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Synthesis of *Pseudomonas aeruginosa* lipopolysaccharide core antigens containing 7-*O*-carbamoyl-L-*glycero*-α-D-*manno*-heptopyranosyl residues☆

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Abstract

The monosaccharide allyl 7-*O*-carbamoyl-L-*glycero*-α-D-*manno*-heptopyranoside, the reducing disaccharide 7-*O*-carbamoyl-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)-L-*glycero*-D-*manno*-heptopyranose and the disaccharides allyl 7-*O*-carbamoyl-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)-L-*glycero*-β- and α-D-*manno*-heptopyranoside were prepared in good yields. The 7-*O*-carbamoyl substituent was regioselectively introduced via NH_3–NH_4HCO_3 treatment of a 6,7-*O*-carbonate group. Glycosylation steps were carried out using Me_3SiOTf or $BF_3{\cdot}Et_2O$ promoted coupling of allyl alcohol with trichloroacetimidate or fluoride glycosyl donors, respectively. The deprotected allyl glycosides were reacted with cysteamine to afford spacer glycosides which were subsequently linked to bovine serum albumin. The artificial antigens which are related to the dephosphorylated heptose region of the lipopolysaccharide core region from *Pseudomonas aeruginosa* classified into RNA group I may be used for the characterization of monoclonal antibodies directed against inner core epitopes of human-pathogenic *Pseudomonas* species.

Keywords: *Pseudomonas aeruginosa*; Lipopolysaccharide; Heptose; Carbamate; Neoglycoprotein

1. Introduction

Lipopolysaccharides (LPS)—also termed endotoxins—are structurally complex amphipathic and microheterogeneous glycolipids located in the external membrane of the outer cell wall of Gram-negative bacteria [1]. LPS are highly potent toxins leading to multiple organ failure as a consequence of Gram-negative sepsis. In this context, *Pseudomonas aeruginosa* has emerged as a common bacterial nosocomial pathogen with increasing resistance to antibiotic treatment, thus causing 10–20% of infections in hospitals at a high mortality rate [2]. LPS of *Pseudomonas* differ from enterobacterial LPS with respect to the reduced chain length of the fatty acids of lipid A, the high number and different types of phosphate residues and the presence of L-alanine amide-linked to galactosamine in the outer core region [3,4]. The structural elucidation of the phosphate substitution pattern and the nature of the individual phosphate substituents, which most likely include triphosphates, diphosphates,

☆ Dedicated to Professor Aleksander Zamojski on the occasion of his 70th birthday.

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2-aminoethyl-diphosphate and phosphate monoesters, respectively, has been partially accomplished [5]. In addition, recent investigations revealed the occurrence of a 7-*O*-carbamoyl residue within the heptose region [6], which is present in rough mutants as well as smooth-type LPS of *Pseudomonas* spp. classified into RNA group I, thereby constituting a taxonomic marker [7]. Furthermore, antibodies against the core and lipid A region have been raised, their immunodominant epitopes have been defined [8,9] and a cross-reactive monoclonal antibody mAb (7-4)—which still retains its reactivity in the presence of O-chains—has been expressed in an *E. coli* system [10]. Consequently, these core-determinants may serve as candidate antigens for the production of vaccines [11]. As a first part of synthetic studies on inner-core LPS determinants of *Pseudomonas* species, we report on the synthesis of the dephosphorylated heptose region comprising 7-*O*-carbamoyl-substituted L-*glycero*-D-*manno*-heptopyranosyl monosaccharide and α-(1→3)-linked disaccharide allyl glycosides. In continuation of previous investigations directed toward the characterization of epitope specificities of monoclonal antibodies against LPS core antigens, the allyl glycosides were converted into neoglycoproteins following introduction of a cysteamine spacer group [12,13].

2. Results and discussion

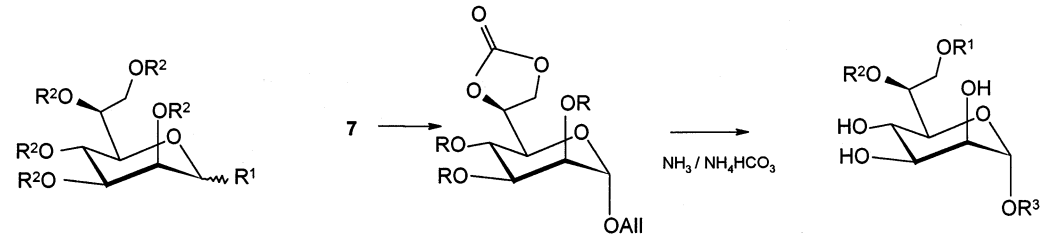
For the synthesis of the monosaccharide allyl 7-*O*-carbamoyl-L-*glycero*-α-D-*manno*-heptapyranoside (**11**) and the disaccharides allyl 7-*O*-carbamoyl-L-*glycero*-α-D-*manno*-heptapyranoside-(1→3)-L-*glycero*-β- and α-D-heptapyranoside (**29** and **30**, respectively), the previously described [14] allyl L-*glycero*-α-D-*manno*-heptopyranoside derivative **5** was employed as the starting material. The reaction of the trichloroacetimidate donor **2**—prepared from the reducing heptopyranose derivative **1** [15]—with allyl alcohol in the presence of trimethylsilyl trifluoromethanesulfonate afforded the allyl α-glycoside **5** in 70% yield and the β isomer **6** in 11% yield, isolated after reacetylation of partially O-deacetylated intermediates. The reduced stereoselectivity of the Me_3SiOTf-mediated glycosylation might be due to enhanced acid-catalyzed transesterification of the 2-*O*-acetyl group with allyl alcohol, thereby eliminating the neighboring group participation for the transition state [16]. Alternatively, the anomeric fluoride donors **3** and **4** were synthesized in 58 and 33% yield, respectively, by treatment of **1** with diethylaminosulfur trifluoride [17]. Under promotion of the glycosylation reaction with $BF_3{\cdot}Et_2O$ using either fluoride donor, the allyl α-glycoside derivative **5** was produced in a similar yield (85%); however, the formation of the β isomer **6** could not be observed by TLC. Zemplén O-deacetylation of the protected monosaccharide derivatives **5** and **6** furnished the crystalline allyl α-glycoside **7** and the β isomer **8** in high yields. Measurement of the heteronuclear coupling constant $J_{C\text{-}1,H\text{-}1}$ for C-1 of compound **8** (162.6 Hz) confirmed the assignment of the anomeric configuration (Scheme 1).

As a suitable precursor for the carbamate moiety, the 6,7-*O*-carbonate group was chosen, which could be used as a temporary protecting group of the side chain diol as well. Thus, by using trichloromethyl chloroformate–*sym*-collidine–oxolane under carefully controlled conditions (slow addition of reagent at −30 °C), the O-deacetylated derivative **7** was converted in a regioselective fashion into the 6,7-*O*-carbonate derivative **9** in 76% yield [18]. The formation of the cyclic diester was evident from the downfield shift of H-6 (5.28 ppm) and the geminal protons at C-7 (4.70 and 4.58 ppm, respectively) and after subsequent O-acetylation of the remaining hydroxy groups with acetic anhydride–pyridine to give the 2,3,4-tri-*O*-acetyl heptopyranoside derivative **10** in quantitative yield. The formation of the 7-*O*-carbamate was achieved in excellent yield (84%) and in high selectivity by treatment of **9** with NH_3–NH_4HCO_3–oxolane to give the crystalline allyl 7-*O*-carbamoyl-L-*glycero*-α-D-*manno*-heptopyranoside (**11**) and a small proportion of the 6-*O*-carbamoyl derivative **12** (9%). In addition, the feasibility of the selective carbamate formation with concomitant hydrolysis of the *O*-acetyl protecting groups was demonstrated for the 2,3,4-tri-*O*-acetyl derivative **10**

as well, which gave **11** in 89% yield and a small proportion (9%) of the 6-*O*-substituted isomer **12**. The structural assignment of **11** was based on the downfield shift observed for C-7 (66.41 ppm) in the ^{13}C NMR spectrum (Table 1). The carbamoyl group at O-7 was prone to slow acyl migration to O-6 also under neutral conditions. Thus, upon standing of an aqueous solution of **11** for 2 weeks at room temperature (rt), formation of compound **12** (in ~10% yield) was evident from the characteristic downfield shift of H-6 (to 5.13 ppm) in the 300 MHz ^1H NMR spectrum.

The allyl glycoside **11** was transformed in high yield into the 3-(2-aminoethylthio)propyl spacer derivative **13** by radical addition of cysteamine hydrochloride under UV-irradiation [13]. The spacer compound **13**, which was isolated as the acetate salt by silica gel chromatography, was subsequently activated with thiophosgene and coupled to the ϵ-amino groups of lysine residues of bovine serum albumin (BSA) to give the neoglycoconjugate **14**.

For the synthesis of the reducing disaccharide derivative **20** (Scheme 2), the previously reported [19] α-benzyl heptofuranoside derivative **15** was subjected to O-deacetylation with sodium methoxide giving **16**, which was converted regioselectively into the 6′,7′-*O*-carbonyl derivative **17** in 95% overall yield similar to the preparation of **9**. Since H-6′ and the geminal protons at C-7 did not display a significant downfield shift upon carbonate formation, probably due to the presence of the benzyl groups of the heptofuranosyl unit, the remaining hydroxy groups of the pyranose ring were O-acetylated with acetic anhydride–pyridine–*N*,*N*-dimethylaminopyridine, which afforded the 2′,3′,4′-tri-*O*-acetyl derivative **18** in 98% yield. The observed downfield shift of the ^1H NMR signals of H-2′, H-3′ and H-4′ (5.27–5.35 ppm) confirmed the structural assignments. Treatment of **17** with NH_3–NH_4HCO_3 in methanol afforded the 7′-*O*-carbamoyl derivative **19** in 66% yield, which was isolated by HPLC following removal of compound **16**, originating from hydrolysis of the carbonate ester group. Finally, hydrogenolysis of the benzyl protecting groups of **19** furnished the reducing disaccharide **20** as a 3:1 α/β mixture in quantitative yield. The ^{13}C NMR data of compound **20** revealed a downfield shift for C-7′ (to 66.22 ppm for the α and 66.12 ppm for the β



9 R = H

10 R = Ac

	R^1	R^2
1	α-OH	Ac
2	α-OC(=NH)CCl_3	Ac
3	α-F	Ac
4	β-F	Ac
5	α-OAll	Ac
6	β-OAll	Ac
7	α-OAll	H
8	β-OAll	H

hν
$HSCH_2CH_2NH_2$. HCl

i. $CSCl_2$ / $NaHCO_3$
ii. BSA

	R^1	R^2	R^3
11	$CONH_2$	H	All
12	H	$CONH_2$	All
13	$CONH_2$	H	$(CH_2)_3S(CH_2)_2NH_2$. HOAc
14	$CONH_2$	H	$(CH_2)_3S(CH_2)_2NHCSNH$-BSA

Scheme 1.

Table 1
^{13}C NMR data[a] for mono- and disaccharides **7**, **8**, **11**, **20**, **29** and **30**

Residue	Carbon	δ (ppm)						
		7	**8**	**11**	**20α**	**20β**	**29**	**30**
α-Hep*p*	1			100.02	103.18	102.78	102.95	103.11
	2			70.88	70.86	70.86	70.88	70.84
	3			71.67	71.29	71.29	71.37	71.32
	4			67.02	66.75	66.75	66.78	67.04
	5			71.88	72.02	72.02	72.39	72.40
	6			66.74	67.09	67.01	66.98	66.71
	7			66.41	66.22	66.12	66.05	66.05
Hep*p*	1	99.90	99.86		94.87	94.60	99.72	99.77
	2	70.87	71.40		71.47	71.92	71.07	70.84
	3	71.69	74.07		78.10	79.95	80.19	78.14
	4	66.90	66.78		66..83	66.66	66.67	66.71
	5	72.14	75.41		72.41	75.22	75.24	72.40
	6	69.59	69.69		69.50	69.37	69.59	69.04
	7	63.77	63.67		63.82	63.54	63.70	63.80
Allyl	1	68.87	70.99	68.95			70.96	69.49
	2	134.04	134.38	134.05			134.46	134.29
	3	119.26	119.23	119.11			119.17	118.88
$CONH_2$				160.02	159.93	159.93	159.84	159.75

[a] Spectra (75.47 MHz) were recorded at 297 K and referenced to 1,4-dioxane (67.40). Assignments are based on HMQC-spectra.

isomer), an upfield shift of ~2 ppm for C-6 as well as the characteristic signal of the carbamoyl carbon at ~159.9 ppm (Table 1).

Next, the synthesis of a heptosyl donor with the carbonate group present in the side chain, which will be used for further glycosylation of the Kdo region, was elaborated. Compound **17** was hydrogenolyzed with 10% Pd–C to remove the benzyl groups followed by O-acetylation (acetic anhydride–pyridine) to give the acetylated heptobiose derivative **21** in 91% yield as a 5:1 α/β mixture. The anomeric acetyl group was selectively cleaved by the action of hydrazine acetate [20] to afford **22** in 91% yield, which was subsequently transformed with K_2CO_3–trichloroacetonitrile into the α-trichloroacetimidate disaccharide donor **23** in 86% yield. Reaction of **23** with 2 equivalents of allyl alcohol promoted by 0.5 equivalents of trimethylsilyl trifluoromethanesulfonate in dichloromethane proceeded with low stereoselectivity to give the disaccharide allyl α-glycoside **25** in 23% yield. The anomeric configuration of the disaccharide **25** was unambiguously established on the basis of the heteronuclear coupling constant $J_{C\text{-}1,H\text{-}1}$ (172.0 Hz) for C-1 of compound **25**. In addition, the partially O-deacetylated products **26** and **27** were formed in 30 and 33% yield, respectively. The structural assignments of **26** and **27** were based upon the high-field shift displayed by the signal attributable to H-2 (4.14 and 4.13 ppm), which upon O-acetylation (acetic anhydride–pyridine)—to afford the 2-*O*-acetyl derivatives **25** and **28**—experienced a downfield shift to 5.22 and 5.41 ppm, respectively. By using a smaller amount of Me_3SiOTf (0.05 equivalents) the formation of exo/endo orthoester derivatives (~3:1 ratio) was observed (based on ^1H NMR data of exo/endo CH_3 signals). By employing boron trifluoride etherate as the promoter of the glycosylation step, the anomeric selectivity was improved (α/β ratio 4:1).

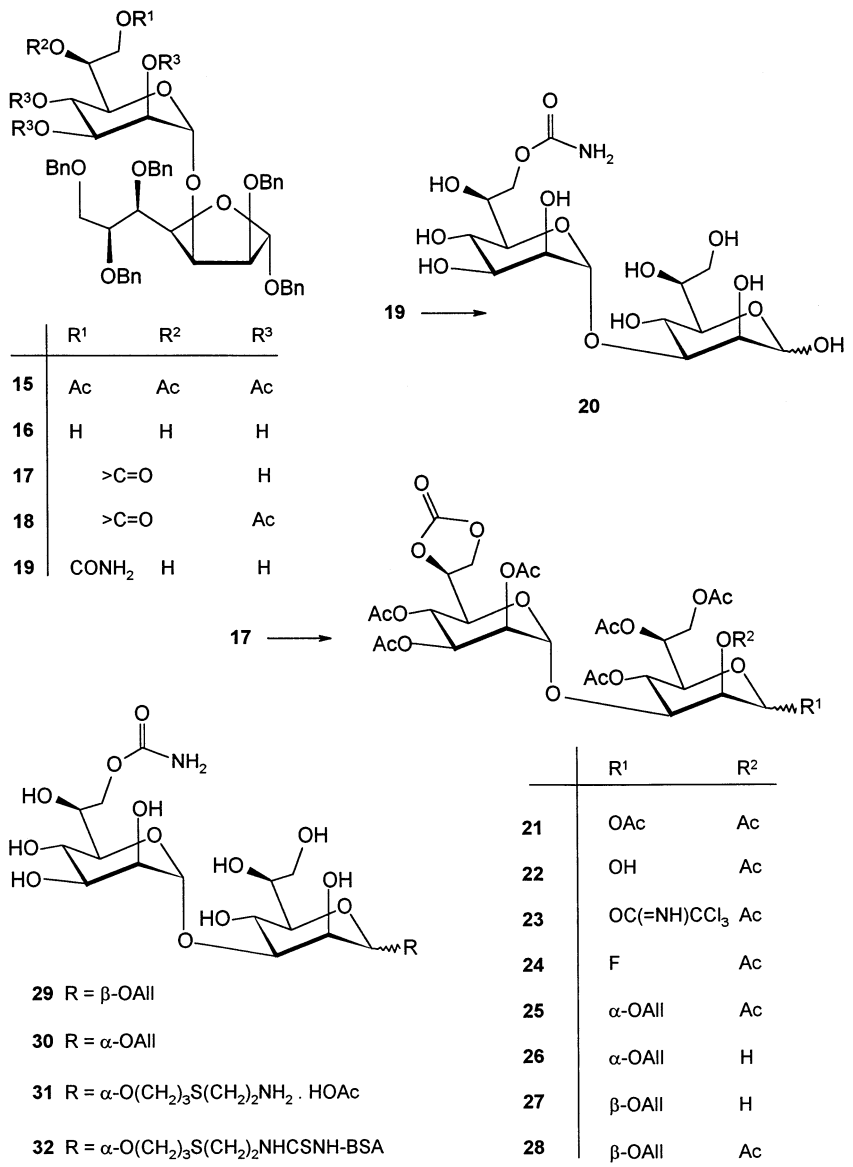
As an alternative to the trichloroacetimidate donor **23**, the disaccharide fluoride donor **24** was prepared in 90% yield by treatment of the reducing disaccharide **22** with diethylaminosulfur trifluoride. Reaction of the α-fluoride donor **24** with allyl alcohol in the presence of boron trifluoride etherate in dichloromethane afforded the disaccharides in an α/β ratio of 10:1.

O-Deacetylation of the β-allyl glycoside **28** with simultaneous formation of the 7′-*O*-carbamate was accomplished with NH_3–NH_4HCO_3 in 1:1 methanol–oxolane, which gave the disaccharide derivative **29** in 63% yield after final purification on Sephadex LH-20.

Deprotection of both α-anomeric disaccharide derivatives **25** and **26** was performed under similar conditions to afford the 6′-*O*-carbamoyl derivative (20% by integration of ^{1}H NMR signals) and the target disaccharide allyl glycoside **30** isolated by silica gel chromatography in 64 and 63% yield, respectively. Introduction of the spacer group by reaction of cysteamine hydrochloride with the allyl glycoside **30** gave compound **31** in high yield, which was isolated by ion-exchange chromatography. Subsequent coupling of the isothiocyanate-activated ligand to BSA furnished the neoglycoprotein **32**. Immunochemical results obtained with the glycoconjugates will be published elsewhere.

3. Experimental

General methods.—Melting points were determined with a Kofler hot stage and are uncorrected. Optical rotations were measured



	R1	R2	R3
15	Ac	Ac	Ac
16	H	H	H
17	>C=O		H
18	>C=O		Ac
19	$CONH_2$	H	H

	R1	R2
21	OAc	Ac
22	OH	Ac
23	$OC(=NH)CCl_3$	Ac
24	F	Ac
25	α-OAll	Ac
26	α-OAll	H
27	β-OAll	H
28	β-OAll	Ac

Scheme 2.

with a Perkin–Elmer 243 B polarimeter. $[\alpha]_D^{20}$ values are given in units of 10^{-1} deg cm^2/g. ^1H NMR spectra were recorded at 297 K with a Bruker DPX instrument operating at 300 MHz for ^1H using $CDCl_3$ as solvent and Me_4Si as standard, unless stated otherwise. ^{13}C NMR spectra were measured at 75.47 MHz and referenced to 1,4-dioxane (δ 67.40). Homo- and heteronuclear 2D NMR spectroscopy was performed with Bruker standard software. TLC was performed on E. Merck precoated plates (5×10 cm, layer thickness 0.25 mm, Silica Gel $60F_{254}$); spots were detected by spraying with anisaldehyde–H_2SO_4. For column chromatography, silica gel (0.040–0.063 mm, 10 μm for HPLC) was used. Concentration of solutions was performed at reduced pressure at temperatures < 40 °C. Elemental analyses were provided by Dr J. Theiner, Mikroanalytisches Laboratorium, Institut für Physikalische Chemie, Universität Wien.

2,3,4,6,7-Penta-O-*acetyl*-L-glycero-α-D-manno-*heptopyranosyl fluoride* (**3**) *and 2,3,4,6,7-penta*-O-*acetyl*-L-glycero-β-D-manno-*heptopyranosyl fluoride* (**4**).—DAST (37 μL, 0.28 mL) was added to a solution of **1** (58.8 mg, 0.14 mmol) in dry CH_2Cl_2 (3 mL) at -30 °C under Ar. The solution was warmed to rt and stirred for 4 h. Methanol (0.5 mL) was added at -30 °C, and the solution was diluted with CH_2Cl_2 (30 mL), washed with aq $NaHCO_3$, dried ($MgSO_4$) and concentrated. Purification of the residue on silica gel (2:1 → 0:1 hexane–Et_2O) afforded **3** (34 mg, 58%) as a syrup; $[\alpha]_D^{20}$ −8° (*c* 1.0, $CHCl_3$); ^1H NMR: δ 5.59 (dd, 1 H, $J_{1,2}$ 1.8, $J_{1,F}$ 48.3 Hz, H-1), 5.42 (m, 1 H, H-2), 5.36 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 5.34 (dd, 1 H, $J_{2,3}$ 3.5 Hz, H-3), 5.32 (ddd, 1 H, $J_{5,6}$ 2.0 Hz, H-6), 4.34 (dd, 1 H, $J_{6,7a}$ 5.3, $J_{7a,7b}$ 11.5 Hz, H-7a), 4.20 (dd, 1 H, $J_{6,7b}$ 7.7 Hz, H-7b), 4.22 (dd, 1 H, H-5), 2.20, 2.14, 2.06, 2.04 and 2.01 (5 s, each 3 H, 5 Ac); ^{13}C NMR: δ 105.2 (C-1, $J_{1,F}$ 243 Hz), 71.2 (C-5), 68.7 (C-3), 68.0 (C-2), 67.0 (C-6), 64.5 (C-4), 62.4 (C-7), 21.2–20.0 (5 Ac). Anal. Calcd for $C_{17}H_{23}FO_{11}$: C, 48.35; H, 5.49. Found: C, 48.12; H, 5.46.

Further elution afforded a mixture of **3** and **4** (4 mg, 6%), then **4** (19.5 mg, 33%) as a syrup; $[\alpha]_D^{20}$ −21° (*c* 1.0, $CHCl_3$); ^1H NMR: δ 5.58 (ddd, 1 H, $J_{1,2}$ 1.2, $J_{2,3}$ 4.0, $J_{2,F}$ 5.5 Hz, H-2), 5.45 (dd, 1 H, $J_{1,F}$ 46.6 Hz, H-1), 5.34 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 5.32 (ddd, 1 H, $J_{5,6}$ 2.3 Hz, H-6), 5.11 (dd, 1 H, $J_{3,F} < 1$ Hz, H-3), 4.38 (dd, 1 H, $J_{6,7a}$ 5.1, $J_{7a,7b}$ 11.8 Hz, H-7a), 4.26 (dd, 1 H, $J_{6,7b}$ 7.3 Hz, H-7b), 3.83 (dd, 1 H, H-5), 2.23, 2.16, 2.08, 2.05, and 2.03 (5 s, each 3 H, 5 Ac); ^{13}C NMR: δ 105.3 (C-1, $J_{1,F}$ 260 Hz), 73.2 (C-5), 70.4 (C-3), 67.8 (C-2), 66.2 (C-6), 65.0 (C-4), 62.6 (C-7), 20.0–21.0 (5 Ac).

Allyl 2,3,4,6,7-penta-O-*acetyl*-L-glycero-α-D-manno-*heptopyranoside* (**5**).—Solutions of **3** (19.2 mg, 0.045 mmol) and **4** (15.5 mg, 0.037 mmol) in dry CH_2Cl_2 (3 mL) containing allyl alcohol (9.5 and 7.5 μL, respectively) were stirred with 4 Å molecular sieves (200 mg) for 1 h at rt under N_2. Boron trifluoride etherate (40 μL, 0.18 mmol) was added in four portions over a period of 3 h to each batch. After 1 h, the conversion of **4** into **3** was observed using TLC. After stirring for 5 h at rt, the reaction was stopped by addition of triethylamine (0.1 mL). The solutions were combined, filtered over a pad of Celite and concentrated to give a syrup which was treated with Ac_2O (0.1 mL) in pyridine (2 mL) for 20 h at rt. The solution was concentrated and purified on silica gel (3:1 → 0:1 hexane–Et_2O), followed by chromatography on Sephadex LH-20 (2:1 CH_2Cl_2–MeOH) which gave **5** as a syrup. Yield: 32 mg (85%). ^1H NMR data were in agreement with published data [14].

Allyl 2,3,4,6,7-penta-O-*acetyl*-L-glycero-β-D-manno-*heptopyranoside* (**6**).—A solution of **2** (420 mg, 0.74 mol) in dry CH_2Cl_2 (10 mL) containing allyl alcohol (0.1 mL, 1.47 mmol) was stirred with 4 Å molecular sieves (200 mg) for 60 min at rt under Ar, then Me_3SiOTf (15 μL) was added. The suspension was stirred for 15 h, diluted with CH_2Cl_2 (20 mL) and filtered over Celite. The filtrate was concentrated and treated with Ac_2O (0.1 mL) as described before. Purification on silica gel (1:1 EtOAc–toluene) afforded **5** (240 mg, 70%), followed by **6** as the less-mobile isomer. Yield for **6**: 37 mg (11%); $[\alpha]_D^{20}$ −45° (*c* 0.6, $CHCl_3$); ^1H NMR: δ 5.87 (m, 1 H, –CH=), 5.50 (dd, 1 H, $J_{1,2}$ 1.1, $J_{2,3}$ 3.5 Hz, H-2), 5.30 (t, 1 H, $J_{3,4} = J_{4,5}$ 10.1 Hz, H-4), 5.28 (dq, 1 H, $=CH_{2\,trans}$),

5.27 (ddd, 1 H, $J_{5,6}$ 2.3, $J_{6,7a}$ 5.1, $J_{6,7b}$ 7.5 Hz, H-6), 5.25 (dq, 1 H, $=CH_{2\ cis}$), 5.04 (dd, 1 H, H-3), 4.67 (d, 1 H, H-1), 4.44 (t, 1 H, $J_{7a,7b}$ 11.2 Hz, H-7a), 4.58 (dd, 1 H, H-7b), 4.35 and 4.12 (m, 2 H, OCH_2), 4.18 (dd, 1 H, H-7b), 3.66 (dd, 1 H, H-5), 2.22, 2.13, 2.06, 2.01 and 1.98 (5 s, each 3 H, 5 Ac). Anal. Calcd for $C_{20}H_{28}O_{12}$: C, 52.17; H, 6.13. Found: C, 52.46; H, 5.98.

Allyl L-*glycero*-β-D-manno-*heptopyranoside* (**8**).—A solution of **6** (6.4 mg, 0.014 mmol) in dry MeOH (2 mL) was stirred with 0.1 M methanolic NaOMe (0.2 mL) for 2 h at rt. The solution was deionized by adding Dowex 50 (H^+) resin, filtered and evaporated to dryness to give **8** as a colorless syrup. Yield: 3.1 mg (89%); $[\alpha]_D^{20}$ −45° (*c* 0.3, H_2O); ^1H NMR (D_2O): δ 5.98 (m, 1 H, –CH=), 5.35 (dq, 1 H, $=CH_{2\ trans}$), 5.28 (dq, 1 H, $=CH_{2\ cis}$), 4.70 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 4.34 and 4.20 (m, 2 H, OCH_2), 4.02 (ddd, 1 H, $J_{5,6}$ 1.7, $J_{6,7a}$ 6.4, $J_{6,7b}$ 7.0 Hz, H-6), 3.99 (dd, 1 H, $J_{2,3}$ 3.4 Hz, H-2), 3.83 (t, 1 H, $J_{3,4} = J_{4,5}$ 10.0 Hz, H-4), 3.73 (dd, 1 H, H-7a), 3.73 (dd, 1 H, H-7b), 3.64 (dd, 1 H, H-3) and 3.31 (dd, 1 H, H-5).

Allyl 6,7-O-*carbonyl*-L-glycero-α-D-manno-*heptopyranoside* (**9**).—A solution of **5** (175 mg, 0.38 mmol) in dry MeOH (5 mL) was stirred with 0.1 M methanolic NaOMe (1 mL) for 2 h at rt. The pH of the solution was neutralized by addition of Dowex 50 (H^+) resin, the resin was filtered off and the filtrate was evaporated to give allyl L-*glycero*-α-D-*manno*-heptopyranoside (**7**) as colorless needles; mp 130 °C (EtOH). NMR data were in agreement with published data [14]. A solution of **7** (92 mg, 0.37 mmol) and *sym*-collidine (3.8 mmol, 0.5 mL) in dry THF (10 mL) was cooled to −30 °C under argon. A solution of diphosgene (40 μL, 0.33 mmol) in THF (4 mL) was added during 5 h. The reaction was quenched by addition of MeOH (0.5 mL), the suspension was diluted with THF (5 mL), centrifuged, and the supernatant was concentrated. Purification of the residue on silica gel (4:4:1 toluene–EtOAc–EtOH) afforded **9** as colorless syrup. Yield: 78 mg (76%); $[\alpha]_D^{20}$ +103° (*c* 0.7, H_2O); ^1H NMR (CD_3OD–$CDCl_3$): δ 5.95 (m, 1 H, –CH=), 5.32 (dq, 1 H, $=CH_{2\ trans}$), 5.28 (dq, 2 H, $=CH_{2\ cis}$, H-6), 4.95 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.70 (t, 1 H, $J_{7a,7b} = J_{7a,6}$ 8.5 Hz, H-7a), 4.58 (dd, 1 H, $J_{7b,6}$ 6.0 Hz, H-7b), 4.22 and 4.10 (m, 2 H, OCH_2), 3.98 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-2), 3.83 (t, 1 H $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4), 3.78 (dd, 1 H, H-3) and 3.72 (dd, 1 H, $J_{5,6}$ 2.2 Hz, H-5). Anal. Calcd for $C_{11}H_{16}O_8 \cdot 0.5H_2O$: C, 46.31; H, 5.99. Found: C, 46.46; H, 5.81.

Allyl 2,3,4-*tri*-O-*acetyl*-6,7-O-*carbonyl*-L-glycero-α-D-manno-*heptopyranoside* (**10**).—A solution of **9** (20 mg, 0.072 mmol) in pyridine (8 mL) was stirred with Ac_2O (0.25 mL) and a catalytic amount of DMAP for 4 h at rt. Methanol (0.5 mL) was added, and the solution was coevaporated three times with toluene and concentrated. Purification of the residue on silica gel (1:1 toluene–EtOAc) gave **10** as a syrup (29 mg, ~quant.); $[\alpha]_D^{20}$ +117° (*c* 1.0, $CHCl_3$); ^1H NMR: δ = 5.89 (m, 1 H, –CH=), 5.50 (t, 1 H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4), 5.39 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-3), 5.32 (dq, 1 H, $=CH_{2\ trans}$), 5.27 (dq, 2 H, $=CH_{2\ cis}$, H-6), 5.22 (dd, 1 H, $J_{1,2}$ 1.9 Hz, H-2), 4.93 (d, 1 H, H-1), 4.79 (ddd, 1 H, $J_{7a,6}$ 8.3, $J_{6,5}$ 1.3, $J_{7b,6}$ 5.5 Hz, H-6), 4.52 (t, 1 H, $J_{7a,7b}$ 8.3 Hz, H-7a), 4.40 (dd, 1 H, H-7b), 4.18 and 4.07 (m, 2 H, OCH_2), 3.84 (dd, 1 H, H-5), 2.17, 2.07 and 2.02 (3 s, each 3 H, 3 Ac). Anal. Calcd for $C_{17}H_{22}O_{11}$: C, 50.75; H, 5.51. Found: C, 50.50; H, 5.24.

Allyl 7-O-*carbamoyl*-L-glycero-α-D-manno-*heptopyranoside* (**11**)

Method A. To a solution of **9** (38 mg, 0.138 mmol) in THF (3 mL) solid NH_4HCO_3 (250 mg) was added, followed by addition of two portions of 25% aq NH_3 (0.5 mL each) during 2 h at rt. The solution was concentrated and coevaporated three times with MeOH. The residue was crystallized from EtOH to give **11** as colorless needles. Yield: 23 mg (57%), mp 154 °C (EtOH); $[\alpha]_D^{20}$ +56° (*c* 0.5, H_2O); ^1H NMR (D_2O): δ 5.94 (m, 1 H, –CH=), 5.32 (dq, 1 H, $=CH_{2\ trans}$), 5.24 (dq, 1 H, $=CH_{2\ cis}$), 4.89 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.19 (ddd, 1 H, $J_{7a,6}$ 8.0, $J_{6,5}$ 1.5, $J_{7b,6}$ 5.5 Hz, H-6), 4.18–4.13 (m, 1 H, OCH_2), 4.14–4.12 (m, 2 H, H-7a, 7b), 3.91 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-2), 3.84 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.6 Hz, H-4), 3.77 (dd, 1 H, H-3) and 3.59 (dd, 1 H, H-5). Anal. Calcd for $C_{11}H_{19}NO_8$: C, 45.05; H, 6.53; N, 4.78. Found: C, 44.82; H, 6.30; N, 4.61.

The mother liquor was evaporated to dryness giving 15 mg (37%) of a 3:1 mixture of **11** and **12**. ^{1}H NMR (D_2O) for **12**: δ 5.13 (ddd, 1 H, $J_{7a,6} \sim J_{7b,6} \sim 5.8$, $J_{6,5}$ 1.6 Hz, H-6), 4.90 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1), 3.68 (dd, 1 H, $J_{4,5}$ 9.6 Hz, H-5); ^{13}C NMR: δ 72.78 (C-6), 71.49 (C-3), 70.62 (C-2), 69.09 (OCH_2), 66.91 (C-4), 61.47 (C-7).

Method B. A solution of **10** (11.4 mg, 0.028 mmol) in dry THF (3 mL) was stirred with NH_4HCO_3 (160 mg) and 25% aq NH_3 (1.6 mL) for 24 h at ambient temperature. Additional NH_4HCO_3 (150 mg) and 25% aq NH_3 (1.5 mL) were added and stirring was continued for 24 h. The solution was concentrated and the residue was extracted with MeOH. Evaporation of the methanolic solution afforded 8.2 mg (98%) of a 10:1 mixture of **11** and **12**.

*3-(2-Ammoniumethylthio)propyl 7-*O*-carbamoyl*-L-glycero-α-D-manno-*heptopyranoside acetate* (**13**).—A solution of **11** (7.5 mg, 0.026 mmol) and cysteamine hydrochloride (8.4 mg, 0.074 mmol, recrystallized from MeOH) in water (0.1 mL) was placed in a quartz vial and irradiated at 254 nm for 90 min at rt. The solution was purified by chromatography on silica gel (7:2:1 EtOAc–HOAc–H_2O) to remove excess of reagent followed by elution with 3:2:1 EtOAc–HOAc–H_2O to give the spacer compound **13**, which was further purified on Sephadex LH-20 (MeOH). Yield: 11.5 mg ($\sim$quant.), colorless syrup; $[\alpha]_D^{20}$ + 29° (*c* 0.3, H_2O); ^{1}H NMR (D_2O): δ 4.87 (d, 1 H, H-1), 4.25–4.20 (m, 2 H, H-6, 7a), 4.15 (dd, 1 H, $J_{7a,7b}$ 11.9, $J_{7b,6}$ 8.7 Hz, H-7b), 3.92 (dd, 1 H, $J_{1,2}$ 1.7, $J_{2,3}$ 3.3 Hz, H-2), 3.87 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4), 3.78 (dd, 1 H, H-3), 3.74 (t, 1 H, OCH_2), 3.59 (dd, 1 H, H-5), 3.59 (t, 1 H, OCH_2), 3.21 (t, 2 H, NCH_2), 2.85 (t, 2 H, SCH_2), 2.67 (dt, 2 H, SCH_2), 1.95 (s, 3 H, Ac) and 1.92 (m, 2 H, CH_2); ^{13}C NMR (D_2O): δ 182.32 (CO), 160.06 ($CONH_2$), 100.65 (C-1), 72.01, 71.66 (C-5,3), 70.85 (C-2), 67.08 (C-4), 66.84 (C-6), 66.68 (C-7, OCH_2), 39.10 (NCH_2), 28.95, 28.22 (2 SCH_2), 27.83 (CH_2) and 24.05 (Ac). Anal. Calcd for $C_{13}H_{26}N_2O_8S{\cdot}CH_3COOH{\cdot}0.5H_2O$: C, 40.99; H, 7.11; N, 6.37. Found: C, 40.48; H, 6.96; N, 6.14.

*Benzyl (6,7-*O*-carbonyl*-L-glycero-α-D-manno-*heptopyranosyl)-(1→3)-2,5,6,7-tetra*-O-*benzyl*-L-glycero-α-D-manno-*heptofuranoside* (**17**).—A solution of **15** (120 mg, 0.11 mmol) in dry MeOH (3 mL) was stirred with 0.1 M methanolic MeONa (0.3 mL) for 2 h at rt. The reaction mixture was neutralized with Dowex 50 (H^+) resin, filtered and concentrated under reduced pressure to give crude benzyl L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)-2,5,6,7-tetra-*O*-benzyl-L-*glycero*-α-D-*manno*-heptofuranoside (**16**). The residue was coevaporated with dry toluene (10 mL) and redissolved in dry CH_2Cl_2 (5 mL). *sym*-Collidine (182 mg, 0.19 mL, 1.5 mmol) was added, and the solution was cooled to −40 °C under N_2. A solution of trichloromethyl chloroformate (0.09 mmol, 11 μL) in CH_2Cl_2 (8 mL) was added dropwise under vigorous stirring at −40 °C under N_2 during 4 h at a rate of 2 mL/h. The reaction was stopped by addition of dry MeOH (0.2 mL), and the reaction mixture was concentrated to dryness. Purification of the residue by chromatography on silica gel (99:1→9:1 CH_2Cl_2–MeOH) gave **17** as a colorless syrup. Yield 93 mg (95%); $[\alpha]_D^{20}$ +44.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 7.39–7.22 (m, 25 H, 5 Ph), 5.24 (d, 1 H, $J_{1,2}$ 4.6 Hz, H-1), 4.85 (s, 1 H, $J_{1',2'}$ 1.1 Hz, H-1′), 4.77 (d, 1 H, *J* 11.2 Hz, CHPh), 4.75 (d, 1 H, *J* 11.8 Hz, CHPh), 4.68 (d, 1 H, *J* 12.1 Hz, CHPh), 4.66 (d, 1 H, *J* 11.6 Hz, CHPh), 4.62 (d, 1 H, *J* 11.8 Hz, CHPh), 4.55 (d, 1 H, *J* 12.1 Hz, CHPh), 4.53 (s, 1 H, CHPh), 4.53 (d, 1 H, *J* 11.9 Hz, CHPh), 4.52 (s, 1 H, CHPh), 4.50 (ddd, 1 H, $J_{5',6'}$ 2.2, $J_{6',7a'}$ 6.2, $J_{6',7b'}$ 8.5 Hz, H-6′), 4.47 (dd, 1 H, $J_{3,4}$ 2.1, $J_{4,5}$ 8.2 Hz, H-4), 4.40 (d, 1 H, *J* 1.1 Hz, CHPh), 4.24 (dd, 1 H, $J_{2,3}$ 4.6 Hz, H-3), 4.05–3.96 (m, 5 H, H-2, 5, 6, 5′, 7a′), 3.82 (m, 1 H, H-2′), 3.76 (d, 2 H, H-7a, 7b), 3.70 (m, 2 H, H-3′, 4′), 3.51 (dd, 1 H, H-7b′); ^{13}C NMR ($CDCl_3$): δ 155.05 (CO), 105.07 (C-1′), 100.92 (C-1). Anal. Calcd for $C_{50}H_{54}O_{14}{\cdot}0.5H_2O$: C, 67.63; H, 6.24. Found: C, 67.82; H, 6.22.

*Benzyl (2,3,4-tri-*O*-acetyl-6,7-*O*-carbonyl*-L-glycero-α-D-manno-*heptopyranosyl)-(1→3)-2,5,6,7-tetra*-O-*benzyl*-L-glycero-α-D-manno-*heptofuranoside* (**18**).—A solution of **17** (10.5 mg, 0.012 mmol), 4-dimethylaminopyridine (2 mg, 0.01 mmol), and Ac_2O (10 μL) in dry

pyridine (2 mL) was stirred for 20 h at rt. The reaction mixture was concentrated, co-evaporated with toluene (15 mL) and the residue was purified on silica gel (93:7 → 85:15 toluene–EtOAc) affording **18** as a syrup. Yield 11.8 mg (98%); $[\alpha]_D^{20}$ +60° (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 7.37–7.15 (m, 25 H, 5 Ph), 5.35 (d, 1 H, $J_{1,2}$ 4.8 Hz, H-1), 5.35–5.27 (m, 2 H, H-2′, 3′), 5.27 (dd, 1 H, $J_{3',4'} \sim J_{4',5'}$ 9.1 Hz, H-4′), 4.83 (d, 1 H, *J* 11.5 Hz, CHPh), 4.79 (d, 1 H, *J* 11.6 Hz, CHPh), 4.78 (s, 1 H, H-1′), 4.73 (d, 1 H, *J* 10.6 Hz, CHPh), 4.71 (d, 1 H, *J* 12.1 Hz, CHPh), 4.68 (d, 1 H, *J* 11.5 Hz, CHPh), 4.57 (d, 1 H, *J* 11.7 Hz, CHPh), 4.56 (s, 3 H, 3 CHPh), 4.48 (dd, 1 H, H-4), 4.36 (d, 1 H, *J* 10.6 Hz, CHPh), 4.30 (dd, 1 H, $J_{4',5'}$ 9.1 Hz, H-5′), 4.25 (dd, 1 H, $J_{3,4}$ 2.0 Hz, H-3), 4.11 (dd, 1 H, $J_{1,2}$ 4.7, $J_{2,3}$ 4.5 Hz, H-2), 4.07 (ddd, 1 H, $J_{6,7a}$ 6.0, $J_{6,7b}$ 8.3 Hz, H-6), 3.99 (dd, 1 H, $J_{4,5}$ 8.7, $J_{5,6}$ 2.0 Hz, H-5), 3.98 (dd, 1 H, H-7a′), 3.88 (ddd, 1 H, $J_{6',7a'}$ 6.0, $J_{6',7b'}$ 8.5, $J_{6',5'}$ 1.4 Hz, H-6′), 3.81–3.79 (m, 2 H, H-7a, 7b), 3.42 (t, 1 H, $J_{7a',7b'}$ 8.4 Hz, H-7b′), 2.05, 2.02, and 1.99 (3 s, each 3 H, 3 Ac). Anal. Calcd for $C_{56}H_{60}O_{17}$: C, 66.92; H, 6.02. Found: C, 66.83; H, 5.86.

Benzyl (7-O-*carbamoyl*-L-glycero-α-D-manno-*heptopyranosyl)-(1→3)-2,5,6,7-tetra*-O-*benzyl*-L-glycero-α-D-manno-*heptofuranoside* (**19**).—To a stirred solution of **17** (32.8 mg, 0.037 mmol) in MeOH (5 mL), ammonium hydrogenocarbonate (300 mg) and 25% aq NH_3 (1 mL) were added. The suspension was vigorously stirred for 24 h at rt, the reaction mixture was concentrated under reduced pressure. Purification of the residue on silica gel (99:1 → 93:7 CH_2Cl_2–MeOH), followed by a second purification by HPLC (10:1 $CHCl_3$–MeOH), afforded **19** as a solid. Yield: 22 mg (66%); $[\alpha]_D^{20}$ +38° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$–CD_3OD): δ 7.34–7.24 (m, 25 H, 5 Ph); 5.14 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 4.95 (d, 1 H, $J_{1',2'}$ 1.6 Hz, H-1′), 4.75 (d, 1 H, *J* 11.3 Hz, CHPh), 4.68 (d, 1 H, *J* 12.2 Hz, CHPh), 4.66 (d, 1 H, *J* 11.0 Hz), 4.63 (s, 3 H, 3 CHPh), 4.62 (s, 1 H, CHPh), 4.50 (s, 1 H, CHPh), 4.49 (s, 1 H, CHPh), 4.50 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 8.2 Hz, H-4), 4.44 (d, 1 H, *J* 12.0 Hz, CHPh), 4.42 (m, 1 H, H-3), 4.13 (dd, 1 H, $J_{6',7a'}$ 8.9, $J_{7a',7b'}$ 12.3 Hz, H-7a′), 4.04 (ddd, 1 H, $J_{5,6}$ 2.3, $J_{6,7a} = J_{6,7b}$ 6.1 Hz, H-6), 4.01 (dd, 1 H, H-5), 4.00 (dd, 1 H, $J_{2,3}$ 4.5 Hz, H-2), 3.91–3.84 (m, 4 H, H-2′, 3′, 5′, 6′), 3.79 (m, 2 H, H-4′, 7b′), 3.70 (m, 2 H, H-7a, 7b); ^{13}C NMR ($CDCl_3$): δ 157.40 ($CONH_2$), 104.59 (C-1′), 99.92 (C-1). Anal. Calcd for $C_{50}H_{57}NO_{14}\cdot H_2O$: C, 65.70; H, 6.51; N, 1.53. Found: C, 66.24; H, 6.62; N, 1.54.

7-O-*Carbamoyl*-L-glycero-α-D-manno-*heptopyranosyl-(1→3)*-L-glycero-D-manno-*heptopyranose* (**20**).—Compound **19** (14 mg, 0.016 mmol) was dissolved in MeOH (10 mL) and hydrogenated over 10% Pd–C (0.05 g) under H_2 at atmospheric pressure at ambient temperature for 16 h. The solution was filtered and concentrated under reduced pressure. The residue was taken up in water and purified on a Bio-gel P-2 column (2.5 × 100 cm, 1% aq NaCl, 1% aq NH_4HCO_3) to afford **20** as a white solid after lyophilization.Yield 6.8 mg (98%); $[\alpha]_D^{20}$ +17° (*c* 0.4, H_2O); ^{1}H NMR (D_2O) for the α anomer: δ 5.20 (d, 1 H, H-1′), 5.13 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.21 (m, 1 H, H-6′), 4.18 (dd, 1 H, $J_{6',7a'}$ 6.7 Hz, H-7a′), 4.11 (dd, 1 H, $J_{6',7b'}$ 7.9 Hz, H-7b′), 4.07 (dd, 1 H, $J_{1',2'}$ 1.7 Hz, H-2′), 4.01 (ddd, 1 H, H-6), 3.97 (dd, 1 H, $J_{1,2}$ 1.7 Hz, H-2), 3.93 (dd, 1 H, H-4), 3.92 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 10.5 Hz, H-3), 3.91 (dd, 1 H, $J_{4',5'}$ 6.0 Hz, H-4′), 3.90 (dd, 1 H, $J_{2',3'}$ 2.3, $J_{3',4'}$ 6.8 Hz, H-3′), 3.77 (dd, 1 H, $J_{5',6'}$ 1.1 Hz, H-5′), 3.71 (dd, 1 H, $J_{4,5}$ 10.2, $J_{5,6}$ 1.3 Hz, H-5), 3.69 (dd, 1 H, $J_{6,7a}$ 7.2 Hz, H-7a), 3.67 (dd, 1 H, $J_{7a,7b}$ 10.8, $J_{6,7b}$ 5.8 Hz, H-7b); ^{1}H NMR (D_2O) for the β anomer: δ 5.22 (d, 0.3 H, H-1′), 4.87 (d, 0.3 H, $J_{1,2}$ 1.0 Hz, H-1), 3.35 (dd, 0.3 H, $J_{4,5}$ 9.8, $J_{5,6}$ 1.8 Hz, H-5). Anal. Calcd for $C_{15}H_{27}NO_{14}\cdot 2H_2O$: C, 37.43; H, 6.49; N, 2.91. Found: C, 37.42; H, 6.28; N, 2.78.

(2,3,4-Tri-O-*acetyl-6,7*-O-*carbonyl*-L-glycero-α-D-manno-*heptopyranosyl)-(1→3)-1,2,4,-6,7-penta*-O-*acetyl*-L-glycero-D-manno-*heptopyranose* (**21**).—Compound **17** (88 mg, 0.1 mmol) was dissolved in MeOH (10 mL) and hydrogenated under atmospheric pressure of H_2 over 10% Pd–C (0.1 g) for 16 h at 20 °C. The solution containing crude (6,7-*O*-carbonyl-L-*glycero*-α-D-*manno*-heptopyranosyl)-(1→3)-L-*glycero*-D-*manno*-heptopyranose was

filtered over Celite and concentrated under reduced pressure. The residue was coevaporated with dry toluene (5 mL), redissolved in pyridine (2 mL) and treated with Ac_2O (0.2 mL) overnight at rt. The reaction mixture was concentrated, and coevaporated with toluene (10 mL). Purification of the residue on silica gel (1:1 → 2:3 toluene–EtOAc) afforded **21** as a 5:1 α/β mixture. Yield 68 mg (91%); ^{1}H NMR for the α isomer: δ 5.97 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 5.38 (dd, 1 H, $J_{3',4'} = J_{4',5'}$ 9.8 Hz, H-4′), 5.27 (dd, 1 H, $J_{3,4} = J_{4,5}$ 10.0 Hz, H-4), 5.14 (dd, 1 H, H-2), 5.11 (dd, 1 H, $J_{2',3'}$ 3.5 Hz, H-3′), 5.10 (ddd, 1 H, $J_{5,6}$ 1.9, $J_{6,7a}$ 5.5, $J_{6,7b}$ 7.0 Hz, H-6), 4.99 (d, 1 H, $J_{1',2'}$ 2.0, H-1′), 4.84 (dd, 1 H, H-2′), 4.67 (ddd, 1 H, $J_{5',6'}$ 1.8, $J_{6',7b'}$ 5.3, $J_{6',7a'}$ 8.3 Hz, H-6′), 4.38 (dd, 1 H, $J_{7a',7b'}$ 8.5 Hz, H-7a′), 4.28 (dd, 1 H, H-7b′), 4.18 (dd, 1 H, $J_{7a,7b}$ 11.5 Hz, H-7a), 4.08 (dd, 1 H, H-7b), 4.07 (dd, 1 H, $J_{2,3}$ 3.5 Hz, H-3), 3.95 (dd, 1 H, H-5), 3.87 (dd, 1 H, H-5′), 2.27, 2.19, 2.16, 2.15, 2.13, 2.08, 2.04, and 2.01 (8 s, each 3 H, 8 Ac). Anal. Calcd for $C_{31}H_{40}O_{22}$: C, 48.70; H, 5.27. Found: C, 48.98; H, 5.09.

(2,3,4-Tri-O-*acetyl-6,7*-O-*carbonyl*-L-glycero-α-D-manno-*heptopyranosyl)-(1→3)-2,4,6,-7-tetra*-O-*acetyl*-L-glycero-α-D-manno-*heptopyranose trichloroacetimidate* (**23**).—A solution of **21** (50 mg, 0.065 mmol) and hydrazine acetate (6.4 mg. 0.07 mmol) in dry DMF (2 mL) was stirred for 4 h. The reaction mixture was dissolved in CH_2Cl_2, and was sequentially washed with water, 10% aq $NaHCO_3$, and water. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The product was purified by silica gel column chromatography (3:2 → 7:3 EtOAc–toluene), which afforded (2,3,4-tri-*O*-acetyl-6,7-*O*-carbonyl-L-*glycero*-α-D-*manno*-heptopyranosyl)-(1→3)-2,4,6,7-tetra-*O*-acetyl-L-*glycero*-D-*manno*-heptopyranose (**22**) as a syrup. Yield: 43 mg (91%). A suspension of **22** (40 mg, 0.055 mmol), K_2CO_3 (70 mg) and trichloroacetonitrile (70 μL) in dry CH_2Cl_2 (5 mL) was stirred for 20 h at ambient temperature. The reaction mixture was diluted with toluene (5 mL), filtered and the filtrate was concentrated. The residue was purified by flash column chromatography on silica gel (1:1 → 2:3 toluene–EtOAc) to give **23** as a syrup. Yield: 41 mg (86%); $[\alpha]_D^{20}$ + 57° (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 8.85 (s, 1 H, NH), 6.25 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.50 (dd, 1 H, $J_{3',4'} = J_{4',5'}$ 9.8 Hz, H-4′), 5.46 (dd, 1 H, $J_{3,4} = J_{4,5}$ 10.0 Hz, H-4), 5.42 (m, 1 H, H-2), 5.25–5.20 (m, 2 H, H-3′, H-6), 5.11 (d, 1 H, $J_{1',2'}$ 1.8 Hz, H-1′), 4.94 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-2′), 4.77 (ddd, 1 H, $J_{5',6'}$ 1.8, $J_{6',7b'}$ 5.3 Hz, H-6′), 4.50 (dd, 1 H, $J_{6',7a'}$ 8.5, $J_{7a',7b'}$ 8.5 Hz, H-7a′), 4.36 (dd, 1 H, H-7b′), 4.29-4.14 (m, 4 H, H-3,5,7a,7b), 4.02 (dd, 1 H, H-5′), 2.32 (s, 3 H), 2.17 (s, 3 H), 2.16 (s, 6 H), 2.09 (s, 3 H) and 2.03 (s, 6 H, total 7 Ac).

(2,3,4-Tri-O-*acetyl-6,7*-O-*carbonyl*-L-glycero-α-D-manno-*heptopyranosyl)-(1→3)-2,4,-6,7-tetra*-O-*acetyl*-L-glycero-α-D-manno-*heptopyranose fluoride* (**24**).—To a solution of **22** (22 mg, 0.03 mmol) in CH_2Cl_2 (3 mL) under nitrogen, diethylaminosulfur trifluoride (20 μL, 0.15 mmol) was added at −30 °C. After stirring for 3 h at 20 °C, the mixture was cooled (−30 °C), excess DAST was quenched with MeOH (0.1 mL), and the mixture was concentrated under reduced pressure. The resulting oil was taken up in CH_2Cl_2 (10 mL), washed with H_2O, 10% aq $NaHCO_3$, H_2O, dried over $MgSO_4$, filtered and concentrated to give an oil. Purification on silica gel (1:1 → 0:1 hexane–Et_2O) gave **24** as a syrup (13 mg, 59%), a 1:1 mixture of **24** and α-fluoride (6 mg, 27%) and β-fluoride (1 mg, 4%); $[\alpha]_D^{20}$ + 42° (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ = 5.57 (dd, 1 H, $J_{1,2}$ 1.0, $J_{1,F}$ 49.2 Hz, H-1), 5.49 (dd, 1 H, $J_{3',4'} = J_{4',5'}$ 9.8 Hz, H-4′), 5.40 (dd, 1 H, $J_{3,4} = J_{4,5}$ 10.2 Hz, H-4), 5.36 (m, 1 H, H-2), 5.26 (ddd, 1 H, $J_{5,6}$ 1.9 Hz, H-6), 5.21 (dd, 1 H, $J_{3',4'}$ 10, $J_{2',3'}$ 3.2 Hz, H-3′), 5.07 (d, 1 H, $J_{1',2'}$ 1.7, H-1′), 4.91 (dd, 1 H, H-2′), 4.77 (ddd, 1 H, $J_{5',6'}$ 1.3 Hz, H-6′), 4.55 (dd, 1 H, $J_{6',7a'} = J_{7a',7b'}$ 8.5 Hz, H-7a′), 4.40 (dd, 1 H, $J_{6',7b'}$ 5.2 Hz, H-7b′), 4.32 (dd, 1 H, $J_{6,7a}$ 5.6, $J_{7a,7b}$ 11.4 Hz, H-7a), 4.19 (dd, 1 H, $J_{6,7b}$ 7.6 Hz, H-7b), 4.14 (dd, 1 H, H-5), 4.13 (dd, 1 H, H-3), 3.95 (dd, 1 H, H-5′), 2.36, 2.28, 2.15, 2.14, 2.08, 2.07 and 2.01 (7 s, each 3 H, 7 Ac); ^{13}C NMR: δ 170.43, 170.38, 170.12, 170.11, 169.65, 169.58, 169.32 (7 CO, $COCH_3$), 154.32 (CO), 105.14 (C-1, $J_{C\text{-}1,F}$ 223 Hz), 99.10 (C-1′), 74.56 (C-3), 72.94 (C-6′), 71.16 (C-5), 70.52 (C-5′), 69.59 (C-2′), 68.69 (C-2, $J_{C\text{-}2,F}$ 40.7 Hz), 67.65 (C-3′), 66.37 (C-6), 65.75 (C-4′), 65.40 (C-7′), 65.30 (C-4), 61.80 (C-7); ^{1}H NMR data for the

the β-fluoride: δ 5.20 (m, 1 H, H-2), 5.47 (dd, 1 H, $J_{3',4'} = J_{4',5'}$ 9.9 Hz, H-4′), 5.42 (dd, 1 H, $J_{1,2}$ 1.0, $J_{1,F}$ 47.7 Hz, H-1), 5.35 (dd, 1 H, $J_{3,4} = J_{4,5}$ 10.1 Hz, H-4), 5.26 (ddd, 1 H, $J_{5,6}$ 1.9 Hz, H-6), 5.22 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-3′), 5.03 (d, 1 H, $J_{1',2'}$ 2.0 Hz, H-1′), 4.93 (dd, 1 H, H-2′), 4.77 (ddd, 1 H, $J_{5',6'}$ 1.9 Hz, H-6′), 4.56 (dd, 1 H, $J_{6',7a'} = J_{7a',7b'}$ 8.5 Hz, H-7a′), 4.48 (dd, 1 H, $J_{6',7b'}$ 5.4 Hz, H-7b′), 4.37 (dd, 1 H, $J_{6,7a}$ 5.3, $J_{7a,7b}$ 11.7 Hz, H-7a), 4.26 (dd, 1 H, $J_{6,7b}$ 6.9 Hz, H-7b), 3.96 (dd, 1 H, H-5′), 4.13 (dd, 1 H, $J_{2,3}$ 4.0, H-3), 3.73 (dd, 1 H, H-5), 2.31 (s, 3 H), 2.17 (s, 6 H), 2.14, 2.09, 2.08, and 2.02 (3 s, each 3 H, total 7 Ac).

Allyl (2,3,4-tri-O-*acetyl-6,7*-O-*carbonyl*-L-glycero-α-D-manno-*heptopyranosyl)-(1→3)-2,4,6,7-tetra*-O-*acetyl*-L-glycero-α-D-manno-*heptopyranoside* (**25**), *allyl (2,3,4-tri*-O-*acetyl-6,7*-O-*carbonyl*-L-glycero-α-D-manno-*heptopyranosyl)-(1→3)-4,6,7-tri*-O-*acetyl*-L-glycero-α-D-manno-*heptopyranoside* (**26**) *and allyl (2,3,4-tri*-O-*acetyl-6,7*-O-*carbonyl*-L-glycero-α-D-manno-*heptopyranosyl)-(1→3)-4,6,7-tri*-O-*acetyl*-L-glycero-β-D-manno-*heptopyranoside* (**27**)

Method A. A solution of glycosyl donor **23** (35 mg, 0.04 mmol) and allyl alcohol (4.8 mg, 5.6 μL, 0.08 mmol) in dry CH_2Cl_2 (5 mL) was stirred at ambient temperature with powdered 4 Å molecular sieves for 1 h under N_2. A solution of Me_3SiOTf (2.1 μmol, 0.47 mg, 0.4 μL) in CH_2Cl_2 (100 μL) was added to the mixture, followed by addition of the same amount every 30 min until the formation of three products was observed (total amount of Me_3SiOTf: 10.5 μmol, 2.4 mg, 2.0 μL). The reaction mixture was neutralized with Na_2CO_3 (200 mg), filtered over a pad of Celite, and the filtrate was concentrated. The residue was taken up in CH_2Cl_2 (100 mL), extracted with water, 10% aq $NaHCO_3$, water, dried over $MgSO_4$, and concentrated to an oil. Purification by chromatography on silica gel (7:3→2:3 toluene–EtOAc), followed by a second purification on silica gel (100:0→97:3 $CHCl_3$–MeOH), afforded **25** (7 mg, 23%) as a syrup (R_f 0.5 in 1:2 toluene–EtOAc), followed by **26** (9 mg, 30%) as a syrup (R_f 0.2) and **27** (10 mg, 33%) as a syrup (R_f 0.3).

Compound **25** was finally purified by HPLC (1:1 toluene–EtOAc). Yield: 6.8 mg (22%); $[\alpha]_D^{20}$ −0.5° (*c* 0.7, $CHCl_3$); ^{1}H NMR: δ 5.88 (m, 1 H, –CH=), 5.49 (dd, 1 H, $J_{3',4'} = J_{4',5'}$ 9.9 Hz, H-4′), 5.37 (dd, 1 H, $J_{3,4}$ 9.9, $J_{4,5}$ 9.8 Hz, H-4), 5.32 (dq, 1 H, $=CH_{2\ trans}$), 5.26 (dq, 1 H, $=CH_{2\ cis}$), 5.22 (dd, 1 H, $J_{1,2}$ 1.6 Hz, H-2), 5.25–5.20 (m, 2 H, H-6, H-3′), 5.06 (d, 1 H, $J_{1',2'}$ 1.9 Hz, H-1′), 4.93 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-2′), 4.87 (d, 1 H, H-1), 4.78 (ddd, 1 H, $J_{5',6'}$ 1.3 Hz, H-6′), 4.55 (dd, 1 H, $J_{6',7a'}$ 8.5, $J_{7a',7b'}$ 8.4 Hz, H-7a′), 4.44 (dd, 1 H, $J_{6',7b'}$ 5.3 Hz, H-7b′), 4.30 (dd, 1 H, $J_{6,7a}$ 6.2, $J_{7a,7b}$ 11.2 Hz, H-7a), 4.23 (dd, 1 H, $J_{6,7b}$ 7.4 Hz, H-7b), 4.22 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-3), 4.18–4.15 (m, 1 H, OCH_2), 4.02 (dd, 1 H, $J_{5,6}$ 1.7 Hz, H-5), 4.02–3.96 (m, 1 H, OCH_2), 3.96 (dd, 1 H, H-5′), 2.26, 2.17, 2.16, 2.14, 2.09, 2.07, and 2.02 (7 s, each 3 H, 7 Ac). 'Gated decoupled' ^{13}C NMR: δ 99.50 ($^1J_{C\text{-}1',H\text{-}1'} \sim 172.0$ Hz), 97.20 ($^1J_{C\text{-}1,H\text{-}1} \sim 171.0$ Hz); ^{13}C NMR: δ 170.8–169.0 (7 $COCH_3$), 160.0 (CO), 133.50 (–CH=), 119.0 ($=CH_2$), 99.48 (C-1′), 97.22 (C-1), 75.82 (C-3), 73.30 (C-6′), 71.06 (C-2), 73.50 (C-5), 70.08 (C-2′), 68.24 (C-5′), 68.04 (OCH_2), 68.0 (C-3), 67.55 (C-6), 66.48 (C-4), 66.05 (C-4′), 65.85 (C-7′), 61.82 (C-7), 21.15–20.05 (7 CH_3). Anal. Calcd for $C_{32}H_{42}O_{21}$: C, 50.40; H, 5.55. Found: C, 50.65; H, 5.31.

Final purification of crude **26** by chromatography (99:1→95:5 CH_2Cl_2–MeOH) furnished pure **26** as a syrup. Yield: 8.5 mg (29%); $[\alpha]_D^{20}$ +11.6° (*c* 0.2, $CHCl_3$); ^{1}H NMR: δ 5.88 (m, 1 H, –CH=), 5.49 (dd, 1 H, $J_{3',4'} = J_{4',5'}$ 10.0 Hz, H-4′), 5.33 (dd, 1 H, $J_{3,4}$ 10.4, $J_{4,5}$ 10.2 Hz, H-4), 5.33–5.25 (m, 2 H, $=CH_2$), 5.27 (ddd, 1 H, $J_{5,6}$ 1.8, $J_{6,7a}$ 5.9, $J_{6,7b}$ 7.2 Hz, H-6), 5.26 (dd, 1 H, H-3′), 5.07 (dd, 1 H, $J_{1',2'}$ 2.1, $J_{2',3'}$ 5.2 Hz, H-2′), 5.06 (d, 1 H, H-1′), 4.94 (d, 1 H, $J_{1,2} < 1.0$ Hz, H-1), 4.74 (ddd, 1 H, $J_{5',6'}$ 1.3, $J_{6',7a'}$ 4.9, $J_{6',7b'}$ 8.4 Hz, H-6′), 4.51 (dd, 1 H, H-7a′), 4.46 (dd, 1 H, $J_{7a',7b'}$ 8.5 Hz, H-7b′), 4.30 (dd, 1 H, $J_{6,7a}$ 5.9 Hz, H-7a), 4.21 (dd, 1 H, $J_{6,7b}$ 7.2, $J_{7a,7b}$ 11.1 Hz, H-7b), 4.16–4.20 (m, 1 H, OCH_2), 4.10 (dd, 1 H, H-5′), 4.04 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-3), 4.03 (m, 1 H, H-2), 4.01 (dd, 1 H, H-5), 3.99–3.96 (m, 1 H, OCH_2), 2.17 (s, 3 H), 2.14 (s, 6 H), 2.08, 2.07, and 2.01 (3 s, each 3 H, total 6 Ac).

Compound **27** was finally purified by chromatography on silica gel (98:2→93:7 CH_2Cl_2–MeOH). Yield: 8 mg (27%); $[\alpha]_D^{20}$

$-7°$ (c 0.5, $CHCl_3$); ^1H NMR: δ 5.91 (m, 1 H, –CH=), 5.53 (dd, 1 H, $J_{3',4'}$ 9.8 Hz, H-4′), 5.40 (dd, 1 H, $J_{3,4}$ 9.8 Hz, H-4), 5.32 (dq, 1 H, $=CH_{2\ trans}$), 5.29 (ddd, 1 H, H-6), 5.28–5.26 (m, 1 H, $=CH_{2\ cis}$), 5.22 (dd, 1 H, H-3′), 5.10 (dd, 1 H, $J_{2',3'}$ 3.1 Hz, H-2′), 4.99 (d, 1 H, $J_{1',2'}$ 2.1 Hz, H-1′), 4.74 (ddd, 1 H, H-6′), 4.58 (dd, 1 H, $J_{6',7a'}$ 4.8, $J_{7a',7b'}$ 8.4 Hz, H-7a′), 4.55 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 4.45 (dd, 1 H, $J_{6',7b'}$ 8.8 Hz, H-7b′), 4.41 (dd, 1 H, $J_{6,7a}$ 5.4 Hz, H-7a), 4.35–4.37 (m, 1 H, OCH_2), 4.24 (dd, 1 H, $J_{5',6'}$ 1.1, $J_{4',5'}$ 10.0 Hz, H-5′), 4.18–4.20 (m, 1 H, OCH_2), 4.14 (dd, 1 H, $J_{6,7b}$ 7.3, $J_{7a,7b}$ 11.1 Hz, H-7b), 4.13 (dd, 1 H, H-2), 3.65 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-3), 3.58 (dd, 1 H, $J_{5,6}$ 2.4, $J_{4,5}$ 10.1 Hz, H-5), 2.16 (s, 3 H), 2.13 (s, 6 H), 2.08, 2.07, and 1.99 (3 s, each 3 H, total 6 Ac).

Method B. The trichloroacetimidate donor **23** was dried by coevaporation with toluene (3 mL). A solution of glycosyl donor **23** (14.2 mg, 0.016 mmol) and allyl alcohol (6.8 μL, 0.1 mmol) in dry CH_2Cl_2 (3 mL) was stirred at ambient temperature with powdered 4 Å molecular sieves for 1 h under N_2. Boron trifluoride etherate (0.02 mmol, 2.5 μL) was added. After 2 h at 20 °C the reaction was stopped with triethylamine (0.1 mL), the reaction mixture was diluted with CH_2Cl_2 (10 mL). The solids were removed by filtration over a pad of Celite and the filtrate was concentrated. The residue was dried by coevaporation with dry toluene (3 mL) and purified as described above to afford **25** (2.5 mg, 20%), **26** (4.6 mg, 38%) and **27** (1.8 mg, 15%). Compound **26** was dissolved in pyridine (1 mL) and stirred for 24 h at ambient temperature with Ac_2O (20 μL). The reaction mixture was concentrated and coevaporated with toluene (3 mL). Purification by chromatography on silica gel (10:0 → 5:1 CH_2Cl_2–acetone) afforded the additional α-allyl glycoside **25** (4.7 mg). Overall yield for **25**: 7.1 mg (58%).

Method C. The fluoride **24** was dried by coevaporation with dry toluene (3 mL). A solution of glycosyl donor **24** (12.5 mg, 0.017 mmol) and allyl alcohol (5.8 mg, 6.8 μL, 0.1 mmol) in dry CH_2Cl_2 (5 mL) was stirred at ambient temperature with powdered 4 Å molecular sieves for 1 h under N_2. Boron trifluoride etherate (0.08 mmol, 10 μL) was added. TLC analysis, after 3 h at 20 °C, revealed the formation of two main products **25**, **26** and a minor amount of **27**. The reaction was stopped with triethylamine (0.05 mL), the reaction mixture was diluted with CH_2Cl_2 (10 mL). The solids were removed by filtration over a pad of Celite and the filtrate was concentrated. Purification by chromatography on silica gel (10:0 → 5:1 CH_2Cl_2–acetone) afforded α-allyl glycoside **25** (3.5 mg, 25%), **26** (3.4 mg, 28%) and **27** (0.6 mg, 5%). Compound **26** (3.4 mg) was dissolved in pyridine (1 mL) and stirred for 24 h at ambient temperature with Ac_2O (10 μL). The reaction mixture was concentrated, coevaporated with toluene (3 mL) and purified by chromatography on silica gel (7:3 → 1:1 toluene–EtOAc), followed by purification on the column of Sephadex LH-20 (2:1, CH_2Cl_2–MeOH), to give compound **25** (3.5 mg, 97%). Overall yield of **25**: 53%.

Acetylation of **26**.—A solution of **26** (7.0 mg, 9.6 μmol) and Ac_2O (20 μL) in dry pyridine (1.5 mL) was stirred for 24 h at ambient temperature. The reaction mixture was taken to dryness, coevaporated twice with toluene (10 mL), and the residue was purified by chromatography (100:0 → 97:3 CH_2Cl_2–MeOH) to afford compound **25** as a solid. Yield 6.5 mg (90%).

Allyl (2,3,4-tri-O-*acetyl-6,7*-O-*carbonyl*-L-glycero-α-D-manno-*heptopyranosyl)-(1 → 3)-2,4,6,7-tetra*-O-*acetyl*-L-glycero-β-D-manno-*heptopyranoside* (**28**).—A solution of **27** (8 mg, 11 μmol) in dry pyridine (1.5 mL) was stirred with Ac_2O (0.05 mL) for 12 h at rt. The reaction mixture was evaporated, coevaporated twice with toluene (10 mL), and purified by silica gel chromatography (7:3 → 1:1 toluene–EtOAc) which afforded **28** as a syrup. Yield 7.5 mg (90%); R_f 0.55 (1:2 toluene–EtOAc); $[\alpha]_D^{20}$ $-1°$ (c 0.3, $CHCl_3$); ^1H NMR: δ 5.91 (m, 1 H, –CH=), 5.49 (dd, 1 H, $J_{3',4'}$ 9.8 Hz, H-4′), 5.41 (dd, 1 H, $J_{1,2} < 1.0$ Hz, H-2), 5.33 (dd, 1 H, $J_{3,4} = J_{4,5}$ 10.2 Hz, H-4), 5.30 (dq, 1 H, $=CH_{2\ trans}$), 5.25 (ddd, 1 H, H-6), 5.26–5.22 (m, 1 H, $=CH_{2\ cis}$), 5.21 (dd, 1 H, $J_{2',3'}$ 3.1 Hz, H-3′), 5.01 (d, 1 H, $J_{1',2'}$ 2.0 Hz, H-1′), 4.92 (dd, 1 H, H-2′), 4.76 (ddd, 1 H, H-6′), 4.63 (d, 1 H, H-1), 4.56 (dd, 1 H, $J_{6',7a'}$ 8.4 Hz, H-7a′), 4.50 (dd, 1 H, $J_{6',7b'}$ 5.6, $J_{7a',7b'}$ 8.8 Hz, H-7b′), 4.45 (dd, 1 H, $J_{6,7a}$ 5.7

Hz, H-7a), 4.34–4.40 (m, 1 H, OCH_2), 4.17 (dd, 1 H, $J_{6,7b}$ 7.0, $J_{7a,7b}$ 11.4 Hz, H-7b), 4.10–4.15 (m, 1 H, OCH_2), 3.97 (dd, 1 H, $J_{5',6'}$ 1.5, $J_{4',5'}$ 10.2 Hz, H-5′), 3.86 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 10.2 Hz, H-3), 3.58 (dd, 1 H, $J_{5,6}$ 2.4 Hz, H-5), 2.16 (s, 6 H), 2.08 (s, 6 H), 2.06 (s, 6 H), and 2.02 (s, 3 H, total 7 Ac).

*Allyl (7-*O*-carbamoyl-*L*-glycero-α-*D*-manno-heptopyranosyl)-(1→3)-*L*-glycero-β-*D*-manno-heptopyranoside* (**29**).—To a suspension of **28** (7.5 mg, 10 μmol) and ammonium hydrogencarbonate (500 mg) in 1:1 MeOH–THF (8 mL) 25% aq NH_3 (2 mL) was added. Stirring was continued for 60 h at ambient temperature, the reaction mixture was diluted with MeOH, the salts were removed by filtration and the filtrate was concentrated to dryness. The residue was purified by chromatography on Sephadex LH-20 using MeOH as an eluent giving **29** as amorphous solid. Yield 3.0 mg (63%); $[\alpha]_D^{20}$ +8° (*c* 0.3, H_2O); ^{1}H NMR (D_2O): δ 5.96 (m, 1 H, –CH=), 5.33 (dq, 1 H, $=CH_{2\ trans}$), 5.26 (dq, 1 H, $=CH_{2\ cis}$), 5.20 (d, 1 H, $J_{1',2'}$ 1.6 Hz, H-1′), 4.75 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.35–4.27 (m, 1 H, OCH_2), 4.23–4.15 (m, 2 H, H-6′, OCH_2), 4.15 (dd, 1 H, $J_{6',7a'}$ 8.3 Hz, H-7a′), 4.12 (dd, 1 H, $J_{6',7b'}$ 9.0 Hz, H-7b′), 4.08 (dd, 1 H, $J_{2',3'}$ 2.9 Hz, H-2′), 4.03–3.97 (m, 2 H, H-2, 6), 3.94 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 3.91 (dd, 1 H, $J_{4',5'}$ 8.4 Hz, H-4′), 3.90 (dd, 1 H, H-3′), 3.92 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.8 Hz, H-3), 3.75–3.68 (m, 3 H, H-7a, 7b, 5′) and 3.34 (dd, 1 H, $J_{5,6}$ 1.7 Hz, H-5).

*Allyl (7-*O*-carbamoyl-*L*-glycero-α-*D*-manno-heptopyranosyl)-(1→3)-*L*-glycero-α-*D*-manno-heptopyranoside* (**30**)

Method A. To a stirred suspension of **25** (7.0 mg, 9 μmol) and ammonium hydrogencarbonate (800 mg) in 2:1 MeOH–THF (12 mL) 25% aq NH_3 (3 mL) was added. Stirring was continued for 72 h at ambient temperature, the reaction mixture was diluted with MeOH, the salts were removed by filtration and the filtrate was concentrated to dryness. The residue was purified by silica gel chromatography (80:20:0→75:25:3 THF–acetone–H_2O), followed by a second chromatography (3:1→3:2 acetone–EtOH). Final purification was performed on Sephadex LH-20 using MeOH as an eluent giving **30** as amorphous solid. Yield 3.0 mg (67%), R_f 0.4 (5:5:1 THF–acetone–H_2O); $[\alpha]_D^{20}$ +76° (*c* 0.5, H_2O); ^{1}H NMR (D_2O): δ 5.98 (m, 1 H, –CH=), 5.33 (dq, 1 H, $=CH_{2\ trans}$), 5.25 (dq, 1 H, $=CH_{2\ cis}$), 5.18 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1′), 4.86 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.24-4.18 (m, 1 H, H-6′), 4.21–4.17 (m, 1 H, OCH_2), 4.15 (dd, 1 H, $J_{6',7a'}$ 6.5 Hz, H-7a′), 4.10 (dd, 1 H, $J_{6',7b'}$ 9.2 Hz, H-7b′), 4.08–4.01 (m, 2 H, H-6, OCH_2), 4.07 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-2′), 4.00 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 3.98 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.6 Hz, H-4), 3.93–3.88 (m, 2 H, H-3′, 4′), 3.92 (dd, 1 H, H-3), 3.76–3.65 (m, 3 H, H-7a, 7b, 5) and 3.63 (dd, 1 H, $J_{4',5'}$ 9.5 Hz, H-5′). Anal. Calcd for $C_{18}H_{31}NO_{14}$: C, 44.54; H, 6.44; N, 2.89. Found: C, 44.21; H, 6.17; N, 2.92.

Method B. To a stirred suspension of **26** (7.5 mg, 10 μmol) and ammonium hydrogencarbonate (500 mg) in 1:1 MeOH–THF (8 mL) 25% aq NH_3 (2 mL) was added. Stirring was continued for 60 h at ambient temperature, the reaction mixture was diluted with MeOH, salts were removed by filtration and the filtrate was concentrated to dryness. Purification as described on Sephadex LH-20 using MeOH as an eluent afforded **30**. Yield 3.0 mg (63%).

*3-(2-Ammoniumethylthio)propyl (7-*O*-carbamoyl-*L*-glycero-α-*D*-manno-heptopyranosyl)-(1→3)-*L*-glycero-β-*D*-manno-heptopyranoside acetate* (**31**).—A solution of **30** (2.5 mg, 0.005 mmol) and cysteamine hydrochloride (4.9 mg, 0.043 mmol) in water (0.1 mL) was treated as described for **7** to give the spacer compound **31**, which was further purified on Sephadex LH-20 (MeOH) followed by chromatography on Bio-Rad Econo-Pac CM ion-exchange cartridges using 0.01 M NH_4Ac as eluent. Yield: 3.2 mg (99%), colorless syrup; $[\alpha]_D^{20}$ +41° (*c* 0.3, H_2O); ^{1}H NMR (D_2O): δ 5.19 (d, 1 H, $J_{1',2'}$ 1.4 Hz, H-1′), 4.81 (d, 1 H, H-1), 4.20–4.16 (m, 2 H, H-6′, 7a′), 4.06 (dd, 1 H, H-7b′), 4.07 (dd, 1 H, $J_{2',3'}$ 3.6 Hz, H-2′), 4.04–3.99 (m, 2 H, H-2, 6), 3.93 (t, 1 H, H-3), 3.90 (m, 2 H, H-3′, 4′), 3.77–3.67 (m, 4 H, H-7a, 7b, 5′, OCH_2), 3.63 (dd, 1 H, $J_{4,5}$ 9.9, $J_{5,6}$ 1.7 Hz, H-5), 3.55 (t, 1 H, OCH_2), 3.21 (t, 2 H, NCH_2), 2.85 (t, 2 H, SCH_2), 2.67 (dt, 2 H, SCH_2), 1.92 (m, 2 H, CH_2), and 1.91 (s, 3 H,

Ac); ^{13}C NMR (D_2O): δ 159.5 ($CONH_2$), 102.59 (C-1′), 99.97 (C-1), 77.34 (C-3), 71.83 (C-5,5′), 70.89, 70.38 (C-2, 2′, 3′), 69.00 (C-6), 66.29 (C-4,4′,6′,7′, OCH_2), 63.33 (C-7), 38.76 (NCH_2), 28.69, 28.08 (2 SCH_2, CH_2) and 23.65 (Ac).

Synthesis of BSA-conjugates **14** *and* **32**.—A solution of thiophosgene (2 μL, 26 μmol) in $CHCl_3$ (1 mL) was added to a solution of compound **13** (3.0 mg, 19 μmol) in 0.1 M aq $NaHCO_3$ (1.5 mL) and the mixture was vigorously stirred for 3 h at rt. The organic phase was separated and the aqueous phase was washed three times with $CHCl_3$ (2 mL portions) and finally purged with N_2 until a clear solution was obtained. The solution was transferred to a solution of BSA (Sigma®, 4.0 mg) in 0.1 M aq $NaHCO_3$–0.3 M NaCl (1 mL) and stirred for 48 h at ambient temperature, then passed through a Sephadex G-25 column (1.6×50 cm, 0.01 M aq $NaHCO_3$). Ninhydrin-positive fractions were combined and dialysed twice with water (2 L) for 24 h. Lyophilization gave the BSA-conjugate **14** as a fluffy white powder. Yield: 4.6 mg.

BSA-conjugate **32** was prepared in a similar manner using 3.2 mg of **31** and 3.0 mg of BSA to give 3.2 mg of the neoglycoprotein **32**.

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