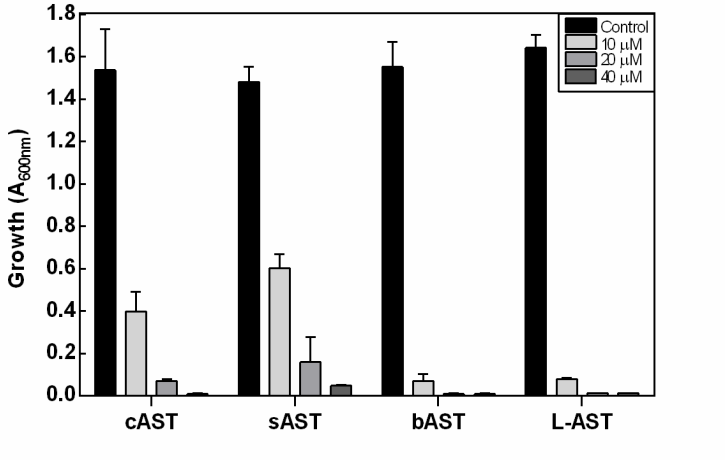


Figure 16. AST inhibits growth of *E. coli*. Cells were cultured in M9 medium in the absence or presence of the indicated concentrations of D/L-cAST, D/L-sAST, L-bAST, L-AST. Growth was estimated from the A_{600nm} after 16 h. Data are the mean ± SE (n = 3)

Purified PpArsN1 (ArsN1 from *Pseudomonas putida* KT2440) nearly completely converted bAST to an arsenic species predicted to be AcAST, while only 50% of racemic cAST or sAST were converted to the putative species and the other half was unmodified (Figure 15), consistent with only the L-enantiomer being the substrate of ArsN1, as predicted from L-AST-bound ArsN1 crystal structures.

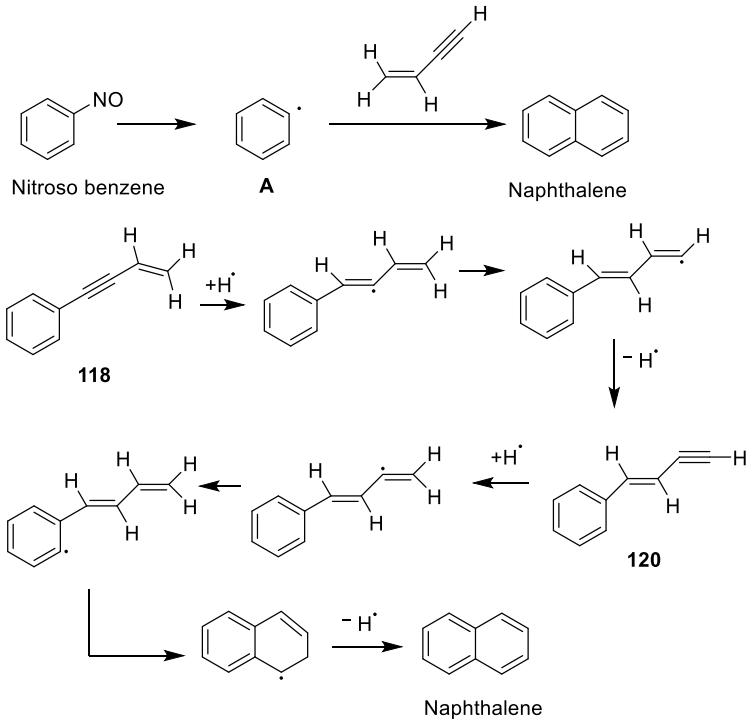
Table 4. Inhibition of *E. coli* glutamine synthetase by AST

AST	K_i (μM)
Chemically synthesized (D/L-cAST)	0.75 ± 0.20
Semisynthetic (D/L-sAST)	0.65 ± 0.20
Biogenic (L-bAST)	0.30 ± 0.10

3.5. Synthesis of precursors and calibration compounds for the gas phase synthesis of polyaromatic hydrocarbons (PAH)

3.5.1. Synthesis of 4-phenylvinylacetylene and *trnas*-1-phenylvinylacetylene.

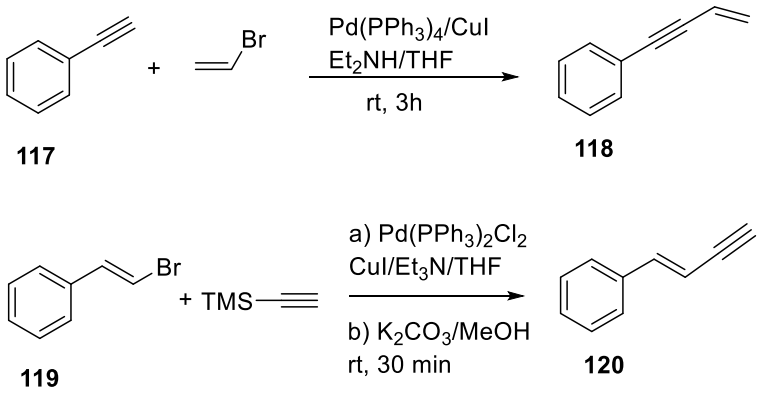
It was proposed that the naphthalene, 4-phenylvinylacetylene **118**, and trans-1-phenylvinylacetylene **120** are the *predominant* $C_{10}H_8$ isomers formed in the reaction between phenyl radical **A**, pyrolytically generated from nitrosobenzene, and vinylacetylene (C_4H_4) at elevated temperatures of 1600 K within the pyrolytic reactor.[133]



Scheme 38. Gas phase synthesis of naphthalene from nitrosobenzene and vinyl acetylene

The 4-phenylvinylacetylene is produced via the phenyl radical addition to the terminal acetylenic carbon of vinylacetylene (Scheme 38) followed by the H loss atom. Similarly, *trans*-1-phenylvinylacetylene is produced by phenyl addition to terminal vinylic carbon. The pathway to form naphthalene involving two H migrations and a six-member ring closure through immediate H loss which is most favorable due to the formation aromatic ring.

To confirm this pathway and to validate theoretical calculation the enynes **118** and **120** were prepared. The 4-phenylvinylacetylene **118** was synthesized by CuI/$Pd(PPh_3)_4$-mediated Sonogashira coupling between phenylacetylene **117** and vinyl bromide in 83% yield in THF at rt for 3 h (Scheme 39). The *trass*-1-phenylvinylacetylene **120** was prepared by CuI/$Pd(PPh_3)_2Cl_2$ mediated coupling between *β*-bromostyrene **119** and (trimethylsilyl)acetylene followed by desilylation of the resulting TMS protected alkyne with the yield of 95%.



Scheme 39. Synthesis of 4-phenylvinylacetylene and *trnas*-1-phenylvinylacetylene

3.5.2. Synthesis of haloindenes

Recently, it was demonstrated that pyrolysis of different bromoindenes at 1500 K produces resonance-stabilized and thermodynamically most stable 1-indenyl π radical,

which was found not to be an effective precursor for the further growth of polycyclic aromatic hydrocarbons (PAH) through the hydrogen abstraction-acetylene (or vinylacetylene) addition.[167] However, radical-radical reaction between 1-indenyl radical and methyl radical form naphthalene took place predominantly to convert for the first time five-membered ring to six-membered rings (Figure 17).[137] Alternatively, it was anticipated that pyrolysis of 5-, or 6-iodoindene isomers might lead to the formation of σ radicals localized in the phenyl ring of indene because C-I bond is weaker than C-Br bond. These 5- and 6-indenyl radicals might then act as precursors for growth of non-planar PAH molecules containing five-member rings. To confirm this pathway and to validate theoretical calculation different haloindene precursors and their enyne derivatives were prepared.

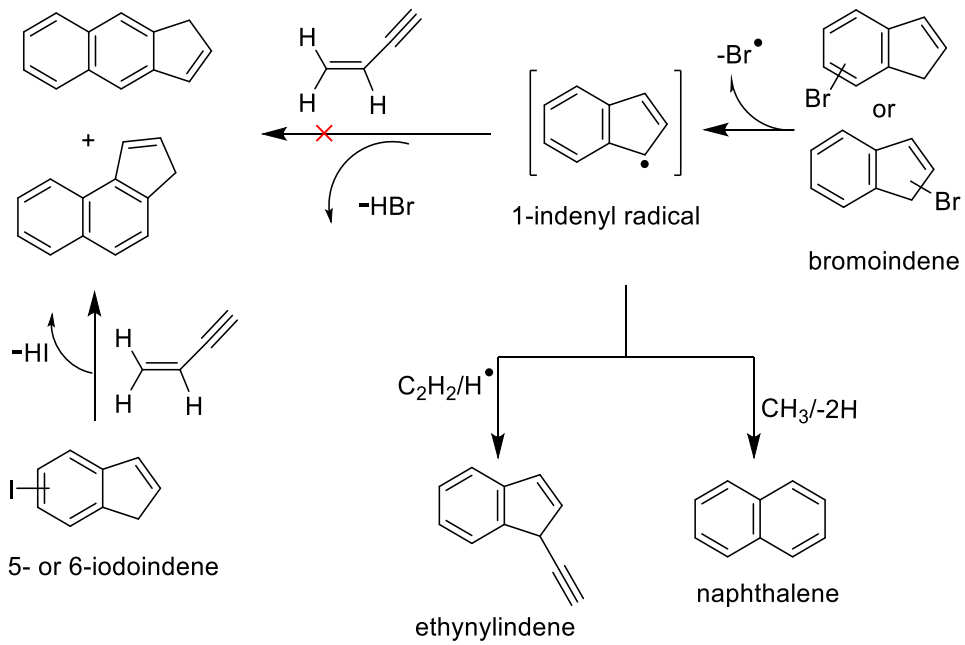
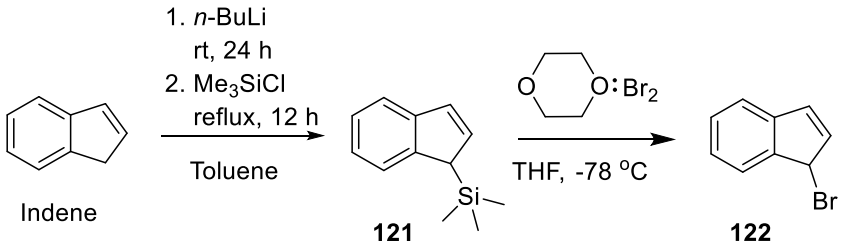


Figure 17. Generation of indenyl radical from haloindenes and its further reactions

3.5.2.1. Synthesis of 1-bromoindene.

The 1-bromoindene **122** was synthesized from commercially available indene with *n*-BuLi and trimethylchlorosilane (TMSCl) followed by substitution of TMS group with dioxane dibromide of the resulting 1-trimethylsilylindene **121** in 68% yield (Scheme 40).



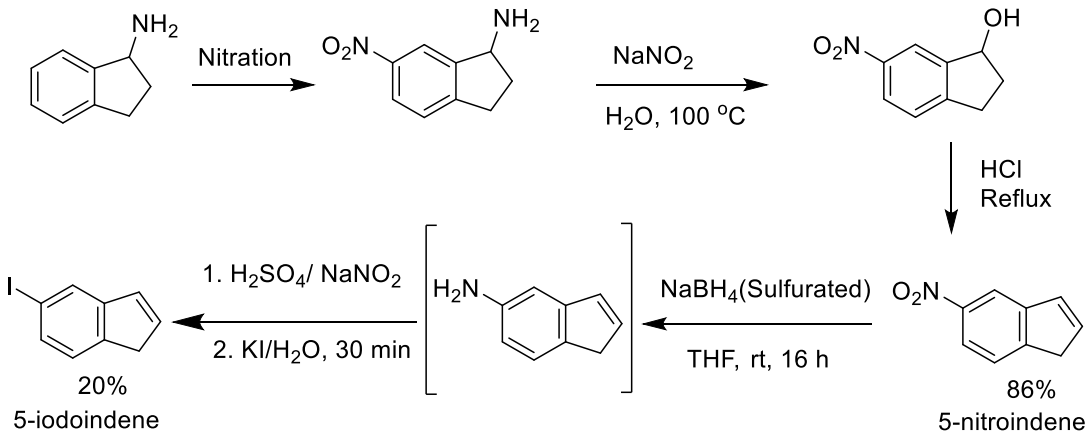
Scheme 40. Synthesis of 1-bromoindene from indene

3.5.2.2. Synthesis of iodoindene and its derivatives

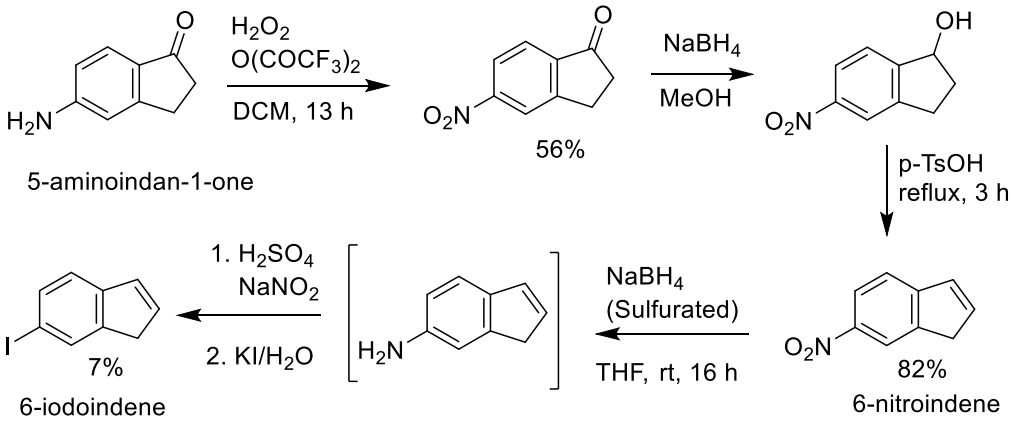
The work in Section 3.5.2.2 and part of experimental section 4.4 was published as an original research paper in the *Tetrahedron Letters* for which I was the first authors. Adapted with permission from Howlader, A. H.; Diaz, K.; Mebel, A. M.; Kaiser, R. I.; Wnuk, S. F. Iodoindenes: Synthesis and application to cross-coupling. *Tetrahedron Lett.* **2020**, *61*, 152427. *Copyright © 2020, Elsevier Ltd.*

Although there are several reports for the synthesis of 5- or 6-chloro- and bromoindenes,[168-171] there is only one method for the preparation of more the reactive 5- or 6-iodoindene which requires expensive intermediates and several steps.[170] Furthermore, reported yields for 5- or 6-iodoindenes obtained by the reduction of the corresponding 5- or 6-nitroindene followed by diazotization-iodination of the resulting unstable 5- or 6-aminoindene were only 20% (Schemes 41) and 7% (Scheme 42).[170] The 5-nitroindene and 6-nitroindene precursors were prepared from 1-aminoindane[172] or 5-aminoindan-1-one,[170] respectively. Therefore, we have undertaken efforts to develop a general method for the

synthesis of iodoindenes which employs iodoindan-1-ones as convenient precursors and avoids the use of expensive nitroindenes, unstable aminoindenes and potentially explosive trifluoroperacetic acid.



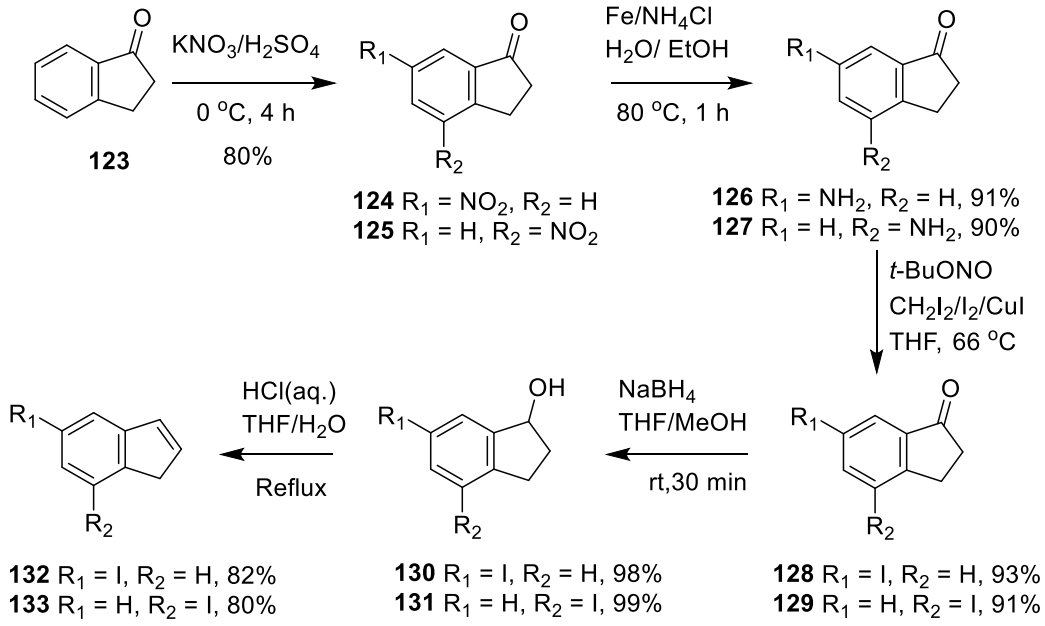
Scheme 41. Reported synthesis of 5-iodoindene[170,172]



Scheme 42. Reported synthesis of 6-iodoindene[170]

Electrophilic nitration of indan-1-one **123** with KNO_3/H_2SO_4 afforded separable mixture of 6-nitro- **124** and 4-nitroindan-1-one **125** (80%, 4:1 ratio; Scheme 43).[173,174] Selective reduction **124** or **125** with Fe powder/NH_4Cl [175] gave 6-amino- **126** and 4-aminoindan-1-one **127** in excellent yield. Subsequent, diazotization-iodination of **126** or **127** with *t*-BuONO/CH_2I_2/I_2/CuI afforded 6-iodo- **128** and 4-iodoindanones **129** (>90%) in addition

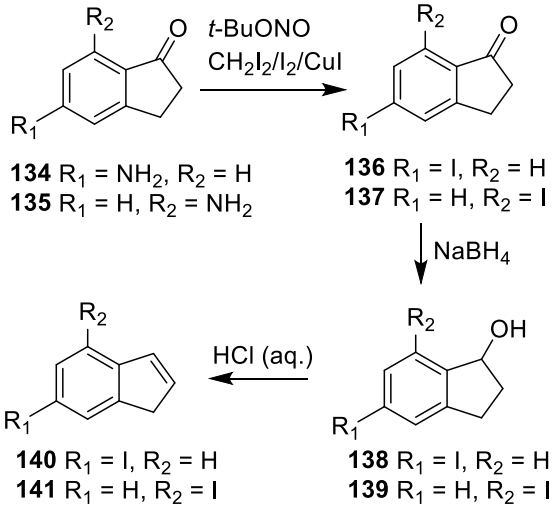
to diiodo substituted by-products (~4%). Reduction of **128** or **129** with $NaBH_4$ provided secondary alcohols **130** and **131** (>98%). Subsequent dehydration with aqueous HCl in THF/H_2O yielded selectively 5- and 7-iodoindenes, **132** and **133** (>80%). Isomerization to different indene isomers was not observed during this reaction sequence. It is noteworthy that dehydration of **130** or **131** with *p*-toluenesulfonic acid in refluxed toluene, used successfully for dehydration of the corresponding nitroindanoles,[170] failed to produce expected iodoindenes. The general method describing here allows preparation of expensive 5-iodoindene and unreported 7-iodoindene in high yields utilizing readily available and cost-effective reagents.



Scheme 43. Synthesis of 5-iodoindene and 7-iodoindene

Subjection of the commercially available 5-aminoindan-1-one **134** to the same sequence of diazotization-iodination followed by the reduction and dehydration yielded 6-iodoindene **140** in 71% overall yield (Scheme 44). This represents a significant improvement to the reported five-step procedure which gave **140** from **134** in 3% overall

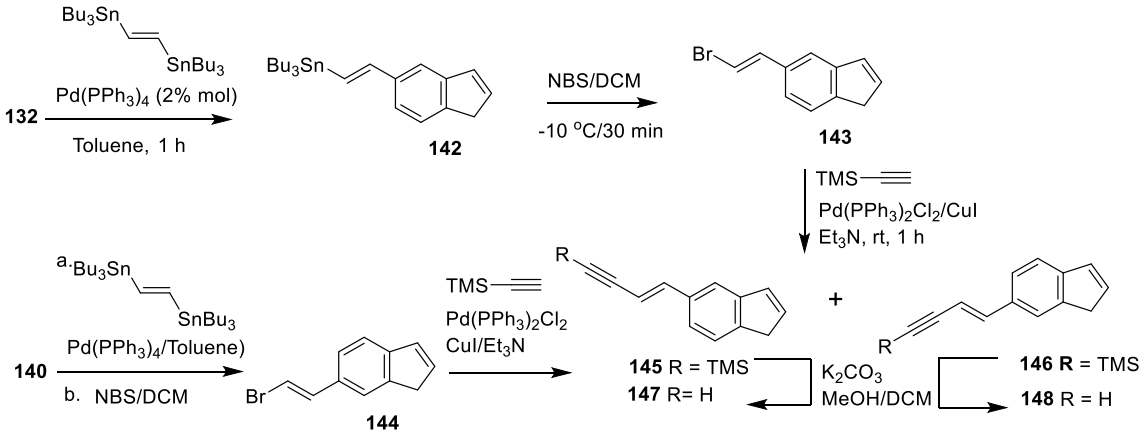
yield.[170] The method describing here avoids oxidation of **134** to 5-nitroindan-1-one with trifluoroperacetic acid and does not require reduction of 6-nitroindene to unstable 6-aminoindene intermediate[170] (Scheme 42). Analogous diazotization-iodination of 7-aminoindan-1-one **135** gave 7-iodoindan-1-one **137** (51%) as a major product in addition to 4-iodoindan-1-one **129** (5.5%) and a diiodo byproduct (20%) which was tentatively assigned as 4,7-diiodoindan-1-one. Analogous treatment of **135** with tert-butyl nitrite at ambient temperature for 8 h gave a similar distribution of products. Reduction and dehydration of **137** afforded 4-iodoindene **141** in 79% yield.



Scheme 44. Synthesis of 6-iodoindene and 4-iodoindene

Stille coupling of **132** with *trans*-1,2-bis(tributylstannyl)ethylene in the presence of catalytic $Pd(PPh_3)_4$ in toluene (100 ^oC/1 h) afforded regio- and stereoselectively the *E*-vinylstannane **142** with no isomerization of the indene five-membered double bond (Scheme 45). Compound **142** was directly used in the next step since attempted purification on silica gel column resulted in protiodestannylation yielding 5-vinylindene instead. Treatment of crude **142** with NBS in DCM (-10 °C/30 min) gave 5(*E*)-(2-

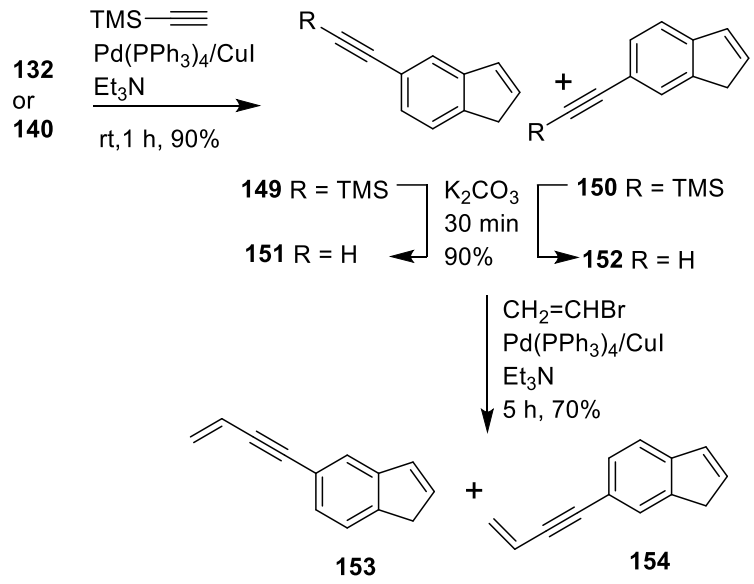
bromovinyl)indene **143** (70% from **132**) as a single product. Similarly, Stille coupling of **140** yielded selectively 6(*E*)-(2-bromovinyl)indene **144** also with no isomerization which would lead to **143**. Treatment of **143** or **144** with trimethylsilylacetylene in the presence of catalytic $Pd(PPh_3)_2Cl_2$/CuI in Et_3N at rt gave the TMS-protected enyne as an inseparable mixture of 5-enyneindene, **145** and 6-enyneindene, **146** (91%; 1:1.5). Desilylation of mixture **145** and **146** with anhydrous K_2CO_3 in MeOH/DCM (1:1) afforded a mixture of 5- and 6-enyneindenes **147** and **148** (92%; 1:1.5). The ratio of enynes in mixtures **145/146** or **147/148** was assigned based on the chemical shift pattern in ^1H NMR and differences in the chemical shift values (e.g., H4 in **132** (7.75 ppm) and H7 in **140** (7.81 ppm)).



Scheme 45. Regioselective bromovinylation of iodoindenes, and subsequent alkynylation

Sonogashira alkynylation of **132** with trimethylsilylacetylene in the presence of catalytic $Pd(PPh_3)_4$/CuI in Et_3N produced 5-alkynylindene **149** and 6-alkynylindene **150** as 1:1.5 isomeric mixture in 90% yield (Scheme 46). Analogous treatment of **140** gave an identical mixture of **149** and **150**. Attempted coupling of **132** (or **140**) with TMS-acetylene in the presence of 2.0 equiv. of Et_3N in dry THF resulted only in the isomerization of substrate **132** (or **140**) to a 1:1 mixture of **132/140**. Desilylation of mixture of **149/150** (1:1.5) with anhydrous K_2CO_3 in MeOH/DCM yielded mixture of **151/152** (90%; 1:1.7).

Separations of either protected **149/150** or deprotected **151/152** enynes on silica gel columns were not successful because of identical mobility in several eluting systems. Pd-catalyzed coupling of **151/151** mixture (1:1.7) with vinylbromide (CuI/Et_3N/rt/5 h) gave mixture of 5-enyne- **153** and 6-enyneindene **154** (70%) in 1:1.5 ratio.



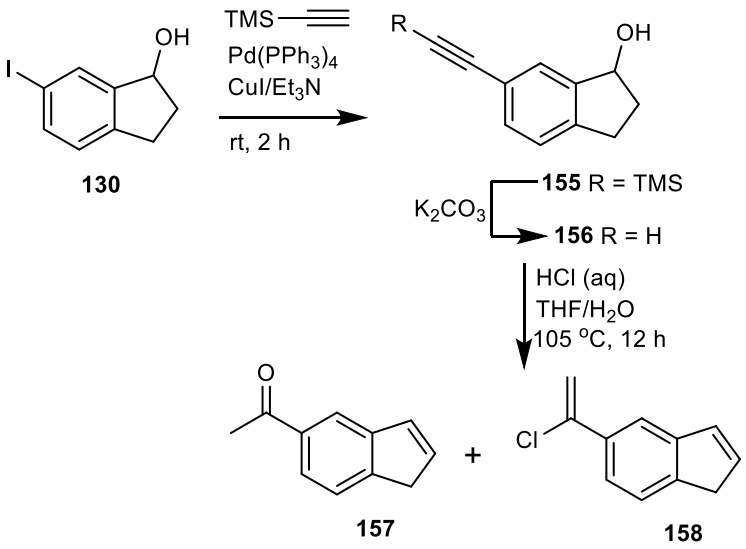
Scheme 46. Synthesis of isomeric enyneindenes via Sonogashira coupling

Stirring of pure **132** or **140** in the presence of Et_3N in THF at rt for 1h resulted in the formation of 1:1 isomeric mixture of **132/140** confirming that substituted indenes are prone to base-catalyzed isomerization.[176] Moreover, when 2:1 mixture of **132/140** was subjected to the similar experiments a 1:1 ratio was also observed at equilibrium. These results demonstrate that the double bond in the five-member ring of the substituted indenes can shift in the presence of base leading to the observed isomers under the conditions of the coupling reactions.

To avoid isomerization of indene ring during Sonogashira coupling and in order to get regioselective access to indenyl alkynes, we attempted synthesis of single **151** from 6-

iodoindan-1-ol **130**. Thus, coupling of **130** with trimethylsilylacetylene provided the trimethylsilylalkyne **155** (90%) as the sole product from which the trimethylsilyl group was removed with K_2CO_3 to give 6-ethynylindan-1-ol **156**. (Scheme 47). Dehydration of either **155** or **156** with aqueous HCl led to the formation of indene products without isomerization of a double bond in cyclopentadiene ring of indene but the simultaneous addition of water or HCl to the triple bond gave acetyl **157** (80%) and 1-chlorovinyl **158** (20%) products.

The iodoindenes **132** and **140** were used as precursors and enyne derivatives **147**, **148**, **153** and **154** were as calibration compounds for gas phase formation of cyclopentanaphthalene (benzindene) isomers via reactions of 5- and 6-indenyl radicals with vinylacetylene.[141,177]



Scheme 47. Regioselective synthesis of indene derivatives

3.5.3. Synthesis of Benzindene ($C_{13}H_{10}$) isomers

It was proposed from experiments and computational calculations that major products 3*H*-benz[e]indene **163** and 1H-benz[e]indene **167** along with minor products 2-(propa-1,2-

dien-1-yl)naphthalene **160**, 2-(prop-2-yn-1-yl)naphthalene **161**, 2-(prop-1-yn-1-yl)naphthalene and 1H-benz[f]indene **168** are forming during the gas phase radical reaction between 2-naphthyl radical and allene or methyl acetylene (Figure 18). Since compound **167** and **168** are commercially available, I designed the protocols to synthesize the other isomers.

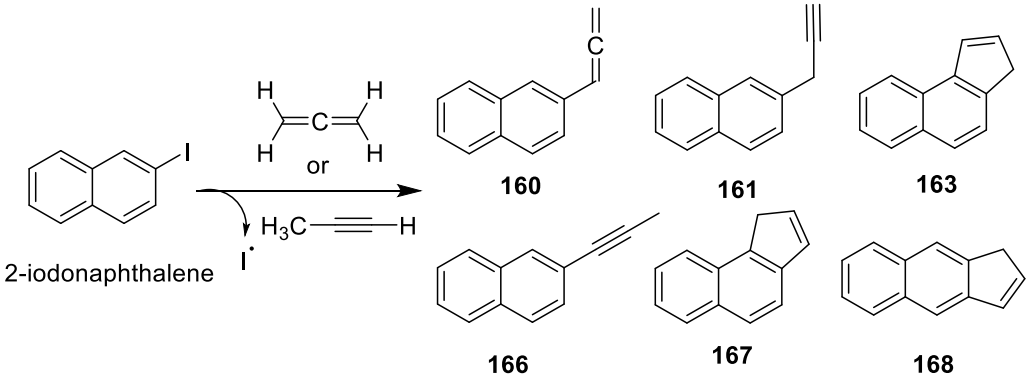


Figure 18. Formation of benzindenes and other isomers from 2-naphthylradical with allene or methyl acetylene

However, it was proposed from experiments and computational calculations that 1*H*-Phenalene **173** and 1-methylacenaphthalene **176** and 3*H*-benz[a]indene **163** are forming during the gas phase radical reaction between 1-naphthyl radical and alene or methyl acetylene (Figure 19).

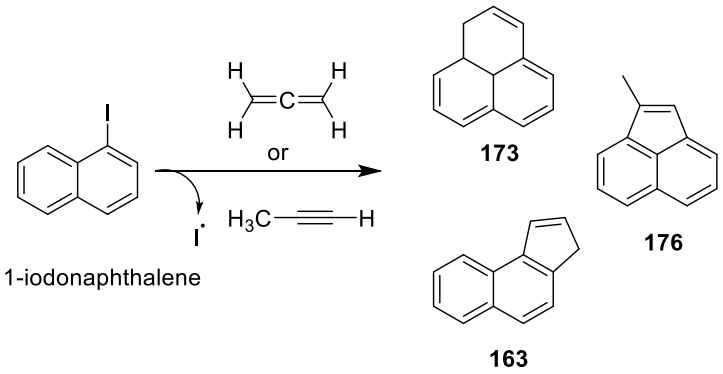
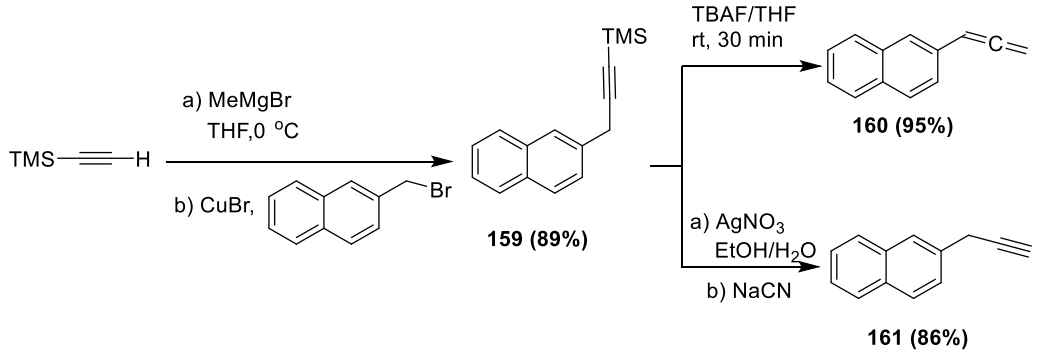


Figure 19. Formation of 1*H*-Phenalene and other isomers from 1-naphthylradical with allene or methyl acetylene

3.5.3.1. Synthesis of 2-(propa-1,2-dien-1-yl)naphthalene and 2-(prop-2-yn-1-yl)naphthalene

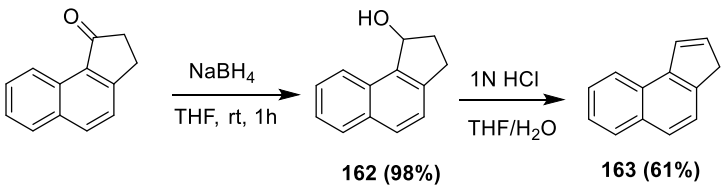
The 2-(propa-1,2-dien-1-yl)naphthalene **160** and 2-(prop-2-yn-1-yl)naphthalene **161** were synthesized from the commercially available 2-bromomethylnaphthalene by modifying reported protocols.[178,179] Thus, treatment of trimethylsilylacetylene with MeMgBr generate alkynide which was reacted with 2-bromomethylnaphthalene (Scheme 48) in presence of CuBr to give **159**. Then treatment of **159** with TBAF in THF at rt gave expected product **160.** On the other hand, treatment of **159** with $AgNO_3$/NaCN in $EtOH/H_2O$ at rt gave expected isomeric product **161.**



Scheme 48**.** Synthesis of 2-(propa-1,2-dien-1-yl)naphthalene and 2-(prop-2-yn-1-yl)naphthalene

3.5.3.2. Synthesis of 3*H*-benz[e]indene

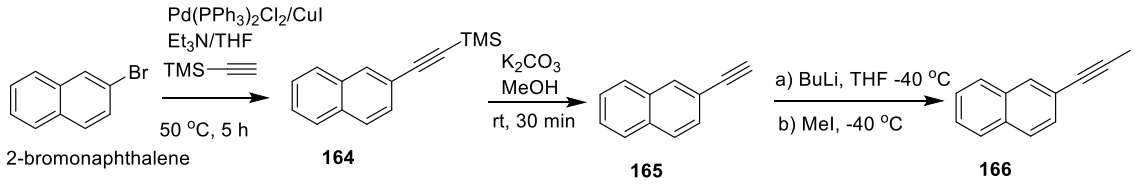
The 3*H*-benz[a]indene[180,181] **163** was synthesized by $NaBH_4$ reduction of commercially available 2,3-dihydro-1*H*-cyclopenta[a]naphthalene-1-one (Scheme 49) and *β*-elimination of the resulted secondary alcohol **162** with aqueous HCl.



Scheme 49**.** Synthesis of 3*H*-cyclopenta[a]naphthalene

3.5.3.3. Synthesis of 2-(prop-1-yn-1-yl)naphthalene

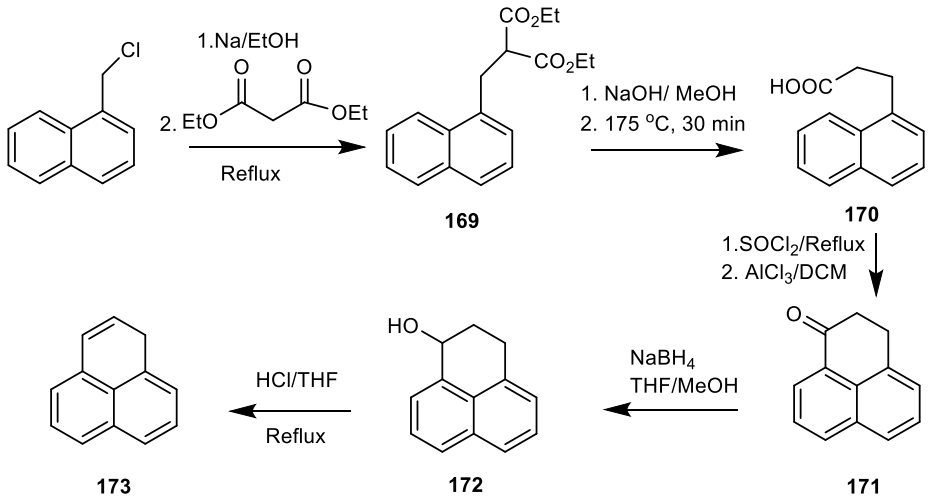
The 2-(prop-1-yn-1-yl)naphthalene **166** was synthesized by Sonogashira coupling between 2-bromonaphthalene and TMS-acetylene (Scheme 50) followed by desilylation of **164**. The resulted 2-ethynylnaphthalene **165** was converted to alkynide with BuLi and methylated with MeI yielding 2-(prop-1-yn-1-yl)naphthalene **166**.[182]



Scheme 50. Synthesis of 2-(prop-1-yn-1-yl)naphthalene

3.5.3.4. Synthesis of 1*H*-Phenalene

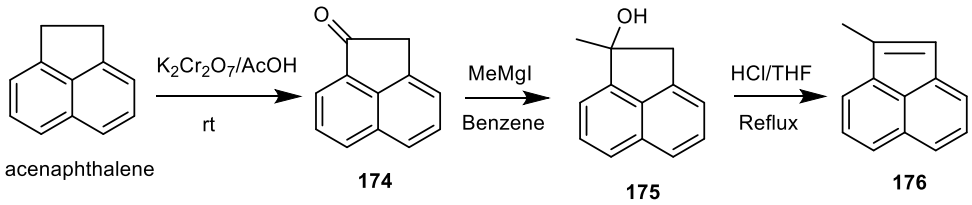
The 1*H*-phenalene **173** was synthesized from commercially available 1-chloromethylnaphthalene by modifying reported protocols.[183,184] Shortly, treatment of 1-chloromethylnaphthalene with sodium ethoxide and malonic ester under reflux afforded diethyl 2-(naphthalene-1-ylmethyl)malonate **169** (Scheme 51). Hydrolysis and subsequent decarboxylation of **169** gave 3-(naphthalene-1-yl)propanoic acid **170**.[185] Acylation of **170** with $SOCl_2$ and cyclization with $AlCl_3$ provided perinaphthanone-7 **171** in 75% yield. Reduction of ketone **171** with $NaBH_4$ and dehydration of the resulting **172** with HCl gave 1*H*-Phenalene **173** in high overall yields (67%). Described here method uses $SOCl_2/AlCl_3$ instead of HF for the cyclization of **170** to **171**.



Scheme 51**.** Synthesis of 1*H*-phenalene

3.5.3.5. Synthesis of 1-methylacenaphthalene

The 1-methylacenaphthalene **176** was prepared from commercially available acenaphthalene by modifying reported protocols.[185,186] Shortly, oxidation of acenaphthalene with $K_2Cr_2O_7$ in acetic acid afforded acenaphthylenone **174** (Scheme 52). Reaction of ketone **174** with MeMgI in dry THF led to the mixture of unidentified products. However, treatment of **174** with MeMgI in dry benzene[187] gave tertiary alcohol **175** in 81% yield. Dehydration of **175** with aqueous HCl in THF under reflux provided 1-methylacenaphthalene **176** in high overall yield (80%)**.**



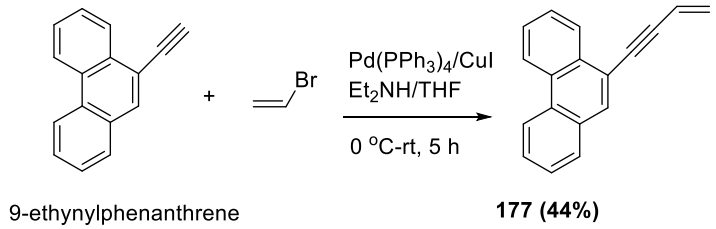
Scheme 52**.** Synthesis of 1-methylacenaphthalene

The benzindene isomers **160**, **161**, **163** and **166** were used as calibration compounds for the project "How to add a five-membered ring to polycyclic aromatic hydrocarbons (PAHs) – molecular mass growth of the 2-naphthyl radical ($C_{10}H_7$) to benzindenes ($C_{13}H_{10}$)

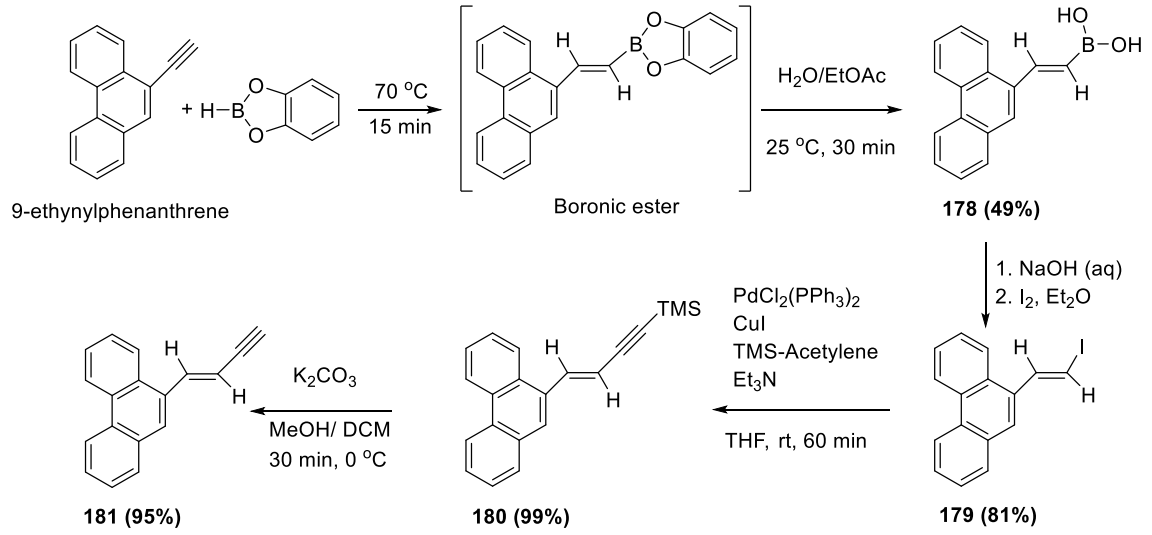
as a case study".[140] While compound **173** and **176** were used as calibration compounds for the project "Gas phase formation of phenalene via 10π aromatic, resonantly stabilized free radical intermediates".[188]

3.5.4. Synthesis of calibration compound of triphenylene isomers

Next, I synthesized two novel vinyl acetylene isomers of triphenylene as they are important intermediates for the gas phase synthesis of triphenylene between 9-phenanthrenyl radical ($C_{14}H_9$) and vinylacetylene (C_4H_4). Experiments and computational calculations suggested that 4-phenathrylvinylacetylene **177** and *trans*-1-phenathrylvinylacetylene **181** are forming during that gas phase radical reactions. The 4-phenathrylvinylacetylene **177** was synthesized by Sonogashira coupling of commercially available 9-ethynylphenanthrene with vinyl bromide (Scheme 53). Slow addition of 9-ethynylphenanthrene solution in THF via a syringe pump into a solution of vinyl bromide containing CuI/$Pd(PPh_3)_4$ and Et_2NH in THF was required to produce **177**.



Scheme 53. Synthesis of 4-phenathrylvinylacetylene **177**

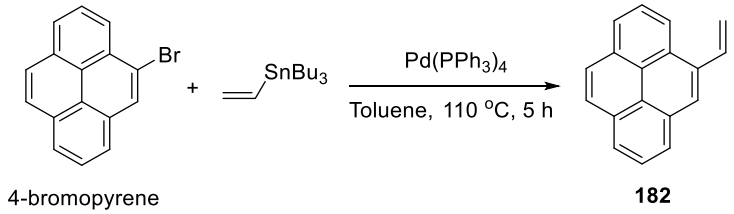


Scheme 54**.** Synthesis of *trans*-1-phenathrylvinylacetylene

The *trans*-1-phenathrylvinylacetylene 1**81** was synthesized by stereoselective conversion of 9-ethynylphenanthrene into *trans*-1-alkenyliodide via hydroboration-hydrolysis-iodination[189] sequence followed by Sonogashira cross-coupling reaction with (trimethylsilyl)acetylene (Scheme 54). Thus, treatment of 9-ethynylphenanthrene with catecholborane at 70 ^{o}C form intermediary boronic ester, which was hydrolyzed with H_2O to give *trans*-alkenylboronic acid, **178** in 49% yield after purification on silica gel column. Treatment of the purified (catechol free) **178** in ether solution with iodine (1.2 equiv) in the presence of aqueous NaOH (3.0 equiv) at 0 ^{o}C provided alkenyliodide **179** (81%). Subsequent Sonogashira coupling of **179** with (trimethylsilyl)acetylene yielded **170** (99%), which on desilylation with K_2CO_3 in MeOH/DCM gave desired *trans*-1-phenathrylvinylacetylene, **181** (95% yield). The compound **177** and **181** were used as calibration compounds for the gas-phase synthesis of triphenylene ($C_{18}H_{12}$).[134]

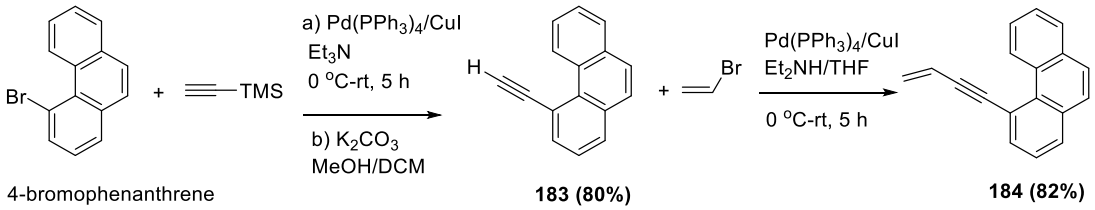
3.5.5. Synthesis of calibration compound of 4-helicene isomers

I synthesized the novel 4-vinylpyrene **182**, 4-(but-3-en-1-yn-1-yl)-phenanthrene **184** and (*E*)-4-(but-1-en-3-yn-1-yl)-phenanthrene **187** isomers as critical intermediates for developing [4]-Helicene via the radical ring annulation in gas phase. It was proposed that during synthesis of [4]-Helicene from 4-phenathrenyl radical (generated from 4-bromophenantrene) and vinylacetylene, the aforementioned three isomers are also formed by varying amount. The 4-vinylpyrene **182** was successfully synthesized by Pd-catalyzed Stille cross coupling reaction between commercially available 4-bromopyrene with Bu_3Sn(vinyl) in toluene at 110 ^oC in 83% yield (Scheme 55).



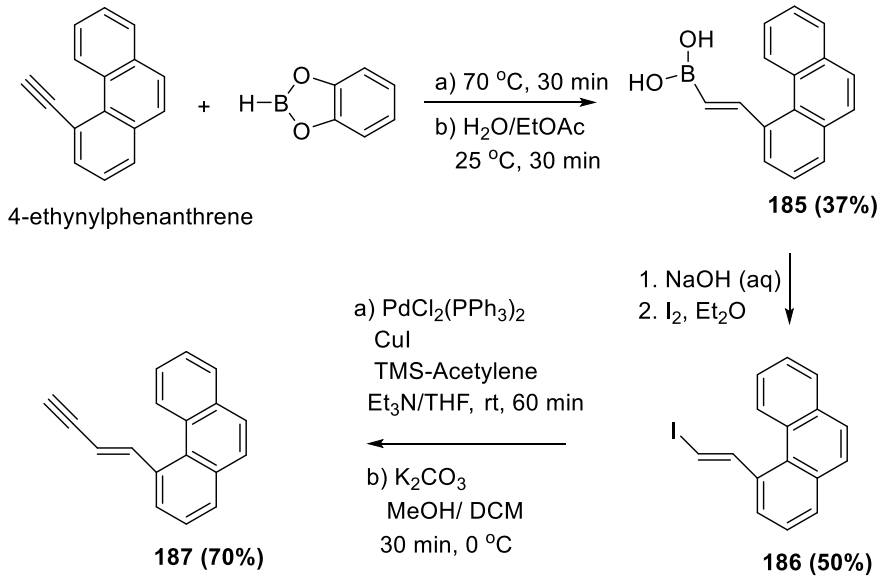
Scheme 55**.** Synthesis of 4-vinylpyrene

For the synthesis of 4-(but-3-en-1-yn-1-yl)-phenanthrene **184** and (*E*)-4-(but-1-en-3-yn-1-yl)-phenanthrene **187**, we started with commercially available 4-bromophenanthrene, which was converted to 4-ethynylphenanthrene **183** (Scheme 56) by CuI/$Pd(PPh_3)_4$-mediated Sonogashira coupling with (trimethylsilyl)acetylene and desilylation with K_2CO_3. Succeeding Sonogashira coupling between 4-ethynylphenanthrene **183** and vinyl bromide resulted in the formation of 4-(but-3-en-1-yn-1-yl)-phenanthrene **184**.



Scheme 56. Synthesis of 4-(But-3-en-1-yn-1-yl)-phenanthrene

While (*E*)-4-(But-1-en-3-yn-1-yl)-phenanthrene **187**, was synthesized using similar a approach to that shown in Scheme 54**.** Stereoselective conversion of 4-ethynylphenanthrene into *trans*-1-alkenylboronic acid **185** via hydroboration-hydrolysis and *trans*-1-alkenyliodide **186** via iodination sequence (Scheme 57) followed by Sonogashira cross-coupling reaction with (trimethylsilyl)acetylene and desilylation gave (*E*)-4-(but-1-en-3-yn-1-yl)-phenanthrene **187**. The compound **182**, **184 and 187** were used as calibration compounds for the gas-phase synthesis of [4]-helicene.[135]



Scheme 57 . Synthesis of (*E*)-4-(But-1-en-3-yn-1-yl)-phenanthrene

3.5.6. Synthesis of 7-ethynylfluoranthene

Corannulene ($C_{20}H_{10}$) a molecular building block for 3D nanostructures such as buckybowls and buckyballs can be synthesized in the gas phase through the reactions of 7-fluoranthenyl ($C_{16}H_9^{\bullet}$) and benzo[*ghi*]fluoranthen-5-yl ($C_{18}H_9^{\bullet}$) radicals with acetylene (C_2H_2) mimicking conditions in carbon-rich circumstellar envelopes (Figure 20).[139]

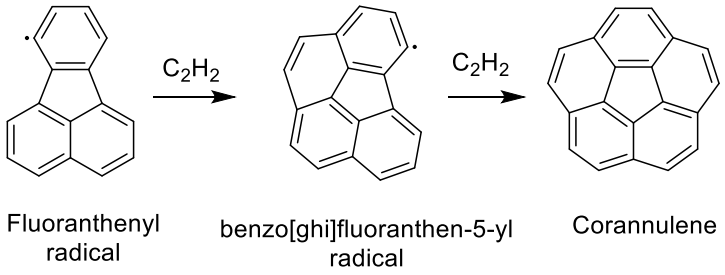
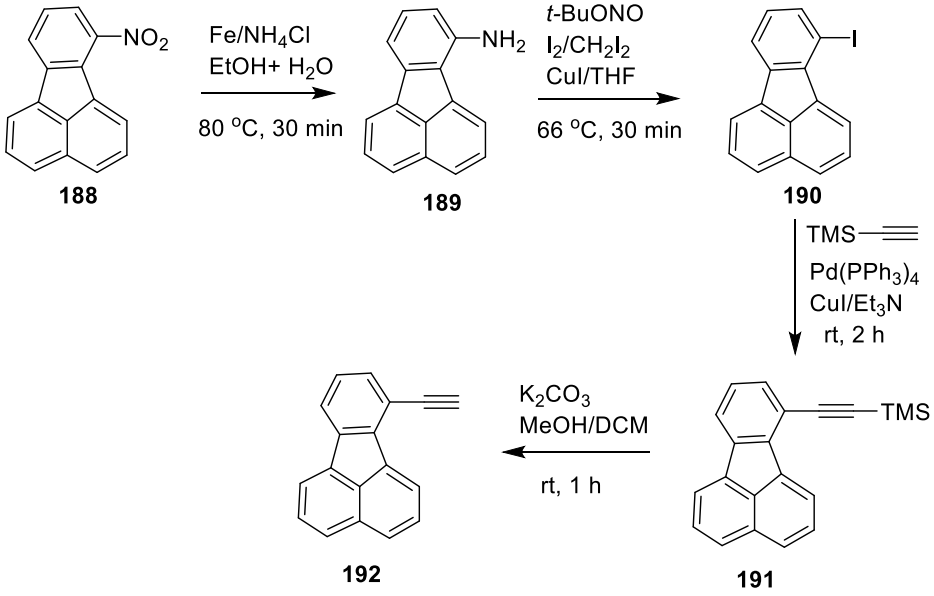


Figure 20. Gas phase synthesis of corannulene

I designed the synthetic protocol to prepare a novel calibration compound 7-ethynylfluoranthene **192**. The 7-ethynylfluoranthene **192** has been synthesized from 7-nitrofluoranthene **188** which has been prepared by reported protocol.[190] Thus, reduction of **188** with Fe powder/NH_4Cl gave 7-aminofluoranthene **189** (Scheme 58). Subsequent, diazotization-iodination of **189** with *t*-BuONO/CH_2I_2/I_2/CuI afforded 7-iodofluoranthene **190**. Sonogashira coupling of **190** with TMS-acetylene in presence of $Pd(PPh_3)_4$/CuI in Et_3N gave TMS protected 7-ethynylfluoranthene **191** which on desilylation with K_2CO_3 provided 7-ethynylfluoranthene **192**.



Scheme 58**.** Synthesis of 7-Ethynylfluoranthene

3.5.7. Synthesis of benzocorannulene

The corannulene ring even can be expand to additional ring via HAVA method. Thus, reaction between corannulenyl radical and vinyl acetylene will produce benzocorannulene (Figure 21).

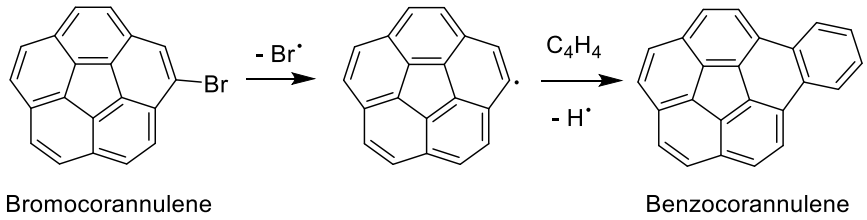
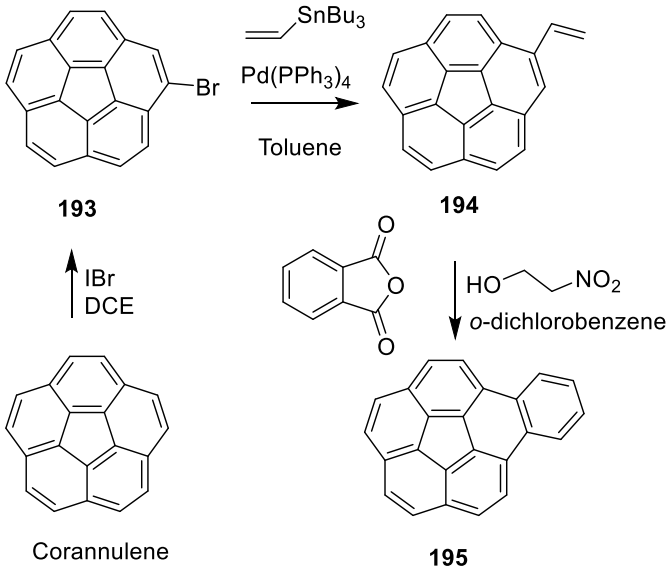


Figure 21. Gas phase formation of benzocorannulene

The benzocorannule **195** has been synthesized from bromocorannulene **193** which has been prepared by reported protocol.[191] Thus, Pd-catalyzed Stille cross coupling reaction between brocorannulene with Bu_3Sn(vinyl) in toluene at 100 ^oC for 3 h afforded **194** in 52% yield (Scheme 59). Subsequent reaction of **194** with nitroethanol in presence of phthalic anhydride in *o*-dichlorobenzene at 180 ^oC for 3 days provided **195** in 28% yield.



Scheme 59**.** Synthesis of benzocorannulene

3.5.8. Synthesis of 1-bromotriphenylene or 1-iodotriphenylene

Coronene ($C_{24}H_{12}$) a molecular building block for planar carbon sheet such as graphene. The proposed gas phase synthesis of coronene, propagated by acetylene, is shown in Figure 22. In order to verify the proposed gas phase formation of coronene, the bromo precursors are needed. None of the bromo precursor's synthesis is not reported. Therefore, at first I started the synthesis of 1-bromotriphenyle **201** which will be useful to determine the validity of the gas phase pathway for the formation of coronene.

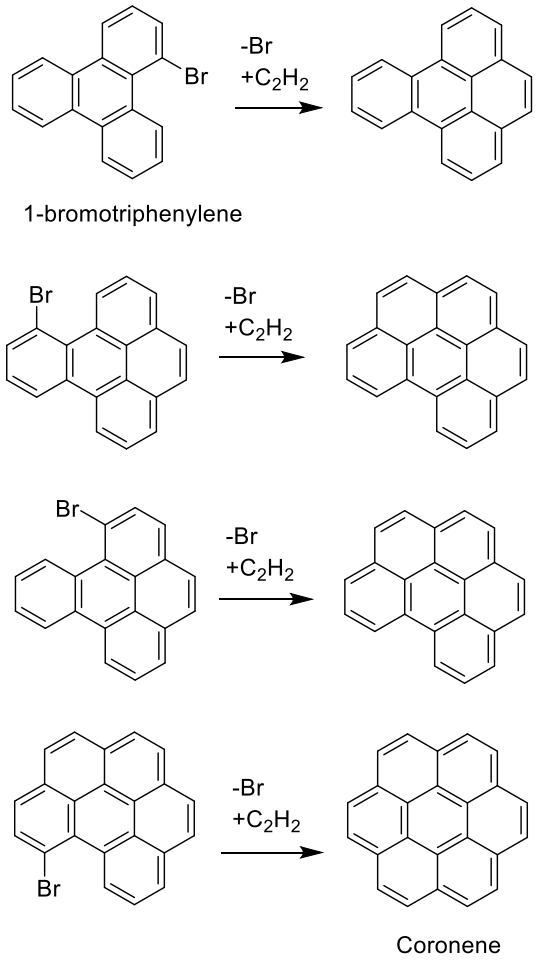
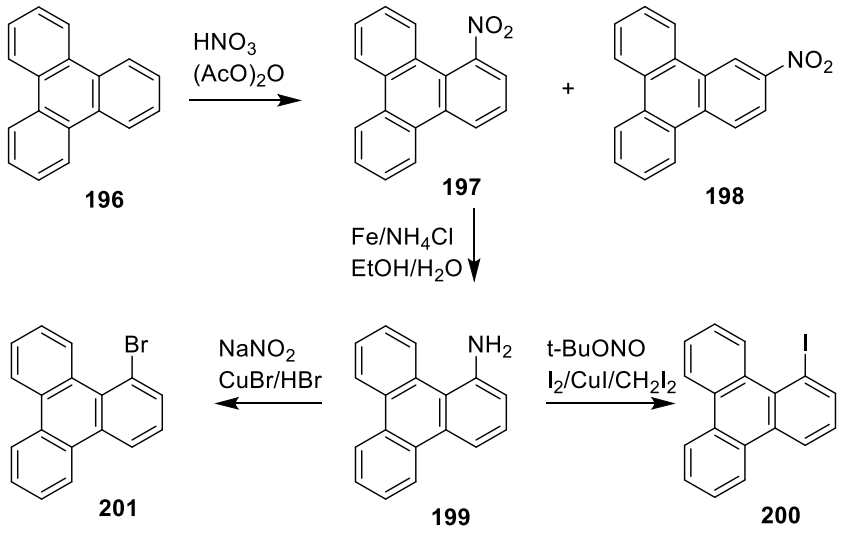


Figure 22. Proposed formation pathway of coronene via consecutive acetylene addition

We started the synthesis of 1-bromotriphenylene **201** from commercially available triphenylene **196**; although direct nitration of triphenylene always gives substitution at 2-position. However, nitration of **196** with $HNO_3/(AcO)_2O$ gives a mixture of 1-nitrotriphenylene **197** and 1-nitrotriphenylene **198,** which are separable in silica column (Scheme 60). Upon reduction of **197** with Fe powder/NH_4Cl gave 1-aminotriphenylene **199**. Subsequent, diazotization-iodination of **199** with *t*-BuONO/CH_2I_2/I_2/CuI afforded 1-iodotriphenylene **200**. Similarly, diazotization-bromination of **198** with $NaNO_2$/ CuBr/HBr provided mixture of 1-bromotriphenylene **201**/2-bromotriphenylene (77:23).



Scheme 60**.** Synthesis of 1-bromotriphenylene and 1-iodotriphenylene

3.5.9. Synthesis of "enyne" and "ynene" isomers of pyridine

Finally, we moved to the study of the gas phase synthesis of polyaromatic compound bearing nitrogen atom in the ring. Experiments and computational calculations suggested that reaction of *o*-, *m*- and *p*-pyridinyl radicals ($C_5H_4N^{\bullet}$) with vinylacetylene (C_4H_4) also follow Hydrogen Abstraction – Vinylacetylene Addition (HAVA) mechanism to form quinolone or isoqunoline as the major products as well as other enyne isomers of pyridine

(Figure 23). *o*-, *m*- and *p*-pyridinyl radicals are generated from the pyrolysis of corresponding iodopyridines (C_5H_4NI) precursors. Therefore, I designed the protocols to synthesize all the enyne isomers of pyridine.

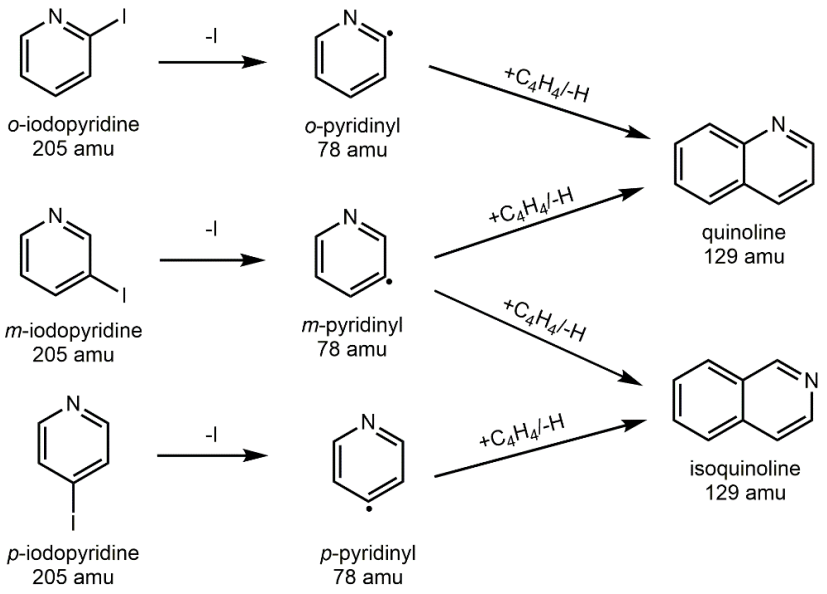
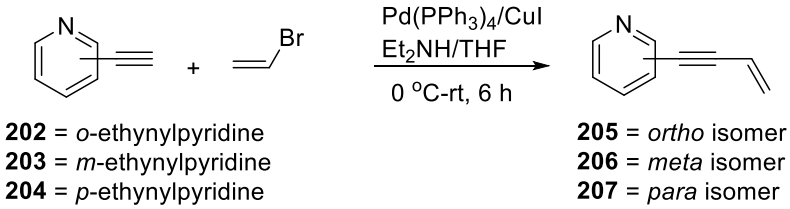


Figure 23. Formation of quinoline (C_9H_7N) and isoquinoline (C_9H_7N) from the reaction of *o*-, *m*- and *p*-pyridinyl radicals ($C_5H_4N^{\bullet}$) with vinylacetylene (C_4H_4)

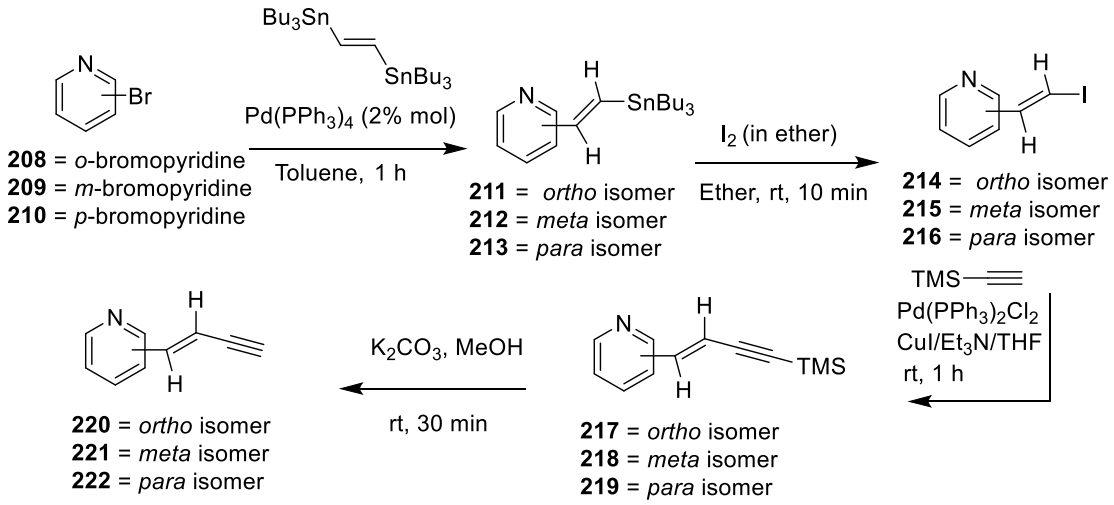
The enyne isomers of pyridine **205**, **206**, and **207** were synthesized by CuI/$Pd(PPh_3)_4$-mediated Sonogashira coupling between *o*-ethynylpyridine **202**, *m*-ethynylpyridine **203**, *p*-ethynylpyridine **204** and vinyl bromide/Et_2NH in THF respectively (Scheme 61).



Scheme 61. Synthesis of *ortho*, *meta*, and *para* enyne isomers of pyridine

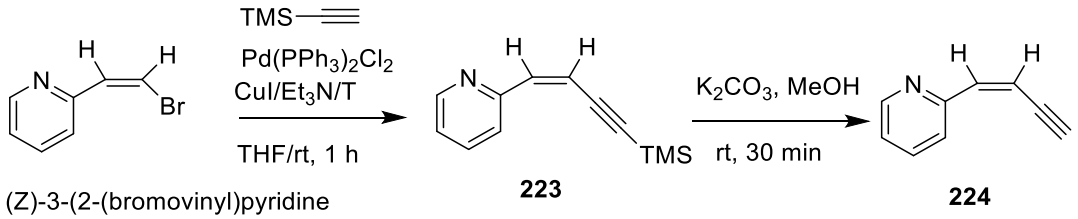
The ynene isomers of pyridine **220**, **221**, and **222** were synthesized by Pd-catalyzed Stille cross coupling reaction between *ortho*, *meta*, and *para* isomers of bromopyridine

(e.g., **208**) and *trans*-1,2-Bis(tri-n-butylstannyl)ethylene yielded trans-vinylstannane (e.g., **211**) which were converted to *trans*-1-alkenyliodide (e.g., **214**) via iodination followed by Sonogashira cross-coupling with (trimethylsilyl)acetylene and desilylation of resulting protected alkyne(e.g., **217**) with K_2CO_3 (Scheme 62) gave desired products.



Scheme 62**.** Synthesis of *ortho*, *meta*, and *para* (*E*)-*ynene* isomers of pyridine analogue

The (*Z*)-3-(but-1-en-3-yn-1-yl)pyridine **224** was synthesized by CuI/$Pd(PPh_3)_2Cl_2$-mediated Sonogashira cross-coupling of commercially available (*Z*)-3-(2-(bromovinyl)pyridine with (trimethylsilyl)acetylene and desilylation of **223** with K_2CO_3 (**Scheme 63**).



Scheme 63**.** Synthesis of (*Z*)-3-(but-1-en-3-yn-1-yl)pyridine

4. EXPERIMENTAL SECTION

4.1. General Information

^{1}H NMR spectra at 400 MHz and ^{13}C NMR at 101 MHz were recorded in DMSO-d_6 unless otherwise noted. All chemical shift values are reported in parts per million (ppm) and referenced to the residual solvent peaks of $CDCl_3$ (7.26 ppm), DMSO-d_6 (2.50 ppm), MeOD-d_4 (3.31 ppm) and D_2O (4.79 ppm) for ^{1}H NMR and $CDCl_3$ (77.16 ppm), DMSO-d_6 (39.52 ppm) and MeOD-d_4 (49.00 ppm) peaks for ^{13}C NMR spectra, with coupling constant (*J*) values reported in Hz. HRMS were recorded in TOF negative or positive mode unless otherwise noted. Reaction progress was monitored by TLC on Merck Kieselgel 60-F_{254} sheets with product detection by 254-nm light. Products were purified by column chromatography using Merck Kiselgel 60 (230-400 mesh). Reagent grade chemicals were used and solvents were purchased from commercial suppliers and used without further purification unless otherwise specified.

4.2. Synthesis of purine nucleosides analogs at C8 or C2 positon and their 5'-triphosphates

2',3',5'-Tri-*O*-(*tert*-butyldimethylsilyl)-8-ethynyladenosine (37). *Step a*. $Pd(PPh_3)_2Cl_2$ (193 mg, 0.27 mmol) and Cu(I)I (105 mg, 0.55 mmol) were added to dry DMF (100 mL) and Et_3N (10.0 mL, 7.26 g, 71.7 mmol) in a flame-dried flask equipped with a stirring bar under N_2 at rt. Then 8-bromo-2', 3', 5'-tri-*O*-(*tert*-butyldimethylsilyl)adenosine[192] (9.5 g, 13.79 mmol) was added followed by TMS-acetylene (3.82 mL, 2.71 g, 27.6 mmol). The resulting mixture was stirred for 6 h at 80 ^{o}C. Volatiles were evaporated and the residue was purified by column chromatography (20 → 50% EtOAc/hexane) to give 2',3',5'-tri-*O*-(*tert*-butyldimethylsilyl)-8-(2-

trimethylsilyl)ethynyladenosine as a brown solid (8.76 g, 90%, with ^{1}H NMR as reported[53]). *Step b.* The product from *step a* was dissolved in anhydrous MeOH (100 mL) and stirred at 0 ºC. Anhydrous K_2CO_3 (8.6 g, 62.2 mmol) was added portion wise and the mixture was stirred at rt. After 30 min, volatiles were evaporated and the residue was extracted with EtOAc. The solvent was removed at reduced pressure and the residue was recrystallized with hexane to give **37** (7.52 g, 96%) as an off-white solid: ^{1}H NMR ($CDCl_3$) δ -0.40 (s, 3H), -0.11 (s, 3H), -0.04 (s, 3H), 0.00 (s, 3H), 0.13 (s, 3H), 0.14 (s, 3H), 0.74 (s, 9H), 0.82 (s, 9H), 0.93 (s, 9H), 3.70 (dd, *J* = 10.8, 4.8 Hz, 1H), 3.94–3.98 (m, 1H),), 4.05 (dd, *J* = 10.8, 7.4 Hz, 1H), 4.55 (dd, *J* = 4.4, 2.8 Hz, 1H), 5.00 (s, 1H), 5.42 (dd, *J* = 6.2, 4.2 Hz, 1H), 5.98 (d, *J* = 6.20Hz, 1H), 7.58 (s, 2H), δ 8.15 (s, 1H)**;** ^{13}C NMR ($CDCl_3$) δ -5.70, -5.68, -5.64, -5.54, -5.50, -4.84, -4.77, -4.70, 17.44, 17.75, 17.89, 25.31, 25.46, 25.55, 25.66, 25.72, 25.79, 61.97, 71.39, 71.92, 72.50, 84.97, 87.12, 88.38, 119.05, 132.93, 148.68, 153.64, 156.10.

8-(1-Chloro-2-tosylvinyl)adenine (38). *p*-Toluenesulfonyl hydrazide (173.2 mg, 0.93 mmol), $FeCl_3 \cdot 6H_2O$ (160.5 mg, 0.62 mmol), and TBHP (70% in water; 155 μL, 1.24 mmol) were added to a stirring solution of 3′, 5′-di-*O*-(*tert*-butyldimethylsilyl)-8-ethynyl-2'-deoxyadenosine[50] (**36**; 155 mg, 0.31 mmol) in CH_3CN at rt. The resulting mixture was stirred at 80 °C for 4 h. The volatiles were evaporated, and the residue was purified by column chromatography ($MeOH/CHCl_3$; 0 → 5%) to give **38** (96 mg, 89%) as a white solid: ^{1}H NMR δ 2.40 (s, 3H), 7.45 (d, *J* = 8.0 Hz, 4H), 7.81 (d, *J* = 8.0 Hz, 2H), 7.90 (s, 1H), 8.18 (s, 1H), 13.54 (s, 1H); ^{13}C NMR δ 21.19, 119.26, 128.14, 130.01, 135.22, 136.37, 145.11, 150.53, 156.26.

3',5'-Di-*O*-(*tert*-butyldimethylsilyl)-8-(1-iodo-2-tosylvinyl)-2'-deoxyadenosine (42). Sodium acetate (244 mg, 2.97 mmol), sodium *p*-toluenesulfinate (1061 mg, 5.96 mmol), and I_2 (755 mg, 2.97 mmol) were added to a stirring solution of **36** (1.0 g, 1.98 mmol) in MeCN. The resulting mixture was stirred at 80 °C for 1.5 h. Upon completion of the reaction, the reaction mixture was quenched by saturated aqueous $Na_2S_2O_3$. The volatiles were removed under reduced pressure and the residue extracted with EtOAc. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (20 → 40% EtOAc/hexane) to give **42** (1.01 g, 65%) as an off-white solid: ^{1}H NMR δ -0.04 (s, 2H), 0.0 (s, 2H), 0.06 (s, 2H), 0.10 (s, 1H), 0.12 (s, 5H), 0.82 (s, 6H), 0.86 (s, 3H), 0.89 (s, 3H), 0.90 (s, 6H), 2.12–2.23 (m, 1H), 2.40 (s, 3H), 3.38–3.43 (m, 1H), 3.68 (dd, *J* = 10.4, 4.8 Hz, 0.7H), 3.79 (dd, *J* = 10.4, 4.8 Hz, 0.3H), 3.83–3.87 (m, 0.7H), 3.91–4.02 (m, 1.3H), 4.63–4.67 (m. 0.3H), 4.81–4.83 (m, 0.7H), 5.99 (t, *J* = 6.9 Hz, 1H), 7.39–7.54 (m, 4H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.88 (d, *J* = 8.0 Hz, 0.7H), 8.14 (d, *J* = 6.8 Hz, 1H), δ 8.21 (s, 0.33H), 8.30 (s, 0.67H); ^{13}C NMR δ -5.44, -5.34, -5.32, 4.88, -4.84, -4.72, 17.79, 17.99, 21.22, 25.62, 25.74, 25.77, 25.83, 36.39, 36.44, 62.43, 62.82, 72.30, 72.64, 85.06, 85.15, 87.16, 87.49, 98.60, 99.15, 119.18, 119.26, 127.86, 128.45, 130.16, 130.21, 135.77, 135.84, 145.11, 145.30, 145.64, 146.11, 146.46, 148.88, 149.35, 152.72, 152.81, 156.11, 156.16. HRMS (TOF, ESI) *m/z* calcd for $C_{31}H_{49}IN_5O_5SSi_2$ 786.2032 $[M + H]^+$, found 786.2028.

2',3',5'-Tri-*O*-(*tert*-butyldimethylsilyl)-8-(1-iodo-2-tosylvinyl)adenosine (43). Treatment of solution of **37** (1.63 g, 2.57 mmol) with sodium *p*-toluenesulfinate (1374 mg, 7.71 mmol), and I_2 (980 mg, 3.86 mmol) in presence of sodium acetate (309 mg, 3.86 mmol), as described for **42**, gave **43** (1.18 g, 50%) as an off-white solid: ^{1}H NMR δ -0.28

(s, 2H), -0.23 (s, 2H), -0.09 (s, 1H), -0.08 (s, 1H), -0.04 (s, 1H), -0.03 (s, 1H), 0.08 (s, 4H), 0.12 (s, 4H), 0.18 (s, 2H), 0.71 (s, 3H), 0.75 (s, 6H), 0.82 (s, 6H), 0.85 (s, 3H), 0.92 (s, 6H), 0.94 (s, 3H), 2.39 (s, 2H), 2.42 (s, 1H), 3.59–4.12 (m, 3H), 4.31 (d, *J* = 4.0 Hz, 0.64 H), 4.90 (brs, 0.25H), 5.53 (d, *J* = 44.6 Hz, 0.67 H), 5.60 (brs, 0.3H), 5.73 (brs, 0.34H), 5.82–5.88 (m, 0.66H), 7.40–7.55 (m, 4H), 7.81 (d, *J* = 7.6 Hz, 0.66 H), 7.89 (d, *J* = 8.0 Hz, 1.47 H), 8.16 (s, 1.28H), 8.25 (s, 0.63H); ^{13}C NMR δ -5.70, -5.63, -5.42, -5.36, -5.03, -4.97, -4.84, -4.81, -4.78, -4.68, -4.66, -4.58, -4.52, 4.45, 17.59, 17.69, 17.71, 17.81, 17.87, 17.93, 21.15, 21.18, 25.44, 25.57, 25.67, 25.79, 25.91, 26.06, 62.35, 70.33, 72.47, 72.63, 85.05, 85.38, 87.15, 97.05, 119.37, 128.17, 128.24, 128.39, 128.40, 129.87, 130.03, 135.88, 136.14, 144.90, 144.98, 145.91, 146.01, 146.92, 149.13, 149.46, 152.61, 156.10, 156.18.

8-(1-Iodo-2-tosylvinyl)-2'-deoxyadenosine (45). Procedure A. CH_3COOH (2.2 mL, 2.31 g, 38.5 mmol), and NH_4F (1.18 g, 31.8 mmol) were added to a stirring solution of **42** (503 mg, 0.64 mmol) in MeOH at rt. The resulting mixture was stirred at 60 °C for 3.0 h. The volatiles were evaporated at reduced pressure and co-evaporated with acetonitrile (3x5 mL) yielding an off-white solid, which is suspended in 20% MeOH in CH_2Cl_2. The white precipitate was removed by vacuum filtration and the mother liquor was evaporated at reduced pressure. The residue was purified by column chromatography (5 → 10% MeOH/$CHCl_3$) to give **45** (239 mg, 67%) as a white solid: ^{1}H NMR δ 2.19 (dd, *J* = 13.6, 6.0 Hz, 1H), 2.41 (s, 3H), 2.88–2.96 (m, 0.37H), 3.03-3.10 (m, 0.63H), 3.54–3.65 (m, 1H), 3.71–3.81 (m, 1H), 3.97 (brs, 0.64H), 4.02 (brs, 0.37H), 4.43, (brs, 0.37), 4.47 (brs, 0.63H), 5.38 (d, *J* = 3.6 Hz, 1H), 5.70 (dd, *J* = 9.2, 3.6 Hz, 0.66H), 5.92–5.99 (m, 0.67H), 6.03 (dd, *J* = 8.8, 6.0 Hz, 0.68H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.61 (s, 2H), 7.74 (d, *J* = 8.0 Hz, 1.25H), 7.86 (d, *J* = 8.2 Hz, 0.75H), 8.15 (s, 1H), 8.24 (s, 0.37H), 8.29 (s, 0.63H); ^{13}C NMR δ

21.10, 21.25, 38.11, 38.59, 54.52, 54.96, 61.34, 62.41, 70.02, 71.49, 84.93, 86.33, 88.83, 88.98, 98.59, 99.21, 104.10, 112.66, 119.34, 128.02, 128.44, 130.28, 135.74, 143.73, 145.31, 145.36, 146.00, 146.08, 148.53, 148.60, 152.58, 156.35. HRMS (TOF, ESI) *m/z* calcd for $C_{19}H_{21}IN_5O_6S$ 558.0303 $[M + H]^+$, found 558.0291.

8-(1-Iodo-2-tosylvinyl)adenosine (46). Treatment of **43** (605 mg, 0.66 mmol) with CH_3COOH (2.3 mL, 2.41 g, 40.2 mmol), and NH_4F (1.22 g, 32.9 mmol) by **Procedure A** (column chromatography; 5 → 10% MeOH/$CHCl_3$) gave **46** (189 mg, 50%) as a white solid: ^{1}H NMR δ 2.41 (s, 3H), 3.53–3.65 (m, 1H), 3.68–3.82 (m, 1H), 3.96 (q, *J* = 4.0 Hz, 0.4H), 4.07 (q, *J* = 2.8 Hz, 0.6H), 4.16 (d, *J* = 5.4 Hz, 1H), 4.16 (d, *J* = 5.6 Hz, 0.6H), 4.95 (qd, *J* = 6.7, 6.1, 4.4 Hz, 1H), 4.07 (q, *J* = 4.8 Hz, 0.4H), 4.92–4.97 (m, 1H), 5.15 (d, *J* = 4.0 Hz, 0.6H), 5.20 (d, *J* = 4.8 Hz, 0.4H), 5.37 (t, *J* = 6.6 Hz, 1H), 5.42 (t, *J* = 6.6 Hz, 1H), 5.60 (d, *J* = 5.6 Hz, 0.4H), 5.90–5.96 (m, 0.6H), 7.45 (d, *J* = 8.4 Hz, 0.8H), 7.47 (d, *J* = 8.0 Hz, 1.2H), 7.57 (s, 1.2H), 7.61 (m, 0.8H), 7.87 (d, *J* = 8.0 Hz, 1.2H), 7.90 (d, *J* = 8.4 Hz, 0.8H), 8.16 (s, 1.4H), 8.21 (s, 0.6H); ^{13}C NMR δ 21.21, 62.11, 62.39, 70.76, 71.11, 72.15, 72.19, 85.89, 86.87, 89.63, 90.31, 97.58, 98.33, 109.24, 119.09, 119.30, 128.44, 128.51, 130.08, 130.23, 135.71, 135.96, 145.04, 145.25, 145.95, 146.09, 146.79, 148.58, 148.79, 152.46, 152.70, 156.27. HRMS (TOF, ESI) *m/z* calcd for $C_{19}H_{21}IN_5O_6S$ 574.0252 $[M + H]^+$, found 574.0249.

(*Z*)-3',5'-Di-*O*-(*tert*-butyldimethylsilyl)-8-(1-amino-2-tosylvinyl)-2'-deoxyadenosine (47). Procedure B. The iodovinylsulfone **42** (400 mg, 0.51 mmol) was dissolved in methanolic ammonia (10 mL) and the resulting mixture was stirred at rt for 1h. The volatiles were evaporated, and the residue was column-chromatographed (40 → 50% EtOAc/hexane) to give products **47** (309 mg, 90%) as an off-white solid: ^{1}H NMR δ

-0.11 (s, 3H), -0.06 (s, 3H), 0.11 (s, 6H), 0.77 (s, 9H), 0.91 (s, 9H), 2.20 (ddd, *J* = 13.2, 6.8, 4.0 Hz, 1H), 2.40 (s, 3H), 3.47 (dt, *J* = 12.8, 6.4 Hz, 1H), 3.60 (dd, *J* = 10.8, 4.8 Hz, 1H), 3.77 (q, *J* = 4.8 Hz, 1H), 3.85 (dd, *J* = 10.8, 6.0 Hz, 1H), 4.79 (q, *J* = 4.8 Hz, 1H), 5.17 (s, 1H), 6.21 (t, *J* = 6.8 Hz, 1H), 7.23 (s, 2H), 7.41 (d, *J* = 8.0 Hz, 2H), 7.43 (s, 2H), 7.83 (d, *J* = 8.4 Hz, 2H), 8.12 (s, 1H); ^{13}C NMR δ -5.58, -5.49, -4.90, -4.83, -4.74, -4.67, 17.78, 17.92, 21.02, 25.61, 25.71, 25.79, 36.41, 62.17, 72.06, 84.42, 86.86, 94.49, 118.47, 125.78, 125.87, 129.64, 129.79, 141.19, 143.09, 145.65, 145.98, 149.79, 152.94, 156.38.

(*Z*)-2',3',5'-Tri-*O*-(*tert*-butyldimethylsilyl)-8-(1-amino-2-tosylvinyl)adenosine (48).Treatment of **43** (500 mg, 0.55 mmol) with methanolic ammonia (10 mL) by **Procedure B** (column chromatography; 30 → 50% EtOAc/hexane) gave **48** (395 mg, 90%) as a white solid: ^{1}H NMR δ -0.47 (s, 3H), -0.15 (s, 3H), -0.08 (s, 3H), -0.02 (s, 3H), 0.12 (s, 3H), 0.14 (s, 3H), 0.67 (s, 9H), 0.79 (s, 9H), 0.92 (s, 9H), 2.38 (s, 3H), 3.67 (dd, *J* = 11.2, 4.8 Hz, 1H), 3.89–3.94 (m, 1H), 4.08 (dd, *J* = 11.2, 6.8 Hz, 1H), 4.62 (t, *J* = 4.0 Hz, 1H), 5.06 (s, 1H), 5.50 (t, *J* = 4.8 Hz, 1H), 5.80 (d, *J* = 5.6 Hz, 1H), 7.15 (s, 2H), 7.39 (d, *J* = 8.0 Hz, 2H), 7.53 (s, 2H), 7.81 (d, *J* = 8.0 Hz, 2H), 8.13 (s, 1H); ^{13}C NMR δ -5.66, -5.50, -5.46, -5.32, -5.00, -4.80, -4.72, -4.67, -4.60, 17.42, 17.81, 17.90, 21.04, 25.29, 25.41, 25.54, 25.67, 25.82, 25.92, 61.62, 71.39, 71.63, 84.48, 88.63, 94.79, 118.54, 125.69, 125.80, 129.65, 129.81, 141.06, 143.09, 144.89, 146.15, 149.83, 153.04, 156.49.

(Z)-8-(1-Amino-2-tosylvinyl)-2'-deoxyadenosine (49). Method 1. NH_4F (282 mg, 7.6 mmol) was added to a stirring solution of **42** (200 mg, 0.25 mmol) in MeOH (10 mL) at rt. The resulting mixture was stirred at 60 °C for 3.0 h. The volatiles were evaporated at reduced pressure and evaporated with acetonitrile (3x5 mL) yielding an off-white solid, which is suspended in 20% MeOH in CH_2Cl_2. The white precipitate was removed by

vacuum filtration and the mother liquor was evaporated at reduced pressure. The residue was purified by column chromatography (5 → 10% MeOH/$CHCl_3$) to give **49** (56 mg, 50%) as a white solid: ^{1}H NMR δ 2.17 (dd, J = 13.2, 6.4 Hz, 1H), 2.41 (s, 3H), 3.10 (ddd, *J* = 13.6, 8.4, 5.6 Hz, 1H), 3.51 (ddd, *J* = 12.0, 8.4, 4.0 Hz, 1H), 3.66 (dt, *J* = 12.0, 4.0 Hz, 1H), 3.86–3.92 (m, 1H),), 4.42–4.48 (m, 1H), 5.16 (s, 1H), 5.30 (d, *J* = 3.6 Hz, 1H), 5.55 (dd, *J* = 8.4, 3.6 Hz, 1H), 6.20 (dd, *J* = 8.8, 6.0 Hz, 1H), 7.27 (s, 2H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.54 (s, 2H), 7.84 (d, *J* = 8.4 Hz, 2H), 8.13 (s, 1H); ^{13}C NMR δ 20.99, 37.92, 62.20, 71.27, 79.22, 85.68, 94.58, 118.59, 125.77,125.88, 129.65, 129.81, 141.04, 143.13, 145.60, 145.63, 149.25, 152.74, 156.48. HRMS (TOF, ESI) *m/z* calcd for $C_{19}H_{22}N_6O_5S$ 447.1445 $[M + H]^+$, found 4470.1454.

Method 2. Treatment of **45** (200 mg, 0.36 mmol) with methanolic ammonia (10 mL) by **Procedure B** (column chromatography; 5 → 10% MeOH/$CHCl_3$) gave **49** (140 mg, 87%) as a white solid.

Method 3. Procedure C. TBAF [1.30 mL, 1.3 mmol (1.0 M in THF)] was added to a stirring solution of **47** (400 mg, 0.59 mmol) in THF (10 mL) at 0 ^{o}C and the resulting mixture was stirred at rt for 1h. The volatiles were evaporated, and the residue was column-chromatographed (5 → 10% MeOH/$CHCl_3$) gave **49** (237 mg, 90%) as a white solid.

(Z)-8-(1-Amino-2-tosylvinyl)adenosine (50). Treatment of **48** (600 mg, 0.75 mmol) with TBAF [2.50 mL, 2.5 mmol (1.0 M in THF)] by **Procedure C** (column chromatography; 5 → 10% MeOH/$CHCl_3$) gave **50** (295 mg, 85%) as a white solid: ^{1}H NMR δ 2.40 (s, 3H), 3.54 (dt, *J* = 12.8, 6.0 Hz, 1H), 3.68 (dt, *J* = 12.0, 3.6 Hz, 1H), 3.96 (q, *J* = 3.2 Hz, 1H), 4.20 (t, *J* = 5.2 Hz, 4.96 (q, *J* = 6.0 Hz, 1H), 5.18 (d, *J* = 4.0 Hz, 1H), 5.22 (s, 1H), 5.46 (d, *J* = 5.6 Hz, 1H), 5.71–5.78 (m, 1H), 5.82 (d, *J* = 6.8 Hz, 1H), 7.16 (s,

2H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.62 (s, 2H), 7.85 (d, *J* = 8.0 Hz, 8.14 (s, 1H); ^{13}C NMR δ 21.09, 62.18, 71.04, 71.78, 86.75, 89.18, 94.66, 118.60, 125.85, 125.95, 129.72, 129.88, 141.09, 143.13, 145.15, 146.03, 149.39, 152.94, 156.60. HRMS (TOF, ESI) *m/z* calcd for $C_{19}H_{22}N_6O_6SNa$ 485.1230 $[M + Na]^+$, found 485.1230.

3',5'-Di-*O*-(*tert*-butyldimethylsilyl)-8-(2-tosylacetyl)-2'-deoxyadenosine (51). **Procedure D**. CH_3COOH/H_2O (1:1, 1.0 mL) was added to a stirring solution of **47** (400 mg, 0.59 mmol) in MeOH (10 mL) at rt. The resulting solution was stirred at rt for 8.0 h. The volatiles were evaporated at reduced pressure and the residue was dissolved in EtOAc. The solution was extracted with H_2O, $NaHCO_3$ and brine. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (40 → 50% EtOAc/hexane) to give **51** (279 mg, 70%) as a white solid: ^{1}H NMR ($CDCl_3$) δ -0.05 (s, 3H), -0.01 (s, 3H), 0.13 (s, 6H), 0.82 (s, 9H), 0.93 (s, 9H), 2.22 (ddd, *J* = 13.0, 7.0, 4.4 Hz, 1H), 2.38 (s, 3H), 3.40 (dt, *J* = 13.0, 6.4 Hz, 1H), 3.67–3.74 (m, 1H), 3.87–3.95 (m, 2H), 4.83 (dt, *J* = 6.4, 4.2 Hz, 1H), 4.94 (d, *J* = 13.6 Hz, 1H), 5.15 (d, *J* = 13.2 Hz, 1H), 5.95 (s, 2H), 7.04 (t, *J* = 6.6 Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 2H), 7.78 (d, *J* = 8.4 Hz, 2H), 8.33 (s, 1H); ^{13}C NMR ($CDCl_3$) δ -5.32, -5.26, -4.59, -4.50, 18.15, 18.48, 21.76, 25.96, 26.00, 38.00, 63.01, 64.00, 72.48, 85.26, 87.84, 120.03, 128.75, 129.85, 136.32, 143.80, 145.40, 151.51, 155.64, 157.23, 181.25.

Note: 5'-*O*-(*Tert*-butyldimethylsilyl)-8-(2-tosylacetyl)-2'-deoxyadenosine (**55**; 99 mg, 30%) was also isolated as a more polar compound: ^{1}H NMR ($CDCl_3$) δ 0.11 (s, 3H), 0.12 (s, 3H), 0.94 (s, 9H), 2.27 (ddd, *J* = 12.8, 5.6, 1.2 Hz, 1H), 2.40 (s, 3H), 2.81 (ddd, *J* = 12.8, 9.2, 5.6 Hz, 1H), 3.76 (t, *J* = 11.4 Hz, 1H), 3.96 (d, *J* = 13.2 Hz, 1H), 4.12 (d, *J* = 1.6 Hz, 1H), 4.69 (d, *J* = 5.4 Hz, 1H), 4.95 (d, *J* = 13.4 Hz, 1H), 5.15 (d, *J* = 13.4 Hz, 1H), 6.02 (s,

2H), 6.45 (d, *J* = 11.2 Hz, 1H), 7.11 (dd, *J* = 9.2, 5.6 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 2H), 7.78 (d, *J* = 8.4 Hz, 2H), 8.34 (s, 1H); ^{13}C NMR ($CDCl_3$) δ -4.59, -4.53, 18.15, 18.48, 21.79, 25.94, 41.53, 63.11, 64.02, 73.69, 87.75, 90.43, 120.30, 128.68, 129.94, 136.26, 143.40, 145.63, 150.71, 155.36, 157.56, 181.45.

2', 3', 5'-Tri-*O*-(*tert*-butyldimethylsilyl)-8-(2-tosylacetyl)adenosine (52). Treatment of **48** (500 mg, 0.62 mmol) with CH_3COOH/H_2O (1:1, 1.2 mL) by **Procedure D** (column chromatography; 40 → 50% EtOAc/hexane) gave **52** (450 mg, 90%) as a white solid: ^{1}H NMR ($CDCl_3$) δ -0.35 (s, 3H), -0.10 (s, 3H), -0.06 (s, 3H), -0.01 (s, 3H), 0.15 (s, 6H), 0.77 (s, 9H), 0.81 (s, 9H), 0.97 (s, 9H), 2.40 (s, 3H), 3.65–3.75 (m, 1H), 3.96–4.08 (m, 2H), 4.56–4.65 (m, 1H), 4.92 (dd, *J* = 13.4, 2.6 Hz, 1H), 5.14 (d, *J* = 13.4 Hz, 1H), 5.49 (t, *J* = 5.0 Hz, 1H), 5.91 (s, 1H), 5.98 (s, 1H), 6.72 (d, *J* = 5.2 Hz, 1H), 7.30 (d, *J* = 8.4 Hz, 2H), 7.78 (d, *J* = 8.4 Hz, 2H), 8.35 (s, 1H); ^{13}C NMR ($CDCl_3$) δ -5.41, -5.28, -4.95, -4.50, -4.41, -4.30, 18.02, 18.27, 18.41, 21.78, 25.85, 25.95, 26.09, 62.40, 63.91, 72.29, 72.59, 85.25, 89.63, 119.96, 128.85, 129.83, 136.19, 144.21, 145.33, 151.65, 155.67, 157.28, 180.91.

8-(2-Tosylacetyl)-2'-deoxyadenosine (53). Method 1. Procedure E. CH_3COOH/H_2O (1:1, 2.0 mL) was added to a stirring solution of **49** (402 mg, 0.9 mmol) in MeOH (10 mL) at rt. The resulting solution was stirred at rt for 8.0 h. The volatiles were evaporated at reduced pressure and co-evaporated with acetonitrile (3x5 mL). The residue was purified by column chromatography (5 → 10% MeOH/$CHCl_3$) to give **53** (363 mg, 90%) as a white solid: ^{1}H NMR δ 2.14 (ddd, *J* = 12.8, 6.4, 2.4 Hz, 1H), 2.34 (s, 3H), 2.97 (ddd, *J* = 13.6, 8.4, 6.0 Hz, 1H), 3.51 (ddd, *J* = 12.4, 8.8, 4.0 Hz, 1H), 3.68 (dt, *J* = 12.0, 4.0 Hz, 1H), 3.89 (q, *J* = 3.6 Hz, 1H), 4.45 (q, *J* = 3.2 Hz, 1H), 5.28 (d, *J* = 14.4 Hz, 1H), 5.30 (d, *J* = 4.0 Hz, 1H), 5.39 (d, *J* = 14.4 Hz, 1H), 5.55 (dd, *J* = 8.8, 4.0 Hz, 1H), 6.95 (dd, *J* = 8.0, 6.0 Hz,

1H), 7.40 (d, *J* = 8.0 Hz, 2H), 7.77 (d, *J* = 8.2 Hz, 2H), 7.96 (s, 2H), 8.21 (s, 1H); ^{13}C NMR *δ* 20.99, 38.31, 62.20, 62.61, 71.33, 85.59, 88.49, 119.07, 128.05, 129.69, 136.21, 142.40, 144.79, 150.42, 155.28, 157.67. 181.45. HRMS (TOF, ESI) *m/z* calcd for $C_{19}H_{21}N_5O_6S$ 448.1285 $[M + H]^+$, found 448.1259.

Method 2. Treatment of **51** (350 mg, 0.52 mmol) with TBAF [1.30 mL, 1.30 mmol (1.0 M in THF)] by **Procedure C** (column chromatography; 5 → 10% MeOH/$CHCl_3$) gave **53** (208 mg, 90%) as a white solid.

8-(2-Tosylacetyl)adenosine (54). Method 1. Treatment of **50** (420 mg, 0.91 mmol) with CH_3COOH/H_2O (1:1, 2.0 mL) by **Procedure E** (column chromatography; 5 → 10% MeOH/$CHCl_3$) gave **54** (370 mg, 88%) as a white solid: ^{1}H NMR *δ* 2.33 (s, 3H), 3.52 (ddd, *J* = 12.8, 8.8, 4.4 Hz, 1H), 3.68 (dt, *J* = 12.4, 3.6 Hz, 1H), 3.94 (q, *J* = 3.6 Hz, 1H), 4.18–4.23 (m, 1H), 4.97 (q, *J* = 6.0 Hz, 1H), 5.17 (d, *J* = 4.4 Hz, 1H), 5.31 (d, *J* = 6.4 Hz, 1H), 5.32 (d, *J* = 14.4 Hz, 1H), 5.39 (d, *J* = 14.4 Hz, 1H), 5.44 (dd, *J* = 8.8, 3.6 Hz, 1H), 6.60 (d, *J* = 6.4 Hz, 1H), 7.39 (d, *J* = 8.0 Hz, 2H), 7.77 (d, *J* = 8.0 Hz, 2H), 7.99 (s, 2H), 8.23 (s, 1H); ^{13}C NMR *δ* 21.07, 62.21, 62.71, 70.92, 71.80, 86.35, 89.22, 119.04, 128.11, 128.23, 129.65, 129.81, 136.13, 142.57, 144.80, 150.59, 155.60, 157.68, 181.24. HRMS (TOF, ESI) *m/z* calcd for $C_{19}H_{21}N_5O_7SNa$ 486.1074 $[M + Na]^+$, found 486.1074.

Method 2. Treatment of **52** (404 mg, 0.50 mmol) with TBAF [1.75 mL, 1.75 mmol (1.0 M in THF)] by **Procedure C** (column chromatography; 5 → 10% MeOH/$CHCl_3$) gave **54** (209 mg, 90%) as a white solid.

3',5'-Di-*O*-(*tert*-butyldimethylsilyl)-8-ethynyl-2'-deoxyguanosine (56). TBDMSCl (1.46 g, 9.69 mmol) was added to a mixture of 8-[2-(trimethylsilyl)ethynyl]- 2'-deoxyguanosine[52] (1.60 g, 4.40 mmol) and imidazole (899 mg, 13.2 mmol) in dry DMF

(15 mL) at 0 ˚C. The reaction mixture was warmed to rt and stirred for 16 h. The volatiles were removed under reduced pressure and extracted with EtOAc. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (2 → 5% MeOH/DCM) to give 3',5'-di-*O*-(*tert*-butyldimethylsilyl)-8-[(2-trimethylsilyl)ethynyl]-2'-deoxyguanosine (2.34 g, 90%) as a brown solid. This material was dissolved in MeOH (10 mL) and methanolic ammonia (5 mL) was added and the resulting solution was stirred at rt for 1 h. Volatiles were evaporated, and the residue was extracted with EtOAc. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. Recrystallization from with hexane/EtOAc to give **56** (1.6 g, 70% overall) as an off-white solid: ^{1}H NMR δ 0.00 (s, 3H), 0.01 (s, 3H), 0.11 (s, 6H), 0.84 (s, 9H), 0.89 (s, 9H), 2.16 (ddd, J = 13.2, 6.8, 3.2 Hz, 1H), 3.20–3.28 (m, 1H), 3.63–3.69 (m, 1H), 3.71–3.80 (m, 2H), 4.50–4.59 (m, 1H), 4.79 (s, 1H), 6.24 (t, J = 7.2 Hz, 1H), 6.55 (s, 2H), 10.89 (s, 1H); ^{13}C NMR δ -5.42, -5.40, -4.89, -4.71, 17.71, 17.98, 25.69, 25.77, 36.45, 62.91, 72.59, 73.97, 83.25, 85.50, 87.04, 116.90, 128.50, 150.91, 153.98, 156.04.

3',5'-Di-*O*-(*tert*-butyldimethylsilyl)-8-(1-iodoethynyl)-2'-deoxyguanosine (57). Sodium acetate (23 mg, 0.29 mmol), sodium *p*-toluenesulfinate (97 mg, 0.58 mmol), and I_2 (73 mg, 0.29 mmol) were added to a stirring solution of **56** (100 mg, 0.19 mmol) in MeCN (5 mL). The resulting mixture was stirred at 80 °C for 5 h. The reaction mixture was quenched by saturated aqueous sodium thiosulfate ($Na_2S_2O_3$). The volatiles were removed under reduced pressure and extracted with EtOAc. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (1 → 2% MeOH/DCM) to give **57** (61 mg, 50%) as an off-

white solid: ^{1}H NMR δ 0.04 (s, 6H), 0.12 (s, 3H), 0.12 (s, 3H), 0.86 (s, 9H), 0.90 (s, 9H), 2.16 (ddd, J = 13.2, 6.8, 3.2 Hz, 1H), 3.00–3.08 (m, 1H), 3.67–3.80 (m, 3H), 4.47 (dt, J = 6.8, 3.6 Hz, 1H), 6.22 (t, J = 7.2 Hz, 1H), 6.57 (s, 2H), 10.82 (s, 1H); ^{13}C NMR δ -5.26, -5.24, -4.76, -4.61, 17.74, 18.07, 25.74, 25.86, 26.76, 37.11, 63.33, 72.63, 82.58, 83.49, 86.86, 116.37, 128.86, 150.75, 154.14, 156.02. HRMS (TOF, ESI) *m/z* calcd for $C_{24}H_{41}IN_5O_4Si_2$ 646.1736 $[M + H]^+$, found 646.1724.

8-(1-Iodo-2-tosylvinyl)guanine (58). $TsNHNH_2$ (32.4 mg, 0.17 mmol), I_2 (7.5 mg, 0.3 mmol), and *t*-butyl hydroperoxide (TBHP, 70% in H_2O) (16 µL, 0.12 mmol) were added to a stirring solution of **56** (100 mg, 0.19 mmol) in MeCN (5 mL) at rt. The resulting mixture was stirred at 80 °C for 2 h. The volatiles were removed under reduced pressure and the residue was purified by column chromatography (2 → 5% MeOH/DCM) to give **58** (17.2 mg, 65%) as a yellow solid: ^{1}H NMR δ 2.40 (s, 3H), 6.47 (s, 2H), 7.42 (d, J = 8.0 Hz, 2H), 7.78 (d, J = 8.4 Hz, 2H), 7.87 (s, 1H), 10.65 (s, 1H), 12.63 (s, 1H); ^{13}C NMR δ 21.17, 101.71, 123.75, 127.89, 128.15, 129.86, 136.54, 142.90, 144.81, 153.80, 156.25, 159.32. HRMS (TOF, ESI) *m/z* calcd for $C_{14}H_{12}N_5O_3SNa$ 479.9609 $[M + Na]^+$, found 479.9609.

3',5'-Di-*O*-(*tert*-butyldimethylsilyl)-8-(1-Iodo-2-tosylvinyl)-2'-deoxyguanosine (60). $TsNHNH_2$ (269 mg, 1.44 mmol), KI (159 mg, 0.96 mmol), and benzoyl peroxide (BPO) (232.5 mg, 0.96 mmol) were added to a stirring solution of **56** (500 mg, 0.96 mmol) in DMSO (10 mL) at rt. The resulting mixture was stirred for another 15 h. The reaction mixture was diluted with EtOAc (50 mL) and extracted with water (3 x 50 mL), $NaHCO_3$, and brine. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (1 → 2% MeOH/DCM)

to give **60** (462 mg, 60%) as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 0.04 (s, 3H), 0.05 (s, 3H), 0.14 (s, 3H), 0.15 (s, 3H), 0.88 (s, 9H), 0.94 (s, 9H), 2.26-2.35 (m, 1H), 2.32 (s, 3H), 3.15–3.42 (m, 1H), 3.78 (s, 1H), 3.91 (t, *J* = 8.4 Hz, 1H), 4.01 (s, 1H), 4.68 (s, 1H), 6.09 (s, 1H), 6.37 (s, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.54 (s, 1H), 7.69 (d, *J* = 7.8 Hz, 2H), 12.17 (s, 1H); ^{13}C NMR ($CDCl_3$) δ -4.99, -4.92, -4.40, -4.33, 18.32, 18.65, 21.96, 26.12, 26.22, 36.99, 63.29, 73.08, 86.28, 88.22, 97.13, 118.12, 128.76, 130.36, 134.55, 136.05, 146.16, 147.52, 151.71, 153.63, 159.20. HRMS (TOF, ESI) *m/z* calcd for $C_{31}H_{49}IN_5O_6SSi_2$ 802.1981 $[M + H]^{+,}$ found 802.2029.

8-(1-Iodo-2-tosylvinyl)-2'-deoxyguanosine (62). Treatment of **60** (200 mg, 0.25 mmol) with CH_3COOH (856 µL, 900 mg, 21.0 mmol), and NH_4F (463 mg, 12.5 mmol) by **Procedure A** (column chromatography; 5 → 15% MeOH/$CHCl_3$) gave **62** (100 mg, 70%) as an off- white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.11 (dd, *J* = 13.6, 6.0 Hz, 1H), 2.41 (s, 3H), 2.95–3.07 (m, 1H), 3.55–3.63 (m, 1H), 3.66–3.72 (m, 1H), 3.88 (brs, 1H), 4.39 (brs, 1H), 5.00–5.12 (m, 1H), 5.28 (d, *J* = 4.0 Hz, 1H), 5.89 (dd, *J* = 8.8, 6.0 Hz, 1H), 6.44 (s, 2H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.74 (s, 2H), 8.12 (s, 1H), 10.85 (s, 1H); ^{13}C NMR (101 MHz, DMSO) δ 21.18, 37.28, 62.19, 71.30, 85.46, 88.24, 99.97, 117.60, 128.03, 128.16, 130.03, 130.18, 136.05, 142.35, 145.19, 145.43, 150.69, 153.42, 156.35. HRMS (TOF, ESI) *m/z* calcd for $C_{19}H_{20}N_5O_6SNa$ 596.0100 $[M + Na]^{+}$, found 596.0100.

(*Z*)-3',5'-Di-*O*-(*tert*-butyldimethylsilyl)-8-(1-amino-2-tosylvinyl)-2'-deoxyguanosine (63). Treatment of **60** (200 mg, 0.25 mmol) with methanolic ammonia (10 mL) by **Procedure B** (column chromatography; 2 → 5% MeOH/DCM) gave **63** (147 mg, 85%) as a yellow solid: ^{1}H NMR δ 0.03 (s, 6H), 0.08 (s, 3H), 0.09 (s, 3H), 0.84 (s, 9H), 0.88 (s, 9H), 2.06 (ddd, *J* = 13.6, 7.2, 3.6 Hz, 1H), 2.39 (s, 3H), 3.18–3.26 (m 1H),

3.69 (dtd, *J* = 19.8, 8.8, 4.0 Hz, 3H), 3.63–3.76 (m,3H), 4.44 (quint, *J* = 3.2 Hz, 1H), 5.14 (s, 1H),), 6.10 (t, *J* = 7.2 Hz, 1H), 6.48 (s, 2H), 7.06 (s, 2H), 7.40 (d, *J* = 8.0 Hz, 2H), 7.80 (d, *J* = 8.0 Hz, 2H), 10.81 (s, 1H); ^{13}C NMR δ -5.41,- 5.37, -5.34, -4.92, -4.86, -4.73, -4.67, 17.71, 18.01, 21.05, 25.66, 25.74, 25.83, 36.41, 62.99, 72.49, 83.78, 87.30, 93.22, 116.60, 125.61, 125.71, 129.62, 129.76, 141.32, 142.18, 142.96, 145.77, 152.36, 153.59, 156.49.

(*Z*)-8-(1-Amino-2-tosylvinyl)-2'-deoxyguanosine (**64**). **Method 1**. Treatment of **63** (118 mg, 0.17 mmol) with TBAF [427 µL, 0.43 mmol (1.0 M in THF)] by **Procedure C** (column chromatography; 5 → 15% MeOH/DCM) gave **64** (63 mg, 80%) as a yellow solid: ^{1}H NMR δ 2.04 (ddd, *J* = 13.2, 6.8, 2.8 Hz, 1H), 2.40 (s, 3H), 2.98–3.08 (m, 1H), 3.47–3.54 (m, 1H), 3.57–3.64 (m, 1H), 3.76 (q, *J* = 4.8 Hz, 1H), 4.29–4.36 (m, 1H), 4.91 (t, *J* = 5.6 Hz, 1H), 5.15 (s, 1H), 5.21 (d, *J* = 4.4 Hz, 1H), 6.11 (t, *J* = 7.4 Hz, 1H), 6.53 (s, 2H), 7.07 (s, 2H), 7.41 (d, *J* = 8.0 Hz, 2H), 7.81 (d, *J* = 8.0 Hz, 2H), 10.81 (s, 1H); ^{13}C NMR δ 21.06, 36.95, 61.83, 70.78, 84.13, 87.77, 93.22, 116.68, 125.66, 125.77, 129.65, 129.81, 141.25, 142.16, 143.00, 145.77, 152.11, 153.58, 156.44. HRMS (TOF, ESI) *m/z* calcd for $C_{19}H_{22}N_6O_6SNa$ 485.1230 $[M + Na]^+$, found 485.1230.

Method 2. Treatment of **62** (80 mg, 0.14 mmol) with methanolic ammonia (2 mL) by **Procedure B** (column chromatography; 5 → 15% MeOH/ DCM) gave **64** (55 mg, 85%) as a white solid.

3',5'-Di-*O*-(*tert*-butyldimethylsilyl)-8-(2-Tosylacetyl)-2'-deoxyguanosine (65). Treatment of **64** (104 mg, 0.15 mmol) with CH_3COOH/H_2O (1:1, 400 µL) by **Procedure D** (column chromatography; 2 → 5% MeOH/DCM) gave **65** (88 mg, 85%) as an off-white solid: ^{1}H NMR δ 0.00 (s, 6H), 0.10 (s, 3H), 0.10 (s, 3H), 0.84 (s, 9H), 0.89 (s, 9H), 2.03 (ddd, *J* = 12.8, 6.8, 3.2 Hz, 1H), 2.38 (s, 3H), 3.18–3.26 (m, 1H), 3.65 (dd, *J* = 9.6, 4.8 Hz,

1H), 3.72–3.82 (m, 2H), 4.53 (quint J = 3.2 Hz, 1H), 5.12 (d, J = 14.4 Hz, 1H), 5.21 (d, J = 14.4 Hz, 1H), 6.82 (q, J = 10.2, 7.2 Hz, 3H), 6.80 (t, J = 7.2 Hz, 1H), 6.82 (s, 2H), 7.40 (d, J = 8.0 Hz, 2H), 7.74 (d, J = 8.4 Hz, 2H), 11.07 (s, 1H); ^{13}C NMR δ -5.40, -5.36, -5.32, -4.86, -4.79, -4.70, -4.64, 17.72, 18.00, 21.09, 25.67, 25.75, 25.85, 36.75, 62.38, 63.28, 72.94, 83.98, 87.44, 118.41, 127.94, 128.06, 129.60, 129.75, 136.58, 140.49, 144.54, 153.86, 154.70, 156.65, 179.47.

8-(2-Tosylacetyl)-2'-deoxyguanosine (66). Method 1. Treatment of **64** (50 mg, 0.108 mmol) with CH_3COOH/H_2O (1:1, 200 µL) by **Procedure E** (column chromatography; 10 → 15% MeOH/DCM) gave **66** (41 mg, 82%) as an off-white solid: ^{1}H NMR δ 2.03 (ddd, J = 12.8, 6.8, 3.2 Hz, 1H), 2.39 (s, 3H), 3.00 (dt, J = 13.7, 7.2 Hz, 1H), 2.96-3.03 (m, 1H), 3.45–3.56 (m, 1H), 3.60–3.68 (m, 1H), 3.74–3.80 (m, 1H), 4.36–4.42 (m, 1H), 4.84 (t, J = 6.0 Hz, 1H), 5.10–5.25 (m, 3H), 6.84 (t, J = 7.2 Hz, 1H), 6.94 (s, 2H), 7.42 (d, J = 7.6 Hz, 2H), 7.75 (d, J = 8.0 Hz, 2H), 11.17 (s, 1H); ^{13}C NMR δ 21.10, 37.32, 62.17, 62.36, 71.31, 84.38, 88.07, 118.58, 127.96, 128.06, 129.68, 129.83., 136.60, 140.34, 144.63, 153.64, 154.77, 156.67, 179.63. HRMS (TOF, ESI) *m/z* calcd for $C_{19}H_{21}N_5O_7SNa$ 486.1076 $[M + Na]^+$, found 486.1076.

Method 2. Treatment of **65** (77 mg, 0.11 mmol) with TBAF [280 µL, 0.28 mmol (1.0 M in THF)] by **Procedure C** (column chromatography; 10 → 15% $MeOH/CHCl_3$) gave **66** (43 mg, 83%) as a white solid.

8-(2-Benzyl-2-tosylacetyl)adenosine (67). Aqueous NaOH solution (1 M, 128 µL, 0.13 mmol) was added to a stirred solution of **54** (0.065 mmol) in MeOH (2 mL) at rt. After 30 min, BnBr (15.4 µL, 22.3 mg, 0.13 mmol) was added and the resulting mixture was stirred for 24 h. The reaction mixture was then neutralized with dil. HCl to pH ~7, and the

volatiles were evaporated. The residue was column chromatographed (5 → 10% MeOH/DCM) to give **67** (25 mg, 70%) as white solid: ^{1}H NMR (MeOD-d_4) δ 2.17 (s, 1.5H), 2.19 (s, 1.5H), 3.47–3.57 (m, 2H), 3.70–3.78 (m, 1H), 3.85 (dd, J = 12.4, 2.4 Hz, 0.5H), 3.92 (dd, J = 12.4, 2.4 Hz, 0.5H), 4.15 (q, J = 2.6 Hz, 0.5H), 4.19 (q, J = 2.6 Hz, 0.5H), 4.35–4.39 (m, 1H), 4.84–4.91 (m, 1H), 6.10–6.22 (m, 1H), 6.81 (d, J = 6.8 Hz, 0.5H), 6.85 (d, J = 7.0 Hz, 0.5H), 7.09 – 7.14 (m, 1H), 7.16 – 7.24 (m, 6H), 7.63 (d, J = 8.4 Hz, 1H), 7.73 (d, J = 8.3 Hz, 1H), 8.13 (s, 0.5H), 8.14 (s, 0.5H); ^{13}C NMR (MeOD-d_4) δ 21.43, 31.62, 31.69, 63.96, 64.06, 72.73, 72.75, 73.02, 73.24, 74.64, 88.43, 88.47, 91.11, 91.13, 120.87, 120.91, 128.02, 128.06 129.74, 130.05, 130.09, 130.16, 130.57, 130.71, 130.92, 135.58, 135.74, 137.55, 137.57, 144.53, 145.14, 147.03, 151.38, 151.43, 156.15, 156.27, 159.07, 159.12, 185.22, 185.36.

8-(2-Allyl-2-tosylacetyl)adenosine (68). Aqueous NaOH solution (1 M, 128 µL, 0.13 mmol) was added to a stirred solution of **54** (0.065 mmol) in MeOH (2 mL) at rt. After 30 min, allyl bromide (11.2 µL 15.7 mg, 0.13 mmol) was added and the resulting mixture was stirred for 24 h. The reaction mixture was then neutralized with dil. HCl to pH ~7, and the volatiles were evaporated. The residue was column chromatographed (5 → 10% MeOH/DCM) to give **68** (16.4 mg, 50%) as white solid: ^{1}H NMR (MeOD-d_4) δ 2.20 (s, 1.3H), 2.21 (s, 1.7H), 2.89–2.99 (m, 2H), 3.75 (ddd, J = 12.4, 8.0, 2.8 Hz, 1H), 3.86 – 3.96 (m, 1H), 4.18 (dq, J = 12.4, 2.4 Hz, 1H), 4.38 (dt, J = 5.6, 2.0 Hz, 1H), 4.89–7.96 (m, 1H), 5.03 (ddt, J = 10.2, 2.8, 1.2 Hz, 1H), 5.11–5.18 (m, 1H), 5.72–5.82 (m, 1H), 5.95–5.99 (m, 1H), 6.80 (d, J = 6.8 Hz, 0.44H), 6.88 (d, J = 6.8 Hz, 0.56H), 7.19–7.26 (m, 2H), 7.61 (d, J = 8.4 Hz, 0.9H), 7.70 (d, J = 8.4 Hz, 1.1H), 8.18 (s, 0.44H), 8.19 (s, .55H); ^{13}C NMR (MeOD-d_4) δ 21.44, 30.23, 30.32, 64.01, 64.07, 71.03, 71.24, 72.77, 74.66, 74.74, 88.47,

88.51, 91.14, 91.21, 118.99, 119.06, 120.92, 120.95, 130.18, 130.58, 130.70, 130.90, 133.64, 133.67, 133.72, 133.74, 135.55, 135.75, 144.65, 145.23, 147.01, 147.04, 151.51, 151.57, 156.20, 156.31, 159.20, 159.25, 185.34, 185.45.

3', 5'-di-*O*-(*tert*-butyldimethylsilyl)-2-ethynyl-2'-deoxyadenosine (73). $Pd(PPh_3)_2Cl_2$ (70.0 mg, 0.10 mmol) and Cu(I)I (38.1 mg, 0.2 mmol) were added to dry DMF (50 mL) and Et_3N (5.0 mL, 3.63 g, 35.9 mmol) in a flame-dried flask equipped with a stirring bar under N_2 at rt. Then **71**[61] (3.0g, 4.95 mmol) was added followed by TMS-acetylene (1.41 mL, 973 mg, 9.90 mmol). The resulting mixture was stirred for 6 h at 80 ^oC. Volatiles were evaporated and the residue was purified by column chromatography (20 → 50% EtOAc/hexane) to give silyl protected alkyne of **73** as a brown solid which was dissolved in anhydrous MeOH (50 mL) and stirred at 0 ^oC. Anhydrous K_2CO_3 (3.2 g, 23.1 mmol) was added portion wise at room temperature and the mixture was stirred at rt. After 30 min, volatiles were evaporated and the residue was extracted with EtOAc. The solvent was removed at reduced pressure and the residue was recrystallized with hexane to give **73** (2.25 g, 90%, overall) as an off-white solid: ^1H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 6.47 (t, J = 6.4 Hz, 1H), 6.07 (s, 2H), 4.61 (dt, J = 6.0, 4.0 Hz, 1H), 3.99 (q, J = 3.6 Hz, 1H), 3.90 (dd, J = 11.2, 4.0 Hz, 1H), 3.77 (dd, J = 11.2, 3.0 Hz, 1H), 3.00 (s, 1H), 2.58 (dt, J = 12.4, 6.1 Hz, 1H), 2.44 (ddd, J = 13.2, 6.4, 4.2 Hz, 1H), 0.92 (s, 9H), 0.91 (s, 9H), 0.10 (s, 6H), 0.09 (s, 6H).

2', 3', 5'-tri-*O*-(*tert*-butyldimethylsilyl)-2-ethynyladenosine (74). Treatment of **72**[60] (5.0 g, 6.79 mmol) with TMS-acetylene (1.93 mL, 1.33 g, 13.6 mmol) in presence of $Pd(PPh_3)_2Cl_2$ (71.5 mg, 0.102 mmol), Cu(I)I (38.8 mg, 0.204 mmol) and Et_3N (5.0 mL, 3.63 g, 35.9 mmol) as described for **73**, gave **74** (3.79 g, 88%, overall) as an off-white

solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ -0.21 (s, 3H), -0.04 (s, 3H), 0.10 (s, 3H), 0.10 (s, 3H),), 0.13 (s, 3H), 0.14 (s, 3H), 0.81 (s, 8H), 0.93 (s, 9H), 0.95 (s, 9H), 2.93 (s, 1H), 3.78 (dd, *J* = 11.2, 2.8 Hz, 1H), 4.06 (dd, *J* = 11.2, 4.8 Hz, 1H), 4.10-4.14 (m, 1H), 4.31 (t, *J* = 3.8 Hz, 1H), 4.70 (t, *J* = 4.8 Hz, 1H), 6.00 (d, *J* = 5.2 Hz, 1H), 8.19 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ -5.24, -5.24, -4.98, -4.62, -4.58, -4.24, 18.02, 18.24, 18.64, 25.84, 25.99, 26.22, 62.60, 72.08, 72.93, 75.79, 82.66, 85.69, 88.84, 120.07, 140.84, 145.27, 149.79, 155.39.

3', 5'-di-*O*-(*tert*-butyldimethylsilyl)-2-(1-iodoethynyl)-2'-deoxyadenosine (75). Sodium acetate (7.4 mg, 0.09 mmol), sodium *p*-toluenesulfinate (32.1 mg, 0.18 mmol), and I_2 (22.8 mg, 0.09 mmol) were added to a stirring solution of **73** (31 mg, 0.06 mmol) in MeCN at rt. The resulting mixture was stirred at 80 °C for 1.5 h. The reaction mixture was quenched by saturated aqueous $Na_2S_2O_3$. The volatiles were removed under reduced pressure and extracted with EtOAc. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (30 → 50% EtOAc/hexane) to give **75** (30 mg, 80%) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.10 (s, 12H), 0.91 (s, 9H), 0.92 (s, 9H), 2.43 (ddd, *J* = 13.2, 6.0, 4.4 Hz, 1H), 2.56-2.65 (m, 1H), 3.77 (dd, *J* = 11.6, 3.2 Hz, 1H), 3.90 (dd, *J* = 11.2, 4.0 Hz, 1H), 3.99 (q, *J* = 3.4 Hz, 1H), 4.61 (dt, *J* = 6.0, 3.6 Hz, 1H), 5.92 (s, 2H), 6.45 (t, *J* = 6.4 Hz, 1H), 8.22 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ -5.37, -5.31,-5.26, -5.20, -4.71, -4.65, -4.54, -4.47, 18.18, 18.58, 24.84, 25.90, 25.96, 26.09, 26.14, 41.62, 62.78, 71.85, 84.69, 88.09, 93.73, 119.76, 140.46, 145.40, 149.31, 154.92.

3', 5'-di-*O*-(*tert*-butyldimethylsilyl)-2-(1-iodo-2-tosylvinyl)-2'-deoxyadenosine (76). **Procedure F**. NaOAc (342 mg, 4.17 mmol) and freshly prepared TsI (784 mg, 2.78

mmol) were added to a stirring solution of **73** (700 mg, 1.39 mmol) in THF (15 mL) at rt. The resulting mixture was stirred for 40 h. The reaction mixture was quenched by saturated aqueous sodium thiosulfate ($Na_2S_2O_3$). The volatiles were removed under reduced pressure and extracted with EtOAc. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (30 → 50% EtOAc/hexane) to give **76** (885 mg, 81%) as an off-white solid: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 0.11 (m, 12H), 0.92 (s, 9H), 0.93 (s, 9H), 2.31 (s, 3H), 2.44(ddd, *J* = 13.2, 6.0, 4.0 Hz, 1H), 2.68-2.76 (m, 1H), 3.79 (dd, *J* = 10.8, 3.2 Hz, 1H), 3.92 (dd, *J* = 11.2, 4.4 Hz, 1H), 4.03 (q, *J* = 3.6 Hz, 1H), 4.63 (dt, *J* = 6.0, 3.6 Hz, 1H), 6.22 (s, 2H), 6.41 (t, *J* = 6.4 Hz, 1H), 7.22 (d, *J* = 7.8 Hz, 2H), 7.23 (s, 1H), 7.75, (d, *J* = 8.4 Hz, 2H), 8.19 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ -5.17, -5.08, -4.53, -4.36, 18.27, 18.68, 21.18, 26.04, 26.24, 41.46, 63.12, 72.12, 84.89, 88.29, 110.84, 119.33, 128.56, 129.86, 137.22, 140.54, 140.63., 144.94, 149.08, 155.16,158.29.

2', 3', 5'-tri-*O*-(*tert*-butyldimethylsilyl)-2-(1-iodo-2-tosylvinyl)adenosine (77). Treatment of **74** (1.5 g, 2.36 mmol) with NaOAc (582 mg, 7.09 mmol) and freshly prepared TsI (1.33 g, 4.72 mmol) by **Procedure F** (column chromatography; 30 → 50% EtOAc/hexane) gave **77** (1.99 g, 92%) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ -0.07 (s, 3H), 0.02 (s, 3H), 0.09 (s, 3H), 0.11 (s, 3H), 0.16 (s, 3H), 0.17 (s, 3H), 0.84 (s, 9H), 0.93 (s, 9H), 0.97 (s, 9H), 2.30 (s, 3H), 3.83 (dd, *J* = 11.2, 2.6 Hz, 1H), 4.10 (dd, *J* = 11.2, 4.4 Hz, 1H), 4.16 (td, *J* = 4.8, 2.8 Hz, 1H), 4.32 – 4.34 (m, 1H), 4.60 (t, *J* = 4.0 Hz, 1H), 5.93 (d, *J* = 4.0 Hz, 1H), 6.28 (s, 2H), 7.18 – 7.23 (m, 3H), 7.73 (d, *J* = 8.4 Hz, 2H), 8.26 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ -5.17, -5.09, -4.67, -4.60, -4.51, -4.14, 18.07,

18.23, 18.72, 21.70, 25.97, 26.01, 26.29, 62.25, 71.31, 75.83, 84.93, 89.29, 110.06, 119.24, 128.53, 129.74, 137.02, 140.40, 140.68, 144.83, 149.13, 155.04, 158.34.

2-(1-Iodo-2-tosylvinyl)-2'-deoxyadenosine (78). Treatment of **76** (400 mg, 0.51 mmol) with CH_3COOH (1.7 mL, 1.78 g, 29.6 mmol), and NH_4F (944 mg, 25.5 mmol) in MeOH (15 mL) by **Procedure A** (column chromatography; 5 → 10% MeOH/CH_2Cl_2) gave **78** (222 mg, 78%) as a white solid: ^{1}H NMR (400 MHz, MeOD-d_4) δ 2.37 (s, 3H), 2.43 (ddd, J = 13.6, 6.4, 3.2 Hz, 1H), 2.77-2.86 (m, 1H), 3.75 (dd, J = 12.0, 3.6 Hz, 1H), 3.87 (dd, J = 12.4, 3.2 Hz, 1H), 4.09 (q, J = 3.2 Hz, 1H), 4.60 (quint, J = 2.8 Hz, 1H), 6.42 (t, J = 6.8 Hz, 1H), 7.32 (d, J = 8.0 Hz, 2H), 7.45 (s, 1H), 7.71 (d, J = 8.0 Hz, 2H), 8.38 (s, 1H); ^{13}C NMR (101 MHz, MeOD-d_4) δ 21.64, 41.57, 63.64, 73.04, 86.78, 89.85, 110.85, 119.80, 129.43, 130.82, 138.06, 141.36, 142.38, 146.53, 149.69, 156.90, 160.11. HRMS (TOF, ESI) *m/z* calcd for $C_{19}H_{20}IN_5O_5SNa$ 580.0159 $[M + Na]^+$, found 580.0159.

2-(1-iodo-2-tosylvinyl)adenosine (79). Treatment of **77** (800 mg, 0.87 mmol) with CH_3COOH (3.1 mL, 3.25 g, 54.2 mmol), and NH_4F (1.61 g, 43.5 mmol) in MeOH (20 mL) by **Procedure A** (column chromatography; 5 → 10% MeOH/ CH_2Cl_2) gave **79** (499 mg, 80%) as a white solid: ^{1}H NMR (400 MHz, MeOD -d_4) δ 2.35 (s, 3H), 3.75 (dd, J = 12.6, 3.0 Hz, 1H), 3.92 (dd, J = 12.6, 2.6 Hz, 1H), 4.20 (q, J = 2.8 Hz, 1H), 4.36 (dd, J = 5.0, 3.0 Hz, 1H), 4.75 (dd, J = 6.2, 5.0 Hz, 1H), 5.98 (d, J = 6.4 Hz, 1H), 7.31 (d, J = 8.0 Hz, 2H), 7.46 (s, 1H), 7.70 (d, J = 8.4 Hz, 2H), 8.38 (s, 1H); ^{13}C NMR (101 MHz, MeOD -d_4) δ 21.64, 63.38, 72.59, 75.66, 87.97, 90.97, 110.37, 119.98, 129.49, 130.83, 137.81, 141.48, 142.74, 146.56, 149.72, 156.96, 160.13. HRMS (TOF, ESI) *m/z* calcd for $C_{19}H_{20}IN_5O_6SNa$ 596.0090 $[M + Na]^+$, found 596.0090.

(Z)-2', 3', 5'-tri-*O*-(*tert*-butyldimethylsilyl)-2-(1-amino-2-tosylvinyl)adenosine (81). The iodovinylsulfone **77** (303 mg, 0.33 mmol) was dissolved in methanolic ammonia

(10 mL) and the resulting mixture was stirred at rt for 2h. The volatiles were evaporated, and the residue was dissolved in EtOAc (50 mL) and extracted with water, and brine. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated to give **81** (256 mg, 96%) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ -0.18 (s, 3H), -0.05 (s, 3H), 0.07 (s, 3H), 0.09 (s, 3H), 0.14 (s, 3H), 0.16 (s, 3H), 0.79 (s, 9H), 0.92 (s, 9H), 0.96 (s, 9H), 2.39 (s, 3H), 3.81 (dd, *J* = 11.6, 2.0 Hz, 1H), 4.01 (dd, *J* = 11.6, 2.8 Hz, 1H), 4.11-4.13 (m, 1H), 4.29 (t, *J* = 4.6 Hz, 1H), 4.32 (t, *J* = 4.0 Hz, 1H), 5.62 (s, 2H), 6.04 (d, *J* = 3.6 Hz, 1H), 6.27 (s, 1H), 6.9 (s, 2H), 7.27 (d, *J* = 7.6 Hz, 2H), 7.85 (d, *J* = 8.4 Hz, 2H), 8.38 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ -5.23, -5.18, -4.93, -4.67, -4.65, -4.14, 17.96, 18.24, 18.72, 21.64, 25.75, 25.98, 26.27, 62.16, 71.20, 77.02, 84.73, 88.32, 92.90, 120.19, 126.37, 129.62, 140.74, 141.87, 143.04, 149.84, 149.98, 153.41, 154.64.

(Z)-2-(1-amino-2-tosylvinyl)-2'-deoxyadenosine (82). Treatment of **78** (100 mg, 0.18 mmol) with methanolic ammonia (5 mL) by **Procedure B** (column chromatography; 5 → 15% MeOH/CH_2Cl_2) gave **82** (56 mg, 70%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.30 (ddd, *J* = 13.6, 6.4, 3.6 Hz, 1H), 2.37 (s, 3H), 2.63 – 2.70 (m, 1H), 3.49 – 3.59 (m, 2H), 3.82-2.87 (m, 1H), 4.40 (s, 1H), 4.93 (s, 1H), 5.34 (s, 1H), 6.00 (s, 1H), 6.40 (t, *J* = 6.8 Hz, 1H),), 6.98 (s, 2H), 7.39 (d, *J* = 7.6 Hz, 2H), 7.55 (s, 2H), 7.80 (d, *J* = 8.3 Hz, 2H), 8.45 (s, 1H); ^{13}C NMR (101 MHz, MeOD-d_4) δ 21.46, 41.34, 63.12, 72.47, 85.62, 89.22, 91.72, 120.50, 127.00, 130.66, 142.23, 143.07, 144.65, 150.71, 152.29, 154.60, 156.75.

3', 5'-di-*O*-(*tert*-butyldimethylsilyl)-2-(2-tosylacetyl)-2'-deoxyadenosine (84). Treatment of **80** (150 mg, 0.22 mmol) with CH_3COOH/H_2O (1:1, 500 µL) by **Procedure D** (column chromatography; 1 → 2% MeOH/CH_2Cl_2) gave **84** (129 mg, 86%) as a white

solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.11 (s, 6H), 0.12 (s, 3H), 0.12 (s, 3H), 0.92 (s, 9H), 0.93 (s, 9H), 2.36 (s, 3H), 2.46 – 2.54 (m, 2H), 3.80 (dd, *J* = 11.2, 2.8 Hz, 1H), 3.89 (dd, *J* = 11.2, 3.6 Hz, 1H), 4.03 (q, *J* = 3.2 Hz, 1H), 4.57 – 4.62 (m, 1H), 5.10 (s, 2H),), 6.42 (t, *J* = 6.2 Hz, 1H), 6.69 (s, 2H), 7.26 (d, *J* = 8.0 Hz, 2H), 7.77 (d, *J* = 8.4 Hz, 2H), 8.34 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ -5.35, -5.25, -4.66, -4.46, 18.14, 18.55, 21.72, 25.89, 26.09, 42.16, 62.69, 62.80, 71.70, 84.44, 88.12, 121.08, 128.77, 129.75, 136.42, 141.63, 145.09, 148.75, 153.54, 155.82, 187.75.

2', 3', 5'-tri-*O*-(*tert*-butyldimethylsilyl)-2-(2-tosylacetyl)adenosine (85). Treatment of **81** (250 mg, 0.31 mmol) with CH_3COOH/H_2O (1:1, 1.0 mL) by **Procedure D** (column chromatography; 1 → 2% MeOH/CH_2Cl_2) gave **85** (213 mg, 85%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ -0.19 (s, 3H), -0.10 (s, 3H), 0.10 (s, 3H), 0.12 (s, 3H), 0.16 (s, 3H), 0.18 (s, 3H), 0.81 (s, 9H), 0.94 (s, 9H), 0.98 (s, 9H), 2.35 (s, 3H), 3.84 (dd, *J* = 11.6, 2.4 Hz, 1H), 4.05 (dd, *J* = 11.6, 3.0 Hz, 1H), 4.15-4.18 (m, 1H), 4.32 (t, *J* = 4.4 Hz, 1H), 4.37 (t, *J* = 4.4 Hz, 1H), 4.76 (d, *J* = 14.0 Hz, 1H), 5.38 (d, *J* = 14.0 Hz, 1H), 5.98 (d, *J* = 4.0 Hz, 1H), 6.78 (s,2H), 7.25 (d, *J* = 8.0 Hz, 2H), 7.76 (d, *J* = 8.4 Hz, 2H), 8.46 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ -5.23, -5.19, -4.72, -4.65, -4.58, -4.15, 17.96, 18.24, 18.70, 21.70, 25.75, 25.98, 26.26, 62.37, 62.64, 71.54, 77.06, 85.18, 88.25, 121.22, 128.78, 129.75, 136.40, 141.93, 145.14, 148.95, 153.54, 155.98, 187.62.

2-(2-Tosylacetyl)-2'-deoxyadenosine (86). Treatment of **84** (101 mg, 0.15 mmol) with TBAF [330 µL, 0.33 mmol (1.0 M in THF)] by **Procedure C** (column chromatography; 5 → 15% MeOH/$CHCl_3$) gave **86** (56 mg, 84%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.27 – 2.32 (m, 1H), 2.35 (s, 3H), 2.66 – 2.73 (m, 1H), 3.49-3.55 (m 1H), 3.56-3.63 (m 1H), 3.88 (q, *J* = 4.4 Hz, 1H), 4.38-3.44 (m, 1H), 4.94 (t, *J* = 5.6 Hz, 1H),

5.30 (d, *J* = 2.4 Hz, 2H), 5.37 (d, *J* = 4.0 Hz, 1H), 6.33 (t, *J* = 6.8 Hz, 1H), 7.38 (d, *J* = 8.0 Hz, 2H), 7.68 (s, 2H), 7.73 (d, *J* = 8.4 Hz, 2H), 8.56 (s, 1H); ^{13}C NMR (101 MHz, MeOD-d_4) δ 21.50, 41.64, 54.81, 63.26, 72.62, 86.28, 89.53, 121.66, 129.58, 130.72, 137.78, 143.82, 146.60, 150.04, 154.60, 157.05, 189.15.

2-(2-Tosylacetyl)adenosine (87). Treatment of **85** (202 mg, 0.25 mmol) with TBAF [827 µL, 0.83 mmol (1.0 M in THF)] by **Procedure C** (column chromatography; 5 → 15% MeOH/$CHCl_3$) gave **87** (95 mg, 82%) as a white solid: ^{1}H NMR (400 MHz, MeOD-d_4) δ 2.31 (s, 3H), 3.79 (dd, *J* = 12.6, 3.4 Hz, 1H), 3.91 (dd, *J* = 12.4, 2.8 Hz, 1H), 4.16 (q, *J* = 3.2 Hz, 1H), 4.35 (t, *J* = 4.4 Hz, 1H), 4.63 (t, *J* = 5.6 Hz, 1H), 6.00 (d, *J* = 5.6 Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 2H), 7.72 (d, *J* = 8.0 Hz, 2H), 8.52 (s, 1H); ^{13}C NMR (101 MHz, MeOD-d_4) δ 21.49, 62.86, 71.93, 76.07, 87.38, 90.59, 121.79, 129.62, 130.73, 137.70, 143.96, 146.65, 150.28, 154.66, 157.11, 189.07.

2-(2-Benzyl-2-tosylacetyl)adenosine (88). Aqueous NaOH solution (1 M, 128 µL, 0.13 mmol) was added to a stirred solution of **87** (0.065 mmol) in MeOH (2 mL) at rt. After 30 min, BnBr (15.4 µL, 22.3 mg, 0.13 mmol) was added and the resulting mixture was stirred for 24 h. The reaction mixture was then neutralized with dil. HCl to pH ~7, and the volatiles were evaporated. The residue was column chromatographed (5 → 10% MeOH/DCM) to give **88** (23 mg, 65%) as white solid: ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.47 (d, *J* = 5.2 Hz, 1H), 7.66 (dd, *J* = 12.4, 8.0 Hz, 2H), 7.29 – 7.06 (m, 7H), 6.45 – 6.52 (m, 1H), 5.98 (t, *J* = 5.2 Hz, 1H), 4.61 (t, *J* = 5.2 Hz, 1H), 4.34 (ddd, *J* = 6.8, 5.2, 3.6 Hz, 1H), 4.18 (dq, *J* = 11.6, 3.2 Hz, 1H), 3.92 (ddd, *J* = 12.4, 5.6, 2.8 Hz, 1H), 3.78 (ddd, *J* = 12.4, 7.2, 3.2 Hz, 1H), 3.49 (dt, *J* = 8.6, 3.2 Hz, 2H), 2.24 (d, *J* = 11.9 Hz, 3H); ^{13}C NMR (101 MHz, MeOD-d_4) δ 192.47, 192.26, 156.89, 155.13, 154.99, 150.12, 150.06, 146.92,

144.05, 144.02, 137.65, 137.62, 137.60, 137.57, 136.16, 136.09, 130.69, 130.48, 130.36, 130.05, 130.03, 129.63, 127.93, 127.90, 121.66, 90.71, 90.65, 87.50, 87.46, 75.98, 75.90, 72.11, 72.02, 70.39, 63.07, 62.97, 32.72, 32.44, 21.46, 21.44.

8-(2-Tosylacetyl)-2'-deoxyadenosine-5'-*O*-triphosphate (**89**). $(MeO)_3PO$ (1.0 mL; dried over 3A molecular sieves) was added to the flame-dried flask containing 8-(*β*-keto)sulfone **53** [30 mg, 0.067 mmol; dried in vacuum (40 °C, over P_2O_5)] and proton sponge (21.5 mg, 0.10 mmol), and the resulting solution was stirred at 0 °C for 5 min under an Ar atmosphere. Freshly distilled $POCl_3$ (8.1 μL, 13.4 mg, 0.087 mmol) was then added, and stirring was continued for 1.0 h at 0 °C. Then, mixture of tributylammonium pyrophosphate (TBAPP; 0.5 M/dimethylformamide (DMF); 670 μL, 0.34 mmol) and Bu_3N (49.7 mg, 0.27 mmol) was added and stirred for another 30 min at 0 °C. The reaction mixture was quenched by adjusting pH to 7.5−7.8 with triethylammonium bicarbonate (TEAB) buffer (2 M, several drops). The residue was dissolved in water (5 mL) and was extracted with EtOAc (3 × 5 mL). The water layer was evaporated and co-evaporated (three times) with a mixture of EtOH/H_2O (1:1, 5 mL). The residue was chromatographed on a DEAE-Sephadex A-25 column with TEAB (0.1 → 0.6 M), and the appropriate fractions (TLC, R_f 0.28; *i*-PrOH/H_2O/NH_4OH, 5:2:3) were evaporated in vacuum and co-evaporated three times with a mixture of EtOH/H_2O (1:1, 10 mL) to remove excess of TEAB salt to give **89** (19.4 mg, 45%) as a triethylammonium salt: ^{1}H NMR (D_2O) δ 2.11 (s, 3H), 2.39 (ddd, J = 13.6, 7.2, 5.2 Hz, 1H), 2.93–3.01 (m, 1H), 4.12–4.24 (m, 2H), 4.27–4.34 (m, 1H), 4.81–4.91 (m, 1H), 6.94 (t, J = 7.2 Hz, 1H), 7.19 (d, J = 8.0 Hz, 2H), 7.55 (d, J = 8.0 Hz, 2H), 8.23 (s, 1H); ^{13}C NMR (D_2O) δ 8.18, 20.48, 36.66, 42.17, 46.60, 58.77, 65.75, 71.29, 84.53, 119.21, 127.85, 128.02, 129.81, 129.96, 133.14, 142.34, 146.98, 150.03, 155.69,

156.56, 181.36; ^{31}P NMR (162 MHz, D_2O) δ -23.26 (t, *J* = 19.8, Hz, 1P), -11.31 (d, *J* = 19.9, Hz, 1P), -10.72 (d, *J* = 19.8, Hz, 1P). HRMS (TOF, ESI) *m/z* calcd for $C_{19}H_24N_5O_{15}P_3S$ 686.0123 $[M + H]^+$, found 686.0136.

2'-Deoxyadenosine-2-carboxylic acid 5'-*O*-Triphosphate (**90**) Treatment of **86** (30 mg, 0.067 mmol) with $(MeO)_3PO$ (1.0 mL), proton sponge (21.5 mg, 0.10 mmol), and freshly distilled $POCl_3$ (8.1 μL, 13.4 mg, 0.087 mmol) followed by mixture of tributylammonium pyrophosphate (TBAPP; 0.5 M/ DMF; 670 μL, 0.34 mmol) and Bu_3N (49.7 mg, 0.27 mmol) as described for **89** gave **90** (11.0 mg, 25%) as a triethylammonium salt: ^{1}H NMR (400 MHz, D_2O) *δ* 8.56 (s, 1H), 6.61 (s, 1H), 4.89 (s, 1H), 4.32 – 4.13 (m, 3H), 2.85 – 2.74 (m, 1H), 2.69 – 2.58 (m, 1H); ^{31}P NMR (162 MHz, D_2O) *δ* -10.89 (d, *J* = 19.8 Hz), -11.44 (d, *J* = 20.1 Hz), -23.24 (t, *J* = 19.8 Hz).

Adenosine-2-carboxylic acid 5'-*O*-Triphosphate (**91**). Treatment of **87** (31 mg, 0.067 mmol) with $(MeO)_3PO$ (1.0 mL), proton sponge (21.5 mg, 0.10 mmol), and freshly distilled $POCl_3$ (8.1 μL, 13.4 mg, 0.087 mmol) followed by mixture of tributylammonium pyrophosphate (TBAPP; 0.5 M/dimethylformamide (DMF); 670 μL, 0.34 mmol) and Bu_3N (49.7 mg, 0.27 mmol) as described for **89** gave **91** (9.6 mg, 22%) as a triethylammonium salt: ^{1}H NMR (400 MHz, D_2O) *δ* 8.63 (s, 1H), 6.23 (s, 1H), 4.75 (s, 1H), 4.62 (d, *J* = 4.7 Hz, 1H), 4.40 (s, 1H), 4.29 (dd, *J* = 19.6, 13.3 Hz, 2H); ^{31}P NMR (162 MHz, D_2O) *δ* -5.57 (d, *J* = 19.4 Hz), -10.90 (d, *J* = 19.1 Hz), -21.34 (t, *J* = 19.2 Hz).

Methyl Adenosine-2-carboxylate (95). A mixture of **93** (100 mg, 0.34 mmol) and NaOMe (5.4 M; 95 μL, 0.51 mmol) in MeOH (10 mL) was stirred at rt for 15 h. After neutralization with dowex 50 (H+) and filtration of resin, the volatiles was at reduced pressure. The residue was dissolve in mixture of MeOH/H_2O (10 mL; 1:1) and 1.0 M HCl

(340 µL, 0.34 mmol) was added. The mixture was stirred at rt for 2 h. After neutralization 1 M NaOH, the volatiles were removed at reduced pressure. The residue was column chromatographed (10 → 20% MeOH/DCM) to give **95** (89 mg, 80%) as white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (s, 1H), 7.71 (s, 2H), 5.93 (d, J = 6.4 Hz, 1H), 5.47 (d, J = 6.0 Hz, 1H), 5.22 (d, J = 4.7 Hz, 1H), 5.05 (dd, J = 6.4, 5.2 Hz, 1H), 4.62 – 4.56 (m, 1H), 4.15 (td, J = 4.8, 2.8 Hz, 1H), 3.96 (q, J = 3.6 Hz, 1H), 3.84 (s, 3H), 3.71 – 3.64 (m, 1H), 3.56 (ddd, J = 12.0, 6.4, 4.0 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.47, 156.15, 150.61, 149.47, 141.85, 120.12, 87.41, 86.08, 73.84, 70.73, 61.67, 52.57.

4.2.1. Polymerase-catalyzed incorporation into DNA

Material for Enzymatic Reactions

All DNA oligonucleotides were synthesized by Integrated DNA Technologies (IDT) (Coralville, IA). T4 polynucleotide kinase was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Radionucleotides [^{32}P] ATP (3000 Ci/mmol) was purchased from Perkin Elmer Inc. (Boston, MA, USA). Micro Bio-Spin TM 6 Columns were purchased from Bio-Rad (Hercules, CA). All other chemicals used were purchased from Sigma-Aldrich (St. Louis, MO) and Thermo Scientific (Pittsburgh, PA). Recombinant human DNA polymerase β (pol β) was expressed and purified as described previously.[193]

Table 5. Oligonucleotide Substrates for incorporation of 89 into DNA

Oligonucleotide	**nt**	**Sequence (5'-3')**
Upstream	33	GCAGTCCTCTAGTCGTAGTAGCAGATCATCAAC
Downstream	37	ACCGGCATTAGGTGTAGTAGCTAGACTTACTCATTGC
Template strand	71	GCAATGAGTAAGTCTAGCTACTACACCTAATGCCGGT **T**GTTGATGATCTGCTACTACGACTAG AGGACTGC

The one-nucleotide gap substrate was constructed by annealing the 5'-^{32}P-labeled upstream strand and the downstream strand containing a 5'-phosphate group with the template strand at a molar ratio of 1:2:2. The sequences of oligonucleotides for constructing the substrate are listed in Table 5.

Incorporation of the 89 nucleotide analog by DNA polymerase β

The incorporation of **89** by human DNA polymerase was performed by incubating different concentrations of pol β with 5 nM ^{32}P-labeled substrates at 37 °C for 30 min as described previously.[194,195] The enzymatic reactions were assembled in the presence of **89** (50 μM). The use of one nucleotide gap allowed us to examine whether **89** can be incorporated into duplex DNA. The substrate was separated from pol β DNA synthesis product by a 15% urea-denaturing polyacrylamide gel electrophoresis and detected by a Pharos FX Plus PhosphorImager (Bio-Rad Laboratory, Hercules, CA).

Incorporation of the 90 nucleotide analog by DNA polymerase β

The incorporation of **90** by pol β was examined with the substrate containing a 1 nt-gap with a 5'-phosphate at the downstream primer (Table 6). The substrate was constructed by annealing the 5'-end ^{32}P-labeled upstream primer and downstream primer with a template at the ratio of 1:2:2. Pol β incorporation of **90** was measured by incubating 25 nM substrate with increasing concentrations of pol β (5-100 nM) in the presence of 50 μM of the nucleotide analog at 37 °C for 30 minutes. The reaction mixture was assembled in 10 μl-reaction buffer containing 50 mM Tris-HCl, pH 7.5, 50 mM KCl, 0.1 mM EDTA, 0.1 mg/ml bovine serum albumin, and 0.01% Nonidet P-40. The enzyme reaction was stopped using 2x stopping buffer containing 95% formamide and 10 mM EDTA, 0.05% (w/v) bromophenol blue and 0.05% (w/v) xylene cyanol, followed by incubation at 95°C for 5

minutes. Substrates and products were separated by 15% urea-denaturing polyacrylamide gel and detected by a phosphorimager. All experiments were repeated at least three times independently.

Table 6. Oligonucleotides sequence for incorporation of 90 into DNA

Oligonucleotide	**nt**	**Sequence (5'-3')**
Upstream	22	GTCCTAATAAGGACTTAGATTG
Downstream	23	GAAAGACCGCCCCCTCTGAGAAG
Template strand	46	CTTCTCAGAGGGGGCGGTCTTTCT**T**CAATCTAAGTCCT TATTAGGAC

4.3. Synthesis of organoarsenical antibiotic arsinothricin (AST)

(2-Hydroxyethyl)arsonic acid (97). 12 M aqueous NaOH (155 mL, 74.5 g, 1.86 mol) was slowly added with stirring into the cooled (ice-bath) suspension of As_2O_3 (62.5 g, 0.31 mol) in H_2O (100 mL) in a round bottom flask over 20 min. Then 2-chloroethanol (**96**; 42 mL, 50 g, 0.621 mol) was slowly added into the resulting homogeneous mixture so that temperature did not rise above 20 ^oC. The reaction mixture was stirred at 20 ^oC for 30 min and then at ambient temperature for 12 h. The white precipitate of NaCl was removed by vacuum filtration. Evaporation of volatiles from the filtrate at reduced pressure afforded the disodium salt of crude **97**[196] (115 g, 86%) as a white solid: ^1H NMR (D_2O) δ 2.84 (t, *J* = 6.2 Hz, 2H), 3.86 (t, *J* = 6.2 Hz, 2H); ^{13}C NMR (D_2O) δ 39.2, 54.7. HRMS calcd for $C_2H_6AsO_4$ $[M-H]^-$ 168.9487, found 168.9483.

(2-Chloroethyl)arsonic acid (100). Method 1. (Step *a*). Conc. HCl (500 mL) was slowly added into the stirring solution of crude **97** (70 g, 0.33 mol; dissolved in 130 mL H_2O) over 10 min. A catalytic amount of KI (500 mg, 3 mmol) was then added, and SO_2

gas (1.0 mol) was bubbled into the solution for 1 h with continuous stirring. The resulting mixture was extracted with dichloromethane (3 x100 mL) and dried over anhydrous Na_2SO_4. The volatiles were evaporated under reduced pressure to afford dichloro(2-hydroxyethyl)arsine **98** (59.3 g, 94%) as a yellowish oil: ^{1}H NMR ($CDCl_3$) δ 2.78 (t, *J* = 6.5 Hz, 2H), 4.2 (t, *J* = 6.1 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 48.0, 58.7. (Step *b*). Anhydrous $CHCl_3$ (60 mL) was added into a flask containing **98** (40 g, 0.21 mol), and the resulting solution was stirred for 20 min at 0 ^{o}C (ice-bath). Next $SOCl_2$ (38 mL, 62.4 g, 0.525 mol) was slowly added over 20 min with continuous stirring. The resulting mixture was allowed to warm to ambient temperature (1 h), and stirring was continued for 12 h. The resulting mixture was concentrated under reduced pressure at ambient temperature, and distilled at (110 – 120 ^{o}C) under reduced pressure (water vacuum, 16 mmHg) to yield dichloro(2-chloroethyl)arsine **99** (27.9 g, 64.7%) as a light pink liquid: ^{1}H NMR ($CDCl_3$) δ 2.90 (t, *J* = 7.2 Hz, 2H), 4.07 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 40.4, 47.3. (Step *c*) Compound **99** (22 g, 0.11 mol) was suspended in 100 mL of H_2O, and the suspension was placed in an ice-bath for 20 min. H_2O_2 (42 mL 30% aqueous solution) was slowly added into the suspension (0 ^{o}C, ice-bath) with continuous stirring for 20 min. The resulting solution was allowed to warm to ambient temperature (1 h) with continuous stirring. Volatiles were evaporated under reduced pressure at approximately 50 ^{o}C, and the residue was dissolved in 15 mL hot acetone (~45 ^{o}C). The product was crystallized by adding diethyl ether (10 mL) and cooled in ice-bath for 30 min. Vacuum filtration afforded **100**[196] (13.4 g, 64.8%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 2.77 (t, *J* = 7.5 Hz, 2H), 3.89 (t, *J* = 8.1 Hz, 2H), 6.40–6.45 (br, 2H); ^{13}C NMR (DMSO-d_6) δ 36.6, 37.7; HRMS calcd for $C_2H_5AsClO_3$ $[M-H]^-$ 186.9149, found 186.9149.

Method 2. 6 M aqueous NaOH (100 mL, 24 g, 0.6 mol) was added over 20 min into the cooled (ice-bath) suspension of As_2O_3 (20.1 g, 0.1 mol) in H_2O (50 mL) in a round bottom flask with continuous stirring. Then 1,2-dichloroethane (**104**; 16 mL, 20 g, 0.2 mol) was slowly added into the resulting homogeneous mixture. The reaction mixture was stirred at 60 ^oC for 48 h with continuous stirring. The mixture was then concentrated to100 mL at reduced pressure and the pH of the solution was adjusted to ~2.0 with 4 M HCl. The off-white precipitate was removed by vacuum filtration. Evaporation of volatiles from the filtrate at reduced pressure gave 30 g of a white solid, which was suspended in isopropyl alcohol. The white precipitate was removed by vacuum filtration. Evaporation of volatiles from the filtrate at reduced pressure afforded crude **100** (3.0 g, 12.8%) as a transparent gummy solid containing ~10% of the vinylarsonic acid: ^1H NMR (600 MHz, D_2O) δ 4.06 (t, *J* = 6.4 Hz, 2H), 2.88 (t, *J* = 6.4 Hz, 2H); 6.48 (d, *J* = 18.4 Hz, 0.1H), 6.56 (t, *J* = 11.6 Hz, 0.1H), 6.65 (dd, *J* = 18.4, 11.6 Hz, 0.1H); HRMS calcd for $C_2H_6AsO_4$ $[M-H]^-$ 186.9149, found 186.9151.

2-Amino-4-arsonobutanoic acid (AST-OH, 103). *Via esterification of **100***. (Step *a*): 1-Propanol (25 mL) was added into a round bottom flask containing **100** (5 g, 26.5 mmol; from Method 1), and the resulting mixture was refluxed in an oil bath at 115 ^oC for 48 h. Volatiles were evaporated under reduced pressure to give dipropyl (2-chloroethyl)arsonate **101** (5.3 g, 73%) as a gummy solid containing ~30% of dipropyl vinylarsonate, as judged by the NMR signals for the vinylic protons at 6.15-6.72 ppm: ^1H NMR (DMSO-d_6) δ 0.83 (t, *J* = 7.2 Hz, 6H), 1.41 (sext, *J* = 7.4 Hz, 4H), 2.76 (t, *J* = 7.5 Hz, 2H), 3.34 (t, *J* = 7.0 Hz, 4H), 3.89 (t, *J* = 7.5 Hz, 2H). (Step *b*) Sodium (170 mg, 7.4 mmol) was added into a dry flask containing 3 mL of anhydrous EtOH and the mixture

was stirred at ambient temperature until the sodium dissolved. Then diethylacetamidomalonate (1.2 g, 5.5 mmol) was added, and the resulting mixture was stirred for 5 min, followed by addition of a freshly prepared solution of **101** containing ~30% of dipropyl vinylarsonate (500 mg, 1.84 mmol dissolved in 2 mL EtOH). The resulting mixture was stirred at 70 ^{o}C in an oil bath for 4 h. Volatiles were evaporated under reduced pressure, yielding crude diethyl 2-acetamido-2-(2-dipropoxyarsoryl)ethyl)malonate **102** as a brownish solid (~2 g), which was directly used in next step. (Step *c*) 6 M HCl (6 mL) was added into the crude **102,** and the resulting mixture was refluxed at 120 ^{o}C in an oil bath for 3 h. Volatiles were evaporated, and the residue was dissolved in 20 mL H_2O. The solution was applied to a Dowex$^{(R)}$ 50WX8 (H^+ form) column (30 x 1 cm, 10 g), which was washed with 50 mL of H_2O. The product was eluted with a solution of NH_4OH (0.5 M, 100 mL). Fractions from the ammonium elution (~100 mL) were evaporated under reduced pressure, and the residue was dissolved in 40 mL H_2O. The diluted solution was passed through a (Dowex$^{(R)}$ 50WX8 H^+ form) column (30 x 1 cm, 12 g) equilibrated with a weakly acidic triethylammonium acetate (TEAA) buffer solution (acetic acid 30 mM and triethylamine 15 mM). Compound **103** (TLC, R_f 0.35, *i*-PrOH/H_2O/NH_4OH, 5:2:3; identified by staining with 1% ninhydrin solution) eluted with the same buffer followed by glycine byproduct (TLC, R_f 0.55). The appropriate fractions were evaporated and co-evaporated (3x) with a mixture of EtOH/H_2O (1:1, 12 mL) to afford **103**[196] (100 mg, 24% from **101**) as a white solid: ^{1}H NMR (D_2O) δ 2.13 - 2.28 (m, 4H), 3.81 (t, J = 5.2 Hz, 1H); ^{13}C NMR (D_2O) δ 23.85, 28.83, 54.63, 173.41; HRMS calcd for $C_4H_9AsNO_5$ $[M-H]^-$ 225.9702, found 225.9703.

*Via Direct Condensation of **100***. Treatment of **100** (500 mg, 5.65 mmol; from Method 1) with sodium (244 mg, 10.6 mmol) and diethylacetamidomalonate (1.74 g, 8.0 mmol) in 10 mL anhydrous EtOH as described above in steps b and c gave **103** (337 mg, 56% from **100**): HRMS $[M-H]^-$ found 225.9704.

Subjection of **100** (800 mg, 4.25 mmol; from Method 2) to the same protocol as described above (steps b and c) also gave **103** (95 mg, 10% from **100**): HRMS $[M-H]^-$ found 225.9704.

2-Hydroxyethyl(methyl)arsinic acid (106). (*a*) *Preparation of diiodo(methyl)arsine (MeAsI$_2$).* A solution of KI (41.5 g, 0.252 mol) in H_2O (40 mL) was added into the solution of monosodium salt of the commercially available methylarsonate (MeAs(O)(OH)ONa; 0.126 mol, 3.15 M/H_2O, 40 mL). Conc. HCl (30 mL) was slowly added into the mixtures with continuous stirring. Then SO_2 gas was passed into the mixtures for 30 min. The resulting mixture was extracted with CH_2Cl_2 (3 x 70 mL) and dried over anhydrous Na_2SO_4. The volatiles were evaporated to afford $MeAsI_2$ (46 g, 97%) as orange liquid.[163] (*b*) *Condensation with 2-chloroethanol*. 12 M aqueous NaOH (40 mL, 19.2 g, 0.48 mol) was slowly added into $MeAsI_2$ (46 g, 0.122 mol) placed in a round bottom flask (0 ^oC, ice bath) over a 20 min with vigorous stirring. During the addition of NaOH, the yellow color was disappeared resulting in colorless solution. The resulting $MeAs(ONa)_2$ solution was stirred for 15 min and then 2-chloroethanol (**96**; 8.2 mL, 9.8 g, 0.122 mol) was slowly added over 10 min. The mixture was allowed to warm to ambient temperature (approximately 1 h) and stirring was continued for 12 h. The mixture was acidified with 6 M HCl to pH~4 and white precipitate was filtered out. The filtrate was evaporated at reduced pressure yielding a white solid, which was suspended in MeOH. The white

precipitate was removed by vacuum filtration and the mother liquor was evaporated at reduced pressure to give **106** (19.9 g, 86%) as a white solid: ^{1}H NMR δ 1.92 (s, 3H), 2.55 (t, J = 6.4 Hz, 2H), 3.97 (t, J = 6.4 Hz, 2H); ^{13}C NMR δ 18.06, 37.30, 55.63; HRMS *m/z* calcd for $C_3H_8AsO_3$ $[M-H]^-$ 166.9694, found 166.9693.

Note: Removal of volatiles from the reaction mixture without neutralization with HCl and purification on silica column with 10-30 % MeOH/CH_2Cl_2 afforded iodide-free sodium salt of **106**.

2-Hydroxyethyl(chloro)(methyl)arsine (107). Conc. HCl (37%, 70 mL) was slowly added into the stirring solution of **106** (15 g, 79 mmol) in H_2O (40 mL) over a 10 min at rt. Next, catalytic amount of KI (200 mg, 1.2 mmol) was added and SO_2 gas was passed into this solution for 30 min. with continuous stirring. The mixtures were extracted with CH_2Cl_2 (3 x 70 mL) and dried over anhydrous Na_2SO_4. The volatiles were evaporated under reduced pressure to give **107** (12.3 g, 92%) as yellowish oil: ^{1}H NMR ($CDCl_3$) δ 1.72 (brs, 1H), 2.04 (s, 3H), 2.50 (t, J = 6.8 Hz, 2H), 4.01 (t, J = 6.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 22.0, 35.8, 61.4. Molecular ion for **107** was not detected in HRMS experiment instead molecular ion for **106** was observed (HRMS: *m/z* calcd for $C_3H_{10}AsO_3$ $[M+H]^+$ 168.9840, found 168.9840).

Note: Treatment (0 °C to rt, 3 h) of the stirred solution of **107** (12 g, 70.4 mmol) in anhydrous CH_2Cl_2 (60 mL) with $SOCl_2$ (12.3 mL, 20.1 g, 0.17 mol) followed by removal of volatiles under reduced pressure provided dichloro-(2-hydroxyethyl)arsine **98** (8.7 g, 65%) as brown liquid with the ^{1}H and ^{13}C NMR spectra as reported[165] (HRMS *m/z* calcd for $C_2H_4AsOCl_2$ $[M-H]^-$ 188.8860 found 188.8862) instead of desired chloro(2-chloroethyl)(methyl)arsine **108**.

2-Chloroethyl(methyl)arsinic acid (109). **Method A**. $SOCl_2$ (10 mL) was added to an iodide free sodium salt of **106** (500 mg, 2.63 mmol) in a dry flask at 0 ^oC (ice-bath) and was stirred at rt for 15 h. The reaction was quenched by adding 20 mL water and extracted with CH_2Cl_2. The aqueous phase was separated and volatiles were evaporated at reduced pressure. The residue was purified by column chromatography (20 → 30% MeOH/ CH_2Cl_2) to give mixture of **109** and **106** as a sticky solid (450 mg; 80:20). Second silica column purification afforded pure **109** (20 mg) as colorless gummy solid in addition to mixture of **109** and **105** as a gummy solid (400 mg; 85:15); ^1H NMR δ 2.08 (s, 2H), 2.94 (t, J = 6.6 Hz, 1H), 3.98 (t, J = 6.8 Hz, 1H); ^{13}C NMR δ 17.47, 36.86, 36.97; HRMS *m/z* calcd for $C_3H_9AsClO_2$ $[M+H]^+$ 186.9501, found 186.9500.

Method B. Trimethylsilyl chloride (755 µL, 647 mg, 5.96 mmol) was added to a stirring solution of sodium salt of **106** (500 mg, 2.98 mmol) in DMSO (1 mL) at rt. The resulting mixture was stirred at rt for 14 h. The reaction was quenched by adding 10 mL water and extracted with EtOAc (5 x 10 mL) to remove DMSO. The aqueous phase was separated, and volatiles were evaporated at reduced pressure to give a gummy solid (~400 mg) containing **109** (8%, based on ^1H NMR and HPLC-ICP-MS) and unchanged **106** (92%). HRMS *m/z* calcd for $C_3H_9AsClO_2$ $[M+H]^+$ 186.9501, found 186.9500 for **109** and *m/z* calcd for $C_3H_{10}AsO_3$ $[M+H]^+$ 168.9840, found 168.9840 for **106**.

***N*-[1-Carboxy-3-(hydroxyl(methyl)arsonyl)propan-1-yl]-*N*,*N*,*N*-trimethylammonium hydroxide (112).** *(a) Reduction*. AST-OH[165] (**103**; 50 mg, 0.22 mmol) was dissolved in the mixture of concentrated HCl and water (1:1, 5 mL). Then catalytic amount of KI (2.2 mg, 0.013 mmol) was added and SO_2 gas was passed into this solution for 15 min at rt. The pH was then adjusted around ~11 with 6 M NaOH (aq)

solution under N_2. *(b) Methylation*. To the solution from *step a*, MeI (1.1 mL) was added and the mixture was stirred at 50 ^oC for 2 h. After 2 h, the pH of the reaction mixture showed around 6.5. The volatiles were evaporated under reduced pressure and the residue was suspended in methanol. The off-white precipitate was removed by vacuum filtration. Evaporation of volatiles from the filtrate at reduced pressure gave brown solid. The residue was dissolved in 2 mL H_2O and applied to a Dowex 50W X 8 (H^+ form) column (30 x 1 cm, 10 g) which was washed with 50 mL of H_2O. The product was eluted with a solution of NH_4OH (0.5 M, 50 mL). The appropriate fractions (TLC, R_f 0.70, i-PrOH/H_2O/NH_4OH, 5:2:3; identified by staining with 1% ninhydrin solution) from the ammonium elution (~20 mL) were evaporated under reduced pressure to afford **112** (41 mg, 70%) as an off-white solid: ^{1}H NMR δ 1.69 (s, 3H), 1.90-2.06 (m, 2H), 2.09-2.19 (m, 1H), 2.23-2.32 (m, 1H), 3.19 (s, 9H), 3.68 (dd, J = 11.6, 3.6 Hz, 1H); ^{13}C NMR δ 15.84, 19.61, 28.99, 51.95, 78.62, 170.65; HRMS *m/z* calcd for $C_8H_{19}AsNNaO_4$ $[M+H]^+$ 290.0344, found 290.0346.

Ethyl-2-Acetamido-2-ethoxycarbonyl-4-(hydroxymethylarsinoyl)butanoate (113). Sodium (1.46 mg, 63.6 mmol) was added into a dry flask containing 40 mL of anhydrous EtOH and the mixture was stirred at ambient temperature until the sodium dissolved. Then diethyl acetamidomalonate (10.4 g, 47.7 mmol) was added, and the resulting mixture was stirred for 1 h. During this time, the reaction was turned into milky white mixture. To the above mixture solid **100** (3.0 g, 15.9 mmol) was added and the resulting mixture was stirred at 70 °C in an oil bath for 4 h. Volatiles were evaporated under reduced pressure to give crude **113** as a brownish solid, which was suspended in H_2O and transferred to a separatory funnel. The mixture was extracted with CH_2Cl_2 (6 x 50 mL) to remove excess malonate and aqueous layer was collected. The volatiles were evaporated

under reduced pressure and residue was suspended in 30% MeOH in CH_2Cl_2. The off-white precipitate was removed by vacuum filtration. Evaporation of the volatiles at reduced pressure gave brown solid which was loaded on the silica column. The residual malonate was removed by 5-10% MeOH in CH_2Cl_2 until all the yellow color eluted. Then the arsenic compound was eluted by 30% MeOH. Only the colorless fractions were collected. Volatiles were evaporated under reduced pressure to afford 6.0 g white solid which on ^{1}H NMR spectra showed approximate 10% malonate impurity. Second silica column purification gave pure **113** (5.2 g, 89%) as white solid: ^{1}H NMR δ 1.25 (t, J = 7.2 Hz, 6H), 1.99-2.04 (m, 2H), 2.07 (s, 3H), 2.54-2.58 (m, 2H), 4.29 (q, J = 7.2 Hz, 4H); ^{13}C NMR δ 13.73, 22.14, 26.80, 27.88, 64.77, 67.41, 168.97, 174.21; HRMS *m/z* calcd for $C_{11}H_{21}AsNO_8$ $[M+H]^+$ 370.0478, found 370.0475.

2-Amino-4-(hydroxymethylarsinoyl)butanoic acid (AST, 105). Method 1. *From Enzymatic methylation of AST-OH (103).* Chemically synthesized AST-OH **103** was reduced to trivalent As(III)T-OH as described previously.[8] Briefly, 27 mM $Na_2S_2O_3$, 66 mM $Na_2S_2O_5$, and 82 mM H_2SO_4 were added to and mixed well with 2.5 mM **103** (Method 1), on a one-by-one basis, to reduce it to As(III)T-OH , immediately followed by adjustment of the pH to 6 with NaOH. HPLC-ICP-MS analysis suggests that roughly 80% of **103** was converted to As(III)T-OH (Figure 14, line A). 100 µM As(III)T-OH (25 mL, 0.5 mg) was methylated by incubation with 0.75 mM *S*-adenosylmethionine and 10 µM CmArsM in a buffer consisting of 50 mM MOPS, 0.15 M KCl and 3 mM tris(2-carboxyethyl)phosphine (TCEP), 1 mM cysteine, pH 7.0, at 40 °C overnight in aerobic conditions with shaking at 180 rpm. HPLC-ICP-MS analysis suggests that roughly 70% of **103** was converted to **105** (Figure 14, line B). Another five batches of reaction were further

carried out. In total, 3 mg of As(III)T-OH (100 μM, 150 mL) were used. The reaction solution was filtered using an Amicon Ultra Centrifugal Filter with K cutoff membrane (MilliporeSigma) to remove protein. The filtrate was concentrated to 10 mL by rotary evaporator at reduced pressure and applied to a Dowex 50WX8 (H^+ form) column (30 x 1 cm, 10 g) which was washed with 100 mL of H_2O to remove most of the inorganic salts. The product was eluted with a solution of NH_4OH (0.5 M, 100 mL). The appropriate fractions (TLC, R_f 0.70, i-PrOH/H_2O/NH_4OH, 5:2:3; identified by staining with 1% ninhydrin solution) from the ammonium elution (~100 mL) were evaporated under reduced pressure. The residue was dissolved in 5 mL H_2O andwas applied onto a size-exclusion chromatography with a glass Econo-Column (25-mm ID x 1000 mm) packed with Sephadex LH-20 (GE Healthcare) with a mobile phase 70% (v/v) EtOH at a flow rate of 1.0 mL/min. Arsenic species in each fraction was analyzed by HPLC-ICP-MS. Fractions containing AST with high purity (>90%) were combined and concentrated by a rotary evaporator. The concentrated AST solution was applied again onto a Sephadex LH-20 size-exclusion chromatography for further purification. Fractions containing AST with high purity (>95%) were combined and concentrated by a rotary evaporator to afford **105** (0.6 mg, 24% from **103**) as an off-white solid.^{1}H NMR (D_2O) δ 1.96 (s, 3H), 2.21-2.28 (m, 2H), 2.32-2.47 (m, 2H), 3.84 (t, J = 6.0 Hz, 1H); ^{13}C NMR (D_2O) δ 15.49, 22.90, 28.59, 54.38, 173.09; HRMS *m/z* 226.0054 $[M+H]^+$ (calcd for $C_5H_{13}AsNO_4$, 226.0055).

Method 2 (*from 113***)**. *(a) Reduction.* Compound **113 (**500 mg, 1.35 mmol) was dissolved in the mixture of concentrated hydrochloric acid and water (1:1, 14 mL). Then catalytic amount of KI (13.5 mg, 0.08 mmol) was added and SO_2 gas was passed into this solution for 15 min at ambient temperature. The pH was then adjusted to around 11 with 6

M NaOH (aq) solution under N_2 to give crude **114** which was directly used in next step. *(b) Methylation*. To the product from *step a*, MeI (7 mL) was added and the mixture was stirred at 50 ^oC for 4 h. The reaction progress was monitored by HPLC-ICP-MSA. After 4h, the pH of the reaction mixture showed around 6.7. The volatiles were evaporated under reduced pressure and the residue was suspended in methanol. The off-white precipitate was removed by vacuum filtration. Evaporation of volatiles from the filtrate at reduced pressure gave brown solid crude **110**. *(c) Deprotection and decarboxylation*. 6 M HCl (20 mL) was added into the crude **110**, and the resulting mixture was refluxed at 120 °C in an oil bath for 3 h. The mixture was neutralized with 6 M HCl around pH~7 and white precipitate was filtered out. The volatiles were evaporated under reduced pressure and the residue was suspended in methanol. The off-white precipitate was removed by vacuum filtration. Evaporation of volatiles from the filtrate at reduced pressure gave brown solid crude **105**. The residue was dissolved in 15 mL H_2O and applied to a Dowex 50WX8 (H^+ form) column (30 x 1 cm, 10 g) which was washed with 100 mL of H_2O. The product was eluted with a solution of NH_4OH (0.5 M, 100 mL). The appropriate fractions (TLC, R_f 0.70, i-PrOH/H_2O/NH_4OH, 5:2:3; identified by staining with 1% ninhydrin solution) from the ammonium elution (~40 mL) were evaporated under reduced pressure to afford **105** as an off-white solid with some impurities. The residue was dissolved in 5 mL H_2O and was applied onto a Sephadex LH-20 (GE Healthcare) column with a mobile phase 70% (v/v) EtOH at a flow rate of 1.0 mL/min. Arsenic species in each fraction was analyzed by HPLC-ICP-MS. Fractions containing AST with high purity (>95%) were combined and concentrated by a rotary evaporator to afford **105**[123,165] (200 mg, 65% from **113**) as an off-white solid. ^1H NMR δ 2.03 (s, 3H), 2.27-2.34 (m, 2H), 2.42-2.48 (m, 1H), 2.52-2.58 (m,

1H), 3.97 (t, J = 6.4 Hz, 1H); ^{13}C NMR δ 16.39, 23.34, 29.39, 54.39, 172.93; HRMS *m/z* calcd for $C_5H_{13}AsNO_4$ $[M+H]^+$ 226.0055, found 226.0055.

Note: Use of pure **113** free of diethyl acetamidomalonate impurities is critical since during deprotection and decarboxylation steps these impurities are converted to glycine that is difficult to separate from the AST product during purification on Dowex.

Method 3 (*from 109*). *(a) Condensation.* Sodium (198 mg, 8.60 mmol) was added into a dry flask containing anhydrous EtOH (5 mL) and the mixture was stirred at rt until the sodium dissolved. Then diethylacetamidomalonate (1.40 g, 6.45 mmol) was added, and the resulting mixture was stirred for 30 min, followed by addition of a mixture **109** and **106** (85:15, 400 mg from Method A) dissolved in 2 mL EtOH. The resulting mixture was stirred at 70 °C for 4 h. Volatiles were evaporated under reduced pressure, yielding crude **110**. (*b) Deprotection and decarboxylation*. Subjection of crude **110** to the same protocol as described for Method 1 (*step c*) also gave **105** (72 mg, 17%**)** as an off-white solid: ^{1}H NMR δ 1.97 (s, 3H), 2.21-2.27 (m, 2H), 2.33-2.41 (m, 1H), 2.43-2.51 (m, 1H), 3.83 (t, J = 6.2 Hz, 1H); ^{13}C NMR δ 16.17, 23.53, 29.26, 55.01, 173.73; HRMS *m/z* calcd for $C_5H_{13}AsNO_4$ $[M+H]^+$ 226.0055, found 226.0055.

2-Acetamido-4-(hydroxymethylarsinoyl)butanoic acid (Ac-AST, 115): For separation of L-AST from racemicAST, a larger amount of D/L-cAST **105** (0.9 mM, 35 mL, 7 mg) was incubated overnight with 1 mM AcCoA and 20 µM PpArsN1 in a buffer consisting of 20 mM Tris-HCl, pH 7.4 at 37 °C. The reaction solution was filtered using an Amicon Ultra centrifugal filter with a 3K cutoff membrane to remove protein. The filtrate was concentrated to 5 mL by rotary evaporation at reduced pressure and separated by Sephadex LH-20 size-exclusion chromatography gave L-AcAST **115** and D-AST with

little impurities. Arsenic species in each fraction was analyzed by HPLC-ICP-MS. Fractions containing putative L-AcAST with high purity (>90%) were combined and concentrated by a rotary evaporation. The concentrated L-AcAST solution was applied again to Sephadex LH-20 size-exclusion chromatography for further purification. Fractions containing purified L-AcAST (>95%) were combined and concentrated to give **115** (3.0 mg, 36%): ^{1}H NMR δ 1.92 (s, 3H), 19.8 – 2.07 (m, 1H), 2.04 (s, 3H), 2.24 – 2.15 (m, 1H), 2.37 – 2.26 (m, 2H), 4.24 (dd, J = 8.2, 4.6 Hz, 1H); ^{13}C NMR δ 15.22, 21.89, 24.07, 29.42, 55.01, 173.80, 177.32; HRMS *m/z* calcd for $C_7H_{15}AsNO_5$ $[M+H]^+$ 268.0161, found 268.0162.

Note: Fractions containing D-AST (>95%) were combined and concentrated to give D-AST (2.1 mg, 30%): HRMS *m/z* calcd for: $C_5H_{13}AsNO_4$ $[M+H]^+$ 226.0055, found 226.0059.

(S)-2-Amino-4-(hydroxymethylarsinoyl)butanoic acid (L-AST, 116): 2 M HCl (5 mL) was added into **115** (3.0 mg, 0.013 mmol) in round bottom flask, and the resulting mixture was refluxed at 120 °C (oil bath) for 3 h. Volatiles were evaporated at reduced pressure. The residue was dissolved in 2 mL H_2O and separated by Sephadex LH-20 size-exclusion chromatography. Fractions containing L-AST (>95%) were combined and concentrated at reduced pressure to give **116** (1.9 mg, 75%) as a white solid; ^{1}H NMR δ 1.73 (s, 3H), 2.08-2.29 (m, 4H), 3.83 (t, *J* = 5.6 Hz, 1H); ^{13}C NMR δ 15.66, 23.35, 28.80, 54.70, 173.52; HRMS *m/z* calcd for $C_5H_{13}AsNO_4$ $[M+H]^+$ 226.0055, found 226.0056.

4.3.1. Arsenic Speciation by HPLC-ICP-MS

Arsenic species, including trivalent and pentavalent forms of AST-OH and AST were analyzed by high pressure liquid chromatography (HPLC) (Series 2000; PerkinElmer,

Waltham, MA) coupled to inductively coupled plasma mass spectrometry (ICP-MS) (ELAN DRC-e; PerkinElmer), as described previously,[S123,160] with minor modifications. Briefly, arsenic species in samples were separated by HPLC on a BioBasic™ 18 LC column (250 mm × 4.6 mm, 5 μm, 300 Å) (Thermo Fisher Scientific, Waltham, MA) using a mobile phase consisting of 3 mM malonic acid and 5% methanol (v/v) (pH 5.6 adjusted with tetrabutylammonium hydroxide) with a flow rate of 1 mL min^{-1} at 25 °C. Arsenic was monitored by ICP-MS. Arsenic species were determined from the HPLC retention time of known standards.

4.3.2. Enzymatic methylation of AST-OH to form AST

AST-OH (**103**) was methylated by the enzyme CmArsM. CmArsM was expressed and purified as described previously.[9] Briefly, cells of *E. coli* BL21(DE3) pET28-arsM7B were grown at 37 °C in LB medium supplemented with 25 μg/mL kanamycin to an absorbance of 0.5 at 600 nm, at which time 0.3 mM isopropyl β-D-1-thiogalactopyranoside was added to induce expression of CmArsM. The cells were grown for another 4 h, harvested by centrifugation (5,000 x g) at 4 °C for 20 min, washed once with Buffer A (50 mM morpholinopropane-1-sulfonic acid (MOPS), pH 7.5, containing 20% (wt/vol) glycerol, 0.5 M NaCl, 20 mM imidazole, and 10 mM 2-mercaptoethanol), and suspended in 5 mL of Buffer A per g of wet cells. The cells were lysed by a single pass through a French pressure cell at 20,000 psi, and 2.5 μL per g wet cell of the protease inhibitor diisopropyl fluorophosphate was added immediately. Membranes and unbroken cells were removed by centrifugation at 150,000 x *g* for 1 h, and the supernatant solution was loaded at a flow rate of 0.5 ml/min onto a Ni(II)-NTA column preequilibrated with Buffer A. The column was then washed with 150 mL of Buffer A, followed by elution with 60 mL of Buffer A with a

concentration gradient of imidazole from 0 to 0.2 M. CmArsM was identified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Fractions containing CmArsM were concentrated by centrifugation using a 10-kDa-cutoff Amicon Ultrafilter (MilliporeSigma). Protein concentrations were estimated by the method of Bradford using BSA (MilliporeSigma) as a standard.

4.3.3. Assay of Antibiotic Activity

Single colonies of *E. coli* W3110 were inoculated in M9 medium (47.7 mM Na_2HPO_4, 22 mM KH_2PO_4, 8.6 mM NaCl, 18.7 mM NH_4Cl, 2 mM $MgSO_4$ and 0.1 mM $CaCl_2$) supplemented with 0.2% glucose (w/v) and cultured in the absence or presence of the indicated concentrations of chemically synthesized AST (cAST), semisynthetic AST (sAST)[165], biogenic AST (bAST)[123], and L-AST for 16 h at 37 °C. The A_{600nm} was determined to compare the antibiotic activity of each compound.

4.3.4. Assay of Glutamine Synthetase (GS) Inhibition

Inhibition of GS activity by bAST, sAST, cAST or L-AST was analyzed by a coupled assay using GS from *E. coli* (Millipore-Sigma), as described previously[124] with minor modifications. Briefly, the GS reaction was initiated by addition of L-glutamate (5, 10, 20 or 50 mM) to the reaction mixture (100 mM Tris-acetate (pH 8.6), 9 mM ATP, 1 mM phosphoenolpyruvate, 60 mM Mg_2Cl, 19 mM KCl, 45 mM NH_4Cl, 0.25 mM NADH, 13-20 units of L-lactic dehydrogenase, 8-14 units of pyruvate kinase and 1 unit of GS) and incubated at 37 °C in the absence or presence of 0.5 or 2.0 μM of bAST, sAST, cAST or L-AST and the decrease in $A_{340\ nm}$ was monitored to quantify oxidation of NADH to NAD^+ using an extinction coefficient 6230 $M^{-1}cm^{-1}$. Activities were corrected with the values from control assays without enzyme. The inhibition constant (K_i) for each AST was

determined based on the apparent K_m of GS using Sigma Plot (Systat Software, Inc., Sun Jose, CA).

4.3.5. Enzymatic *N*-acetylation of AST

PpArsN1, the AST-selective *N*-acetyltransferase from *Pseudomonas putida* KT2440, was purified as described previously.[124] 10 µM of **105** (cAST, sAST bAST, or L-AST) was incubated in a buffer consisting of 20 mM Tris-HCl (pH 7.4), 1 mM ethylenediaminetetraacetic acid, 0.2 mM acetyl coenzyme A (AcCoA) at 37 °C for 30 min, with or without 0.2 µM PpArsN1. The reaction solution was filtered using an Amicon Ultra centrifugal filter with a 3K cutoff membrane (MilliporeSigma), and arsenic species in the filtrate were analyzed by HPLC-ICP-MS.

4.4. Synthesis of polycyclic aromatic hydrocarbons

4-Phenylvinylacetylene (118). $Pd(PPh_3)_4$ (15.6 mg, 0.01 mmol) and Cu(I)I (7.6 mg, 0.04 mmol) were placed in the flame-dried flask under N_2 at 0 ^oC (ice-bath). Then Et_2NH (1 mL, 707 mg, 9.67 mmol) followed by phenylacetylene (220 µL, 204 mg, 2.0 mmol) and vinyl bromide (2.6 mL; 1.0 M in THF, 2.6 mmol) were added and the resulting mixture was allowed to warm up to ambient temperature and was stirred for 3h [progress of the reaction was monitored by TLC (hexane)]. The reaction mixture was partitioned between water (5 mL) and *iso*pentane/diethyl ether (5 mL; 1:1, v/v). The The organic layer was separated and the aqueous layer was extracted with *iso*pentane/diethyl ether twice. The combined organic layer was washed with 1 M HCl, dried ($NaSO_4$) and carefully evaporated (below 30 ^oC). The residue was column chromatographed (hexane) to give **118**[197] (212 mg, 83%) as colorless liquid: ^1H NMR: ($CDCl_3$, 400 MHz) δ 5.55 (dd, J = 11.2, 2.0 Hz, 1H); 5.74 (dd, J = 17.6, 2.0 Hz, 1H); 6.03 (dd, J = 17.6, 11.2 Hz, 1H); 7.32-7.30 (m, 3H); 7.47-

7.33 (m, 2H); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 88.22, 90.10, 117.32, 123.28, 127.04, 128.40, 128.46, 128.52, 131.63, 131.80.

***trans*-1-Phenylvinylacetylene (120).** *Step a.* $Pd(PPh_3)_2Cl_2$ (10.9 mg, 0.016 mmol) and Cu(I)I (5.9 mg, 0.031 mmol) were added to dry THF (5 mL) flame-dried round bottom flask equipped with a stir bar under N_2. Then β-bromostyrene (100 µL, 142 mg, 0.78 mmol) was added followed by TMS-acetylene (161.3 µL, 114 mg, 1.16 mmol) and Et_3N (216 µL, 157 mg, 1.56 mmol). The resulting mixture was stirred at ambient temperature for 5 h (progress of the reaction was monitored by TLC (hexane)]. The reaction mixture was then diluted with EtOAc and filtered through a short pad of silica. Volatiles were evaporated and the residue was column chromatographed (n-hexane) to give TMS-protected of **120** as colorless liquid (150 mg, 96%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.23 (s, 9H); 6.18 (d, J = 16.4 Hz, 1H); 7.01 (d, J = 16.4 Hz, 1H), 7.28-7.39 (m, 5H). *Step b.* K_2CO_3 (100 mg, 0.72 mmol) was added to a stirred solution of the product from *step a* (145 mg, 0.72 mmol) in MeOH (5.0 mL) at room temperature. After for 30 min, volatiles were evaporated in vacuo and the residue was column chromatographed (*n*-hexane) to give **120**[198] (91 mg, 99%) as colorless liquid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.06 (d, J = 2.4 Hz, 1H); 6.14 (dd, 16.4, J = 2.4 Hz, 1H); 7.05 (d, J = 16.4 Hz, 1H); 7.30-7.32 (m, 3H); 7.34-7.40 (m, 2H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 79.33, 83.00, 107.13, 126.38, 126.59, 128.96, 136.00, 143.31.

1-Trimethylsilylindene (121). The indene (5.0 g, 43 mmol) and dry toluene (30 mL) were placed in the flame-dried flask under N_2 at 0 ^{o}C (ice-bath). Then butyllithium (1.6 M/hexane, 28 mL, 44.8 mmol) was added slowly into the stirring solution. The reaction mixture was allowed to warm to room temperature and stirring was continued for 30 h. During this time, the reaction mixture became pale yellow in color. Then

trimethylchlorosilane (5.7 mL, 4.88 g, and 44.9 mmol) was added slowly to cooled reaction mixture at -10 ^{o}C (ice/NaCl). The reaction mixture was refluxed under N_2 at 115 ^{o}C for 12 h and was poured into crushed ice and extracted with EtOAc. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated at reduced pressure. The residue was column chromatographed (*n*-hexane) to give light yellow liquid, which upon ^{1}H NMR analysis, showed to be mixture of product and indene substrate (70:30). Distillation of this mixture at reduced pressure gave pure **121**[199] (4.86 g, 60%) as pale yellow liquid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ -0.04 (s, 9 H), 3.35 (s, 1H), 6.65 (dd, J = 5.6, 2.0 Hz, 1H), 6.92 (d, J = 6.0 Hz, 1H), 7.16-7.26 (m, 2H), 7.46 (t, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ -2.41, 46.62, 121.16, 122.87, 123.67, 124.92, 128.96, 135.86, 144.23, 145.50.

1-Bromoindene (122). The 1-trimethylsilylindene (**121**; 4.80 g, 25.5 mmol) was dissolved in dry THF (100 mL) in foil-covered flame dry flask and cooled to -78 ^{o}C. Dioxane dibromide (6.95 g, 28.0 mmol) was dissolved in dry THF (25 mL) and was added dropwise via addition funnel. The reaction mixture was allowed to warm to ambient temperature and volatiles were evaporated at reduced pressure. The residue was column chromatographed (*n*-hexane) to give **122**[199] (3.38 g, 68%) as pale yellow liquid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.49 (s, 1H), 6.47 (dd, J = 5.6, 2.0 Hz, 1H), 6.82 (d, J = 5.6 Hz, 1H), 7.23-7.32 (m, 3H), 7.56 (d, J = 6.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 47.04, 121.97, 124.95, 126.53, 128.76, 132.88, 136.60, 141.71, 144.70.

6-Amino-1-indanone (126). Procedure G. Iron powder (6.14 g, 110 mmol) was added to the solution of NH_4Cl (5.89 g, 110 mmol) in H_2O/EtOH (80 mL, 1:1) in 250 mL flask equipped with a stir bar. The mixture was stirred at 60 ^{o}C for 30 min to activate the iron

powder. Then 6-nitro-1-indanone **124**[173] (3.0 g, 16.9 mmol) was added and the temperature of reaction mixture was raised to 80 ºC and stirring was continued for another 45 min. The mixture was cooled with ice-bath, basified with dilute aqueous NaOH to pH ~12 and was filtered to remove solid residue. The filtrate was concentrated under reduced pressure and extracted with EtOAc. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (20 → 40% EtOAc/hexane) to give **126**[175] (2.27 g, 91%) as a yellow solid: ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.52–2.56 (m, 2H), 2.86–2.93 (m, 2H), 5.28 (s, 2H), 6.75 (d, J = 2.4 Hz, 1H), 6.92 (dd, J = 7.8, 2.4 Hz, 1H), 7.21 (d, J = 7.8 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 24.51, 36.51, 105.47, 122.28, 127.01, 137.55, 143.02, 148.26, 206.75.

4-Amino-1-indanone (127). Treatment of **125**[173] (1.12 g, 6.3 mmol) with Iron powder/NH_4Cl by **Procedure A** (column chromatography; 20 → 40% EtOAc/hexane) gave **127**[200] (837 mg, 90%) as a yellow solid: ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.55–2.62 (m, 2H), 2.78–2.84 (m, 2H), 5.38 (s, 2H), 6.81 (d, J = 7.8 Hz, 1H), 6.82 (d, J = 7.2 Hz, 1H), 7.10 (t, J = 7.8 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 22.90, 35.85, 110.04, 117.77, 128.29, 137.37, 140.09, 146.37, 207.45; HRMS (TOF, APCI) *m/z* calcd for C_9H_8NO 146.0600 $[M - H]^-$, found 146.0601.

6-Iodo-1-indanone (128). Procedure H. Iodine (5.17 g, 20.4 mmol), CuI (4.66 g, 24.5 mmol), CH_2I_2 (4.93 mL, 61.2 mmol), and tert-butyl nitrite (7.3 mL, 61.2 mmol) were added to a solution of **126** (3.0 g, 20.4 mmol) in dry THF (40 mL). The reaction mixture was stirred at 66 ºC for 30 min, cooled to rt, and filtered. The filtrate was concentrated under reduced pressure and extracted with EtOAc. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by column

chromatography (10 → 20% EtOAc/hexane) to give **128** (4.9 g, 93%) as a white solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.69–2.72 (m, 2H), 3.07–3.11 (m, 2H), 7.25 (d, J = 8.4, 1H), 7.87 (dd, J = 7.8, 1.8 Hz, 1H), 8.09 (d, J = 1.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.73, 36.34, 92.67, 128.67, 133.11, 139.34, 143.14, 154.36, 205.27; HRMS (TOF, APCI) *m/z* calcd for C_9H_6IO 256.9469 $[M - H]^-$, found 256.9470.

Note: A byproduct which was tentatively assigned as 2,6-diiodo-1-indanone (312 mg, 4.0%) was also isolated from the reaction mixture: ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.41 (dd, J = 18.3, 2.6 Hz, 1H), 3.82 (dd, J = 18.3, 7.5 Hz, 1H), 4.94 (dd, J = 7.2, 2.4 Hz, 1H), 7.21 (d, J = 8.4 Hz, 1H), 7.94 (dd, J = 8.4, 1.5 Hz, 1H), 8.19 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.39, 39.56, 93.46, 128.38, 134.25, 135.00, 144.16, 150.45, 199.97.

4-Iodo-1-indanone (129). Treatment of **127** (736 mg, 5.0 mmol) with tert-butyl nitrite by **Procedure H** (column chromatography; 10 → 20% EtOAc/hexane) gave **129** (1.17 g, 90%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.71–2.76 (m, 2H), 2.95–3.02 (m, 2H), 7.14 (t, J = 7.6 Hz, 1H), 7.74 (d, J = 7.6 Hz, 1H), 8.00 (d, J = 7.6 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 31.04, 36.57, 96.80, 123.57, 129.36, 138.91, 143.75, 158.91, 206.64; HRMS (TOF, APCI) *m/z* calcd for C_9H_6IO 256.9469 $[M - H]^-$, found 256.9469.

6-Iodo-1-indanol (130). Procedure I. $NaBH_4$ (2.1 g, 55.5 mmol) was added portion wise to a stirred solution of **128** (3.62 g, 14.0 mmol) in dry MeOH/THF (60 mL, 2;1) at 0 ºC (ice-bath). After 5 min, the reaction mixture was allowed to warm to ambient temperature and stirring was continued for 30 min. Water (10 mL) was then added to quench the reaction. The mixture was concentrated under reduced pressure and extracted with EtOAc. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (20 → 40% EtOAc/hexane) to give

130 (3.57 g, 98%) as a white solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.74 (brs, 1H), 1.90–1.96 (m, 1H), 2.45–2.51 (m, 1H), 2.73–2.79 (m, 1H), 2.99 (ddd, J = 16.2, 8.4, 4.2 Hz, 1H), 5.21 (t, J = 6.2 Hz, 1H), 7.01 (d, J = 7.8 Hz, 1H), 7.56 (dd, J = 7.8, 1.8 Hz, 1H), 7.74 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 29.62, 36.18, 76.12, 91.65, 127.00, 133.61, 137.31, 143.06, 147.75; HRMS (TOF, APCI) *m/z* calcd for C_9H_8IO 258.9625 $[M - H]^-$, found 258.9626.

4-Iodo-1-indanol (131). Treatment of **128** (220 mg, 0.77 mmol) with $NaBH_4$ by **Procedure I** (column chromatography; 20 → 40% EtOAc/hexane) gave **131**[201] (198 g, 99%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.77 (brs, 1H), 1.89–2.00 (m, 1H), 2.46–2.55 (m, 1H), 2.73–2.83 (m, 1H), 3.00 (ddd, J = 16.4, 8.8, 4.4 Hz, 1H), 5.34 (dd, J = 6.8, 5.2 Hz, 1H), 6.97 (t, J = 7.6 Hz, 1H), 7.37 (d, J = 7.6 Hz, 1H), 7.66 (d, J = 8.0 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 34.80, 35.26, 77.82, 94.53, 124.11, 128.86, 137.71, 146.02, 147.85; HRMS (TOF, APCI) *m/z* calcd for C_9H_8IO 258.9625 $[M - H]^-$, found 258.9625.

5-Iodoindene (132). **Procedure J**. Alcohol **130** (3.0 g, 11.5 mmol) was dissolved in THF/H_2O (40 mL, 1:1). Aqueous 6 N HCl (10.0 mL, 60 mmol) was then added and the resulting mixture was refluxed at 105 ºC for 24 h. The reaction mixture was concentrated under reduced pressure to approximately 20 mL and was transferred to a separatory funnel and extracted with EtOAc. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated at reduced pressure. The residue was purified by column chromatography (n-hexane) to give **132**[170] (2.28 g, 82%) as a white solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.34–3.36 (m, 2H), 6.56 (dt, J = 5.4, 1.8 Hz, 1H), 6.80–6.82 (m, 1H), 7.22 (d, J = 7.8 Hz, 1H), 7.51 (dd, J = 7.8, 1.8 Hz, 1H), 7.75 (d, J = 1.2 Hz, 1H); ^{13}C NMR (75

MHz, $CDCl_3$) δ 39.00, 91.76, 125.59, 130.15, 131.25, 133.40, 135.60, 143.19, 147.42: HRMS (TOF, APCI) *m/z* calcd for C_9H_6I 240.9519 $[M - H]^-$, found 240.9518.

7-Iodoindene (133). Treatment of **131** (180 mg, 0.69 mmol) with 6 N HCl by **Procedure J** (column chromatography; n-hexane) gave **133** (134 g, 80%) as a white solid: ^1H NMR (600 MHz, $CDCl_3$) δ 3.32–3.33 (m, 2H), 6.61 (dt, J = 5.4, 1.8 Hz, 1H), 6.95–7.04 (m, 2H), 7.37 (dd, J = 7.8, 1.2 Hz, 1H), 7.55 (dd, J = 7.8, 1.2 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 44.58, 92.48, 120.94, 128.34, 132.52, 133.99, 134.49, 145.30, 148.52; HRMS (TOF, APCI) *m/z* calcd for C_9H_6I 240.9519 $[M - H]^-$, found 240.9517.

5-Iodo-1-indanone (136). Treatment of **134** (2.5 g, 19.0 mmol) with tert-butyl nitrite by **Procedure H** (column chromatography; 10 → 20% EtOAc/hexane)gave **136**[202] (4.42 g, 90%) as a white solid: ^1H NMR (600 MHz, DMSO-d_6) δ 2.56–2.62 (m, 2H), 3.06–3.11 (m, 2H), 7.39 (d, J = 7.8 Hz, 1H), 7.78 (d, J = 7.8 Hz, 1H), 8.05 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 25.30, 35.94, 103.93, 124.65, 136.28, 136.33, 136.52, 157.57, 206.26; HRMS (TOF, APCI) *m/z* calcd for C_9H_6IO 256.9469 $[M - H]^-$, found 256.9470.

7-Iodo-1-indanone (137). Treatment of **135** (200 mg, 1.36 mmol) with tert-butyl nitrite by **Procedure H** (column chromatography; 10 → 20% EtOAc/hexane) gave **137**[203] (179 mg, 51%) as a white solid: ^1H NMR (400 MHz, $CDCl_3$) δ 2.73–2.76 (m, 2H), 3.03–3.06 (m, 2H), 7.23 (t, J = 7.6 Hz, 1H), 7.46 (dd, J = 7.6, 0.8 Hz, 1H), 7.85 (dd, J = 7.6, 0.8 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 24.58, 37.31, 90.81, 126.73, 135.13, 136.48, 139.40, 158.06, 204.46.; HRMS (TOF, APCI) *m/z* calcd for C_9H_6IO 256.9469 $[M - H]^-$, found 256.9470.

Note: Also isolated during column chromatography were **129** (19 mg, 5.5%) and diiodo byproduct which was tentatively assigned as 4,7-diiodo-1-indanone (105 mg, 20%): ^1H

NMR (400 MHz, $CDCl_3$) δ 2.76–2.81 (m, 2H), 2.87–2.92 (m, 2H), 7.59 (d, J = 8.0 Hz, 1H), 7.63 (d, J = 8.0 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 29.99, 37.49, 90.76, 96.86, 137.96, 141.06, 144.07, 161.39, 204.12.

5-Iodo-1-indanol (138). Treatment of **136** (3.36 g, 13.0 mmol) with $NaBH_4$ by **Procedure I** (column chromatography; 20 → 40% EtOAc/hexane) gave **138** (3.31 g, 98%) as a white solid: ^1H NMR (600 MHz, DMSO-d_6) δ 1.71–1.78 (m, 1H),), 2.26–2.32 (m, 1H), 2.66–2.73 (m, 1H), 2.88 (ddd, J = 15.6, 8.4, 3.6 Hz, 1H), 4.98 (dd, J = 12.6, 6.0 Hz, 1H), 5.28 (d, J = 6.0 Hz, 1H), 7.13 (d, J = 7.8 Hz, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.59 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 29.00, 35.32, 73.93, 93.19, 126.45, 133.25, 134.85, 145.70, 146.31; HRMS (TOF, APCI) *m/z* calcd for C_9H_8IO 258.9625 $[M - H]^-$, found 258.9626.

7-Iodo-1-indanol (139). Treatment of **137** (118 mg, 0.46 mmol) with $NaBH_4$ by **Procedure I** (column chromatography; 20 → 40% EtOAc/hexane) gave **139** (115 mg, 97%) as a gummy solid: ^1H NMR (400 MHz, $CDCl_3$) δ 2.11–18 (m, 1H), 2.26–2.45 (m, 2H), 2.91 (ddd, J = 16.4, 8.8, 3.2 Hz, 1H), 3.26 (dt, J = 16.0, 8.0 Hz, 1H), 5.18 (dd, J = 6.8, 2.4 Hz, 1H), 6.97 (t, J = 7.6 Hz, 1H), 7.23 (dd, J = 7.2, 0.4 Hz, 1H), 7.59 (dq, J = 8.0, 0.8 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 31.40, 33.21, 79.35, 93.37, 125.17, 130.53, 136.39, 145.85, 147.85; HRMS (TOF, APCI) *m/z* calcd for C_9H_8IO 258.9625 $[M - H]^-$, found 258.9626.

6-Iodoindene (140). Treatment of **138** (3.0 g, 11.5 mmol) with 6 N HCl by **Procedure J** (column chromatography; n-hexane) gave **140**[170] (2.23 g, 80%) as a white solid: ^1H NMR (600 MHz, $CDCl_3$) δ 3.37 (s, 2H), 6.51 (dt, J = 5.4, 1.8 Hz, 1H), 6.83 (dd, J = 5.4, 2.4 Hz, 1H), 7.16 (d, J = 7.8 Hz, 1H), 7.60 (d, J = 7.8 Hz, 1H), 7.81 (s, 1H); ^{13}C NMR (75 MHz,

$CDCl_3$) δ 39.12, 89.94, 122.74, 131.75, 132.97, 134.68, 135.36, 144.53, 146.26: HRMS (TOF, APCI) *m/z* calcd for C_9H_6I 240.9519 $[M - H]^-$, found 240.9518.

4-Iodoindene (141). Treatment of **139** (100 mg, 0.38 mmol) with 6 N HCl by **Procedure J** (column chromatography; n-hexane) gave **141** (75.2 g, 82%) as a clear oil: ^1H NMR (400 MHz, $CDCl_3$) δ 3.52–3.54 (m, 2H), 6.63 (dt, J = 5.6, 2.0 Hz, 1H), 6.86 (dtd, J = 5.6, 2.0, 0.8 Hz, 1H),), 6.92 (t, J = 7.6 Hz, 1H), 7.41 (dp, J = 7.2, 0.8 Hz, 1H), 7.63 (dd, J = 8.0, 0.8 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 40.96, 88.22, 123.52, 126.47, 135.22, 135.31, 135.82, 144.45, 149.03 ; HRMS (TOF, APCI) *m/z* calcd for C_9H_6I 240.9519 $[M - H]^-$, found 240.9518.

(*E*)-5-(2-Bromovinyl)indene (143). Procedure K. A flame dry round bottom flask equipped with a magnetic stirrer was charged with 5-iodoindene **132** (484.1 mg, 2.0 mmol), trans-1,2-bis(tri-*n*-butylstannyl)ethylene (1.3 mL, 1470 mg, 2.4 mmol), dry toluene (10 mL) and $Pd(PPh_3)_4$ (46.2 mg, 0.04 mmol) and the resulting mixture was degassed with N_2 for 20 min. The reaction mixture was then heated (oil bath) at 100 ^oC for 1 h. Removal of volatiles under reduced pressure afforded crude (*E*)-5-(2-(tributylstannyl)vinyl)indene **142**, which was directly used in the next bromodestannylation step without further purification. NBS (534 mg, 3.0 mmol) was added portion wise to a stirred solution of all crude **142** in dry DCM (10 mL) at -10 ^oC and was stirred for 30 min. The volatiles were evaporated and the residue was purified by column chromatography (n-hexane) to give **143** (310 mg, 70%) as an off-white solid: ^1H NMR (600 MHz, $CDCl_3$) δ 3.37–3.40 (m, 2H), 6.58–6.60 (m, 1H), 6.80 (d, J = 15.0 Hz, 1H), 6.85–6.87 (m, 1H), 7.13 (dd, J = 7.8, 1.8 Hz, 1H), 7.35 (d, J = 1.2 Hz, 1H), 7.41 (d, J = 7.8 Hz, 1H), 7.48 (d, J = 15.0 Hz, 1H); ^{13}C NMR (101 MHz,

$CDCl_3$) δ 39.12, 75.52, 118.42, 123.00, 123.94, 131.90, 135.32, 136.27, 144.15, 145.54, 145.60; HRMS (TOF, APCI) *m/z* calcd for $C_{11}H_8{}^{79}Br$ 218.9815 $[M - H]^-$, found 218.9814.

(*E*)-6-(2-Bromovinyl)indene (144). Treatment of 6-iodoindene **140** (100 mg, 0.41 mmol) with trans-1,2-bis(tri-n-butylstannyl)ethylene and NBS by **Procedure K** (column chromatography; n-hexane) gave **144** (64 mg, 70%) as an off-white solid: ^1H NMR (600 MHz, $CDCl_3$) δ 3.40–3.41 (m, 2H), 6.59–6.63 (m, 1H), 6.77 (d, J = 14.8 Hz, 1H),), 6.85–6.88 (m, 1H), 7.19–7.22 (m, 1H), 7.34 (d, J = 7.6 Hz, 1H), 7.42–7.43 (m, 1H), 7.47 (d, J = 14.8 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 39.19, 74.86. 121.13, 121.31, 124.88, 131.97, 134.51, 135.52, 144.31, 145.39, 145.60; HRMS (TOF, APCI) *m/z* calcd for $C_{11}H_8{}^{79}Br$ 218.9815 $[M - H]^-$, found 218.9814.

(*E*)-5-(But-1-en-3-yn-1-yl)indene (147) and (*E*)-6-(But-1-en-3-yn-1-yl)indene (148). Procedure L. (Step a) $Pd(PPh_3)_2Cl_2$ (31.7 mg, 0.04 mmol) and Cu(I)I (17.2 mg, 0.08 mmol) were added to dry Et_3N (5 mL) in a flame-dried flask equipped with a stirring bar under N_2 at rt. Then **143** (250 mg, 1.13 mmol) was added followed by TMS-acetylene (322 μL, 222.0 mg, 2.26 mmol). The resulting mixture was stirred for 1h at rt [progress of the reaction was monitored by TLC (n-hexane)]. Volatiles were evaporated and the residue was purified by column chromatography (0 → 5% EtOAc/hexane) to give an inseparable mixture of (*E*)-5-[4-(trimethylsilyl)but-1-en-3-yn-1-yl]indene **145** and (*E*)-6-[4-(trimethylsilyl)but-1-en-3-yn-1-yl]indene **146** as light yellow liquid (245 mg, 91%; 1:1.5): ^1H NMR (400 MHz, $CDCl_3$) δ 0.23 (s, 9H), 3.39–3.43 (m, 2H), 6.16 (d, J = 16.4 Hz, 0.6H), 6.19 (d, J = 16.4 Hz, 0.4H), 6.59 (tt, J = 5.6, 2.0 Hz, 1H), 6.85–6.88 (m, 1H), 7.06 (d, J = 16.0 Hz, 0.6H), 7.07 (d, J = 16.4 Hz, 0.4H), 7.19–7.22 (m, 0.4H), 7.27–7.30 (m, 0.6H), 7.34 (d, J = 7.8 Hz, 0.6H), 7.41 (d, J = 7.4 Hz, 0.4H), 7.41 (d, J = 0.8 Hz, 0.4H), 7.50 (d, J

= 0.8 Hz, 0.6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 0.15, 0.16, 39.14, 39.19, 94.46, 104.89, 105.07, 106.57, 107.15, 118.61, 121.20, 121.49, 123.47, 124.02, 125.39, 131.94, 132.02, 132.94, 134.66, 135.24, 135.67, 143.25, 143.30, 144.34, 144.73, 145.59, 145.99. (Step b) Anhydrous K_2CO_3 (200 mg, 1.45 mmol) was added to a stirred solution of mixture of **145** and **146** (230 mg, 0.96 mmol) in dry MeOH/DCM (10 mL, 1:1) at room temperature. After 30 min, volatiles were evaporated and the residue was column chromatographed (0 → 5% EtOAc/hexane) to give inseparable mixture of **147** and **148** as light yellow liquid (147.5 mg, 92%; 1:1.5): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.05 (t, J = 2.4 Hz, 1H), 3.40–3.43 (m, 2H), 6.12 (dd, J = 16.4, 2.4 Hz, 0.6H), 6.15 (dd, J = 16.4, 2.4 Hz, 0.4H), 6.60 (tt, J = 5.6, 2.0 Hz, 1H), 6.86–6.89 (m, 1H), 7.10 (d, J = 16.4 Hz, 0.6H), 7.11 (d, J = 16.4 Hz, 0.4H), 7.22 (dd, J = 7.6, 1.6 Hz, 0.4H), 7.30 (dd, J = 8.0, 1.6 Hz, 0.6H), 7.36 (d, J = 7.6 Hz, 0.7H), 7.43 (d, J = 8.4 Hz, 0.8H), 7.52 (d, J = 0.8 Hz, 0.5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 39.14, 39.18, 78.88, 83.36, 83.53, 105.53, 106.11, 118.65, 121.21, 121.53, 123.49, 124.03, 125.41, 131.90, 132.01, 132.66, 134.39, 135.29, 135.75, 143.97, 144.00, 144.36, 144.86, 146.10, 145.62; HRMS (TOF, APCI) *m/z* calcd for $C_{13}H_{11}$ 167.0855 $[M + H]^+$, found 167.0855. Subjection of **144** (32 mg, 0.14 mmol) to **Procedure L** also gave mixture of **147** and **148** (19.2 mg, 80%; 1:1.5) with identical spectroscopic data.

5-Ethynylindene (151) and 6-Ethynylindene (152). Treatment of 5-iodoindene **132** (400 mg, 1.65 mmol) with TMS-acetylene (470 μL, 324 mg, 3.3 mmol) and $Pd(PPh_3)_4$ by **Procedure L** (step a) gave a mixture of 5-[2-(trimethylsilyl)ethynyl]indene **149** and 6-[2-(trimethylsilyl)ethynyl]indene **150** as a light yellow liquid (315 mg, 90%; 1:1.5): ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.26 (s, 9H), 3.39 (t, J = 2.2 Hz, 1.2H), 3.40 (t, J = 2.2 Hz, 0.8H), 6.58 (dt, J = 5.4, 1.8 Hz, 0.4H), 6.62 (dt, J = 5.4, 1.8 Hz, 0.6H), 6.83–6.84 (m, 0.4H), 6.86–

6.88 9 (m, 0.6H), 7.32 (d, J = 7.8 Hz, 1H), 7.40 (t, J = 7.8 Hz, 1H), 7.52 (s, 0.4H), 7.58 (s, 0.6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 0.21, 0.23, 39.06, 39.29, 93.02, 93.40, 106.10, 106.38, 119.17, 102.84, 121.05, 123.62, 124.58, 127.36, 128.70, 130.61, 131.74, 132.05, 135.14, 135.90, 143.60, 144.33, 144.96, 145.41. Treatment of the mixture of **149** and **150** (300 mg, 1.41 mmol) with anhydrous K_2CO_3 (293 mg, 2.12 mmol) by **Procedure L** (step b) gave a mixture of **151** and **152** as light yellow liquid (178 mg, 90%; 1:1.7): ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.04 (s, 0.39H), 3.07 (s, 0.61H), 3.40 (t, J = 2.4 Hz, 1.22H), 3.42 (t, J = 2.2 Hz, 0.80H), 6.60 (dt, J = 5.4, 2.4 Hz, 0.37H), 6.64 (dt, J = 5.4, 2.4 Hz, 0.63H), 6.84–6.86 (m, 0.37H), 6.87–6.89 (m, 0.63H), 7.35 (d, J = 7.8 Hz, 1H), 7.42 (t, J = 7.8 Hz, 1H), 7.54 (s, 0.37H), 7.60 (s, 0.63H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 39.10, 39.30, 76.24, 76.54, 84.54, 84.81, 118.10, 120.00, 120.93, 123.74, 124.71, 127.49, 128.82, 130.76, 131.67, 131.99, 135.33, 136.06, 143.70, 144.63, 145.07, 145.69; HRMS(TOF, APCI) *m/z* calcd for $C_{11}H_9$ 141.0699 $[M + H]^+$, found 141.0699.

Subjection of **140** (200 mg, 0.83 mmol) by **Procedure L** gave also mixture of **151** and **152** (89 mg, 92%; 1:1.7) with identical spectroscopic data.

5-(But-3-en-1-yn-1-yl)indene (153) and 6-(But-3-en-1-yn-1-yl)indene (154). $Pd(PPh_3)_4$ (37.1 mg, 0.032 mmol) and Cu(I)I (12.2 mg, 0.064 mmol) were placed in the flame-dried flask under N_2 at 0 ^{o}C (ice-bath). Then dry Et_3N (5 mL) and vinyl bromide (1.0 M in THF; 1.4 mL, 1.4 mmol) were added following by slow addition of mixture of **151** and **152** (150 mg, 1.07 mmol) dissolved in dry Et_3N (2 mL) via a syringe pump (over 3 h). The resulting mixture was allowed to warm up to ambient temperature (30 min) and was stirred for another 2 h. Volatiles were evaporated and the residue was purified by column chromatography (hexane) to give mixture of **153** and **154** as light yellow liquid (125 mg,

70%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.40–3.42 (m, 2H), 5.53 (dd, J = 11.2, 2.0 Hz, 0.6H), 5.54 (dd, J = 11.2, 2.0 Hz, 0.4H), 5.72 (dd, J = 17.2, 2.0 Hz, 0.6H), 5.74 (dd, J = 17.6, 2.0 Hz, 0.4H), 6.04 (dd, J = 17.6, 11.2 Hz, 0.4H), 6.09 (dd, J = 17.6, 11.2 Hz, 0.6H), 6.60 (dt, J = 5.6, 2.0 Hz, 0.4H), 6.63 (dt, J = 5.6, 2.0 Hz, 0.6H), 6.84–6.89 (m, 1H), 7.29–7.43 (m, 2H), 7.49 (d, J = 0.8 Hz, 0.4H), 7.54–7.57 (m, 0.6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 38.10, 38.28, 86.31, 86.73, 89.90, 90.20, 116.52, 116.56, 118.18, 119.95, 120.04, 122.75, 123.15, 125.40, 125.59, 125.95, 127.30, 129.22, 130.74, 131.04, 134.21, 134.82, 142.75, 143.15, 144.28, 144.08; HRMS (TOF, APCI) m/z calcd for $C_{13}H_{11}$ 167.0855 $[M + H]^+$, found 167.0856.

6-Ethynylindan-1-ol (156). Treatment of **130** (40 mg, 0.194 mmol) with TMS-acetylene (55 μL, 38 mg, 0.39 mmol) and $Pd(PPh_3)_4$ by **Procedure L** (step a; column chromatography (10 → 20% EtOAc/hexane)) gave **155** (40 mg, 90%) as light yellow gummy solid: ^{1}H NMR (400 MHz $CDCl_3$) *δ* 0.24 (s, 9H), 1.89 (d, *J* = 6.8 Hz, 1H), 1.90–1.98 (m, 1H), 2.42–2.52 (m, 1H), 2.74–2.85 (m, 1H), 3.03 (ddd, *J* = 16.4, 8.4, 4.8 Hz, 1H), 5.19 (q, *J* = 6.4 Hz, 1H), 7.17 (d, *J* = 8.0 Hz, 1H), 7.36 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.50–7.52 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 0.14, 29.98, 36.01, 76.17, 93.54, 105.49, 121.59, 124.90, 127.98, 132.21, 144.12, 145.27. Treatment of **155** (40 mg, 0.174 mmol) with anhydrous K_2CO_3 (96 mg, 0.7 mmol) by **Procedure L** (step b; column chromatography (20 → 30% EtOAc/hexane))) gave **156** as pale yellow solid (26 mg, 95%): ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 1.90–1.99 (m, 2H), 2.45–2.53 (m, 1H), 2.76–2.86 (m, 1H), 3.00–3.08 (m, 2H), 5.20 (t, *J* = 6.4 Hz, 1H), 7.20 (d, *J* = 8.0 Hz, 1H), 7.39 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.51–7.55 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 29.98, 36.04, 76.14, 76.66, 83.98, 120.54,

125.03, 128.14, 132.41, 144.46, 145.37; HRMS (TOF, ESI) *m/z* calcd for $C_{11}H_9$ 141.0699 $[M - H_2O + H]^+$, found 141.0693.

5-Acetylindene (157) and 6-(1-Chlorovinyl)indene (158). The alcohol **156** (10 mg, 0.06 mmol) was dissolved in THF/H_2O (4 mL, 1:1). Aqueous 6 N HCl (200 µL, 1.2 mmol) was then added and the reaction mixture was refluxed at 105 °C for 12 h. The reaction mixture was concentrated under vacuum to approximately 2 mL and extracted with EtOAc. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated at reduced pressure. The residue was purified by column chromatography (5 → 10% EtOAc/hexane) to give **157** (6 mg, 63%) and **158** (2.5 mg, 24%) as white solids. The more polar **157** had: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.64 (s, 3H), 3.46 (td, J = 2.0, 0.8 Hz, 2H), 6.63–6.66 (m, 1H), 6.92–6.95 (m, 1H), 7.54 (dp, J = 7.6, 0.8 Hz, 1H), 7.83 (dd, J = 8.0, 1.6 Hz, 1H), 7.99 (d, J = 1.2 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 26.99, 39.39, 120.85, 123.80, 125.39, 131.95, 135.53, 136.07, 145.44, 149.21, 198.61; HRMS (TOF, DART) *m/z* calcd for $C_{11}H_{11}O$ 159.0804 $[M + H]^+$, found 159.0808. The less polar **158** had: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.41–3.43 (m, 2H), 5.50 (d, J = 1.6 Hz, 1H), 5.76 (d, J = 2.0 Hz, 1H), 6.59–6.63 (m, 1H), 6.88–6.90 (m, 1H), 7.43–7.50 (m, 2H), 7.66 (d, J = 1.2 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 37.89, 111.10, 118.03, 112.07, 123.37, 130.78, 134.22, 134.46, 139.54, 143.75, 144.08; HRMS (TOF, APCI) *m/z* calcd for $C_{11}H_8{}^{35}Cl$ 175.0320 $[M - H]^-$, found 175.0390.

Trimethyl(3-(naphthalene-yl)prop-1-yn-yl)silane (159). To a stirred solution of trimethylsilylacetylene (1.4 mL, 966.0 mg, 10.0 mmol) in dry THF (5 mL) was added dropwise MeMgBr (3 M/Et_2O, 3.4 mL, 10.0 mmol) at 0 °C under N_2. The stirring was continued for 30 min at 0 °C and another 30 min at room temperature. Then CuBr (212.2

mg, 1.5 mmol) was added and stirring was continued for 30 min. Next, 2-bromomethylnaphthalene was added and the resulting mixture was refluxed (80 ^{o}C, oil bath) for 5 h. After being cooled to room temperature, the mixture was poured into a saturated aqueous solution of NH_4Cl and extracted with Et_2O. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated at reduced pressure. The residue was column chromatographed (*n*-hexane) to give trimethyl(3-(naphthalene-yl)prop-1-yn-1-yl)silane **159** (530 mg, 89%) as a white solid: ^{1}H NMR δ 0.22 (s, 9H), 3.82 (s, 2H), 7.45-7.50 (m, 3H), 7.80-7.84 (m, 4H); ^{13}C NMR δ 0.30, 26.54, 87.36, 104.38, 125.65, 125.75, 126.31, 126.41, 126.50, 126.63, 127.81, 132.47, 133.63, 133.98.

2-(Propa-1,2-dien-1-yl)naphthalene (160). The trimethylsilane product **159** (160 mg, 0.67 mmol) was dissolved in THF (5 mL) under N_2. A solution of tetra-*n*-butylammonium fluoride (TBAF) in THF (1 M/THF, 810 µL, 0.81 mmol) was added dropwise and stirring was continued for 30 min at room temperature. During this time, the reaction mixture turned to deep pink color. The mixture was poured into a saturated aqueous solution of NH_4Cl and extracted with Et_2O. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated at reduced pressure. The residue was column chromatographed (*n*-hexane) to give 2-(propa-1,2-dien-1-yl)naphthalene **160** (106 mg, 95%) as a white solid: ^{1}H NMR δ 5.23 (d, J = 6.8 Hz, 2H), 6.35 (t, J = 6.8 Hz, 1H), 7.41-7.52 (m, 3H), 7.67 (s, 1H), 7.77-7.81 (m, 3H); ^{13}C NMR δ 79.25, 94.45, 124.71, 124.83, 125.49, 125.60, 126.31, 127.84, 128.42, 131.55, 132.74, 133.82, 210.48.

2-(Prop-2-yn-1-yl)naphthalene (161). A stirred solution of trimethylsilane product **159** (190 mg, 0.80 mmol) in EtOH (4 mL) was treated with $AgNO_3$ (0.35 M, 3.5 mL, 1.23 mmol) in EtOH/H_2O (2.3:1). The resulting mixture was covered with aluminum foil and

stirred for 2 h at room temperature (a white solid was precipitated during this time). An aqueous solution of NaCN (7.6 M, 1 mL, 7.6 mmol) was then added and stirring was continued until the disappearance of white precipitate. The reaction mixture extracted with Et_2O. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated at reduced pressure. The residue was column chromatographed (*n*-hexane) to give 2-(prop-2-yn-1-yl)naphthalene **161** (110 mg, 83%) as a white solid: ^{1}H NMR δ 2.26 (t, J = 2.4 Hz, 1H), 3.78 (d, J = 2.0 Hz, 2H), 7.44-7.50 (m, 3H), 7.81-7.84 (m, 4H); ^{13}C NMR δ 25.51, 70.90, 82.03, 125.74, 126.28, 126.36, 126.51, 127.74, 127.81, 128.33, 128.42, 132.50, 133.65.

2,3-dihydro-1*H*-cyclopenta[a]naphthalene (162). $NaBH_4$ (98 mg, 2.58 mmol) was added portion wise to a stirred solution of commercially available 2,3-dihydro-1*H*-cyclopenta[a]naphthalene-1-one (470 mg, 2.58 mmol) in dry MeOH/THF (2;1) at 0 ^{o}C (ice-bath). After 5 min, the reaction mixture was allowed to warm to ambient temperature and stirring was continued for 1 h. Water (1 mL) was then added to quench the reaction. The mixture was concentrated under reduced pressure and extracted with EtOAc. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was column chromatographed (EtOAc in hexane 10-20%) to give 2,3-dihydro-1*H*-cyclopenta[a]naphthalene **162** (465 mg, 98%) as a white solid: ^{1}H NMR δ 1.80 (s, 1H), 2.15-2.23 (m, 1H), 2.56-2.65 (m, 1H), 2.95-3.02 (m, 1H), 3.26-3.34 (m, 1H), 5.79 (d, J = 4.8 Hz, 1H), 7.40 (d, , J = 8.4 Hz, 1H), 7.44-7.48 (m, 1H), 7.52-7.56 (m, 1H), 7.79 (d, J = 8.4 Hz, 1H), 7.88 (d, J = 8.4 Hz, 1H), 7.16 (d, J = 8.0 Hz, 1H); ^{13}C NMR δ 31.06, 35.48, 75.97, 123.52, 124.02, 125.29, 126.73, 128.66, 129.61, 130.32, 133.19, 139.29, 141.78.

3*H*-benz[e]indene (163). The secondary alcohol **162** (440 mg, 2.39 mmol) was dissolved in THF/H_2O (20 mL, 1:1). Aqueous 1N HCl (6.0 mL, 6 mmol) was then added and the reaction mixture was refluxed at 105 ºC for 6 h. After removing THF, the reaction mixture was transferred to a separatory funnel and extracted with EtOAc. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated at reduced pressure. The residue was column chromatographed (*n*-hexane) to give 3*H*-benz[a]indene **163** (240 mg, 61%) as a white solid: ^{1}H NMR δ 3.59 (s, 2H), 6.77 (d, J = 5.6 Hz, 1H), 7.46-7.56 (m, 3H), 7.66 (d, J = 8.0 Hz, 1H), 7.72 (d, J = 8.4 Hz, 1H), 7.92 (d, J = 8.4 Hz, 1H), 8.15 (d, J = 8.4 Hz, 1H); ^{13}C NMR δ 40.51, 122.57, 123.95, 124.89, 125.02, 125.70, 127.99, 128.50, 129.70, 132.74, 134.40, 141.12, 141.38.

Trimethyl(naphthalen-2-ylethynyl)silane (164). $Pd(PPh_3)_2Cl_2$ (35.1 mg, 0.05 mmol) and Cu(I)I (19.1 mg, 0.1mmol) were added to anhydrous THF (10 mL) and anhydrous Et_3N (1.5 mL, 1090 mg, 10.7 mmol) placed in flame-dried round bottom flask equipped with a stir bar. Then 2-bromonaphthalene (1035 mg, 5.0 mmol) was added followed by TMS-acetylene (832 µL, 575 mg, 5.85 mmol). The resulting mixture was stirred at 50 ºC for 5 h [progress of the reaction was monitored by TLC (hexane)]. The reaction mixture was then diluted with hexane and filtered through a short pad of silica. Volatiles were evaporated and the residue was column chromatographed (n-hexane) to give trimethyl(naphthalen-2-ylethynyl)silane **164** (500 mg, 45%) as a light yellow solid: ^{1}H NMR δ 0.29 (s, 9H), 7.46-7.52 (m, 3H), 7.75-7.78 (m, 3H), 8.00 (s, 1H); ^{13}C NMR δ 0.17, 94.69, 105.62, 120.61, 126.65, 126.87, 127.89, 127.94, 128.00, 128.74, 132.15, 133.05, 133.07.

2-Ethynylnaphthalene (165). Anhydrous K_2CO_3 (300 mg, 2.2 mmol) was added to a stirred solution of **164** (480 mg, 2.14 mmol) in 10 mL MeOH at room temperature. After

for 30 min, volatiles were evaporated and the residue was column chromatographed (*n*-hexane) to give **165** (300 mg, 92%) as a light yellow solid: ^{1}H NMR δ 3.15 (s, 1H), 7.49-7.55 (m, 3H), 7.78-7.84 (m, 3H), 8.04 (s, 1H); ^{13}C NMR δ 77.53, 84.17, 119.56, 126.76, 127.05, 127.92, 127.93, 128.17, 128.70, 132.46, 133.00, 133.21.

2-(Prop-1-yn-1-yl)naphthalene (166). A stirring solution of terminal alkyne **165** (204 mg, 1.34 mmol) in dry THF (10 mL) was cooled to -40 ºC and *n*-BuLi (1.6 M/hexane, 1.70 mL, 2.72 mmol) was added. After 1 h, iodomethane (176 µL, 400 mg, 2.82 mmol) was added dropwise at -40 ºC and stirred for 1 h at room temperature. The mixture was poured into a saturated aqueous solution of NH_4Cl and extracted with Et_2O. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated at reduced pressure. The residue was column chromatographed (*n*-hexane) to give 2-(Prop-1-yn-1-yl)naphthalene **166** (160 mg, 74%) as a gummy solid: ^{1}H NMR δ 2.12 (s, 2H), 7.45-7.51 (m, 3H), 7.76-7.82 (m, 3H), 7.93 (s, 1H); ^{13}C NMR δ 4.54, 80.25, 86.34, 121.54, 126.38, 126.48, 127.72, 127.82, 127.96, 128.80, 131.14, 132.63, 133.22.

Diethyl 2-(naphthalen-1-ylmetyl)malonate (169). Sodium (1.1 g, 47.9 mmol) was added to a stirred absolute EtOH (22 mL) at rt for 20 min and the resulting solution was cooled to 0 ºC. Then malonic ester (12.8 g, 80 mmol) was then added slowly, followed by 1-chloromethylnaphthalene (5.0 g, 28.3 mmol). After 4 h of refluxing at 90 ºC, the mixture was diluted with 20 mL water and neutralized with dilute HCl and extracted with EtOAc. The organic layer was washed with $NaHCO_3$ and brine. The EtOAc layer was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was column chromatographed (0 → 5 % EtOAc/hexane) to give **169**[204] (6.8 g, 80%) as a clear liquid: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.06 (dq, *J* = 8.6, 0.9 Hz, 1H), 7.87 (dt, *J* = 8.0, 0.8

Hz, 1H), 7.77-7.73 (m, 1H), 7.55 (ddd, *J* = 8.5, 6.8, 1.5 Hz, 1H), 7.50 (ddd, *J* = 8.1, 6.8, 1.3 Hz, 1H), 7.40-7.36 (m, 2H), 4.22 – 4.12 (m, 4H), 3.86 (dd, *J* = 8.1, 7.0 Hz, 1H), 3.73 (d, *J* = 7.5 Hz, 2H), 1.20 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.13, 134.00, 133.92, 131.68, 129.06, 127.76, 127.26, 126.37, 125.74, 125.49, 123.31, 61.60, 52.97, 31.90, 14.09.

3-(Naphthalen-1-yl)propanoic acid (170). A mixture of ester **169** (5 g, 16.6 mmol) in the solution of MeOH (5 mL) and NaOH (20 mL; 4 g, 0.1 mol) was refluxed at 110 ^{o}C for 3 h. After adding another 10 mL of hot water, the pH of the solution was made below 2.0 with 4 M HCl (10 mL). The precipitated solid was filtered and washed with cold water and dried in piston under the reduced pressure. The white powder was placed in round-bottom flask and was heated at 175 ^{o}C (oil bath) while evolution of carbon dioxide was observed. After 30 min, the heating was stopped and the flask was cooled to rt. Recrystallization from mixture of MeOH/benzene gave **170**[183] (3.0 g, 90%) as a white powder: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.04 (dq, *J* = 8.8, 1.0 Hz, 1H), 7.87 (dt, *J* = 8.0, 0.8 Hz, 1H), 7.75 (dt, *J* = 8.0, 1.2 Hz, 1H), 7.52 (dddd, *J* = 19.2, 8.1, 6.8, 1.4 Hz, 2H), 7.44 – 7.36 (m, 2H), 3.49 – 3.42 (m, 2H), 2.88 – 2.81 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.48, 136.24, 134.04, 131.69, 129.09, 127.43, 126.33, 126.07, 125.81, 125.73, 123.42, 34.88, 27.95.

2,3-Dihydro-1*H*-phenalen-1-one (171). Compound **170** (2.8 g, 13.98 mmol) was dissolved in $SOCl_2$ (40 mL) and the solution was refluxed at 85 ^{o}C for 1 h. The $SOCl_2$ was distilled off under reduced pressure. The resulted acid chloride was dissolved in 10 mL of dry dichloromethane and then added dropwise to a cold (-10°C) solution of 4.47 g (33.5 mmol) of aluminum chloride in 100 mL of dry dichloromethane. After stirring for 30 min at 0 ^{o}C, the mixture was poured into 100 mL of ice/water and the resulting solution was

extracted with dichloromethane. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was column chromatographed (5 → 10 % EtOAc/hexane) to give **171**[183] (1.91 g, 75%) as off-white solid: ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.20 (dd, *J* = 7.2, 1.3 Hz, 1H), 8.09 (dd, *J* = 8.2, 1.2 Hz, 1H), 7.80 (d, *J* = 8.1 Hz, 1H), 7.60 (t, *J* = 7.7 Hz, 1H), 7.50 (t, *J* = 7.5 Hz, 1H), 7.48 – 7.45 (m, 1H), 3.44 (t, *J* = 7.2 Hz, 2H), 3.01 – 2.97 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.78, 134.22, 133.51, 133.37, 131.76, 129.93, 126.88, 126.41, 125.83, 125.68, 125.20, 38.65, 28.69.

2,3-Dihydro-1*H*-phenalen-1-ol (172). $NaBH_4$ (748 mg, 19.8 mmol) was added portion wise to a stirred solution of **171** (1.8 g, 9.88 mmol) in dry MeOH/THF (40 mL, 2;1) at 0 ^{o}C (ice-bath). After 5 min, the reaction mixture was allowed to warm to rt and stirring was continued for 30 min. Water (10 mL) was then added to quench the reaction. The mixture was concentrated under reduced pressure and extracted with EtOAc. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was column chromatographed (EtOAc in hexane 20 → 40%) to give **172**[184] (1.64 g, 90%) as a white solid: ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.80 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.56 (d, *J* = 7.0 Hz, 1H), 7.50 – 7.46 (m, 1H), 7.43 (dd, *J* = 8.2, 6.9 Hz, 1H), 7.31 (dd, *J* = 7.0, 1.5 Hz, 1H), 5.09 (dd, *J* = 6.8, 3.7 Hz, 1H), 3.33 (ddd, *J* = 16.1, 8.9, 4.6 Hz, 1H), 3.09 (ddd, *J* = 16.2, 7.3, 4.6 Hz, 1H), 2.24 – 2.14 (m, 2H), 1.96 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.53, 135.21, 133.73, 128.79, 128.15, 126.01, 125.79, 125.72, 124.50, 123.59, 69.48, 31.41, 26.37.

1*H*-Phenalene (173). The secondary alcohol **172 (**1.0 g, 5.4 mmol) was dissolved in the mixture of THF/H_2O (20 mL, 1:1). Aqueous 4 N HCl (10.0 mL, 40 mmol) was then added and the reaction mixture was refluxed at 105 ^{o}C for 6 h. The reaction mixture was

concentrated under vacuum to approximately 20 mL and was transferred to a separatory funnel and extracted with EtOAc. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was column chromatographed (*n*-hexane) to give **173**[184] (673 mg, 75%) as a light yellow solid: ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.56 (d, *J* = 8.2 Hz, 1H), 7.52 (d, *J* = 8.3 Hz, 1H), 7.36 (t, *J* = 7.6 Hz, 1H), 7.30 – 7.23 (m, 2H), 6.98 (d, *J* = 6.9 Hz, 1H), 6.60 (dt, *J* = 9.8, 2.3 Hz, 1H), 6.05 (dt, *J* = 9.8, 4.1 Hz, 1H), 4.09 – 4.06 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 134.36, 133.70, 132.13, 129.59, 127.91, 127.76, 126.82, 126.40, 126.17, 125.19, 125.04, 122.22, 32.19.

Acenaphthylen-1(*2H*)-one (174). Suspension of $K_2Cr_2O_7$ (9.53 g, 32.4 mmol) in acetic acid (40 mL) was added to an acetic acid (40 mL) solution of acenaphthalene (5.0 g, 32.4 mmol) at rt for overnight. The resulting mixture was poured into 200 mL of H_2O and extracted with 5 x 50 mL EtOAc. The combined organic portions were washed with saturated $NaHCO_3$ solution followed by brine. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was column chromatographed (0 → 10 % EtOAc/hexane) to give **174**[185] (3.27 g, 60%) as off-white solid: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.09 (dd, *J* = 8.1, 0.7 Hz, 1H), 7.96 (dd, *J* = 7.1, 0.8 Hz, 1H), 7.82 (dd, *J* = 8.4, 0.8 Hz, 1H), 7.71 (dd, *J* = 8.1, 7.0 Hz, 1H), 7.60 (dd, *J* = 8.4, 6.8 Hz, 1H), 7.46 (dq, *J* = 6.9, 1.0 Hz, 1H), 3.82 (t, *J* = 0.9 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 203.11, 143.06, 135.13, 134.81, 131.61, 131.07, 128.50, 128.12, 124.08, 121.57, 121.16, 42.13.

1-Methyl-1,2dihydroacenaphthylen-1-ol (175). A solution of **174** (505 mg, 3.0 mmol) in benzene (10 mL) was added dropwise for 10 min to a cooled (ice-bath) solution of MeMgI (3 M, 1.5 mL, 4.5 mmol), and stirring was continues for an additional 10 min at rt. The reaction mixture was then refluxed at 85 ^{o}C for 2 h [progress of the reaction was

monitored by TLC]. The reaction mixture was poured in ice-water and saturated NH_4Cl was added to quench the reaction. The mixture was extracted with EtOAc and organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was column chromatographed (0 → 20% EtOAc/hexane) to give **175**[187] (450 mg, 81.4%) as off-white solid: ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.76 (d, *J* = 8.1 Hz, 1H), 7.70 – 7.67 (m, 1H), 7.57 (dd, *J* = 8.1, 6.9 Hz, 1H), 7.52 (dd, *J* = 8.2, 6.8 Hz, 1H), 7.48 (d, *J* = 6.9 Hz, 1H), 7.31 (dq, *J* = 6.8, 1.1 Hz, 1H), 3.62 – 3.47 (m, 2H), 1.87 (brs, 1H), 1.77 (s, 3H).

1-Methylacenaphthalene (176). The tertiary alcohol **175 (**400 mg, 2.17 mmol) was dissolved in THF/H_2O (10 mL, 1:1). Aqueous 6 N HCl (3.0 mL, 18 mmol) was then added and the reaction mixture was refluxed at 105 ^{o}C for 12 h. The reaction mixture was concentrated to ~6 mL and was transferred to a separatory funnel and extracted with EtOAc. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated at reduced pressure. The residue was column chromatographed (*n*-hexane) to give **176**[187] (356 mg, 98.6%) as a light orange liquid: ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.79 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.71 (d, *J* = 8.0 Hz, 1H), 7.65 (d, *J* = 6.7 Hz, 1H), 7.58 – 7.47 (m, 3H), 6.72 (t, *J* = 1.8 Hz, 1H), 2.45 (t, *J* = 1.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.17, 140.46, 139.95, 129.10, 127.99, 127.90, 127.50, 127.27, 125.98, 125.41, 122.48, 121.83, 13.31.

9-(But-3-en-1-yn-1-yl)phenanthrene (177). $Pd(PPh_3)_4$ (5.8 mg, 0.005 mmol) and Cu(I)I (3.8 mg, 0.02 mmol) were placed in the flame-dried flask under N_2 at 0 ^{o}C (ice-bath). Then Et_2NH (0.65 mL, 460 mg, 6.29 mmol) and vinyl bromide (1.0 M in THF; 0.65 mL, 0.65 mmol) were added. Next, commercially available 9-ethynylphenanthrene (101.1 mg, 0.5 mmol) dissolved in dry THF (1 mL) was added slowly via a syringe pump (over 3

h) and the resulting mixture was allowed to warm up to ambient temperature (30 min) and was stirred for another 2 h. Volatiles were evaporated and the residue was dissolved in EtOAc and filtered. The filtrate was collected and solvent was evaporated. The residue was column chromatographed (*n*-hexane) to give **177** (50 mg, 44%) as white powder: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.65 (dd, J = 11.2, 2.0 Hz, 1H), 5.89 (dd, J = 17.6, 2.0 Hz, 1H), 6.20 (dd, J = 17.2, 11.2 Hz, 1H), 7.58-7.62 (m, 1H), 7.65-7.73 (m, 3H), 7.85 (d, J = 7.6 Hz, 1H), 8.01 (s, 1H), 8.42-8.45 (m, 1H), 8.65-8.70 (m, 2H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 88.39, 92.75, 117.42, 117.47, 119.70, 122.66, 122.82, 122.98, 127.14, 127.39, 127.70, 128.74, 130.23, 130.45, 131.21, 131.37, 132.05, 132.09.

(*E*)-(2-(Phenanthren-9-yl)vinyl)boronic acid (178). 9-Ethynylphenanthrene (505.5 mg, 2.5 mmol) and catecholborane (266 µL, 300 mg, 2.5 mmol) were placed in a flame-dried flask under N_2 at ambient temperature and the reaction mixture were stirred for 20 min at 70 ^{o}C. Within this 20 min, the reaction mixture form a small lump and was kept few minutes at ambient temperature. Then H_2O/EtOAc (1:1; 10 mL) were added into the reaction mixture and stirred for 30 min at 25 ^{o}C to effect the hydrolysis of boronic ester. The reaction mixture was extracted with EtOAc, organic layer separated and the aqueous layer was extracted with EtOAc two more times. The combined organic layer was dried (Na_2SO_4) and evaporated. The residue was column chromatographed (20-40% EtOAc in hexane) to give **178** (301 mg, 49%) as white powder: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 6.32 (d, J = 18.0 Hz, 1H), 7.63-7.76 (m, 4H), 7.98 (s, 2H), 8.04 (d, J = 8.8 Hz, 2H), 8.13 (d, J = 18.0 Hz, 1H), 8.32-8.34 (m, 1H), 8.79-8.82 (m, 1H), 8.88-8.90 (m, 1H); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 122.66, 122.86, 123.36, 123.55, 124.12, 124.15, 126.98, 127.05, 128.90, 129.81, 129.85, 129.88, 131.23, 134.26, 143.09, 145.27.

(*E*)-9-(2-iodovinyl)phenanthrene (179). The boronic acid **178** (100 mg, 0.40 mmol) was dissolved in 5 mL Et_2O in a 50 mL flask and cooled to 0 ^oC. Then aqueous NaOH (400 µL, 3 N, 1.2 mmol) was added dropwise followed by elemental iodine (121.8 mg, 0.48 mmol) dissolved in 5 mL Et_2O, while stirring at 0 ^oC. The reaction mixture was stirred for 30 min at 0 ^oC. The excess I_2 was destroyed by aqueous $Na_2S_2O_3$ solution. The reaction mixture was extracted with Et_2O and organic layer was separated and the aqueous layer was extracted with Et_2O twice. The combined organic layer was dried (Na_2SO_4) and evaporated. The residue was column chromatographed (*n*-hexane) to give **179** (108 mg, 81%) as white powder: ^1H NMR ($CDCl_3$, 400 MHz) δ 6.93 (d, J = 14.8 Hz, 1H), 7.58-7.71 (m, 4H), 7.75 (s, 1H), 7.89 (d, J = 7.6 Hz, 1H), 8.09 (d, J = 8.0 Hz, 1H), 8.16 (d, J = 14.4 Hz, 1H), 8.65 (d, J = 8.4 Hz, 1H), 8.72 (d, J = 7.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 122.63, 122.85, 123.13, 123.37, 124.72, 125.53, 125.66, 127.06, 128.94, 129.67, 130.49, 130.55, 131.65, 134.85, 143.58, 143.61.

(*E*)-Trimethyl(4-(phenanthren-9-yl)but-3-en-1-yn-yl)silane (180). $Pd(PPh_3)_2Cl_2$ (8.4 mg, 0.012 mmol) and Cu(I)I (4.6 mg, 0.024 mmol) were added to dry THF (5 mL) in a flame-dried round bottom flask equipped with a stir bar under N_2 at 0 ^oC (ice-bath). Then iodovinylphenanthrene, **179** (100 mg, 0.30 mmol) was added followed by TMS-acetylene (62 µL, 44 mg, 0.45 mmol) and Et_3N (84 µL, 61 mg, 0.60 mmol). The resulting mixture was allowed to warm up to ambient temperature and was stirred for 1h. [progress of the reaction was monitored by TLC (*n*-hexane)]. Volatiles were evaporated and the residue was column chromatographed (*n*-hexane) to give **180** as light yellow gummy solid (90 mg, 99%).^1H NMR: ($CDCl_3$, 400 MHz) δ 0.30 (s, 9H), 6.34 (d, J = 16.0 Hz, 1H), 7.58-7.67 (m,

4H), 7.81 (d, *J* = 16.0 Hz, 1H), 7.85 (s, 1H), 7.88 (d, *J* = 7.6 Hz, 1H), 8.17 (d, *J* = 9.2 Hz, 1H), 8.65 (d, *J* = 8.0 Hz, 1H), 8.72 (d, *J* = 9.2 Hz, 1H).

[(*E*)-9-But-1-en-3-yn-1-yl)phenanthrene (181). Anhydrous K_2CO_3 (41 mg, 0.3 mmol) was added to a stirred solution of **180** (80 mg, 0.27 mmol) in 4 mL MeOH/DCM (1:1) at room temperature. After for 30 min, volatiles were evaporated and the residue was column chromatographed (*n*-hexane) to give **181** (58 mg, 95%) as light yellow powder. ^{1}H NMR: ($CDCl_3$, 400 MHz) δ 3.12 (d, *J* = 2.4 Hz, 1H), 6.29 (dd, *J* = 16.0, 2.4 Hz, 1H), 7.58-7.67 (m, 4H), 7.83-7.90 (m, 3H), 8.13-8.16 (m, 1H), 8.66 (d, *J* = 8.0 Hz, 1H), 8.72-8.74 (m, 1H); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 79.17, 83.13, 110.41, 122.61, 122.85, 123.21, 123.38, 124.55, 125.10, 126.99, 129.07, 130.10, 130.53, 131.80, 131.57, 132.57, 141.05, 141.13.

4-Vinylpyrene (182). The 4-Bromopyrene (56.3 mg, 0.2 mmol) was dissolved in dry toluene in a flame-dried flask. Then $Pd(PPh_3)_4$ (11.6 mg, 0.01 mmol) and Bu_3Sn(vinyl) (64.3 µL, 69.8 mg, 0.22 mmol) were added at rt. The reaction mixture was heated at 110 ^{o}C and stirred for 5 h [progress of the reaction was monitored by TLC (hexane)]. Volatiles were evaporated and the residue was column chromatographed (n-hexane) to give **182** (38 mg, 83%) as a white powder: ^{1}H NMR δ 5.63 (dd, *J* = 10.8, 1.6 Hz, 1H), 6.02 (dd, *J* = 17.2, 1.6 Hz, 1H), 7.65 (dd, *J* = 17.2, 11.2 Hz, 1H), 7.99-8.09 (m, 4H), 8.16-8.21 (m, 4H), 8.43 (d, *J* = 7.6 Hz, 1H); ^{13}C NMR δ 117.81, 121.72, 124.52, 124.85, 124.91, 125.09, 125.39, 125.91, 126.18, 127.41, 127.75, 129.99, 131.10, 131.17, 131.59, 135.09, 135.22, 135.45.

4-Ethynylphenanthrene (183). *(Step a)* $Pd(PPh_3)_4$ (43.3 mg, 0.037 mmol) and Cu(I)I (6.9 mg, 0.036 mmol) were added to dry Et_3N (10 mL) flame-dried round bottom flask equipped with a stir bar. Then 4-bromophenanthrene (300 mg, 1.17 mmol) was added

followed by TMS-acetylene (292 μL, 207 mg, 2.11 mmol). The resulting mixture was stirred at ambient temperature for 5 h [progress of the reaction was monitored by TLC (hexane)]. The reaction mixture was then diluted with dichloromethane (DCM) and filtered through a short pad of silica. Volatiles were evaporated and the residue was column chromatographed (n-hexane) to give trimethyl(phenanthren-4-ylethynyl)silane as a light yellow solid (257 mg, 80%): ^{1}H NMR δ 0.39 (s, 9H), 7.52 (t, J = 7.6 Hz, 1H), 7.61-7.66 (m, 2H), 7.67-7.76 (m, 2H), 7.86-7.93 (m, 3H), 10.43-10.47 (m, 1H); ^{13}C NMR δ 0.04, 100.80, 108.23, 119.34, 125.56, 125.67, 126.65, 127.08, 127.48, 128.03, 128.45, 130.11, 130.13, 130.83, 133.16, 133.18, 135.62. *Step b.* Anhydrous K_2CO_3 (250 mg, 1.8 mmol) was added to a stirred solution of trimethyl(phenanthren-4-ylethynyl)silane (412 mg, 1.5 mmol) in 10 mL MeOH/DCM (1:1) at room temperature. After for 30 min, volatiles were evaporated and the residue was column chromatographed (*n*-hexane) to give **183** (249 mg, 82%) as a light yellow solid: ^{1}H NMR δ 3.70 (s, 1H), 7.54 (t, J = 7.8 Hz, 1H), 7.63-7.68 (m, 2H), 7.69-7.78 (m, 2H), 7.89-7.97 (m, 3H), 10.34-10.38 (m, 1H); ^{13}C NMR δ 83.59, 86.54, 118.29, 125.63, 126.09, 126.35, 127.19, 127.42, 128.13, 128.55, 130.26, 130.42, 130.69, 133.15, 133.22, 136.27.

4-(But-3-en-1-yn-1-yl)phenanthrene (184). $Pd(PPh_3)_4$ (8.1 mg, 0.007 mmol) and Cu(I)I (5.3 mg, 0.028 mmol) were placed in the flame-dried flask under N_2 at 0 ^{o}C (ice-bath). Then Et_2NH (0.90 mL, 636 mg, 8.70 mmol) and vinyl bromide (1.0 M in THF; 0.90 mL, 0.90 mmol) were added. Next, 4-ethynylphenanthrene **183** (140. mg, 0.69 mmol) dissolved in dry THF (2 mL) was added slowly via a syringe pump (over 3 h) and the resulting mixture was allowed to warm up to ambient temperature (30 min) and was stirred for another 2 h. Volatiles were evaporated and residue was column chromatographed (*n*-

hexane) to give **184** [13 mg, 8.3%; TLC (hexane) R*f* = 0.6] as a clear liquid. ^{1}H NMR : δ 5.63 (dd, *J* = 11.2, 2.0 Hz, 1H), 5.89 (dd, *J* = 17.2, 2.0 Hz, 1H), 6.25 (dd, *J* = 17.6, 11.2 Hz, 1H), 7.53 (t, *J* = 7.6 Hz, 1H), 7.62-7.69 (m, 2H), 7.70-7.77 (m, 2H), 7.87-791 (m, 3H), 10.23-10.25 (m, 1H); ^{13}C NMR : δ 92.96, 94.00, 117.79, 119.36, 125.67, 126.12, 126.33, 126.99, 127.04, 127.48, 128.03, 128.53, 129.92, 130.84, 133.19, 133.28, 135.07.

Note. Also isolated from the reaction mixture was a dimer resulting from homocoupling of **183**.

(*E*)-4-(2-iodovinyl)phenanthrene (186). *Step a.* The 4-ethynylphenanthrene **183** (101.1 mg, 0.5 mmol) and catecholborane (53.2 µL, 60 mg, 0.5 mmol) were placed in a flame-dried flask under N_2 at ambient temperature and the reaction mixture were stirred for 60 min at 70 ^{o}C. Then H_2O/EtOAc (1:1; 10 mL) were added and stirring was continued for 30 min at 25 ^{o}C to effect the hydrolysis of boronic ester. The reaction mixture was extracted with EtOAc, organic layer separated and the aqueous layer was back extracted with EtOAc twice. The combined organic layer was dried (Na_2SO_4) and evaporated. The residue was column chromatographed (20-40% EtOAc in hexane) to give boronic acid **185** (45 mg, 37%) as a gummy solid, which was directly used for the next step. *Step b.* The boronic acid **185** (45 mg, 0.18 mmol) was dissolved in 5 mL Et_2O in a 25 mL flask and cooled to 0 ^{o}C. Then aqueous NaOH (180 µL, 3 N, 0.54 mmol) was added dropwise followed by elemental iodine (54.8 mg, 0.22 mmol) dissolved in 5 mL Et_2O, while stirring at 0 ^{o}C. The reaction mixture was stirred for 30 min at 0 ^{o}C. The excess I_2 was destroyed by addition of few drops of aqueous $Na_2S_2O_3$ solution. The reaction mixture was extracted with Et_2O and the organic layer was separated and the aqueous layer was back extracted with Et_2O twice. The combined organic layer was dried (Na_2SO_4) and evaporated. The

residue was column chromatographed (*n*-hexane) to give **186** (30 mg, 50%) as a white powder: ^{1}H NMR δ 6.85 (d, J = 14.8 Hz, 1H), 7.53-7.59 (m, 2H), 7.62-7.70 (m, 2H), 7.72-7.77 (m, 2H), 7.87-7.94 (m, 2H), 8.17 (d, J = 14.8 Hz, 1H), 8.72 (d, J = 8.4 Hz, 1H); ^{13}C NMR δ 125.67, 126.24, 126.27, 126.62, 127.35, 127.78, 127.98, 128.16, 128.73,128.74, 129.33, 130.62, 133.36, 133.54, 137.36, 149.46.

(*E*)-4-(But-1-en-3-yn-1-yl)phenanthrene (187). *Step a.* $Pd(PPh_3)_2Cl_2$ (4.2 mg, 0.006 mmol) and Cu(I)I (2.3 mg, 0.012 mmol) were added to dry THF (2 mL) in a flame-dried round bottom flask equipped with a stir bar under N_2 at 0 ^{o}C (ice-bath). Then iodovinylphenanthrene **186** (30 mg, 0.09 mmol) was added followed by TMS-acetylene (18.6 μL, 13.2 mg, 0.14 mmol) and Et_3N (25.2 μL, 18.3 mg, 0.18 mmol). The resulting mixture was allowed to warm up to ambient temperature and was stirred for 1h [progress of the reaction was monitored by TLC (*n*-hexane)]. Volatiles were evaporated and the residue was column chromatographed (*n*-hexane) to give **(***E*)-Trimethyl(4-(phenanthren-4-yl)but-3-en-1-yn-yl)silane as a light yellow gummy solid (23 mg, 85%): ^{1}H NMR δ 0.30 (s, 9H), 6.28 (d, J = 16.0 Hz, 1H), 7.53-7.69 (m, 4H), 7.71-7.79 (m, 3H), 7.83-7.87 (m, 1H), 7.91-7.94 (m, 1H), 8.72 (d, J = 8.4 Hz, 1H); ^{13}C NMR δ 0.20, 96.30, 104.73, 109.08, 126.11, 126.22, 126.58, 127.33, 127.66, 128.31, 128.53, 128.57, 129.34, 130.76, 133.35, 133.52, 135.67, 139.39, 146.46. *Step b.* Anhydrous K_2CO_3 (12 mg, 0.09 mmol) was added to a stirred solution of (*E*)-Trimethyl(4-(phenanthren-4-yl)but-3-en-1-yn-yl)silane (23 mg, 0.08 mmol) in 2 mL MeOH/DCM (1:1) at room temperature. After for 30 min, volatiles were evaporated and the residue was column chromatographed (*n*-hexane) to give **187** (16 mg, 92%) as a clear liquid: ^{1}H NMR δ 3.13 (d, J = 2.0 Hz, 1H), 6.23 (dd, J = 16.0, 2.4 Hz, 1H), 7.56-7.69 (m, 4H), 7.72-7.77 (m, 2H), 7.85-7.89 (m, 2H), 7.91-7.94 (m, 1H), 8.67 (d,

J = 7.6 Hz, 1H); ^{13}C NMR δ 78.75, 83.24, 107.98, 126.13, 126.23, 126.64, 127.34, 127.71, 128.30, 128.41, 128.62, 128.91, 129.48, 130.72, 133.38, 133.55, 135.42,147.43.

7-Aminofluoranthene (189). Iron powder (1.10 g, 19.7 mmol) was added to the solution of NH_4Cl (1.05 g, 19.7 mmol) in H_2O/EtOH (30 mL, 1:1) in 100 mL flask equipped with a stir bar. The mixture was stirred at 60 ^oC for 30 min to activate the iron powder. Then 7-nitrofluoranthene **188**[190] (742 mg, 3.0 mmol) was added and the temperature of reaction mixture was raised to 80 °C and stirring was continued for another 30 min. The mixture was cooled with ice-bath, basified with dilute aqueous NaOH to pH ~12 and was filtered to remove solid residue. The filtrate was concentrated under reduced pressure and extracted with EtOAc. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (20 → 30% EtOAc/hexane) to give **189** (534 mg, 82%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.93 (d, J = 6.8 Hz 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.81–7.75 (m, 2H), 7.66–7.59 (m, 2H), 7.45 (dd, J = 7.4, 0,8 Hz, 1H), 7.23 (t, J = 7.6 Hz, 1H), 6.76 (dd, J = 8.0, 0.8 Hz, 1H), 4.22 (s, 2H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ = 143.2, 140.8, 137.4, 137.0, 132.3, 130.1, 128.83, 128.1, 128.0, 126.7, 125.6, 123.7, 121.0, 120.3, 116.6, 113.0 ppm.

7-Iodofluoranthene (190). Iodine (467 mg, 1.84 mmol), CuI (420 g, 2.20 mmol), CH_2I_2 (445 µL, 5.52 mmol), and tert-butyl nitrite (660 µL, 5.55 mmol) were added to a solution of **189** (400 mg, 1.84 mmol) in dry THF (15 mL). The reaction mixture was stirred at 66 °C for 30 min, cooled to 24 °C, and filtered. The filtrate was concentrated under reduced pressure and extracted with EtOAc. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (5 → 10% EtOAc/hexane) to give **190** (428 mg, 71%) as an off-white

solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.84 (d, J = 7.2 Hz, 1H), 7.97 (d, J = 6.8 Hz, 1H), 7.95–7.89 (m, 3H), 7.82 (dd, J = 8.0, 0.8 Hz, 1H), 7.74 (dd, J = 8.0, 6.8 Hz, 1H), 7.67 (dd, J = 8.0, 6.8 Hz, 1H), 7.09 (t, J = 7.6 Hz, 1H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ = 142.0, 141.2, 138.7, 137.4, 135.4, 132.5, 130.0, 128.5, 128.0, 127.8, 127.6, 127.5, 123.1, 121.1, 120.2, 90.0 ppm.

7-[2-(Trimethylsilyl)ethynyl]fluoranthene (191). $Pd(PPh_3)_4$ (28.2 mg, 0.024 mmol) and Cu(I)I (9.1 mg, 0.048 mmol) were added to dry Et_3N (10 mL) in a flame-dried flask equipped with a stirring bar under N_2 at 24 °C. Then **190** (400 mg, 1.22 mmol) was added followed by TMS-acetylene (347 μL, 2.44 mmol). The resulting mixture was stirred for 2 h at 24 °C [progress of the reaction was monitored by TLC (n-hexane)]. Volatiles were evaporated and the residue was purified by column chromatography (0 → 5% EtOAc/hexane) to give **191** as light yellow solid (331 mg, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.57 (d, J = 6.8 Hz, 1H), 7.95 (d, J = 7.2 Hz, 1H), 7.92–7.86 (m, 3H), 7.72–7.63 (m, 2H), 7.49 (dd, J = 7.6, 1.2 Hz, 1H), 7.34 (t, J = 7.6 Hz, 1H), 0.42 (s, 9H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ = 139.7, 139.7, 136.5, 135.8, 132.6, 131.8, 129.9, 128.2, 127.9, 127.3, 127.18, 127.1, 123.5, 121.9, 120.3, 117.7, 104.0, 99.2, 0.2 ppm.

7-Ethynylfluoranthene (192). Anhydrous K_2CO_3 (370 mg, 2.68 mmol) was added to a stirred solution of mixture of **191** (200 mg, 0.67 mmol) in dry MeOH/DCM (10 mL, 1:1) at 24 °C. After 2 h, volatiles were evaporated and the residue was column chromatographed (0 → 5% EtOAc/hexane) to give **192** as an off-white solid (140 mg, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.58 (d, J = 7.2 Hz, 1H), 7.96 (d, J = 6.8 Hz, 1H), 7.93 (dd, J = 7.4, 1.0 Hz, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.89 (d, J = 8.0 Hz, 1H), 7.7–7.63 (m, 2H), 7.52 (dd, J = 7.8, 1.0 Hz, 1H), 7.36 (t, J = 7.8 Hz, 1H), 3.57 (s, 1H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$)

δ = 140.0, 139.8, 136.2, 135.7, 132.5, 132.2, 130.0, 128.3, 127.93, 127.4, 127.3, 127.2, 123.5, 122.1, 120.4, 116.7, 82.6, 81.8 ppm.

1-Vinyl corannulene (**194**). The 1-Bromocorannulene **193**[191] (198 mg, 0.6 mmol) was dissolved in dry toluene in a flame-dried flask. Then $Pd(PPh_3)_4$ (23.2 mg, 0.02 mmol) and Bu_3Sn(vinyl) (210 µL, 227 mg, 0.72 mmol) were added at rt. The reaction mixture was heated at 110 ^oC and stirred for 5 h [progress of the reaction was monitored by TLC (hexane)]. Volatiles were evaporated and the residue was column chromatographed (5 → 10% EtOAc/hexane) to give **194** (86 mg, 52%) as a light yellow powder:^{1}H NMR (400 MHz, $CDCl_3$) δ 5.60 (dd, J = 10.8, 1.2 Hz, 1H), 6.13 (dd, J = 17.2, 1.2 Hz, 1H), 7.36 (ddd, J = 17.6, 10.8, 1.2 Hz, 1H), 7.74 – 7.88 (m, 8H), 8.08 (d, J = 8.8 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 118.24, 122.21, 124.46, 125.66, 127.01, 127.10, 127.17, 127.22, 127.37, 127.43, 127.57, 129.05, 130.79, 130.89, 130.94, 131.05, 134.81, 134.83, 135.54, 135.64, 135.79, 136.02, 136.35, 137.88.

Benzocorannulene (**195**). 193 (80 mg, 0.29 mmol) was dissolved in mixture of o-dichlorobenzene (15 mL) and nitroethanol (1.0 mL, 1.27 g, 13.9 mmol) in a pressurized flask. Phthalic anhydride (2.1 g, 14.2 mmol) was added at rt. The reaction mixture was heated at 180 ^oC and stirred for 3 days. Volatiles were evaporated and the residue was extracted with EtOAc. The organic layer washed with 1. 0 M NaOH (aq), brine, and anhydrous Na_2SO_4. Volatiles were evaporated and the residue was column chromatographed (5 → 10% EtOAc/hexane) to give **195** (24.4 mg, 28%) as a light yellow powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (dd, J = 6.0, 3.2 Hz, 1H), 7.81 – 7.87 (m, 2H), 7.95 (d, J = 8.8 Hz, 1H), 8.25 (d, J = 8.8 Hz, 1H), 8.67 (dd, J = 6.0, 3.2 Hz, 1H); ^{13}C NMR

(101 MHz, $CDCl_3$) δ 124.41, 125.22, 127.10, 127.28, 127.46, 127.68, 129.00, 130.61, 130.89, 133.29, 134.76, 135.55, 137.75.

1-Nitrotriphenylene (197) and **2-Nitrotriphenylene (198).** The triphenylene **196** (10.0 g, 43.8 mmol) was dissolved in acetic anhydride (60 mL) in a flame-dried flask. HNO_3 was added into the mixture dropwise for 10 minutes. The reaction mixture was stirred at 60 ^oC for 1 h. The reaction mixture transfer into a separatory funnel and EtOAc (100 mL) was added which was extracted with H_2O (100 x 5). Then extracted with saturated $NaHCO_3$ (50 mLx5). The organic layer washed with brine and anhydrous Na_2SO_4. Volatiles were evaporated and the residue was column chromatographed (5 → 20% EtOAc/hexane) to give **197** (3.6 g, 30%) and **198** (2.4 g, 20%) as light yellow powder. More polar compound **197** had: ^1H NMR (400 MHz, $CDCl_3$) δ 7.51 (t, J = 7.8 Hz, 1H), 7.59 – 7.71 (m, 4H), 7.79 (d, J = 7.6 Hz, 1H), 8.00 (d, J = 8.4 Hz, 1H), 8.50 (d, J = 7.4 Hz, 1H), 8.57 (d, J = 7.6 Hz, 2H), 8.70 (d, J = 8.2 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 122.44, 123.33, 123.50, 123.62, 127.78, 125.37, 126.33, 126.45, 126.54, 127.16, 127.97, 128.20, 128.66, 128.78, 130.40, 131.11, 132.50, 149.90. Less polar **198** had: ^1H NMR (400 MHz, $CDCl_3$) δ 7.65 – 7.77 (m, 4H), 8.31 (dd, J = 8.8, 2.4 Hz, 1H), 8.51 – 8.62 (m, 5H), 9.35 (d, J = 2.4 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 119.24, 120.99, 123.54, 123.65, 124.35, 124.57, 127.88, 128.02, 128.23, 128.69, 128.75, 129.28, 130.10, 130.24, 131.11, 134.44, 146.49.

1-Aminotriphenylene (**199**). Iron powder (4.38 g, 78.5 mmol) was added to the solution of NH_4Cl (4.2 g, 78.5 mmol) in H_2O/EtOH (60 mL, 1:1) in a flask equipped with a stir bar. The mixture was stirred at 60 ^oC for 30 min to activate the iron powder. Then 1-nitrotriphenyle **197** (742 mg, 3.0 mmol) was added and the temperature of reaction mixture

was raised to 80 °C and stirring was continued for another 30 min. The mixture was cooled with ice-bath, basified with dilute aqueous NaOH to pH ~12 and was filtered to remove solid residue. The filtrate was concentrated under reduced pressure and extracted with EtOAc. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (20 → 30% EtOAc/hexane) to give **199** (2.45 g, 83%) as a yellow solid.^{1}H NMR (400 MHz, $CDCl_3$) δ 4.37 (s, 2H), 6.98 (d, J = 7.6 Hz, 1H), 7.43 (t, J = 8.0 Hz, 1H), 7.55–7.66 (m, 4H), 8.11 (d, J = 8.0 Hz, 1H), 8.55 – 8.67 (m, 3H), 9.22 (d, J = 7.9 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 114.22, 115.99, 118.85, 123.13, 123.70, 123.99, 126.09, 126.32, 126.36, 127.31, 127.38, 130.12, 130.27, 130.42, 130.49, 132.22, 145.20.

1-Iodotriphenylene (**200).** Iodine (52 mg, 0.21 mmol), CuI (47 mg, 0.25 mmol), CH_2I_2 (50 µL, 0.62 mmol), and tert-butyl nitrite (73 µL, .62 mmol) were added to a solution of **199** (50 mg, 0.21 mmol) in dry THF (5 mL). The reaction mixture was stirred at 66 °C for 30 min, cooled to rt, and filtered. The filtrate was concentrated under reduced pressure and extracted with EtOAc. The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (2 → 5% EtOAc/hexane) to give **200** (52 mg, 72%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (dd, J = 8.2, 0.8 Hz, 1H), 7.57 – 7.69 (m, 4H), 8.30 (dd, J = 7.6, 1.2 Hz, 1H), 8.50 – 8.60 (m, 4H), 9.43 (dd, J = 8.4, 1.0 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 90.88, 123.21, 123.27, 123.30, 123.40, 124.81, 127.65, 127.80, 127.97, 128.18, 128.47, 129.56, 129.77, 130.35, 131.00, 132.24, 132.27, 142.38.

1-Bromotriphenylene (**201**). The 1-Aminotriphenylene **199** (200 mg, 0.82 mmol) was dissolved in mixture of MeCN/H2O (16 mL, 1:1). Then pre cooled HBr (48%, 5 mL) was

added and stirred at 0 ^{o}C. Aqueous solution of NaNO2 (85 mg, 1.2 mmol) was added into the mixture dropwise for 2 minutes. The reaction mixture was stirred at 0 ^{o}C for 20 min. Next, pre cooled CuBr (128 mg, 0.9 mmol) solution in HBr (8 mL) solution was added at rt and stirred for 2 h. The reaction mixture transfer into a separatory funnel and EtOAc (20 mL) was added which was extracted with H_2O (10 mL x 5). Then extracted with saturated $NaHCO_3$ (5 mLx2). The organic layer washed with brine and anhydrous Na_2SO_4. Volatiles were evaporated and the residue was column chromatographed (5 → 20% EtOAc/hexane) to give mixture of **201** and 2-bromotriphenylene isomer (100 mg, 40%; 77:23) as off-white powder.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (t, J = 8.0 Hz,, 1H), 7.52 – 7.70 (m, 5.5H), 7.81 (d, J = 8.8 Hz,, 0.3H), 7.94 (dd, J = 7.6, 1.2 Hz, 1H), 8.32 (dd, J = 8.8, 0.8 Hz, 0.3H), 8.39 –8.42 (m, 0.3H), 8.48 – 8.60 (m, 4.9H), 9.31 – 9.34 (m, 0.3H), 9.52 – 9.56 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 120.02, 121.77, 122.61, 123.07, 123.20, 123.26, 123.38, 123.64, 125.08, 125.31, 127.24, 127.62, 127.80, 127.95, 127.98, 128.25, 128.35, 128.40, 128.85, 128.93, 129.02, 129.05, 129.47, 129.49, 130.27, 130.29, 130.33, 131.09, 131.39, 131.45, 131.53, 132.48, 133.22, 134.77.

2-(But-3-en-1-yn-1-yl)pyridine (205). Procedure M. $Pd(PPh_3)_4$ (34.67 mg, 0.03 mmol) and Cu(I)I (22.85 mg, 0.12 mmol) were placed in the flame-dried flask under N_2 at 0 ^{o}C (ice-bath). Then Et_2NH (1.50 mL, 1060 mg, 14.5 mmol) and vinyl bromide (1.0 M in THF; 4.0 mL, 4.0 mmol) were added following by slow addition of commercially available 2-ethynylpyridine **202** (310.0 mg, 3.0 mmol) dissolved in dry THF (2 mL) via a syringe pump (over 3 h). The resulting mixture was allowed to warm up to ambient temperature (30 min) and was stirred for another 2 h. Volatiles were evaporated and the residue was dissolved in EtOAc and filtered. The filtrate was collected and solvent was evaporated. The

residue was column chromatographed (10-30% EtOAc in hexane) to give **205** (300.0 mg, 77%) as a yellow liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.64 (dd, J = 11.2, 2.0 Hz, 1H), 5.85 (dd, J = 17.6, 2.0 Hz, 1H), 6.02 (dd, J = 17.6, 11.2 Hz, 1H), 7.21 (43 (dd, J = 7.2, 5.2 Hz, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.64 (td, J = 7.6, 1.6 Hz, 1H), 8.58 (d, J = 3.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 88.07, 89.14, 116.65, 122.86, 127.11, 129.05, 136.36, 143.45, 150.02.

3-(But-3-en-1-yn-1-yl)pyridine (206). Treatment of commercially available 3-ethynylpyridine **203** (310.0 mg, 3.0 mmol) with vinyl bromide by Procedure **M** (column chromatography; EtOAc in hexane 10-30%) gave **206** (270.0 mg, 70%) as a yellow liquid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 5.72 (dd, J = 11.2, 2.0 Hz, 1H), 5.82 (dd, J = 17.6, 2.0 Hz, 1H), 6.17 (dd, J = 17.6, 11.2 Hz, 1H), 7.43 (dd, J = 8.0, 4.8 Hz, 1H), 7.89 (dt, J = 8.0, 1.6 Hz, 1H), 8.57 (d, J = 4.0 Hz, 1H), 8.67 (s, 1H); ^{13}C NMR (DMSO-d_6, 100.6 MHz) δ 86.70, 91.24, 116.70, 119.36, 123.89, 129.05, 138.40, 149.08, 151.32.

4-(But-3-en-1-yn-1-yl)pyridine (207). Treatment of commercially available 4-ethynylpyridine **204** (310.0 mg, 3.0 mmol) with vinyl bromide by Procedure **M** (column chromatography; EtOAc in hexane 10-30%) gave **207** (290.0 mg, 75%) as a yellow liquid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 5.77 (dd, J = 11.2, 1.6 Hz, 1H), 5.88 (dd, J = 17.6, 1.6 Hz, 1H), 6.18 (dd, J = 17.6, 11.2 Hz, 1H), 7.47 (s, 2H), 8.69 (brs, 2H); ^{13}C NMR (DMSO-d_6, 100.6 MHz) δ 87.22, 92.41, 116.38, 116.44, 125.69, 129.94, 130.14, 130.18, 149.85.

(*E*)-2-(2-(Tributylstannyl)vinyl)pyridine (211). Procedure N. A flame dry round bottom flask equipped with a magnetic stirrer was charged with 2-bromopyridine **208** (191 µL, 316 mg, 2.0 mmol), *trans*-1,2-bis(tri-n-butylstannyl)ethylene (1.3 mL, 1470 mg, 2.4 mmol), dry toluene (10 mL) and $Pd(PPh_3)_4$ (46.2 mg, 0.04 mmol) and the resulting mixture

was deoxygenated with N_2. The reaction mixture was stirred at 100 ºC (oil bath) for 1 h. The volatiles were evaporated, and the residue was column chromatographed (0-10% EtOAc in hexane) to give **211** (633.4 mg, 80%) as a clear oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.90 (t, J = 7.6 Hz, 9H), 0.99 (t, J = 8.0 Hz, 6H), 1.35 (m, 6H), 1.54 (m, 6H), 7.02 (d, J = 19.6 Hz, 1H), 7.13 (m, 1H), 7.38 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 19.6, Hz, 1H), 7.66 (td, J = 7.6, 2.0 Hz, 1H), 8.54 (d, J = 4.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 9.81, 13.82, 27.46, 29.25, 120.87, 122.24, 136.27, 136.72, 145.94, 149.43, 156.18.

(*E*)-3-(2-(Tributylstannyl)vinyl)pyridine (212). Treatment 3-bromopyridine **209** (500 mg, 3.16 mmol) with *trans*-1,2-bis(tri-n-butylstannyl)ethylene by Procedure **N** (column chromatography; EtOAc in hexane 0-10%) gave **212** (1230 mg, 90%) as a clear oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.90 (t, J = 7.2 Hz, 9H), 0.98 (t, J = 8.0 Hz, 6H), 1.35 (m, 6H), 1.54 (m, 6H), 6.85 (d, J = 19.6 Hz, 1H), 6.99 (d, J = 19.6, Hz, 1H), 7.24 (m, 1H), 7.73 (d, J = 8.0 Hz, 1H), 8.45 (br, 1H), 8.61 (br, 1H). ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 9.82, 13.82, 27.41, 29.23, 123.61, 132.46, 133.63, 134.36, 142.55, 148.33, 148.53.

(*E*)-4-(2-(Tributylstannyl)vinyl)pyridine (213). Treatment 4-bromopyridine **210** (500 mg, 3.16 mmol) with *trans*-1,2-bis(tri-n-butylstannyl)ethylene by Procedure **N** (column chromatography; EtOAc in hexane 0-10%) gave **213** (1025 mg, 75%) as a clear oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.90 (t, J = 7.2 Hz, 9H), 0.99 (t, J = 8.0 Hz, 6H), 1.3 (sex, J = 7.2 Hz, 6H), 1.54 (m, 6H), 6.81 (d, J = 19.6 Hz, 1H), 7.18 (d, J = 19.6 Hz, 1H), 7.25 (dd, J = 4.4, 1.2 Hz, 2H), 8.54 (dd, J = 4.4, 1.6 Hz, 2H); ^{13}C NMR δ 9.80, 13.82, 27.40, 29.20, 120.57, 137.20, 143.70, 145.50, 150.26.

(*E*)-2-(2-(Iodovinyl)pyridine (214). Procedure O. The vinyl stannane **211** (600 mg, 1.52 mmol) was dissolved in Et_2O (10 mL) and elemental iodine (386 mg, 1.52 mmol;

dissolved in 5 mL Et_2O) was added dropwise while stirring at room temperature. After completion of addition, the reaction mixture was stirred for another 10 min. The excess I_2 was destroyed by aqueous $Na_2S_2O_3$ solution. The reaction mixture was extracted with Et_2O (30 mL x 3) and organic layer was separated. The combined organic layer was dried (Na_2SO_4) and evaporated. The residue was column chromatographed (0-10% EtOAc in hexane) to give **214** (288 mg, 82%) as a light-yellow liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.18 (m, 2H), 7.50 (s, 2H), 7.65 (td, J = 7.6, 2.0 Hz, 1H), 8.55 (d, J = 4.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 83.81, 121.69, 122.98, 136.84, 144.60, 149.90, 155.37.

(*E*)-3-(2-(Iodovinyl)pyridine (215). Treatment of vinyl stannane **212** (1200 mg, 3.04 mmol) with I_2 by procedure **O** (column chromatography; EtOAc in hexane 0-10%) gave **215** (650 mg, 92%) as a light yellow liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.98 (d, J = 14.8 Hz, 1H), 7.27 (m, 1H), 7.43 (d, J = 15.2 Hz, 1H), 7.62 (d, J = 8.0 Hz, 1H), 8.55 (br, 2H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 79.34, 123.81, 132.66, 133.61, 141.64, 147.60, 149.32.

(*E*)-4-(2-(Iodovinyl)pyridine (216). Treatment of **213** (1000 mg, 2.54 mmol) with I_2 by procedure **O** (column chromatography; EtOAc in hexane 0-10%) gave **216** (440 mg, 75%) as a light yellow liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.16 (dd, J = 4.4, 1.6 Hz, 2H), 7.21 (d, J = 14.8 Hz, 1H), 7.40 (d, J = 14.8 Hz, 1H), 8.57 (dd, J = 4.4, 1.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 83.04, 120.37, 143.02, 145.51, 150.53. This compound is very unstable, decomposes in open air and even in freezer when stored for more than 2 weeks.

(*E*)-2-(4-(Trimethylsilyl)but-1-en-3-yn-1-yl)pyridine (217). Procedure P. $Pd(PPh_3)_2Cl_2$ (32.8 mg, 0.047 mmol) and Cu(I)I (17.8 mg, 0.094 mmol) were added to dry

THF (5 mL) in a flame-dried flask equipped with a stir bar under N_2 at room temperature. Then iodovinylpyridine, **214** (270 mg, 1.17 mmol) was added followed by TMS-acetylene (250 μL, 172.4 mg, 1.76 mmol) and Et_3N (326 μL, 237 mg, 2.34 mmol). The resulting mixture was stirred for 1h [progress of the reaction was monitored by TLC (*n*-hexane)]. Volatiles were evaporated and the residue was column chromatographed (0-10% EtOAc in hexane) to give **217** as light brown liquid (226 mg, 96%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.23 (s, 9H), 6.75 (d, J = 16.0 Hz, 1H), 7.01 (d, J = 16.0 Hz, 1H), 7.16 (ddd, J = 7.6, 4.8, 0.8 Hz, 1H), 7.22 (d, J = 7.6 Hz, 1H), 7.63 (td, J = 7.6, 1.6 Hz, 1H), 8.56 (d, J = 4.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 0.03, 99.52, 104.16, 113.02, 122.43, 123.23, 136.87, 140.99, 149.72, 154.14.

(*E*)-3-(4-(Trimethylsilyl)but-1-en-3-yn-1-yl)pyridine (218). Treatment of iodovinylpyridine **215** (550 mg, 2.38 mmol) with TMS-acetylene by procedure **P (**column chromatography; EtOAc in hexane 0-10%) gave **218** (430 mg, 90%) as light brown liquid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.20 (s, 9H), 6.58 (d, J = 16.8 Hz, 1H), 7.04 (d, J = 16.4 Hz, 1H), 7.39 (m, 1H), 7.98(d, J = 7.6 Hz, 1H), 8.53 (brs, 1H), 8.74 (brs, 1H); ^{13}C NMR (DMSO-d_6, 100.6 MHz) δ 0.21, 97.64, 104.54, 110.15, 123.80, 131.54, 132.72, 139.02, 148.17, 149.60.

(*E*)-4-(4-(Trimethylsilyl)but-1-en-3-yn-1-yl)pyridine (219). Treatment of iodovinylpyridine **216** (462 mg, 2.0 mmol) with TMS-acetylene by procedure **P** (column chromatography; EtOAc in hexane 0-10%) gave **219** (322 mg, 80%) as a gummy solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.23 (s, 9H), 6.36 (d, J = 16.0 Hz, 1H), 6.90 (d, J = 16.0 Hz, 1H), 7.22 (d, J = 6.0 Hz, 2H), 8.55 (d, J = 5.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 0.09, 100.26, 103.23, 113.17, 120.73, 139.55, 143.37, 150.36.

(*E*)-2-(But-1-en-3-yn-1-yl)pyridine (220). Procedure Q. Anhydrous K_2CO_3 (166 mg, 1.2 mmol) was added to a stirred solution of **217** (201 mg, 1.0 mmol) in dry MeOH (5 mL) at room temperature. After for 30 min, volatiles were evaporated and the residue was column chromatographed (0-10% EtOAc in hexane) to give **220** (116 mg, 90%) as light yellow liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.15 (d, J = 2.4 Hz, 1H), 6.72 (dd, J = 16.0, 2.4 Hz, 1H), 7.05 (d, J = 16.0 Hz, 1H), 7.18 (dd, J = 7.2, 4.8, Hz, 1H), 7.23 (d, J = 8.0 Hz, 1H), 7.64 (td, J = 7.6, 1.6 Hz, 1H), 8.56 (d, J = 4.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 83.25, 84.73, 113.76, 124.47, 125.37, 138.76, 144.00, 151.93, 155.90.

(*E*)-3-(But-1-en-3-yn-1-yl)pyridine (221). Treatment Silyl protected compound **218** (403 mg, 2.0 mmol) with K_2CO_3 by Procedure **Q** (column chromatography; EtOAc in hexane 0-10%) gave **221** (243 mg, 94%) as a light yellow liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.11 (d, J = 2.4 Hz, 1H), 6.20 (dd, J = 16.4, 2.4 Hz, 1H), 7.01 (d, J = 16.4 Hz, 1H), 7.27 (dd, J = 8.0, 4.8, Hz, 1H), 7.71 (dt, J = 8.0, 1.6 Hz, 1H), 7.52 (d, J = 3.2 Hz, 1H), 8.61 (s, 1H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 80.61, 82.27, 109.67, 123.77, 131.76, 132.77, 139.45, 148.24, 149.81.

(*E*)-4-(But-1-en-3-yn-1-yl)pyridine (222). Treatment **219** (220 mg, 1.09 mmol) with K_2CO_3 by Procedure **Q** (column chromatography; EtOAc in hexane 10-20%) gave **222** (243 mg, 94%) as a light yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.18 (d, J = 2.4 Hz, 1H), 6.32 (d, J = 16.4 Hz, 1H), 6.95 (d, J = 16.4 Hz, 1H), 7.23 (dd, J = 4.4, 1.6 Hz, 2H), 8.58 (dd, J = 4.4, 1.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 81.91, 112.15, 120.59, 140.55, 143.05, 150.54.

(*Z*)-3-(4-(Trimethylsilyl)but-1-en-3-yn-1-yl)pyridine (223). Treatment of commercially available (*Z*)-3-(2-(bromovinyl)pyridine (300 mg, 1.63 mmol) with TMS-

acetylene by procedure **P** (column chromatography; EtOAc in hexane 0-10%) gave **223** (322 mg, 98%) as light brown liquid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.23 (s, 9H), 5.98 (d, J = 12.4 Hz, 1H), 6.86 (d, J = 12.0 Hz, 1H), 7.43(dd, J = 8.0, 4.8 Hz, 1H), 8.37 (d, J = 8.0 Hz, 1H), 8.52 (br, 1H), 8.95 (br, 1H); ^{13}C NMR (DMSO-d_6, 100.6 MHz) δ 0.46, 103.07, 103.43, 109.40, 123.28, 131.67, 134.60, 136.67, 149.38, 149.73.

(*Z*)-3-(But-1-en-3-yn-1-yl)pyridine (224). Treatment **223** (300 mg, 1.49 mmol) with K_2CO_3 by Procedure **Q** (column chromatography; EtOAc in hexane 0-10%) gave **224** (164 mg, 85%) as a light yellow liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.41 (dd, J = 2.4, 0.8 Hz, 1H), 5.82 (dd, J = 12.0, 2.4 Hz, 1H), 6.70 (d, J = 12.0 Hz, 1H), 7.30 (dd, J = 8.0, 4.8, Hz, 1H), 8.38 (dt, J = 8.0, 1.6 Hz, 1H), 7.52 (d, J = 4.0 Hz, 1H), 8.88 (s, 1H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 81.36, 85.47, 109.19, 123.39, 132.07, 135.16, 136.95, 149.42, 150.31.

5. CONCLUSION

In this dissertation, I have developed two mechanistically different probes attached to the C8 or C2 positions of adenine and C8 position of guanine nucleosides. One is (*β*-iodovinyl)sulfone probe, which efficiently reacts with nucleophiles, such as amonia via the conjugated addition-elimination pathway. The other one is *β*-ketosulfone probe, which traps electrophiles such as alkyl halides. I have also synthesized 8-(2-Tosylacetyl)-dATP and 2-carboxylate-dATP, which were efficiently incorporated into double-stranded DNA using one nucleotide gap substrates by human DNA polymerase. Moreover, I have developed chemical synthesis methods to prepare larger quantities of the novel antibiotic arsinothricin (AST) for further drug development. Additionally, I have described several novel methods to synthesize various polycyclic aromatic hydrocarbons and their "enyne" derivatives which were used as precursors or calibration compounds for the gas phase synthesis of PAHs.

The novel reactive (*β*-iodovinyl)sulfone group at the C8 position of adenine nucleosides (*i.e.* **42** and **43**) were synthesized via iodovinylsulfonation of 8-ethynyl precursors with $TsNa/I_2$ presence of NaOAc in acetonitrile at elevated temperature. The (*β*-iodovinyl)sulfone group at the C8 position of 2'-deoxyguanosine (**60**) was instead prepared via the radical mediated iodovinylsulfonation of 8-ethynyl-2'-deoxyguanosine with $TsNHNH_2$/KI in the presence of benzoyl peroxide in DMSO at ambient temperature. On the other hand (*β*-iodovinyl)sulfone group at the C2 position of adenine nucleosides (*i.e.* **76** and **77**) was installed by iodovinylsulfonation of 2-alkynyl precursors with freshly prepared TsI in presence of NaOAc in THF at room temperature. It was found that unlike terminal vinylsulfones; which react with nucleophiles via Michael addition, (*β*-

iodovinyl)sulfones underwent conjugated addition-elimination reaction with nucleophilic methanolic ammonia at ambient temperature resulting in quantitative conversion to the corresponding enamines (*i.e.* **49**, **50**, **64**, **82** and **83**). Interestingly, although the (*β*-iodovinyl)sulfone substrates possess *E/Z* stereochemistry, the addition-elimination (*β*-aminovinyl)sulfone products are formed only as *Z* isomers. The acetic acid hydrolysis of the intermediary *β*-aminovinylsulfones provided the *β*-ketosulfones motif in high yields.

Since the *α*-hydrogen of methylene unit of *β*-ketosulfone is acidic, in the presence of weak bases, it can generate enolate which can trap electrophiles. It was found that the *β*-ketosulfones underwent efficient reaction with different electrophiles such as benzyl bromide or allyl bromide in the presence of aqueous NaOH at room temperature resulted in a diastereotopic mixture of α-monobenzylated products (*i.e.* **67**, **68** and **88**). These results encouraged us to believe that *β*-ketosulfonyl-dATP might be viable for the bioconjugation with proteins.

The nucleosides with 8-*β*-ketosulfone group were converted to their 5'-triphosphate **(89**) which was efficiently incorporated into double-stranded DNA using one nucleotide gap substrates by human DNA polymerase catalyzed reactions. Although it has been reported that 8-substitued purine nucleotides with bulky group are poor substrates for polymerases, our results indicate that purine nucleotide with bulky *β*-ketosulfone group at C8 position is good substrate for polymerase. Our results also suggest that if 8-(*β*-keto)sulfone-5'-triphosphates are generated in cells, they could be incorporated into genomic DNA by DNA polymerases during DNA replication and repair. For 2-modified adenine nucleosides, we found interesting and unexpected conversion of 2-(2-Tosylacetyl)-dATP/ATP to 2-caroxylate-dATP/ATP (*i.e.* **90** and **91**). The 2-caroxylate-dATP also

efficiently incorporated into double-stranded DNA using one nucleotide gap substrates by human DNA polymerase. Therefore, the modification at C2 can be used to investigate the effect of the functional groups at the position on their insertion by DNA polymerases.

Recently discovered arsenic-containing compound arsinothricin (AST; **105**), synthesized by the rice rhizosphere bacterium, demonstrates broad-spectrum antibiotic activity and is effective against both Gram-positive and Gram-negative bacteria. We developed a semi-synthetic procedure that involves chemical synthesis of the precursor hydroxyarsinothricin (AST-OH; **103**) from sodium arsenite via 2-chloroethyarsonic acid (**100**) intermediate. The AST-OH was then enzymatically methylated to AST using the robust thermostable enzyme CmArsM, the As(III) *S*-adenosylmethionine methyltransferase which is the first report of semi-synthesis of AST.

To reduce effort and complexities associated with synthesis of pure AST from either bacterial culture or enzymatic methylation, we also developed two straightforward chemical methods for the synthesis of racemic AST. One is by reduction of the *N*-acetyl protected analogue (**113**) of AST-OH and subsequent methylation of the resulting trivalent arsenic intermediate with methyl iodide. While the second method involves condensation of the 2-chloroethyl(methyl)arsinic acid (**109**) with diethylacetamidomalonate followed by deprotection and decarboxylation. Enzymatic separation by enantioselective acetylation with enzyme AST *N*-acetyltransferase (ArsN1) was utilized to purify acetylated L-AST from racemic AST. Subsequent chemical deacetylation with aqueous HCl afforded pure L-AST. This convenient chemical synthesis can be scaled up to gram quantities to produce AST in sufficient amounts of this novel antibiotic for future drug development.

I also developed several protocols to synthesize different polycyclic aromatic hydrocarbons (PAHs) containing two to six ring system and their derivatives, which were utilized as precursors or calibration compounds for the gas phase synthesis of PAHs through the exploitation of molecular beam experiments mimicking the conditions of extreme environments from low temperatures settings to high-temperature environments, like circumstellar envelopes of carbon-rich asymptotic giant branch stars. Furthermore, I have developed an expeditious novel synthesis of 4-, 5-, 6-, and 7-iodoindenes isomers from the corresponding aminoindan-1-ones via three-step sequence which involved diazotization-iodination of aminoindan-1-ones followed by the reduction and dehydration. Moreover, I have developed protocols for the synthesis of enyne derivatives of phenanthrene at 4- and 9-positions (i.e. **177**, **181**, **184**, and **187**) applying conventional Sonogashira and boronic acid coupling. Additionally, I have synthesized a novel 7-ethynylfluoranthene (**192**) molecules which was used a calibration compound for gas phase synthesis of corannulene ($C_{20}H_{10}$) a molecular building block for 3D nanostructures.

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3. A. Hasan Howlader, Richard Fernandez, Pawlos S. Tsegay, Yuan Liu and Stanislaw F. Wnuk, Purine Nucleosides with a Reactive (*β*-Iodovinyl)sulfone or (*β*-Keto)sulfone Group at the C2 Positions. 2-Carboxylate dATP as Substrate for Polymerase-Catalyzed Incorporation into DNA. *To be submitted.*

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