(two peaks superimposed), 142.2, 150.6, 154.0, 161.8, 178.7; IR (KBr) 1627, 1614, 1596, 1583, 1568 cm^{-1}; UV (CH_2Cl_2) 246 (log ϵ = 4.9), 334 (4.5) nm; MS, m/e 338 (35), 336 (100), [[M]$^{+\cdot}$], 310 (30), 308 (93), [[M - CO]$^{+\cdot}$]; HRMS m/e calcd for [M]$^{+\cdot}$ 336.0764, obsd 336.0764. Anal. Calcd for $C_{17}H_{17}ClO_5$: C, 60.63; H, 5.09; Cl, 10.53. Found: C, 60.4; H, 5.0; Cl, 10.7.

Single-Crystal X-ray Diffraction Analysis of Tropolone Methyl Ether 2. Crystal data: $C_{17}H_{17}ClO_5$, M_r 336.8, triclinic, space group $P\bar{1}$, a = 10.188 (2) Å, b = 10.720 (1) Å, c = 9.014 (2) Å, α = 107.48 (1)°, β = 95.60 (1)°, γ = 62.09 (1)°, V = 828.66 Å^3, T = 293 ± K, D_{calcd} = 1.35 g cm^{-3}, D_m = 1.37 g cm^{-3} (by flotation in $CHCl_3$/mesitylene), Z = 2, Mo Kα radiation of λ = 0.7107 Å, graphite monochromator, μ = 2.58 cm^{-1}, orange rhomboids. The crystal selected for intensity data collection measured approximately 0.3 × 0.3 × 0.3 mm. Unit cell constants were derived from a least-squares fit to the setting angles of 25 widely dispersed reflections on a Nonius CAD-4 diffractometer. Intensity data were collected by a variable-width, variable-speed $2\theta/\omega$ scan to the practical diffraction limit of θ = 28°. The data were corrected for Lorentz, polarization, and absorption effects (range of transmission factors 1.0000–0.9599). The data set consisted of 3177 unique reflections of which 1820 were deemed observed ($I > 3\sigma(I)$).[19] The structure was solved by using direct methods. The initial E map correctly revealed positions for all 23 non-hydrogen atoms. Refinement was by damped full-matrix least squares. Hydrogen atoms were located in a difference electron density map and their positions refined. Non-hydrogen atoms were assigned anisotropic thermal parameters. The function minimized was $\sum w(|F_o| - |F_c|)^2$. Atomic scattering factors were for neutral atoms. Reflection weights were $w = 3.2968/[\sigma^2(F) + gF^2]$ with final g being 5.51 × 10^{-4}. At convergence, R and Rw were 0.062 and 0.067 respectively. Further results of the crystallographic experiments are available and are described in the supplementary material paragraph.

Biological Studies on Tropolone Methyl Ethers 2 and 23. Biological assays were performed as previously described.[20] In the cell growth experiments (experiment I of Table I), the IC_{50} value cited is the drug concentration that inhibited the growth of L1210 murine leukemia cells by 50%. The percent mitoses values quoted (experiment II) were obtained after 12 h of growth in the presence of the indicated concentration of drug, when cells were harvested and stained and the mitotic index was determined by microscopic examination. Without drug, 4% mitotic figures were observed. In the microtubule assembly experiment (experiment III), reaction mixtures contained 0.1 M 2-morpholinoethanesulfonate (pH 7.0 with NaOH), 0.5 mM $MgCl_2$, 0.4 mM GTP, 1.5 mg/mL (15 μM) tubulin, 0.5 mg/mL microtubule-associated proteins, and 10 μM drug, except that in the experiment indicated by the value in parentheses 100 μM compound **23** was used. In the tubulin polymerization experiment (experiment IV), reaction mixtures contained 1.0 M monosodium glutamate (pH 6.6 with HCl), 1.0 mM $MgCl_2$, 0.4 mM GTP, 1.0 mg/mL (10 μM) tubulin, and 7.5 μM drug, except that in the experiment indicated by the value in parentheses 100 μM compound **23** was used. In the colchicine binding assays (experiment V), reaction mixtures contained 0.1 mg/mL (1 μM) tubulin, 5 μM [^{3}H]colchicine, and 5 μM drug, except that in the experiment indicated by the value in parentheses 50 μM compound **23** was used.

Acknowledgment. We are grateful to Professor A. Brossi (NIH) for his interest and encouragement. M.G.B. thanks the Australian Research Grants Committee for financial support. K.A.H. is grateful to New Zealand Federation of University Women (Auckland branch) for a postgraduate fellowship (1985–1986) and to the University of Melbourne for the provision of a postgraduate scholarship (1986–present). We thank L. Young (University of Auckland) for conducting some preliminary experiments.

Registry No. **2**, 116211-76-8; **6**, 116211-75-7; **7**, 58529-72-9; **10**, 116211-56-4; **10** ((*p*-(tolylsulfonyl)hydrazone), 116211-57-5; **11**, 116211-58-6; **12a**, 116211-59-7; **12b**, 116211-60-0; **12c**, 116211-62-2; **12d**, 116211-65-5; **13a**, 116211-61-1; **13b**, 116211-63-3; **13c**, 116211-64-4; **13d**, 116211-66-6; **14**, 95127-11-0; **15a**, 116211-68-8; **15b**, 116211-70-2; **16a**, 116211-69-9; **16b**, 116211-71-3; **17a**, 116211-67-7; **17b**, 116211-73-5; **18**, 116211-74-6; **19**, 116211-72-4; **22b**, 116211-77-9; **23**, 116211-78-0; 1,2,3-trimethoxybenzene, 634-36-6; hexachlorocyclopropane, 2065-35-2.

Supplementary Material Available: Tables of atomic coordinates, thermal parameters, interatomic distances and angles, least-squares planes, and torsion angles for **2** (6 pages). Ordering information is given on any current masthead page.

General Synthetic Approach to Stable Nitrogen Analogues of *S*-Adenosylmethionine

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Received April 21, 1988

A general synthetic approach to stable nitrogen alkyl SAM (*S*-adenosylmethionine) analogues, in which the sulfur atom is replaced by a nitrogen atom, is described. This procedure permits the methyl group of SAM to be replaced with larger saturated and unsaturated alkyl groups. The key step is the alkylation of a 5′-(alkylamino)-5′-deoxy-2′,3′-*O*-isopropylideneadenosine with methyl 2(*R*,*S*)-(trifluoroacetamido)-4-iodobutyrate. Subsequent deprotection of the trialkylamine intermediate by alkaline and then acidic hydrolysis provided the final compounds. This procedure has been used to prepare the methyl, ethyl, *n*-propyl, allyl, *n*-butyl, *n*-pentyl, and *n*-octyl nitrogen analogues of SAM. Elaboration of this method allows the synthesis of the 6-amino-1-hexyl nitrogen SAM analogue, a novel potential methyltransferase affinity ligand. Alkylation of 5′-[[6-[[(phenylmethoxy)carbonyl]amino]-1-hexyl]amino]-5′-deoxy-2′,3′-*O*-isopropylideneadenosine with methyl 2(*R*,*S*)-(trifluoroacetamido)-4-iodobutyrate provided the key trialkylamine intermediate. Subsequent deprotection by alkaline hydrolysis, catalytic hydrogenation, and finally acidic hydrolysis provided the final product, N^4-(5′-adenosyl)-N^4-(6-amino-1-hexyl)-2(*R*,*S*),4-diaminobutanoic acid, as a dihydrate.

S-Adenosyl-L-methionine (SAM) is well known as the biological equivalent of methyl iodode. SAM (1) has been found to be a methyl group donor in a wide variety of biochemical processes, including both DNA[1] and RNA

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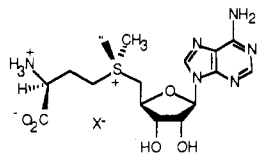
0022-3263/88/1953-4952$01.50/0

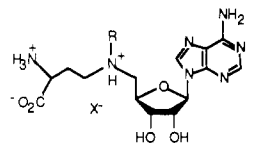


1

methylation,[2] bacterial chemotaxis,[3] and the formation and degradation of the catecholamine neurotransmitters epinephrine[4] and norepinephrine.[5] Consequently, the inhibition of the methyltransferases responsible for these and related processes is of considerable biochemical interest and has been studied by using the potent product inhibitor of this class of enzymes, S-adenosyl-L-homocysteine (SAH), and its many structural analogues.[6] Impeding the development of a wide variety of methyltransferase inhibitors analogous to SAM itself is the inherent chemical instability imparted on the molecule by the sulfonium moiety. This instability assumes the forms of racemization at the sulfonium center[7] and sulfonium group promoted degradation reactions, including an intramolecular cyclization[7a] providing 5′-deoxy-5′-(methylthio)adenosine and L-α-amino-γ-butyrolactone as well as a unique base-catalyzed hydrolysis of the glycoside bond.[7b,8] Replacing the sulfonium moiety of SAM with a tertiary amine should confer stability upon the resulting molecule.

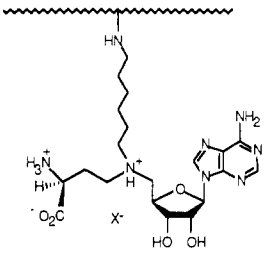
The analogue of SAM in which the sulfur atom is replaced by a nitrogen atom has been reported.[9] This compound (**2a**) will not be susceptible to the decomposition reactions peculiar to SAM.[10] The basic tertiary amino group in this analogue will be protonated at physiological pH and thus conserve the positive charge characteristic of the sulfonium group. Because a dialkylamine is not nearly so good a leaving group as is a dialkyl sulfide, this compound would not be expected to be a methyl group donor in methyltransferase-catalyzed reactions. However, initial testing with human placental homocysteine-N^5-methyltetrahydrofolate methyltransferase indicates that the nitrogen SAM analogue **2a** can serve as a substrate.[9] Other differences between SAM (**1**) and its nitrogen analogues (**2a**) involve bond lengths and bond angles. The average C–N bond length in trialkylammonium compounds is shorter (1.45 Å)[11] than the corresponding C–S bond length in trialkylsulfonium compounds (1.80 Å).[12] Also, the typical C–N–C bond angles (111°) in trialkylammonium compounds[11] are more nearly tetrahedral than the analogous C–S–C bond angles (102°) in trialkylsulfonium compounds.[12]

In light of these differences in structure and stability, the nitrogen SAM analogue **2a** should still compete with SAM for its binding regions in the active sites of methyltransferases. The ethyl, propyl, and higher alkyl nitrogen SAM analogues **2b–g** and **3**, also potential methyltransferase inhibitors, will be much more stable than the S-ethyl and S-propyl analogues of SAM, which are known to inhibit transmethylations,[13] but which can also undergo decompositions similar to those of SAM. Functionalization of the alkyl group of one of the nitrogen SAM analogues described above with an amino group (**3**) would provide a "handle" through which the compound could be immobilized on a suitably activated affinity adsorbent through a stable amide or secondary alkylamine linkage.



2a, R = Me
b, R = Et
c, R = n-Pr
d, R = CH_2=$CHCH_2$
e, R = n-Bu
f, R = n-Pe
g, R = n-Oc

3, R = $H_2N(CH_2)_6$



immobilized **3**

Structure-activity relationship studies have indicated that some amino acid modified (e.g., methyl ester) SAH analogues[6f] will not tightly bind to some methyltransferases, an observation that has implications for the utility of currently available methyltransferase affinity adsorbents

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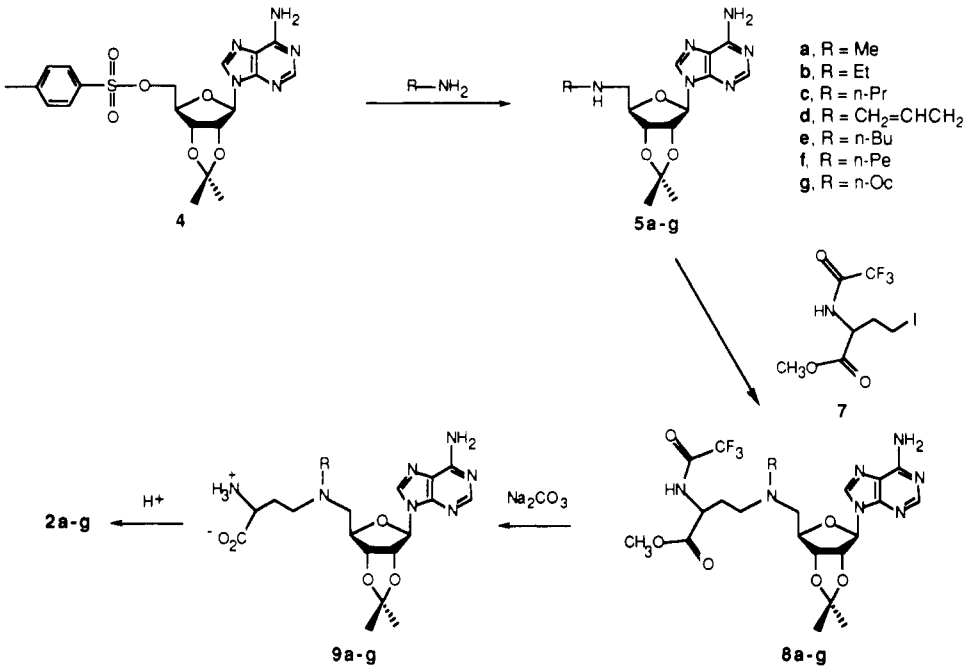
Scheme I



in which SAH is bound through its amino acid moiety. For these reasons, the development of a very general synthesis of nitrogen alkyl SAM analogues, compatible with the incorporation of both saturated and unsaturated alkyl groups, was pursued.

Results and Discussion

Synthesis of Nitrogen Alkyl SAM Analogues 2a–g. The general strategy followed in the synthesis of a series of nitrogen alkyl SAM analogues (**2a–g**) involved two successive monoalkylations of the primary alkylamine replacing the methylsulfonium moiety of SAM. The 5′-adenosyl group was linked to the primary alkylamine in the first alkylation and a homoserine equivalent was coupled to the resulting dialkylamine intermediate in the second alkylation. The trialkylamine product, bearing three protecting groups, was the immediate precursor of the nitrogen alkyl SAM analogues. The amino acid and ribose 2′,3′-diol protecting groups were removed by base and acid hydrolysis, respectively, to provide the final deprotected compounds **2a–g** (see Scheme I).

A derivative of adenosine, activated toward nucleophilic attack at the 5′-position, was needed to conduct the first monoalkylation of the saturated or unsaturated primary alkylamine replacing the methylsulfonium moiety of SAM. Tosylation of the 5′-hydroxy group of 2′,3′-*O*-isopropylideneadenosine with *p*-toluenesulfonyl chloride in pyridine has been shown to activate that position toward attack by a wide variety of nitrogen, sulfur, selenium, and halogen nucleophiles.[15] By dissolving 5′-*O*-tosyl-2′,3′-*O*-isopropylideneadenosine (**4**) in a large excess of a primary alkylamine, a series of 5′-(alkylamino)-5′-deoxy-2′,3′-*O*-isopropylideneadenosine homologues (**5a–g**) were readily obtained in 60–75% yield. (Monoalkylation of the primary alkylamine is favored over di- and trialkylation only when the amine is used in large excess.) Those primary alkylamines that have been used to displace the 5′-*O*-tosyl group in this manner included methylamine (**5a**), ethylamine (**5b**), *n*-propylamine (**5c**), allylamine (**5d**), *n*-butylamine (**5e**), *n*-pentylamine (**5f**), and *n*-octylamine (**5g**). The 5′-(allylamin)-5′-deoxy-2′,3′-*O*-isopropylideneadenosine homologue **5d** is of particular interest because it would ultimately have been hydrogenated to the corresponding propylamino compound if there were no alternative to the existing method[9] for synthesizing nitrogen alkyl SAM analogues. Essentially the same procedure has been used to prepare a group of 5′-(alkylamino)-5′-deoxyadenosine compounds directly from 5′-*O*-tosyladenosine,[16] as well as to prepare the 5′-deoxy-5′-(methylamino)-2′,3′-*O*-isopropylideneadenosine (**5a**) used in the original synthesis of the nitrogen methyl SAM analogue.[9]

Alkylation of the 5′-(alkylamino)-5′-deoxy-2′,3′-*O*-isopropylideneadenosine intermediates **5a–g** with a suitably protected homoserine derivative, activated toward nucleophilic attack at the 4-position, was expected to provide the desired nitrogen alkyl SAM analogues in protected form. Benzyl 2(*R,S*)-azido-4-iodobutyrate was used to alkylate the 5′-(benzylamino)-5′-deoxy-[6e] and 5′-deoxy-5′-(methylamino)-2′,3′-*O*-isopropylideneadenosine[9] (**5a**) compounds in the synthesis of the nitrogen analogues of SAH and SAM, respectively. The catalytic reduction of the azido group of this homoserine derivative to an amino group in a later step was obligatory in both syntheses and would preclude the incorporation of unsaturated alkyl groups elsewhere in the final product. For this reason, another derivative of homoserine, activated toward nucleophilic attack at the 4-position and possessing a suitably protected intact α-amino group, rather than a direct precursor thereof, was needed.

Trifluoroacetylation of methyl 2(*R,S*)-amino-4-iodobutyrate hydrochloride[17] (**6**), prepared in two steps from α-amino-γ-butyrolactone hydrobromide, would provide a compound suitable for the alkylation of the 5′-(alkylamino)-5′-deoxy-2′,3′-*O*-isopropylideneadenosine homologues **5a–g**. After coupling, both alkaline-labile amino acid protecting groups could be removed simultaneously in a one-pot reaction using either sodium carbonate[18] or

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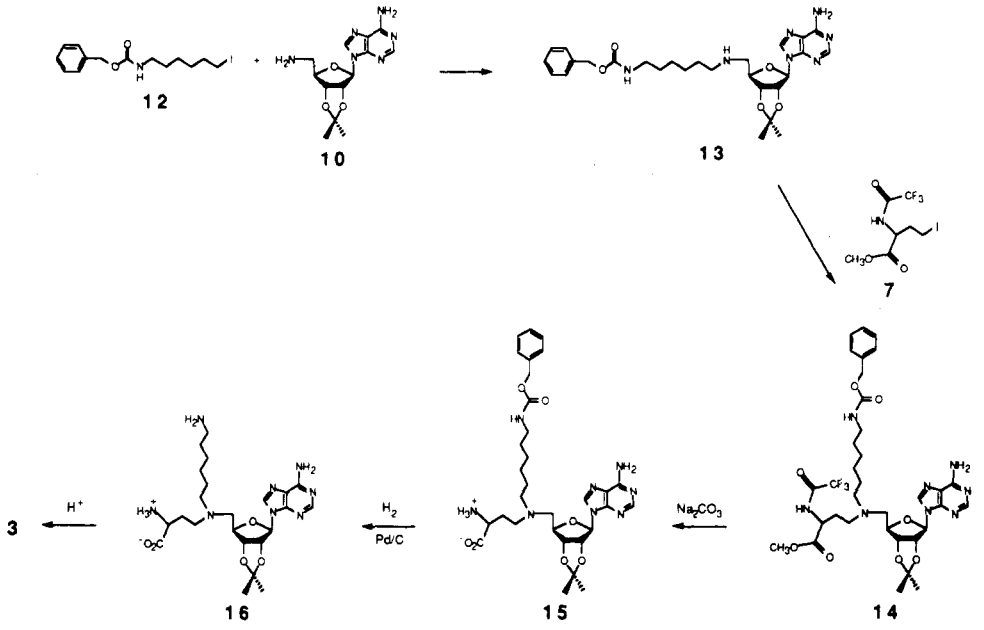
Scheme II



potassium carbonate[19] in aqueous methanol.[20] An adaptation of the procedures described for the formylation of potentially reactive amino acid esters,[21] in which trifluoroacetic anhydride replaced in situ generated formic anhydride, led to the preparation of methyl 2(*R*,*S*)-(trifluoroacetamido)-4-iodobutyrate (**7**) in excellent (90% yield. This procedure complements others that have been used to protect the amino group of methyl 2(*R*,*S*)-amino-4-iodobutyrate hydrochloride (**6**) as benzyl[22] and methyl carbamates.[23]

A small excess of methyl 2(*R*,*S*)-(trifluoroacetamido)-4-iodobutyrate (**7**) was used to alkylate each 5′-(alkylamino)-5′-deoxy-2′,3′-*O*-isopropylideneadenosine homologue **5a–g** in acetonitrile in the presence of *N*,*N*-diisopropylethylamine. The fully protected trialkylamine products **8a–g**, isolated as monohydrates from the crude reaction mixtures by silica gel chromatography, were obtained in low to moderate yields. Although alkylations with benzyl 2(*R*,*S*)-azido-4-iodobutyrate provided the corresponding trialkylamines in higher yield,[6e,9] increasing the scale of the present coupling reactions readily afforded an adequate supply of each protected trialkylamine product **8a–g**.

Analysis by high resolution (500 MHz) proton NMR indicated, as expected, that each fully protected nitrogen alkyl SAM analogue (**8a–g**) was actually present as a 50:50 mixture of two diastereomers, differing only in the stereochemistry around the α-carbon atom in the amino acid moiety. Particularly diagnostic of the presence of diastereomers were the resonances of both isopropylidene methyl groups, the methyl ester methyl group, the amino acid α-carbon hydrogen, the ribose C-2′ and C-3′ hydrogens, and the trifluoroacetamide hydrogen. These resonances, centered at 1.4, 1.6, 3.7, 4.5, 5.0, 5.5, and 9.5 ppm, respectively, were split into two groups of equal intensity, one group for each diastereomer. While many of the other hydrogens elsewhere in these compounds showed similar evidence of diastereomeric nonequivalence, such cases were not so dramatic as those listed above. These diastereomers were not readily separated by either flash or low pressure liquid chromatography on silica gel so as to permit the assignment of absolute stereochemistry at the α-carbon atom. Therefore, each 50:50 mixture of fully protected nitrogen alkyl SAM analogue diastereomers **8a–g** was used directly in the subsequent deprotection steps.

As expected, both amino acid protecting groups of the fully protected nitrogen alkyl SAM analogues **8a–g** could be removed simultaneously by treatment with sodium carbonate in hot 50% aqueous methanol. The loss of both protecting groups was readily monitored by thin layer chromatography of aliquots of the reaction mixture on silica gel, using methanol as the developing solvent. Each N^4-alkyl-N^4-(2′,3′-*O*-isopropylidene-5′-adenosyl)-2(*R*,*S*),4-diaminobutanoic acid homologue **9a–g** in a methanol extract of each neutralized reaction mixture was purified by flash silica gel chromatography in high yield and was used directly in the final deprotection step.

The removal of the final protecting group, the acid-labile isopropylidene ketal blocking the ribose 2′,3′-diol, was conducted in dilute aqueous sulfuric acid under conditions sufficiently mild to avoid any concomitant hydrolysis of the glycoside bond. All N^4-(5′-adenosyl)-N^4-alkyl-2(*R*,*S*),4-diaminobutanoic acid homologues **2a–g** in the neutralized reaction mixtures were obtained as hydrates in high yield by cation exchange chromatography and were characterized by microanalysis, mass spectrometry, and high resolution proton NMR.

Synthesis of 6-Amino-1-hexyl SAM Affinity Ligand (3). The strategy followed in the synthesis of the potential

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SAM affinity ligand, N^4-(5′-adenosyl)-N^4-(6-amino-1-hexyl)-2(*R*,*S*),4-diaminobutanoic acid (**3**), was to use as many of the techniques developed during the synthesis of the nitrogen alkyl SAM analogues **2a–g** as possible. Thus, the alkylation of the secondary alkylamino group of a suitably protected derivative of 5′-[(6-amino-1-hexyl)-amino]-5′-deoxy-2′,3′-*O*-isopropylideneadenosine with methyl 2(*R*,*S*)-(trifluoroacetamido)-4-iodobutyrate (**7**) would provide the key fully protected trialkylamine intermediate. Step-by-step removal of each of the four protecting groups present in this intermediate would ultimately provide the final product (see Scheme II). Immobilization of this compound, through its 6-amino-1-hexyl "tether" onto a suitably activated polymeric matrix, would then be possible. The resulting affinity adsorbent, by having the potential to immobilize any SAM-binding protein temporarily, might eventually become a useful laboratory tool for the purification of methyltransferases.

The most challenging feature of the synthesis of this stable nitrogen analogue of SAM was how to protect exclusively the primary alkylamino group of 5′-[(6-amino-1-hexyl)amino]-5′-deoxy-2′,3′-*O*-isopropylideneadenosine in the presence of a secondary alkylamino group. The latter group must remain unprotected so that it can be selectively alkylated with methyl 2(*R*,*S*)-(trifluoroacetamido)-4-iodobutyrate (**7**) in a subsequent step. An indirect route involving prior protection of the primary amino group of the eventual 6-amino-1-hexyl spacer, followed in a separate step by formation of the secondary amino group, proved to be the most reasonable solution to this problem. The (phenylmethoxy)carbonyl group was found to be the most suitable functionality for the protection of the primary alkylamino group[24] and ultimately made possible the synthesis of the 6-amino-1-hexyl nitrogen SAM affinity ligand **3**, which is described below.

A recent application of the Mitsunobu reaction[25] to nucleoside chemistry now permits 5′-amino-5′-deoxy-2′,3′-*O*-isopropylideneadenosine (**10**) to be prepared from commercially available 2′,3′-*O*-isopropylideneadenosine in two high-yielding steps.[10a] Alkylation of this amino nucleoside (**10**) with phenylmethyl (6-iodohexyl)carbamate (**12**), originally used in the preparation of subsequently immobilized 6-amino-1-hexyl glycosides,[26] was expected to provide the suitably protected nucleoside precursor. Indeed, in the presence of 1 equiv of *N*,*N*-diisopropylethylamine in acetonitrile, the alkylation of 1 equiv of the amino nucleoside **10** with 1 equiv of the iodohexane **12** furnished the desired product, 5′-[[6-[[(phenylmethoxy)-carbonyl]amino]-1-hexyl]amino]-5′-deoxy-2′,3′-*O*-isopropylideneadenosine (**13**), in moderate (48%) yield. The monoalkylation product was readily separated from the unreacted starting materials and the dialkylation side product by flash silica gel chromatography.

The alkylation of the dialkylamine intermediate **13** with the protected homoserine equivalent **7** was carried out under conditions identical with those used in the synthesis of the nitrogen alkyl SAM analogues **2a–g** described above. Again, the protected trialkylamine intermediate **14** was isolated as a monohydrate in low (21%) yield. Analysis of this material by high resolution (500 MHz) proton NMR spectroscopy revealed, as expected, the presence of two diastereomers. Particularly noteworthy was the diastereomeric nonequivalence observed with the resonances of the methyl ester methyl group and the trifluoroacetamide hydrogen at 3.7 and 9.5 ppm, respectively. Unlike the protected nitrogen alkyl SAM analogues **2a–g** above, these diastereomers did exhibit slightly different chromatographic mobilities on silica gel. Upon demonstration [research currently in progress] that the immobilized final product does indeed act in an "affinity" manner, the respective diastereomers of this compound will be separated at this stage and individually studied.

Complete deprotection (see Scheme II) of the amino acid moiety of the trialkylamine intermediate **14** was readily accomplished by starting with mild alkaline hydrolysis of the two amino acid protecting gropus with sodium carbonate in warm 50% aqueous methanol, as described above for the nitrogen alkyl SAM analogues **2a–g**. Subsequent removal of the phenylmethyl carbamate protecting group was achieved by hydrogenation in 50% aqueous formic acid[6e] in the presence of a palladium catalyst. The acidic solvent, which was necessary for the rapid hydrogenolysis of the phenylmethyl carbamate protecting group, was responsible for the slight (approximately 5%) hydrolysis of the isopropylidene ketal but did not hydrolyze the glycoside bond. Complete hydrolysis of the isopropylidene ketal was subsequently conducted in dilute aqueous sulfuric acid. The completely deprotected 6-amino-1-hexyl nitrogen SAM affinity ligand **3** was isolated from the neutralized crude reaction mixture by cation exchange chromatography. Microanalysis, mass spectrometry, and high resolution (500 MHz) proton NMR spectroscopy were all used to confirm the identity of the recovered colorless solid as N^4-(5′-adenosyl)-N^4-(6-amino-1-hexyl)-2(*R*,*S*),4-diaminobutanoic acid dihydrate (**3**).

Biological Testing. Initial rigorous biological testing of the nitrogen alkyl SAM analogues has thus far been conducted with the nitrogen methyl SAM analogue **2a** and the enzymes *E. coli* transfer RNA (uracil-5-)-methyltransferase and *E. coli* B methionine synthase. The former enzyme, probably employing a mechanism of action similar to that described for DNA (cytosine-5-)-methyltransferase,[1a] catalyzes the substitution of the hydrogen at position 5 of a transfer RNA uridine with the methyl group of SAM to provide a thymidine. Testing of the nitrogen SAM analogue **2a** with *E. coli* transfer RNA (uracil-5-)-methyltransferase has revealed that this compound functions as an inhibitor but not as a methyl group donor (i.e., substrate).[27] Interestingly, in the presence of the nitrogen SAM analogue **2a** and in the absence of SAM, the rate of tritium release from the [5-^{3}H]uridine-labeled 5-methyluridine-deficient transfer RNA substrate is essentially the same as that seen under normal turnover conditions when SAM alone is present, but no product is formed.[27] This result may indicate that both SAM and the nitrogen SAM analogue **2a** may induce conformational changes somehow facilitating tritium release.[27]

Methionine synthase is a bacterial enzyme catalyzing the transfer of the methyl group of N^5-methyltetrahydrofolate to L-homocysteine to form L-methionine. SAM is required in only catalytic amounts to activate or "prime" the enzyme at an unknown site.[28,29] Testing with *E. coli* B methionine synthase has indicated that the nitrogen SAM analogue **2a** is a potent inhibitor [competitive with respect to SAM] of normal enzyme activity, with a K_i estimated to be 9 μM.[30] The enzyme cannot be "primed" in the presence

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of this compound and consequently fails to exhibit catalytic activity.

The immobilization of the 6-amino-1-hexyl nitrogen SAM analogue **3** on epoxy-activated Sepharose 6B and activated CH-Sepharose 4B, both commercially available from Pharmacia, has been accomplished.[24] These coupling procedures and the results of the current testing of these coupled gels as potential methyltransferase affinity adsorbents will be reported in the near future.

Experimental Section

Flash silica gel (230–400 mesh), Dowex 50X4-400, and all compounds were purchased from Aldrich. The SP-Sephadex C-25 cation exchanger was obtained from Sigma. Both 5′-*O*-tosyl-2′,3′-*O*-isopropylideneadenosine[15a] (**4**) and 5′-amino-5′-deoxy-2′,3′-*O*-isopropylideneadenosine[10a] (**10**) were prepared from 2′,3′-*O*-isopropylideneadenosine according to literature procedures. Phenylmethyl (6-hydroxyhexyl)carbamate was also prepared by a known method.[26] Precoated Baker 1B2-F silica gel sheets were used for analytical TLC. Low pressure liquid chromatography using a UV detector (280 nm) was carried out on a Lobar Size B (310-25) Lichroprep Si60 silica gel column. All melting points are uncorrected. Ultraviolet spectra were recorded on a spectrophotometer equipped with a Peltier temperature controller. Samples for positive liquid secondary ion mass spectrometry (LSIMS) were run in a matrix of glycerol and 1 M aqueous HCl.

General Preparation of 5′-(Alkylamino)-5′-deoxy-2′,3′-*O*-isopropylideneadenosine Homologues 5a–g. Tosylate **4**[15a] (10.00 g, 21.7 mmol) was dissolved in freshly distilled alkylamine (100 mL, large excess) and stirred at room temperature for 3 days. After evaporation, the residue was partitioned between CH_2Cl_2 (220 mL) and 0.1 M aqueous NaOH (220 mL) and the aqueous layer washed with CH_2Cl_2 (2 × 200 mL). The pooled CH_2Cl_2 extracts were washed with saturated aqueous NaCl (2 × 200 mL), dried ($MgSO_4$), and evaporated. The residue was dissolved in $CHCl_3$ (100 mL), concentrated to approximately 5 mL, and loaded onto a wet-packed flash silica gel column (44 × 3.5 cm, 200 g). The column was eluted with MeOH at a flow rate of 10 mL/min and 28-mL fractions were collected. Pure product (as judged by TLC) generally appeared in fractions 15–80 depending on the alkylamino substituent. The appropriate fractions were pooled, evaporated, twice redissolved in $CHCl_3$ (100 mL), and evaporated to provide a colorless hygroscopic foam. Yields ranged from 65% to 80%.

5′-Deoxy-5′-(methylamino)-2′,3′-*O*-isopropylideneadenosine (5a) was prepared according to the above general procedure with only minor differences. Tosylate **4** was dissolved in anhydrous methylamine (approximately 75 mL) and sealed in a bomb for 3 days. The bomb was cooled in dry ice and opened, and the methylamine was allowed to boil off at room temperature overnight. Treatment of the residue as described in the general procedure above followed by purification on a slightly smaller silica gel column (40 × 3.5 cm, 180 g) provided 5.02 g (70.3%) of pure **5a**: mp 54–56 °C; TLC (MeOH) R_f 0.26 (UV, I_2); 500-MHz ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 3 H, isopropylidene CH_3), 1.62 (s, 3 H, isopropylidene CH_3), 2.44 (s, 3 H, CH_3NH), 2.85 (dd, 1 H, J = 6.2 Hz, 12.5 Hz, H-5′), 2.89 (dd, 1 H, J = 4.4 Hz, 12.5 Hz, H-5′), 4.38 (m, 1 H, J = 3.3 Hz, 4.4 Hz, 6.2 Hz, H-4′), 5.02 (dd, 1 H, J = 3.3 Hz, 6.4 Hz, H-3′), 5.48 (dd, 1 H, J = 3.2 Hz, 6.4 Hz, H-2′), 6.01 (d, 1 H, J = 3.2 Hz, H-1′), 6.19 (br s, 2 H, adenine NH_2), 7.92 (s, 1 H, adenine H-2 or H-8), 8.36 (s, 1 H, adenine H-2 or H-8); UV λ_{max} (MeOH) 259 nm (ϵ 12.5 × 10^3); MS (EI), m/e 305 (M − isopropylidene CH_3)$^+$, 185 (M − adenine)$^+$, 136 (adenine + H)$^+$. Anal. Calcd for $C_{14}H_{20}N_6O_3 \cdot 0.5H_2O$: C, 51.05; H, 6.42; N, 25.52. Found: C, 50.86; H, 6.19; N, 25.52.

Purification details and characterization data for **5b–g** are available as supplementary material.

Methyl 2(*R*,*S*)-amino-4-iodobutyrate hydrochloride (6) was prepared in two steps from α-amino-γ-butyrolactone hydrobromide according to the procedure of Frankel and Knobler.[17] Five recrystallizations from EtOH/ether were necessary to provide **6** as colorless crystals in 58% overall yield (lit.[17] 64%): mp 106–108 °C (lit.[17] mp 125 °C); 240-MHz ^{1}H NMR (D_2O) δ 2.25–2.58 (m, 2 H, $CHCH_2CH_2$), 3.31 (t, 2 H, J = 7.1 Hz, $CHCH_2CH_2$), 3.82 (s, 3 H, CH_3), 4.22 (t, 1 H, J = 6.6 Hz, $CHCH_2CH_2$); MS (positive LSIMS), m/e 244 (M + H)$^+$.

Methyl 2(*R*,*S*)-(trifluoroacetamido)-4-iodobutyrate (7) was prepared by an extension of the formylation procedure originally described by Chen and Benoiten.[21a] 4-Methylmorpholine (9.56 mL, 86.0 mmol) was added to an ice-cold stirred suspension of methyl ester **6** (12.000 g, 43.0 mmol) in CH_2Cl_2 (1.0 L, freshly distilled from P_2O_5). Trifluoroacetic anhydride (30.54 mL, 215.4 mmol) was added approximately 15 min later, and the resulting solution was stirred at 4 °C. for 18 h. The crude reaction mixture was washed with 1 M aqueous HCl (5 × 700 mL), saturated aqueous $NaHCO_3$ (4 × 700 mL), and saturated aqueous NaCl (4 × 700 mL). The CH_2Cl_2 solution was dried over anhydrous $MgSO_4$ for 30 min, filtered, and then evaporated. The residual oil was dissolved in $CHCl_3$ (100 mL), and the resulting solution was concentrated to 20 mL and triturated with ice-cold hexanes (200 mL). All solvent was evaporated to provide 13.19 g (90.5%) of almost pure pale brown **7**. A colorless analytical sample was obtained by filtering the triturated $CHCl_3$–hexanes mixture prior to evaporation: mp 54–58 °C; 240-MHz ^{1}H NMR ($CDCl_3$) δ 2.44 (m, 2 H, CH_2CH_2I), 3.14 (t, 2 H, j = 7.6 Hz, CH_2I), 3.84 (s, 3 H, CH_3), 4.69 (m, 1 H, CH), 7.00 (br s, 1 H, NH); MS (EI), m/e 280 (M − CO_2CH_3)$^+$, 212 (M − I)$^+$. Anal. Calcd for $C_7H_9F_3INO_3$: C, 24.80; H, 2.72; N, 4.13; I, 37.43. Found: C, 24.88; H, 2.72; N, 4.22; I, 37.24.

General Preparation of Methyl N^4-Alkyl-N^4-(2′,3′-*O*-isopropylidene-5′-adenosyl)-2(*R*,*S*)-(trifluoroacetamido)-4-aminobutyrate Homologues 8a–g. The 5′-(alkylamino)-adenosine derivative (**5a–g**) (15.0 mmol) and *N*,*N*-diisopropylethylamine (2.87 mL, 16.5 mmol, 10% excess) were dissolved in MeCN (250 mL) and heated with stirring to 70 °C. A solution of **7** (18.8 mmol, 25% excess) in MeCN (150 mL) was added over approximately 1 h. The mixture was stirred at 70 °C for 3 days, during which time its initial pale brown color progressively deepened. The cooled reaction mixture was evaporated, redissolved in $CHCl_3$ (100 mL), concentrated to 5 mL, and loaded onto a wet-packed flash silica gel column (44 × 3.5 cm, 200 g). The column was eluted with $CHCl_3$/*i*-PrOH (80:20 or 90:10) at a flow rate of 10 mL/min and 28-mL fractions were collected. Partially purified product (as judged by TLC) generally appeared in fractions 12–50, and the appropriate fractions were pooled and concentrated to approximately 15 mL. Further purification was achieved by low pressure liquid chromatography using a Lobar Size B (310–25) LiChroprep Si60 silica gel column. The column was eluted with $CHCl_3$/*i*-PrOH (70:30 or 80:20) at a flow rate of 4.0 mL/min. Injection of 0.70-mL aliquots onto the column under these conditions generally led to the elution (as detected by absorbance at 280 nm) of the product some 30–65 min later. Column eluate containing the product was collected, evaporated, redissolved in a minimal volume (5–10 mL) of the eluting solvent, and reinjected onto the same column until all impurities were removed. Any residual amber color in a $CHCl_3$ solution (5 mL volume) of the product was removed by filtration through a wet-packed silica gel column (40 × 3.5 cm, 180 g). Elution with $CHCl_3$/*i*-PrOH (92:8 or 95:5) at a flow rate of approximately 10 mL/min and collection of 28-mL fractions generally led to the appearance of the product in fractions 19–62. The appropriate fractions were pooled, evaporated, twice dissolved in $CHCl_3$ (100 mL), and evaporated to provide a colorless foam. Yields ranged from 20% to 45%.

Methyl N^4-(2′,3′-*O*-isopropylidene-5′-adenosyl)-N^4-methyl-2(*R*,*S*)-(trifluoroacetamido)-4-aminobutyrate (8a) was prepared from **5a** (5.47 g, 16.6 mmol) and a slightly smaller excess (10%) of **7** (6.37 g, 18.8 mmol). Following initial silica gel [43 × 3.5 cm, 190 g, $CHCl_3$/i-PrOH (90:10)] and low pressure liquid chromatography [3.5 mL/min, $CHCl_3$/*i*-PrOH (70:30), 41–69 min post-injection], final decolorization [$CHCl_3$/*i*-PrOH (92:8)] provided 3.83 g (42.1%) of pure **8a** as a colorless foam: mp 59–61 °C; TLC ($CHCl_3$/*i*-PrOH (90:10)) R_f 0.27 (UV, I_2); 500-MHz ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 1.5 H, isopropylidene CH_3), 1.40 (s, 1.5 H, isopropylidene CH_3), 1.62 (s, 3 H, isopropylidene CH_3), 1.87 (m, 2 H, CH_2CH_2N), 2.23 (s, 1.5 H, CH_3N), 2.24 (s, 1.5 H, CH_3N), 2.43–2.61 (m, 2 H, CH_2CH_2N), 2.71 (m, 2 H, H-5′), 3.64 (s, 1.5 H, CO_2CH_3), 3.77 (s, 1.5 H, CO_2CH_3), 4.33 (m, 1 H,

(30) Matthews, R.; Frasca, V., University of Michigan, unpublished results.

H-4′) (m, 1 H, C*H*CH$_2$CH$_2$N), 4.93 (dd, 0.5 H, J = 3.9 Hz, 6.4 Hz, H-3′), 5.00 (dd, 0.5 H, J = 3.7 Hz, 6.4 Hz, H-3′), 5.46 (dd, 0.5 H, J = 1.8 Hz, 6.4 Hz, H-2′), 5.51 (dd, 0.5 H, J = 1.8 Hz, 6.4 Hz, H-2′), 5.77 (br s, 1 H, adenine NH$_2$), 5.78 (br s, 1 H, adenine NH$_2$), 6.04 (d, 0.5 H, J = 1.8 Hz, H-1′), 6.05 (d, 0.5 H, J = 1.8 Hz, H-1′), 7.85 (s, 0.5 H, adenine H-2 or H-8), 7.91 (s, 0.5 H, adenine H-2 or H-8), 8.33 (s, 0.5 H, adenine H-2 or H-8), 8.34 (s, 0.5 H, adenine H-2 or H-8), 9.46 (br d, 0.5 H, J = 6.7 Hz, NHCOCF$_3$), 9.49 (br d, 0.5 H, J = 6.8 Hz, NHCOCF$_3$); UV λ_{max} (MeOH) 259 nm (ϵ 14.9 × 10^3); MS (positive LSIMS), m/e 532 (M + H)$^+$, 397 (M + H − adenine)$^+$. Anal. Calcd for $C_{21}H_{28}F_3N_7O_6 \cdot H_2O$: C, 45.90; H, 5.50; N, 17.84. Found: C, 45.62; H, 5.25; N, 17.56.

Purification details and characterization data for **8b–g** are available as supplementatary material.

General Preparation of N^4-(5′-Adenosyl)-N^4-alkyl-2-(*R*,*S*),4-diaminobutanoic Acid Homologues 2a–g. A solution of Na_2CO_3 (663 mg, 6.25 mmol) in 50% aqueous MeOH (30 mL) was added to the protected nitrogen alkyl SAM analogue (**8a–g**) (2.50 mmol). This mixture was stirred at 60 °C for 6 h, after which time it was neutralized with 1 M aqueous HCl and evaporated. The residue was extracted with MeOH (4 × 25 mL) and the combined extracts were concentrated to approximately 5 mL before being loaded onto a wet-packed flash silica gel column (44 × 3.5 cm, 200 g). The column was eluted first with $CHCl_3$/MeOH (70:30 or 50:50, 2.0 L) followed by 100% MeOH (1.0 L) at a flow rate of approximately 10 mL/min and 28-mL fractions were collected. Those fractions containing the UV-absorbing product, which generally appeared in fractions 55–110, were pooled and evaporated. The intermediate N^4-alkyl-N^4-(2′,3′-*O*-isopropylidene-5′-adenosyl)-2(*R*,*S*),4-diaminobutanoic acid (**9a–g**) was generally obtained in very high yields and was sufficiently pure to be used directly in the next step.

The crude intermediate (**9a–g**) was dissolved in 0.1 M aqueous H_2SO_4 (75 mL) and the mixture stirred at room temperature for 11 days. The acid was neutralized by the addition of an equivalent amount of Ba(OH)$_2$·8H$_2$O and the $BaSO_4$ precipitate removed by centrifugation. After the filtered aqueous solution was evaporated, the residue was redissolved in water (300 mL) and loaded at 4 °C onto a Dowex 50X4-400 (NH_4^+ form) cation exchange column (44 × 3.5 cm, 450 g) at a flow rate of 2 mL/min and 28-mL fractions were collected. Once the column was washed with water (700 mL), elution was begun with a gradient of aqueous NH_4HCO_3 (5.0 L, 0–1.3 M) beginning with fraction 31 at a flow rate of 3 mL/min. The UV-absorbing product was eluted from the column at buffer concentrations ranging from 0.70 ω 1.20 M depending on the length (i.e., hydrophobicity) of the N^4-alkyl group. [The only exception to this generalization was the N^4-(1-octyl) homologue **2g**, which could only be eluted from the column with 1 M aqueous NH_4OH.] The appropriate fractions were pooled in preparation for desalting and reloaded onto the Dowex 50X4-400 column (44 × 3.5 cm, 450 g, freshly regenerated into the NH_4^+ form after being washed with 1 M aqueous HCl and 1 M aqueous NH_4Cl) at a flow rate of 2 mL/min and 28-mL fractions were collected. The column was washed with water (2.0 L) at a flow rate of 3 mL/min and the adsorbed product eluted with 1 M aqueous NH_4OH. Those fractions containing the desalted product were pooled and evaporated. Product not initially adsorbed onto the Dowex due to overloading was reloaded onto the same column (after washing with water) and washed, eluted, and evaporated as described above. The product was redissolved in water (10 mL) and lyophilized to provide a colorless powder. Overall yields were 60–80%.

N^4-(5′-Adenosyl)-N^4-methyl-2-(*R*,*S*),4-diaminobutanoic acid (2a) was prepared by starting with the alkaline hydrolysis of **8a** (4.395 g, 8.01 mmol). Flash silica gel chromatography [47 × 3.5 cm, 230 g, $CHCl_3$/MeOH (50:50)] provided 3.83 g (97.4%) of crude intermediate **9a** as a colorless solid: mp 120–123 °C; TLC (MeOH) R_f 0.29 (UV, I_2, ninhydrin); 500-MHz ^{1}H NMR (D_2O) δ 1.45 (s, 3 H, isopropylidene CH$_3$), 1.66 (s, 3 H, isopropylidene CH$_3$), 1.79–1.98 (m, 2 H, CHC*H*$_2$CH$_2$N), 2.22 (s, 1.5 H, CH$_3$N), 2.23 (s, 1.5 H, CH$_3$N), 2.52–2.77 (m, 4 H, overlapping H-5′ and CHCH$_2$C*H*$_2$N), 3.65 (m, 1 H, C*H*CH$_2$CH$_2$N), 4.49 (m, 1 H, H-4′), 5.03 (m, 1 H, H-3′), 5.56 (m, 1 H, H-2′), 6.25 (d, 0.5 H, J = 2.5 Hz, H-1′), 6.26 (d, 0.5 H, J = 2.6 Hz, H-1′), 8.24 (s, 1 H, adenine H-2 or H-8), 8.29 (s, 1 H, adenine H-2 or H-8); MS (positive LSIMS), m/e (M + H)$^+$.

Subsequent acidic hydrolysis of crude **9a** followed by cation exchange chromatography [peak elution of **2a** at 0.76 M] provided 2.17 g (66.5% overall) of pure **2a** as a colorless solid: mp 173–176 °C; TLC (EtOH/concentrated NH_4OH (90:10)) R_f 0.37 (UV, I_2, ninhydrin); 500-MHz ^{1}H NMR (D_2O) δ 1.95 (m, 1 H, CHC*H*$_2$CH$_2$N), 2.04 (m, 1 H, CHC*H*$_2$CH$_2$N), 2.35 (s, 1.5 H, CH$_3$N), 2.36 (s, 1.5 H, CH$_3$N), 2.67–2.80 (m, 2 H, CHCH$_2$C*H*$_2$N), 2.84–2.93 (m, 2 H, H-5′), 3.71 (m, 1 H, C*H*CH$_2$CH$_2$N), 4.22 (q, 1 H, H-3′), 4.31 (m, 1 H, H-4′), 4.75 (m, 1 H, H-2′), 5.97 (d, 0.5 H, J = 2.4 Hz, H-1′), 5.98 (d, 0.5 H, J = 2.5 Hz, H-1′), 8.05 (s, o.5 H, adenine H-2 or H-8), 8.06 (s, 0.5 H, adenine H-2 or H-8), 8.18 (s, 0.5 H, adenine H-2 or H-8), 8.19 (s, 0.5 H, adenine H-2 or H-8); UV λ_{max} (H_2O) 259 nm (ϵ 14.2 × 10^3); MS (positive LSIMS), m/e 382 (M + H)$^+$. Anal. Calcd for $C_{15}H_{23}N_7O_5 \cdot 1.5H_2O$: C, 44.11; H, 6.42; N, 24.01. Found: C, 44.46; H, 6.23; N, 24.34.

N^4-(5′-Adenosyl)-N^4-ethyl-2(*R*,*S*),4-diaminobutanoic acid (2b) was prepared by starting with the alkaline hydrolysis of **8b** (1.619 g, 2.90 mmol). Flash silica gel chromatography [$CHCl_3$/MeOH (50:50), then MeOH] provided 1.07 g (84.9%) of crude intermediate **9b** as a colorless solid: mp 112–115 °C; TLC (MeOH) R_f 0.31 (UV, I_2, ninhydrin); 500-MHz ^{1}H NMR (D_2O) δ 0.67 (t, 1.5 H, J = 7.1 Hz, C*H*$_3$CH$_2$N), 0.76 (t, 1.5 H, J = 7.1 Hz, C*H*$_3$CH$_2$N), 1.41 (s, 3 H, isopropylidene CH$_3$), 1.61 (s, 3 H, isopropylidene CH$_3$), 1.62–1.84 (m, 2 H, CHC*H*$_2$CH$_2$N), 2.45 (m, 2 H, CH$_3$C*H*$_2$N), 2.51–2.66 (m, 4 H, overlapping H-5′ and CHCH$_2$C*H*$_2$N), 3.46 (m, 1 H, C*H*CH$_2$CH$_2$N), 4.41 (m, 1 H, H-4′), 4.97 (m, 1 H, H-3′), 5.47 (dd, 0.5 H, J = 2.2 Hz, 6.4 Hz, H-2′), 5.51 (dd, 0.5 H, J = 2.0 Hz, 6.3 Hz, H-2′), 6.14 (d, 0.5 H, J = 2.2 Hz, H-1′), 6.16 (d, 0.5 H, J = 2.0 Hz, H-1′), 8.11 (s, 0.5 H, adenine H-2 or H-8), 8.12 (s, 0.5 H, adenine H-2 or H-8), 8.19 (s, 0.5 H, adenine H-2 or H-8), 8.20 (s, 0.5 H, adenine H-2 or H-8); MS (positive LSIMS), m/e 436 (M + H)$^+$.

Subsequent acidic hydrolysis of crude **9b** followed by cation exchange chromatography [peak elution of **2b** at 0.86 M] provided 847 mg (69.3% overall) of pure **2b** as a colorless solid: mp 160–170 °C; TLC (EtOH/concentrated NH_4OH (90:10)) R_f 0.19 (UV, I_2, ninhydrin); 500-MHz ^{1}H NMR (D_2O) δ 1.04 (t, 1.5 H, J = 7.1 Hz, C*H*$_3$CH$_2$N), 1.05 (t, 1.5 H, C*H*$_3$CH$_2$N), 1.96 (m, 1 H, CHC*H*$_2$CH$_2$N), 2.05 (m, 1 H, CHC*H*$_2$CH$_2$N), 2.70–2.84 (m, 2 H, CH$_3$C*H*$_2$N), 2.89 (m, 2 H, CHCH$_2$C*H*$_2$N), 2.99 (m, 2 H, H-5′), 3.70 (m, 1 H, C*H*CH$_2$CH$_2$N), 4.29 (m, 1 H, H-3′), 4.34 (m, 1 H, H-4′), 4.73 (m, 1 H, H-2′), 6.04 (d, 0.5 H, J = 1.9 Hz, H-1′), 6.05 (d, 0.5 H, J = 2.0, Hz, H-1′), 8.17 (s, 1 H, adenine H-2 or H-8), 8.25 (s, 1 H, adenine H-2 or H-8); UV λ_{max} (H_2O) 259 nm (ϵ 13.7 × 10^3); MS (positive LSIMS), m/e 396 (M + H)$^+$. Anal. Calcd for $C_{16}H_{25}N_7O_5 \cdot 1.5H_2O$: C, 45.49; H, 6.68; N, 23.21. Found: C, 45.60; H, 6.47; N, 23.11.

N^4-(5′-Adenosyl)-N^4-(1-propyl)-2(*R*,*S*),4-diaminobutanoic acid (2c) was prepared beginning with the alkaline hydrolysis of **8c** (1.684 g, 2.92 mmol). Flash silica gel chromatography [$CHCl_3$/MeOH (70:30), then MeOH] provided 1.49 g (>100%, unidentified impurity present) of crude intermediate **9c** as a colorless solid: mp 132–137 °C; TLC (MeOH) R_f 0.38 (UV, I_2, ninhydrin); 500-MHz ^{1}H NMR (D_2O) δ 0.49 (t, 1.5 H, J = 7.2 Hz, propyl CH$_3$), 0.53 (t, 1.5 H, J = 7.3 Hz, propyl CH$_3$), 0.78–1.02 (m, 2 H, CH$_3$C*H*$_2$CH$_2$N), 1.40 (s, 3 H, isopropylidene CH$_3$), 1.59 (s, 3 H, isopropylidene CH$_3$), 1.62–1.84 (m, 2 H, CHC*H*$_2$CH$_2$N), 2.20 (m, 2 H, CH$_3$CH$_2$C*H*$_2$N), 2.49–2.65 (m, 4 H, overlapping H-5′ and CHCH$_2$C*H*$_2$N), 3.48 (m, 1 H, C*H*CH$_2$CH$_2$N), 4.42 (m, 1 H, H-4′), 4.97 (m, 1 H, H-3′), 5.53 (dd, 0.5 H, J = 1.7 Hz, 5.3 Hz, H-2′), 5.57 (dd, 0.5 H, J = 1.3 Hz, 5.5 Hz, H-2′), 6.17 (d, 0.5 H, J = 1.8 Hz, H-1′), 6.18 (d, 0.5 H, J = 1.3 Hz, H-1′), 8.11 (s, 0.5 H, adenine H-2 or H-8), 8.13 (s, 0.5 H, adenine H-2 or H-8), 8.20 (s, 1 H, adenine H-2 or H-8); MS (positive LSIMS), m/e 450 (M + H)$^+$.

Subsequent acidic hydrolysis of crude **9c** followed by cation exchange chromatography [peak elution of **2c** at 0.92 M] provided 946 mg (76.0% overall) or pure **2c** as a colorless solid: mp 150–157 °C.; TLC (EtOH/concentrated NH_4OH (90:10)) R_f 0.19 (UV, I_2, ninhydrin); 500-MHz ^{1}H NMR (D_2O) δ 0.78 (t, 3 H, J = 7.3 Hz, propyl CH$_3$), 1.43 (m, 2 H, CH$_3$C*H*$_2$CH$_2$N), 1.97 (m, 1 H, CHC*H*$_2$CH$_2$N), 2.03 (m, 1 H, CHC*H*$_2$CH$_2$N), 2.50–2.65 (m, 2 H, CH$_3$CH$_2$C*H*$_2$N), 2.74–2.91 (m, 2 H, CHCH$_2$C*H*$_2$N), 2.94 (m, 2 H, H-5′), 3.70 (m, 1 H, C*H*CH$_2$CH$_2$N), 4.30 (m, 1 H, H-3′), 4.33 (m, 1 H, H-4′), 4.72 (m, 1 H, H-2′), 6.06 (d, 1 H, J = 4.1 Hz, H-1′), 8.21 (s, 1 H, adenine H-2 or H-8), 8.27 (s, 1 H, adenine H-2 or H-8); UV λ_{max} (H_2O) 259 nm (ϵ 14.6 × 10^3); MS (positive LSIMS),

m/e 410 $(M + H)^+$. Anal. Calcd for $C_{17}H_{27}N_7O_5 \cdot H_2O$: C, 47.77; H, 6.84; N, 22.94. Found: C, 47.79; H, 6.68; N, 22.80.

N^4-(5′-Adenosyl)-N^4-[1-(2-propenyl)]-2(*R*,*S*),4-diaminobutanoic acid (2d) was prepared starting with the alkaline hydrolysis of **8d** (5.720 g, 9.91 mmol). Flash silica gel chromatography [$CHCl_3$/MeOH (70:30), the MeOH] provided 5.44 g (>100%, unidentified impurity present) of crude intermediate **9d** as a colorless solid: mp 133–137 °C; TLC (MeOH) R_f 0.45 (UV, I_2, ninhydrin); 500-MHz ^1H NMR (D_2O) δ 1.39 (s, 1.5 H, isopropylidene CH_3), 1.59 (s, 3 H, isopropylidene CH_3), 1.66–1.86 (m, 2 H, $CHCH_2CH_2N$), 2.45–2.67 (m, 4 H, overlapping H-5′ and $CHCH_2CH_2N$), 2.77–3.00 (m, 2 H, $CH_2{=}CHCH_2N$), 3.50 (m, 1 H, $CHCH_2CH_2N$), 4.40 (m, 0.5 H, H-4′), 4.44 (m, 0.5, H, H-4′), 4.88–4.94 (m, 2 H, $CH_2{=}CHCH_2N$), 4.97 (m, 1 H, H-3′), 5.20 (m, 0.5 H, $CH_2{=}CHCH_2N$), 5.39 (m, 0.5 H, $CH_2{=}CHCH_2N$), 5.50 (dd, 0.5 H, *J* = 21 Hz, 6.5 Hz, H-2′), 5.54 (dd, 0.5 H, *J* = 1.9 Hz, 6.3 Hz, H-2′), 6.15 (d, 0.5 H, *J* = 2.1 Hz, H-1′), 6.17 (d, 0.5 H, *J* = 1.9 Hz, H-1′), 8.09 (s, 0.5 H, adenine H-2 or H-8), 8.10 (s, 0.5 H, adenine H-2 or H-8), 8.17 (s, 1 H, adenine H-2 or H-8); MS (positive LSIMS), *m/e* 448 $(M + H)^+$.

Subsequent acidic hydrolysis of crude **9d** followed by cation exchange chromatography [peak elution of **2d** at 0.81 M] provided 2.80 g (66.5% overall) of pure **2d** as a colorless solid: mp 165–168 °C.; TLC (EtOH/concentrated NH_4OH (90:10) R_f 0.18 (UV, I_2, ninhydrin); 500-MHz ^1H NMR (D_2O) δ 1.92 (m, 1 H, $CHCH_2CH_2N$), 2.00 (m, 1 H, $CHCH_2CH_2N$), 2.64–2.86 (m, 4 H, overlapping H-5′ and $CHCH_2CH_2N$), 3.06–3.26 (m, 2 H, $CH_2{=}CHCH_2N$), 3.68 (m, 1 H, $CHCH_2CH_2N$), 4.22 (m, 1 H, H-3′), 4.29 (m, 1 H, H-4′), 4.73 (m, 1 H, H-2′), 5.16 (m, 2 H, $CH_2{=}CHCH_2N$), 5.69–5.78 (m, 1 H, $CH_2{=}CHCH_2N$), 6.01 (d, 1 H, *J* = 2.9 Hz, H-1′), 8.16 (s, 1 H, adenine H-2 or H-8), 8.21 (s, 0.5 H, adenine H-2 or H-8), 8.22 (s, 0.5 H, adenine H-2 or H-8); UV λ_{max} (H_2O) 259 nm (ε 15.3 × 10^3); MS (positive LSIMS), *m/e* 408 $(M + H)^+$. Anal. Calcd for $C_{17}H_{25}N_7O_5 \cdot H_2O$: C, 47.99; H, 6.40; N, 23.05. Found: C, 48.03; H, 6.22; N, 22.95.

N^4-(5′-Adenosyl)-N^4-(1-butyl)-2(*R*,*S*),4-diaminobutanoic acid (2e) was prepared beginning with the alkaline hydrolysis of **8e** (1.487 g, 2.52 mmol). Flash silica gel chromatography [$CHCl_3$/MeOH (70:30), then MeOH] provided 1.41 g (>100%, unidentified impurity present) of crude intermediate **9e** as a colorless solid: mp 134–138 °C; TLC (MeOH) R_f 0.42 (UV, I_2, ninhydrin); 500-MHz ^1H NMR (D_2O) δ 0.61 (m, 3 H, butyl CH_3), 0.64–0.95 (m, 4 H, $CH_3CH_2CH_2$), 1.41 (s, 1.5 H, isopropylidene CH_3), 1.42 (s, 1.5 H, isopropylidene CH_3), 1.60 (s, 1.5 H, isopropylidene CH_3), 1.61 (s, 1.5 H, isopropylidene CH_3), 1.69–1.85 (m, 2 H, $CHCH_2CH_2N$), 2.15–2.28 (m, 2 H, $CH_2CH_2CH_2N$), 2.50–2.70 (m, 4 H, overlapping H-5′ and $CHCH_2CH_2N$), 3.52 (m, 1 H, $CHCH_2CH_2N$), 4.44 (m, 0.5 H, H-4′), 4.49 (m, 0.5 H, H-4′), 5.02 (dd, 0.5 H, *J* = 3.0 Hz, 6.3 Hz, H-3′), 5.05 (dd, 0.5 H, *J* = 2.6 Hz, 6.2 Hz, H-3′), 5.61 (dd, 0.5 H, *J* = 1.9 Hz, 6.3 Hz, H-2′), 5.65 (dd, 0.5 H, *J* = 1.6 Hz, 6.2 Hz, H-2′), 6.23 (d, 0.5 H, *J* = 1.9 Hz, H-1′), 6.25 (d, 0.5 H, *J* = 1.6 Hz, H-1′), 8.18 (s, 0.5 H, adenine H-2 or H-8), 8.19 (s, 0.5 H, adenine H-2 or H-8), 8.24 (s, 1 H, adenine H-2 or H-8); MS (positive LSIMS), *m/e* 464 $(M + H)^+$.

Subsequent acidic hydrolysis of crude **9e** followed cation exchange chromatography [peak elution of **2e** at 1.03 M] provided 844 mg (75.8% overall) of pure **2e** as a colorless solid: mp 145–152 °C; TLC (EtOH/concentrated NH_4OH (90:10)) R_f 0.25 (UV, I_2, ninhydrin); 500-MHz ^1H NMR (D_2O) δ 0.76 (t, 3 H, *J* = 7.3 Hz, butyl CH_3), 1.14 (m, 2 H, $CH_3CH_2CH_2$), 1.32 (m, 2 H, $CH_2CH_2CH_2N$), 1.93 (m, 1 H, $CHCH_2CH_2N$), 2.04 (m, 1 H, $CHCH_2CH_2N$), 2.51–2.58 (m, 1 H, $CH_2CH_2CH_2N$), 2.61–2.68 (m, 1 H, $CH_2CH_2CH_2N$), 2.74–2.90 (m, 2 H, $CHCh_2CH_2N$), 2.94 (m, 2 H, H-5′), 4.33 (m, 2 H, H-3′ and H-4′), 4.76 (m, 1 H, H-2′), 6.07 (d, 1 H, *J* = 4.2 Hz, H-1′), 8.22 (s, 1 H, adenine H-2 or H-8), 8.28 (s, 0.5 H, adenine H-2 or H-8), 8.29 (s, 0.5 H, adenine H-2 or H-8); UV λ_{max} (H_2O) 259 nm (ε 14.9 × 10^3); MS (positive LSIMS), *m/e* 424 $(M + H)^+$. Anal. Calcd for $C_{18}H_{29}N_7O_5 \cdot H_2O$: C, 48.97; H, 7.08; N, 22.21. Found: C, 49.06; H, 6.95; N, 22.25.

N^4-(5′-Adenosyl)-N^4-(1-pentyl)-2(*R*,*S*),4-diaminobutanoic acid (2f) was prepared beginning with the alkaline hydrolysis of **8f** (1.792 g, 2.96 mmol). Flash silica gel chromatography [$CHCl_3$/MeOH (70:30), then MeOH] provided 1.90 g (>100%, unidentified impurity present) of crude intermediate **9f** as a colorless solid: mp 118–122 °C; TLC (MeOH) R_f 0.44 (UV, I_2, ninhydrin); 500-MHz ^1H NMR (D_2O) δ 0.64 (t, 3 H, *J* = 7.1 Hz, pentyl CH_3), 0.75 (m, 2 H, $CH_3CH_2CH_2$), 0.82–0.98 (m, 4 H, $CH_2CH_2CH_2N$), 1.41 (s, 1.5 H, isopropylidene CH_3), 1.42 (s, 1.5 H, isopropylidene CH_3), 1.60 (s, 1.5 H, isopropylidene CH_3), 1.61 (s, 1.5 H, isopropylidene CH_3), 1.69–1.87 (m, 2 H, $CHCH_2CH_2N$), 2.13–2.25 (m, 2 H, $CH_2CH_2CH_2N$), 2.50–2.69 (m, 4 H, overlapping H-5′ and $CHCH2CH_2N$), 3.52 (m, 1 H, $CHCH_2CH_2N$), 4.42–4.49 (m, 1 H, H-4′), 5.01 (dd, 0.5 H, *J* = 2.9 Hz, 6.3 Hz, H-3′), 5.05 (dd, 0.5 H, *J* = 2.9 Hz, 6.3 Hz, H-3′), 5.60 (dd, 0.5 H, *J* = 1.9 Hz, 6.3 Hz, H-2′), 5.64 (dd, 0.5 H, *J* = 1.7 Hz, 6.3 Hz, H-2′), 6.22 (d, 0.5 H, *J* = 1.6 Hz, H-1′), 6.24 (d, 0.5 H, *J* = 1.7 Hz, H-1′), 8.16 (s, 0.5 H, adenine H-2 or H-8), 8.17 (s, 0.5 H, adenine H-2 or H-8), 8.23 (s, 1 H, adenine H-2 or H-8); MS (positive LSIMS), *m/e* 478 $(M + H)^+$.

Subsequent acidic hydrolysis of crude **9f** followed by cation exchange chromatography, using a 0–1.5 M gradient with a 6.2-L total volume [peak elution of **2f** at 1.11 M], provided 1.02 g (75.3% overall) of pure **2f** as a colorless solid: mp 146–155 °C; TLC (EtOH/concentrated NH_4OH (90:10)) R_f 0.28 (UV, I_2, ninhydrin); 500-MHz ^1H NMR (D_2O) δ 0.62 (t, 3 H, *j* = 5.5 Hz, pentyl CH_3), 0.93–1.01 (m, 4 H, $CH_3CH_2CH_2$), 1.21 (m, 2 H, $CH_2CH_2CH_2N$), 1.87 (m, 1 H, $CHCH_2CH_2N$), 1.97 (m, 1 H, $CHCH_2CH_2N$), 2.42 (m, 1 H, $CH_2CH_2CH_2N$), 2.53 (m, 1 H, $CH_2CH_2CH_2N$), 2.64–2.88 (m, 4 H, overlapping H-5′ and $CHCH_2CH_2N$), 3.63 (m, 1 H, $CHCH_2CH_2N$), 4.25 (m, 2 H, overlapping H-3′ and H-4′), 4.91 (m, 1 H, H-2′), 5.98 (d, 1 H, *J* = 4.8 Hz, H-1′), 8.12 (s, 1 H, adenine H-2 or H-8), 8.21 (s, 1 H, adenine H-2 or H-8); UV λ_{max} (H_2O) 259 nm (ε 14.6 × 10^3); MS (positive LSIMS), *m/e* 438 $(M + H)^+$. Anal. Calcd for $C_{19}H_{31}N_7O_5 \cdot H_2O$: C, 50.10; H, 7.30; N, 21.52. Found: C, 49.88; H, 7.13; N, 21.37.

N^4-(5′-Adenosyl)-N^4-(1-octyl)-2(*R*,*S*),4-diaminobutanoic acid (2g) was prepared by starting with the alkaline hydrolysis of **8g** (2.148 g, 3.32 mmol). Flash silica gel chromatography [$CHCl_3$/MeOH (70:30), then MeOH] provided 2.11 g (>100%, unidentified impurity present) of crude intermediate **9g** as a colorless solid: mp 113–117 °C; TLC (MeOH), R_f 0.57 (UV, I_2, ninhyrin); 500-MHz ^1H NMR (D_2O) δ 0.80 (m, 7 H, $CH_3CH_2CH_2$), 0.91 (m, 2 H, $CH_3CH_2CH_2CH_2$), 0.98 (m, 2 H, $CH_2CH_2CH_2CH_2N$), 1.07 (m, 2 H, $CH_2CH_2CH_2N$), 1.17 (m, 2 H, $CH_2CH_2CH_2N$), 1.42 (s, 3 H, isopropylidene CH_3), 1.62 (s, 1.5 H, isopropylidene CH_3), 1.63 (s, 1.5 H, isopropylidene CH_3), 1.73–1.91 (m, 2 H, $CHCH_2CH_2N$), 2.20–2.35 (m, 2 H, $CH_2CH_2CH_2N$), 2.54–2.77 (m, 4 H, overlapping H-5′ and $CHCH_2CH_2N$), 3.55 (m, 1 H, $CHCH_2CH_2N$), 4.45–4.52 (m, 1 H, H-4′), 5.04 (dd, 0.5 H, *J* = 3.4 Hz, 6.3 Hz, H-3′), 5.08 (dd, 0.5 H, *J* = 2.7 Hz, 6.4 Hz, H-3′), 5.62 (dd, 0.5 H, *j* = 2.0 Hz, 6.3 Hz, H-2′), 5.65 (dd, 0.5 H, *J* = 1.7 Hz, 6.4 Hz, H-2′), 6.27 (d, 0.5 H, *J* = 2.0 Hz, H-1′), 6.28 (d, 0.5 H, *J* = 1.7 Hz, H-1′), 8.23 (s, 0.5 H, adenine H-2 or H-8), 8.24 (s, 0.5 H, adenine H-2 or H-8), 8.27 (s, 0.5 H, adenine H-2 or H-8), 8.28 (s, 0.5 H, adenine H-2 or H-8); MS (positive LSIMS), *m/e* 520 $(M + H)^+$.

Subsequent acidic hydrolysis of crude **9g** followed by cation exchange chromatography, using a 0–1.7 M gradient with a 6.5-L total volume and then 1 M aqueous NH_4OH, provided 1.10 g (66.9% overall) of pure **2g** as a colorless solid: mp 135–152 °C; TLC (EtOH/concentrated NH_4OH (90:10)) R_f 0.32 (UV, I_2, ninhydrin); 500-MHz ^1H NMR (D_2O) δ 0.77 (t, 3 H, *J* = 7.0 Hz, octyl CH_3), 0.85–1.06 (m, 8 H, $CH_3(CH_2)_2$), 1.12 (m, 2 H, $CH_2CH_2CH_2N$), 1.30 (m, 2 H, $CH_2CH_2CH_2N$), 1.93 (m, 1 H, $CHCH_2CH_2N$), 2.05 (m, 1 H, $CHCH_2CH_2N$), 2.55 (m, 1 H, $CH_2CH_2CH_2Ne$, 2.67 (m, 1 H, $CH_2CH_2CH_2N$), 2.75–3.06 (m, 4 H, overlapping H-5′ and $CHCH_2CH_2N$), 3.72 (m, 1 H, $CHCH_2CH_2N$), 4.33 (m, 4 H, overlapping H-3′ and H-4′), 4.84 (m, 1 H, H-2′), 6.06 (d, 1 H, *J* = 3.1 Hz, H-1′), 8.26 (s, 1 H, adenine H-2 or H-8), 8.30 (s, 0.5 H, adenine H-2 or H-8), 8.31 (s, 0.5 H, adenine H-2 or H-8); UV λ_{max} (H_2O) 259 nm (ε 14.7 × 10^3); MS (positive LSIMS), *m/e* 480 $(M + H)^+$. Anal. Calcd for $C_{22}H_{37}N_7O_5 \cdot H_2O$: C, 53.10; H, 7.90; N, 19.70. Found: C, 52.83; H, 7.75; N, 19.47.

Phenylmethyl [6-[[(4-methylphenyl)sulfonyl]oxy]-hexyl]carbamate (11) was prepared from phenylmethyl (6-hydroxyhexyl)carbamate[26] (23.84 g, 95.0 mmol) and *p*-toluenesulfonyl chloride (19.91 g, 104.5 mmol) according to the procedure of Chipowsky and Lee.[26] Purification was carried out by other means involving chromatography. The evaporated reaction mixture was partitioned between $CHCl_3$ (450 mL) and 1 M aqueous HOAc (450 mL), and the $CHCl_3$ extract was washed with additional 1 M aqueous HOAc (450 mL), water (2 × 450 mL) and

saturated aqueous NaCl (2 × 450 mL) and dried ($MgSO_4$). The concentrated $CHCl_3$ extract was chromatographed on flash silica gel (48 × 3.5 cm, 230 g). After washing with EtOAc/hexanes (20:80), the product was eluted with EtOAc/hexanes (50:50) and the appropriate fractions were pooled and evaporated. The residue was twice dissolved in $CHCl_3$ (100 mL) and evaporated to a colorless oil. After several days at −20 °C, this oil crystallized to provide 24.45 g (63.5%) of analytically pure **11**: mp 35–38 °C (lit.[26] mp 46–47 °C); TLC (EtOAc/hexanes (50:50)) R_f 0.43 (UV, I_2); 500-MHz ^{1}H NMR ($CDCl_3$) δ 1.25 (m, 2 H, $NHCH_2CH_2CH_2$), 1.32 (m, 2 H, $CH_2CH_2CH_2O$), 1.45 (m, 2 H, $NHCH_2CH_2$), 1.63 (m, 2 H, CH_2CH_2O), 2.44 (s, 3 H, CH_3), 3.14 (q, 2 H, J = 6.5 Hz, $NHCH_2$), 4.00 (t, 2 H, J = 6.2 Hz, CH_2CH_2O), 4.77 (br s, 1 H, NH), 5.08 (s, 2 H, benzyl CH_2), 7.35 (m, 7 H, overlapping phenyl and tosyl H-3 and H-5), 7.78 (d, 2 H, J = 7.4 Hz, tosyl H-2 and H-6); MS (positive LSIMS), m/e 406 $(M + H)^+$. Anal. Calcd for $C_{21}H_{27}NO_5S$: C, 62.20; H, 6.71; N, 3.45; S, 7.91. Found: C, 62.13; H, 6.79; N, 3.42; S, 7.91.

Phenylmethyl (6-iodohexyl)carbamate (12) was prepared from **11** (24.45 g, 60.4 mmol) and NaI (13.59 g, 90.6 mmol) according to the procedure of Chipowsky and Lee,[26] although its purification was carried out by other means involving chromatography. Pure product from a concentrated $CHCl_3$ extract of the evaporated reaction mixture was eluted from a flash silica gel column (48 × 3.5 cm, 230 g) with EtOAc/hexanes (20:80). After the appropriate fractions were pooled and evaporated, the residue was twice dissolved in $CHCl_3$ (100 mL) and again evaporated to a colorless oil. This oil crystallized upon cooling in dry ice to provide 20.63 g (94.6%) of analytically pure **12** as a colorless solid: mp 45–47 °C (lit.[26] mp 43–44.5 °C); TLC (EtOAc/hexanes (20:80)) R_f 0.32 (UV, I_2); 500-MHz ^{1}H NMR ($CDCl_3$) δ 1.33 (m, 2 H, $CH_2CH_2CH_2I$), 1.41 (m, 2 H, $NHCH_2CH_2CH_2$), 1.51 (m, 2 H, CH_2CH_2I), 1.81 (m, 2 H, $NHCH_2CH_2$), 3.18 (m, 4 H, overlapping $NHCH_2$ and CH_2I), 4.75 (br s, 1 H, NH), 5.09 (s, 2 H, benzyl CH_2), 7.36 (m, 5 H, phenyl); MS (positive LSIMS), m/e 362 $(M + H)^+$. Anal. Calcd for $C_{14}H_{20}INO_2$: C, 46.55; H, 5.58; I, 35.13; N, 3.88. Found: C, 46.49; H, 5.62; I, 35.28; N, 3.79.

5′-[[6-[[(Phenylmethoxy)carbonyl]amino]-1-hexyl]-amino]-5′-deoxy-2′,3′-*O*-isopropylideneadenosine (13). Iodide **12** (3.61 g, 10.0 mmol) was added in one portion to a solution of amino nucleoside **10**[10a] (3.06 g, 10.0 mmol) and *N,N*-diisopropylethylamine (1.74 mL, 10.0 mmol) in MeCN (250 mL). After stirring at 70 °C for 18 h, the evaporated reaction mixture was partitioned between CH_2Cl_2 (200 mL) and 0.1 N aqueous Na_2CO_3 (200 mL). The original and two additional CH_2Cl_2 (200 mL) extracts were washed with saturated aqueous NaCl (2 × 200 mL), dried ($MgSO_4$), and evaporated. Chromatography of the residue on flash silica gel (48 × 3.5 cm, 230 g), eluting with $CHCl_3$/*i*-PrOH (80:20), led to the appearance of partially pure (as judged by TLC) dialkylated side product (2.05 g, 26.2%, yellow oil) in the early fractions. Pure (as judged by TLC) desired monoalkylated product was eluted in subsequent fractions, which were pooled and evaporated. The residue was twice dissolved in $CHCl_3$ (100 mL) and evaporated to provide 2.56 g (47.5%) of pure **13** as a colorless foam: mp 44–46 °C; TLC ($CHCl_3$/*i*-PrOH (80:20)) R_f 0.26 (UV, I_2); 500-MHz ^{1}H NMR ($CDCl_3$) δ 1.28 (m, 4 H, $CH_2CH_2CH_2CH_2N$), 1.39 (m, 5 H, overlapping isopropylidene CH_3 and $CH_2CH_2NHCH_2$), 1.47 (m, 2 H, $CONHCH_2CH_2$), 1.61 (s, 3 H, isopropylidene CH_3), 2.56 (m, 2 H, $CH_2CH_2NHCH_2$), 2.87 (m, 2 H, H-5′), 3.18 (q, 2 H, J = 6.3 Hz, $CONHCH_2$), 4.38 (m, 1 H, H-4′), 5.02 (dd, 1 H, J = 3.2 Hz, 6.3 Hz, H-3′), 5.10 (s, 2 H, benzyl CH_2e, 5.17 (br s, 1 H, CONH), 5.47 (dd, 1 H, J = 2.7 Hz, 6.3 Hz, H-2′), 5.69 (br s, 2 H, adenine NH_2), 6.04 (d, 1 H, J = 2.7 Hz, H-1′), 7.35 (m, 5 H, phenyl), 7.94 (s, 1 H, adenine H-2 or H-8), 8.33 (s, 1 H, adenine H-2 or H-8); UV λ_{max} (MeOH) 259 nm (ε 13.5 × 10^3); MS (positive LSIMS), m/e 540 $(M + H)^+$, 405 $(M + H - adenine)^+$. Anal. Calcd for $C_{27}H_{37}N_7O_5$: C, 60.10; H, 6.91; N, 18.17. Found: C, 59.89; H, 6.93; N, 17.94.

Methyl N^4-[6-[[(Phenylmethoxy)carbonyl]amino]-1-hexyl]-N^4-(2′,3′-*O*-isopropylidene-5′-adenosyl)-2(*R*,*S*)-(trifluoroacetamido)-4-aminobutyrate (14). Dialkylamine **13** (4.753 g, 8.82 mmol) and *N,N*-diisopropylethylamine (1.69 mL, 9.70 mmol, 10% excess) were dissolved in MeCN (125 mL) and heated with stirring to 70 °C. Over approximately 15 min, a solution of **7** (3.737 g 11.03 mmol, 25% excess) in MeCN (50 mL) was added. The mixture was stirred at 70 °C for 3 days during which time its initial pale brown color gradually deepened. Chromatography of the evaporated reaction mixture on flash silica gel (48 × 3.5 cm, 230 g), eluting with $CHCl_3$/*i*-PrOH (80:20), provided partially pure product (as judged by TLC). Appropriate fractions were concentrated to approximately 15 mL in preparation for further purification by low pressure liquid chromatography using a Lobar Size B (310-25) Lichroprep Si60 silica gel column. The injection of 0.70-mL aliquots of the crude product solution onto this column, which was eluted with $CHCl_3$/*i*-PrOH (80:20) at a flow rate of 4.0 mL/min, resulted in the appearance of purified product (as detected by absorbance at 280 nm) in the eluate 24–38 min later. Product-containing eluates from repeated injections were pooled and evaporated, redissolved in the eluting solvent (approximately 15 mL), and reinjected onto the same column under identical conditions to remove the last traces of any contaminants. Decolorization of the recovered amber product (in 5 mL of $CHCl_3$) was achieved by filtration through a wet-packed flash silica gel column (48 × 3.5 cm, 230 g) eluted with $CHCl_3$/*i*-PrOH (95:5). Appropriate fractions containing colorless **14** were pooled and evaporated. The residue was twice dissolved in $CHCl_3$ (100 mL) and evaporated to provide 1.45 g (21.4%) of analytically pure **14** as a colorless foam: mp 50–57 °C; TLC ($CHCl_3$/*i*-PrOH (80:20)) individual diastereomers at R_f 0.57 and 0.63 (UV, I_2); 500-MHz ^{1}H NMR ($CDCl_3$) δ 1.00 (m, 4 H, $CH_2CH_2CH_2CH_2N$), 1.12 (m, 2 H, $CH_2CH_2CH_2N$), 1.31 (m, 2 H, $CONHCH_2H_2$), 1.39 (s, 1.5 H, isopropylidene CH_3), 1.40 (s, 1.5 H, isopropylidene CH_3), 1.61 (s, 1.5 H, isopropylidene CH_3), 1.63 (s, 1.5 H, isopropylidene CH_3), 1.83–1.95 (m, 2 H, $CHCH_2CH_2N$), 2.30–2.42 (m, 2 H, $CH_2CH_2CH_2N$), 2.48–2.60 (m, 2 H, $CHCH_2CH_2N$), 2.63–2.92 (m, 2 H, H-5′), 3.17 (m, 2 H, $CONHCH_2$), 3.66 (s, 1.5 H, CO_2CH_3), 3.75 (s, 1.5 H, CO_2CH_3), 4.30–4.40 (m, 1 H, H-4′), 4.51 (m, 1 H, $CHCH_2CH_2N$), 4.99 (m, 0.5 H, H-3′), 5.03 (m, 0.5 H, H-3′), 5.12 (s, 1 H, benzyl CH_2), 5.14 (s, 1 H, Benzyl CH_2), 5.43 (br s, 0.5 H, $CONHCH_2$), 5.54 (d, 1 H, J = 6.1 Hz, H-2′), 5.61 (br s, 0.5 H, $CONHCH_2$), 5.69 (br s, 2 H, adenine NH_2), 6.05 (d, 1 H, J = 5.5 Hz, H-1′), 7.29–7.40 (m, 5 H, phenyl), 7.82 (s, 0.5 H, adenine H-2 or H-8), 7.86 (s, 0.5 H, adenine H-2 or H-8), 8.30 (s, 0.k H, adenine H-2 or H-8), 8.31 (s, 0.5 H, adenine H-2 or H-8), 9.47 (br d, 0.5 H, J = 4.8 Hz, CF_3CONH), 9.57 (br d, 0.5 H, J = 7.0 Hz, CF_3CONH); UV λ_{max} (MeOH) 259 (ε 14.0 × 10^3); MS (positive LSIMS), m/e 751 $(M + H)^+$, 616 $(M + H - adenine)^+$. Anal. Calcd for $C_{34}H_{45}F_3N_8O_8 \cdot H_2O$: C, 53.12; H, 6.16; N, 14.58. Found: C, 52.92; H, 5.94; N, 14.38.

N^4-[6-[[(Phenylmethoxy)carbonyl]amino]-1-hexyl]-N^4-(2′,3′-*O*-isopropylidene-5′-adenosyl)-2(*R*,*S*),4-diaminobutanoic Acid (15). A solution of Na_2CO_3 (1.074 g, 1.40 mmol) in 50% aqueous MeOH (15 mL) was added to **15** (1.074 g, 1.40 mmol). After being heated with stirring at 60 °C for 6, the cooled reaction mixture was neutralized with 1 M aqueous HCl and evaporated, and the residue was extracted with MeOH (4 × 25 mL). Chromatography of the pooled and concentrated MeOH extracts on flash silica gel (44 × 3.5 cm, 200 g) was performed by first washing with MeOH/$CHCl_3$ (30:70) followed by elution of the product with MeOH. After the appropriate fractions were pooled and evaporated, the residue was twice dissolved in MeOH (50 mL) and again evaporated to provide 908 mg (>100% yield due to the presence of an unidentified contaminant) of **15** as a colorless foam. The recovered material was used directly without further purification: mp 104–111 °C; TLC (MeOH/$CHCl_3$ (30:70)) R_f 0.33 (UV, I_2, ninhydrin); 500-MHz ^{1}H NMR (D_2O) δ 0.74–0.99 (m, 6 H, $CH_2CH_2CH_2CH_2N$), 1.22 (m, 2 H, $CONHCH_2CH_2$), 1.40 (s, 1.5 H, isopropylidene CH_3), 1.41 (s, 1.5 H, isopropylidene CH_3), 1.60 (s, 1.5 H, isopropylidene CH_3), 1.61 (s, 1.5 H, isopropylidene CH_3), 1.76–1.95 (m, 2 H, $CHCH_2CH_2N$), 2.24–2.34 (m, 2 H, $CH_2CH_2CH_2N$), 2.61 (m, 2 H, $CHCH_2CH_2N$), 2.72 (m, 2 H, H-5′), 2.97 (m, 2 H, $CONHCH_2$), 3.65 (m, 1 H, $CHCH_2CH_2N$), 4.46 (m, 1 H, H-4′), 4.85 (m, 1 H, H-3′), 5.05 (s, 2 H, benzyl CH_2), 5.59 (m, 0.5 H, H-2′), 5.62 (m, 0.5 H, H-2′), 6.24 (d, 0.5 H, J = 0.9 Hz, H-1′), 6.26 (d, 0.5 H, J = 1.5 Hz, H-1′), 7.33 (m, 5 H, phenyl), 8.19 (s, 0.5 H, adenine H-2 or H-8), 8.20 (s, 0.5 H, adenine H-2 or H-8), 8.23 (s, 1 H, adenine H-2 or H-8); UV λ_{max} (MeOH) 259 nm (ε 12.9 × 10^3); MS (positive LSIMS), m/e 641 $(M + H)^+$, 506 $(M + H - adenine)^+$.

N^4-(6-Amino-1-hexyl)-N^4-(2′,3′-*O*-isopropylidene-5′-adenosyl)-2(*R*,*S*),4-diaminobutanoic Acid (16). Crude **15** (908 mg, 1.42 mmol) was dissolved in 50% aqueous formic acid (20

mL); 10% Pd/C (908 mg) was added and the mixture hydrogenated with shaking at 42 psi for 5 h. The catalyst was removed by filtration through a glass fiber filter and the clear solution evaporated. The resulting syrup was redissolved in water (100 mL) and neutralized with 1 M aqueous NH_4OH. Ammonium formate present in this solution was removed by desalting on SP-Sephadex C-25 (34 × 2.5 cm, 30 g, NH_4^+ form, prepared by washing with 2 L of 1 M aqueous NH_4Cl followed by 4 L of water). After the solution was loaded at a flow rate of 2 mL/min, the column was washed with water (1 L) and the adsorbed product eluted with 1 M aqueous NH_4OH. Column eluate containing the product was evaporated and the residue dissolved in water (5 mL) and lyophilized to provide 498 mg (69.3%) of **16** as a colorless solid. Due to the presence of a small amount (approximately 5%) of the fully deprotected derivative **3**, the recovered material was used directly without further purification: mp 101–109 °C; TLC (EtOH/concentrated NH_4OH (90:10)) R_f 0.17 (UV, I_2, ninhydrin); 500-MHz ^1H NMR (D_2O) δ 0.80–1.09 (m, 6 H, $CH_2CH_2CH_2CH_2N$), 1.41 (s, 3 H, isopropylidene CH_3), 1.43 (m, 2 H, $NH_2CH_2CH_2$), 1.60 (s, 1.5 H, isopropylidene CH_3), 1.61 (s, 1.5 H, isopropylidene CH_3), 1.62–1.86 (m, 2 H, $CHCH_2CH_2N$), 2.20 (m, 2 H, $CH_2CH_2CH_2N$), 2.52 (m, 2 H, $CHCH_2CH_2N$), 2.59 (m, 2 H, H-5′), 2.85 (m, 2 H, NH_2CH_2), 3.39 (m, 1 H, $CHCH_2CH_2N$), 4.46 (m, 1 H, H-4′), 5.03 (dd, 0.5 H, J = 2.9 Hz, 6.3 Hz, H-3′), 5.05 (dd, 0.5 H, J = 6.3 Hz, H-3′), 5.62 (dd, 0.5 H, J = 1.8 Hz, 6.3 Hz, H-2′), 5.63 (dd, 0.5 H, J = 1.6 Hz, 6.3 Hz, H-2′), 6.24 (d, 0.5 H, J = 1.8 Hz, H-1′), 6.25 (d, 0.5 H, J = 1.6 Hz, H-1′), 8.19 (s, 0.5 H, adenine H-2 or H-8), 8.20 (s, 0.5 H, adenine H-2 or H-8), 8.26 (s, 1 H, adenine H-2 or H-8); UV λ_{max} (H_2O) 259 nm (ε 12.5 × 10^3); MS (positive LSIMS), m/e $(M + H)^+$.

N^4-(5′-Adenosyl)-N^4-(6-amino-1-hexyl)-2(*R*,*S*),4-diaminobutanoic Acid (3). Crude **16** (498 mg, 0.98 mmol) was dissolved in 0.1 M aqueous H_2SO_4 (75 mL) and stirred at room temperature for 8 days. The reaction mixture was neutralized with $Ba(OH)_2 \cdot 8H_2O$ (2.37 g, 7.50 mmol) and the precipitated $BaSO_4$ removed by centrifugation and filtration. The clear solution was evaporated, redissolved in water (300 mL), and loaded onto an SP-Sephadex C-25 column (71 × 2.5 cm, 60 g, NH_4^+ form, prepared by washing with 4 L of 1 M aqueous NH_4Cl and 6 L of water) at a flow rate of 2 mL/min and 28-mL fractions were collected. After washing with water (700 mL), a gradient of aqueous NH_4HCO_3 (0–0.7 M, 4.3 L) was used to elute the product from the column. The peak elution of the product occurred at a buffer concentration of 0.29 M. The appropriate fractions were diluted with an equal volume of water and desalted on the same SP-Sephadex C-25 column, freshly regenerated in the NH_4^+ form. After loading at a flow rate of 2 mL/min and washing with water (3 L), the adsorbed product was eluted with 1 M aqueous NH_4OH. The column eluate containing the product was evaporated and the residue dissolved in water (5 mL) and lyophilized to provide 434 mg (87.9%) (based on the fully protected derivative **14**, 61.4%) of analytically pure **3** as a colorless solid: mp 136–141 °C; TLC (EtOH/concentrated NH_4OH (90:10)) R_f 0.06 (UV, I_2, ninhydrin); 500-MHz ^1H NMR (D_2O) δ 1.05–1.15 (m, 4 H, $CH_2CH_2CH_2CH_2N$), 1.28 (m, 2 H, $CH_2CH_2CH_2N$), 1.47 (m, 2 H, $NH_2CH_2CH_2$), 1.79 (m, 1 H, $CHCH_2CH_2N$), 1.88 (m, 1 H, $CHCH_2CH_2N$), 2.46 (m, 1 H, $CH_2CH_2CH_2N$), 2.50 (m, 1 H, $CH_2CH_2CH_2N$), 2.65 (m, 2 H, $CHCH_2CH_2N$), 2.85 (m, 4 H, overlapping H-5′ and NH_2CH_2), 3.43 (m, 1 H, $CHCH_2CH_2N$), 4.28 (m, 1 H, overlapping H-3′ and H-4′), 4.85 (m, 1 H, H-2′), 6.03 (d, 1 H, J = 4.4 Hz, H-1′), 8.21 (s, 1 H, adenine H-2 or H-8), 8.28 (s, 1 H, adenine H-2 or H-8); UV λ_{max} (H_2O) 259 nm (ε 14.0 × 10^3); MS (positive LSIMS), m/e 467 $(M + H)^+$. Anal. Calcd for $C_{20}H_{34}N_8O_5 \cdot H_2O$: C, 47.80; H, 7.62; N, 22.30. Found: C, 47.92; H, 7.43; N, 22.06.

Biological Testing. The assay used to study the inhibition of *E. coli* transfer RNA (uracil-5-)-methyltransferase by the nitrogen SAM analogue **2a** is described in detail by Santi and Hardy.[27] The general procedure used to monitor the inhibition of *E. coli* B methionine synthase by the same compound is described by Frasca et al.[31]

Acknowledgment. We appreciate support for this work by NIH Grant AR-17323, NIH Training Grant GMO-7175, and patent funds administered by the Graduate Division of the University of California, San Francisco. We also thank Patricia Bethel, Vincent Powers, and George Garcia for the mass spectra. The HPLC and NMR technical assistance provided by Ann Nguyen and Thomas Marschner is gratefully acknowledged.

Supplementary Material Available: Purification details and characterization data for intermediates **5b–g** and **8b–g** (9 pages). Ordering information is given on any current masthead page.

(31) Frasca, V.; Riazzi, B. S.; Matthews, R. G. *J. Biol. Chem.* **1986**, *261*, 15823.