Tethered Bisubstrate Derivatives as Probes for Mechanism and as Inhibitors of Aminoglycoside 3′-Phosphotransferases

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Aminoglycoside 3′-phosphotransferases [APH(3′)s] phosphorylate aminoglycoside antibiotics, a reaction that inactivates the antibiotics. These enzymes are the primary cause of resistance to aminoglycosides in bacteria. APH(3′)-Ia operates by a random-equilibrium BiBi mechanism, whereas APH(3′)-IIIa catalyzes its reaction by the Theorell−Chance mechanism, a form of ordered BiBi mechanism. Hence, both substrates have to be present in the active site prior to the transfer of phosphate by both mechanisms. Four bisubstrate analogues, compounds **1**−**4**, were designed and synthesized as inhibitors for APH(3′)s. These compounds are made of adenosine linked covalently to the 3′-hydroxyl of neamine (an aminoglycoside) via all-methylene tethers of 5−8 carbons. The K_i values measured for these compounds indicated that affinities of APH(3′)-Ia and APH(3′)-IIa for compounds **2** and **3** (six- and seven-carbon tethers, respectively) were the best, and the inhibition constants for the two were comparable.

The most common mechanism for resistance to aminoglycoside antibiotics is the structural modification of these antibiotics by aminoglycoside-modifying enzymes.[1,2] Among these enzymes, aminoglycoside 3′-phosphotransferases [APH(3′)s], which catalyze the transfer of the γ-phosphoryl group of ATP to the 3′-hydroxyl of aminoglycosides, are most common, and indeed these enzymes were the cause of the demise of kanamycin treatment in the clinic.

Aminoglycoside 3′-phosphotransferases types Ia and IIIa [APH(3′)-Ia and APH(3′)-IIIa] are the most common members of this family of enzymes in Gram-negative and Gram-positive bacteria, respectively.[1,2] Inhibitors for these enzymes are highly sought.[1] Although there are a handful of known inhibitors for these enzymes,[3−5] none appear to be exceptionally effective. A combination therapy of an inhibitor for APH(3′)s and an aminoglycoside antibiotic that would otherwise be a substrate for the resistance enzymes would revive clinical utility of obsolete drugs such as kanamycin. Such a combination drug would expand our therapeutic options in the face of the serious needs in fighting multiply resistant bacteria in the clinic.[6,7] We hasten to add that such a combination therapy has been implemented successfully to counter the effect of β-lactamases, which are resistance determinants for β-lactam antibiotics.[8]

Mechanisms of both APH(3′)-Ia and APH(3′)-IIIa have been investigated. APH(3′)-Ia operates by a random-equilibrium BiBi mechanism,[9] whereas APH(3′)-IIIa catalyzes its reaction by the Theorell−Chance mechanism, a form of ordered BiBi mechanism.[10] Both mechanisms stipulate that the two substrates (aminoglycoside and ATP) should be present in the active site prior to the transfer of the phosphate group, and they refute the possibility for a double-displacement ping-pong mechanism for the enzymes. In essence, one would expect that there should be specific binding sites for both the aminoglycoside and ATP in the active site, and that the enzyme would stabilize the transition state for the transfer of the γ-phosphoryl group from the latter to the former. Figure 1 depicts an energy-minimized ternary complex for APH(3′)-IIIa, kanamycin A, and MgATP.

We set out to prepare bisubstrate analogues as inhibitors for APH(3′)s. Such molecules would bind to both subsites for substrate binding in the enzyme active site and should give potent inhibition of APH(3′)s. We have synthesized four conjoint molecules (compounds **1**−**4**) that incorporate structures of adenosine and neamine (an aminoglycoside) into one compound (Figure 2).

Experimental Section

1,1-Dimethoxycyclohexane was purchased from the TCI America Co. Pyruvate kinase (PK), lactic dehydrogenase (LD),

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(1) Wright, G. D.; Berghuis, A. M.; Mobashery, S. In *Resolving the Antibiotic Paradox: Progress in Understanding Drug Resistance and Development of New Antibiotics*; Rosen, B. P., Mobashery, S., Eds; Plenum Press: New York, 1998; pp 27−69.

(2) Shaw, K. J.; Rather, P. N.; Hare, R. S.; Miller, G. H. *Microbiol. Rev.* **1993**, *57*, 138−163.

(3) Roestamadji, J.; Mobashery, S. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3483−3488.

(4) Roestamadji, J.; Grapsas, I.; Mobashery, S. *J. Am. Chem. Soc.* **1995**, *117*, 80−84.

(5) Daigle, D. M.; McKay, G. A.; Wright, G. D. *J. Biol. Chem.* **1997**, *272*, 24755−24758.

(6) Cohn, M. A. *Science* **1992**, *257*, 1050−1054.

(7) The World Health Report. *Fighting Disease Fostering Development*; World Health Organization: Geneva, 1996.

(8) Bush, K.; Mobashery, S. in *Resolving the Antibiotic Paradox: Progress in Understanding Drug Resistance and Development of New Antibiotics*; Rosen, B. P., Mobashery, S., Eds; Plenum Press: New York, 1998; pp 71−98.

(9) Siregar, J. J.; Miroshnikov, K.; Mobashery, S. *Biochemistry* **1995**, *34*, 12681−12688.

(10) Wright, G. D.; McKay, G. A. *J. Biol. Chem.* **1995**, *270*, 24686−24692.

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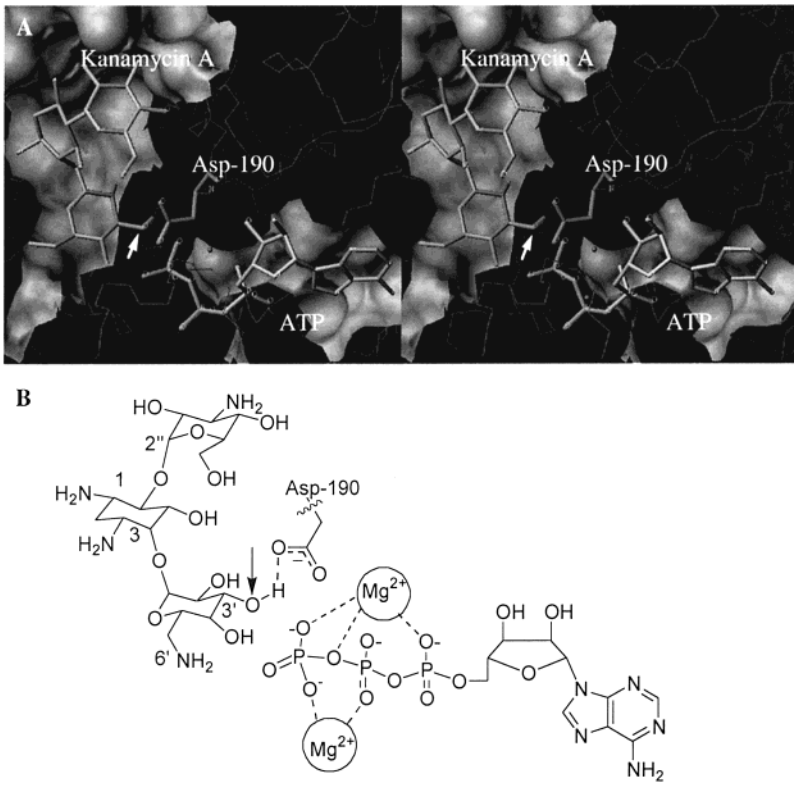


Figure 1. The energy-minimized computational model for the ternary complex of APH(3′)-IIIa: (A) Stereoview of the active site of APH(3′)-IIIa (Hon, W. C.; McKay, G. A.; Thompson, P. R.; Sweet, R. M.; Yang, D. S.; Wright, G. D.; Berghuis, A. M. *Cell* **1997**, *89*, 887−895.) occupied by the substrates kanamycin A and ATP. A portion of the binding sites for kanamycin A and ATP in the active site are shown as gray surfaces, which are rendered as Connolly water-accessible surfaces. Two magnesium ions, depicted as gray spheres, are coordinated to the phosphate backbone of ATP. The backbone of the enzyme is depicted as a gray wire, and kanamycin A, the catalytic residue Asp-190, and ATP are shown as capped-sticks. The 3′-hydroxyl moiety of kanamycin A is marked by an arrow. A hydrogen bond, depicted as a broken line, is shown between the carboxyl moiety of Asp-190 and the 3-hydroxyl moiety of kanamycin A. A schematic of the interactions observed in the energy-minimized model between kanamycin A, ATP, Asp-190, and the Mg^{2+} ions are shown in panel B. The 3′-hydroxyl moiety is shown by an arrow, which forms a hydrogen bond with the side chain of Asp-190.

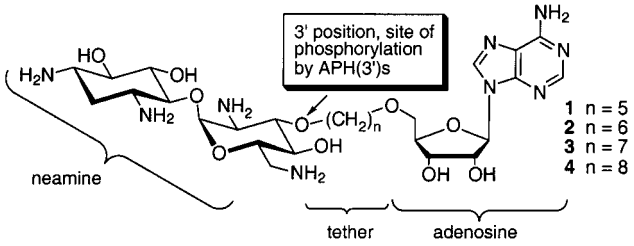


Figure 2. The chemical structures for the tethered bisubstrate probes for aminoglycoside 3′-phosphotransferases. The attachement of the tether is to the 3′-hydroxyl of aminoglycoside, the site of phosphorylation by APH(3′)s, and to the 6′-hydroxyl of adenosine. The tether serves as a surrogate for the triphosphoryl group of ATP. A somewhat different kind of tether for a bisubstrate analogue for a fucosyltransferase has been described previously (Palcic, M. M.; Heerze, L. D.; Srivastava, O. P.; Hindsgaul, O. *J. Biol. Chem.* **1989**, *262*, 17174−17181).

phospho(enol)pyruvate (PEP), ATP, and NADH were purchased from the Sigma Chemical Co. All other reagents were purchased from the Aldrich Chemical Co. All calculations were performed by the MS Excell program. Aminoglycoside 3′-phosphotransferase type Ia and IIa were purified according to the procedures developed by Siregar et al.[9,11]

Determination of Kinetic Parameters. The kinetic parameters for inhibition of APH(3′)s by the four inhibitors (**1**, **2**, **3**, and **4**) were determined by the coupled spectrophotometric assay described by Goldman and Northrop.[12] A typical assay mixture contained phosphoenol pyruvate (5 mM), magnesium chloride (10 mM), NADH (0.3 mM), lactate dehydrogenase (21 units), pyruvate kinase (6 units), and the concentrations of the inhibitors were varied from 2 μM to 1 mM in 25 mM HEPES buffer at pH 7.4. The final volume for each assay mixture was 500 μL. The reactions were performed either at saturating concentration of kanamycin A (200 μM) and varied concentrations of ATP (100 and 200 μM), or at saturating concentration of ATP (800 μM) and varied concentrations of kanamycin A (30 and 60 μM). The reaction was initiated by the addition of APH(3′)-Ia or APH(3′)-IIa at final concentrations of 800 and 400 nM, respectively. The change in optical density at 340 nm was monitored. The K_i values were calculated from Dixon plots.[13]

Assessment of the Potential for Slow-Binding Inhibition. To determine whether **1**, **2**, **3**, or **4** were slow-binding inhibitors for APH(3′)s, assays were carried out with and without preincubation of the enzymes with the inhibitors. The shapes of the reaction progress curves were compared to that obtained in the absence of inhibitor over a period during which the reaction progress was linear in the absence of inhibitor. For reactions without preincubation with inhibitor, the same components of the spectrophotometric assay described above were mixed including kanamycin A and each of the inhibitors

(11) Siregar, J. J.; Lerner, S. A.; Mobashery, S. *Antimicrob. Agents Chemother.* **1994**, *38*, 641−647.

(12) Goldman, P. R.; Northrop, D. B. *Biochem. Biophys. Res. Commun.* **1976**, *69*, 230−236.

(13) Dixon, M. 1953 *Biochem. J.* **1953**, *55*, 161−170.

(**1**, **2**, **3**, or **4**). The reaction was initiated by the addition of 800 nM of APH(3′)-Ia or 400 nM of APH(3′)-IIa. The reaction curves at three different inhibitor concentrations (ranging from 2 to 400 μM, depending on their respective K_i values) were obtained for APH(3′)-Ia and APH(3′)-IIa. In the presence of preincubation with inhibitor, the enzyme was mixed with each inhibitor (**1**, **2**, **3**, or **4**), at concentrations three times their K_i values, and the mixture was incubated for 20 min, 1 h, and 3 h. The preincubation mixtures contained all the components of the assay described above, except kanamycin A. The reactions were then initiated by the addition of kanamycin A to a final concentration of 50 μM in the case of APH(3′)-Ia, and to 25 μM for APH(3′)-IIa.

Molecular Modeling. The X-ray structure of kanamycin A was obtained from Cambridge Structural Database (KNMYSL). The coordinates for the three-dimensional structure of APH(3′)-IIIa with ADP, and two Mg^{2+} ions bound in the active site of the enzyme were kindly provided by Professor Albert Berghuis.[14] The model was constructed from the X-ray structure, as described below. ADP was modified to ATP by the addition of the γ-phosphate and kanamycin A was docked in the active site of the enzyme in such a way that the 3′-hydroxyl, the site of phosphorylation, approached the terminal phosphate group of ATP. The resulting complex was energy-minimized using AMBER 5.0 software (Oxford Molecular, Inc.).[15] For nonstandard residues, MNDO ESP charges were used, and the energy minimization was performed for 25000 iterations. The energy-minimized complex was visualized and analyzed using the Sybyl molecular modeling software.[16] Other modeling tasks that are mentioned in this manuscript were also performed using the Sybyl software.

1,3,2′,6′-Tetra-*N*-*tert*-butoxycarbonyl-3′-*O*-*tert*-butyldimethylsilyl-5,6-*O*-cyclohexylideneneamine (8). To a mixture of compound **7** (2.25 g, 2.81 mmol) and imidazole (243 mg, 3.57 mmol) in 20 mL of anhydrous *N*,*N*-dimethylformamide (DMF) was added *tert*-butyldimethylsilyl chloride (493 mg, 3.27 mmol) at room temperature under a nitrogen atmosphere, and the mixture was stirred overnight. The reaction was quenched by addition of water (3 mL), extracted with CH_2Cl_2 (3 × 20 mL), back extracted with water and then brine, dried over $MgSO_4$, and concentrated to dryness in vacuo. The residue was purified on a column (SiO_2, 2:1 hexane/ethyl acetate) to furnish compound **8** (1.91 g, 74%) as a pure white solid. mp 140−142 °C; IR (film) 3451, 3356, 2977, 1721, 1703, 1507 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.28 (s, 1 H, NH), 6.20 (d, 1 H, J = 5.7 Hz, NH), 6.02 (d, 1 H, J = 8.1 Hz, NH), 5.44 (s, 1 H, NH), 5.12 (s, 1 H, H-1′), 4.88 (d, 1 H, J = 3.0 Hz, OH), 3.80−3.46 (m, 9 H), 3.17 (d, 2 H, J = 9.0 Hz), 1.65−1.28 (m, 47 H), 0.86 (s, 9 H), 0.07 (s, 6 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.2, 155.4, 155.1, 111.4, 97.7, 81.1, 79.0, 78.3, 77.9, 77.2, 72.9, 71.8, 71.4, 55.7, 50.7, 48.76, 40.9, 37.1, 36.4, 36.2, 28.1, 27.9, 27.8, 27.8, 25.8, 25.0, 24.0, 23.8, 18.2, −4.3, −5.4; MS (FAB, NBA) 917 (MH^+); HRMS (FAB, NBA) calcd for $C_{44}H_{81}O_{14}N_4Si$ (MH^+) 917.5519, found 917.5521.

1,3,2′,6′-Tetra-*N*-benzyl-1,3,2′-tri-*N*-*tert*-butoxycarbonyl-3′-*O*-*tert*-butyldimethylsilyl-6′-*N*,4′-*O*-carbonyl-5,6-*O*-cyclohexylideneneamine (9). To an ice-cold solution of **8** (842 mg, 0.92 mmol) in anhydrous DMF (7 mL) was added sodium hydride (60% dispersion in mineral oil, 441 mg, 11.03 mmol), and the mixture was allowed to stir at room temperature under an atmosphere of nitrogen. After 2 h, benzyl bromide (2.18 mL, 18.33 mmol) was added dropwise over 30 min and stirring was continued for another 6 h. The reaction was quenched with a 25% aqueous HOAc solution (2 mL), diluted with water (3 mL), and extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was washed with water, dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a crude product, which was purified by column chromatography (SiO_2, 10:1 hexane/ethyl acetate) to give compound **9** (861 mg, 79%) as a white solid. mp 101−102 °C; IR (film) 2933, 1697 cm^{-1}; ^1H NMR (CD_3COCD_3, 500 MHz) δ 7.60−6.80 (m, 20 H), 5.45 (m, 1 H), 5.00 (m, 2 H), 4.60 (m, 2 H), 4.50−4.00 (m, 11 H), 3.80 (m, 3 H), 3.40 (m, 2 H), 1.80−1.00 (m, 39 H), 0.95 (s, 9 H), 0.25 (s, 6 H); ^{13}C NMR (CD_3COCD_3, 125 MHz) δ 156.7, 155.4, 152.0, 141.4, 139.7, 137.1, 128.7−126.2, 111.6, 96.9, 80.4, 79.9, 79.4, 75.7, 72.4, 68.3, 67.8, 61.2, 59.3, 54.2, 52.0, 47.7, 47.6, 45.8, 36.5, 36.4, 36.1, 32.0, 27.9, 27.7, 27.5, 25.7, 24.9, 23.8, 23.7, 18.0, −3.6, −5.3; MS (FAB, NBA) for $C_{68}H_{94}O_{13}N_4$ ($M + Na^+$) 1226.

1,3,2′,6′-Tetra-*N*-benzyl-3′-*O*-*tert*-butyldimethylsilyl-6′-*N*,4′-*O*-carbonylneamine (10). A stirred solution of compound **9** (100 mg, 0.08 mmol) in a mixed solvent system of CH_2Cl_2 (2 mL) and distilled water (0.01 mL) was treated with trifluoroacetic acid (2 mL) at room temperature for 1 h. The solvent was evaporated to dryness in vacuo, and the residue was purified by column chromatography (SiO_2, 20:1:0.1 $CHCl_3$/MeOH/NH_4OH,) to afford the title compound (45 mg, 66%) as a white powder. mp 67−69 °C; IR (film) 3327, 2950, 1710 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 7.42−7.22 (m, 20 H), 5.10 (d, 1 H, J = 3.3 Hz), 4.38 (d, 2 H, J = 15.0 Hz), 4.18 (dq, 1 H, J = 3.3, 6.3, 10.2 Hz), 3.99 (dd, 1 H, J = 8.7, 9.6 Hz), 3.97 (d, 2 H, J = 13.3 Hz), 3.83 (d, 1 H, J = 13.2 Hz), 3.82 (dd, 2 H, J = 9.6, 12.9 Hz), 3.71 (d, 1 H, J = 12.6 Hz), 3.45 (t, 1 H, J = 9.0 Hz), 3.28 (t, 1 H, J = 9.0, 9.3 Hz), 2.96 (t, 1 H, J = 10.5 Hz), 2.78 (dd, 1 H, J = 6.6, 10.5 Hz), 2.69 (dd, 1 H, J = 3.3, 9.9 Hz), 2.60 (m, 1 H), 2.58 (m, 1 H), 2.41 (m, 1 H), 0.92 (s, 9 H), 0.24 (s, 3 H), 0.18 (s, 3 H); ^{13}C NMR (CD_3OD, 75 MHz) δ 141.5, 141.3, 139.6, 137.1, 128.8, 128.7, 128.4, 128.3, 128.3, 128.2, 128.1, 127.9, 127.6, 127.2, 126.8, 126.6, 101.5, 89.2, 79.0, 77.1, 76.0, 71.7, 62.7, 62.3, 57.2, 56.5, 53.1, 52.2, 51.0, 48.0, 32.6, 25.7, 18.1, −5.08, −4.37; MS (FAB, NBA) for $C_{47}H_{62}O_7N_4Si$ (M^+) 823; ($M + Na^+$) 846.

1,3,2′,6′-Tetra-*N*-benzyl-1,3,2′-tri-*N*-*tert*-butoxycarbonyl-6′-*N*,4′-*O*-carbonyl-5,6-*O*-cyclohexylideneneamine (11). To an ice-cold solution of compound **9** (756 mg, 0.63 mmol) in dry THF (5 mL) was added a 1.0 M solution of tetra-*n*-butylammonium fluoride in THF (2.5 mL, 2.5 mmol). The mixture was stirred for 10 min, quenched with ice−water, and concentrated to dryness in vacuo. The residue was dissolved in CH_2Cl_2, extracted with water and then brine, dried (Na_2SO_4), concentrated, and purified on a column (SiO_2, 8:1 hexane/ethyl acetate) to afford the desired compound **11** as a white solid (453 mg, 66%). mp 123−125 °C; IR (film) 3405, 2933, 1697 cm^{-1}; ^1H NMR (CD_3COCD_3, 500 MHz) δ 7.55−6.95 (m, 20 H), 5.40 (m, 1 H), 4.96−4.82 (m, 2 H), 4.73−3.14 (m, 16 H), 2.99 (m, 2 H), 2.39 (m, 2 H), 1.72−1.18 (m, 38 H); ^{13}C NMR (CD_3COCD_3, 125 MHz) δ 156.8, 155.6, 155.1, 152.5, 141.6, 139.8, 138.6, 137.2, 137.0, 128.7−126.1, 111.6, 97.1, 81.2, 80.9, 80.2, 80.0, 79.5, 75.7, 72.4, 66.0, 61.9, 61.2, 61.3, 60.3, 58.8, 54.5, 52.7, 47.8, 47.5, 45.8, 36.6, 36.4, 36.2, 31.9, 27.8, 27.7, 27.5, 25.0, 23.8; MS (FAB, NBA) for $C_{62}H_{80}N_4O_{13}$ ($M + Na^+$) 1112.

1,3,2′,6′-Tetra-*N*-benzyl-3′-*O*-(5″-bromopentyl)-1,3,2′-tri-*N*-*tert*-butoxycarbonyl-6′-*N*,4′-*O*-carbonyl-5,6-*O*-cyclohexylideneneamine (12a). To a stirred solution of compound **11** (926 mg. 0.85 mmol) and 1,5-dibromopentane (2.44 mL, 17.91 mmol) in DMSO (12 mL) was added powdered potassium hydroxide (238 mg, 4.25 mmol). The resulting mixture was vigorously stirred at room temperature under a nitrogen atmosphere for 30 min. The reaction was quenched with cold water (4 mL), extracted by CH_2Cl_2 (3 × 50 mL), dried (Na_2SO_4), and concentrated in vacuo to furnish a syrup, which was purified by column chromatography (SiO_2, 3:1 hexane/ethyl acetate) to give the title compound (971 mg, 92%) as a white solid. mp 96−97 °C; IR (film) 2975, 2934, 1693 cm^{-1}; ^1H NMR (CD_3COCD_3, 500 MHz) δ 7.51−6.90 (m, 20 H), 5.45 (m, 1 H), 4.93 (m, 1 H), 4.80−4.02 (m, 9 H), 3.93−3.80 (m, 5 H), 3.54−3.49 (m, 5 H), 3.37 (m, 1 H), 3.13 (m, 1 H), 1.89 (m, 2 H), 1.61−1.17 (m, 44 H); ^{13}C NMR (CD_3COCD_3, 125 MHz) δ 156.6, 155.5, 152.0, 141.2, 141.1, 139.6, 137.0, 128.6−126.0, 111.5, 96.6, 80.3, 80.2, 80.1, 80.1, 79.5, 79.4, 73.6, 72.4, 72.1, 70.9, 61.3, 61.2, 58.7, 57.2, 54.4, 54.3, 52.0, 47.4, 45.7, 36.3, 36.0, 36.0, 34.0, 33.9, 32.6, 32.5, 31.9, 29.3, 27.8, 27.6, 27.5, 24.8, 24.5, 24.4,

(14) Hon, W. C.; McKay, G. A.; Thompson, P. R.; Sweet, R. M.; Yang, D. S.; Wright, G. D.; Berghuis, A. M. *Cell* **1997**, *89*, 887−895.

(15) Case, D. A.; Pearlman, D. A.; Caldwell, J. W.; Cheatham III, T. E.; Ross, W. S.; Simmerling, C. L.; Darden, T. A.; Merz, K. M.; Stanton, R. V.; Cheng, A. L.; Vincent, J. J.; Crowley, M.; Ferguson, D. M.; Radmer, R. J.; Seibel, G. L.; Singh, U. C.; Weiner, P. K.; Kollman, P. A. 1997, AMBER 5. University of California, San Francisco, CA.

(16) Tripos Associates, St. Louis, MO.

23.7; MS (FAB, NBA) 1236, 1238 (M^+); HRMS (FAB, NBA) calcd for $C_{67}H_{89}BrN_4O_{13}$ (M^+) 1236.5610, 1238.5610, found 1236.5603, 1238.5556.

1,3,2′,6′-Tetra-*N*-benzyl-3′-*O*-(6″-bromohexyl)-1,3,2′-tri-*N*-*tert*-butoxycarbonyl-6′-*N*,4′-*O*-carbonyl-5,6-*O*-cyclohexylideneneamine (12b). The title compound was prepared according to the procedure described for **12a** from the reaction of **11** (1.18 g, 1.08 mmol) and 1,6-dibromohexane to afford compound **12b** as a white solid (1.23 g, 91%). mp 90−92 °C; IR (film) 2973, 2932, 1694 cm^{-1}; ^1H NMR (CD_3COCD_3, 500 MHz) δ 7.49−6.96 (m, 20 H), 5.43 (m, 1 H), 4.94 (dd, 1 H), 4.80−4.11 (m, 9 H), 3.92 (m, 5 H), 3.50 (m, 3 H), 3.38 (m, 2 H), 3.18 (m, 2 H), 1.87 (m, 2 H), 1.61−1.17 (m, 46 H); ^{13}C NMR (CD_3COCD_3, 125 MHz) δ 157.4, 156.2, 152.7, 142.0, 140.4, 137.8, 129.7−126.7, 112.2, 97.3, 81.4, 81.0, 80.9, 80.8, 80.2, 74.3, 73.1, 72.8, 71.8, 62.0, 59.4, 58.0, 55.1, 55.0, 52.7, 48.2, 46.4, 37.0, 36.7, 34.7, 33.5, 30.7, 28.5, 28.2, 28.4, 28.2, 25.6, 25.5, 24.4; MS (FAB, NBA) 1250, 1252 (M^+); HRMS (FAB, NBA) calcd for $C_{68}H_{91}BrN_4O_{13}$ (M^+) 1250.5770, 1252.5770, found 1250.5770, 1252.5780.

1,3,2′,6′-Tetra-*N*-benzyl-3′-*O*-(7″-bromoheptyl)-1,3,2′-tri-*N*-*tert*-butoxycarbonyl-6′-*N*,4′-*O*-carbonyl-5,6-*O*-cyclohexylideneneamine (12c). The title compound was prepared according to the procedure described for **12a** from the reaction of **11** (1.03 g, 0.95 mmol) and 1,7-dibromoheptane to afford compound **12c** as a white solid (1.06 g, 88%). mp 85−86 °C; IR (film) 2972, 2931, 1695 cm^{-1}; ^1H NMR (CD_3COCD_3, 500 MHz) δ 7.48−6.95 (m, 20 H), 5.46 (m, 1 H), 4.99 (m, 1 H), 4.80−4.09 (m, 9 H), 3.89 (m, 5 H), 3.48 (m, 3 H), 3.37 (m, 2 H), 3.21 (m, 2 H), 1.86 (m, 2 H), 1.63−1.38 (m, 48 H); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ 157.4, 156.2, 155.7, 152.7, 142.0, 140.4 137.8, 129.4−126.7, 112.2, 97.4, 81.4, 81.2, 81.0, 80.9, 80.2, 80.1, 74.3, 73.2, 72.9, 72.3, 71.9, 62.3, 62.0, 59.5, 58.0, 55.2, 52.8, 48.2, 46.5, 37.1, 36.8, 34.7, 34.7, 33.5, 30.9, 29.1, 28.7, 28.5, 28.4, 26.4, 26.3, 25.6, 24.4; MS (FAB, NBA) 1264, 1266 (M^+); HRMS (FAB, NBA) calcd for $C_{69}H_{93}BrN_4O_{13}$ (M^+) 1264.5920, 1266.5920, found 1264.5926, 1266.5914.

1,3,2′,6′-Tetra-*N*-benzyl-3′-*O*-(8″-bromooctyl)-1,3,2′-tri-*N*-*tert*-butoxycarbonyl-6′-*N*,4′-O-carbonyl-5,6-*O*-cyclohexylideneneamine (12d). The title compound was prepared according to the procedure described for **12a** from the reaction of **11** (0.92 g, 0.83 mmol) and 1,8-dibromooctane to afford compound **12d** as a white solid (1.06 g, 98%). mp 84−86 °C; IR (film) 2971, 2929, 1694 cm^{-1}; ^1H NMR (CD_3COCD_3, 400 MHz) δ 7.67−6.93 (m, 20 H), 5.46 (m, 1 H), 4.95 (m, 1 H), 4.78−4.08 (m, 9 H), 3.86 (m, 5 H), 3.48 (m, 3 H), 3.36 (m, 2 H), 3.20 (m, 2 H), 1.86 (m, 2 H), 1.63−1.38 (m, 50 H); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ 157.8, 156.7, 156.6, 156.1, 153.1, 153.0, 142.4, 142.3, 140.8 138.2, 138.1, 138.0, 129.8−127.0, 112.6, 97.7, 81.8, 81.4, 81.3, 81.2, 80.6, 77.1, 74.7, 73.5, 73.2, 72.8, 72.4, 62.4, 59.8, 58.4, 55.5, 55.4, 53.1, 48.6, 46.8, 37.4, 37.2, 37.1, 35.2, 33.9, 31.3, 29.7, 29.1, 29.0, 28.8, 28.7, 26.8, 25.9, 24.8, 24.7; MS (FAB, NBA) for $C_{70}H_{95}BrN_4O_{13}$ ($M + Na^+$) 1303.

Compound 14a. To a mixture of **12a** (784 mg, 0.63 mmol), N^6-benzoyl-2′,3′-*O*-isopropylideneadenosine **13a** (266 mg, 0.64 mmol), and tetrabutylammonium iodide (469 mg, 1.26 mmol) in anhydrous DMSO (10 mL) was added powdered potassium hydroxide (80 mg, 1.43 mmol). The mixture was vigorously stirred at room temperature under a nitrogen atmosphere for 1 h, quenched with cold water, and extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was dried (Na_2SO_4) and concentrated to a syrup in vacuo. The residue [**19a**, MS (FAB, NBA) 1594 ($M + Na^+$)] was subsequently treated with an equal volume of 3 M aqueous sodium hydroxide and 1,4-dioxane (1:1, 3 mL) at 50 °C for 24 h. The mixture was cooled and extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo to give a residue, which was purified by column chromatography (SiO_2, 50:1 $CHCl_3$/MeOH) to afford the desired compound **14a** as a white solid (214 mg, 24%). mp 96−97 °C; IR (film) 3329, 3186, 2934, 1695, 1639, 1162 cm^{-1}; ^1H NMR (CD_3COCD_3, 500 MHz) δ 8.26 (s, 1 H), 8.21 (s, 1 H), 7.48−7.14 (m, 20 H), 6.74 (m, 1 H), 6.22 (s, 1 H), 5.63 (s, 1 H), 5.47 (dd, 1 H, J = 1.5, 6.5 Hz), 5.42 (s, 1 H), 5.06 (dd, 1 H, J = 1.5, 5.0 Hz), 4.41 (m, 1 H), 4.80−2.80 (m, 25 H) 2.46 (m, 1 H), 1.64−1.19 (m, 50 H); ^{13}C NMR (CD_3COCD_3, 125 MHz) δ 156.11, 155.50, 152.91, 149.44, 141.75, 139.39, 128.52−125.67, 119.53, 113.25, 111.29, 96.27, 90.70, 86.03, 85.89, 84.85, 81.85, 79.47, 78.96, 78.33, 76.79, 75.19, 72.03, 71.85, 71.15, 70.76, 59.01, 57.47, 54.52, 53.89, 51.19, 50.96, 47.43, 46.83, 46.42, 36.33, 36.01, 30.20, 27.85, 27.65, 27.50, 26.68, 24.80, 24.71, 23.67, 22.72; MS (FAB, NBA) 1439 (MH^+); HRMS (FAB, NBA) calcd for $C_{79}H_{108}N_9O_{16}$ (MH^+) 1438.7990, found 1438.7987.

Compound 14b. The title compound was prepared according to the procedure described for **14a** from compound **12b** (800 mg, 0.64 mmol) and **13a** (268 mg, 0.65 mmol) to give the desired compound **14b** as a white solid (250 mg, 27%). mp 95−96 °C; IR (film) 3328, 3184, 2934, 1694, 1639, 1162 cm^{-1}; ^1H NMR (CD_3COCD_3, 500 MHz) δ 8.22 (s, 1 H), 8.21 (s, 1 H), 7.47−7.14 (m, 20 H), 6.74 (m, 1 H), 6.20 (s, 1 H), 5.47 (dd, 1 H, J = 2.5, 5.5 Hz), 5.36 (s, 1 H), 5.06 (d, 1 H, J = 5.0 Hz), 4.33 (m, 1 H), 4.94−2.91 (m, 25 H), 2.46 (br, 1 H), 1.64−1.19 (m, 52 H); ^{13}C NMR (CD_3COCD_3, 125 MHz) δ 156.9, 153.5, 150.2, 140.3, 129.2−126.4, 120.4, 113.9, 112.0, 97.1, 91.6, 86.8, 85.2, 82.8, 80.2, 77.3, 75.7, 71.8, 71.4, 59.7, 58.2, 55.2, 54.6, 51.8, 48.0, 47.1, 37.0, 36.7, 31.0, 28.6, 28.4, 28.2, 27.3, 26.4, 25.5, 25.4, 24.4; MS (FAB, NBA) 1453 (MH^+); HRMS (FAB, NBA) calcd for $C_{80}H_{110}N_9O_{16}$ (MH^+) 1452.8100, found 1452.8095.

Compound Test 14c. The title compound was prepared according to the procedure described for **14a** from compound **12c** (1.04 g, 0.82 mmol) and **13a** (407 mg, 0.98 mmol) to give the desired compound **14c** as a white solid (322 mg, 27%). mp 90−92 °C; IR (film) 3327, 3185, 2935, 1693, 1638, 1163 cm^{-1}; ^1H NMR (CD_3COCD_3, 400 MHz) δ 8.25 (s, 1 H), 8.24 (s, 1 H), 7.48−7.15 (m, 20 H), 6.84 (m, 1 H), 6.23 (d, 1 H, J = 1.6 Hz), 5.50 (d, 1 H, J = 2.4 Hz), 5.49 (s, 1 H), 5.08 (dd, 1 H, J = 2.4, 6.0 Hz), 4.99−2.88 (m, 26 H), 2.48 (br, 1 H), 1.76−1.25 (m, 54 H); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ 157.8, 157.3, 156.6, 153.8, 150.6, 141.7, 140.7, 129.7−126.8, 120.8, 114.3, 112.4, 97.4, 92.1, 91.2, 85.7, 83.2, 80.6, 80.4, 80.1, 79.5, 77.7, 76.7, 72.2, 72.2, 71.8, 71.6, 71.4, 60.2, 58.7, 55.6, 55.0, 52.4, 52.2, 48.5, 47.9, 47.5, 37.5, 37.1, 34.0, 33.0, 31.5, 30.5, 29.0, 28.8, 28.6, 27.8, 27.0, 26.0, 25.8, 24.8; MS (FAB, NBA) for $C_{81}H_{111}N_9O_{16}$ (M^+) 1466, ($M + Na^+$) 1489.

Compound 14d. The title compound was prepared according to the procedure described for **14a** from compound **12d** (744 mg, 0.58 mmol) and **13a** (241 mg, 0.58 mmol) to give the desired compound **14d** as a white solid (290 mg, 34%). mp 89−90 °C; IR (film) 3329, 3183, 2932, 1694, 1638, 1164 cm^{-1}; ^1H NMR (CD_3COCD_3, 400 MHz) δ 8.35 (s, 1 H), 8.33 (s, 1 H), 7.59−7.26 (m, 20 H,), 6.94 (m, 1 H), 6.32 (d, 1 H, J = 1.6 Hz), 5.57 (m, 2 H, J = 2.8, 6.0 Hz), 5.48 (s, 1 H), 5.17 (dd, 1 H, J = 2.4, 6.0 Hz), 5.02−3.03 (m, 26 H), 2.57 (br, 1 H), 1.75−1.30 (m, 56 H); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ 157.9, 157.4, 156.7, 154.0, 150.6, 142.7, 141.8, 140.8, 129.7−126.9, 120.9, 114.4, 112.5, 97.5, 92.1, 87.2, 85.8, 83.3, 81.7, 80.7, 80.5, 80.2, 77.9, 77.0, 76.5, 73.5, 73.2, 71.9, 71.6, 71.4, 60.9, 60.3, 58.7, 55.7, 55.1, 54.8, 52.6, 52.4, 48.6, 47.6, 37.5, 37.2, 33.2, 31.6, 30.6, 29.1, 28.9, 28.7, 27.9, 27.1, 26.0, 25.9, 24.9; MS (FAB, NBA) for $C_{83}H_{113}N_9O_{16}$ ($M - H^+ + Na^+$) 1502, (M^+) 1480.

Compound 15a. To a solution of **14a** (100 mg, 0.07 mmol) in a mixed solvent of CH_2Cl_2 (2 mL) and water (0.05 mL) was added trifluoroacetic acid (2 mL), and the resulting solution was stirred at room temperature for 1 h. The solvent was subsequently evaporated to dryness in vacuo, and the residue was purified by column chromatography (SiO_2, 20:1:0.1 $CHCl_3$/MeOH/NH_4OH) to afford compound **15a** as a white powder (65 mg, 92%). mp 77−79 °C; IR (KBr) 3389, 3329, 2924, 1644, 1028 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 8.59 (s, 1 H), 8.48 (s, 1 H), 7.51−7.23 (m, 20 H), 6.10 (d, 1 H, J = 4.2 Hz), 5.95 (d, 1 H, J = 3.9 Hz), 4.55−3.04 (m, 28 H), 2.77 (m, 1 H), 2.24 (m, 1 H), 1.69−1.28 (m, 6 H); ^{13}C NMR (CD_3OD, 300 MHz) δ 159.2, 144.3, 141.8, 130.9, 130.7, 130.5, 130.1, 129.8, 129.4, 129.2, 128.9, 128.7, 103.5, 95.1, 88.9, 84.2, 77.1, 75.8, 75.7, 75.2, 71.4, 71.0, 70.4, 69.7, 57.6, 55.7, 54.7, 54.2, 50.7, 46.3, 29.5, 29.0, 24.1, 22.4; MS (FAB, NBA) for $C_{55}H_{71}N_9O_{10}$ (M^+) 1018.

Compound 15b. The title compound was prepared as described for **15a** from compound **14b** (230 mg, 0.16 mmol) to afford the desired compound **15b** (125 mg, 77%). mp 76−77 °C; IR (KBr) 3390, 3331, 2926, 1646, 1029 cm^{-1}; ^1H NMR (CD_3-

OD, 500 MHz) δ 8.37 (s, 1 H), 8.18 (s, 1 H), 7.35−7.16 (m, 20 H), 6.05 (d, 1 H, J = 4.5 Hz), 4.96 (d, 1 H, J = 3.3 Hz), 4.55 (t, 1 H, J = 4.5 Hz), 4.34 (t, 1 H, J = 4.5 Hz), 4.19 (m, 1 H), 3.94−3.24 (m, 23 H), 2.58 (m, 2 H), 2.47 (m, 1 H), 2.36 (m, 1 H), 1.57 (m, 4 H), 1.36 (m, 4 H); ^{13}C NMR (CD_3OD, 125 MHz) δ 153.2, 141.0, 139.9, 139.7, 138.0, 129.0, 129.0, 128.9, 128.8, 128.7, 127.7, 127.6, 127.4, 102.0, 89.3, 89.2, 84.6, 81.7, 77.1, 75.8, 73.6, 73.0, 72.7, 71.9, 71.2, 70.4, 61.4, 56.8, 55.2, 54.2, 53.2, 50.9, 50.3, 50.1, 30.8, 30.4, 30.0, 26.5, 26.4; MS (FAB, NBA) 1032 (MH^+); HRMS (FAB, NBA) calcd for $C_{56}H_{74}N_9O_{10}$ (MH^+) 1032.5590, found 1032.5586.

Compound 15c. The title compound was prepared as described for **15a** from compound **14c** (320 mg, 0.20 mmol) to afford the desired compound **15c** (198 mg, 86%). mp 75−76 °C; IR (KBr) 3389, 3330, 2926, 1644, 1029 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 8.40 (s, 1 H), 8.21 (s, 1 H), 7.37−7.18 (m, 20 H), 6.09 (d, 1 H, J = 4.4 Hz), 5.07 (d, 1 H, J = 3.2 Hz), 4.57 (t, 1 H, J = 4.4 Hz), 4.38 (t, 1 H, J = 4.8 Hz), 4.23 (m, 1 H), 3.98−3.32 (m, 23 H), 2.67 (m, 2 H), 2.56 (m, 1 H), 2.46 (m, 1 H), 1.59 (m, 4 H), 1.34 (m, 6 H); ^{13}C NMR (CD_3OD, 125 MHz) δ 156.5, 153.2, 149.8, 140.0, 139.8, 139.6, 139.2, 139.1, 129.0, 129.0, 128.8, 127.9, 127.8, 127.6, 119.6, 101.5, 89.4, 88.3, 84.6, 81.6, 77.1, 75.8, 75.6, 73.5, 73.2, 72.4, 72.0, 71.2, 70.4, 61.2, 56.9, 55.4, 53.9, 53.1, 50.8, 50.4, 49.9, 30.8, 30.3, 30.0, 29.7, 26.4; MS (FAB, NBA) 1046 (MH^+); HRMS (FAB, NBA) calcd for $C_{57}H_{76}N_9O_{10}$ (MH^+) 1046.5720, found 1046.5703.

Compound 15d. The title compound was prepared as described for **15a** from compound **14d** (290 mg, 0.20 mmol) to afford the desired compound **15d** (197 mg, 95%). mp 74−75 °C; IR (KBr) 3388, 3331, 2927, 1645, 1030 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 8.37 (s, 1 H), 8.19 (s, 1 H), 7.34−7.16 (m, 20 H), 6.08 (d, 1 H, J = 4.5 Hz), 5.07 (d, 1 H, J = 3.5 Hz), 4.56 (t, 1 H, J = 4.5, 5.0 Hz), 4.35 (t, 1 H, J = 4.5 Hz), 4.20 (m, 1 H), 3.95−3.31 (m, 23 H), 2.66 (m, 2 H), 2.52 (m, 1 H), 2.38 (m, 1 H), 1.57 (m, 4 H), 1.29 (m, 8 H); ^{13}C NMR (CD_3OD, 125 MHz) δ 156.5, 153.2, 152.8, 149.8, 141.3, 140.0, 139.8, 139.6, 139.0, 138.8, 129.0, 128.9 (3C), 127.9, 127.8 (2C), 127.7, 119.7, 101.4, 89.4, 88.0, 84.7, 81.6, 77.1, 75.8, 75.5, 73.5, 73.2, 72.3, 72.0, 71.2, 70.5, 61.2, 56.9, 55.4, 53.9, 53.1, 50.8, 50.5, 49.8, 30.9, 30.3, 30.1, 29.9, 29.8, 26.4; MS (FAB, NBA) for $C_{58}H_{77}N_9O_{10}$ (M^+) 1060, ($M + Na^+$) 1083.

Compound 1. To a suspension of 10% palladium on carbon (10 mg) and 20% palladium hydroxide on carbon (14 mg) in anhydrous methanol was added a solution of compound **15a** (15 mg, 0.01 mmol) in anhydrous methanol−acetic acid (4 mL, 3:1), and the resulting mixture was stirred under a hydrogen atmosphere for 48 h. The mixture was filtered through Celite and the filtrate was concentrated in vacuo to give the title compound as a white solid (8 mg, 83%). IR (KBr) 3403, 2931, 1643, 1573, 1413, 1097, 1048 cm^{-1}; ^{1}H NMR (D_2O, 400 MHz) δ 8.23 (s, 1 H), 8.07 (s, 1 H), 5.93 (d, 1 H, J = 4.4 Hz), 5.84 (d, 1 H, J = 3.6 Hz), 4.63 (t, 1 H, J = 4.8, 4.8 Hz), 4.31 (t, 1 H, J = 5.2, 4.8 Hz), 4.18 (m, 1 H), 3.93 (m, 1 H), 3.87 (t, 1 H, J = 10.0 Hz), 3.80−3.73 (m, 2 H), 3.70−3.55 (m, 4 H), 3.49 (t, 1 H, J = 9.6, 10.0 Hz), 3.41 (m, 4 H,), 3.31 (dd, 1 H, J = 3.6, 13.6 Hz), 3.30 (dd, 1 H, J = 3.6, 10.8 Hz), 3.25 (m, 1 H), 3.12 (dd, 1 H, J = 7.6, 13.6 Hz), 2.41 (m, 1 H), 1.83 (m, 1 H), 1.45 (m, 4 H), 1.18 (m, 2 H); ^{13}C NMR (D_2O, 100 MHz) δ 155.7, 152.7, 149.6, 141.2, 96.8, 88.9, 84.5, 78.5, 77.2, 76.3, 75.0, 74.6, 73.6, 72.5, 72.1, 71.4, 70.6, 64.4, 53.8, 50.8, 49.4, 41.1, 30.1, 29.4, 22.8; MS (FAB, NBA) 658 (MH^+); HRMS (FAB, NBA) calcd for $C_{27}H_{48}N_9O_{10}$ (MH^+) 658.3525, found 658.3538.

Compound 2. The title compound was prepared as described for **1** from compound **15b** (80 mg, 0.08 mmol) to give the desired compound **2** (35 mg, 67%) as a white solid. IR (KBr) 3415, 2931, 1641, 1572, 1412, 1097, 1049 cm^{-1}; ^{1}H NMR (D_2O, 400 MHz) δ 8.18 (s, 1 H), 8.02 (s, 1 H), 5.90 (d, 1 H, J = 4.4 Hz), 5.84 (d, 1 H, J = 3.2 Hz), 4.60 (t, 1 H, J = 4.4 Hz), 4.29 (t, 1 H, J = 5.2 Hz), 4.14 (m, 1 H), 3.92 (m, 1 H, J = 3.2, 7.6 Hz), 3.86 (t, 1 H, J = 10.4, 9.2 Hz), 3.77 (t, 1 H, J = 10.0 Hz), 3.70−3.35 (m, 4 H), 3.47 (t, 1 H, J = 10.0 Hz), 3.44 (m, 1 H), 3.42 (t, 1 H, J = 9.6 Hz), 3.34 (t, 4 H, J = 6.4, 5.6 Hz), 3.30 (d, 1 H, J = 3.6 Hz), 3.22 (m, 1 H), 3.11 (dd, 1 H, J = 7.6, 13.6 Hz), 2.39 (m, 1 H), 1.81 (m, 1 H), 1.37 (m, 4 H), 1.08 (m, 4 H); ^{13}C NMR (D_2O, 100 MHz) δ 155.8, 152.8, 149.6, 141.0, 119.5, 96.7, 88.9, 84.6, 78.4, 77.2, 76.3, 75.1, 74.8, 73.6, 72.7, 72.1, 71.4, 70.6, 70.5, 53.8, 50.8, 49.4, 41.1, 30.3, 29.6, 29.4, 26.2, 25.9; MS (FAB, NBA) 672 (MH^+); HRMS (FAB, NBA) calcd for $C_{28}H_{50}N_9O_{10}$ (MH^+) 672.3681, found 672.3678.

Compound 3. The title compound was prepared as described for **1** from compound **15c** (187 mg, 0.18 mmol) to give the desired compound **3** (109 mg, 89%) as a white solid. IR (KBr) 3419, 2926, 1641, 1576, 1421, 1097, 1048 cm^{-1}; ^{1}H NMR (D_2O, 400 MHz) δ 8.25 (s, 1 H), 8.11 (s, 1 H), 5.96 (d, 1 H, J = 4.4 Hz), 5.87 (d, 1 H, J = 3.6 Hz), 4.68 (t, 1 H, J = 4.8 Hz), 4.34 (t, 1 H, J = 5.2 Hz), 4.18 (m, 1 H), 3.97 (m, 1 H), 3.91 (t, 1 H, J = 9.6 Hz), 3.84−3.73 (m, 2 H), 3.70−3.57 (m, 3 H), 3.51 (t, 1 H, J = 10.4, 9.2 Hz), 3.48 (m, 1 H), 3.47 (t, 1 H, J = 9.2, 8.2 Hz), 3.38 (t, 4 H, J = 7.2, 5.6 Hz), 3.35 (d, 1 H, J = 3.6 Hz), 3.27 (m, 1 H), 3.16 (dd, 1 H, J = 7.6, 13.2 Hz), 2.43 (m, 1 H), 1.83 (m, 1 H), 1.43 (m, 2 H), 1.35 (m, 2 H), 1.19 (m, 1 H), 1.08 (m, 5 H); ^{13}C NMR (D_2O, 100 MHz) δ 156.0, 154.9, 152.2, 143.8, 119.5, 99.2, 91.5, 87.2, 80.9, 79.6, 78.7, 77.5, 77.4, 76.0, 75.2, 74.5, 73.9, 73.1, 72.9, 56.3, 53.2, 51.9, 43.5, 32.8, 32.0, 31.8, 28.7, 28.8, 25.3; MS (FAB, NBA) 686 (MH^+); HRMS (FAB, NBA) calcd for $C_{29}H_{52}N_9O_{10}$ (MH^+) 686.3837, found 686.3849.

Compound 4. The title compound was prepared as described for **1** from compound **15d** (200 mg, 0.19 mmol) to give the desired compound **4** (85.4 mg, 65%) as a white solid. IR (KBr) 3403, 2930, 1642, 1576, 1420, 1097, 1047 cm^{-1}; ^{1}H NMR (D_2O, 500 MHz) δ 8.12 (s, 1 H), 7.98 (s, 1 H), 5.85 (d, 1 H, J = 4.0 Hz), 5.85 (d, 1 H, J = 3.0 Hz), 4.59 (t, 1 H, J = 4.5 Hz), 4.24 (t, 1 H, J = 5.0, 4.5 Hz), 4.07 (m, 1 H), 3.85 (m, 1 H), 3.79 (t, 1 H, J = 10.0, 9.5 Hz), 3.69 (t, 1 H, J = 9.5, 10.0 Hz), 3.64 (t, 1 H, J = 7.0, 7.5 Hz), 3.57−3.46 (m, 3 H), 3.42−3.34 (m, 3 H), 3.27 (m, 4 H), 3.24 (d, 1 H, J = 3.5 Hz), 3.16 (m, 1 H), 3.07 (dd, 1 H, J = 7.0, 13.5 Hz), 2.32 (m, 1 H), 1.72 (m, 1 H), 1.32 (m, 2 H), 1.22 (m, 2 H), 1.09 (m, 1 H), 0.95 (m, 7 H); ^{13}C NMR (D_2O, 125 MHz) δ 155.7, 152.9, 149.4, 140.6, 96.4, 88.4, 84.3, 78.3, 76.8, 75.8, 74.5, 73.1, 72.3, 71.6, 71.0, 70.2, 69.9, 53.4, 50.4, 49.0, 40.6, 29.9, 29.1, 29.0, 28.9, 25.8, 25.5, 25.0; MS (FAB, NBA) 700 (MH^+); HRMS (FAB, NBA) calcd for $C_{30}H_{54}N_9O_{10}$ (MH^+) 700.3994, found 700.3987.

1,3,2′,6′-Tetra-*N*-benzyl-4′-*O*-benzyl-1,3,2′,6′-tetra-*N*-*tert*-butoxycarbonyl-3′-*O*-*tert*-butyldimethylsilyl-5,6-*O*-cyclohexylideneneamine (16). To an ice-cold solution of **8** (4.74 g, 5.17 mmol) in anhydrous DMF (40 mL) was added sodium hydride (60% dispersion in mineral oil, 2.48 g, 0.06 mol), and benzyl bromide (12.40 mL, 18.33 mmol) was subsequently added in one portion. The mixture was stirred at room temperature under a nitrogen atmosphere for 2 h. The reaction was quenched with a 25% aqueous HOAc solution (5 mL), diluted with water (20 mL), and extracted with CH_2Cl_2 (3 × 50 mL). The organic layer was washed with water, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to afford a crude product, which was purified by column chromatography (SiO_2, 10:1 hexane/ethyl acetate) to give compounds **16** (3.00 g, 48%) and **9** (2.40 g, 34%) as white solids. Compound **16**: R_f 0.62 (8:1 hexane/ethyl acetate); mp 104−105 °C; IR (film) 2931, 1695, 1143 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 500 MHz) δ 7.55−7.24 (m, 25 H), 5.51 (m,1 H), 5.04−3.05 (m, 22 H), 1.65−1.19 (m, 47 H), 0.93 (s, 9 H), 0.25 (s, 6 H); ^{13}C NMR (CD_3COCD_3, 125 MHz) δ 156.9, 156.0, 152.8, 142.3, 140.0, 139.4, 129.1, 128.8, 128.7, 128.5, 127.8, 127.4, 127.0, 126.5, 112.1, 95.4, 80.1, 72.3, 70.9, 59.4, 55.1, 48.0, 36.9, 28.5, 28.2, 28.1, 26.6, 26.4, 25.5, 24.4, 18.5, −3.31; MS (FAB, NBA) for $C_{79}H_{110}N_4O_{14}Si$ ($M + Na^+$) 1390.

1,3,2′,6′-Tetra-*N*-benzyl-4′-*O*-benzyl-1,3,2′,6′-tetra-*N*-*tert*-butoxycarbonyl-5,6-*O*-cyclohexylideneneamine (17). To a solution of compound **16** (5.70 g, 4.73 mmol) in dry THF (50 mL) was added a 1.0 M solution of tetra-*n*-butylammonium fluoride in THF (14.6 mL, 14.6 mmol) at 0 °C. The mixture was stirred for 10 min under a nitrogen atmosphere, quenched with ice water, and concentrated to dryness in vacuo. The residue was dissolved in CH_2Cl_2, extracted with water, then with brine and purified on a column (SiO_2, 9:1 hexane/ethyl acetate) to afford the desired compound **17** as a white solid (3.28 g, 63%): R_f 0.48 (6:1 hexane/ethyl acetate); mp 112−114 °C; IR (film) 3450, 2975, 2934, 1693 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 500 MHz) δ 7.51−7.10 (m, 25 H), 5.52 (m, 1 H), 4.80−

4.69 (m, 6 H), 4.49−4.24 (m, 5 H), 3.57−3.24 (m, 3 H), 2.40 (m, 1 H), 1.67−1.21 (m, 47 H); ^{13}C NMR (CD_3COCD_3, 125 MHz) δ 157.6, 156.2, 142.6, 139.9, 129.2−126.5, 112.1, 96.2, 81.8, 80.3, 79.8, 75.6, 75.0, 73.0, 70.3, 61.9, 61.2, 59.7, 55.2, 50.2, 47.4, 46.8, 37.0, 36.9, 32.6, 28.5, 28.4, 28.3, 25.6, 24.4; MS (FAB, NBA) for $C_{73}H_{96}N_4O_{14}$ (M^+) 1253.

1,3,2′,6′-Tetra-*N*-benzyl-4′-*O*-Benzyl-1,3,2′,6′-tetra-*N*-*tert*-butoxycarbonyl-3′-*O*-(5″-bromopentyl)-5,6-*O*-cyclohexylideneneamine (18a). To a stirred solution of compound **17** (1.50 g, 1.20 mmol) and 1,5-dibromopentane (3.54 mL, 25.32 mmol) in DMSO (15 mL) was added powdered potassium hydroxide (336 mg, 6.00 mmol). The resulting mixture was vigorously stirred at room temperature under a nitrogen atmosphere for 30 min. The reaction was quenched with cold water (5 mL), extracted with CH_2Cl_2 (3 × 50 mL), dried (Na_2SO_4), and concentrated in vacuo to furnish a syrup, which was purified by column chromatography (SiO_2, 9:1 hexane/ethyl acetate) to give the title compound (1.36 g, 81%) as a white solid: R_f 0.71 (6:1 hexane/ethyl acetate); mp 89−90 °C; IR (film) 2934, 1693 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 500 MHz) δ 7.54−7.23 (m, 25 H), 5.51 (m, 1 H), 4.91−3.07 (m, 24 H), 1.79 (m, 2 H), 1.64−1.22 (m, 53 H); ^{13}C NMR (CD_3COCD_3, 125 MHz) δ 157.2, 156.2, 142.3, 142.1, 139.7, 129.1−126.5, 112.1, 96.1, 81.7, 80.9, 80.0, 78.2, 76.8, 75.3, 73.2, 72.8, 59.8, 58.2, 55.1, 50.0, 47.7, 47.1, 37.0, 36.8, 34.4, 33.4, 30.3, 28.5, 28.4, 28.2, 25.5, 25.2, 24.4, 24.4; MS (FAB, NBA) for $C_{78}H_{105}BrN_4O_{14}$ (M + H^+) 1403.

3′-*O*-(6″-Bromohexyl)-4′-*O*-benzyl-5,6-*O*-cyclohexylidene-tetra-*N*-benzyl-tetra-*N*-*tert*-butoxycarbonylneamine (18b). The title compound was prepared according to the procedure described for **18a** from the reaction of **17** (1.18 g, 1.08 mmol) and 1,6-dibromohexane to afford compound **18b** (1.06 g, 59%) as a white solid: R_f 0.73 (6:1 hexane/ethyl acetate 6:1); mp 86−88 °C; IR (film) 2933, 1693 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 500 MHz) δ 7.47−7.24 (m, 25 H), 5.53 (m, 1 H), 4.93−3.05 (m, 24 H), 1.79 (m, 2 H), 1.66−1.20 (m, 55 H); ^{13}C NMR (CD_3COCD_3, 125 MHz) δ 157.6, 156.2, 155.7, 142.4, 142.1, 140.3, 139.5, 129.2−126.5, 112.1, 96.1, 81.8, 80.8, 80.1, 80.0, 78.2, 76.9, 75.2, 73.1, 72.8, 72.5, 59.9, 58.2, 55.1, 54.8, 50.2, 47.8, 47.2, 37.0, 36.9, 34.6, 33.3, 31.1, 28.7, 28.6, 28.5, 28.3, 25.8, 25.6, 24.5, 24.4; MS (FAB, NBA) for $C_{79}H_{107}BrN_4O_{14}$ (M^+) 1416, (M + H^+ + Na^+) 1440.

Compound 19b. To a mixture of **12a** (100 mg, 0.08 mmol), N^6,N^6-dibenzyl-2′,3′-*O*-isopropylideneadenosine **13c** (48 mg, 0.10 mmol), and tetrabutylammonium iodide (60 mg, 0.16 mmol) in anhydrous DMSO (8 mL) was added powdered potassium hydroxide (40 mg, 0.71 mmol). The mixture was vigorously stirred at room temperature under a nitrogen atmosphere for 4 h, quenched with cold water, and extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was dried (Na_2SO_4) and concentrated to a syrup in vacuo to give a residue, which was purified by column chromatography (SiO_2, 5:2 hexane/ethyl acetate) to afford the desired compound **19b** as a white solid (48 mg, 36%): R_f 0.19 (hexanes:EtOAc, 5:2); mp 84−86 °C; IR (film) 2933, 1696, 1579, 1158 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 500 MHz) δ 8.34 (s, 1 H), 8.24 (s, 1 H), 7.44−7.09 (m, 30 H), 6.94 (d, 1 H, J = 6.5 Hz), 6.28 (s, 1 H), 5.45 (dd, 1 H, J = 2.5, 6.0 Hz), 5.08 (dd, 1 H, J = 2.5, 6.5 Hz), 4.99 (br, 1 H), 4.90 (t, 2 H, J = 15.0 Hz), 4.41 (d, 1 H, J = 2.5 Hz), 4.77−3.11 (m, 27 H), 1.61−1.18 (m, 50 H); ^{13}C NMR (CD_3COCD_3, 125 MHz) δ 154.7, 152.3, 150.8, 141.2, 139.7, 138.3, 137.0, 128.6, 128.0, 127.8, 127.7, 127.6, 127.4, 127.1, 126.7, 126.5, 126.2, 125.9, 119.7, 113.3, 111.5, 96.6, 90.5, 85.9, 84.6, 82.0, 80.2, 80.1, 79.4, 73.5, 72.1, 71.1, 71.0, 70.7, 61.2, 58.7, 57.2, 54.4, 52.0, 47.4, 45.7, 36.3, 35.9, 34.8, 29.9, 27.8, 27.6, 27.5, 26.7, 24.7, 23.6, 22.3, 22.2; MS (FAB, NBA) for $C_{94}H_{117}N_9O_{17}$ (M^+) 1645, (M + Na^+) 1668.

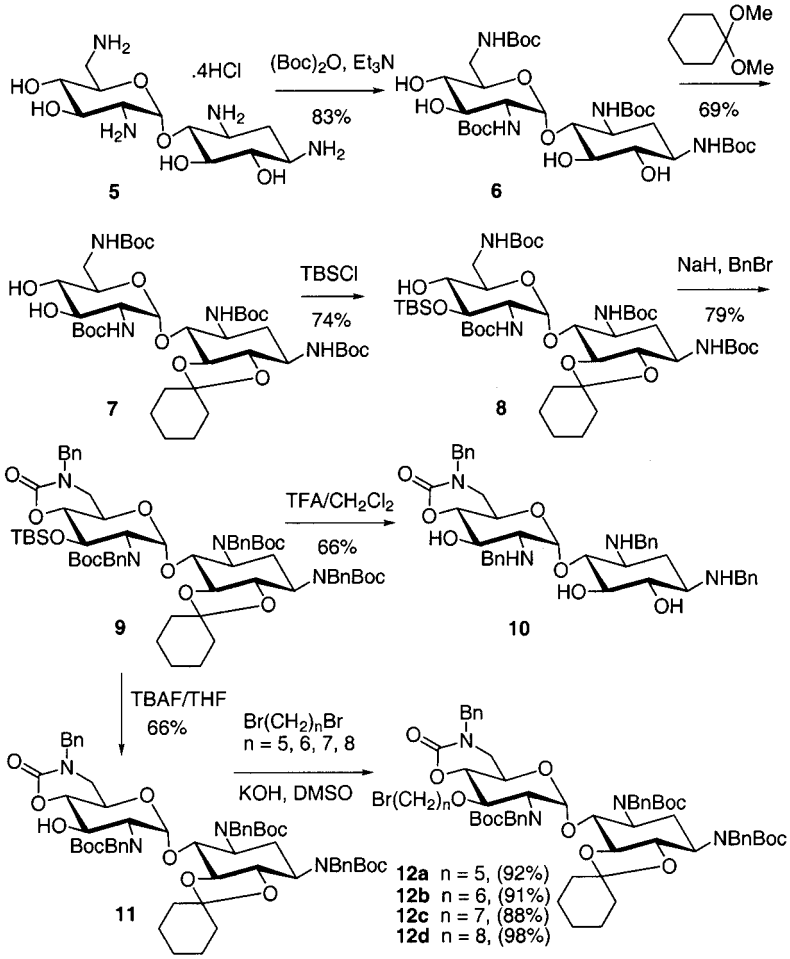
Alternative Route to Compound 14a. To a mixture of **12a** (100 mg, 0.08 mmol), N,$^6N^6$-di-benzoyl-2′,3′-*O*-isopropylideneadenosine **13b** (36 mg, 0.07 mmol), and tetrabutylammonium iodide (60 mg, 0.16 mmol) in anhydrous DMSO (4 mL) was added powdered potassium (20 mg, 0.34 mmol). The mixture was vigorously stirred at room temperature under a nitrogen atmosphere for 1 h, quenched with cold water, extracted with CH_2Cl_2 (3 × 10 mL), dried, and concentrated to dryness in vacuo. The resulting residue was subsequently treated with an equal volume of 3 M aqueous sodium hydroxide and 1,4-dioxane (1:1, 2 mL) at 50 °C for 24 h. The mixture was cooled and extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was washed with water and with brine, dried over $MgSO_4$, concentrated, and purified on a column (SiO_2, 50:1 $CHCl_3$/MeOH) to afford **14a** as a white solid (55 mg, 48%), which was identical with the title compound prepared by the alternative procedure (prepared from **12a** and **13a**) in all respects.

Compound 21a. To a mixture of **18a** (1.35 g, 0.96 mmol), N,$^6N^6$-dibenzoyl-2′,3′-*O*-isopropylideneadenosine **13b** (595 mg, 1.16 mmol), and tetrabutylammonium iodide (1.42 g, 3.84 mmol) in anhydrous DMSO (40 mL) was added powdered potassium hydroxide (270 mg, 4.82 mmol). The mixture was vigorously stirred at room temperature under a nitrogen atmosphere for 1 h, quenched with cold water, extracted with CH_2Cl_2 (3 × 50 mL), dried, and concentrated in vacuo to give a residue, which was purified by column chromatography (SiO_2, 70:1 $CHCl_3$/MeOH) to give the desired compound **21a** (801 mg, 48%) as a white solid: R_f 0.44 (50:1 $CHCl_3$/MeOH); mp 99−100 °C; IR (film) 3303, 2932, 1694, 1163 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 400 MHz) δ 8.69 (s, 1 H), 8.49 (s, 1 H), 8.15 (d, 2 H, J = 7.6 Hz), 7.65 (t, 1 H, J = 7.2 Hz), 7.56 (t, 2 H, J = 7.6 Hz), 7.47−7.20 (m, 25 H), 6.35 (d, 1 H, J = 2.0 Hz), 5.50 (m, 2 H), 5.10 (d, 1 H, J = 3.6 Hz), 4.94−3.00 (m, 29 H), 1.67−1.20 (m, 59 H); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ 166.1, 157.7, 156.6, 156.0, 153.0, 152.9, 151.4, 142.7, 139.0, 135.2, 133.4, 129.6−126.1, 114.5, 112.5, 96.5, 92.1, 87.2, 85.8, 83.2, 82.1, 81.2, 80.5, 80.4, 79.6, 78.6, 77.2, 75.6, 73.2, 72.0, 71.8, 62.6, 60.2, 58.7, 55.5, 50.5, 48.2, 47.6, 42.9, 37.4, 37.3, 33.9, 32.9, 31.6, 31.4, 30.4, 29.0, 28.8, 28.7, 27.8, 25.9, 24.9, 24.8, 23.5; MS (FAB, NBA) for $C_{98}H_{125}N_9O_{19}$ (M^+) 1733, (M + Na^+) 1756.

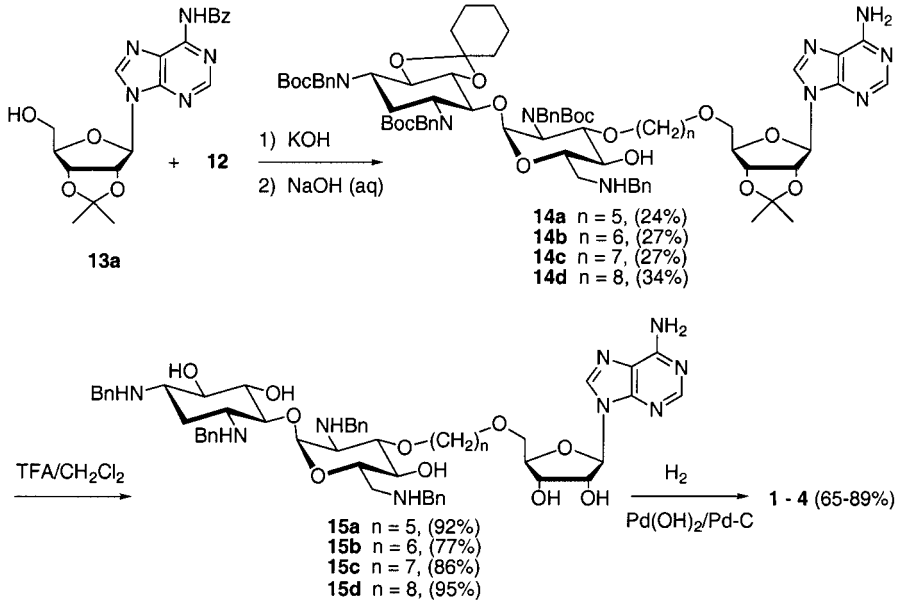
Compound 21b. The title compound was prepared as described for **21a** from compound **18b** (927 mg, 0.65 mmol) to give compound **21b** (736 mg, 64%): R_f 0.46 (50:1 $CHCl_3$/MeOH); mp 93−95 °C; IR (film) 3305, 2934, 1695, 1164 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 500 MHz) δ 8.67 (s, 1 H), 8.60 (s, 1 H), 8.15 (d, 2 H, J = 8.0 Hz), 7.60 (t, 1 H, J = 7.5 Hz), 7.51 (t, 2 H, J = 7.5 Hz), 7.47−7.10 (m, 25 H), 6.35 (d, 1 H, J = 2.0 Hz), 5.48 (m, 2 H), 5.09 (m, 1 H), 4.91−3.00 (m, 29 H), 1.65−1.19 (m, 61 H); ^{13}C NMR (CD_3COCD_3, 125 MHz) δ 166.1, 157.3, 156.3, 155.7, 152.6, 152.5, 151.0, 143.3, 142.4, 142.2, 140.3, 139.7, 134.8, 133.2, 129.3−125.5, 114.2, 112.2, 96.2, 91.6, 86.8, 85.5, 82.8, 81.72, 80.8, 80.2, 80.1, 79.8, 78.2, 76.8, 75.4, 75.2, 73.1, 72.8, 71.9, 71.5, 62.3, 60.5, 59.9, 58.2, 55.1, 50.2, 47.3, 42.9, 36.9, 33.5, 32.5, 31.6, 31.3, 31.4, 28.7, 28.6, 28.4, 27.6, 26.6, 26.5, 26.2, 25.7, 24.4; MS (FAB, NBA) for $C_{99}H_{127}N_9O_{19}$ (M + Na^+) 1756.

Compound 22a. To a solution of **21a** (801 mg, 0.46 mmol) in dry MeOH was added potassium carbonate (200 mg, 1.44 mmol), and the resulting mixture was stirred at room temperature under a nitrogen atmosphere for 6 h. The mixture was filtered and concentrated to dryness in vacuo. The residue was dissolved in CH_2Cl_2, extracted with water (10 mL) and then with brine, dried (Na_2SO_4), and concentrated to give a crude product, which was subsequently treated with trifluoroacetic acid (10 mL) in a mixture of CH_2Cl_2 (10 mL) and water (0.5 mL). The reaction mixture was allowed to stir at room temperature for 1 h. The mixture was concentrated to dryness in vacuo and purified on a column (SiO_2, 20:1:0.1 $CHCl_3$/MeOH/NH_4OH) to afford compound **22a** (270 mg, 53%) as a white powder: R_f 0.25 (20:1:0.1 $CHCl_3$/MeOH/NH_4OH); mp 75−77 °C; IR(KBr) 3323, 2926, 2861, 1641, 1601, 1072 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) δ 8.36 (s, 1 H), 8.17 (s, 1 H), 7.34−7.10 (m, 25 H), 6.03 (d, 1 H, J = 4.2 Hz), 4.98 (d, 1 H, J = 3.6 Hz), 4.55 (d, 1 H, J = 11.4 Hz), 4.49 (t, 1 H, J = 4.8 Hz), 4.32 (t, 1 H, J = 4.8 Hz), 4.17 (m, 1 H), 3.95−3.32 (m, 23 H), 2.63−2.34 (m, 4 H), 1.70−1.61 (m, 4 H), 1.43 (m, 2 H); ^{13}C NMR (CD_3OD, 75 MHz) δ 152.3, 139.3, 139.1, 138.0, 128.1−127.8 (m), 127.3, 127.2, 126.9, 126.8, 126.7, 126.6, 118.8, 101.0, 88.5, 87.6, 83.7, 81.6, 79.8, 76.4, 74.9, 74.1, 72.8, 71.4, 70.9, 70.2, 69.6, 60.6, 56.1, 54.6, 53.2, 52.3, 50.2, 49.8, 30.0, 29.7, 29.2, 22.7; MS (FAB, NBA) for $C_{62}H_{77}N_9O_{10}$ (M^+), (M + Na^+) 1131.

Scheme 1

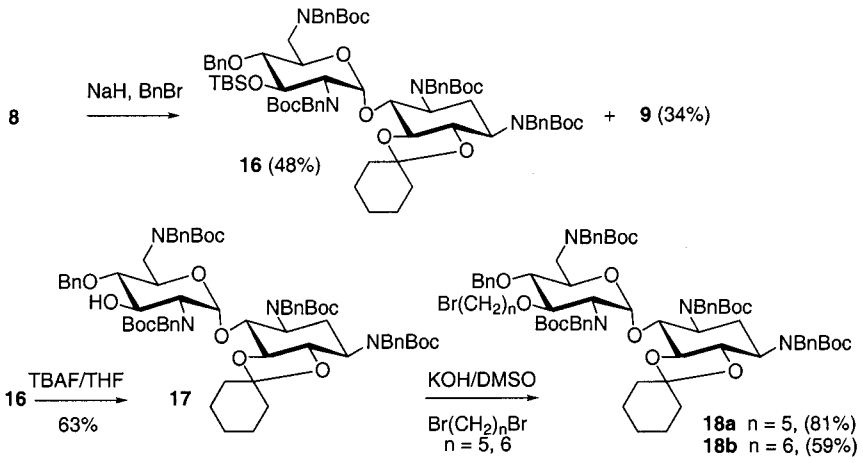


Scheme 2



Compound 22b. The title compound was prepared as described for **22a** from compound **21b** (736 mg, 0.42 mmol) to give the desired compound **22b** (261 mg, 55%) as a white powder: R_f 0.28 (20:1:0.1 $CHCl_3$/MeOH/NH_4OH); mp 76−78 °C; IR(KBr) 3322, 2926, 2860, 1642, 1602, 1071 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 8.36 (s, 1 H), 8.18 (s, 1 H), 7.35−7.12 (m, 25 H), 6.07 (d, 1 H, J = 4.5 Hz), 5.10 (d, 1 H, J = 3.5 Hz), 4.54 (d, 1 H, J = 11.0 Hz), 4.53 (t, 1 H, J = 4.5 Hz), 4.34 (t, 1 H, J = 4.5 Hz), 4.20 (m, 1 H), 3.96−3.29 (m, 23 H), 2.65−2.36 (m, 4 H), 1.54 (m, 4 H), 1.33 (m, 4 H); ^{13}C NMR (CD_3OD, 500 MHz) δ 156.5, 153.0, 149.8, 140.6, 140.0, 139.8, 139.1, 139.0, 138.8, 129.2−128.7 (m), 128.2, 128.1, 127.9, 127.8, 127.8, 127.6, 119.7, 101.2, 89.4, 87.4, 84.6, 82.2, 80.6, 77.2, 75.9, 75.8, 75.5, 74.9, 73.6, 71.9, 71.8, 71.2, 70.5, 61.43, 57.0, 55.6, 53.8, 53.0, 50.8, 50.7, 30.9, 30.4, 30.0, 26.6, 26.5; MS (FAB, NBA) for $C_{63}H_{79}N_9O_{10}$ (M^+) 1122, ($M + Na^+$) 1145.

Scheme 3



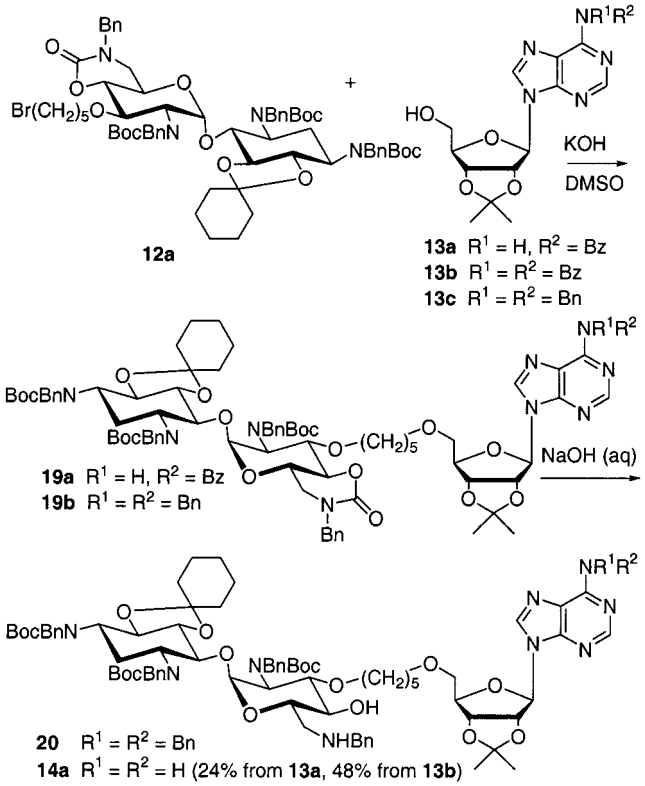
Results and Discussion

We envisioned that the 5′-hydroxyl of adenosine could be tethered to the 3′-hydroxyl of neamine, to bridge the gap between the subsites where the two substrates bind. The closer the length of the tether comes to the distance of the separation between the two subsites, the better should be the affinity for the conjoint molecule with the given tether. If the length of the tether is either shorter or longer than optimal, the enzyme affinity for the compound should decrease. In principle, the binding of one substrate (i.e., one-half of the molecule) drives the binding of the other for entropic reasons. Therefore, when and if the length of the given tether might match the separation of the gap between the two subsites, there exists the potential for strong inhibition of the enzyme, despite the fact that most tethers cannot be expected to be a close structural mimic of the triphosphoryl moiety of ATP and would not have the specific interactions in existence between APH(3′) and ATP.

We have used molecular modeling to investigate what the proper length of the tether should be for a hypothetical phosphotransferase reaction. This analysis takes into account the design criterion for the inhibitors in terms of the tether length, but no assumption regarding conformations is made, because it is not necessary to do so. We constructed molecular models of various lengths of carbon chains to span such distances. We also envisioned that the attachement of the tether to the aminoglycoside and adenosine should be via ether linkages, which are stable in acqueous solution. Compounds **1**−**4** were four such inhibitors of our design, which were synthesized by two routes according to the following procedures given in Schemes 1−5.

Synthetic Route A. Neamine hydrochloride (**5**) was prepared from methanolysis of the commercially available neomycin sulfate, as reported earlier.[17,18,19] Treatment of this compound with di-*tert*-butyl dicarbonate afforded the tetra-*N*-Boc protected derivative **6**. Selective protection of C-5 and C-6 hydroxyl groups via treatment by 1,1-dimethoxycyclohexane[20] resulted in the formation of the 5,6-cyclohexylidene derivative **7**. The 3′-hydroxyl group of **7** was selectively protected with *tert*-butyldimethylsilyl (TBS) group to yield the silyl derivative **8**. Several attempts to protect the C-4′-hydroxyl group of **8** by an ether linkage failed to give any acceptable result. However, treatment of **8** with excess of benzyl bromide and sodium hydride produced the cyclic carbamate derivative **9**. This compound was treated with trifluoroacetic acid to remove the cyclohexylidene and the Boc groups to afford **10**, which proved to be useful in assignment of the sites of modifications by NMR. Removal of the silyl group of **9** with tetrabutylammonium fluoride provided the intermediate **11** having the 3′ hydroxyl group free for further structural manipulation. The reaction of this compound with the corresponding dibromo compounds $Br(CH_2)_nBr$ (n = 5−8), in the presence of powdered potassium hydroxide by application of the modified William's protocol,[21] afforded in high yields the 3′-*O*-bromoalkyl derivatives **12a**−**d**, respectively

Scheme 4



(17) Dutcher, J.; Donin, M. *J. Am. Chem. Soc.* **1952**, *74*, 3420−3422.
(18) Ford, J. H.; Bergy, M. E.; Brooks, A. A.; Garrett, E. R.; Alberti, J.; Dryer, J. R.; Carter, H. E. *J. Am. Chem. Soc.* **1955**, *77*, 5311−5314.
(19) Grapsas, I.; Cho, Y. I.; Mobashery, S. *J. Org. Chem.* **1994**, *59*, 1918−1922.
(20) Tohma, S.; Yoneta, T.; Fukatsu, S. *J. Antibiot.* **1980**, *33*, 671−673.

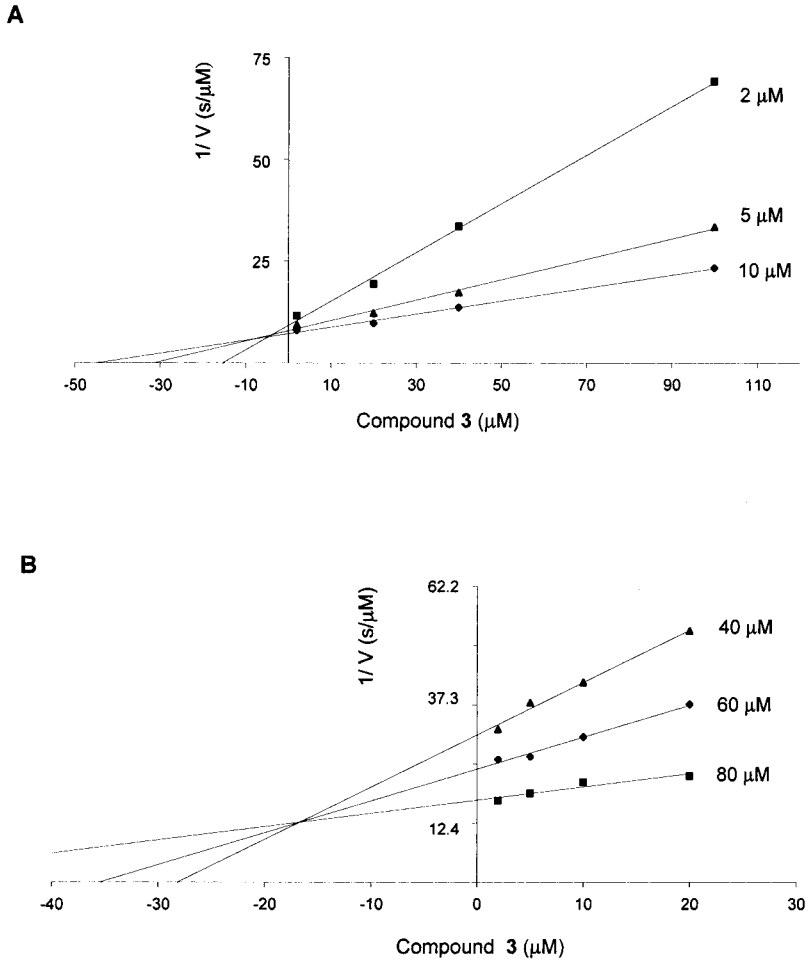
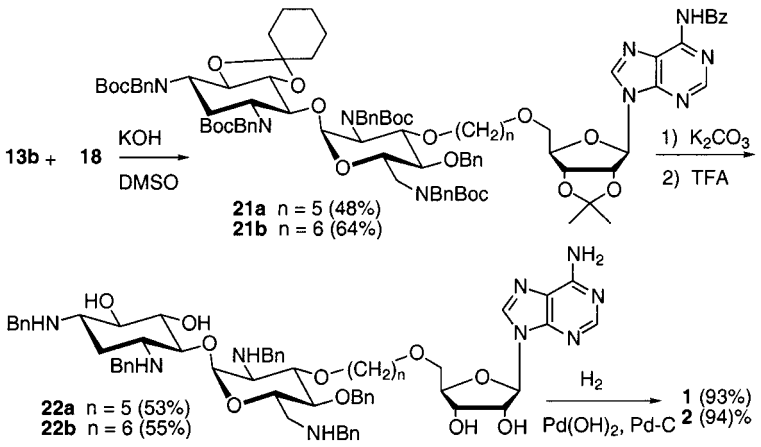


Figure 3. Dixon plots showing competitive inhibition of APH(3′)-IIa by compound **3** against substrates ATP (panel A) and kanamycin A (panel B). Substrate concentrations are indicated to the right for each set of determinations.

Scheme 5



(Scheme 1). We encountered many difficulties in coupling of compounds **12** with the adenosine derivative **13a** (prepared by literature procedures).[22,23] We solved this problem by the use of excess tetrabutylammonium iodide as a promoter. Because of the tedious separation of the coupling products, the subsequent basic hydrolysis with aqueous sodium hydroxide[24,25] was performed to give the partially deprotected compounds **14a**−**d**, which were purified by silica gel chromatography. The acid-labile protective groups, *tert*-butoxycarbonyl, cyclohexylidene, and isopropylidene groups, were simultaneously removed by treatment of **14** with 95% trifluoroacetic acid to give the tetra-*N*-benzyl derivatives **15a**−**d**, respectively. Finally, hydrogenolysis of compounds **15** over palla-

(21) Johnstone, R. A. W.; Rose M. E. *Tetrahedron* **1979**, *35*, 2169−2173.

(22) Maguire A. R.; Meng W.-D.; Robert S. M.; Willetts A. *J. Chem. Soc., Perkin Trans. 1* **1993**, *15*, 1795−1808.

(23) Vrudhula, V. M.; Kappler, F.; Afshar, C.; Gnell, S. L.; Lessinger, L.; Hampton, A. *J. Med. Chem.* **1989**, *32*, 885−890.

(24) Takahashi, Y.; Tsuneda, S.; Tsuchiya, T.; Koyama, Y.; Umezawa, S. *Carbohydr. Res.* **1992**, *232*, 89−105.

(25) Kobayashi, Y.; Tsuchiya, T.; Umezawa, S.; Yoneta, T.; Fukatsu, S.; Umezawa, H. *Bull. Chem. Soc. Jpn.* **1987**, *62*, 713−720.

Table 1. Kinetic Parameters for Inhibition of APH(3′)-Ia and APH(3′)-IIa by Compounds 1−4[a]

inhibitor	variable substrate	K_i (μM) APH(3′)-Ia	K_i (μM) APH(3′)-IIa
1	ATP	558 ± 65	10 ± 4
	kanamycin A	32 ± 5	18 ± 11
2	ATP	10 ± 8	3 ± 2
	kanamycin A	3 ± 2	5 ± 3
3	ATP	22 ± 14	6 ± 5
	kanamycin A	9 ± 1	17 ± 4
4	ATP	35 ± 26	9 ± 5
	kanamycin A	224 ± 61	14 ± 2

[a] The K_m for kanamycin A and ATP for APH(3′)-Ia are 2.8 ± 0.3 μM and 77 ± 4 μM, respectively. The K_m for kanamycin A and ATP for APH(3′)-IIa are 3.1 ± 0.5 μM and 69 ± 13 μM, respectively.

dium−C and palladium hydroxide−C in methanol and acetic acid[26,27] afforded the desired compounds **1**−**4** in good to high yields (Scheme 2).

Synthetic Route B. One of the key steps in the synthesis of compounds **1**−**4** is the coupling reaction of the appropriately protected adenosine derivative and its aminoglycoside counterpart. During the course of synthesis of these compounds we found that the *N*,*N*-dibenzoyl-protected adenosine derivative (**13b**) and the bromoalkylated neamines (**18**) are the best building blocks for synthesis of these conjount molecules in good yields. Therefore a slightly modified procedure was employed to prepare the neamine derivative **16**. To make this compound, benzyl bromide was added to a solution of neamine derivative **8** and NaH in one portion, and the reaction was stopped after 2 h (in contrast to the first synthetic route in which benzyl bromide was added dropwise over 30 min). Products of this reaction were compounds **9** and **16** in 34% and 48% yields, respectively (Scheme 3). Desilylation of **16** with tetrabutylammonium fluoride gave the key intermediate **17**, which was bromoalkylated with dibromoalkanes (n = 5 and 6) in the presence of powdered potassium hydroxide to give 3-*O*-bromoalkyl derivatives **18a** and **18b** in good yields. In the most difficult step of the synthesis, which was the coupling of **18** with the adenosine moiety, the major byproduct of the reaction was elimination of hydrogen bromide and formation of 3′-*O*-(4”-alkenyl) neamine derivatives. To find the optimal condition for the coupling reaction, three adenosine derivatives **13a**−**c** having various protecting groups on the amino group at the C4 position were prepared[22,23,28] and used in the above reaction (Scheme 4). Reaction of **12a** with either **13a** or **13b** in the presence of tetrabutylammonium iodide afforded adduct **19a**, which was subsequently treated with aqueous sodium hydroxide to give the benzoyl-deprotected derivative **14a**. The overall yield for the two-step for these reactions were 24% from **13a** and 48% from **13b**, respectively. Treatment of **12a** with **13c** furnished the tethered compound **19b** in 36% yield. Unfortunately **19b** did not undergo a facile and complete 4-*N*-benzyl group deprotection to give the desired compound **1**.

These findings showed that the *N*,*N*-dibenzoyl-protected adenosyl derivative **13b** is the proper coupling counterpart for the reaction with the neamine derivatives. Therefore, this compound was chosen to undergo reaction with **18a** and **18b** to provide the N^6-benzoyl protected adenosyl derivatives **21a** and **21b** in 48% and 64% yields, respectively (Scheme 5). Subsequent benzoyl group deprotection of these compounds with potassium carbonate in anhydrous methanol, followed by treatment with trifluoroacetic acid furnished the tetra-*N*-benzyl-protected derivatives **22a** and **22b**, in good yields. Finally, hydrogenolysis of these compounds in the presence of the cocatalysts, palladium and palladium hydroxide on carbon, gave the desired compounds **1** and **2** in excellent yields (Scheme 5).

Compounds **1**−**4** were tested with APH(3′)-Ia and APH(3′)-IIa as potential inhibitors. All four compounds were competitive inhibitors for the enzyme. Regardless of which substrate (kanamycin A or ATP) was varied in the kinetics experiments, in general, the best inhibition was afforded by compounds **2** and **3** (Table 1; Figure 3). Furthermore, the K_i values measured for **2** and **3** were relatively within a few folds of each other.

The tethers in compounds **1**−**4** are straight chain hydrocarbons. We note that the X-ray structure for the complex of APH(3′)-IIIa with Mg^{2+}-ADP indicated approximately a half-dozen electrostatic interactions by the enzyme with the magnesium-diphosphoryl moiety of ADP (Figure 1). These interactions would obviously be absent in a simple hydrocarbon tether in interactions of compound **1**−**4** with the enzyme. These observations in part explain why the affinities of the enzymes for our inhibitors are only slightly better than for the substrates themselves, despite the favorable entropic factor for binding to molecules such as **1**−**4**. Nonetheless, these compounds are the best known inhibitors for APH(3′)s to date, which occupy both subsites within the active site. These compounds were investigated for slow binding to APH(3′)s, both with and without preincubation of the inhibitors. They all served as linear competitive inhibitors with no evidence (data not shown) for slow binding.

Availability of these compounds allows us to attempt to study by X-ray analysis the complexes of the APH(3′)s with these inhibitors. This effort should generate a structural basis for the design of the next generation of these molecules that should enjoy more favorable inhibition of these important bacterial enzymes. These efforts are currently under way.

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Note Added in Proof: While this manuscript was in review, a report that addressed inhibition of aminoglycoside-modifying enzymes appeared in the literature (Sucheck, S. J.; Wang, A. L.; Koeller, K. M.; Boehr, D. D.; Draker, K.; Sears, P.; Wright, G. D.; Wang, C. H. *J. Am. Chem. Soc.* **2000**, *122*, 5230−5231).

JO000589K

(26) Pearlman, W. M. *Tetrahedron. Lett.* **1967**, 1663−1664.
(27) Kozikowski, A. P.; Xia, Y.; Reddy, E. R.; Tückmantel, W.; Hanin, I.; Tang, X. C. *J. Org. Chem.* **1991**, *56*, 4636−4645.
(28) (a) Kato T.; Arakawa E.; Ogawa S.; Suzumura Y. *Chem. Pharm. Bull.* **1986**, *34*, 3635−3643. (b) Takeshi E.; Zemlicka J. *J. Org. Chem.* **1979**, *44*, 3652−3656.