Further Study toward Amipurimycin: Synthesis of the Northern Part

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Complex nucleoside antibiotics are challenging natural compounds for organic chemists because they combine the structural feature of nucleosides, higher monosaccharides, branched monosaccharides, disaccharides, and peptides.[1] They exhibit a variety of biological activities such as antifungal, herbicidal, insecticidal, antiviral, and antitumoral.

Amipurimycin (**1**) is a representative of this class of compounds, which was isolated from *Streptomyces novoguineensis* and which is strongly active against *Pyricularia Oryzae*, responsible for the rice blast disease.[2,3] Although the absolute configurations of C-6′ and of the *cis*-cyclopentylamino acid were not determined, the following structure was proposed for **1** by Goto *et al.*[4] (Chart 1).

The synthesis of this molecule would imply the formations of bonds (a) and (b) on a suitably protected and activated central glycosidic moiety **3** (Chart 1).

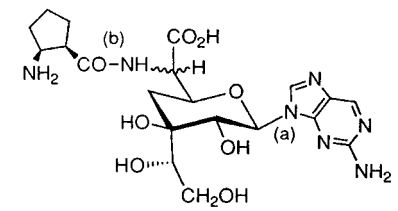
The success of this endeavor would be highly dependent on the choice of the following: (i) protecting groups compatible with the conditions of formation of bonds (a) and (b); (ii) conditions of *N*-glycosylation affording the correct regioselectivity on the 2-aminopurine base in the presence of desactivating functionalities (N*P* and COOMe) on the glycosyl donor, (iii) a correct order for these transformations,[1] and (iv) possibility of final deprotection.[1]

As parts of a program devoted to the synthesis of **1**, we have already studied the stereocontrolled transformation of the primary alcohol into the α-amino acid[5] and the construction of the hydroxylated chain at C-3[6] on methyl 4-deoxy-α-D-*xylo*-hexopyranoside derivatives.

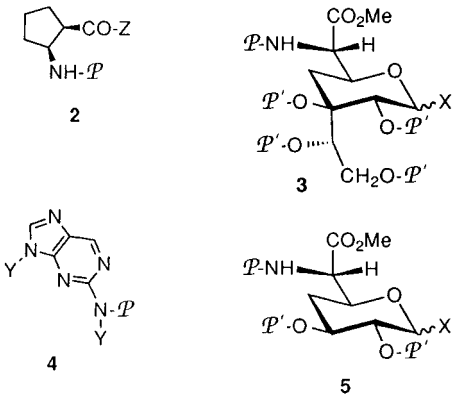
Before undertaking the synthesis of the glycosyl donor **3**, answers to the above-mentioned questions were needed, and we decided to evaluate our strategy on a simplified synthon such as **5** (Chart 1). In order to avoid the presence of several Lewis bases (amide functions) during the glycosylation step, we decided to establish bond (a) before bond (b).

We report here the synthesis of a model for the glycosyl donor **3** and a successful glycosylation of protected 2-aminopurine (a) followed by formation a peptidic linkage (b) leading to the northern part of amipurimycin (**14**) after deprotection.[7]

Chart 1



Amipurimycin **1**



$\mathcal{P}$, $\mathcal{P}'$: protecting groups

X: activating group for the glycosyl donor

Y: activating group for the base

Z: activating group for the amino-acid

In our previous work,[5] compound **6** was obtained efficiently by stereocontrolled ethynylation of methyl 2,3-di-*O*-benzyl-4-deoxy-α-D-*xylo*-hexadialdo-1,5-pyranoside[8] followed by azidation. For convenience and efficiency, we decided to try to preserve the azido group along the whole sequence of reactions and introduce the *cis*-cyclopentylamino acid at the end of the synthesis.

Because they are not compatible with strongly acidic conditions required for *N*-glycosylation of a heterocyclic base, the benzyl ethers present in **6** have to be replaced by ester groups. Furthermore, a participating group at C-2 is necessary to control the formation of the desired β-anomer. Since, in our previous work,[5] the oxidative cleavage of the triple bond of **6** to a carboxylic ester was not very efficient,[9] the benzyl ethers of **6** were replaced before oxidation. Hydrogenolysis was not possible in the presence of a triple bond and an azido group, so they were cleaved by boron trichloride at low temperature.[11] The glycosidic bond was also cleaved, and acetylation of the resulting mixture under acidic conditions afforded triacetates **7a,b** in 81% yield (Scheme 1). An α/β mixture (70:30) was obtained as indicated by ^{1}H-NMR spectroscopy ($\delta = 6.3$ ppm, $J = 3.5$ Hz, H-1$_\alpha$, 70%; $\delta = 5.55$ ppm, $J = 8.0$ Hz, H-1$_\beta$, 30%) and could be employed for further transformation without purification.

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(5) Czernecki, S.; Valéry, J. M.; Wilkens, R. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 1.
(6) Rauter, A. P.; Fernandes, A. C.; Czernecki, S.; Valéry, J.-M. *J. Org. Chem.* **1996**, *61*, 3594.
(7) This work was communicated (in part) at the 211th National Meeting of the American Chemical Society, New Orleans, LA, Mar 24−29, 1996.
(8) Czernecki, S.; Horns, S.; Valéry, J.-M. *J. Carbohydr. Chem.* **1995**, *14*, 157.
(9) The best reagent[10] ($RuCl_3$ under basic conditions) for the oxidative cleavage of the triple bond cannot be employed in the presence of benzyl ethers.
(10) Carlsen, H.; Katsuki, T.; Martin, U. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936.
(11) (a) Seela, F.; Markhoff, S. *Liebigs Ann. Chem.* **1982**, 813. (b) Perdomo, G. A.; Krepinsky, J. J. *Tetrahedron Lett.* **1987**, *28*, 5595.

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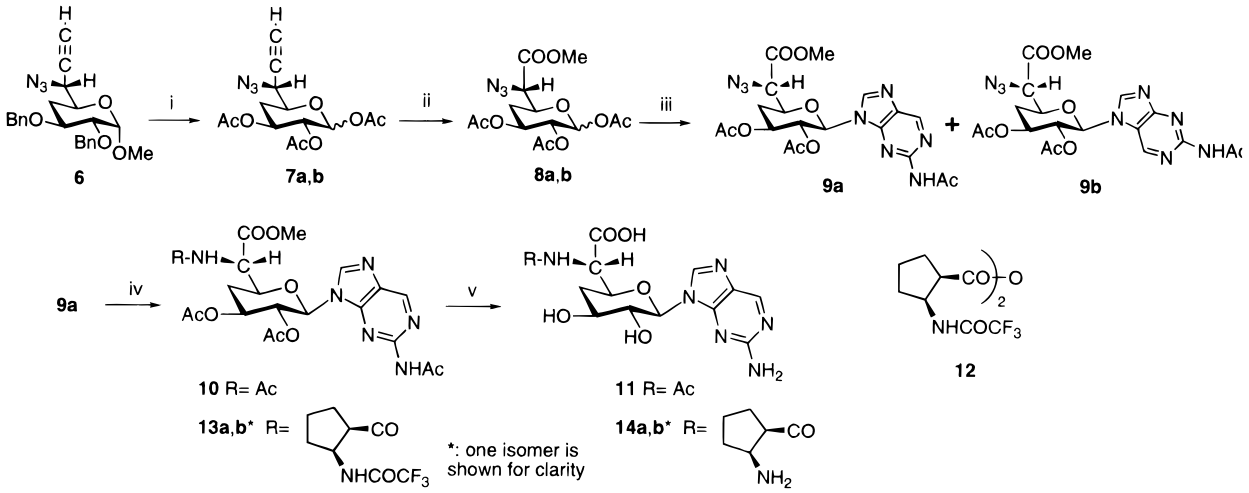
Scheme 1[a]



[a] Reagents and conditions: (i) BCl_3, CH_2Cl_2, −78 °C then Ac_2O, H_2SO_4, AcOH; (ii) $RuCl_3$, $NaIO_4$, $CCl_4/CH_3CN/H_2O$ then CH_2N_2, Et_2O; (iii) bis(trimethylsilyl) derivative of 2-(*N*-acetylamino)purine, $SnCl_4$, $ClCH_2CH_2Cl$, 135 °C; (iv) H_2, Raney Ni, Ac_2O or **12**, THF; (v) LiOH, THF/H_2O, 0 °C.

Oxidative cleavage of the triple bond of **7a,b** with a catalytic amount of $RuCl_3$ (20%) in the presence of $NaIO_4$[10] proceeded smoothly. The formed carboxylic acids were not isolated but directly transformed into their methyl ester with diazomethane. Compounds **8a,b** were obtained in 75% yield after flash chromatography. The ^{1}H-NMR spectrum of the crude product indicated the presence of α and β anomers and good purity suitable for *N*-glycosylation of 2-aminopurine.

For the protection of NH_2 groups of the amino acid and of 2-aminopurine, protecting groups removable without damage to the peptidic linkage (b) were chosen as trifluoroacetate and acetate, respectively.

2-(*N*-acetylamino)purine[12] was activated by bis-trimethylsilylation[13] and directly reacted with **8a,b** in 1,2-dichloroethane in the presence of tin tetrachloride. Two nucleosides were formed (84%) and separated by chromatography (**9a**, 77%, and **9b**, 8%). In both of them, the glycosidic bond was β as indicated by the chemical shift and the high coupling constant of H-1′ (δ = 5.7 ppm, J = 9.4 Hz).

The sugar position on the base was determined by a nuclear Overhauser effect between protons of the base and of the sugar. For the major isomer **9a**, no NOE was detected between H-6 and any proton of the sugar ring, indicating *N*-glycosylation at N-9. For the minor one **9b**, a NOE was observed between H-6 and H-1′. This result is in agreement with previous work with simple glucopyranose derivatives.[13]

Establishement of the peptidic bond (b) was done under mild and neutral conditions. It was first verified that the *N*-acetyl derivative **10** could be obtained by reduction of the azido group in the presence of acetic anhydride (71%) and deprotected (LiOH, H_2O) to afford **11** after neutralization with dilute HCl.

Since the absolute configuration of the cyclic amino acid present in **1** is not known, racemic *cis*-2-aminocyclopentanecarboxylic acid[14] was employed to prepare the trifluoroacetamido anhydride **12**.

A mixture (1:1) of the two diastereoisomers **13a,b** was obtained (61%). The final deprotection proceeded smoothly as described for **10** to afford the northern part of amipurimycin (**14a,b**).

Experimental Section

Melting points were measured with a Thomas-Hoover apparatus and are uncorrected. IR spectra were recorded with a Unicam spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at 250.13 and 62.89 MHz, respectively, on a Brucker ARX 250 spectrometer in $CDCl_3$ with TMS as internal standard unless otherwise noticed. Optical rotations were measured on a Perkin-Elmer 141 polarimeter in a 10 cm cell at 22 °C. Analytical TLC was performed on Merck aluminum precoated plates of silica gel 60 F-254 with detection by UV and by spraying with 6 N H_2SO_4 and heating about 2 min at 300 °C. Evaporation of solvents was carried out under reduced pressure at 40 °C. Merck silica gel 60 (230−400 Mesh) and anhydrous solvents were employed for flash chromatography. Elemental analyses were performed at the Service de microanalyse of Pierre et Marie Curie University.

1,2,3-Tri-*O*-acetyl-6-azido-4,6,7,8-tetradeoxy-D-*gluco*-oct-7-ynopyranose (7a,b). A solution of compound **6**[5] (1.26 g, 3.1 mmol) in dry CH_2Cl_2 (50 mL) was cooled to −78 °C. Boron trichloride (23 mL of a molar solution in CH_2Cl_2, 7.5 equiv) was added drop by drop under stirring. The temperature of the reaction mixture was allowed to rise to −50 °C (3 h were necessary), and the solvent was evaporated under reduced pressure. To the gummy residue was added a mixture of Ac_2O/$AcOH/H_2SO_4$ (17.8 mL, 7/3/1 v/v) at 0 °C, and stirring was maintained overnight at rt. The reaction mixture was then partitioned between CH_2Cl_2 (50 mL) and water (50 mL). The aqueous phase was repeatedly extracted with CH_2Cl_2 (3 × 30 mL), and the combined organic layers were washed successively with 5% aqueous $NaHCO_3$ (2 × 45 mL) and water (2 × 30 mL) and dried ($MgSO_4$). Evaporation of the solvent under reduced pressure afforded a residue that was purified by flash chromatography (hexane−ether, 6:4) to yield **7a,b** (mixture of anomers) as a foamy solid (843 mg, 81%). An analytical sample of the major component (α-anomer) was obtained from the appropriate fractions: mp 132−133 °C dec; $[\alpha]^{20}_D$ + 46.5° (*c* 1.02, $CHCl_3$); ^{1}H NMR δ 1.75 (q, 1H, J = 12 Hz), 1.95, 2.0, and 2.1 (3s, 3 × 3H), 2.3 (ddd, 1H, J = 12.8, 5.1, and 2.1 Hz), 2.55 (d, 1H, J = 2.3 Hz), 3.95−4.2 (m, 2H), 5.0 (dd, 1H, J = 10.2, 3.5 Hz), 5.25 (dt, 1H, J = 11.6, 5.1 Hz), 6.3 (d, 1H, J = 3.5 Hz); ^{13}C NMR δ 20.5, 20.8, 20.9, 30.9, 54.8, 66.9, 69.9, 71.3, 75.1, 77.5, 90.0, 168.8, 170.3. Anal. Calcd for $C_{14}H_{17}N_3O_7$: C, 49.56; H, 5.05; N 12.38. Found: C, 49.38; H, 5.12; N, 12.39.

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1,2,3-Tri-*O*-acetyl-6-azido-4,6-dideoxy-D-*gluco*-heptopyranosiduronic Acid Methyl Esters (8a,b). To a solution of **7a,b** (685 mg, 2 mmol) and $NaIO_4$ (2.78 g, 13 mmol, 6.5 equiv) in a mixture of $CCl_4/CH_3CN/H_2O$ (90 mL, 1/1/1.5 v/v) was added ruthenium trichloride monohydrate (80 mg, 0.35 mmol). After 10 min of stirring at rt, 40% aqueous $NaHSO_3$ (35 mL) was added, and the resulting mixture was extracted successively with CH_2Cl_2 (3 × 25 mL) and AcOEt (2 × 25 mL). The combined organic extracts were dried ($MgSO_4$) and the solvents evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (5.5 mL), and a freshly prepared solution of diazomethane in ether[15] was added drop by drop at 0 °C until a pale yellow color was persistent. After 30 min of strirring at rt, the excess of CH_2N_2 was destroyed by addition of AcOH and the solvent evaporated under reduced pressure. Flash chromatography (hexane−ether, 2:1) afforded **8a,b** as a mixture of anomers (560 mg, 75%). For the major α anomer: ^{1}H NMR δ 1.8 (q, 1H, J = 11.8 Hz), 1.95, 2.0, and 2.1 (3s, 3 × 3H), 2.05−2.12 (m, 1H), 3.75 (s, 3H), 4.5 (d, 1H, J = 4.6 Hz), 4.35 (ddd, 1H, J = 12.0, 4.6, and 2.6 Hz), 4.95 (dd, 1H, J = 10.2, 3.6 Hz), 5.25 (ddd, 1H, J = 11.4, 10.2, 5.2 Hz), 6.25 (d, 1H, J = 3.6 Hz); ^{13}C NMR δ 20.4, 20.7, 20.8, 30.7, 53.0, 64.1, 66.7, 69.7, 69.7, 89.8, 167.5, 168.8, 170.1. Anal. Calcd for $C_{14}H_{19}N_3O_9$: C, 45.04; H, 5.13; N, 11.26. Found: C, 45.11; H, 5.11; N, 11.36.

[*N*-9-(2,3-Di-*O*-acetyl-6-azido-4,6-dideoxy-β-D-*gluco*-heptopyranosyl)-2-(*N*-acetylamino)purinyl]uronic Acid Methyl Ester (9a) and Its *N*-7 Isomer (9b). 2-(*N*-acetylamino)-purine[12] (355 mg, 2 mmol) was bis-trimethylsilylated according to ref 13. The crude compound was dissolved into MeCN (3.5 mL), and the resulting solution was added to a mixture of **8a,b** (375 mg, 1 mmol) in 1,2-dichloroethane (10 mL) under argon. Tin tetrachloride (2.3 mL of a molar solution in CH_2Cl_2) was added, and the reaction mixture was heated at 135 °C (bath temperature) during 1 h and then allowed to cool at rt. The solution was treated with 5% aqueous $NaHCO_3$ (15 mL) and the aqueous phase extracted with CH_2Cl_2 (5 × 5 mL). The combined organic layers were dried ($MgSO_4$), and the solvent was evaporated under reduced pressure to afford a syrupy residue from which TLC analysis (AcOEt−MeOH, 9:1) revealed two spots. Flash chromatography (Et_2O−MeOH, 10:1) afforded compound **9a** (380 mg, 77.5%) as the faster moving product: $[\alpha]^{20}_D$ + 14° (*c* 0.64, $CHCl_3$); ^{1}H NMR δ 1.7 (s, 3H), 2.00 (q + s, 1H + 3H, J = 12.9 Hz), 2.20 (ddd, 1H, J = 12.9, 5.3, 1.8 Hz), 2.55 (s, 3H), 3.75 (s, 3 H), 4.2−4.3 (m, 2H), 5.2 (ddd, 1H, J = 11.4, 9.4, 5.3 Hz), 5.5 (t, 1H, J = 9.4 Hz), 5.7 (d, 1H, J = 9.4 Hz), 8.1 (s, 1H), 8.85 (bs, 1H), 8.9 (s, 1H); ^{13}C NMR δ 20.1, 20.1, 25.2, 29.6, 53.1, 63.9, 70.1, 70.6, 74.4, 80.7, 130.2, 142.3, 150.1, 152.1, 153.4, 167.0, 169.2, 169.9, 171.3. Anal. Calcd for $C_{19}H_{22}N_8O_8$: C, 46.53; H, 4.52; N, 22.85. Found: C, 46.70; H, 4.70; N, 22.86. The slower moving compound **9b** (39 mg, 8%) was obtained as an oil: $[\alpha]^{20}_D$ −5.7° (*c* 0.7, $CHCl_3$); ^{1}H NMR δ 1.85 and 2.05 (2s, 2 × 3H), 2.1 (m, 1H), 2.3 (ddd, 1H, J = 12.7, 4.7, 2.0 Hz), 2.6 (s, 3H), 3.8 (s, 3H), 4.3 (m, 2H), 5.25 (m, 2H), 5.5 (d, 1H, J = 8.8 Hz), 8.2 (s, 1H), 8.5 (bs, 1H), 8.95 (s, 1H); ^{13}C NMR δ 20.1, 20.7, 25.2, 30.9, 53.3, 63.9, 69.8, 70.9, 74.6, 84.6, 120.5, 142.6, 146.4, 154.0, 162.2, 167.2, 168.7, 170.0, 170.06. Anal. Calcd for $C_{19}H_{22}N_8O_8$: C, 46.53; H, 4.52; N, 22.85. Found: C, 46.82; H, 4.38; N, 22.91.

[*N*-9-(2,3-Di-*O*-acetyl-6-(*N*-acetylamino)-4,6-dideoxy-β-D-*gluco*-heptopyranosyl)-2-(*N*-acetylamino)purinyl]uronic Acid Methyl Ester (10). Compound **9a** (40 mg, 0.08 mmol) was added to a suspension of Raney Nickel (*ca.* 30 mg previously rinsed successively with EtOH and THF) in dry THF (3 mL) containing Ac_2O (220 μL, 1.7 mmol). The reaction mixture was stirred under H_2 at 1 atm during 1 h. Filtration, evaporation of the solvent under reduced pressure, and flash chromatography (AcOEt−MeOH, 9:1) yielded **10** (30 mg, 70%): mp 147−148 °C; $[\alpha]^{20}_D$ + 18.6° (*c* 0.5, $CHCl_3$); ^{1}H NMR δ 1.7 (s, 3H), 2.0 and 2.05 (2s, 2 × 3H), 1.95−2.1 (m, 1H), 2.45 (ddd, 1H, J = 11.9, 4.8, 1.7 Hz), 2.55 (s, 3H), 3.8 (s, 3H), 4.3−4.35 (m, 1H), 4.95 (dd, J = 7.7, 2.3 Hz), 5.4 (ddd, 1H, J = 11.9, 9.4, 4.8 Hz), 5.6 (t, 1H, J = 9.5 Hz), 5.85 (d, 1H, J = 9.2 Hz), 6.5 (bs, 1H), 8.25 (s, 1H), 8.8 (bs, 1H), 9.1 (s, 1H); ^{13}C NMR δ 20.1, 20.8, 22.7, 29.7, 32.7, 52.6, 54.6, 70.3, 71.1, 76.1, 80.8, 129.8, 142.5, 149.6, 151.3, 153.1, 168.9, 169.1, 169.8, 170.1. Anal. Calcd for $C_{21}H_{26}N_6O_9$: C, 49.80; H, 5.17; N, 16.59. Found: C, 49.75; H, 5.22; N, 17.03.

[*N*-9-[6-(*N*-Acetylamino)-4,6-dideoxy-β-D-*gluco*-heptopyranosyl]-2-aminopurinyl]uronic Acid (11). Compound **10** (20 mg, 39 μmol) was dissolved in a mixture of THF (2.15 mL) and water (0.6 mL). Lithium hydroxide monohydrate (13 mg, 0.31 mmol) was added at 0 °C, and the reaction was stirred for 12 h at rt. After neutralization (10% aqueous HCl) and evaporation under reduced pressure, the residue was purified by flash chromatography ($CHCl_3$−MeOH−H_2O, 5:4:1) to yield **11** (12 mg, 85%) as a white amorphous solid: $[\alpha]^{20}_D$ +27.3° (*c* 0.2, MeOH); ^{1}H NMR δ 2.0 (m, 2H), 2.05 (s, 3H), 3.95 (m, 2H), 4.25 (ddd, 1H, J = 10.1, 4.3, 2.1 Hz), 4.65 (d, 1H, J = 4.3 Hz), 5.55 (d, 1H, J = 8.8 Hz), 8.3 (s, 1H), 8.65 (s, 1H); ^{13}C NMR (D_2O) δ 21.0, 24.2, 48.2, 61.3, 63.6, 65.3, 73.5, 133.3, 140.15, 140.8, 145.1, 148.8, 166.1, 167.5. Anal. Calcd for $C_{14}H_{18}N_6O_6 \cdot 0.5H_2O$: C, 44.80; H, 5.10; N, 23.38. Found: C, 45.02; H, 5.07; N, 23.12.

[*N*-9-[2,3-Di-*O*-acetyl-4,6-dideoxy-6-[*N*-[*cis*-2-[*N*-(trifluoroacetyl)amino]cyclopentyl] carboxamido)-β-D-*gluco*-heptopyranosyl]-2-(*N*-acetylamino)purinyl]uronic Acid Methyl Ester (13a,b). Racemic *cis*-2-[*N*-(trifluoroacetyl)amino]-cyclopentanecarboxylic acid[15] (110 mg, 0.49 mmol) was treated with DCC (50 mg, 0.245 mmol) in THF (3.5 mL) overnight at rt. After filtration and evaporation of the solvent under reduced pressure, crude anhydride **12** was obtained (100 mg) and employed without purification. Reduction of **9a** (218 mg, 0.44 mmol) in the presence of crude **12** as indicated for the preparation of **10** afforded a mixture containing **13** and *cis*-2-[*N*-(trifluoroacetyl)amino]cyclopentanecarboxylic acid. This mixture was dissolved in CH_2Cl_2 (5 mL) and the resulting solution successively washed with 5% aqueous $NaHCO_3$ (3 × 25 mL) and then saturated brine (2mL) and dried ($MgSO_4$). Evaporation of the solvent afforded **13** (180 mg, 61%) as a mixture of diasteroisomers: ^{1}H NMR (55 °C) δ 1.75, 2.05 and 2.5 (3s, 3 × 3H), 1.55−2.05 (m, 8H), 2.2 and 2.9 (2m, 1H), 3.75 (s, 3H), 3.95 and 4.15 (2d, 2H, J = 11.7 Hz), 4.35 (m, 1H), 4.7 (m, 1H), 5.2 (m,1H), 5.4−5.7 (m, 2H), 6.95 (bs, 1H), 7.4 (m, 1H), 8.05 (s, 1H), 8.85 (s, 1H); FAB-MS (3-nitrobenzylic alcohol matrix) 672 (M + 1). Anal. Calcd for $C_{27}H_{32}F_3N_7O_{10}$: C, 48.29; H, 4.80; N, 14.6. Found: C, 48.17; H, 4.73; N, 14.83.

[*N*-9-[4,6-Dideoxy-6-[*N*-(*cis*-2-aminocyclopentyl)carboxamido]-β-D-*gluco*-heptopyranosyl]-2-aminopurinyl]uronic Acid (14a,b). Compound **13** (50 mg, 74 μmol) was deprotected as described for **10**. Purification was performed on Sephadex LH-20 and yielded **14a,b** (19 mg, 60%) as a mixture of diasteroisomers: mp 150−152 °C; $[\alpha]^{20}_D$ + 13.2° (*c* 1.0, H_2O); ^{1}H NMR (D_2O) δ 1.5−2.2 (m, 8H), 3.0 (m, 1H), 3.6 and 3.7 (2m, 1H), 3.95 (m, 1H), 4.1 (pseudo t, 1H, J = 9.1 Hz), 4.3 (m, 1H), 4.5 (d, 1H, J = 4.0 Hz), 5.55 (d, 1H, J = 9.1 Hz), 8.25 and 8.45 (2s, 1H), 8.6 and 8.65 (2s, 1H). Anal. Calcd for $C_{18}H_{25}N_7O_6 \cdot H_2O$: C, 47.68; H, 6.0; N, 21.62. Found: C, 47.99; H, 5.89; N, 21.45.

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(15) *Vogel's Text Book of Practical Organic Chemistry*, 4th ed.; Longman: London 1978; Chapter II, p 291.