Synthesis and Antiviral Activity of the α-Analogues of 1,5-Anhydrohexitol Nucleosides (1,5-Anhydro-2,3-dideoxy-D-ribohexitol Nucleosides)

Nafizal Hossain, Jef Rozenski, Erik De Clercq, and Piet Herdewijn*

Rega Institute for Medical Research, Laboratory of Medicinal Chemistry, Minderbroedersstraat 10, B-3000 Leuven, Belgium

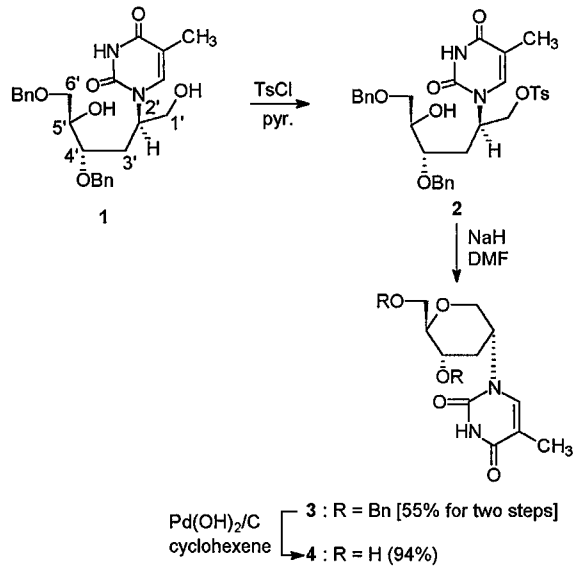
Received October 22, 1996[⊗]

1,5-Anhydro-2,3-dideoxy-D-ribohexitol nucleosides were synthesized starting from 4,6-di-*O*-benzyl-1,5-di-*O*-pivaloyl-3-deoxy-D-glucitol using a ring closure procedure. The target nucleoside adopts a $^4C_1$ conformation as also demonstrated for the corresponding 1,5-anhydrohexitol nucleosides with β-configuration of the base-substituted carbon atom. The cytosine congener demonstrated a moderate but selective activity against Herpes simplex virus types 1 and 2.

Introduction

The research on antiviral nucleosides with a six-membered carbohydrate moiety has been far behind the research on biological active nucleosides with modified five-membered ring structures. This is due to the fact that six-membered rings are conformationally less flexible than five-membered rings, and conformational flexibility of a nucleoside is important for the metabolic activation and for the interaction with the target enzyme. With nonflexible molecules, a perfect steric and electronic fit is necessary between enzyme and substrate, and this is difficult to obtain. Recently, however, a new series of nucleosides with a six-membered carbohydrate moiety has been discovered showing potent antiviral activity.[1] This class of nucleosides also shows an increased RNA duplex stability when used as building blocks for oligonucleotides.[2,3] These nucleosides have a 2,3-dideoxy-1,5-anhydro-D-mannitol moiety with a heterocyclic base situated in the 2β-position. A particular characteristic of these nucleosides is an axial orientation of the base moiety, which is due to the fact that nucleosides with a six-membered carbohydrate moiety prefer a conformation avoiding as much as possible unfavorable 1,3-diaxial interactions. As part of the investigation on the structural requirements of anhydrohexitols for antiviral activity, we synthesized the α-analogues of the aforementioned 2,3-dideoxy-1,5-anhydro-D-mannitol nucleosides starting from either 4,6-dibenzyl-2,3-dideoxy-2-D-mannitylthymine or 4,6-di-*O*-benzyl-1,5-di-*O*-pivaloyl-3-deoxy-D-glucitol. These nucleosides adopt a $^4C_1$ conformation, and the cytosine congener demonstrates activity against Herpes simplex virus types 1 and 2.

Scheme 1



Results and Discussion

Recently, we described[4] the synthesis of a new series of acyclic nucleoside analogues (**1**) starting from commercially available 2-deoxy-D-ribose. These nucleoside analogues can theoretically be used for the preparation of the target compounds. Therefore, compound **1** (Scheme 1) was treated with *p*-toluenesulfonyl chloride in pyridine at room temperature to afford intermediate **2**, which upon treatment with NaH in DMF at room temperature smoothly cyclized to give **3** in 55% yield in two steps.

Benzyl groups were removed from **3** upon treatment[5] with $Pd(OH)_2$ on C to give **4** in 94% yield. The ring-closure reaction with adenine or 6-chloro-2-aminopurine as base, however, was unsuccessful, using either the Mitsunobu reaction or simple nucleophilic displacement, and resulted in a complex mixture.

It appears that this synthetic scheme is limited to the synthesis of certain pyrimidine nucleoside derivatives. To develop a more general scheme that can be used for the synthesis of pyrimidine as well as purine analogues,

[⊗] Abstract published in *Advance ACS Abstracts,* March 15, 1997.

(1) (a) Verheggen, I.; Van Aerschot, A.; Toppet, S.; Snoeck, R.; Janssen, G; Balzarini, J.; De Clercq, E.; Herdewijn, P. *J. Med. Chem.* **1993**, *36*, 2033−2040. (b) Verheggen, I.; Van Aerschot, A.; Van Meervelt, L.; Rozenski, J.; Wiebe, L.; Snoeck, R.; Andrei, G.; Balzarini, J.; Claes, P.; De Clercq, E.; Herdewijn, P. *J. Med. Chem.* **1995**, *38*, 826−835. (c) Tino, J. A.; Bisacchi, G. S.; Ahmad, S. U.S. Patents 5,314,893 and 5,414,000.

(2) Herdewijn, P.; De Winter, H.; Doboszewski, B.; Verheggen, I.; Augustyns, K.; Hendrix, C.; Saison-Behmoaras, T.; De Ranter, C.; Van Aerschot, A. *Carbohydrate Modifications in Antisense Research*; American Chemical Society Symposium Series No. 580; American Chemical Society: Washington, DC, 1994; pp 80−99.

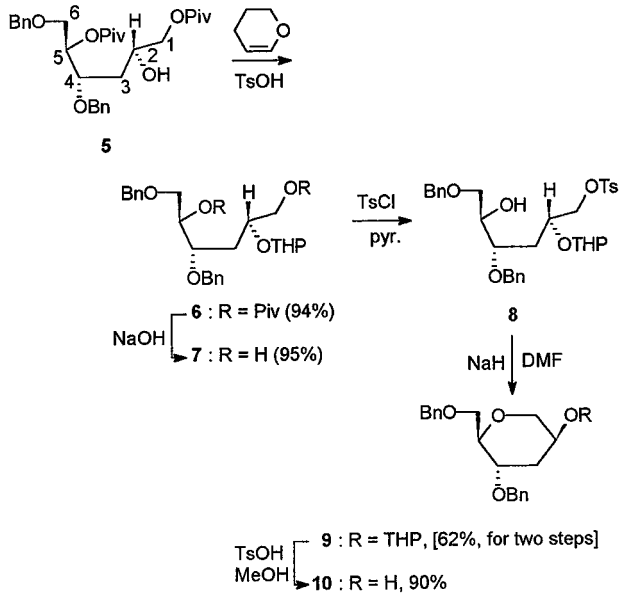
(3) Van Aerschot, A.; Verheggen, I.; Hendrix, C.; Herdewijn, P. *Angew. Chem.* **1995**, *34*, 1338−1339.

(4) Hossain, N.; Rozenski, J.; De Clercq, E.; Herdewijn, P. *Tetrahedron* **1996** 52, 13655−13670.

(5) Tippie, M. A.; Martin, J. C.; Smee, D. F.; Matthews, T. R.; Verheyden, J. P. H. *Nucleosides Nucleotides* **1984**, *3*, 525−535.

S0022-3263(96)01982-2 CCC: $14.00

Scheme 2

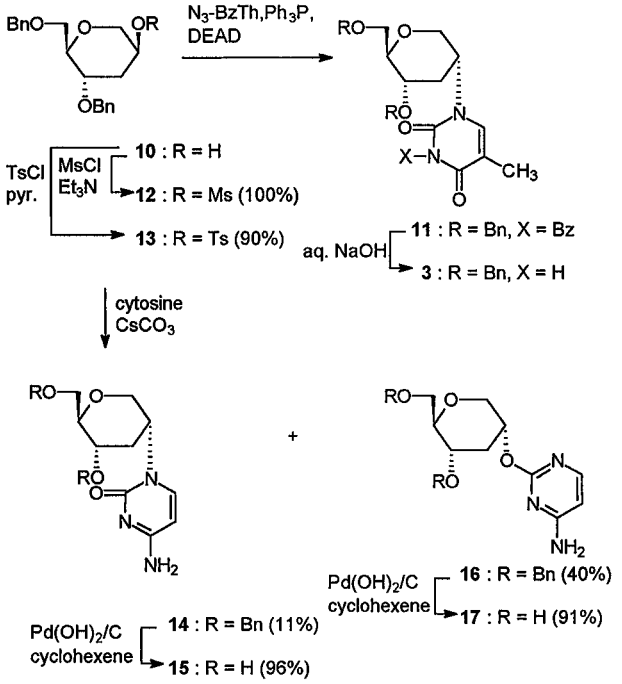


it proved necessary to first transform the pentofuranose sugar to an anhydrohexitol sugar derivative before introducing the base moiety. Conditions for alkylation of the nucleobases, however, are dependent on the base considered.

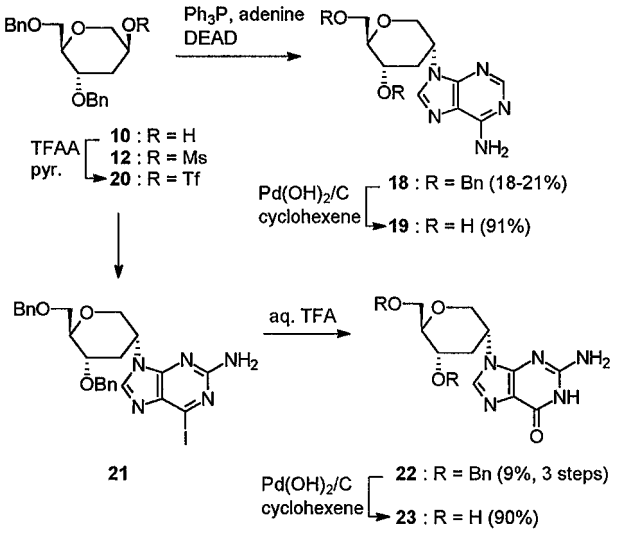
The synthetic strategy is given in Scheme 2. A base-stable protecting group for the 2-hydroxyl group of **5** is needed during removal of the pivaloyl groups at positions 1 and 5 and also during the ring-closure reaction of **8** to **9**. To this end, the tetrahydropyran-1-yl group[6] could be used. Treatment of **5** with dihydropyran in dichloromethane in the presence of a catalytic amount of *p*-toluenesulfonic acid at room temperature for 30 min gave **6** in 94% yield. The pivaloyl groups of **6** were removed with aqueous NaOH (N) and dioxane at 55 °C for 30 h to give **7** in 95% yield. These pivaloyl groups are surprisingly resistant to hydrolysis upon treatment with aqueous NaOH (N) in dioxane at room temperature and were insufficient for hydrolysis. Attempted ring-closure reaction of **7** under Mitsunobu conditions was unsuccessful, in contrast to a simple nucleophilic substitution reaction. Compound **7** was treated with *p*-toluenesulfonyl chloride in pyridine at room temperature to afford **8**, which was not isolated but used directly for the next step after usual workup. Treatment of **8** with NaH in DMF gave **9** in 62% yield. The yield of the acidic hydrolysis of the tetrahydropyran-1-yl group in **9** using aqueous acetic acid never exceeded 60%. When **9** was treated with *p*-toluenesulfonic acid[7] in methanol at room temperature for 30 min, however, **10** was isolated in 90% yield. Compound **10** can be used as a general starting material for the synthesis of both pyrimidine and purine nucleoside analogues.

Treatment of **10** with N^3-benzoylthymine[8] (Scheme 3), triphenylphosphine, and diethylazodicarboxylate (DEAD)[9] in dioxane gave **11**, which was directly treated with aqueous NaOH in dioxane to give **3** (precursor of **4**) in 47% yield (two steps). Compound **10** was treated either

Scheme 3



Scheme 4



with methanesulfonyl chloride in the presence of triethylamine in dichloromethane to give **12** (100%) or with *p*-toluenesulfonyl chloride in pyridine at room temperature to give **13** in 90% yield. Treatment of **12** or **13** with cytosine and cesium carbonate[10] in DMF at 120 °C gave **14** (11%) and **16** (40%). The use of **12** or **13** does not make any difference in yield of **14** and **16**. Unfortunately, Mitsunobu reaction conditions[9] in combination with protected cytosine did not improve the yield. The benzyl groups in **14** and **16** were removed to give **15** (96%) and **17** (91%), respectively.

An analogous procedure was used for the synthesis of the purine derivatives (Scheme 4). The adenine analogue may be obtained either from **10** using triphenylphosphine, adenine, and DEAD in dioxane (21% yield) or from **12** using adenine in the presence of NaH in DMF at 90 °C (18% yield). This low yield is due to steric hindrance,

(6) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 2nd ed.; John Wiley & Sons, Inc.: New York, 1991; pp 31−34.

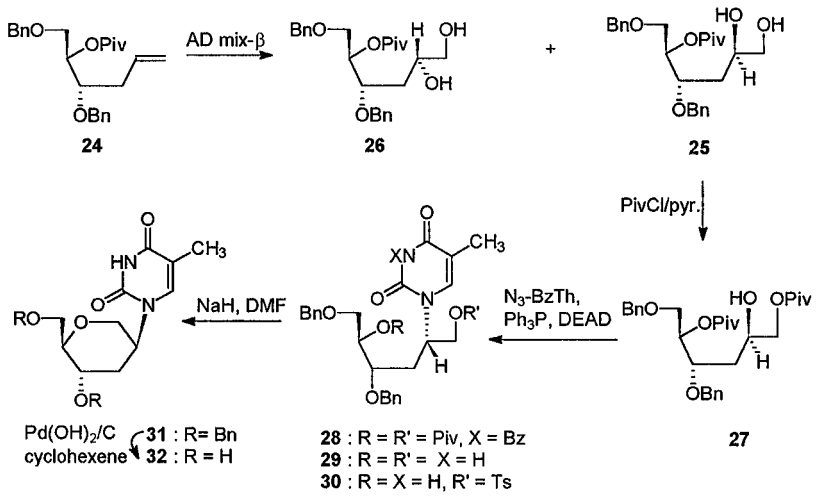
(7) Corey, E. J.; Niwa, H.; Knolle, J. *J. Am. Chem. Soc.* **1978**, *100*, 1942−1943.

(8) Cruickshank, K. A.; Jiricny, J.; Reese, C. B. *Tetrahedron Lett.* **1984**, *25*, 681−684.

(9) Mitsunobu, O. *Synthesis* **1981**, 1−28.

(10) (a) Vandendriessche, F.; Snoeck, R.; Janssen, G.; Hoogmartens, J.; Van Aerschot, A.; De Clercq, E.; Herdewijn, P. *J. Med. Chem.* **1992**, *35*, 1458−1465. (b) Bronson, J. J.; Ghazzouli, I.; Hitchcock, M. J. M; Webb, R. R.; Martin, J. C. *J. Med. Chem.* **1989**, *32*, 1457−1463.

Scheme 5



which is consistent with previously published work.[11] The benzyl groups in **18** were removed to give **19** in 91% yield. Treatment of the triflate derivative **20** with the tetrabutylammonium salt of 6-iodo-2-aminopurine[12] in dichloromethane at 0 °C and with aqueous CF_3COOH[13] at room temperature gave **22** in 9% yield (three steps). Mainly elimination reactions occurred. The benzyl groups from **22** were removed to give **23** in 90% yield.

To be able to unambiguously determine the configuration of compounds **4**, **15**, **19**, and **23**, we repeated the synthetic scheme for the thymine analogue using the C-2 epimeric starting material **27** (Scheme 5). In this way, the anhydrohexitol nucleoside[1] with a β-configuration at position 2 was obtained. Previously, we have described[4] the synthesis of **24**−**26** using AD-mix α. In order to achieve better stereoselectivity in favor of **25**, we have tried AD-mix β, but the effort was unsuccessful. In case of AD-mix β, we used the same procedure as described before[4] using **24**, AD-mix β[14] in 2-methyl-2-propanol, and water to give **25** (46%) and **26** (46%) in a combined yield of 92%. We have performed this reaction on a 7.6 mmol scale using the same experimental conditions as described previously.[4] The spectroscopic properties of **25** and **26** were identical to those of earlier work.[4] Compound **27**[4] was treated with N^3-benzoylthymine, DEAD, and Ph_3P in dioxane to afford crude **28**, which was directly treated with aqueous NaOH (N) in dioxane to give **29** in 45% yield (two steps). Compound **29** was treated with *p*-toluenesulfonyl chloride in pyridine, and after usual workup the residue was treated with NaH in DMF solution to give the cyclized product **31** in 55% yield (two steps). Finally, the benzyl groups in **31** were removed to give **32** in 94% yield. The site of alkylation of the nucleobase in **3**, **4**, **14**−**23**, and **31**−**32** is determined on the basis of ^{13}C-NMR spectra. In case of O^2-alkylated pyrimidine nucleoside derivatives the sugar C attached to O^2 of the nucleobase resonates at lower field

Table 1. Cytotoxicity and Antiviral Activity of the Cytosine Analogue 15 in E_6SM Cell Cultures[15,16] (ED_{50} Values Are Given in μg/mL)

	minimum inhibitory concentration[b]	
	compd **15**	acyclovir
cell morphology[a]	>400	400
Herpes simplex virus-1 (KOS)	7	0.02
Herpes simplex virus-2 (G)	20	0.07
Vaccinia virus	20	>200
Herpes simplex virus-1 TK^-(B2006)	10	10
Herpes simplex virus-1 (TK^-/TK^+) (VMW 1837)	4	1

[a] Minimum cytotoxic concentration or concentration required to cause a microscopically detectable alteration of normal cell morphology. [b] Required to reduce virus-induced cytopathogenecity by 50%.

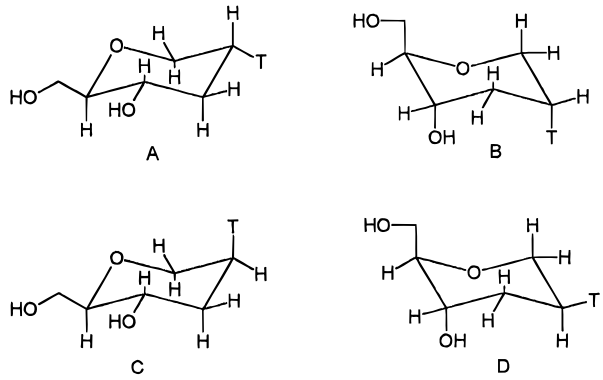


Figure 1.

(60−70 ppm), whereas the C-atom attached to N^1 of the heterocyclic base resonates more upfield (45−55 ppm). All of these data are in agreement with our earlier work.[4]

The configuration and conformation of **4**, **15**, **19**, and **23** were determined on the basis of their ^{1}H-NMR spectra. This is described here using the thymine analogue **4** as a representative example (Figure 1). The ^{1}H-NMR spectrum of **4** shows large $^3J_{HH}$ coupling constants, $^3J_{2',3''} = 11.2$ Hz, $^3J_{3'',4'} = 9.8$ Hz, $^3J_{4',5'} = 9.4$ Hz, and $^3J_{1'',2'} = 12.7$ Hz. These large $^3J_{HH}$ coupling constants are expected for diaxial orientation of the respective protons (structure A). In structure B or D, the $^3J_{4'eq,5'eq}$ should be small, which was not observed in the ^{1}H-NMR spectrum of **4**. In C, the $^3J_{1''ax,2'eq}$ and $^3J_{2'eq,3''ax}$ should differ from our observed $^3J_{HH}$ coupling constants. From these observations it is clear that the large $^3J_{HH}$ coupling

(11) Pérez-Pérez, M.-J.; Rozenski, J.; Busson, R.; Herdewijn, P. *J. Org. Chem.* **1995**, *60*, 1531−1537.
(12) Bisacchi, G. S.; Singh, J.; Godfrey, J. D., Jr.; Kissick, T. P.; Mitt, T.; Malley, M. F.; Di Marco, J. D.; Gougoutas, J. Z.; Mueller, R. H.; Zahler, R. *J. Org. Chem.* **1995**, *60*, 2902−2905.
(13) Jindrich, J.; Holy, A.; Dvorakova, H. *Collect. Czech. Chem. Commun.* **1993**, *58*, 1645−1667.
(14) Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K.-S.; Kwong, H.-L.; Morikawa, K.; Wang, Z.-M.; Xu, D.; Zhang, X.-L. *J. Org. Chem.* **1992**, *57*, 2768−2771.
(15) De Clercq, E.; Descamps, J.; Verhelst, G.; Walker, R. T.; Jones, A. S.; Torrence, P. F.; Shugar, D. *J. Infect. Dis.* **1980**, *141*, 563−574.
(16) De Clercq, E.; Holy, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P. C. A. *Nature* **1986**, *323*, 464−467.

constants of **4** fit well with structure A. This together with the independent synthesis of the C-2 epimer of **4** (i.e., **32**) proves the configuration of the synthesized compounds. Note that the precursors of **1** and **29** were obtained as diastereomeric mixtures by Sharpless dihydroxylation of **24**. The obtained results also confirmed the previously determined configuration of both starting materials.[4]

The antiviral activity of the synthesized compounds was determined against herpes simplex virus type 1, herpes simplex virus type 2, vaccinia virus, vesicular stomatitis virus, Coxsackie virus type B4, respiratory syncytial virus, parainfluenza-3 virus, reovirus-1, Sindbis virus, and Punta Toro virus. Except for **15**, none of the compounds demonstrated any antiviral activity. The antiviral activity and cytotoxicity data for **15** are presented in Table 1 in comparison with acyclovir. Although the antiviral activity was moderate, the compound proved selective in its antiviral action, as it showed no toxicity to the host cells. Moreover, the compound was as active against thymidine kinase positive (TK^+) as against thymidine kinase negative (TK^-) herpesvirus strains (Table 1). The observed activity is remarkable as it proves that even the α-isomers of this anomalous class of nucleosides are able to selectively interfere with viral specific processes. Further studies are needed to unravel the mode of action of this compound.

Experimental Section

Analytical instruments used were described previously.[4] All concentrations were done in a vacuum, and organic layers were dried over anhydrous Na_2SO_4. Chemical shifts are reported in δ units.

1,5-Anhydro-4,6-di-*O*-benzyl-2,3-dideoxy-2-(thymin-1-yl)-D-ribohexitol (3). A mixture of **1**[4] (170 mg, 0.37 mmol) and *p*-toluenesulfonyl chloride (85 mg, 0.45 mmol) in pyridine (3 mL) was kept at room temperature overnight and then concentrated. After addition of water (3 mL), the mixture was extracted with dichloromethane (3 × 20 mL). The combined organic layer was concentrated and coevaporated with toluene (3 × 10 mL). The residue was dissolved in dichloromethane (40 mL) and washed successively with aqueous saturated $NaHCO_3$ (3 × 5 mL) and water (5 mL). The organic layer was dried, filtered, and concentrated. The residue was dissolved in DMF (1 mL), added to a suspension of NaH (80%) (32 mg, 1.1 mmol) in DMF (4 mL), and kept at room temperature overnight. The mixture was concentrated, and after addition of aqueous saturated $NaHCO_3$ (3 mL) it was extracted with ethyl acetate (3 × 20 mL). The combined organic layer was washed with water (3 mL), dried, filtered, and concentrated. The residue was purified by silica gel column chromatography (2.5−50% EtOAc an hexane) to give **3** (90 mg, 55%). **Alternative Method.** Compound **3** was also prepared by the Mitsunobu reaction. Thus, to a mixture of **10** (80 mg, 0.24 mmol), Ph_3P (126 mg, 0.48 mmol), and N^3-benzoylthymine (109 mg, 0.48 mmol) in dioxane (5 mL) was added DEAD (74 μL) in dioxane (10 mL) over a period of 60 min at room temperature, and the reaction mixture was kept at room temperature overnight. The reaction mixture was concentrated to give crude **11**, which was directly treated with aqueous NaOH (N) in dioxane at room temperature for 4 h. The reaction mixture was adjusted to pH 7.0 by addition of dilute aqueous HCl, and the volume was reduced to 1/3 of its original volume and extracted with ethyl acetate (2 × 20 mL). The combined organic layer was washed with water, dried, filtered, and concentrated. The residue was purified by silica gel column chromatography (2.5−50% EtOAc in hexane) to afford **3** (50 mg, 47% in two steps). ^{1}H-NMR ($CDCl_3$): 9.10 (br s, 1H); 7.41−7.15 (m, 10 H); 6.98 (s, 1H); 4.78−4.38 (m, 5H); 4.05 (m, J = 4.6, 10.6 Hz, 1H); 3.81−3.58 (m, 3H); 3.49−3.35 (m, 2H); 2.48 (m, 1H); 1.90 (s, 3H); 1.81 (m, 1H). ^{13}C-NMR ($CDCl_3$): 163.5, 150.8, 138.0, 137.7, 128.5, 128.0, 127.9, 111.3, 80.1, 73.7, 72.4, 71.5, 69.0, 68.7, 50.3, 34.2; 12.7. HRMS: calcd for $C_{25}H_{29}N_2O_5$ $(M + H)^+$ 437.2076, found 437.2075.

1,5-Anhydro-2,3-dideoxy-2-(thymin-1-yl)-D-ribohexitol (4). Compound **3** (80 mg, 0.18 mmol) was treated with $Pd(OH)_2$ on C(20%) (80 mg) in methanol (10 mL) and cyclohexene (3 mL) at reflux temperature for 24 h. The reaction mixture was filtered and concentrated to give a white solid, which was washed with hexane and dichloromethane to afford pure **4** (44 mg, 94%). Mp: 220−222 °C. ^{1}H-NMR (DMSO-d_6): 11.91 (br s, 1H); 7.51 (s, 1H); 5.05 (d, J = 5.0 Hz, 1H); 4.46 (t, J = 5.8 Hz, 1H); 4.35 (m, J = 12.7 Hz, 1H); 3.73 (ddd, J = 4.5, 10.3, 1.8 Hz, 1H); 3.69 (ddd, J = 2.0, 14.8, 5.8 Hz, 1H); 3.42 (m, 2H); 3.38 (m, 1H); 3.03 (ddd, J = 6.2, 9.4, 2.0 Hz, 1H); 2.04 (m, J = 4.3, 4.9, 11.8 Hz, 1H); 1.86 (m, J = 11.2, 9.8 Hz, 1H); 1.75 (s, 3H). ^{13}C-NMR (DMSO-d_6): 164.8, 152.0, 138.9, 110.1, 83.9, 68.6, 66.3, 62.3, 51.0, 37.7, 13.1. HRMS: calcd for $C_{11}H_{17}N_2O_5$ $(M + H)^+$ 257.1137, found 257.1154. Anal. Calcd for $C_{11}H_{16}N_2O_5 \cdot 0.3C_6H_{14}$: C, 54.50; H, 7.22; N, 9.93. Found: C, 54.37; H, 6.98; N, 9.68.

3-Deoxy-4,6-di-*O*-benzyl-1,5-di-*O*-pivaloyl-2-*O*-(tetrahydropyran-1-yl)-D-glucitol (6). Compound **5** (7.3 g, 14.2 mmol) was treated with dihydropyran (6.0 g) and *p*-toluenesulfonic acid (24 mg) in dichloromethane (100 mL) for 30 min at room temperature. The reaction mixture was diluted with dichloromethane (150 mL) and washed successively with aqueous saturated $NaHCO_3$ (10 mL) and water (5 mL). The organic layer was dried, filtered, and concentrated. The residue was purified by silica gel column chromatography (0−15% EtOAc in hexane) to give **6** (8.0 g, 94%). Compound **6** is a mixture of two diastereoisomers due to the chirality of the tetrahydropyran-1-yl (THP) group. ^{1}H-NMR ($CDCl_3$): 7.41−7.19 (m, 10 H); 5.45 (m, 1H); 5.00−4.50 (m, 4H); 4.41−3.38 (m, 9H); 1.98−1.41 (m, 8H); 1.25 and 1.20 (2 × s, 18 H). HRMS: calcd for $C_{35}H_{50}O_8$ (M + Na) 621.3403, found 621.3434.

3-Deoxy-4,6-di-*O*-benzyl-2-*O*-(tetrahydropyran-1-yl)-D-glucitol (7). A mixture of **6** (7.0 g, 11.7 mmol), aqueous NaOH (N) (150 mL), and dioxane (150 mL) was kept at 55 °C for 30 h. The reaction mixture was cooled to 0 °C and adjusted to pH 7 by addition of dilute aqueous HCl. The reaction mixture was reduced to one third of its volume, and it was extracted with ethyl acetate (3 × 200 mL). The organic layer was dried, filtered, and concentrated. The residue was purified by silica gel column chromatography (2.5−60% EtOAc in hexane) to afford **7** (4.8 g, 95%), which is a mixture of two diastereoisomers due to the chirality of THP group. ^{1}H-NMR ($CDCl_3$ + D_2O): 7.41−7.20 (m, 10 H); 4.58−4.48 (m, 5H); 4.18−3.38 (m, 9H); 1.99−1.38 (m, 8H). HRMS: calcd for $C_{25}H_{35}O_6$ $(M + H)^+$ 431.2433, found 431.2447.

1,5-Anhydro-3-deoxy-4,6-di-*O*-benzyl-2-*O*-(tetrahydropyran-1-yl)-D-arabinohexitol (9). Compound **7** (4.7 g, 10.9 mmol) was treated with *p*-toluenesulfonyl chloride (2.27 g, 11.9 mmol) in pyridine (100 mL) at room temperature overnight, and an additional 400 mg of *p*-toluenesulfonyl chloride was added and kept at room temperature for another 5 h. After addition of methanol

(5 mL), the mixture was concentrated. The residue was dissolved in ethyl acetate (200 mL) and washed with water (20 mL). The combined organic layer was concentrated and coevaporated with toluene (3 × 20 mL) and the residue redissolved in ethyl acetate (200 mL) and washed successively with aqueous saturated $NaHCO_3$ (3 × 20 mL) and water (10 mL). The organic layer was dried, filtered, and concentrated. The residue was dissolved in DMF (5 mL) and added to a suspension of NaH (80%) (650 mg, 21.7 mmol) in 75 mL of DMF. The reaction mixture was kept at room temperature overnight. After concentration, saturated aqueous NH_4Cl (20 mL) was added, and the mixture was extracted with ethyl acetate (3 × 60 mL). The combined organic layer was washed with water (20 mL), dried, and concentrated. The residue was purified by silica gel column chromatography (2.5−40% EtOAc in hexane) to give **9** (2.8 g, 62%). This compound is a mixture of two diastereoisomers due to the chirality of the tetrahydropyran-1-yl group. ^{1}H-NMR ($CDCl_3$): 7.41−7.18 (m, 10 H); 4.71−4.37 (m, 5H); 4.10−3.38 (m, 9H); 2.55−2.30 (m, 1H); 1.91−1.39 (m, 7H).

1,5-Anhydro-3-deoxy-4,6-di-*O*-benzyl-d-arabinohexitol (10). Compound **9** (2.8 g, 6.8 mmol) was treated with *p*-toluenesulfonic acid (1.52 g, 8.0 mmol) in methanol (60 mL) at room temperature for 30 min. After concentration, the residue was dissolved in dichloromethane (200 mL) and washed successively with aqueous saturated $NaHCO_3$ (3 × 25 mL) and water (20 mL). The combined organic layer was dried, filtered, and concentrated. The residue was purified by silica gel column chromatography (2.5−50% EtOAc in hexane) to give **10** (2.0 g, 90%). ^{1}H-NMR ($CDCl_3$): 7.41−7.18 (m, 10 H); 4.65−4.32 (m, 4H); 3.98 (m, 1H); 3.90−3.35 (m, 6H); 2.45 (m, J = 4.3, 13.2 Hz, 1H); 2.22 (d, J = 7.6 Hz, 1H); 1.55 (m, 1H). ^{13}C-NMR ($CDCl_3$): 138.0, 128.3, 128.0, 127.9, 127.6, 80.6, 73.5, 72.1, 71.0, 69.8, 69.5, 66.7 and 36.0. HRMS: calcd for $C_{20}H_{25}O_4$ $(M + H)^+$ 329.1752, found 329.1768.

1,5-Anhydro-3-deoxy-4,6-di-*O*-benzyl-2-*O*-mesyl-D-arabinohexitol (12). Compound **10** (328 mg, 1.0 mmol) was treated with methanesulfonyl chloride (116 μL, 1.5 mmol) in the presence of triethylamine (208 μL, 1.5 mmol) in dichloromethane (10 mL) at 0 °C for 2 h. After addition of 2 mL of water the two layers were separated. The organic layer was washed successively with aqueous saturated $NaHCO_3$ (2 × 3 mL) and water (3 mL). The organic layer was dried, filtered, and concentrated to give **12** (406 mg, 100%), which was used in the next step without further purification. ^{1}H-NMR ($CDCl_3$): 7.39−7.18 (m, 10 H); 4.98 (m, 1H); 4.67−4.38 (m, 4H); 4.15 (dt, J = 2.3, 13.3 Hz, 1H); 3.83−3.57 (m, 4H); 3.45 (m, 1H); 3.02 (s, 3H); 2.65 (m, J = 4.6, 14.1 Hz, 1H); 1.72 (m, 1H). ^{13}C-NMR ($CDCl_3$): 138.0, 137.8, 128.4, 127.8, 127.7, 80.4, 76.0, 73.6, 71.5, 69.3, 39.0 and 34.5.

1,5-Anhydro-3-deoxy-4,6-di-*O*-benzyl-2-*O*-tosyl-D-arabinohexitol (13). A mixture of **10** (656 mg, 2.0 mmol) and *p*-toluenesulfonyl chloride (572 mg, 3.0 mmol) in 10 mL of pyridine was stirred at room temperature for 24 h. After concentration, the residue was dissolved in dichloromethane (30 mL) and washed with water (5 mL). The organic layer was concentrated and coevaporated with toluene (3 × 10 mL). The residue was dissolved in dichloromethane (30 mL) and washed successively with aqueous saturated $NaHCO_3$ (3 × 5 mL) and water (5 mL). The organic layer was dried, filtered, and concentrated. The residue was purified by silica gel column chromatography (0−30% EtOAc in hexane) to give pure **13** (870 mg, 90%). ^{1}H-NMR ($CDCl_3$): 7.79 (m, 2H); 7.40−7.11 (m, 12 H); 4.76 (m, 1H); 4.62−4.28 (m, 4H); 3.90 (br d, J = 2.0, 12.9 Hz, 1H); 3.78−3.57 (m, 3H); 3.46 (br d, 1H); 3.38 (m, 1H); 2.48 (m, 1H); 2.42 (s, 3H); 1.60 (m, 1H). ^{13}C-NMR ($CDCl_3$): 144.8, 138.1, 137.8, 129.9, 128.3, 127.7, 80.2, 76.8, 73.5, 71.4, 69.6, 69.5, 69.0, 34.1, 21.6.

1,5-Anhydro-2-(cytosin-1-yl)-2,3-dideoxy-4,6-di-*O*-benzyl-D-ribohexitol (14) and 1,5-Anhydro-2-(cytosin-2-*O*-yl)-2,3-dideoxy-4,6-*O*-dibenzyl-D-ribohexitol (16). Compound **13** (400 mg, 0.83 mmol) was treated with cytosine (150 mg, 1.35 mmol) in the presence of cesium carbonate (588 mg, 1.80 mmol) in DMF (10 mL) at 120 °C for 14 h. The reaction mixture was cooled to room temperature and concentrated. After addition of dichloromethane (20 mL) and methanol (1 mL), the mixture was filtered, concentrated, and purified by silica gel column chromatography (0.5−6.0% MeOH in CH_2Cl_2) to give **14** (40 mg, 11%) and **16** (140 mg, 40%). **Compound 14.** ^{1}H-NMR ($CDCl_3$): 7.40−7.18 (m, 11 H); 5.88 (d, J = 7.1 Hz, 1H); 4.75 (m, 1H); 4.68−4.38 (m, 4H); 4.05 (m, J = 4.2, 10.5 Hz, 1H); 3.81−3.55 (m, 3H); 3.46−3.28 (m, 2H); 2.48 (m, 1H), 1.85 (m, 1H). ^{13}C-NMR ($CDCl_3$): 165.1, 156.2, 141.5, 138.0, 137.9, 128.4, 127.9, 127.8, 127.7, 95.2, 80.1, 73.6, 72.6, 71.3, 69.3, 50.9, 34.4. HRMS: calcd for $C_{24}H_{28}N_3O_4$ $(M + H)^+$ 422.2079, found 422.2075. **Compound 16.** ^{1}H-NMR ($CDCl_3$): 8.00 (d, J = 5.6 Hz, 1H); 7.41−7.18 (m, 10 H); 6.09 (d, J = 5.6 Hz, 1H); 5.07 (m, 1H); 4.89 (br s, 2H); 4.70−4.36 (m, 4H); 4.25 (m, J = 5.1, 10.6 Hz, 1H); 3.82−3.55 (m, 3H); 3.48−3.28 (m, 2H); 2.71 (m, 1H); 1.68 (m, 1H). ^{13}C-NMR ($CDCl_3$): 164.7, 157.8, 138.2, 128.4, 127.9, 127.7, 127.6, 99.5, 80.0, 73.6, 72.2, 71.0, 69.4, 69.3, 68.7, 35.4. HRMS: calcd for $C_{24}H_{28}N_3O_4$ $(M + H)^+$ 422.2079, found 422.2079.

1,5-Anhydro-2-(cytosin-1-yl)-2,3-dideoxy-D-ribohexitol (15). The reaction was performed as described for **4** using **14** (40 mg, 0.095 mmol) and $Pd(OH)_2$ on C (20%) (40 mg) in methanol (10 mL) and cyclohexene (3 mL) to give pure **15** (22 mg, 96%). ^{1}H-NMR (DMSO-d_6): 7.67 (d, J = 7.4 Hz, 1H), 7.18, 7.00 (2 × br s, 2H); 5.69 (d, J = 7.4 Hz, 1H); 5.18 (d, J = 4.9 Hz, 1H); 4.59 (t, J = 5.8 Hz, 1H); 4.45 (m, 1H); 3.69 (m, 2H); 3.49−3.13 (m, 3H, under DMSO peak); 3.01 (m, 1H); 2.01 (m, 1H); 1.78 (m, 1H). ^{13}C-NMR (DMSO-d_6): 165.3, 155.6, 142.5, 93.8, 82.9, 68.3, 65.2, 61.2, 50.5, 37.0. HRMS: calcd for $C_{10}H_{16}N_3O_4$ $(M + H)^+$ 242.1140, found 242.1124. Anal. Calcd for $C_{10}H_{15}N_3O_4 \cdot 0.5CH_2Cl_2 \cdot 2.0H_2O$: C, 39.44; H, 6.30; N, 13.14. Found: C, 39.98; H, 6.22; N, 13.17.

1,5-Anhydro-2-(cytosin-2-*O*-yl)-2,3-dideoxy-D-ribohexitol (17). The reaction was performed as described for **4** using **16** (125 mg, 0.30 mmol), $Pd(OH)_2$ on C (20%) (125 mg) in cyclohexene (3 mL), and methanol (10 mL) to give **17** (65 mg, 91%). ^{1}H-NMR (DMSO-d_6): 7.82 (d, J = 5.8 Hz, 1H); 7.01 (br s, 2H); 6.08 (d, J = 5.8 Hz, 1H); 4.98 (d, J = 3.5 Hz, 1H); 4.82 (m, 1H); 4.50 (br s, 1H); 4.04 (m, J = 5.0, 10.0 Hz, 1H); 3.72−3.22 (m, 3H, under DMSO peak); 3.15−2.93 (m, 2H), 2.38 (m, 1H, under DMSO peak); 1.45 (m, 1H). ^{13}C-NMR (DMSO-d_6): 165.6, 163.5; 155.2; 99.6; 83.2; 68.4; 64.4; 61.2; 38.3. HRMS: Calcd for $C_{10}H_{16}N_3O_4$ $(M + H)^+$ 242.1140, found 242.1153. Anal. Calcd for $C_{10}H_{15}N_3O_4 \cdot 0.1CH_3OH \cdot 0.7H_2O$: C, 47.19; H, 6.59; N, 16.35. Found: C, 47.46; H, 6.29; N, 16.01.

2-(Adenin-9-yl)-1,5-anhydro-4,6-di-*O*-benzyl-2,3-dideoxy-D-ribohexitol (18). Diethyl azodicarboxylate (129 μL) in dioxane (10 mL) was added to a mixture of **10** (140 mg, 0.43 mmol), Ph_3P (220 mg, 0.84 mmol), and adenine (113 mg, 0.84 mmol) in dioxane (10 mL) over a period of 60 min at room temperature. After 20 h, the

reaction mixture was concentrated and the residue purified by silica gel column chromatography (0.5−6.0% MeOH in CH_2Cl_2) to give pure **18** (40 mg, 21%). This compound was also prepared by a nucleophilic displacement reaction as follows: a mixture of adenine (270 mg, 2.0 mmol) and NaH (80%) (60 mg, 2.0 mmol) in DMF (10 mL) was kept at 90 °C for 60 min. To the sodium salt of adenine was added **12** (406 mg, 1.0 mmol) in DMF (1 mL) and the mixture left at 90 °C for 24 h. The reaction mixture was cooled to room temperature. The solvent was removed, and aqueous saturated $NaHCO_3$ (10 mL) was added and extracted with ethyl acetate (3 × 30 mL). The combined organic layer was washed with water (10 mL), dried, filtered, and concentrated. The residue was purified by silica gel column chromatography (0.5−6% MeOH in CH_2Cl_2) to give pure **18** (80 mg, 18%). ^{1}H-NMR ($CDCl_3$): 8.38 (s, 1H); 7.80 (s, 1H); 7.41−7.18 (m, 10 H); 5.76 (br s, 2H); 4.72 (m, 1H); 4.66−4.41 (m, 4H); 4.20 (ddd, J = 1.9, 4.7, 10.7 Hz, 1H); 3.85−3.51 (m, 5H); 2.72 (m, 1H); 2.24 (m, 1H). ^{13}C-NMR ($CDCl_3$): 155.5, 153.0, 150.0, 138.4, 138.0, 137.7, 128.4, 127.9, 127.8, 119.9, 80.2, 73.6, 72.2, 71.5, 69.7, 69.0, 50.4, 35.3. HRMS: calcd for $C_{25}H_{28}N_5O_3$ $(M + H)^+$ 446.2192, found 446.2199.

2-(Adenin-9-yl)-1,5-anhydro-2,3-dideoxy-D-ribohexitol (19). The reaction was performed as described for **4** using **18** (70 mg, 0.16 mmol) and $Pd(OH)_2$ on C (20%) (70 mg) in methanol (10 mL) and cyclohexene (3 mL) to give **19** (38 mg, 91%). ^{1}H-NMR (DMSO-d_6): 8.28, 8.16 (2 × s, 2H); 7.31 (br s, 2H); 5.17 (br s, 1H); 4.53 (m, 2H); 3.98 (m, 1H); 3.71 (m, 2H); 3.58−3.28 (m, 2H, under DMSO peak); 3.16 (m, 1H); 2.28 (m, 2H). ^{13}C-NMR (DMSO-d_6): 155.8, 152.3, 149.4, 139.9, 119.0, 83.2, 68.7, 64.9, 61.3, 50.4, 37.9. HRMS: calcd for $C_{11}H_{16}N_5O_3$ $(M + H)^+$ 266.1253, found 266.1256. Anal. Calcd for $C_{11}H_{15}N_5O_3 \cdot 0.8CH_3OH \cdot H_2O$: C, 45.88; H, 6.59; N, 22.67. Found: C, 45.92; H, 6.15; N, 22.46.

1,5-Anhydro-4,6-di-*O*-benzyl-2,3-dideoxy-2-(guanin-9-yl)-D-ribohexitol (22). The reaction was performed following a literature procedure[12] using **10** (280 mg, 0.85 mmol), trifluoromethanesulfonic anhydride (214 μL in 3 mL of CH_2Cl_2), and pyridine (136 μL). After workup,[12] the filtrate was dried over Na_2SO_4 at 0 °C and directly filtered onto a solution of the tetrabutylammonium salt of 6-iodo-2-aminopurine (502 mg, 1.0 mmol) in CH_2Cl_2 (2 mL) at 0 °C and stirred for 8 h. The residue was concentrated and treated with 80% CF_3COOH in water (3 mL) at room temperature for 48 h. The solvent was removed. The residue was treated with NH_4OH (1 mL) in methanol (3 mL). After concentration, the residue was purified by silica gel column chromatography (0.5−7.0% MeOH in CH_2Cl_2) to give **22** (35 mg, 9% in three steps). ^{1}H-NMR (DMSO-d_6): 8.95 (br s, 1H); 7.80 (s, 1H); 7.41−7.20 (m, 10 H); 6.50 (br s, 2H); 4.68−4.37 (m, 4H); 4.30 (m, 1H); 3.90 (m, 1H); 3.80−3.40 (m, 4H); 3.09 (m, 1H); 2.60 (m, 1H); 2.15 (m, 1H). HRMS: calcd for $C_{25}H_{28}N_5O_4$ $(M + H)^+$ 462.2141, found 462.2151.

1,5-Anhydro-2-(guanin-9-yl)-2,3-dideoxy-D-ribohexitol (23). The reaction was performed as described for **4** using **22** (20 mg, 0.043 mmol), $Pd(OH)_2$ on C (20%) (20 mg) in methanol (6 mL), and cyclohexene (2 mL) to give pure **23** (11 mg, 90%). ^{1}H-NMR (DMSO-d_6): 10.62 (br s, 1H); 7.80 (s, 1H); 6.58 (br s, 2H); 5.18 (d, J = 4.8 Hz, 1H); 4.58 (t, J = 5.5 Hz, 1H); 4.30 (m, 1H); 3.90 (m, 1H), 3.71 (m, 1H); 3.58−3.30 (m, 3H, under DMSO peak); 3.10 (m, 1H); 2.28 (m, 1H); 2.08 (m, 1H). ^{13}C-NMR (DMSO-d_6): 156.9, 153.6, 150.9, 135.4, 116.7, 83.0, 68.7, 64.8, 61.2, 49.4, 37.9. HRMS: calcd for $C_{11}H_{16}N_5O_4$ $(M + H)^+$ 282.1202, found 282.1240.

1-(2,3-Dideoxy-4,6-di-*O*-benzyl-2-D-glucityl)thymine (29). This reaction was performed as described for **11** (see alternative procedure for the preparation of **3**) using **27**[4] (900 mg, 1.75 mmol), Ph_3P (915 mg, 3.48 mmol), N^3-benzoylthymine (796 mg, 3.48 mmol), and DEAD (540 μL in 20 mL dioxane) in dioxane (20 mL) to give crude **28**, which was directly treated, as described previously,[4] with 20 mL of NaOH (N) and dioxane (20 mL) at room temperature for 24 h to give **29** (360 mg, 45%). The workup and purification is identical to the procedure previously described.[4] ^{1}H-NMR ($CDCl_3$): 9.68 (br s, 1H); 7.41−7.18 (m, 10 H); 7.00 (s, 1H); 4.68 (m, 1H); 4.58−4.30 (m, 4H); 3.95 (m, 1H), 3.80−3.53 (m, 4H); 3.42 (m, 1H); 2.08 (m, 1H); 1.85 (m, 1H); 1.69 (s, 3H). ^{13}C-NMR ($CDCl_3$): 164.2, 151.9, 139.3, 137.6, 128.6, 128.0, 110.5, 77.0, 73.6, 72.6, 71.1, 70.8, 64.0, 56.6, 29.5, 12.5. HRMS: calcd for $C_{25}H_{31}N_2O_6$ $(M + H)^+$ 455.2181, found 455.2184.

1,5-Anhydro-4,6-di-*O*-benzyl-2,3-dideoxy-2-(thymin-1-yl)-D-arabinohexitol (31). Compound **29** (227 mg, 0.5 mmol) was treated with *p*-toluenesulfonyl chloride (114 mg, 0.6 mmol) in pyridine (3 mL) at room temperature overnight. After addition of water (3 mL), it was extracted with ethyl acetate (2 × 20 mL). The combined organic layer was concentrated and coevaporated with toluene (3 × 10 mL). The residue was dissolved in ethyl acetate (20 mL) and washed successively with aqueous saturated $NaHCO_3$ (2 × 5 mL) and water (3 mL). The organic layer was dried, filtered, and concentrated to give crude **30**. The residue was dissolved in DMF (2 mL) and added to a suspension of NaH (80%, 45 mg, 1.5 mmol) in DMF (5 mL). The reaction mixture was stirred at room temperature overnight. The solvent was removed, and the residue was dissolved in ethyl acetate (20 mL) and washed successively with saturated aqueous $NaHCO_3$ (2 × 5 mL) and water (5 mL). The organic layer was dried, filtered, and concentrated. The residue was purified by silica gel column chromatography (2.0−60% EtOAc in hexane) to give pure **31** (120 mg, 55% in two steps). ^{1}H-NMR ($CDCl_3$): 9.10 (br s, 1H); 7.92 (s, 1H); 7.42−7.18 (m, 10 H); 4.75 (m, 1H); 4.61−4.36 (m, 4H); 4.16 (m, 1H); 3.88 (dd, J = 3.3, 13.5 Hz, 1H); 3.82−3.68 (m, 3H); 3.52 (m, 1H); 2.48 (m, 1H); 1.88 (m, 1H); 1.78 (s, 3H). ^{13}C-NMR ($CDCl_3$): 163.8, 151.1, 138.9, 138.0, 137.6, 128.5, 127.9, 127.8, 127.7, 110.6, 79.9, 73.5, 71.4, 69.1, 68.5, 68.4, 50.6, 33.0, 12.5. HRMS: calcd for $C_{25}H_{29}N_2O_5$ $(M + H)^+$ 437.2076, found 437.2050. Experimental procedure for the preparation of **32** is analogous to **4**. The spectroscopic properties of **32** were identical with that of the previously synthesized compound.[1]

Acknowledgment. The authors thank Mieke Vandekinderen for editorial help, Anita Van Lierde and Frieda De Meyer for technical assistance, and Professor W. Pfleiderer for elemental analysis. This work was supported by the grant GOA.97/11. We also thank referees for constructive criticisms.

Supporting Information Available: Copies of ^{1}H and (or) ^{13}C and (or) attached proton test (APT) NMR spectra for compounds **3**, **4**, **6**, **7**, **9**, **10**, **12**−**19**, **22**, **23**, **29**, and **31** (36 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO961982M