Synthesis and Biochemical Evaluation of Adenosylspermidine, a Nucleoside–Polyamine Adduct Inhibitor of Spermidine Synthase

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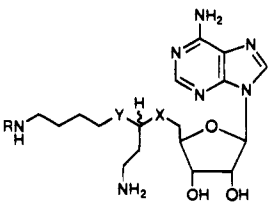
The synthesis of a new class of multisubstrate adduct inhibitors of polyamine biosynthesis has been investigated. The first target compound, designed to inhibit spermidine synthase, was obtained and proved to be a very potent inhibitor of that enzyme. Two synthetic routes to effect the coupling of the polyamine spermidine to the nucleoside adenosine were studied. The first route involved a proposed Wittig or Julia olefination reaction to form the critical 5′-6′ carbon–carbon bond between the nucleoside and polyamine moieties This route failed due to a facile β-elimination of a portion of the side chain from a carbanion intermediate during either coupling reaction. A second route involved a reductive amination approach and proved to be successful. The new inhibitor, given the trivial name adenosylspermidine, is the most potent inhibitor of spermidine synthase prepared to date.

During the past two decades, considerable progress has been made on the design and synthesis of potent, specific enzyme inhibitors based on mechanistic enzymology.[1,2] A major portion of our research effort has focused on enzyme-catalyzed alkyl transfer reactions including those catalyzed by the methyltransferases and aminopropyltransferases. These enzymes use *S*-adenosylmethionine (AdoMet) and the decarboxylated derivative dcAdoMet as electrophilic substrates which donate methyl and aminopropyl groups, respectively, to a variety of cellular nucleophiles. On the basis of kinetic[3] and stereochemical[4] data, we concluded that the methyltransferase-catalyzed reactions proceed via a ternary complex consisting of the enzyme and the two substrates. Similarly, research on the stereochemical course of the aminopropyltransferase reaction[5] provided strong evidence that it also proceeds via a ternary complex. Not surprisingly, analogues of *S*-adenosylhomocysteine (AdoHcy) and 5′-deoxy-5′-methylthioadenosine (MTA), the common nucleoside products of all reactions catalyzed by methyltransferases and aminopropyltransferases, respectively, show a lack of specificity as inhibitors of these enzymes.[6,7] However, for enzymes which bind two ligands or substrates in a ternary complex, such as the alkyltransferases, one can use the "multisubstrate adduct" approach.[2,8] We have applied this to the methyltransferases catechol *O*-methyltransferase (COMT, EC 2.1.1.6)[9–11] and phenethanolamine *N*-methyltransferase (PNMT, EC 2.1.1.28)[11] and to the aminopropyltransferases spermidine synthase (putrescine aminopropyltransferase, PAPT, EC 2.5.1.16)[12] and spermine synthase (spermidine aminopropyltransferase SAPT, EC 2.5.1.22).[13] The potent and specific inhibitors of PAPT and SAPT, *S*-adenosyl-1,8-diamino-3-thiooctane (AdoDATO, **1a**) and *S*-adenosyl-1,12-diamino-3-thio-9-azadodecane (AdoDATAD, **1b**), respectively, have been especially useful as modulators of polyamine biosynthesis in intact mammalian cells[14,15] and have revealed new mechanisms for the regulation of polyamine biosynthesis.[16]

In this paper, we describe the synthesis of 3-(*RS*)-(5′-deoxy-5′-carbaadenos-6′-yl)spermidine (adenosylspermidine, AdoSpd, **1c**) and provide preliminary biochemical data which demonstrate that **1c** is a potent and selective inhibitor of spermidine synthase. This compound was chosen as a synthetic target for three reasons: (1) The proposed transition state shown below for the APT-catalyzed reactions[5] places partial positive charge on the attacking primary amine of putrescine (PAPT) or spermidine (SAPT). This charged transition state is apparently stabilized by the enzyme, thus leading to the large rate enhancements,[17] and protonation of the appropriately positioned nitrogen of **1c** may lead to tight binding of this ligand, resulting in potent enzyme inhibition. (2) These proposed "second-generation" multisubstrate (MSA) inhibitors of PAPT (**1c**) or SAPT (**1d**) are substituted polyamines and, as such, may be substrates for the polyamine transport protein,[18] thus facilitating their transport across cellular membranes. (3) Earlier work in our laboratory[10] and elsewhere[19] provided a synthetic method for the coupling of complex side chains to adenosine via a 5′-6′ carbon–carbon bond using Wittig chemistry.



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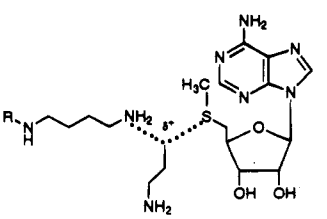
a. R = H, Y = CH_2, X = S (AdoDATO)
b. R = $H_2N(CH_2)_3$, Y = CH_2, X = S (AdoDATAD)
c. R = H, Y = NH, X = CH_2 (AdoSpd)
d. R = $H_2N(CH_2)_3$, Y = NH, X = CH_2 (AdoSpm)



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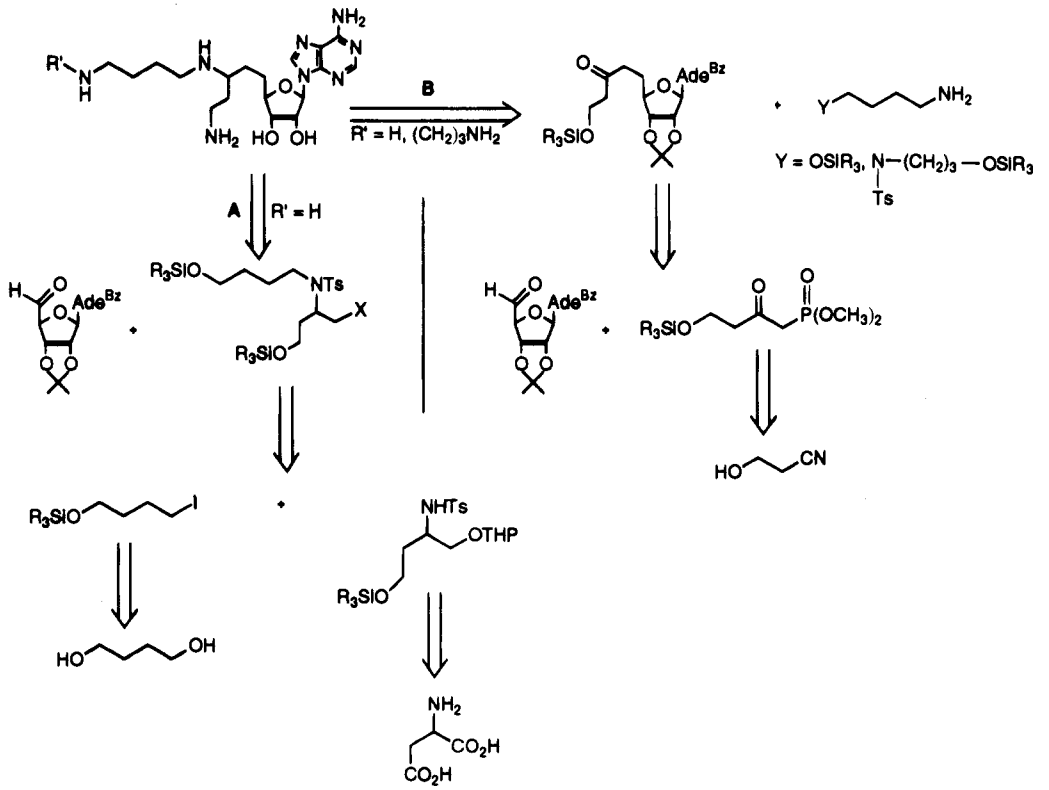
Scheme 1

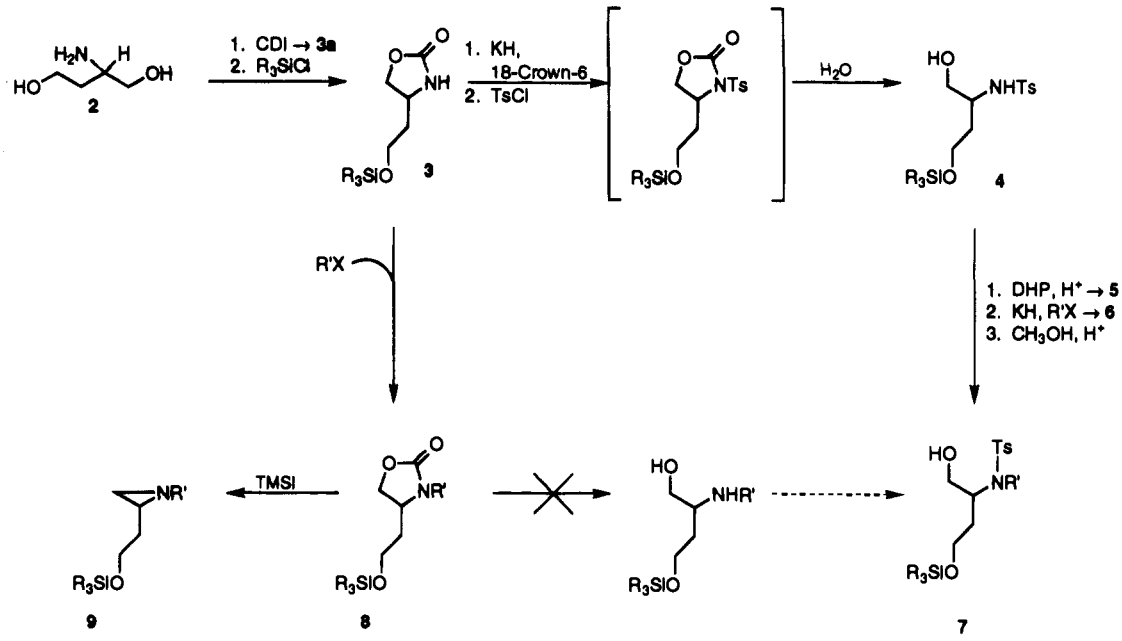


Chemistry

Our retrosynthetic analysis is shown in Scheme 1 and suggests two routes to this class of compounds, i.e., **1c,d**. In route A, formation of the key 5′-6′ C–C bond is effected via an olefination procedure, such as a Wittig reaction (X = PPh_3)[20] or Julia coupling (X = SO_2Ph),[21] using a protected adenosine-5′-carboxaldehyde as the electrophilic partner. The nucleophilic partner, a protected 3-substituted-4-aza-1,8-octanediol, is in turn derived ultimately from 1,4-butanediol and aspartic acid. The possible use of either D- or L-aspartic acid as a starting material was an important factor in the synthetic design. If successful, this approach should allow for the stereospecific synthesis of both diastereomers of **1c** and **1d**. We have already reported that the *R*- and *S*-diastereomers of AdoDATO (**1a**) have different inhibitory properties as inhibitors of spermidine synthase[22] as well as different rates of transport in Chinese hamster ovary (CHO) cells.[23] Thus, the opportunity to effect a stereospecific synthesis of both isomers was a key factor in favor of this approach (route A) vs a less convergent, achiral route. In the alternative route (route B), the key 5′-6′ C–C bond is formed via a Horner–Wadsworth–Emmons (HWE) Wittig reaction between a β-keto phosphonate and the same adenosine-5′-carboxaldehyde used in route A. Reductive amination of the resultant 7′-ketonucleoside involves a suitably protected butylamine derivative where Y = OSiTBDP or N(Ts)$(CH_2)_3$OSiTBDP for the synthesis of **1c** and **1d**, respectively. The β-keto phosphonate is derived from 3-hydroxypropionitrile.

Our initial research focused on route A, and the initial steps of the synthesis are outlined in Scheme 2. Cyclization of the aspartidiol **2**[24] to oxazolidinone **3** was accomplished in 57% overall yield using carbonyldiimidazole (CDI) in DMF. The intermediate alcohol was silylated *in situ* using *tert*-butyldiphenylsilyl chloride (TBDPSiCl). This procedure facilitated the purification of the cyclization product **3** since the intermediate alcohol **3a** is water soluble and therefore is lost in an organic–aqueous workup of the reaction. The five-member ring structure of **3** was confirmed by desilylation using tetrabutylammonium fluoride (TBAF) to regenerate the intermediate alcohol **3a** in pure form, suitable for ^1H NMR decoupling experiments. In the absence of certain proton resonances of the TBDPSi protecting group, all the protons in **3a** could be unambiguously assigned. The furthest downfield protons located α to the oxazolidinone ring oxygen are strongly coupled to the ring methine proton. This would not be the case if **3a** were the six-member ring product. Additionally, mass spectra, using electron impact ionization, show a distinct loss of hydroxyethyl from the parent ion rather than hydroxymethyl expected from the six-member carbamate. The oxazolidinone **3** was a key intermediate in the synthesis strategy. The oxazolidinone ring nitrogen could be directly alkylated with a variety of alkyl halides (**3** → **8**), but subsequent opening of the ring proved to be especially difficult. Tosylation of the ring nitrogen of **3** proved to be the best method for both protection of the nitrogen and subsequent ring opening, thereby leading to the alcohol **4** in 65% yield. The electron-withdrawing effect of the *N*-tosyl group destabilizes the oxazolidinone ring enough to allow for ring opening during workup of the reaction ($MeOH/H_2O$). 4-[(*tert*-Butyldiphenylsilyl)oxy]-1,4-butanediol[25] was converted to the corresponding iodide TBDPSiO$(CH_2)_4$I by reaction with triphenoxymethylphosphonium iodide. Direct aminoalkylation of **4** with the resultant iodide using potassium hydride in THF proved to be unsatisfactory. A rapid alkoxide-induced elimination of iodide gave 4-[(*tert*-butyldiphenylsilyl)-

Scheme 2[a]



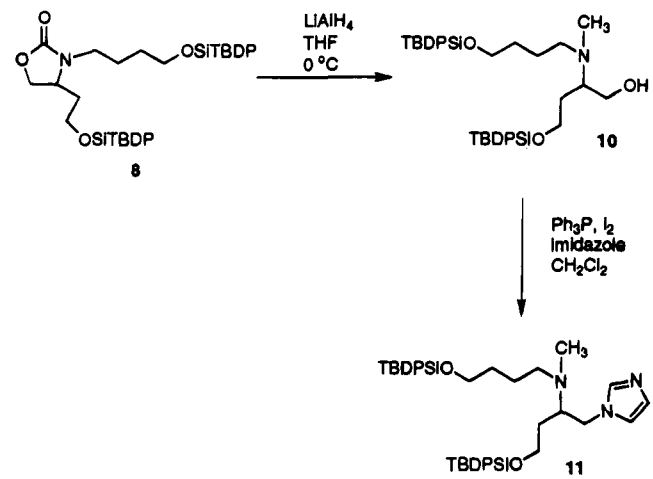
[a] R′ = $(CH_2)_4OSiR_3$; R_3Si = TBDPSi.

oxy]-1-butene in quantitative yield. This elimination reaction was prevented by protection of the hydroxyl function of **4** using DHP to give the THP ether **5** in 90% yield. Subsequent aminoalkylation resulted in the fully protected side chain **6** in 64% yield. Deprotection of **6** using catalytic tosic acid in $MeOH/CH_2Cl_2$ gave the alcohol **7** in 87% yield.

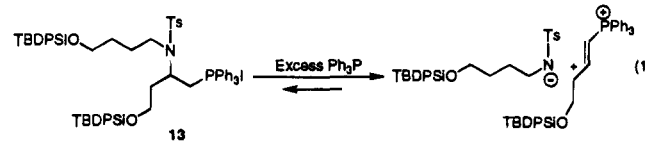
The formation of a 2-substituted *N*-tosylaziridine as a minor product was observed on workup of the reaction of **3** and excess TsCl. It occurred to us that a suitably substituted *N*-tosylaziridine might function as a useful phosphonium salt precursor (e.g., Scheme 4, **9** → **13**). Although reaction of **8** and TMSBr failed to produce the expected 2-bromoamine aziridine precursor, facile conversion of **8** to the desired aziridine **9** was observed on reaction with TMSI. Unfortunately, this substituted aziridine could not be converted to the analogous phosphonium salt by sequential reaction with TsCl and Ph_3P in a limited set of experiments. However, reduction of **8** with $LiAlH_4$ gave the β-hydroxy *N*-methylamine **10** which, on reaction with Ph_3P, I_2, and imidazole, provided a 93% yield of the β-imidazolyl amine **11**, presumably arising from nucleophilic attack by imidazole on an N-CH_3 aziridinium intermediate (Scheme 3). Thus, the use of aziridinium salts, generated *in situ*, and their reaction with nucleophiles (e.g., RSH) for the formation of olefination reagents remains a possibility.

Iodination of alcohol **7** using triphenylphosphine, I_2, and imidazole gave the iodo intermediate **12** in 84% yield (Scheme 4). The iodinated compound **12** is another key intermediate and provides for flexibility in the olefination reaction. The iodide **12** can be converted to the phosphonium salt **13** (Wittig) or the thioether **14** for eventual conversion to sulfone **15** (Julia). The Wittig route was pursued first by conversion of **12** to the phosphonium salt **13** in 39% optimized yield. This low yield was obtained in a neat melt of 1.1 equiv of Ph_3P. If the number of equivalents of Ph_3P was increased, a competing elimination reaction dominated the reaction

Scheme 3

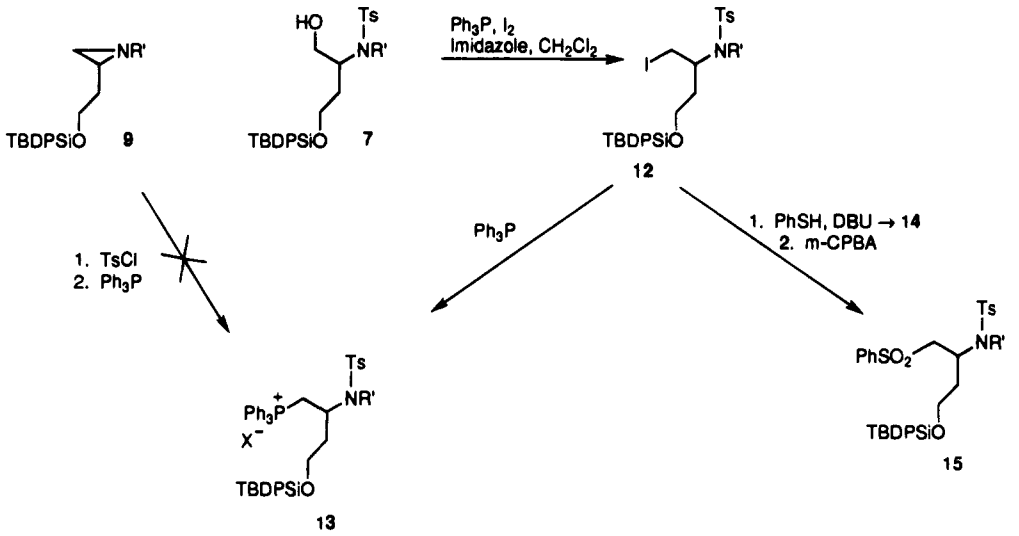


course (eq 1). Apparent from this reaction is the



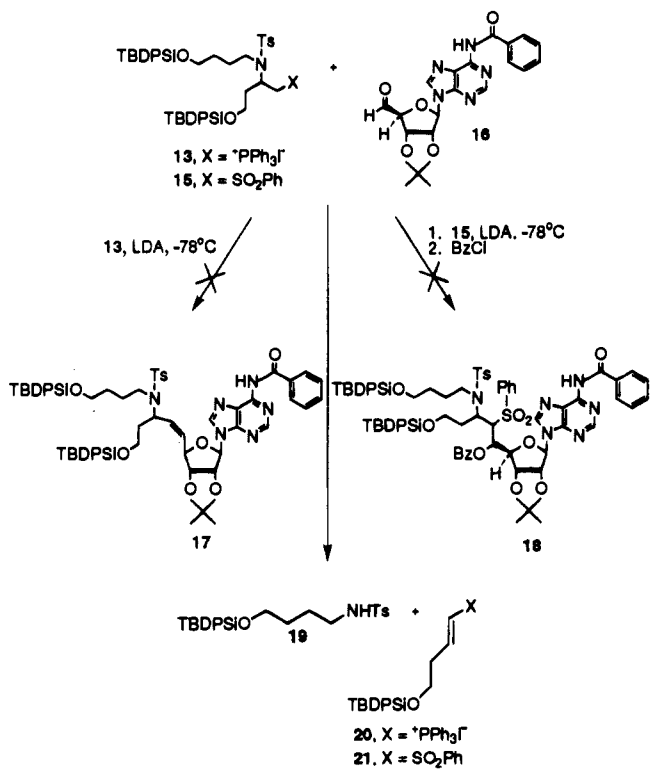
stability of the *N*-tosyl anion as a leaving group, and with hindsight, this is a serious concern in the Wittig and Julia olefination reactions to be discussed later. The strenuous conditions necessary for generating the phosphonium salt were perplexing. It may be that β-branched alkyl halides such as **12** are too hindered for S_N2 attack. Kocienski reported a similar problem generating a phosphonium salt from a β-alkyl-branched iodide.[26] They resorted to a Julia olefination to synthesize their target molecule. Prompted by Kocienski's results, the alternate Julia olefination procedure was also attempted in the present work. Reaction of iodide **12** with thiophenol in the presence of DBU at room temperature smoothly gives thioether **14** in 70% yield (Scheme 4). Oxidation of **14** to sulfone **15** with *m*-CPBA was effected

Scheme 4[a]



[a] R′ = $(CH_2)_4OSiTBDP$; X = I, Cl.

Scheme 5



in 70% yield. This completed the synthesis of both side chain olefination reagents.

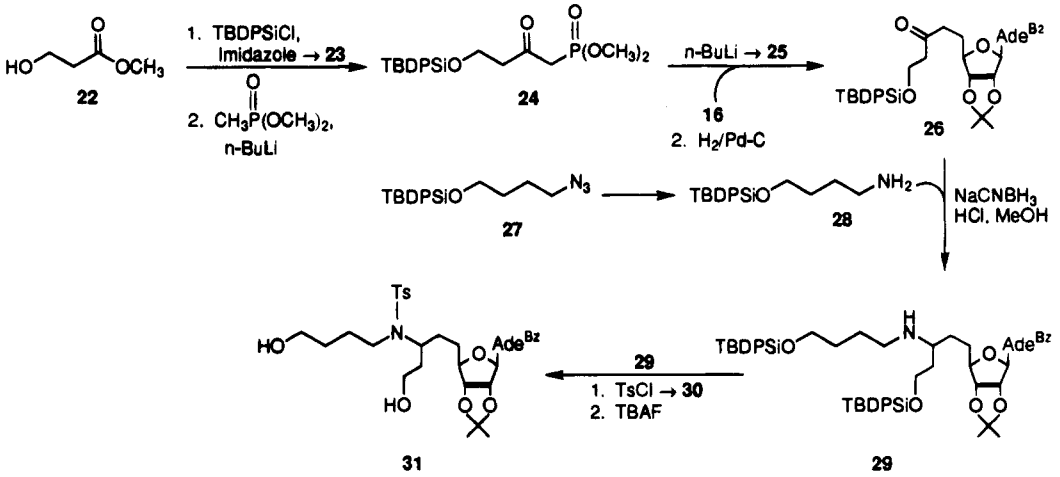
Unfortunately, attempts to react **13** or **15** in either a Wittig or Julia olefination reaction, respectively, failed to give any of the desired products (Scheme 5). The only product isolated from the Wittig reaction, the *N*-tosylamine **19**, resulted from a β-elimination reaction. The aldehyde **16** is not stable to silica gel chromatography and could not be recovered; apparently the vinyl phosphonium salt **20** decomposes as well. However, **19** was isolated in quantitative yield, accounting for all the potential ylide. Likewise, **19** and the corresponding vinyl sulfone **21** were isolated in quantitative yield from the attempted Julia olefination. It appears that the tosylamide protecting group of **13** and **15** sufficiently stabilizes a negative charge on nitrogen so that the conjugate base of **19** is a good leaving group.

With the problems encountered in the synthesis described above, a new disconnection of the target compound **1c** was devised. The new route would eliminate the possibility of β-elimination during the key 5′-6′ C−C bond formation step, taking advantage of β-keto phosphonates as well-established synthons, e.g., in the synthesis of various compactin analogues.[27-29] The proposed alternative synthesis is based on the retrosynthetic analysis shown in Scheme 1 (route B) and has already been discussed briefly. Key features include the use of β-keto phosphonates to alkylate the 5′-nucleoside aldehyde without the possibility of β-elimination of the side chain as in the previous synthesis. Also, this route relies principally on a reductive amination of a ketone with a protected (substituted) aminobutyl side chain. In theory, it would be advantageous to have all the side chain amino groups attached in protected form so it would be unnecessary to introduce them later. This strategy results in some protecting group problems which are detailed below.

β-Alanine methyl ester appeared to be an ideal starting material for the synthesis of a properly functionalized 7′-ketonucleoside derivative. Unfortunately, attempts to construct an appropriate β-keto phosphonate containing a δ-amino functionality were thwarted.[30] At this point, a hydroxyl group was considered as a suitably stable amine precursor functionality for synthesis of the β-keto phosphonate. The hydroxyl function could then be converted to an amine later in the synthesis. Protection of the terminal alcohol function of **22**[31] was accomplished using *tert*-butyldiphenylsilyl chloride to give the protected ester **23** in 50% yield (Scheme 6). The low yield encountered at this step results from β-elimination of silanol, probably catalyzed by imidazole, to produce methyl acrylate. Strong odors of methyl acrylate were present upon workup of the reaction. This problem was reported in a similar system by Heathcock[27] and Karanewsky[29] in the synthesis of compactin analogues. Practically, this is not a problem since at this early stage of the synthesis the scale is sufficiently large so as to offset the low yield.

Dimethyl methylphosphonate was then treated with *n*-butyllithium and condensed with **23** to give the elongated β-keto phosphonate **24** in 38% yield. Elimi-

Scheme 6



nation of silanol was a problem again in this reaction in addition to low reactivity of the ester **23**. However, the unreacted ester could be recovered and recycled in the reaction. HWE Wittig coupling of 5′-nucleoside aldehyde **16** with excess **24** was effective and gave α,β-unsaturated ketone **25** in what appeared to be good yield from mass recovery. This yield was difficult to determine due to contamination from unreacted β-keto phosphonate **24**. The β-keto phosphonate coelutes with the ketone **25** under normal silica gel chromatography. ^{1}H NMR evaluation of the product shows a vinyl proton coupling constant of 16 Hz which supports exclusive formation of the *trans* double-bond stereochemistry, as expected from a reaction involving a stabilized ylide. Hydrogenation of the mixture of **24** and **25** gave **26** (and unreacted **24**) in quantitative mass recovery. Preparative HPLC gave pure **26** in 53% yield overall from **24**. The excess unreacted β-keto phosphonate **24** was recovered (63%) and recycled. Interestingly, β-keto phosphonates are quite often used in HWE Wittig couplings run at ambient temperature using aliphatic amines as bases. The mixture of starting materials is stirred together overnight in a procedure which is operationally convenient. When this procedure was applied to the coupling of stoichiometric amounts of phosphonate **24** and aldehyde **16**, an isomerized β,γ-unsaturated ketonucleoside was obtained in 60% yield. Presumably, the desired α,β-unsaturated ketone **25** forms initially followed by isomerization of the double bond to the more substituted alkene in the presence of triethylamine. The β,γ-unsaturated product was resistant to catalytic reduction.

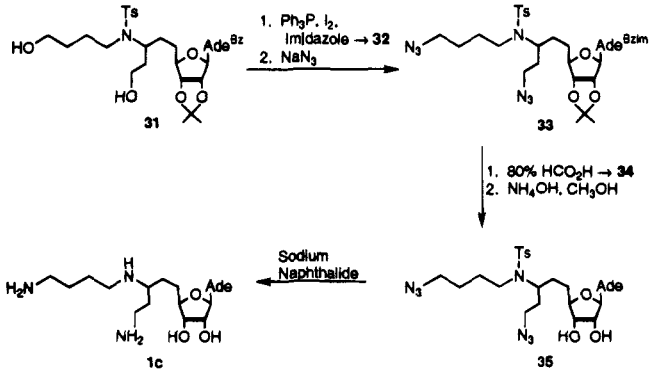
Having successfully completed the synthesis of **26**, it was necessary to construct the side chain amine for reductive coupling as envisioned in our retrosynthetic analysis (Scheme 1). 4-Chlorobutanol was reacted with sodium azide in DMF to generate 4-azidobutanol which was directly protected with *tert*-butyldiphenylsilyl chloride *in situ* to give the corresponding silyl ether **27** in 41% overall yield. The modest yield obtained in this step is most likely due to cyclization of the azidobutanol to form THF in the presence of imidazole. The azide function of **27** was then reduced under hydrogenation conditions to give the free amine **28** in quantitative yield.

With the side chain amine **28** synthesized, reductive amination of the ketone **26** was investigated (Scheme 6). Reductive amination of **26** with **28** proved to be difficult under a variety of conditions. Catalytic hydrogenation of **26** and **28** under basic conditions failed to give any coupling product. Unreacted starting materials were detected by TLC. These two compounds (**26** and **28**) decomposed when concentrated together. Apparently debenzoylation of **26** becomes a significant side reaction on removal of the reaction solvent in vacuo in the presence of an amine such as **28**. Since reductive amination of ketones suffers from an unfavorable ketone–imine equilibrium under basic conditions, an attempt to preform the imine was attempted. Azeotropic removal of water from the reaction using dry benzene (Dean–Stark apparatus) with a catalytic amount of PPTS failed to give any imine-like products.

Aza–Wittig reactions are often used to form imines under somewhat milder conditions using triphenylphosphine and azides.[32,33] This methodology appeared to be useful for this synthesis. Mixing azide **27** with triphenylphosphine under neat conditions rapidly evolved N_2 gas. In more dilute solutions in benzene, a somewhat longer reaction time under reflux conditions was necessary to consume all of azide **27**. However, addition of ketone **26** to the preformed (presumed) phosphoimine followed by overnight stirring at ambient temperature failed to show any consumption of **26**. It may be that the phosphoimine of **27** is inherently unstable and cyclizes to form THF before reaction with ketone **26**. However, heating the concentrated mixture at 85 °C consumed all of **26** in 3 h. The crude reaction mixture was then reduced under hydrogenation conditions overnight in an attempt to form the desired secondary amine **29**. TLC of this crude reaction product did not indicate that any of amine **29** was formed under these conditions.

Reductive amination was then attempted according to the procedure described by Borch et al.[34] Ketone **26** was reacted with amine **28** in MeOH using sodium cyanoborohydride and HCl to form amine **29** in 63% yield. The key to this successful reaction is careful control of the ambient pH in the reaction. An internal indicator, bromothymol blue, was used to titrate the proper amount of HCl (saturated solution in MeOH) into the reaction. Imine formation is highly sensitive to pH and is optimized between pH 6 and 7. Protection of the central nitrogen of **29** was accomplished with tosyl chloride to give tosylamide **30** in 61% yield, and removal

Scheme 7



of the silicon protecting groups using TBAF gave the diol **31** in 71% yield (Scheme 6).

As shown in Scheme 7, iodination of diol **31** gave an unusual product which proved to be a N^6-benzoylimidazole (BzIm)[35] derivative in 76% yield.[36] The BzIm derivative results from the addition of imidazole to the benzoyl carbonyl with subsequent dehydration to give the imine. This result is somewhat surprising given that the N^6-adenosine amine has a relatively low pK_a of 3.5 and hence is a good leaving group. Iodine must act as a Lewis acid to catalyze this reaction since imine formation is disfavored at higher pH. Evidence for the assigned structure of the N^6-benzoylimidazole is derived from mass spectral and UV analysis. The observed molecular ion results from the addition of imidazole and loss of H_2O from the expected mass of the N^6-benzoyl derivative. Facile fragmentation results in loss of 68 mass units which corresponds to the formula weight of imidazole. UV spectra show broad absorption into the 300+ nm range in contrast to the relatively sharp 280 nm absorption of N^6-benzoyladenosines. This extension of absorption into the visible range is confirmed by a slight yellow color of the N^6-BzIm derivative, resulting from the extended conjugation of the π electrons from the adenine ring into the imidazolyl and benzoyl moieties. The diiodido compound **32** could be converted into the diazido compound **33** by reaction with excess NaN_3 in DMF at ambient temperature overnight but in only 38% yield. Apparently, the modified N^6 protecting group is unstable, and the reaction led to a mixture of two other compounds along with the desired product **33**.[37]

Stirring compound **33** in 80% formic acid rapidly converts the modified N^6 protecting group to the N^6-benzoyl group as shown by TLC. This intermediate then loses the isopropylidene group to form diol **34** which has a UV absorption maximum at 280 nm. This UV absorption is consistent with a N^6-benzoyladenine. Reaction of **34** with NH_4OH in MeOH at ambient temperature overnight results in deprotection at the N^6 position to give compound **35** which has a UV absorbance maximum at 254 nm. All of these transformations were monitored by a HPLC diode array spectrophotometer and gave very clean products which were taken on without purification. Finally, nucleoside **35** was treated with sodium naphthalide in dry DME to remove the N-tosyl group. HPLC results indicated that all of nucleoside **35** was converted to a more polar compound, consistent with the expected chromatographic behavior of the desired product **1c**. ^1H NMR analysis after purification by preparative scale HPLC is also consistent with the structure of **1c**. Unfortunately, the HPLC purification failed to remove all the inorganic salts remaining from the final reaction.

Table 1. Effects of **1** on Enzymes of Polyamine Metabolism

	IC_{50}, nM		
enzyme	AdoSpd (**1c**)	AdoDATO (**1a**)	AdoDATAD (**1b**)
PAPT[a]	14 (3)[b]	100 (250)	>10 000
SAPT	750	>200 000	~50
ODC	NI[c]	ND[d]	
AdoMetDC	NI[c]	ND[d]	

[a] Rat liver; *E. coli* in parentheses. [b] No effect of 40 mM NaCl, NaO_2CH, or NH_4O_2CH. [c] NI, not inhibited. [d] ND, not determined.

Biochemical Results

Enzyme Assays. Putrescine aminopropyltransferase (PAPT) and spermidine aminopropyltransferase (SAPT) were both assayed with AdoSpd (**1c**). AdoSpd showed potent concentration-dependent inhibition of mammalian (rat liver) PAPT, IC_{50} = 14 nM (Table 1). A prokaryotic (*Escherichia coli*) PAPT was similarly inhibited, IC_{50} = 3 nM (Table 1). SAPT was less potently inhibited, IC_{50} = 750 nM. These IC_{50} values for PAPT using AdoSpd are quite remarkable when compared to the IC_{50} of 100 nM for AdoDATO (**1a**; Table 1). As discussed earlier, AdoDATO provided the structural basis for the design of AdoSpd and was the most potent inhibitor of PAPT known to date. AdoSpd is less selective than AdoDATO and inhibits SAPT to a significant extent. AdoDATAD (**1b**) potently and selectively inhibits SAPT, IC_{50} = 50 nM (Table 1). From these results, it may be inferred that the proposed inhibitor analogue AdoSpm (**1d**) may inhibit SAPT more potently than AdoDATAD.[38]

AdoSpd showed no significant inhibition of either ornithine decarboxylase (ODC) or adenosylmethionine decarboxylase (AdoMetDC). These results are consistent with the multisubstrate adduct design of AdoSpd and correlate well with similar data obtained previously for AdoDATO and AdoDATAD. Since the final HPLC purification could not fully remove all the inorganic salts from AdoSpd, several enzyme control experiments were performed to determine if 40 mM salt (NaCl, NaO_2CH, NH_4O_2CH) had any effect on enzyme activity. No effect on the enzymatic activity of PAPT in either the presence or absence of inhibitor was observed (data not shown).

Cell Cultures. The addition of AdoSpd to the culture medium of HT29 human colon carcinoma or COS-7 monkey kidney cells resulted in alterations in the cellular polyamines consistent with its acting as an inhibitor of spermidine synthase (Figure 1A,B). There was a substantial fall in the concentration of spermidine in the cells and a large increase in putrescine. Exposure to 1–5 μM AdoSpd gave a maximal effect, and there was an 80–90% reduction in spermidine. Treatment of Chinese hamster ovary (CHO) cells with AdoSpd also led to a decrease in intracellular spermidine, but these cells were less sensitive and there was only a 40% reduction in the presence of 10 μM AdoSpd (Figure 1C). These differences may reflect in the uptake or metabolism of AdoSpd in the different cell types.

Discussion

The facile β-elimination of the ylide side chain fragments shown in Scheme 5 results in the failure of both

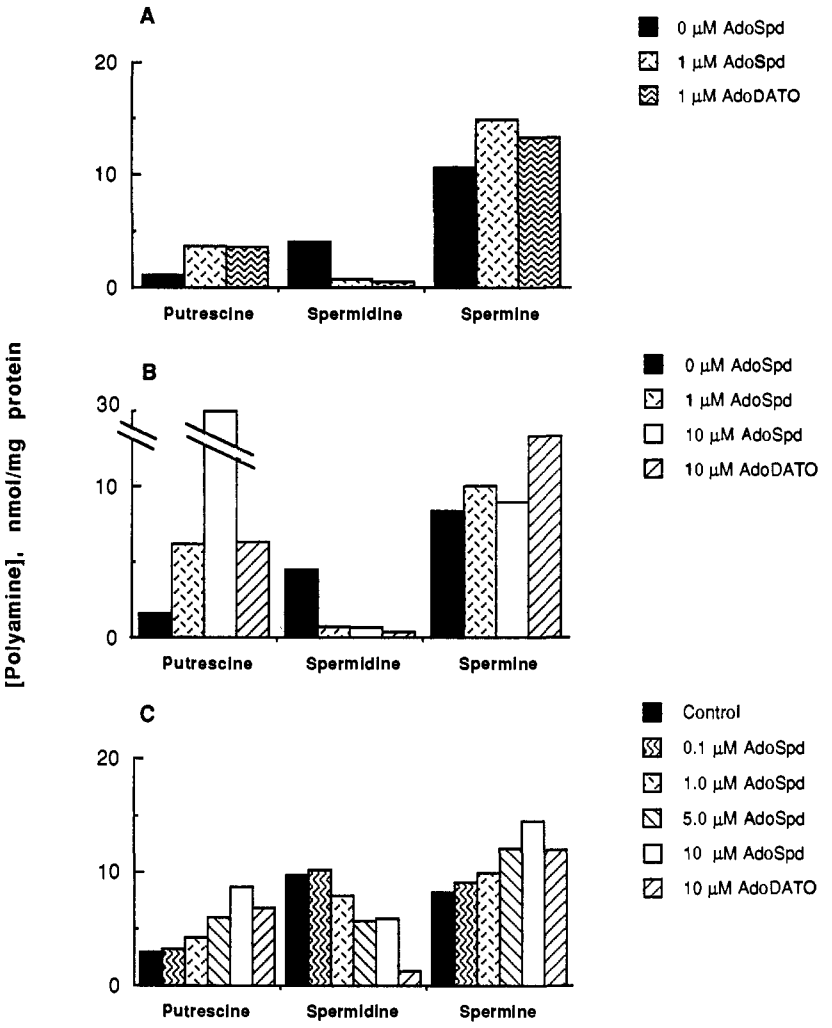


Figure 1. Effects of AdoSpd (**1c**) and AdoDATO (**1a**) on the biosynthesis of polyamines in cultured mammalian cells: (A) HT29 colon carcinoma cells, (B) COS-7 monkey kidney cells, (C) Chinese hamster ovary (CHO) cells.

the Julia and Wittig olefination reactions. Apparent from these observations, the electronic nature of the protecting group on the central nitrogen is critical. The successful Wittig olefination using (*N,N*-dimethyl-β-aminoethyl)phosphonium bromide[30] would suggest the use of an electron-donating N-protecting group. In addition, the steric bulk of the protecting group determines (at least in part) the ease of formation of the corresponding phosphonium salt. These observations suggest an N-benzylated amine function or a similar electron-releasing N-protecting group for Wittig or Julia reagents analogous to compounds **13** or **15**.

The second synthetic route, shown in Schemes 6 and 7, was successful in constructing the desired array of functional groups from the 5′ position of a nucleoside. This methodology is more flexible than the previous methods involving Wittig/Julia couplings. In principle, the nucleoside ketone **26** (Scheme 6) could be reductively alkylated with a variety of different amines for the synthesis of additional inhibitor analogues, e.g., a second-generation AdoDATAD analogue, AdoSpm (**1d**).

AdoSpd (**1c**) gives the most potent inhibition of PAPT known (Table 1). The stronger binding of AdoSpd to PAPT supports the synthesis of AdoSpm and further investigation of the inhibition of polyamine biosynthesis in vitro. Finally, these nucleoside-substituted polyamines may represent interesting tools to study the binding requirements of spermidine or spermine synthase and the polyamine transport protein.

Experimental Section

^{1}H NMR and ^{13}C NMR spectra were obtained using Bruker AM 500, AM 300, WM 360, and WM 200 spectrometers with chemical shifts referenced to tetramethylsilane internal standard. ^{1}H NMR spectra recorded in deuterium oxide, unless otherwise indicated, were referenced to tetramethylsilane external standard in $CDCl_3$. ^{13}C NMR spectra in D_2O were referenced to the $CDCl_3$ contained in an external standard. All melting points were obtained using a Mel-Temp apparatus and are uncorrected. IR spectra were recorded on a Nicolet FTIR spectrometer. High-resolution mass spectra were obtained using a VG 70-250S analytical high-resolution MS instrument. Elemental analyses were acquired from Atlantic Microlabs, Atlanta, GA. Analytical thin-layer chromatography was done on either silica gel plates (EM Science, 5554-7) with 254 nm fluorescent indicator or Eastman cellulose plates with fluorescent indicator. Flash column chromatography refers to the method described by Still et al. using silica gel 60.[39] Solvents were purchased in their anhydrous form and used directly or dried by distillation from sodium benzophenone ketyl (tetrahydrofuran), calcium hydride (dichloromethane), or barium oxide (DMF). Unless otherwise indicated, all chemicals were used directly from the supplier without further purification. All experiments were carried out in oven-dried or flame-dried glassware, and reaction solutions were magnetically stirred. Reactions involving air or moisture sensitive material were carried out under a positive pressure of dry nitrogen. The 5′-aldehyde nucleoside **16** was prepared as reported by Yau and

Coward.[10] Methyl 3-hydroxypropionate (**22**) was prepared as described by Ogawa et al.[31] 4-[(*tert*-Butyldiphenylsilyl)oxy]-1,4-butanediol was prepared as described by Freeman et al.[25]

2-Amino-1,4-butanediol (2).[24] $LiAlH_4$ (3 g, 79.1 mmol) was added to a 500 mL round bottom flask followed by 25 mL of dry THF to form a gray slurry. This slurry was cooled to 0 °C with an ice bath. In a separate round bottom flask, D,L-aspartic acid diethyl ester (4.98 g, 26.3 mmol) was dissolved in 20 mL of dry THF. This solution was added in portions via cannula to the stirred $LiAlH_4$ solution. Vigorous bubbling was observed. Once all of the ester was added, the resulting solution was heated at reflux temperature for 30 min. The solution was then allowed to cool to room temperature and then finally to 0 °C with an ice bath. 2-Propanol (35 mL) was then added as fast as bubbling would allow. Following this, H_2O (6.5 equiv to $LiAlH_4$) was added. The resulting mixture was vigorously stirred for 15 min. The solvents were then evaporated in vacuo to give dry aluminum salts. These salts were added into a Soxhlet cup and continuously extracted with 2-propanol overnight. The 2-propanol filtrate was then evaporated in vacuo to give a yellow oil. This oil was then distilled (bulb to bulb) (bp 118 °C, 0.15 mmHg) giving 1.979 g (72%) of **2** as a light yellow oil: ^{1}H NMR (DMSO-d_6) δ 4.5 (s, 1H), 3.45–3.55 (t, 2H), 3.3–3.4 (s, 1H), 3.27–3.23 (m, 1H), 3.15–3.1 (m, 1H), 2.74–2.67 (m, 1H), 1.54–1.46 (m, 1H), 1.30–1.20 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 67.00, 58.95, 50.91, 36.45; MS (CI, NH_3) *m/e* (%) 106 (MH^+, 100), 88 (3), 75 (1); HRMS (CI, NH_3) calcd for $C_4H_{11}NO_2H$ (MH^+) 106.0868, found 106.0872.

2-[2-[(*tert*-Butyldiphenylsilyl)oxy]ethyl]-2-oxazolidinone (3). 2-Aminobutanediol (**2**; 6.45 g, 61.35 mmol) was weighed into a round bottom flask and dissolved in 150 mL of dry DMF. This solution was then cooled to 0 °C. CDI (1.70 g, 10.5 mmol) was then added, and the resulting homogeneous mixture was stirred at 0 °C for 3 h. After this the reaction mixture was allowed to warm to ambient temperature overnight. *tert*-Butyldiphenylsilyl chloride (42.16 g, 153 mmol) was added via syringe to a 50 mL addition funnel attached to the reaction flask. The reaction vessel was cooled to 0 °C followed by dropwise addition of the silyl chloride. The resulting solution was then stirred at ambient temperature overnight. Excess DMF was removed in vacuo (45 °C, 0.1 mmHg). The resulting oily residue was partitioned between H_2O and EtOAc. The organic layer was separated, and the aqueous layer was washed two times with an equivalent volume of EtOAc. The organic layers were combined, dried ($MgSO_4$), filtered, and evaporated in vacuo to give 47 g of crude reddish colored oil. This oil was purified by chromatography on silica gel eluting with 5–10–20% and finally 50% EtOAc in hexane to give 12.87 g (57%) of **3** as a white solid: mp 113–114.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.65–7.58 (m, 4H), 7.35–7.50 (m, 6H), 5.25–5.15 (s, br, 1H, N*H*), 4.55–4.40 (m, 1H), 4.10–4.00 (m, 2H), 3.68–3.80 (m, 2H), 1.90–1.68 (m, 2H, CH_2), 1.10–1.00 (s, 9H, C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$) δ 158.45, 135.47, 132.81, 130.00, 127.89, 70.45, 61.30, 51.37, 37.36, 26.87, 19.05; MS (CI, NH_3) *m/e* (%) 387 [$(M + NH_4)^+$, 7], 370 (MH^+, 9), 312 (43), 292 (100), 234 (38), 214 (27); HRMS (CI, NH_3) calcd for $C_{21}H_{27}NO_3SiH$ (MH^+) 370.1838, found 370.1830. Anal. ($C_{21}H_{27}NO_3Si$) C, H, N.

2-(2-Hydroxyethyl)-2-oxazolidinone (3a). 2-[2-[(*tert*-Butyldiphenylsilyl)oxy]ethyl]-2-oxazolidinone (**3**; 488 mg, 1.32 mmol) was weighed into a round bottom flask and dissolved in AcOH (0.1 mL) in 9.9 mL of THF. Tetra-*N*-butylammonium fluoride (3.96 mL, 3.96 mmol) was added via syringe, and the resulting solution was stirred at ambient temperature overnight. All volatiles were then removed in vacuo, and the resulting oil was purified by column chromatography on silica gel eluting with 100% EtOAc to 10% MeOH in EtOAc giving 136 mg (79%) of **3a** as a white solid: mp 76–78.0 °C; ^{1}H NMR (DMSO-d_6) δ 7.71 (s, 1H, N*H*), 4.46–4.31 (apparent triplet, 1H, H_b), 4.03–3.91 (apparent triplet, 1H, H_a), 3.91–3.78 (m, 1H, methine), 3.60–3.39 (m, 2H, CH_2O), 1.75–1.57 (q, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ 158.45, 59.20, 57.21, 49.64, 37.71; MS (EI, 70 eV) *m/e* (%) 131 (M^+, 5), 113 (13), 86 (100), 42 (68); HRMS (EI, 70 eV) calcd for $C_5H_9NO_3$ (M^+) 131.0582, found 131.0586.

2-[*N*-(Tolylsulfonyl)amino]-4-[(*tert*-butyldiphenylsilyl)oxy]-1,4-butanediol (4). In a glovebag under a positive pressure of dry nitrogen, KH (164 mg, 1.49 mmol) was weighed into a round bottom flask. Dry THF (10 mL) was added via syringe. Then 2-[2-[(*tert*-butyldiphenylsilyl)oxy]ethyl]-2-oxazolidinone (**3**; 353 mg, 0.995 mmol) was added directly as a solid followed by 18-crown-6 (50 mg). The resulting mixture was stirred at ambient temperature for 30 min. In a separate round bottom flask, TsCl (200 mg, 1.09 mmol) was weighed out and dissolved in dry THF (5 mL), and the resulting solution was then added to the reaction mixture which was stirred at ambient temperature overnight. MeOH (10 mL) was added to quench the reaction to form a clear yellow solution. All volatile solvent was then removed in vacuo, and the resulting residue was partitioned between $CHCl_3$ and H_2O. The organic layer was removed, and the aqueous layer was washed two times with an equivalent volume of $CHCl_3$. The combined organic layers were dried ($MgSO_4$), filtered, and evaporated in vacuo to give an oil. Purification by chromatography on silica gel eluting with 20% EtOAc in hexane gave 332 mg (65%) of **4** as an oil: ^{1}H NMR ($CDCl_3$) δ 7.81–7.69 (d, 2H, tosyl), 7.69–7.30 (m, 10H), 7.30–7.18 (d, 2H, tosyl), 5.62–5.51 (d, 1H, N*H*), 3.78–3.50 (m, 2H, CH_2O), 3.50–3.33 (m, 1H, methine), 2.76–2.62 (t, 1H, O*H*), 2.41 (s, 3H, CH_3), 1.75–1.60 (q, 2H), 1.05 (s, 9H, C(CH_3)$_3$); MS (DCI, NH_3) *m/e* (%) 515 [$(M + NH_4)^+$, 100], 498 (MH^+, 81), 420 (69), 344 (26); HRMS (DCI, NH_3) calcd for $C_{27}H_{35}NO_4SSiH$ (MH^+) 498.2134, found 498.2141. Anal. ($C_{27}H_{35}NO_4SSi$) C, H, N.

2-[*N*-(Tolylsulfonyl)amino]-4-[(*tert*-butyldiphenylsilyl)oxy]-1-(tetrahydropyranyl)-1,4-butanediol (5). 2-[*N*-(Tolylsulfonyl)amino]-4-[(*tert*-butyldiphenylsilyl)oxy]-1,4-butanediol (**4**; 350 mg, 0.703 mmol) was added to a round bottom flask and dissolved in dry CH_2Cl_2 (10 mL). Dihydropyran (0.16 mL, 1.76 mmol) was then added via syringe, and the resulting solution was cooled to 0 °C. Toluenesulfonic acid (1.3 mg, 1%) was then added. After 30 min the solution was allowed to warm to ambient temperature over 1.5 h. The reaction solution was then transferred to a separatory funnel and washed one time with an equivalent volume of $NaHCO_3$ and one time with an equivalent volume of H_2O. The organic layer was then dried ($MgSO_4$), filtered, and evaporated in vacuo to give a yellow oil. Purification by chromatography on silica gel eluting with 100% hexane to 10% EtOAc in hexane gave 334 mg (82%) of **5** as an oil: ^{1}H NMR ($CDCl_3$) δ 7.76–7.73 (d, 2H, tosyl aryl), 7.62–7.59 (m, 4H, silyl aryl), 7.43–7.34 (m, 6H, silyl aryl), 7.24–7.21 (d, 2H, tosyl aryl), 5.51 (d, 0.5H, NH, D_2O exchangeable), 5.36 (d, 0.5H, NH, D_2O exchangeable), 4.42–4.41 (m, 0.5H, THP methine), 4.35–4.33 (m, 0.5H, THP methine), 3.74–3.35 (m, 6H), 2.39 (s, 3H, CH_3), 1.76–1.44 (m, 8H), 1.04 (s, 9H, C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$) δ 135.59, 133.63, 129.70, 129.49, 127.70, 127.26, 99.87, 99.45, 69.82, 69.06, 62.97, 62.74, 61.02, 51.72, 34.80, 30.67, 26.97, 25.38, 21.43, 19.89, 19.73, 19.18, 16.15; MS (DCI, NH_3) *m/e* (%) 599 [$(M + NH_4)^+$, 5], 582 (MH^+, 16), 524 (16), 498 (100), 428 (43); HRMS (DCI, NH_3) calcd for $C_{32}H_{43}NO_5SSiH$ (MH^+) 582.2709, found 582.2664. Anal. ($C_{32}H_{43}NO_5SSi$) C, H, N.

4-[(*tert*-Butyldiphenylsilyl)oxy]-1-iodobutane. Triphenoxymethylphosphonium iodide (1.38 g, 3.05 mmol) was weighed into a 100 mL two-neck round bottom flask in a glovebag under positive nitrogen pressure. This flask was then covered with aluminum foil and cooled to −68 °C with a dry ice/2-propanol bath while under positive nitrogen flow. In a separate round bottom flask, 4-[(*tert*-butyldiphenylsilyl)oxy]-1,4-butanediol (503 mg, 1.53 mmol) was added and dissolved in 5 mL of dry dichloromethane. This solution was then added to the stirred phosphonium iodide via cannula. The resulting pink-red solution was stirred at −68 °C for 20 min followed by warming to room temperature overnight. The reaction mixture was diluted to 20 mL with chloroform and then washed three times with 20 mL of 5% NaS_2O_3, three times with 20 mL of H_2O, and one time with 20 mL of brine. The organic layer was then dried ($MgSO_4$), filtered, and evaporated in vacuo to give an oil. This oil was purified by column chromatography on silica gel eluting with cyclohexane to give 428 mg (64%) of the title compound as a clear and colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.70–7.65 (m, 4H), 7.50–7.30 (m, 6H), 3.71–3.63 (t, 2H, CH_2O), 3.23–3.16 (t, 2H, CH_2I), 2.03–1.86 (m, 2H, CH_2), 1.72–

1.55 (m, 2H, CH_2), 1.1–1.0 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 135.54, 133.78, 129.61, 127.65, 62.65, 33.25, 30.13, 26.84, 19.19, 7.10; FTIR (neat, cm^{-1}) 3071, 2929, 2858, 1472, 1426, 1223, 1111, 825, 739, 703; MS (CI, NH_3) *m/e* (%) 456 [$(M + NH_4)^+$, 26), 439 (MH^+, 59), 398 (45), 381 (34), 326 (26), 313 (34), 309 (22), 272 (24), 183 (100); HRMS (CI, NH_3) calcd for $C_{20}H_{27}OISiH$ (MH^+) 439.0954, found 439.0932.

2-[*N*-(Tolylsulfonyl)-*N*-[4-[(*tert*-butyldiphenylsilyl)oxy]-butyl]amino]-4-[(*tert*-butyldiphenylsilyl)oxy]-1-(tetrahydropyranyl)-1,4-butanediol (6). In a glovebag under a positive pressure of dry N_2, KH (189 mg, 1.65 mmol) was added into a 35 mL two-neck round bottom flask. Dry THF (6 mL) was added via syringe, and the resulting solution was cooled to 0 °C. The sulfonamide **5** (480 mg, 0.825 mmol) was weighed into a separate round bottom flask and dissolved in dry THF (1 mL). The solution of **5** was then added via cannula to the KH solution. In yet another round bottom flask, 4-[(*tert*-butyldiphenylsilyl)oxy]-1-iodobutane (513 mg, 1.17 mmol) was weighed out along with 18-crown-6 (50 mg, catalytic). Both of these were then dissolved in THF (1 mL) and added to the KH solution via cannula. The resulting solution was stirred at ambient temperature overnight. Then the reaction mixture was heated at 50 °C for 6 h. MeOH (10 mL) was added to quench the reaction. All volatiles were then removed in vacuo to give an oily residue. The oil was then partitioned between EtOAc and H_2O (equal volumes), and the aqueous layer was separated. The organic layer was washed two times with an equivalent volume of H_2O, and dried ($MgSO_4$), filtered, and evaporated in vacuo to give an oil. The oil was purified by chromatography on silica gel eluting with 100% hexane to 10% EtOAc in hexane to give 470 mg (64%) of **6** as an oil: ^1H NMR ($CDCl_3$) δ 7.75–7.58 (m, 10H), 7.48–7.31 (m, 12H), 7.20–7.08 (d, 2H, tosyl aryl), 4.51–4.43 (m, 0.5H, THP methine), 4.43–4.36 (m, 0.5H, THP methine), 4.19–4.04 (m, 1H, methine), 3.79–3.47 (m, 6H), 3.47–3.32 (m, 2H), 3.22–2.97 (m, 2H), 2.39–2.30 (s, 3H, CH_3), 1.92–1.33 (m, 12H), 1.13–0.94 (m, 18H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 142.57, 138.50, 135.53, 133.93, 133.70, 129.62, 129.55, 129.34, 129.29, 127.63, 127.22, 98.77, 98.66, 68.87, 68.67, 63.55, 63.49, 61.97, 61.00, 55.30, 54.64, 45.23, 44.87, 33.92, 33.52, 30.39, 30.33, 30.14, 27.56, 27.30, 26.85, 25.35, 21.41, 19.18; MS (DCI, CH_4+NH_3) *m/e* (%) 909 [$(M + NH_4)^+$, 3], 892 (MH^+, 0.6), 808 (23), 738 (100), 638 (42); HRMS (DCI, CH_4) calcd for $C_{52}H_{69}NO_6SSi_2H$ (MH^+) 892.4462, found 892.4454.

2-[*N*-(Tolylsulfonyl)-*N*-[4-[(*tert*-butyldiphenylsilyl)oxy]-butyl]amino]-4-[(*tert*-butyldiphenylsilyl)oxy]-1,4-butanediol (7). 2-[*N*-(Tolylsulfonyl)-*N*-[4-[(*tert*-butyldiphenylsilyl)oxy]butyl]amino]-4-[(*tert*-butyldiphenylsilyl)oxy]-1-(tetrahydropyranyl)-1,4-butanediol (**6**; 100 mg, 0.121 mmol) was weighed into a two-neck round bottom flask and dissolved in dry CH_2Cl_2. Dry MeOH (0.015 mL, 0.363 mmol) was then added via syringe. Toluenesulfonic acid (0.23 mg, 1%) was added as a solid, and the resulting solution was stirred at ambient temperature overnight. The reaction was quenched with excess saturated $NaHCO_3$. The aqueous layer was separated, and the organic layer was washed one time with an equivalent volume of dH_2O. The organic layer was dried ($MgSO_4$), filtered, and evaporated to give an oil. Purification by chromatography on silica gel eluting with 100% hexane to 10% EtOAc in hexane gave 86 mg (87%) of **7** as an oil: ^1H NMR ($CDCl_3$) δ 7.71–7.55 (m, 10H), 7.48–7.26 (m, 12H), 7.15–7.11 (d, 2H, tosyl), 4.04–3.80 (quintet, 1H, methine), 3.66–3.60 (m, 4H), 3.43–3.37 (t, 2H), 3.28–3.01 (m, 2H), 2.43–2.37 (t, 1H, O*H*), 2.32 (s, 3H), 1.72–1.43 (m, 6H), 1.03–1.02 (m, 18H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 135.58, 135.52, 133.93, 129.82, 129.62, 127.77, 127.67, 127.14, 63.92, 63.41, 61.05, 57.79, 44.98, 32.75, 30.02, 27.62, 26.87, 26.82, 21.44, 19.08; MS (DCI, NH_3) *m/e* (%) 825 [$(M + NH_4)^+$, 13], 808 (MH^+, 8), 654 (50), 622 (45), 366 (63), 328 (62), 274 (93), 174 (100). Anal. ($C_{47}H_{61}NO_5SSi_2$) C, H, N.

***N*-[4-[(*tert*-Butyldiphenylsilyl)oxy]butyl]-2-[2-[(*tert*-butyldiphenylsilyl)oxy]ethyl]-2-oxazolidinone (8).** In a glovebag under positive nitrogen pressure, KH (35% dispersion) (150 mg, 1.31 mmol) was added to a 50 mL, two-neck round bottom flask. The dispersion was stirred with THF (5 mL). This solution was cooled to 0 °C. Then 2-[2-[(*tert*-butyldiphenylsilyl)oxy]ethyl]-2-oxazolidinone (**3**; 256 mg, 0.693 mmol) was added, and the resulting solution was stirred for 30 min. Then 4-[(*tert*-butyldiphenylsilyl)oxy]-1-iodobutane (334 mg, 0.762 mmol) was dissolved in THF (5 mL) and added to the solution of **3** via cannula. Solid 18-crown-6 (50 mg) was then added. The resulting solution was then stirred at ambient temperature overnight. MeOH (10 mL) was then added to quench the reaction after cooling the solution to 0 °C. All volatiles were evaporated in vacuo leaving an oily residue. The resulting residue was dissolved in EtOAc and washed three times with an equivalent volume of of H_2O. The organic layer was dried ($MgSO_4$), filtered, and evaporated in vacuo to give a thick oil. Purification by chromatography on silica gel (10% EtOAc in hexane) gave 369 mg (78%) of **8** as a white solid. The solid was recrystallized from MeOH: mp 94.8–95.5 °C; ^1H NMR ($CDCl_3$) δ 7.7–7.5 (m, 8H), 7.5–7.2 (m, 12H), 4.35–4.25 (apparent triplet, 1H, CH_aOCO), 4.1–4.0 (apparent triplet, 1H, CH_bOCO), 3.9–3.8 (m, 1H, methine), 3.7–3.5 (m, 4H, CH_2-OSi), 3.45–3.3 (m, 1H, NCH_a), 3.05–2.95 (m, 1H, NCH_b), 2.0–1.9 (m, 1H), 1.7–1.5 (m, 5H), 1.1–0.8 (m, 18H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 158.2, 135.6, 135.5, 133.9, 133.7, 130.0, 129.6, 127.9, 127.7, 67.9, 63.4, 60.3, 53.7, 41.8, 34.6, 29.8, 26.9, 23.9, 19.2, 19.1; MS (CI, NH_3) *m/e* (%) 680 (MH^+, 19), 622 (100), 602 (86), 346 (39), 199 (43); HRMS (CI, NH_3) calcd for $C_{41}H_{53}NO_4Si_2H$ 680.3591, found 680.3602. Anal. ($C_{41}H_{53}NO_4Si_2$) C, H, N.

***N*-[4-[(*tert*-Butyldiphenylsilyl)oxy]butyl]-2-[2-[(*tert*-butyldiphenylsilyl)oxy]ethyl]aziridine (9).** *N*-[4-[(*tert*-Butyldiphenylsilyl)oxy]butyl]-2-[2-[(*tert*-butyldiphenylsilyl)oxy]-ethyl]-2-oxazolidinone (**8**; 200 mg, 0.294 mmol) was added to a 35 mL two-neck round bottom flask fitted with a reflux condenser. Then $CHCl_3$ (5 mL) was added followed by TMSI (0.1 mL, 0.673 mmol) via syringe. The resulting homogeneous mixture was stirred at ambient temperature for 30 h. MeOH (5 mL) was added to quench the reaction, and the resulting yellow solution was stirred at ambient temperature overnight. All volatiles were then removed in vacuo giving a yellow oil. The oil was stirred with a saturated aqueous Na_2CO_3 solution for 3 h. After this time, $CHCl_3$ (5 mL) was added, and the resulting biphasic mixture was stirred for an additional 2 h. The organic layer was separated, and the aqueous layer was washed three times with 5 mL of $CHCl_3$. The combined organic layers were dried ($MgSO_4$), filtered, and evaporated in vacuo to give 200 mg of oil. Purification by chromatography on silica gel (step gradient elution 100% cyclohexane to 10% EtOAc in cyclohexane to 50% EtOAc in cyclohexane) gave 80 mg (43%) of **9** as an oil: ^1H NMR ($CDCl_3$) δ 7.75–7.55 (m, 8H), 7.50–7.30 (m, 12H), 3.81–3.70 (m, 2H, CH_2O), 3.70–3.58 (m, 2H, CH_2O), 2.28–2.09 (m, 2H), 1.73–1.54 (m, 6H), 1.48–1.54 (d, 1H, aziridine ring H_a), 1.48–1.40 (m, 1H, methine), 1.22–1.15 (d, 1H, aziridine ring H_b), 1.05 (s, 18H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 135.6, 129.5, 127.6, 63.9, 62.3, 61.4, 36.7, 36.3, 33.8, 30.5, 26.9, 26.2; MS (EI, 70 eV) *m/e* (%) 635 (MH^+, 1.6), 578 (23), 199 (100).

2-[*N*-Methyl-*N*-[4-(*tert*-butyldiphenylsilyl)oxy]butyl]-amino]-4-[(*tert*-butyldiphenylsilyl)oxy]-1,4-butanediol (10). In a glovebag under a positive pressure of dry nitrogen, $LiAlH_4$ (72 mg, 1.90 mmol) was added to a three-neck round bottom flask. Dry THF (10 mL) was added via syringe. The resulting slurry was cooled to 0 °C, and oxazolidinone **8** (430 mg, 0.632 mmol) was added. The resulting solution was stirred at 0 °C for 2.5 h. The reaction was quenched with H_2O (1.5 mL), and the resulting aluminum salts were removed by filtration. The filtrate was dried ($MgSO_4$), filtered, and evaporated in vacuo to give a yellow oil. Purification by chromatography on silica gel eluting with 10–50% EtOAc in hexane and finally 100% EtOAc gave 253 mg (60%) of **10** as a white crystalline solid: ^1H NMR ($CDCl_3$) δ 7.75–7.56 (m, 8H), 7.50–7.26 (m, 12H), 3.73–3.55 (m, 4H, CH_2O), 3.55–3.41 (m, 1H, CH_aO), 3.32–3.01 (apparent triplet, 1H, CH_bO), 2.98–2.77 (m, 1H, methine), 2.58–2.20 (m, 2H, CH_2N), 2.20–2.08 (s, 3H, NCH_3), 1.89–1.66 (m, 1H), 1.65–1.42 (m, 4H), 1.40–1.12 (m, 1H), 1.12–0.92 (s, 18H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 135.51, 129.66, 129.50, 127.66, 127.57, 63.67, 61.67, 61.26, 61.09, 53.17, 35.79, 30.15, 27.81, 26.82, 24.59, 19.0, 2.0; MS (DCI, NH_3) *m/e* (%) 668 (MH^+, 100), 638 (13), 342 (32), 274 (27), 196 (17).

N^{1m}-[2-[*N*-Methyl-*N*-[4-[(*tert*-butyldiphenylsilyl)oxy]-butyl]amino]-4-[(*tert*-butyldiphenylsilyl)oxy]butyl]imidazole (11). Into a two-neck round bottom flask, Ph_3P (118 mg, 0.449 mmol), imidazole (31 mg, 0.45 mmol), and I_2 (57 mg, 0.225 mmol) were weighed. These were dissolved in dry CH_2Cl_2 and stirred for 15 min at ambient temperature resulting in a yellow solution. Into a separate round bottom flask, the amino alcohol **10** (100 mg, 0.15 mmol), prepared as described above, was weighed and dissolved in dry CH_2Cl_2. The alcohol was then added to the iodinating solution via cannula. A white precipitate formed immediately. This mixture was allowed to stir at ambient temperature overnight. The precipitate was removed by filtration, and the resulting filtrate was evaporated in vacuo to give 299 mg of an oil. Purification by chromatography on silica gel eluting with 10% EtOAc in hexane to 100% EtOAc and finally 10% MeOH in EtOAc gave 100 mg (93%) of **11** as a yellow crystalline solid: ^{1}H NMR ($CDCl_3$) δ 7.69–7.27 (m, 21H), 7.05 (s, 1H), 6.90 (s, 1H), 4.0–3.55 (m, 6H), 3.10–3.00 (m, 1H), 2.40–2.30 (m, 2H), 2.15 (s, 3H), 1.80–1.70 (m, 1H), 1.50–1.30 (m, 5H), 1.00 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 137.43, 135.44, 134.85, 133.90, 133.77, 133.40, 132.87, 132.02, 131.90, 131.72, 129.63, 129.51, 129.43, 129.10, 128.48, 128.35, 127.62, 127.54, 127.50, 127.36, 119.32, 63.56, 62.46, 61.29, 60.83, 53.68, 53.18, 47.31, 35.98, 30.15, 29.85, 26.76, 26.57, 24.42, 19.10; MS (DCI, NH_3) *m/e* (%) 718 (MH^+, 100), 636 (35), 342 (24), 279 (97).

2-[*N*-(Tolylsulfonyl)-*N*-[4-[(*tert*-butyldiphenylsilyl)oxy]-butyl]amino]-4-[(*tert*-butyldiphenylsilyl)oxy]-1-iodobutan-4-ol (12). 2-[*N*-(Tolylsulfonyl)-*N*-[4-[(*tert*-butyldiphenylsilyl)oxy]butyl]amino]-4-[(*tert*-butyldiphenylsilyl)oxy]-1,4-butanediol (**7**; 100 mg, 0.124 mmol) was weighed into a 25 mL two-neck round bottom flask along with triphenylphosphine (97 mg, 0.372 mmol) and imidazole (25 mg, 0.372 mmol). This mixture was dissolved in dry CH_2Cl_2. Iodine (47 mg, 0.185 mmol) was then directly added as a solid, and the resulting solution was stirred at ambient temperature overnight. Precipitated triphenylphosphine oxide was removed by filtration, and the resulting filtrate was evaporated in vacuo to give an oil. Purification by chromatography on silica gel eluting with 100% hexane to 20% EtOAc in hexane gave 80 mg (84%) of **12** as an oil: ^{1}H NMR ($CDCl_3$) δ 7.73–7.52 (m, 10H), 7.49–7.28 (m, 12H), 7.20–7.09 (d, 2H, tosyl), 4.12–3.99 (m, 1H, methine), 3.65–3.56 (t, 2H), 3.52–3.38 (m, 2H), 3.29–3.20 (d, 2H, α to iodide), 3.13–2.91 (m, 2H), 2.32 (s, 3H, CH_3), 2.14–1.99 (m, 1H), 1.79–1.54 (m, 3H), 1.53–1.39 (m, 2H), 1.10–0.90 (d, 18H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 143.14, 137.77, 135.55, 135.50, 133.80, 133.40, 129.75, 129.69, 129.59, 129.54, 127.70, 127.63, 127.26, 63.27, 60.58, 57.76, 45.06, 34.70, 29.98, 27.65, 26.84, 21.45, 19.16, 19.10, 8.15; MS (EI, 70 eV) *m/e* (%) 860 [$(M - C_4H_9)^+$, 100], 732 (18); HRMS (EI, 70 eV) calcd for $C_{43}H_{51}NO_4SSi_2I$ [$M - C_4H_9$]$^+$ 860.2122, found 860.2131. Anal. ($C_{47}H_{60}INO_4SSi_2$) C, H, N.

[2-[*N*-(Tolylsulfonyl)-*N*-[4-[(*tert*-butyldiphenylsilyl)-oxy]butyl]amino]-4-[(*tert*-butyldiphenylsilyl)oxy]butyl]-triphenylphosphonium Iodide (13). Iodide **12** (176 mg, 0.192 mmol) was weighed into a 25 mL pear-shaped flask. Triphenylphosphine (55 mg, 0.21 mmol) was added, and the resulting mixture was melted together at 100 °C under nitrogen overnight. The reaction mixture was cooled, and the resulting oil was purified by chromatography on silica gel eluting with 100% EtOAc giving 89 mg (39%) of **13** as a thick oil: ^{1}H NMR ($CDCl_3$) δ 8.00–7.26 (m, 39H), 6.94–6.91 (d, 2H, J = 9 Hz), 4.90–4.80 (m, 1H), 4.67–4.57 (apparent triplet of d, 1H), 3.91–3.76 (apparent triplet of d, 1H), 3.61–3.45 (m, 5H), 3.32–3.18 (apparent triplet of d, 1H), 2.25 (s, 3H), 1.92–1.91 (m, 2H), 1.75–1.23 (m, 4H), 0.98 (s, 9H, $C(CH_3)_3$), 0.97 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 143.38, 137.04, 135.49, 135.37, 135.32, 134.92, 134.30, 134.18, 134.02, 133.31 132.92, 132.09, 131.92, 130.37, 130.23, 129.80, 129.61, 129.35, 128.53, 128.39, 127.77, 127.52, 127.25, 118.57, 117.62, 63.48, 60.60, 51.23, 44.66, 36.28, 29.92, 27.19, 26.96, 26.83, 23.15, 21.41, 19.18; MS (FAB+) *m/e* (%) 1052 (M^+, 100), 571 (27), 277 (48), 135 (72); HRMS (FAB+) calcd for $C_{65}H_{75}INO_4SSi_2P^+$ (M^+) 1052.4693, found 1052.4791. Anal. ($C_{65}H_{75}INO_4PSSi_2 \cdot H_2O$) C, H, N.

2-[*N*-(Tolylsulfonyl)-*N*-[4-[(*tert*-butyldiphenylsilyl)oxy]-butyl]amino]-4-[(*tert*-butyldiphenylsilyl)oxy]butyl Phenyl Thioether (14). Iodide **12** (101 mg, 0.109 mmol) was weighed into a 25 mL round bottom flask and dissolved in dry THF (8 mL). DBU (0.018 mL, 0.12 mmol) was added via syringe followed by thiophenol (0.012 mL, 0.12 mmol). The resulting clear homogeneous solution was stirred at ambient temperature overnight. The THF was removed in vacuo, and the resulting residue was dissolved in $CHCl_3$. The organic layer was washed one time with an equivalent volume of H_2O, dried ($MgSO_4$), filtered, and evaporated in vacuo to give an oil. Purification by chromatography on silica gel eluting with 100% hexane to 10% EtOAc in hexane gave 69 mg (70%) of **14** as an oil: ^{1}H NMR ($CDCl_3$) δ 7.65–7.64 (d, 2H, J = 3.6 Hz), 7.57–7.54 (m), 7.39–7.17 (m), 7.05–7.03 (d, 2H, J = 7.2 Hz), 3.95–3.91 (m, 1H), 3.59–3.56 (t, 2H, J = 7.2 Hz), 3.44–3.36 (m, 2H), 3.20–3.14 (dd, 1H, J = 3.6 Hz), 3.06–2.93 (m, 3H), 2.27 (s, 3H), 2.15–2.09 (m, 1H), 1.76–1.41 (m, 5H), 1.03 (s, 9H, $C(CH_3)_3$), 0.99 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 142.8, 137.9, 135.5, 133.8, 133.5, 129.9, 129.6, 129.3, 128.9, 127.6, 127.1, 126.3, 63.3, 60.7, 55.2, 45.1, 38.2, 34.47, 30.0, 27.28, 26.81, 26.78, 21.38, 19.12, 19.04, 0.98; MS (DCI, NH_3) *m/e* (%) 917 [$(M + NH_4)^+$, 0.6], 900 (MH^+, 1.0), 842 (0.6), 746 (23), 638 (16), 622 (17), 436 (25), 380 (62), 328 (85), 274 (77), 196 (100); HRMS (CI, NH_3) calcd for $C_{53}H_{65}NO_4S_2Si_2$ (MH^+) 900.3917, found 900.3972.

[2-[*N*-(Tolylsulfonyl)-*N*-[4-[(*tert*-butyldiphenylsilyl)-oxy]butyl]amino]-4-[(*tert*-butyldiphenylsilyl)oxy]butyl]-phenylsulfone (15). *m*-CPBA (50–60%) was dissolved in CH_2Cl_2 and washed three times with an equivalent volume of Na_2PO_4 buffer (pH 7.5). The organic layer was dried ($MgSO_4$), filtered, and evaporated in vacuo to give a white solid (100% *m*-CPBA). Phenyl thioether **14** (100 mg, 0.111 mmol) was weighed into a round bottom flask and dissolved in dry CH_2Cl_2 (5 mL). The resulting solution was cooled to 0 °C. The *m*-CPBA (48 mg, 0.278 mmol) was added, and the resulting solution was stirred at 0 °C for 3 h. The reaction was quenched with a saturated solution of $NaHCO_3$. The organic layer was separated, washed two times with an equivalent volume of saturated $NaHCO_3$, dried ($MgSO_4$), filtered, and evaporated to give 115 mg of an oil. Purification by chromatography on silica gel eluting with 100% hexane to 10–20% EtOAc in hexane gave 72 mg (70%) of **15** as an oil: ^{1}H NMR ($CDCl_3$) δ 7.89–7.86 (d, 2H, J = 9 Hz), 7.64–7.33 (m, 25H), 7.10–7.08 (d, 2H, J = 9 Hz), 4.25–4.22 (m, 1H), 3.55–3.51 (t, 2H, J = 6 Hz), 3.44–3.28 (m, 4H), 2.98–2.96 (m, 2H), 2.30 (s, 3H), 2.26–2.25 (m, 1H), 1.80–1.79 (m, 1H), 1.65–1.39 (m, 4H), 1.02 (s, 18H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 143.3, 140, 138, 135.6, 134.1, 133.7, 129.7, 129.4, 128.2, 127.8, 127.4, 63.41, 61.07, 60.45, 51.36, 47, 36.19, 30.01, 26.94, 26.58, 21.36, 19.15; MS (DCI, NH_3) *m/e* (%) 949 [$(M + NH_4)^+$, 14], 932 (MH^+, 11), 778 (23), 468 (96), 404 (100); HRMS (DCI, CH_4) calcd for $C_{53}H_{65}NO_6S_2Si_2$ (MH^+) 932.3870, found 932.3823.

Methyl 3-[(*tert*-Butyldiphenylsilyl)oxy]propionate (23). Methyl 3-hydroxypropionate (**22**; 7.57 g, 73 mmol) was weighed into a three-neck 250 mL round bottom flask and dissolved in 40 mL of dry DMF. Imidazole (10 g, 146 mmol) was then added as a solid along with *tert*-butyldiphenylsilyl chloride (20 g, 73 mmol) via syringe. The resulting solution was stirred at ambient temperature overnight. Excess DMF was removed in vacuo, and the residual oil was dissolved in Et_2O. The organic mixture was washed three times with an equivalent volume of dH_2O. The Et_2O layer was then dried ($MgSO_4$), filtered, and evaporated in vacuo to give an oil. Flash chromatography on silica gel eluting with 1–2% and finally 5% EtOAc in hexane gave 9.02 g (50%) of **23** as a clear and colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.68–7.65 (m, 4H), 7.45–7.36 (m, 6H), 3.96–3.93 (t, 2H, J = 7.2 Hz, CH_2O), 3.68 (s, 3H, CH_3O), 2.58–2.55 (t, 2H, J = 7.2 Hz, CH_2CO_2), 1.03 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 172.14, 135.52, 133.47, 129.65, 127.65, 59.82, 51.51, 37.67, 26.68, 19.12; MS (DCI, NH_3) *m/e* (%) 343 (MH^+, 2), 266 (22), 265 (100), 136 (13); HRMS (CI, NH_3) calcd for $C_{20}H_{26}O_3SiH$ (MH^+) 343.1729, found 343.1717. Anal. ($C_{20}H_{26}O_3Si$) C, H, N.

4-[(*tert*-Butyldiphenylsilyl)oxy]-1-(dimethoxyphosphinyl)-2-oxobutane (24). Dimethyl methylphosphonate (3.77 g, 30.4 mmol) was weighed into a three-neck, 250 mL round

bottom flask and dissolved in 20 mL of dry THF. The resulting solution was cooled to −78 °C. From an attached addition funnel, *n*-BuLi (1.6 M, 19 mL, 30.4 mmol) was slowly added over 15 min, and the resulting mixture was stirred at −78 °C for 75 min. Methyl 3-[(*tert*-butyldiphenylsilyl)oxy]propionate (**23**; 9.02 g, 27.6 mmol) was dissolved in 10 mL of dry THF and transferred into the addition funnel via cannula. The ester **23** was then slowly added over 15 min with the reaction solution turning clear yellow from cloudy white. The reaction solution was stirred for 1 h at −78 °C followed by 1 h at 0 °C and finally at ambient temperature for 20 min. Saturated NH_4Cl solution (2 mL) was added to quench the reaction. The solvents were then evaporated in vacuo, and the residual oil was dissolved in EtOAc. The organic phase was washed three times with an equivalent volume of H_2O, dried ($MgSO_4$), filtered, and evaporated in vacuo to give 11.46 g of oil. Purification by chromatography on silica gel eluting with 50% EtOAc in hexane to 100% EtOAc gave 4.38 g (38%) of **24** as a clear and colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.71−7.61 (m, 4H), 7.48−7.32 (m, 6H), 3.99−3.90 (t, 2H, J = 7.5 Hz, CH_2O), 3.80 (s, 3H, CH_3O), 3.75 (s, 3H, CH_3O), 3.22−3.11 (d, 2H, J = 22.5 Hz, CH_2P), 2.89−2.80 (t, 2H, J = 7.5 Hz, CH_2CO), 1.02 (s, 9H, $C(CH_3)_3$); MS (CI, NH_3) m/e (%) 452 [(M + NH_4)$^+$, 11], 435 (MH^+, 3), 357 (100); HRMS (CI, NH_3) calcd for $C_{22}H_{31}O_5PSiH$ (MH^+) 435.1757, found 435.1751. Anal. ($C_{22}H_{31}O_5PSi\cdot0.5H_2O$) C, H, N.

N^6-Benzoyl-2′,3′-isopropylidene-5′-[2-oxo-4-[(*tert*-butyldiphenylsilyl)oxy]butyl]-5′-deoxyadenosine (26). The 5′-aldehyde nucleoside **16** was obtained by dehydration of the stable hydrate (1.16 g, 2.83 mmol) by azeotropic distillation of H_2O:benzene using a Dean−Stark apparatus.[10] β-Keto phosphonate **24** (1.38 g, 3.18 mmol) was weighed into a 50 mL round bottom flask and dissolved in dry THF (5 mL). The phosphonate solution was cooled to −78 °C followed by addition of *n*-BuLi (1.6 M solution in hexane, 2.4 mL, 3.18 mmol). This mixture was allowed to stir at −78 °C for 15 min. The aldehyde **16** was dissolved in dry THF (5 mL) and added via cannula to the reaction mixture. The resulting solution was stirred at −78 °C for 2 h, −20 °C for 2 h, and finally 0 °C for 15 min. The reaction was then quenched at 0 °C with the reagent MeOH (2 mL). The bulk of solvents was then removed in vacuo, and the residual oil was partitioned between EtOAc and H_2O. The H_2O layer was removed, and the resulting organic layer was washed two times with an equivalent volume of H_2O. The organic layer was then dried ($MgSO_4$), filtered, and evaporated in vacuo to give 1.86 g of crude white foam. A small portion of the product **25** could be purified by flash chromatography on silica gel eluting with 40% EtoAc in hexane to 50% and finally 100% EtOAc to give pure **25** as a white foam: ^{1}H NMR ($CDCl_3$) δ 9.09 (br s, 1H, N*H*), 8.78 (s, 1H, *H*$_8$), 8.09 (s, 1H, *H*$_2$), 8.03−8.00 (d, 2H, benzoyl), 7.64−7.59 (m, 4H), 7.54−7.50 (t, 3H, benzoyl), 7.41−7.35 (m, 6H), 6.87−6.81 (dd, 1H, J(H$_5$′−H$_4$′) = 5.6 Hz, J(H$_5$′−H$_6$′) = 16 Hz, *H*$_5$′), 6.22−6.15 (dd, 1H, J(H$_6$′−H$_4$′) = 1.2 Hz, J(H$_6$′−H$_5$′) = 16 Hz, *H*$_6$′), 6.21−6.20 (d, 1H, J = 1.8 Hz, *H*$_1$′), 5.55−5.53 (dd, 1H, *H*$_2$′), 5.14−5.11 (apparent triplet, 1H, *H*$_3$′), 4.84−4.81 (m, 1H, *H*$_4$′), 3.93−3.89 (t, 2H, J = 4 Hz, CH_2O), 2.69−2.64 (m, 2H, CH_2CO), 1.65 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 0.99 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 198.13, 164.51, 152.80, 151.22, 149.81, 142.28, 141.10, 135.49, 133.44, 133.34, 132.83, 130.61, 129.65, 128.85, 127.85, 127.66, 123.59, 115.04, 90.55, 86.31, 84.21, 83.89, 59.57, 43.18, 27.10, 26.71, 25.33, 19.08.

The crude ketone **25** (1.86 g) was dissolved in EtOAc and added to a 100 mL hydrogenation bottle. Then Pd−C (200 mg, catalytic) was added, and the resulting slurry was hydrogenated under 20 psi of H_2 pressure overnight. The catalyst was removed via filtration through 1 in. Celite powder on a glass frit. The filtrate was evaporated in vacuo to give 1.95 g of a yellow foam. The foam was purified in 300 mg aliquots via preparative HPLC (reverse phase C_{18}, flow 14 mL/min, 50% acetonitrile/H_2O to 100% acetonitrile over 30 min) to give 1.08 g (53%) of **26** as a white foam: ^{1}H NMR ($CDCl_3$) δ 9.34 (br s, 1H, N*H*), 8.75 (s, 1H, *H*$_8$), 8.06 (s, 1H, *H*$_2$), 8.01−7.99 (d, 2H, benzoyl), 7.64−7.32 (m, 13H), 6.08−6.07 (d, 1H, J = 2.4 Hz, *H*$_1$′), 5.46−5.43 (dd, 1H, *H*$_2$′), 4.86−4.82 (m, 1H, *H*$_3$′), 4.22−4.16 (m, 1H, *H*$_4$′), 3.93−3.89 (t, 2H, J = 6 Hz, CH_2O), 2.59−2.54 (m, 4H, CH_2CO), 2.07−1.93 (m, 2H), 1.59 (s, 3H, CH_3), 1.21 (s, 3H, CH_3), 1.00 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 207.51, 164.42, 152.51, 151.21, 149.70, 141.97, 135.31, 133.68, 133.39, 132.40, 129.49, 128.56, 127.73, 127.49, 123.74, 114.72, 90.18, 85.89, 83.98, 59.69, 45.50, 39.07, 27.21, 26.98, 26.79, 25.44, 19.06; MS (FAB+) m/e (%) 720 (MH^+, 68), 240 (100), 135 (61); HRMS (FAB+) calcd for $C_{40}H_{45}N_5O_6SiH$ (MH^+) 720.3217, found 720.3181. Anal. ($C_{40}H_{45}N_5O_6Si$) C, H, N.

4-[(*tert*-Butyldiphenylsilyl)oxy]butyl Azide (27). 4-Chlorobutanol (3 g, 23.5 mmol) was weighed into a 50 mL round bottom flask and dissolved in 20 mL of dry DMF. Sodium azide (4.58 g, 70.5 mmol) was then added in one portion. The resulting slurry was heated to 57 °C and stirred overnight. Excess NaN_3 and NaCl were then removed via filtration. Imidazole (4.0 g, 58.75 mmol) was added to the filtrate along with *tert*-butyldiphenylsilyl chloride (7.11 g, 25.85 mmol) via syringe. The resulting solution was stirred at ambient temperature overnight. DMF was removed in vacuo, and the residual oil was partitioned between Et_2O and H_2O. The aqueous layer was separated, and the organic layer was washed two times with an equivalent volume of dH_2O. The combined organic layers were dried ($MgSO_4$), filtered, and evaporated in vacuo to give 8.96 g of oil. Purification by chromatography on silica gel eluting with 100% hexane to 5% EtOAc in hexane gave 3.39 g (41%) of **27** as a clear and colorless liquid: ^{1}H NMR ($CDCl_3$) δ 7.67−7.65 (m, 4H), 7.43−7.36 (m, 6H), 3.70−3.66 (t, 2H, J = 7.2 Hz, CH_2O), 3.28−3.24 (t, 2H, J = 7.2 Hz, CH_2N_3), 1.73−1.60 (m, 4H), 1.05 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 135.53, 133.80, 129.60, 127.64, 63.14, 51.28, 29.78, 26.84, 25.43, 19.19; FTIR (neat, cm^{-1}) 2099 (N_3); MS (CI, NH_3) m/e (%) 371 [(M + NH_4)$^+$, 8], 354 (MH^+, 100), 341 (49), 326 (80), 276 (55); HRMS (CI, NH_3) calcd for $C_{20}H_{27}N_3OSiH$ (MH^+) 354.2002, found 354.1997.

4-[(*tert*-Butyldiphenylsilyl)oxy]butylamine (28). 4-[(*tert*-Butyldiphenylsilyl)oxy]butyl azide (**27**; 470 mg, 1.33 mmol) was dissolved in 10 mL of absolute ethanol and added to a 100 mL hydrogenation bottle. Then Pd−C (10%, 50 mg, catalytic) was added, and the resulting slurry was hydrogenated at 40 psi of H_2 pressure for 3 h. The catalyst was then filtered out using 1 in. Celite powder on a glass frit. The resulting filtrate was evaporated in vacuo to give 435 mg (100%) of **28** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.66−7.63 (m, 4H), 7.43−7.26 (m, 6H), 5.61 (br s, 2H, N*H*$_2$), 3.70−3.64 (t, 2H, J = 6.0 Hz, CH_2O), 2.86−2.81 (t, 2H, J = 6.6 Hz, CH_2N), 1.73−1.59 (m, 4H), 1.05 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 135.53, 133.99, 129.55, 127.62, 63.45, 40.83, 29.79, 27.06, 19.33; MS (DCI, NH_3) m/e (%) 328 (MH^+, 100), 270 (42); HRMS (DCI, NH_3) calcd for $C_{20}H_{29}NOSiH$ (MH^+) 328.2097, found 328.2085.

N^6-Benzoyl-2′,3′-isopropylidene-5′-[2-[[4-[(*tert*-butyldiphenylsilyl)oxy]butyl]amino]-4-[(*tert*-butyldiphenylsilyl)oxy]butyl]-5′-deoxyadenosine (29). 4-[(*tert*-Butyldiphenylsilyl)oxy]butylamine (**28**; 280 mg, 0.855 mmol) was weighed into a 50 mL round bottom flask along with the nucleoside ketone **26** (205 mg, 0.285 mmol), and both were dissolved in dry MeOH (10 mL). Bromothymol blue (1 mg, indicating amount) was then added to form a dark blue solution. The pH of the reaction mixture was then adjusted to pH 6 by dropwise addition of saturated methanolic HCl (yellow color). After this, $NaCNBH_3$ (18 mg, 0.285 mmol) was added resulting in a darker green solution color. Methanolic HCl was then used to restore the pH of the reaction mixture to 6. After stirring for 24 h, an additional amount of $NaCNBH_3$ (ca. 20 mg) was added along with 4 Å molecular sieves. The solution pH was titrated back to pH 6 (yellow) with methanolic HCl. The resulting solution was stirred at room temperature overnight. The sieve dust was removed via filtration through a glass frit, and the resulting filtrate was evaporated in vacuo. The oily residue was partitioned between EtOAc and H_2O. This biphasic mixture was stirred at ambient temperature for 2 h. The aqueous layer was removed, and the organic layer was washed three times with an equivalent volume of H_2O. The third wash required NaCl(s) to break an emulsion. The organic layer was then dried ($MgSO_4$), filtered, and evaporated in vacuo to give 360 mg of green foam. The foam was purified

by chromatography on silica gel eluting with 50% EtOAc in hexane to 100% EtOAc to give 150 mg (63%) of the HCl salt of **29** as a light green foam. The free base could be obtained as a foam by dissolving the HCl salt in $CHCl_3$/saturated $NaHCO_3$ solution with stirring followed by separation of the organic layer and workup in the usual manner: ^1H NMR ($CDCl_3$) δ 8.80 (s, 1H, H_8), 8.08–8.07 (d, 1H, H_2), 8.01–7.97 (d, 2H, benzoyl), 7.69–7.58 (m, 8H), 7.54–7.48 (m, 3H, benzoyl), 7.42–7.31 (m, 12H), 6.09 (s, 1H, H_1'), 5.47–5.43 (m, 1H, H_2'), 4.81–4.77 (m, 1H, H_3'), 4.17–4.13 (m, 1H, H_4'), 3.78–3.60 (m, 4H, CH_2O), 2.68–2.66 (m, 1H, methine), 2.50–2.45 (t, 2H, J = 6.6 Hz, CH_2N), 1.76–1.37 (m, 17H), 1.05 (d, 18H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 166.19, 152.61, 151.52, 148.92, 142.76, 135.51, 133.86, 133.46, 133.27, 133.17, 132.54, 129.81, 129.56, 129.04, 127.77, 127.61, 123.16, 115.09, 90.48, 90.40, 86.93, 84.20, 83.85, 63.63, 62.01, 55.99, 46.67, 46.55, 39.44, 35.16, 30.25, 29.82, 29.51, 29.33, 27.15, 26.88, 26.86, 26.11, 25.94, 25.38, 19.16, 19.10; MS (FAB+) m/e (%) 1031 (MH^+, 20), 927 (6), 792 (24), 240 (26), 197 (51), 135 (100), 105 (39); HRMS (FAB+) calcd for $C_{60}H_{74}N_6O_6Si_2H$ (MH^+) 1031.5287, found 1031.5203.

N^6-Benzoyl-2',3'-isopropylidene-5'-2-[*N*-[4-[(*tert*-butyldiphenylsilyl)oxy]butyl]-*N*-(tolylsulfonyl)amino]-4-[(*tert*-butyldiphenylsilyl)oxy]butyl]-5'-deoxyadenosine (30). Amino nucleoside **29** (50 mg, 0.047 mmol) was weighed into a round bottom flask and dissolved in dry CH_2Cl_2 (5 mL). Triethylamine (10 mg, 0.103 mmol) was added via syringe. Finally, toluenesulfonyl chloride (10 mg, 0.052 mmol) was added as a solid, and the resulting mixture was stirred at ambient temperature overnight. The reaction solution was then diluted with $CHCl_3$ and washed one time with an equivalent volume of H_2O. The organic layer was then dried ($MgSO_4$), filtered, and evaporated in vacuo to give a yellow oil. The oil was purified by chromatography on silica gel eluting with 50% EtOAc in hexane to 100% EtOAc to give 34 mg (61%) of **30** as a white foam: ^1H NMR ($CDCl_3$) δ 9.05 (s, 1H, N*H*), 8.84 (s, 1H, H_8), 8.08 (s, 1H, H_2), 8.03–7.95 (m, 2H, benzoyl), 7.64–7.26 (m, 25H), 7.08–7.06 (d, 2H, J = 7.2 Hz, tosyl), 6.08–6.05 (d, 1H, H_1'), 5.42–5.37 (m, 1H, H_2'), 4.78–4.73 (m, 1H, H_3'), 4.22–4.20 (m, 0.5H, H_4'), 4.13–4.08 (m, 0.5H, H_4'), 4.05 (m, 0.5H, methine), 3.89 (m, 0.5H, methine), 3.62–3.36 (m, 4H, CH_2O), 2.96–2.86 (m, 2H, CH_2NTs), 2.29 (s, 3H, tosyl CH_3), 1.83–1.31 (m, 16H), 1.04–0.88 (dd, 18H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 164.47, 152.81, 151.23, 149.59, 142.69, 142.65, 141.90, 138.25, 138.19, 135.42, 133.73, 133.54, 133.43, 132.69, 129.63, 129.51, 129.35, 128.77, 127.78, 127.57, 127.05, 127.00, 123.53, 114.93, 114.80, 90.18, 89.98, 86.29, 86.04, 83.99, 83.94, 83.77, 63.26, 60.79, 55.44, 54.76, 51.55, 43.67, 36.03, 30.60, 30.36, 30.12, 30.04, 29.73, 28.94, 27.55, 27.16, 26.77, 25.35, 21.35, 19.07, 18.98, 8.26; MS (FAB+) m/e (%) 1185 (MH^+, 22), 1127 (8), 197 (81), 135 (100). Anal. ($C_{67}H_{80}N_6O_8SSi_2 \cdot H_2O$) C, H, N.

N^6-Benzoyl-2',3'-isopropylidene-5'-[2-[(4-hydroxybutyl)-*N*-(tolylsulfonyl)amino]-4-hydroxybutyl]-5'-deoxyadenosine (31). Silylated nucleoside **30** (28 mg, 0.024 mmol) was weighed into a round bottom flask and dissolved in reagent THF (5 mL). TBAF (1 M solution in THF, 0.05 mL, 0.05 mmol) was added via syringe, and the resulting mixture was stirred at ambient temperature overnight. All volatiles were removed in vacuo to give an oil. The oil was purified by chromatography on silica gel eluting with 100% EtOAc to 10% MeOH in EtOAc to give 12 mg (71%) of **31** as a clear thick oil: ^1H NMR ($CDCl_3$) δ 9.80–9.60 (br s, 1H, N*H*), 8.78 (s, 1H, H_8), 8.16 (s, 1H, H_2), 8.06–8.03 (d, 2H, J = 8.1 Hz, benzoyl), 7.67–7.48 (m, 5H), 7.24–7.18 (apparent t, 2H, tosyl), 6.07–6.05 (m, 1H, H_1'), 5.47–5.37 (dd, 1H, H_2'), 4.67–4.59 (m, 1H, H_3'), 4.01–4.00 (m, 1H, H_4'), 3.83–3.74 (d of multiplets, 1H, methine), 3.59–3.55 (m, 2H, CH_2O), 3.48–3.40 (m, 2H, CH_2O), 3.40–2.70 (m, 4H), 2.35 (s, 3H, tosyl CH_3), 1.58–1.15 (m, 16H); ^{13}C NMR ($CDCl_3$) δ 164.00, 152.58, 151.24, 149.81, 143.10, 142.22, 137.98, 133.57, 132.57, 129.44, 128.62, 127.90, 127.05, 126.98, 123.61, 114.62, 90.43, 90.29, 86.62, 86.35, 83.91, 83.76, 61.89, 61.79, 58.73, 55.05, 43.86, 43.58, 38.53, 36.56, 36.25, 30.61, 30.04, 29.57, 29.22, 27.87, 27.77, 27.19, 25.41, 21.34; MS (FAB+) m/e (%) 709 (MH^+, 33), 240 (80), 136 (100), 105 (71); HRMS (FAB+) calcd for $C_{35}H_{44}N_6O_8SH$ (MH^+) 709.3019, found 709.3024.

N^6-(α-Imidazolylbenzylidene)-2',3'-isopropylidene-5'-[2-[(4-iodobutyl)-*N*-(tolylsulfonyl)amino]-4-iodobutyl]-5'-deoxyadenosine (32). Nucleoside diol **31** (125 mg, 0.176 mmol) was weighed into a round bottom flask and dissolved in dry CH_2Cl_2 (10 mL). Triphenylphosphine (277 mg, 1.06 mmol) was added along with I_2 (134 mg, 0.527 mmol) and imidazole (72 mg, 1.06 mmol). The resulting yellow solution was stirred in darkness overnight. A white precipitate was evident in the reaction solution. The precipitate was removed via filtration, and the resulting filtrate was evaporated in vacuo. The resulting residue was purified by chromatography on silica gel eluting with 100% EtOAc to 10% MeOH in EtOAc to give 130 mg (76%) of **32** as a white foam: broad UV absorbance band from 240 to 350 nm; ^1H NMR ($CDCl_3$) δ 8.67 (s, 1H, H_8), 8.10 (s, 1H, H_2), 7.90 (s, 1H), 7.75–7.00 (m, 11H), 6.04 (s, 1H, H_1'), 5.39–5.30 (m, 1H, H_2'), 4.76–4.69 (m, 1H, H_3'), 4.10–4.05 (m, 1H, H_4'), 3.74–3.63 (m, 1H, methine), 3.18–2.81 (m, 6H, CH_2I, CH_2NTs), 2.40 (s, 3H, tosyl CH_3), 1.88–1.25 (m, 16H); ^{13}C NMR ($CDCl_3$) δ 157.90, 152.69, 143.47, 142.73, 137.99, 137.54, 131.53, 130.72, 129.68, 128.98, 128.64, 127.03, 122.00, 118.31, 114.93, 90.13, 89.96, 85.88, 83.78, 83.61, 59.19, 58.92, 43.15, 42.84, 37.47, 31.87, 31.62, 30.59, 30.17, 28.62, 27.15, 25.33, 21.44, 5.78, 1.48; MS (FAB+) m/e (%) 979 (MH^+, 32), 911 (22), 851 (7), 520 (9), 222 (100).

N^6-(α-Imidazolylbenzylidene)-2',3'-isopropylidene-5'-[2-[(4-azidobutyl)-*N*-(tolylsulfonyl)amino]-4-azidobutyl]-5'-deoxyadenosine (33). Diiodo nucleoside **32** (130 mg, 0.133 mmol) was weighed into a round bottom flask and dissolved in dry DMF (5 mL). Sodium azide (173 mg, 2.66 mmol) was then added, and the resulting slurry was stirred for 48 h at ambient temperature. Excess DMF was then removed in vacuo (40 °C, 0.1 mmHg), and the resulting residue was purified by chromatography on silica gel eluting with 50% EtOAc in hexane to 100% EtOAc to give 39 mg (38%) of **33** as a white foam: broad UV absorbance band from 240 to 350 nm; ^1H NMR ($CDCl_3$) δ 8.65 (s, 1H, H_8), 8.00 (s, 1H, H_2), 7.88 (s, 1H), 7.68–7.16 (m, 11H), 5.99 (s, 1H, H_1'), 5.38–5.33 (m, 1H, H_2'), 4.74–4.67 (m, 1H, H_3'), 4.15–4.00 (m, 1H, H_4'), 3.79–3.65 (m, 1H, methine), 3.35–3.26 (t, 2H, J = 11.7 Hz, CH_2N_3), 3.22–3.10 (m, 2H, CH_2N_3), 3.05–2.83 (m, 2H, CH_2NTs), 2.39–2.37 (d, 3H, tosyl CH_3), 1.63–1.23 (m, 16H); ^{13}C NMR ($CDCl_3$) δ 157.96, 154.30, 152.68, 151.24, 143.43, 142.65, 138.05, 137.61, 131.48, 130.82, 130.33, 129.63, 129.01, 128.60, 127.00, 118.30, 114.96, 90.04, 89.95, 85.84, 83.82, 83.77, 83.62, 55.82, 55.51, 50.85, 50.74, 48.31, 48.26, 43.52, 43.20, 32.60, 32.53, 30.40, 30.23, 29.28, 29.11, 28.56, 28.33, 27.14, 26.40, 26.30, 25.32, 21.41; MS (FAB+) m/e (%) 809 (MH^+, 96), 741 (62), 222 (100).

5'-[2-[(4-Aminobutyl)amino]-4-aminobutyl]-5'-deoxyadenosine (1c). Nucleoside **33** (39 mg, 0.0482 mmol) was added to a 25 mL single-neck round bottom flask and dissolved in 80% formic acid (2 mL). The reaction mixture was stirred at ambient temperature for 5 h. The formic acid was then removed in vacuo to give the desired intermediate **34**: analytical HPLC (C_{18}, 100% H_2O to 100% CH_3CN, 30 min) t_R = 22.4 min, λ_{max} = 280 nm. Crude **34** was then dissolved in reagent MeOH (4 mL) together with NH_4OH (concentrated, 2 mL). The resulting mixture was stirred at ambient temperature overnight to give complete conversion to intermediate **35**: analytical HPLC (C_{18}, 100% H_2O to 100% CH_3CN, 30 min) t_R = 19.5 min, λ_{max} = 254 nm. Crude intermediate **35** (10 mg, 0.016 mmol) was then dissolved in dry DME and cooled to −78 °C. Sodium naphthalide solution (0.1 M in DME, ca. 1.46 mL, 0.146 mmol) was added dropwise via syringe to the rapidly stirred reaction mixture. The naphthalide was added continuously until a dark green color was maintained in the reaction mixture for at least 1 min. The reaction was then quenched with H_2O (2 mL), and all volatiles were then evaporated in vacuo. The resulting residue was partitioned between H_2O and Et_2O to remove excess naphthalene. The organic layer was separated, and the resulting aqueous layer was freeze-dried overnight. The resulting product was then obtained as a mixture in excess salt: analytical HPLC (C_{18}, 100% H_2O to 100% CH_3CN, 30 min) t_R = 8.5, 9.0 min (diastereomers), λ_{max} = 254 nm. Purification using preparative scale HPLC (100% H_2O to 100% CH_3CN, reverse phase C_{18}, 30 min) gave **1c** as a

mixture of two diastereomers together with inseparable inorganic salts: ^{1}H NMR (D_2O) δ 8.68 (s, 1H, H_8), 8.39 (s, 0.5H, H_2), 8.30 (s, 0.5H, H_2), 6.05–6.03 (d, 1H, H_1'), 4.87–4.85 (t, 1H, H_2'), 4.34–4.32 (t, 1H, H_3'), 4.14 (s, 1H, H_4'), 3.45–3.40 (m, 1H, methine), 3.15–2.98 (m, 6H), 2.15–1.64 (m, 10H); analytical HPLC (ion pair method)[39] t_R = 26.8, 27.6 min (diastereomers), λ_{max} = 254 nm. The structurally related nucleoside–polyamine adduct AdoDATO (**1a**) has t_R = 28.8 min under similar ion pair conditions (M. Kwiat and J. K. Coward, unpublished results).

Enzyme Assays. The concentration of stock solutions of AdoSpd (**1c**) was determined by quantitative UV absorbance spectroscopy using ϵ^{260} = 16 000 based on the previously determined value of ϵ^{260} = 15 400 for AdoDATO (**1a**) (K.-C. Tang and J. K. Coward, unpublished results). Aminopropyltransferases were isolated from rat liver or prostate and assayed by following the formation of 5′-methyl[^{35}S]thioadenosine from decarboxylated *S*-adenosyl[^{35}S]methionine as previously described.[40,41] The assay mixture for spermidine synthase contained 100 mM sodium phosphate, pH 7.5, 5 μM decarboxylated *S*-adenosyl[^{35}S]methionine (80 000 dpm), the concentration of putative inhibitor indicated and 0.5 mM putrescine in a total volume of 0.2 mL. For the assay of spermine synthase, the putrescine was replaced by 0.5 mM spermidine. After incubation at 37 °C for 30 min, the 5′-methyl[^{35}S]thioadenosine produced was separated from the substrate by chromatography on cellulose phosphate. Sufficient enzyme was added to ensure that 5–10 000 dpm was incorporated into product when no inhibitor was added.

Polyamine Analysis. Extracts from cells were deproteinized and putrescine, spermidine, and spermine separated and quantified using a slight modification[42] of the method described by Seiler and Knodgen.[43] Results were expressed as nmol of polyamine/mg of cell protein. Protein was measured by the method of Bradford.[44]

Cell Culture. Cell cultures were grown as previously described for CHO cells,[42] HT29 human colon carcinoma cells,[45] and monkey kidney COS-7 cells.[46] In some experiments, aminoguanidine (1 mM) was added to the culture medium to prevent any oxidation of primary amines in the drugs added.

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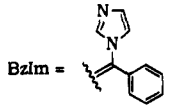
(35) Benzoylimidazole (BzIm) is the trivial name for the following N^6-substituent:



(36) When the iodination was run on small scale (ca. 20 mg of **31**), the expected N^6-benzoyl diiodo product was observed. This product was inseperable from the reaction byproduct $Ph_3P{=}O$. In both small and large scale syntheses, the relative number of equivalents of iodine, Ph_3P, and imidazole remained the same. The N^6-benzoyl diiodo derivative was converted to the N^6-benzoyl diazide which was used for analytical purposes. Removal of the benzoyl (NH_4OH) and isopropylidene (HCOOH) groups gave **35**.

(37) Upon separation, one byproduct appeared to be the N^6-benzoyl-diazido derivative. However, ^1H NMR analysis revealed this byproduct to be a mixture of at least two different compounds, one of which could have been the N^6-benzoyldiazide. Attempted further deprotection of this mixture of side products under acidic conditions resulted in hydrolysis of the glycosidic bond. In a similar fashion, reaction of the crude N^6-benzoyl diazide with NH_4OH results in cleavage of the benzoyl group.

(38) It occurred to us that AdoSpd might act as a SAPT substrate and be converted to AdoSpm. However, incubation of AdoSpd (1−100 μM) with SAPT in the absence of spermidine failed to produce any [^{35}S]MTA from [^{35}S]dcAdoMet. We conclude that AdoSpd is <0.1% the activity of spermidine as a SAPT substrate.

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