Chain-Extension of Carbohydrates. 5.[1] Synthesis of the C-Glycosyl Amino Acid Moiety of Miharamycins Involving Stereocontrolled Ethynylation of Methyl 2,3,4-Tri-*O*-benzyl-α-D-*gluco*-hexodialdo-1,5-pyranoside

Stanislas Czernecki,* Stefan Horns, and Jean-Marc Valery

Laboratoire de Chimie des Glucides, Université Pierre et Marie Curie, 4 Place Jussieu, 75005 Paris, France

Received July 18, 1994 (Revised Manuscript Received November 21, 1994®)

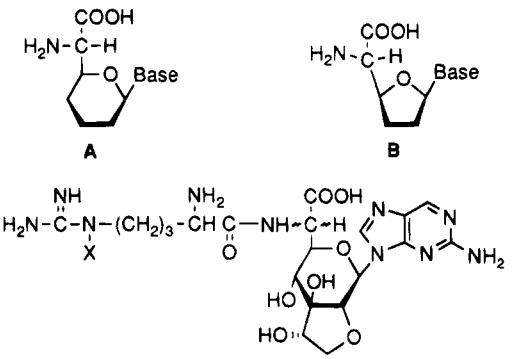
A multistep synthesis of the C-glycosyl amino acid moiety of miharamycins from methyl 2,3,4-tri-*O*-benzyl-α-D-*gluco*-hexodialdo-1,5-pyranoside (**1**) is described. The ethynyl group was employed as a synthetic equivalent of the carboxylic acid function. In the key step, highly diastereoselective ethynylation of compound **1** with the Grignard reagent of (trimethylsilyl)acetylene in the presence of magnesium bromide followed by desilylation afforded acetylenic alcohols **4** and **5** (19:1). The L-*glycero* configuration at C(6) of the major isomer **4** was unambiguously proven by ^{1}H NMR of the 4,6-benzylidene derivative **9**. The amino function was introduced at C(6) by reaction of **4** with zinc azide in the presence of triphenylphosphine and diisopropyl azodicarboxylate. Transformation of the resulting methyl 6-azido-2,3,4-tri-*O*-benzyl-6,7,8-trideoxy-D-*glycero*-α-D-*gluco*-oct-7-ynopyranoside (**10**) into methyl 6-(*N*-acetylamino)-2,3,4-tri-*O*-benzyl-6-deoxy-D-*glycero*-α-D-*gluco*-heptopyranosiduronic acid (**17**) was achieved by two different sequences of reactions: (1) oxidative cleavage of the triple bond, benzylation, reduction of the azido group, and *N*-acetylation or (2) reduction of the azido group, *N*-acetylation, oxidative cleavage of the triple bond, and treatment with phenyldiazomethane. The overall yield of the two sequences was different (41% versus 50%), showing the second method to be superior. Final debenzylation afforded methyl 6-(*N*-acetylamino)-6-deoxy-D-*glycero*-α-D-*gluco*-heptopyranosiduronic acid (**18**). To prepare the epimeric amino acid derivative **28**, the configuration at C(6) of **4** was inverted by a Mitsunobu reaction. The same sequence of reactions was applied to the so-obtained D-*glycero* isomer **5** and methyl 6-(*N*-acetylamino)-6-deoxy-L-*glycero*-α-D-*gluco*-heptopyranosiduronic acid (**28**) was obtained. In this case almost identical overall yields were obtained for the two different transformations of the azidoalkyne **20** to compound **28** (62% versus 63%).

Introduction

The C-glycopyranosylated[2] and C-glycofuranosylated[3] α-amino acid substructures **A** and **B** are present in many natural products. Due to the high antibiotic and antifungal activities of these compounds, many synthetic approaches to such structures have been reported. These syntheses generally start from a suitably protected and functionalized carbohydrate derivative on which the amino acid moiety is introduced[4] except in one case where the three stereogenic centers of the ribofuranose unit were built from a natural L-amino acid.[5] Nevertheless, there is no general and fully stereocontrolled method for the synthesis of such structures (Chart 1).

As part of a program devoted to the synthesis of C-glycosyl amino acids of biological importance we became interested in the synthesis of amipurimycin[2a] and miharamycins[2b] in which a 6-amino-6-deoxyhepturonic acid is present and we examined the stereocontrolled ethynylation of hexodialdo-1,5-pyranose derivatives with two objectives in mind.[6,7] First, a direct and stereoselective route to 6-amino-6-deoxyhepturonic acids of predictable stereochemistry after adequate functionality adjustments had to be found. Second, this could lead to an unambiguous way of determination of the absolute configuration at C(6) of these compounds which could not be assigned in the first structure determination.[2]

Chart 1



Miharamycin A : X = OH
Miharamycin B : X = H

® Abstract published in *Advance ACS Abstracts,* January 1, 1995.

(1) Part 4: Czernecki, S.; Valéry, J. M. *J. Carbohydr. Chem.* **1990**, *9*, 767.

(2) Representative examples: (a) Amipurimycin: Goto, T.; Toya, Y.; Ohgi, T.; Konto, T. *Tetrahedron Lett.* **1982**, *23*, 1271. (b) Miharamycins: Seto, H.; Koyama, M.; Ogino, H.; Tsurnoka, T. *Tetrahedron Lett.* **1983**, *24*, 1805.

(3) Polyoxins: (a) Isono, K.; Asahi, K.; Suzuki, S. *J. Am. Chem. Soc.* **1969**, *91*, 7490. (b) Isono, K.; Suzuki, S. *Heterocycles* **1979**, *13*, 333.

(4) (a) Naka, T.; Hashizune, T.; Nishimura, M. *Tetrahedron Lett.* **1971**, 95. (b) Ohrni, H.; Kuzuhara, H.; Emoto, S. *Tetrahedron Lett.* **1971**, 4267. (c) Damodaran, N. P.; Jones, G. H.; Moffatt, J. G. *J. Am. Chem. Soc.* **1971**, 93, 3812. (d) Paulsen, H.; Brieden, M.; Sinnwell, V. *Liebigs Ann. Chem.* **1985**, 113. (e) Czernecki, S.; Dieulesaint, A.; Valéry J. M. *J. Carbohydr. Chem.* **1986**, *5*, 469. (f) Bessodes, M.; Komiotis, D.; Antonakis, K. *J. Chem. Soc. Perkin Trans. 1* **1989**, 41.

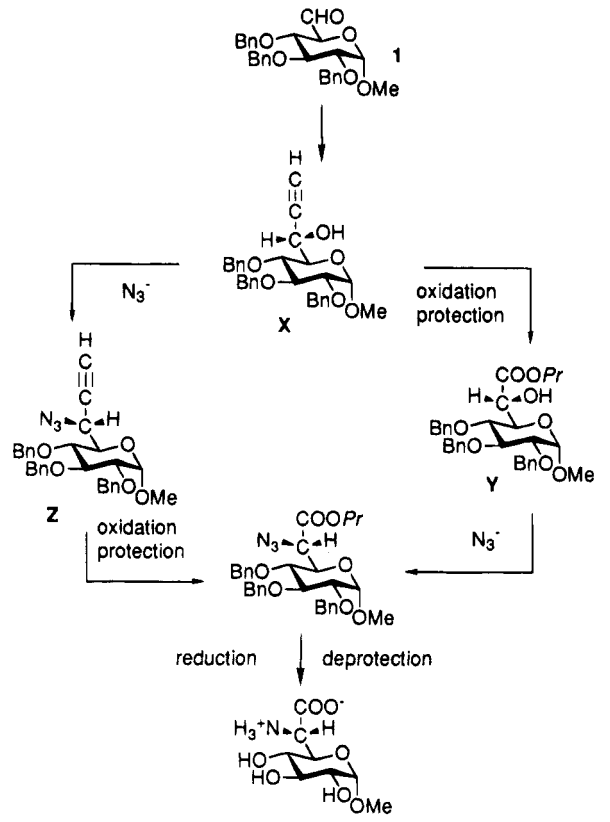
(5) Garner, P.; Park, J. M. *Tetrahedron Lett.* **1989**, *30* , 5065.

(6) Czernecki, S.; Valéry, J. M. *J. Carbohydr. Chem.* **1988**, *7*, 151.

(7) Czernecki, S.; Valéry, J. M. *J. Carbohydr. Chem.* **1989**, *8*, 793.

0022-3263/95/1960-0650$09.00/0

Scheme 1



Pr = protecting group

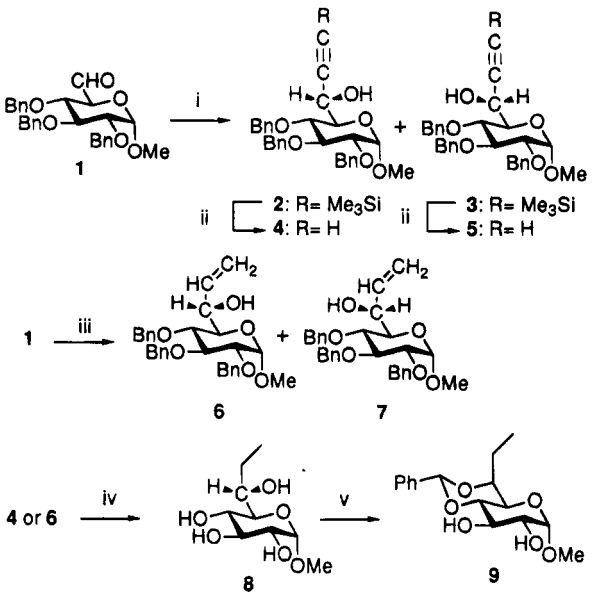
We report herein the syntheses of the D- and L-glycero isomers of the 6-amino-6-deoxyhepturonic acid present in the miharamycins.

The amino acid group was introduced by stereocontrolled ethynylation of methyl 2,3,4-tri-*O*-benzyl-α-D-*gluco*-hexodialdo-1,5-pyranoside **1** to **X**, followed by oxidative cleavage of the triple bond which would afford the carboxylic acid **Y**. The amino function could then be introduced by S_N2 reaction of **Y** with azide ion after activation of the hydroxyl group. Alternatively, cleavage of the triple bond could be performed on **Z** which would be obtained by prior azidation of **1** (Scheme 1).

Results and Discussion

When the dialdosugar **1**[8] was treated with an excess of the Grignard reagent of (trimethylsilyl)acetylene in diethyl ether in the presence of anhydrous magnesium bromide at low temperature (see Experimental Section), **2** (82%) and **3** (5%) were isolated (Scheme 2). The experimental conditions for the condensation are of prime importance to obtain a high diastereoselectivity. The importance of excess magnesium bromide to ensure an efficient chelation of the carbonyl group with O(5) was previously demonstrated.[6] When a more basic solvent such as THF is employed, the chelation is less efficient and the diastereoselectivity is lower.[6,9] Desilylation with tetrabutylammonium fluoride was almost quantitative, and acetylenic alcohols **4** and **5** were obtained (78 and 4% respectively, from **1**).

Scheme 2



[a] Reagents and conditions: (i) $MgBr_2$, 8 equiv, − Et_2O then $Me_3SiC{\equiv}CMgBr$, 4 equiv; (ii) nBu_4NF, THF (82%); (iii) CH2≡CHMgBr, THF (69%); (iv) H_2, Pd/C, MeOH (95%); (v) $PhCH(OMe)_2$, TsOH, DMF (79%).

In analogy to our previous results,[6] the L-*glycero* configuration at C(6) was expected for the major isomer **4** (mp 75 °C, $[\alpha]_D$ +9°, *c* 1, $CHCl_3$). Since the D-*glycero* configuration was assigned to the same compound (mp 75 °C; $[\alpha]_D$ +11°, *c* 2, $CHCl_3$) obtained in a similar way,[9] an unambiguous structure determination was needed for **4** at this stage of our work.

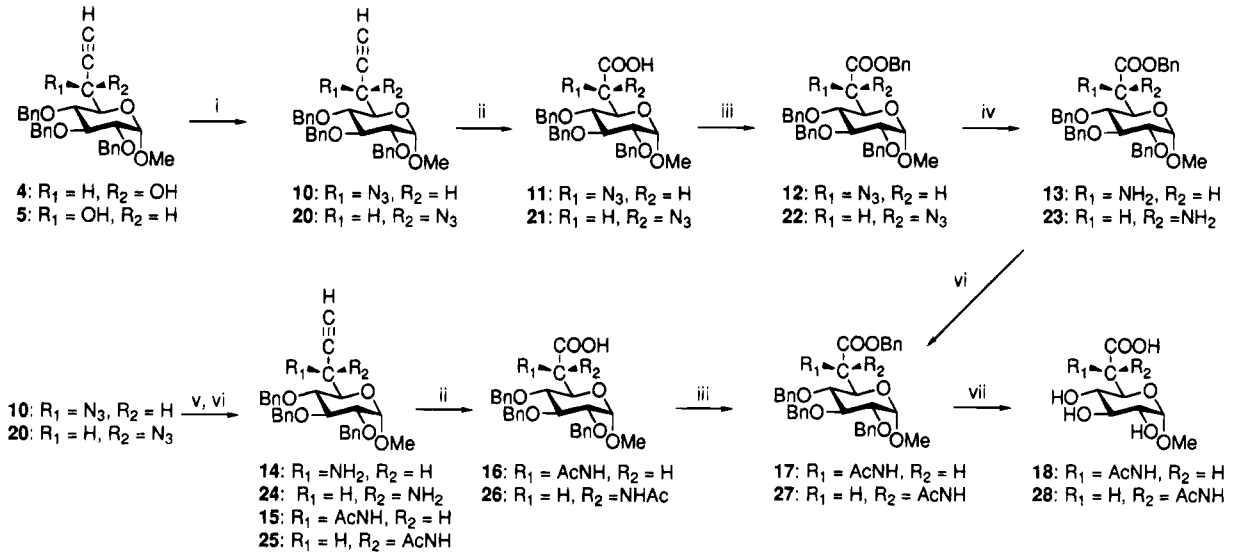
A first indication of the stereochemical outcome of the addition was obtained by comparison with the vinylation of **1** in THF which afforded the two allylic alcohols **6** and **7** with moderate diastereoselectivity (2:1). Since the vinylation of similar hexodialdo-1,5-pyranoses was found to give predominantly the L-*glycero* configuration at C(6),[10,11] the same configuration could be assigned to the major isomer **6**. Furthermore, **6** was obtained by reduction of **4** with lithium aluminohydride.

Finally, the L-*glycero* configuration at C(6) of compounds **4** and **6** was proven by the sequence of reactions depicted in Scheme 2: hydrogenation of **4** and **6** and debenzylation affording **8**, followed by formation of a 4,6-*O*-benzylidene acetal in order to lock the conformation of the side-chain. The ^3J coupling constant between H(5) and H(6) ($J_{5,6}$ = 5.9 Hz) of **9** is too small for a diaxial relationship between these two protons: consequently, H(6) is equatorial which corresponds to the L-*glycero* configuration at C(6).

Since the azido group is readily reduced but rather stable in the presence of oxidants, we decided to introduce it early in the synthesis. The replacement of the hydroxyl group was done under Mitsunobu conditions in presence of zinc azide,[12] and **10** was obtained in high yield (Scheme 3). No propargyl−allenyl transposition occurred during the reaction as indicated by the presence of the acetylenic proton in the IR (ν = 3305 cm^{-1}) and ^1H NMR spectra (δ = 2.58). When the allylic alcohol **6** was treated under

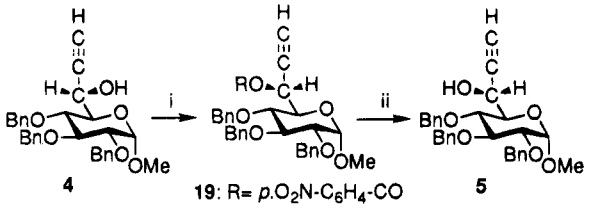
(8) Hashimoto, H.; Asano, K.; Fuji, F.; Yoshimura, J. *Carbohydr. Res.* **1982**, *104*, 87.
(9) Jarosz, S.; Glodek, J.; Zamojski, A. *Carbohydr. Res.* **1987**, *163*, 289.
(10) Jarell, H. C.; Szarek, W. A. *Can. J. Chem.* **1979**, *57*, 924.
(11) Dasser, M.; Chrétien, F.; Chapleur, Y. *J. Chem. Soc., Perkin Trans. 1* **1990**, 3091.
(12) Viaud, M. C.; Rollin, P. *Synthesis* **1990**, 130.

Scheme 3



[a] Reagents and conditions: (i) DIAD, $Zn(N_3)_2$·2py. PPh_3, PhMe (75%); (ii) OsO_4, $NaIO_4$, THF–H_2O; (iii) $PhCHN_2$, CH_2Cl_2 (49% from **10**; 58% from **15**); (iv) H_2, Raney Ni, THF (83%); (v) $HS(CH_2)_3SH$, Et_3N, MeOH; (vi) Ac_2O, pyridine (86% from **10**; quantitative from **13**); (vii) H2 Pd/C, MeOH (quantitative).

Scheme 4



[a] Reagents and conditions: (i) p-$O_2NC_6H_4COOH$, DEAD, PPh_3 THF (95%); (ii) K_2CO_3, MeOH (95%).

the same conditions a mixture of two isomeric allylic azides was obtained resulting from partial allylic rearrangement during the Mitsunobu reaction.

Two routes were evaluated for the transformation of **10** into the protected amino acid **17**. Mild conditions were chosen to avoid epimerization at C(6) and ensure stereospecific transformations which would afford a single stereoisomer.

Oxidative cleavage of a triple bond by ruthenium tetraoxide leading to a carboxylic acid was already reported[13] but this reagent was not compatible with benzyl ethers as protecting groups. When osmium tetraoxide was employed in the presence of sodium periodate, a system generally employed to cleave double bonds,[14] no side reaction with the azido group or benzyl ethers was observed. The carboxylic acid **11** was not isolated but directly reacted with phenyldiazomethane[15] and transformed into a benzyl ester **12** (49% from **10**). The benzyl ester was chosen for a convenient final deprotection. Reduction of the azido group was achieved without debenzylation in the presence of Raney nickel and afforded **13**. The presence of free amino group in **13** could allow the introduction of the other amino acid present in miharamycin. This sequence of reactions is of particular interest because in other natural compounds of this type (amipurimycin,[2a] polyoxins[3]) another amino acid is usually connected to this nitrogen atom. Acetylation of **13** gave **17** which was also obtained by the second route.

In this case selective reduction of the azido group with preservation of the triple bond was achieved by treatment of **10** with excess of 1,3-propanedithiol in the presence of triethylamine,[15] affording **14** which was acetylated to **15**. Oxidative cleavage of the triple bond as described for **10** gave the carboxylic acid **16**. For convenient workup and purification, **16** was directly transformed into its benzyl ester **17** with phenyldiazomethane in 58% yield. Debenzylation of **17** to **18** was quantitative.

The epimeric amino acid **28** could be prepared by the same sequence of reactions carried out from **5**. Since this compound was obtained in too small quantity by ethynylation of **1**, we decided to prepare it from **4** by inversion of configuration at C(6). This was possible by a Mitsunobu reaction[17] on **4** with p-nitrobenzoic acid followed by saponification, and **5** was obtained in high yield (Scheme 4).

The reaction pathway depicted in Scheme 3 was used to convert the D-*glycero*- acetylenic alcohol **5** into the *N*-acetylamino acid **28**. Interestingly, the oxidative cleavage of the triple bond led to better yields when it was performed in this series. So the 6-azido-6-deoxybenzyl ester **22** was obtained in 75% yield from **20** and by following the second route the cleavage of the triple bond of **25** afforded **27** in 82% yield. Further debenzylation gave **28** in 93% yield.

At this stage the comparison of the ^1H NMR spectra of the epimeric amino acids **18** and **28** revealed significant changes in the chemical displacement of H-4 and H-5. The chemical shift of H-4 of the D-*glycero*-isomer **18** was 3.82 ppm whereas the corresponding signal for **28** appeared at 3.37 ppm. The situation is reversed for the H-5 resonance which was slightly more deshielded for the L-*glycero*-epimer **28** (4.21 ppm) than for compound **18** (4.02 ppm). These features can be related to those

(13) Gopal, J.; Gardon, A. J. *Tetrahedron Lett.* **1971**, 2941.
(14) Pappo, R.; Allen, D. S., Jr.; Lemieux, R. U.; Johnson, W. S. *J. Org. Chem.* **1956**, *21*, 478.
(15) Jonezyk, A.; Wlostowska, J. *Synth. Commun.* **1978**, *8*, 569.
(16) Bayley, H.; Standring, D. N.; Knowles, J. R. *Tetrahedron Lett.* **1978**, 3633.
(17) Mitsunobu, O. *Synthesis* **1981**, 1.

which were previously obtained from the α-anomers of the epimeric 6-acetamido-6-deoxyhepturonic acids which were prepared by by different pathways.[4d,f]

Experimental Section

Melting points were measured with a Thomas-Hoover apparatus and are uncorrected. IR spectra were recorded with a Unicam spectrometer. ^{1}H NMR spectra were recorded on Bruker AM 200 or AM 250 spectrometers. 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 60F-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 (300–400) and anhydrous solvents were employed for flash chromatography. Elemental analyses were performed at the Service de microanalyse of Pierre et Marie Curie University.

Methyl 2,3,4-Tri-*O*-benzyl-7,8-dideoxy-L-*glycero*- and D-*glycero*-D-*gluco*-oct-7-ynopyranosides (4 and 5). The reaction was carried out under argon. Two batches of anhydrous $MgBr_2$ (24 mmol each) were prepared from magnesium (0.6 g, 24 mmol) and 1,2-dibromoethane (2.1 mL, 24 mmol) in ether (15 mL) at rt. In a separate flask, (trimethylsilyl)-acetylene (1.92 mL, 13.6 mmol) was added dropwise to a cooled (−5 °C) solution of n-BuLi (7.8 mL of 1.6 M solution in hexane diluted with 15 mL of ether). After being stirred for 30 min, this mixture was added to one batch of freshly prepared anhydrous $MgBr_2$. The resulting white suspension was cooled to −30 °C. The second batch of $MgBr_2$ (24 mmol) was added to dialdosugar **1** (1.43 g, 3.09 mmol) dissolved in ether (70 mL), and the above suspension was added to this mixture at −30 °C. The temperature of the reaction mixture was allowed to attain room temperature before careful hydrolysis by cold saturated aqueous NH_4Cl (50 mL). After decantation, the aqueous phase was extracted with ether (2 × 50 mL), and the combined organic layers were washed with H_2O to neutral pH and dried ($MgSO_4$). Evaporation of the solvent afforded a syrup. Flash chromatography (EtOAc/hexane, 1:4.5 → 1:3) afforded successively the trimethylsilyl derivatives **2** (1.414 g, 82%) and **3** (95 mg, 5.5%) as colorless syrups which were separately desilylated.

Desilylation. To a solution of **2** (1.414 g, 2.52 mmol) in THF (10 mL), was added a solution of Bu_4NF in THF (1.1 M, 4 mL) at rt. After completion of the reaction (15 min at rt), THF was evaporated and the reaction mixture partitioned between water and toluene (70 mL, 2:5). The aqueous layer was extracted with toluene (2 × 10 mL). The organic phase was dried ($MgSO_4$) and evaporated to give a white foam. Flash chromatography (eluting with EtOAc–hexane 1:2) afforded **4** as white crystals (1.180 g, 96%). An analytical sample was obtained by recrystallization from ether–hexane: mp 75 °C; $[\alpha]_D$ +9° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.38 (br s, 1H, OH), 2.43 (d, 1H, $J_{6,8}$ = 2.2 Hz), 3.40 (s, 3H), 3.52 (dd, 1H, $J_{1,2}$ = 3.6 and $J_{2,3}$ = 9.6 Hz), 3.63–3.75 (m, 2H), 4.02 (m, 1H), 4.60 (m, 1H), 4.62 (d, 1H, $J_{1,2}$ = 3.6 Hz), 4.73 (AB, 2H, J_{AB} = 12.1 Hz), 4.79 (AB, 2H, J_{AB} = 10.9 Hz), 4.91 (AB, 2H, J_{AB} = 10.9 Hz), 7.24–7.40 (m, 15H). Anal. Calcd for $C_{30}H_{32}O_6$: C, 73.75; H, 6.60. Found: C, 73.61; H, 6.66.

Epimer **3** (95 mg, 0.17 mmol) was desilylated under the same conditions. Flash chromatography afforded **5** (61 mg, 74%). An analytical sample was obtained by recrystallization from ether–hexane: mp 86–87 °C; $[\alpha]_D$ +41° (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.50 (d, 1H, $J_{6,8}$ = 2.2 Hz), 2.56 (d, 1H, $J_{6,OH}$ = 9.5 Hz), 3.41 (s, 3H), 3.59 (dd, 1H, $J_{1,2}$ = 3.4 and $J_{2,3}$ = 9.6 Hz), 3.63 (dd, 1H, $J_{3,4}$ = 8.9 and $J_{4,5}$ = 10.0 Hz), 3.88 (dd, 1H, $J_{5,6}$ = 3.2 Hz), 4.05 (dd, 1H, $J_{2,3}$ = 9.6 and $J_{3,4}$ = 8.9 Hz), 4.63–5.04 (m, 8H), 7.26–7.42 (m, 15H). Anal. Calcd for $C_{30}H_{32}O_6$: C, 73.75; H, 6.60. Found: C, 73.65; H, 6.59.

Methyl 2,3,4-Tri-*O*-benzyl-7,8-dideoxy-L-*glycero*- and D-*glycero*-α-D-*gluco*-oct-7-enopyranosides (6 and 7). Under argon, to a cooled (-65 °C) solution of **1** (231 mg, 0.50 mmol) in THF (5 mL) was added vinylmagnesium bromide (0.74 M solution in THF, 3.4 mL). The mixture was allowed to warm to 0 °C (2 h) and left overnight at rt. After hydrolysis with saturated aqueous NH_4Cl (10 mL) and decantation, the aqueous phase was extracted with ether (2 × 10 mL). The combined organic layers were dried ($MgSO_4$) and the solvent evaporated. Flash chromatography (elution with EtOAc–hexane, 1:3.5) afforded **6** (114 mg, 46%) as an oil: $[\alpha]_D$ +1.2° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.32 (s, 3H), 3.50 (dd, 1H, $J_{1,2}$ = 3.6 and $J_{2,3}$ = 9.6 Hz), 3.60 (dd, 1H, $J_{4,5}$ = 9.9 and $J_{5,6}$ = 1.2 Hz), 3.69 (dd, 1H, $J_{3,4}$ = 8.4 Hz), 4.00 (dd, 1H, $J_{2,3}$ = 9.6 and $J_{3,4}$ = 8.4 Hz), 4.37 (m, 1H), 4.56 (d, 1H, $J_{1,2}$ = 3.6 Hz), 4.72 (AB, 2H, J_{AB} = 11.9 Hz), 4.76 (AB, 2H, J_{AB} = 10.8 Hz), 4.96 (AB, 2H, J_{AB} = 10.9 Hz), 5.19 (ddd, 1H, $J_{6,8a}$ = 1.4, J_{8ab} = 1.4 and $J_{7,8a}$ = 10.5 Hz), 5.32 (ddd, 1H, $J_{6,8b}$ = 1.4 and $J_{7,8b}$ = 17.2 Hz), 5.94 (ddd, 1H, $J_{6,7}$ = 5.1 Hz), 7.22–7.43 (m, 15H). Anal. Calcd for $C_{30}H_{34}O_6$: C, 73.45; H, 6.93. Found: C, 73.38; H, 7.08.

Further elution with EtOAc–hexane (1:2.5) afforded **7** (56 mg, 23%) as white crystals: mp 84 °C; $[\alpha]_D$ +19.6° (*c* 1, $CHCl_3$) ^{1}H NMR ($CDCl_3$) δ 3.38 (s, 3H), 3.44 (dd, 1H, $J_{3,4}$ = 8.8 and $J_{4,5}$ = 10.0 Hz), 3.49 (dd, 1H, $J_{1,2}$ = 3.6 and $J_{2,3}$ = 9.6 Hz), 3.79 (dd, 1H, $J_{5,6}$ = 3.8 Hz), 4.03 (dd, 1H, $J_{2,3}$ = 9.6 and $J_{3,4}$ = 8.8 Hz), 4.34 (m, 1H), 4.59 (d, 1H), 4.71 (AB, 2H, J_{AB} =12.3 Hz), 4.76 (AB, 2H, J_{AB} = 10.9 Hz), 4.90 (AB, 2H, J_{AB} = 10.9 Hz), 5.17–5.30 (m, 2H), 5.91 (ddd, 1H, $J_{6,7}$= 6.7, $J_{7,8a}$= 10.4 and $J_{7,8b}$ = 17.2 Hz), 7.22–7.45 (m, 15H). Anal. Calcd for $C_{30}H_{34}O_6$: C, 73.45; H, 6.93. Found: C, 73.28; H, 7.11.

Methyl 4,6-*O*-Benzylidene-7,8-dideoxy-L-*glycero*-α-D-*gluco*-octopyranoside (9). To a solution of **4** (50 mg, 0.1 mmol) in methanol (3 mL) was added 10% palladium on charcoal (30 mg), and the mixture was stirred under hydrogen (1 atm). After completion of the reaction (2 h at rt), filtration and evaporation of the solvent afforded **8** as white crystals (21.2 mg, 95%) which were dissolved in anhydrous DMF (1 mL), and benzaldehyde dimethyl acetal (90 μL, 0.6 mmol) and *p*-toluenesulfonic acid (3 mg) were added. After the mixture was stirred at rt until the reaction was complete (2 days), the acid was neutralized with Et_3N (4 drops), and the solvent and excess of reagent were evaporated under reduced pressure (1 Torr). Purification through preparative thin layer chromatography using EtOAc–hexane (3:1) as eluent afforded **9** (23.2 mg, 79%) as white crystals: mp 168 °C; $[\alpha]_D$ + 40.8° (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.07 (t, 3 H, *J* = 7.3 Hz), 1.70 (ddq, 1H, $J_{6,7a}$ = 3.8 and $J_{7a,b}$ = 15.1 Hz), 2.06 (ddq, 1H, $J_{6,7b}$ = 10.8 Hz), 2.25 (d, 1H, $J_{2,OH}$ = 9.1 Hz), 2.72 (s, 1 H), 3.44 (s, 3 H), 3.57 (ddd, 1H, $J_{1,2}$ = 3.8 and $J_{2,3}$ = 8.7 Hz), 3.72 (dd, 1H, $J_{3,4}$ = 9.2 and $J_{4,5}$ = 9.7 Hz), 3.90 (dd, 1H, $J_{2,3}$ = 8.7 and $J_{3,4}$ = 9.2 Hz), 4.06 (dd, 1H, $J_{5,6}$ = 5.9 Hz), 4.17 (ddd, 1H), 4.79 (d, 1 H), 5.74 (s, 1H), 7.32–7.40 (m, 3H), 7.46–753 (m, 2 H). Anal. Calcd for $C_{16}H_{22}O_6$: C, 61.92; H, 7.15. Found: C, 61.96; H, 7.16.

The same procedure applied to **6** (60 mg, 0.12 mmol) afforded **9** (30.2 mg, 80%).

Methyl 6-Azido-2,3,4-tri-*O*-benzyl-6,7,8-trideoxy-D-*glycero*-α-D-*gluco*-oct-7-ynopyranoside (10). To a solution of **4** (171 mg, 0.35 mmol) and Ph_3P (184 mg, 0.70 mmol) in anhydrous toluene (2 mL) under an atmosphere of argon were successively added $Zn(N_3)_2$·2 pyr (81 mg, 0.263 mmol) and diisopropyl azodicarboxylate (DIAD) (138 μL, 0.70 mmol) according to ref 12. After completion of the reaction (30 min at rt), Et_2O (15 mL) was added and the suspension was filtered through a small column of silica gel (2 g) to remove polar impurities. Flash chromatography (elution with EtOAc–hexane, 1:8) afforded **10** as white crystals (135 mg, 75%): mp 64 °C; $[\alpha]_D$ +39.1° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.58 (br s, 1H), 3.43 (s, 3H), 3.55–3.65 (m, 2H), 3.85 (d, 1H, $J_{4,5}$ = 9.9 Hz), 3.99 (t, 1H, *J* = 9.2 Hz), 4.27 (br s, 1H), 4.70–5.00 (m, 7H), 7.22–7.40 (m, 15H). Anal. Calcd for $C_{30}H_{31}N_3O_5$: C, 70.16; H, 6.08; N, 8.18. Found: C, 70.33; H, 6.12; N, 8.08.

Methyl 6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-D-*glycero*-α-D-*gluco*-heptopyranosiduronic Acid Benzyl Ester (12). To a stirred solution of **10** (77 mg, 0.15 mmol) in THF–H_2O (3 mL, 1:1) were successively added an aqueous solution of OsO_4 (4%, 200 μL, 3 μmol) and $NaIO_4$ (160 mg, 0.75 mmol). The mixture was stirred at 45 °C until completion of the reaction (5 h). A 10% aqueous solution of $NaHSO_3$ (10 mL)

and CH_2Cl_2 (10 mL) was added. The mixture was stirred at rt for 30 min. After decantation, the aqueous layer was extracted with CH_2Cl_2 (5 × 10 mL). The organic phase was dried ($MgSO_4$) and evaporated to give a black syrup. The crude product was dissolved in CH_2Cl_2 (3 mL), and a solution of phenyldiazomethane[15] in CH_2Cl_2 was added until completion of esterification (≈0.5 mL). The excess $PhCHN_2$ was destroyed with formic acid (1 drop), and the solvent was evaporated. After coevaporation with toluene (3 × 1 mL), the crude residue was purified by flash chromatography. Eluting with EtOAc/hexane (1:7 then 1:5) afforded **12** as a syrup (45.5 mg, 49%): $[\alpha]_D$ +14.8° (*c* 1, $CHCl_3$); ^1H NMR (C_6D_6) δ 3.12 (s, 3H), 3.38 (dd, 1H, $J_{1,2}$= 3.7 and $J_{2,3}$ = 9.7 Hz), 3.91 (dd, 1 H, $J_{3,4}$ = 8.6 and $J_{4,5}$ = 10.0 Hz), 4.03 (d, 1H, $J_{5,6}$ = 1.9 Hz), 4.14 (dd, 1H, $J_{2,3}$ = 9.7 and $J_{3,4}$ = 8.6 Hz), 4.33 (m, 1H), 4.39 (AB, 2H, J_{AB} = 12.0 Hz), 4.48 (d, 1H), 4.68–5.04 (m, 6H), 6.96–7.28 (m, 20H). Anal. Calcd for $C_{36}H_{37}N_3O_7$: C, 69.33; H 5.98; N. 6.74. Found: C, 69.38; H, 6.13; N, 6.60.

Methyl 6-(*N*-Acetylamino)-2,3,4-tri-*O*-benzyl-6-deoxy-D-*glycero*-α-D-*gluco*-heptopyranosiduronic Acid Benzyl Ester (17) from 12. To a suspension of Raney nickel (1 g) in THF (5 mL) was added **12** (93.5 mg, 0.15 mmol) under stirring. The mixture was stirred under an atmosphere of hydrogen until completion of the reaction (20 h). After filtration on celite and evaporation of the solvent, crude **13** was dissolved in pyridine (0.5 mL), and Ac_2O (50 μL, 50 μmol) was added. After completion of the reaction (30 min at rt), evaporation of the solvent and coevaporation of the residue with toluene (4 × 1 mL) afforded crude **17** which was purified by flash chromatography. Elution with EtOAc/hexane (1.5:1) afforded **17** (81 mg, 84%): $[\alpha]_D$ +27.7° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.94 (s, 3H), 3.33 (m, 4H), 3.62 (dd, 1H, $J_{3,4}$ = 8.9 and $J_{4,5}$ = 10.2 Hz), 3.90–4.00 (m, 2H), 4.53 (d, 1H, $J_{1,2}$ = 3.6 Hz), 4.69 (AB, 2H, J_{AB} = 12.5 Hz), 4.77 (AB, 2H, J_{AB} = 10.9 Hz), 4.86 (AB, 2H, JAB = 10.9 Hz), 5.05 (dd, 1H, $J_{5,6}$ = 2.9 and $J_{6,NH}$ = 8.2 Hz), 5.16 (AB, 2 H, J_{AB} = 11.9 Hz, 6.22 (d, 1H, $J_{6,NH}$ = 8.2 Hz), 7.22–7.40 (m, 20H). Anal. Calcd for $C_{38}H_{41}NO_8$: C, 71.34; H, 6.46; N, 2.19. Found: C, 70.59; H, 6.58; N, 2.26.

Methyl 6-(*N*-Acetylamino)-2,3,4-tri-*O*-benzyl-6,7,8-trideoxy-D-*glycero*-α-D-*gluco*-oct-7-ynopyranoside (15). To a solution of **10** (51.5 mg, 0.1 mmol) in MeOH (2 mL) under an atmosphere of argon were added 1,3-propanedithiol (50 μL, 0.5 mmol) and Et_3N (70 μL, 0.5 mmol) according to ref 15. After completion of the reaction (24 h at 45 °C), the solvent was evaporated. After coevaporation of the residue with toluene (4 × 5 mL), the residue was dissolved in pyridine (2 mL) and Ac_2O (200 μL, 2.1 mmol) was added. After completion of acetylation (3 h at rt), evaporation of the solvent, and coevaporation with toluene (4 × 5 mL), the crude product was purified by flash chromatography. Elution with EtOAc–hexane (3:2) afforded **15** as white crystals (45.6 mg, 86%): mp 136 °C; $[\alpha]_D$ -7.0° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.99 (s, 3H), 2.29 (d, 1H, $J_{6,8}$ = 1.7 Hz), 3.36 (s, 3H), 3.57 (dd, 1H, $J_{1,2}$ = 3.6 and $J_{2,3}$ = 9.6 Hz), 3.68 (dd, 1H, $J_{3,4}$ = 7.9 and $J_{4,5}$ = 10.0 Hz), 3.75 (dd, 1H, $J_{5,6}$ = 2.8 Hz), 4.02 (dd, 1H, $J_{2,3}$ = 9.6 and $J_{3,4}$ = 7.9 Hz), 4.64–5.01 (m, 7H), 5.33 (ddd, 1H, $J_{6,NH}$ = 9.2 Hz), 6.00 (d, 1H), 7.28–7.41 (m, 15H). Anal. Calcd for C_{32} $H_{35}NO_6$: C, 72.57; H, 6.66; N, 2.64. Found: C, 72.49; H, 6.75; N, 2.69.

Methyl 6-(*N*-Acetylamino)-2,3,4-tri-*O*-benzyl-6-deoxy-D-*glycero*-α-D-*gluco*-heptopyranosiduronic Acid Benzyl Ester (17) from 15. Treatment of **15** (26.5 mg, 50 μmol) by OsO_4 and $NaIO_4$ followed by esterification with phenyldiazomethane as described for **10** afforded **17** (18.4 mg, 58%).

Methyl 6-(*N*-Acetylamino)-6-deoxy-D-*glycero*-α-D-*gluco*-heptopyranosiduronic Acid (18). To a solution of **17** (18.4 mg, 29 μmol) in MeOH (2 mL) was added 10% Pd/C (10 mg). The mixture was stirred under hydrogen (1 atm). After completion of the reaction (2 h at rt), filtration, and evaporation of the solvent, **18** was obtained as a syrup (7.7 mg, 95%): $[\alpha]_D$ +168.3° (*c* 1, MeOH); ^1H NMR (D_2O) δ 2.17 (s, 3H), 3.51 (s, 3H), 3.62 (dd, 1H, $J_{1,2}$ = 3.6 Hz, H-2), 3.69–3.83 (m, 2H), 4.02 (dd, 1H, $J_{4,5}$ = 9.6 and $J_{5,6}$ = 2.8 Hz), 4.87 (d, 1H, $J_{1,2}$ = 3.6 Hz), 5.00 (d, 1H). Anal. Calcd for $C_{10}H_{17}NO_8$: C, 43.01; H, 6.14; N, 5.02. Found: C, 42.82; H, 6.26; N, 5.07.

Methyl 2,3,4-Tri-*O*-benzyl-7,8-dideoxy-6-*O*-(*p*-nitrobenzoyl)-D-*glycero*-α-D-*gluco*-hept-7-ynopyranoside (19). To a solution of **4** (489 mg, 1.0 mmol) and Ph_3P (525 mg, 2.0 mmol) in anydrous THF (2.5 mL) were successively added *p*-nitrobenzoic acid (334 mg, 2.0 mmol) and diethylazodicarboxylate (315 μL, 2.0 mmol) under stirring at rt. After completion of the reaction (15 h at rt), THF was evaporated and the residue purified by flash chromatography. Elution with EtOAc/hexane (1:4.5 → 1:4) afforded **19** as a syrup (589 mg, 92%): $[\alpha]_D$ +7.6° (*c* 1.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.55 (d, 1H, $J_{6,8}$ = 2.2 Hz), 3.38 (s, 3H), 3.62 (dd, 1H, $J_{1,2}$ = 3.6 and $J_{2,3}$ = 9.6 Hz), 3.70 (m, 1H), 4.01–4.11 (m, 2H), 4.65–5.05 (m, 7H), 6.03 (dd, 1H, $J_{5,6}$ = 2.5 Hz), 7.30–7.41 (m, 15H), 8.19–8.31 (m, 4H). Anal. Calcd for $C_{37}H_{35}NO_9$: C, 69.69, H, 5.53, N, 2.20. Found: C, 69.62, H, 5.61; N, 2.20.

Methyl 2,3,4-Tri-*O*-benzyl-7,8-dideoxy-D-*glycero*-α-D-*gluco*-oct-7-ynopyranoside (5) from 19. To a solution of **19** (695 mg, 1.09 mmol) in MeOH (20 mL) was added K_2CO_3 (15 mg, 0.11 mmol), and the mixture was stirred at rt. After completion of the reaction (20 min at rt), the solvent was evaporated and the product isolated by flash chromatography. Elution with EtOAc/hexane (1:4 to remove methyl *p*-nitrobenzoate then 1:1) afforded **5** as white crystals (495 mg, 93%): mp 87 °C.

Methyl 6-Azido-2,3,4-tri-*O*-benzyl-6,7,8-trideoxy-L-*glycero*-α-D-glucooct-7-ynopyranoside (20). Treatment of **5** (489 mg, 1.0 mmol) with Zn $(N_3)_2$·2 pyr under the conditions described for **4** and flash chromatography (EtOAc/hexane, 1:6) afforded **20** as a syrup (397 mg, 77%): $[\alpha]_D$ +88.2° (*c* 1, CHCl3); ^1H NMR (C_6D_6) δ 2.07 (d, 1H, $J_{6,8}$ = 2.3 Hz), 3.23 (s, 3H), 3.53 (dd, 1H, $J_{1,2}$ = 3.6 and $J_{2,3}$ = 9.6 Hz), 3.77 (dd, 1H, $J_{3,4}$ = 8.8 and $J_{4,5}$ = 9.8 Hz), 3.93 (dd, 1H, $J_{5,6}$ = 1.7 Hz), 4.18 (dd, 1H), 4.33 (dd, 1H), 4.44 (AB, 2H, J_{AB} = 11.9 Hz), 4.66 (d, 1H, $J_{1,2}$ = 3.6 Hz), 4.78 (AB, 2H, J_{AB} = 11.3 Hz), 4.90 (AB, 2H, J_{AB} = 11.3 Hz), 7.06–7.39 (m, 15H). Anal. Calcd for $C_{30}H_{31}N_3O_5$: C, 70.16; H, 6.08; N, 8.18. Found: C, 70.14; H, 6.26; N, 8.01.

Methyl 6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-L-*glycero*-α-D-*gluco*-heptopyranosiduronic Acid Benzyl Ester (22). Treatment of **20** (150 mg, 0.29 mmol) with OsO_4 in the presence of $NaIO_4$ under the conditions described for **10** followed by esterification with $PhCHN_2$ and flash chromatography (EtOAc/hexane 1:7) afforded **22** as white crystals (137 mg, 75%): mp 79 °C; $[\alpha]_D$ +56.7° (*c* 1, $CHCl_3$); ^1H NMR (C_6D_6) δ 2.95 (s, 3H), 3.54 (dd, 1H, $J_{1,2}$ = 3.7 and $J_{2,3}$ = 9.7 Hz), 3.88 (dd, 1H, $J_{3,4}$ = 8.8 and $J_{4,5}$ = 10.0 Hz), 4.09 (d, 1H, $J_{5,6}$ = 2.3 Hz), 4.23 (dd, 1H, $J_{2,3}$ = 9.7 and $J_{3,4}$ = 8.8 Hz), 4.42 (AB, 2H, J_{AB} = 12.1 Hz), 4.91 (AB, 2H, J_{AB} = 11.2 Hz), 4.97 (AB, 2H, J_{AB} = 12.0 Hz), 7.01–7.40 (m, 20H). Anal. Calcd for $C_{36}H_{37}N_3O_7$: C, 69.33; H, 5.98; N, 6.74. Found: C, 69.30; H, 5.94; N, 6.71.

Methyl 6-(*N*-Acetylamino)-2,3,4-tri-*O*-benzyl-6,7,8-trideoxy-L-*glycero*-α-D-*gluco*-oct-7-ynopyranoside (25). Treatment of **20** (128 mg, 0.25 mmol) with 1,3-propanedithiol under the conditions described for **10** followed by acetylation and flash chromatography (EtOAc/hexane 1:4 then 2:3) afforded **25** as white crystals (102 mg, 77%): mp 149 °C; $[\alpha]_D$ +8.3° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.87 (s, 3H), 2.26 (d, 1H, $J_{6,8}$ = 2.3 Hz), 3.41 (s, 3H), 3.47 (dd, 1H, $J_{3,4}$ = 8.8 and $J_{4,5}$ = 9.9 Hz), 3.51 (dd, 1H, $J_{1,2}$ = 3.7 and $J_{2,3}$ = 9.6 Hz), 3.77 (dd, 1H, $J_{5,6}$ = 1.5 Hz), 4.03 (dd, 1H), 4.62 (d, 1H, $J_{1,2}$ = 3.7 Hz), 4.74 (AB, 2H, J_{AB} = 12.2 Hz), 4.75 (AB, 2H, J_{AB} = 10.3 Hz), 4.93 (AB, 2H, J_{AB} = 10.8 Hz), 5.24 (ddd, 1H, $J_{6,NH}$ = 10.0 Hz), 6.04 (d, 1H), 7.26–7.43 (m, 15H). Anal. Calcd for $C_{32}H_{35}$-NO_6: C, 72.57; H, 6.66; N, 2.64. Found: C, 72.76; H, 6.68; N, 2.67.

Methyl 6-(*N*-Acetylamino)-2,3,4-tri-*O*-benzyl-L-*glycero*-α-D-*gluco*-heptopyranosiduronic Acid Benzyl Ester (27) from 25. Treatment of **25** (79.5 mg, 0.15 mmol) under the conditions described for **15** and flash chromatography (EtOAc/hexane 1:1.5 then 1:1) afforded **27** as white crystals (79 mg, 82%): mp 142 °C; $[\alpha]_D$ -13.8° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.08 (s, 3H), 2.99 (s, 3H), 3.34 (dd, 1H, $J_{3,4}$ = 8.8 and $J_{4,5}$ = 10.0 Hz), 3.43 (dd, 1H, $J_{1,2}$ = 3.5 and $J_{2,3}$ = 9.7 Hz), 4.02 (dd, 1H), 4.18 (dd, 1H, $J_{5,6}$ = 1.5 Hz), 4.45 (d, 1H), 4.70 (AB, 2H, J_{AB} = 9.8 Hz), 4.71 (AB, 2H, J_{AB} = 12.2 Hz), 4.91 (AB, 2H, J_{AB} = 10.7 Hz), 5.16 (AB, 2H, J_{AB} = 12.1 Hz), 5.23 (dd, 1H, $J_{6,NH}$ = 9.9 Hz), 6.04 (d, 1H, $J_{6,NH}$ = 9.9 Hz), 7.23–7.48 (m, 20H).

Anal. Calcd for $C_{38}H_{41}NO_8$ C, 71.34; H, 6.46; N, 2.19. Found: C, 71.16; H, 6.50; N, 2.19.

Methyl 6-(*N*-Acetylamino)-2,3,4-tri-*O*-benzyl-L-*glycero*-α-D-*gluco*-heptopyranosiduronic Acid Benzyl Ester (27) from 22. Treatment of **22** (25.0 mg, 40 mmol) with Raney nickel followed by acetylation as described for **12** afforded **27** (21.2 mg, 83%).

Methyl 6-(*N*-acetylamino)-6-deoxy-L-*glycero*-α-D-*gluco*-heptopyranosiduronic Acid (28). Treatment of **27** (75.5 mg, 0.12 mmol) under the conditions described for **17** afforded **28** (30.6 mg, 93%) as a syrup: $[\alpha]_D$ +107.5° (*c* 1, MeOH); ^1H NMR (D_2O) 2.15 (s, 3H), 3.37 (dd, 1H, $J_{3,4}$ = 8.8 and $J_{4,5}$ = 10.1 Hz), 3.40 (s, 3H), 3.59 (dd, 1H, $J_{1,2}$ = 3.7 and $J_{2,3}$ = 9.8 Hz), 3.74 (m, 1H, $J_{2,3}$ = 9.8 and $J_{3,4}$ = 8.8 Hz), 4.21 (dd, 1H, $J_{5,6}$ = 2.1 Hz), 4.82 (d, 1H), 4.94 (d, 1H). Anal. Calcd for $C_{10}H_{17}NO_8$: C, 43.01; H, 6.14; N. 5.02. Found: C, 42.87; H, 6.17; N, 4.94.

Acknowledgment. The European Economic Community is acknowledged for financial support (project SCI-CT91-0723).

Supplementary Material Available: Elemental analysis data for **4**–**12**, **15**, **17**–**20**, **22**, **25**, **27**, and **28** (2 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.

JO941205R