Synthesis of Phosphorylated Pentasaccharides Found on Asparagine-Linked Carbohydrate Chains of Lysosomal Enzymes

Om P. Srivastava and Ole Hindsgaul*

Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

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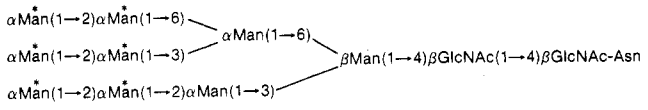
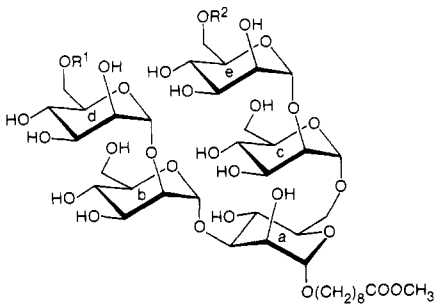
Simple and direct syntheses of 8-(methoxycarbonyl)octyl 3,6-bis-*O*-[2-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyl]-α-D-mannopyranoside (**2**), the corresponding terminally 6-O-diphosphorylated pentasaccharide **3**, and the two terminally 6-O-monophosphorylated derivatives **4** and **5** are reported. The key step in these syntheses involved the selective glycosylation of 8-(methoxycarbonyl)octyl 2,4-di-*O*-benzyl-3,6-bis-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**14**) which occurred preferentially on the α (1→6)-linked sugar residue.

Carbohydrate structures containing D-mannose 6-phosphate residues are the essential component of a recognition marker that directs the intracellular transport of newly biosynthesized acid hydrolases to lysosomes.[1-3] The recognition marker is carried on so-called high-mannose asparagine-linked oligosaccharide chains that may contain as many as nine mannose residues, several of which may be 6-O-phosphorylated in any given structure.[4-6] The largest of these oligosaccharide structures **1** is shown; the mannose residues on which 6-*O*-phosphate groups have been located are indicated by an asterisk. Targeting of lysosomal enzymes to the lysosomes is dependent on the recognition and binding of the phosphorylated oligosaccharides by specific phosphomannosyl receptors.



1, * = 6-O-phosphomonoester



2, $R^1=R^2=H$

3, $R^1=R^2=PO_3Na_2$

4, $R^1=PO_3Na_2$; $R^2=H$

5, $R^1=H$; $R^2=PO_3Na_2$

To clarify the detailed structural features required for specific recognition by phosphomannosyl receptors, we became involved in a synthetic effort to produce 14 phosphorylated oligomannosides, all corresponding to partial structures of **1**. We have recently reported[7-10] the syntheses of 11 di- and trimannosides in this series. Here we describe a simple direct preparation of the branched pentamannoside arm of **1**, αMan(1→2)αMan(1→6)-[αMan(1→2)αMan(1→3)]αManOR (**2**), the corresponding diphosphorylated pentasaccharide **3**, and the two terminally monophosphorylated derivatives **4** and **5**. These structures were prepared as their 8-(methoxycarbonyl)octyl glycosides,[11-13] R = $(CH_2)_8COOCH_3$, to allow their eventual covalent attachment to proteins and solid supports. The synthesis of the carbohydrate sequence of pentasaccharide **2**, as the methyl glycoside, has previously been reported by Ogawa et al.[14] The preparation of a fully protected diphosphorylated pentasaccharide with the sequence **3** has been reported by Lönngren and Ottosson[15] but was not deprotected.

General strategies for synthesis of oligomannosides are now well developed and have been reviewed by Ogawa et al.[16] The challenges in such syntheses now lie in reducing the number of overall steps required to produce a given series of compounds. In oligosaccharide synthesis these steps involve mainly selective protection/deprotection schemes and glycosylation reactions, the latter frequently producing complex mixtures of products. Convenient syntheses of structures such as **2–5** should therefore minimize the number of glycosylation reactions and keep the protection strategy as simple as possible to avoid the tedious preparation of highly asymmetric building blocks.

With these considerations in mind, the simplest route to pentasaccharides **2–5** appeared to begin with the diglycosylation of the protected monosaccharide **11** using the well-known glycosyl chloride **12**,[14,17] followed by removal of both acetyl groups to produce the trisaccharide diol **14**. The completion of the syntheses of the symmetrically substituted **2** and **3** would then simply require further diglycosylation using the glycosyl donors **15** and **16**,[9,18] respectively, then subsequent deprotection. Preparation of the nonsymmetrically phosphorylated **4** and **5** would require either the selective glycosylation of one of the two hydroxyl groups in **14** (with either **15** or **16**) or a random

(1) Sahagian, G. G. *Biol. Cell.* **1984**, *51*, 207.
(2) Creek, K. E.; Sly, W. S. *Lysosomes Biol. Pathol.* **1984**, *7*, 63.
(3) Lang, L.; Reitman, M.; Tang, J.; Roberts, R. M.; Kornfeld, S. *J. Biol. Chem.* **1984**, *259*, 14663.
(4) Varki, A.; Kornfeld, S. *J. Biol. Chem.* **1980**, *255*, 10847.
(5) Natowicz, M.; Baenziger, J. U.; Sly, W. S. *J. Biol. Chem.* **1982**, *257*, 4412.
(6) Varki, A.; Kornfeld, S. *J. Biol. Chem.* **1982**, *258*, 2808.
(7) Srivastava, O. P.; Hindsgaul, O. *Carbohydr. Res.* **1986**, *155*, 57.
(8) Srivastava, O. P.; Hindsgaul, O. *Can. J. Chem.* **1986**, *64*, 2324.
(9) Srivastava, O. P.; Hindsgaul, O. *Carbohydr. Res.* **1987**, *161*, 195.
(10) Srivastava, O. P.; Hindsgaul, O. *Carbohydr. Res.* **1987**, *161*, 324.
(11) Lemieux, R. U.; Bundle, D. R.; Baker, D. A. *J. Am. Chem. Soc.* **1975**, *97*, 4076.
(12) Lemieux, R. U.; Baker, D. A.; Bundle, D. R. *Can. J. Biochem.* **1977**, *55*, 507.
(13) Lemieux, R. U.; Baker, D. A.; Weinstein, W. M.; Switzer, C. M. *Biochemistry* **1981**, *20*, 199.
(14) Ogawa, T.; Katano, K.; Matsui, M. *Carbohydr. Res.* **1978**, *64*, C-3.
(15) Lönngren, J.; Ottosson, H. Proceedings of the 7th International Symposium on Glycoconjugates, Lund-Ronneby, July 17–23, 1983; p 374.
(16) Ogawa, T.; Yamamoto, H.; Nukada, T.; Kitajima, T.; Sugimoto, M. *Pure Appl. Chem.* **1984**, *56*, 779.
(17) Garegg, P. J.; Maron, L. *Acta Chem. Scand., Ser. B* **1979**, *33*, 39.
(18) Matta, K. L.; Chowdhary, M. S.; Jain, R. K.; Abbas, S. A. *Carbohydr. Res.* **1986**, *150*, C1.

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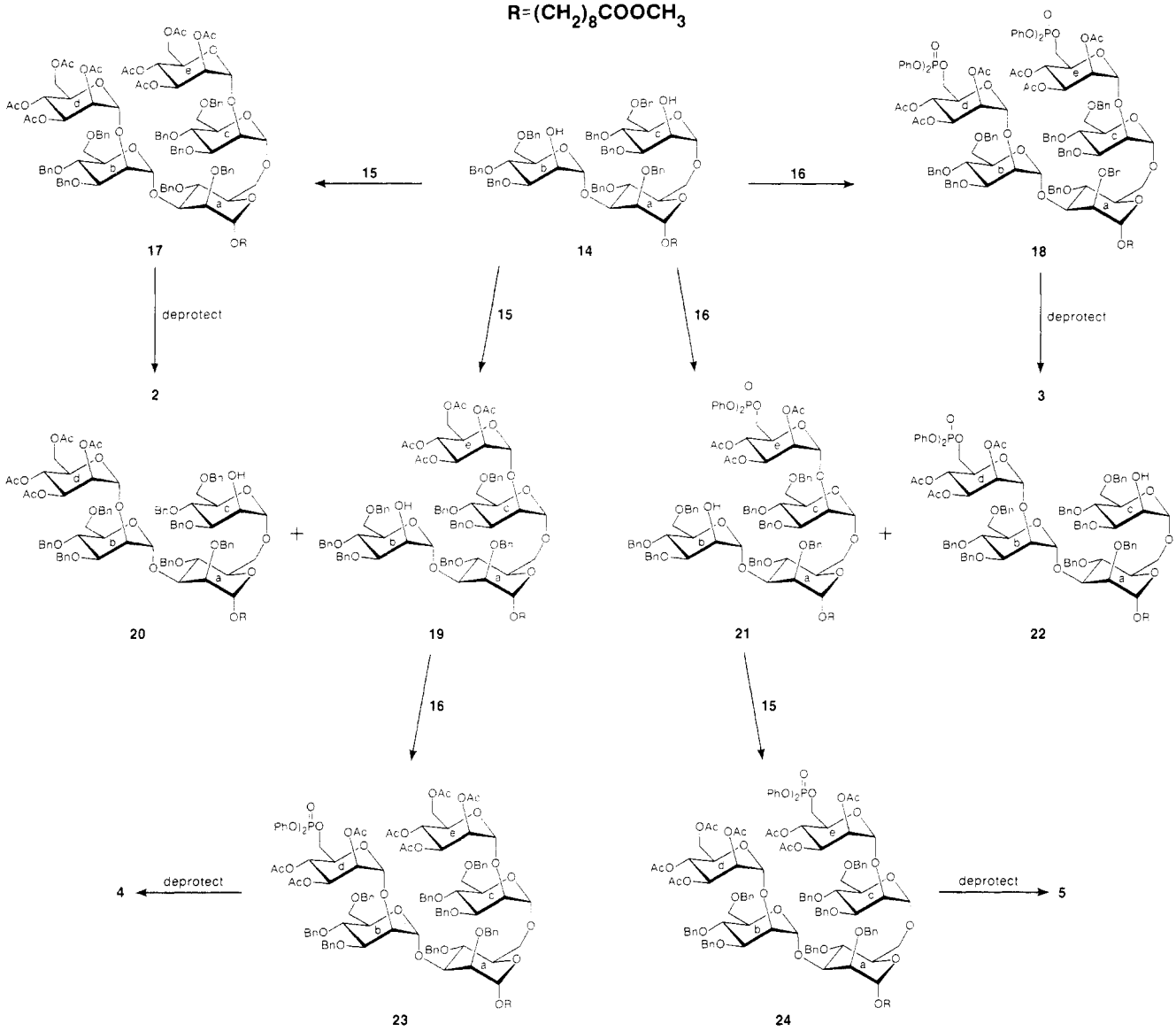
Scheme I

$R=(CH_2)_8COOCH_3$



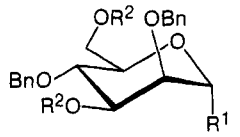
glycosylation followed by separation of the product tetrasaccharides and further glycosylation with the alternate **16** or **15**. Since the objective of this work was to provide well-characterized samples of **2–5** in quantities (10–20 mg) sufficient to assess their biological activity, the yields obtainable in the selective reactions were not critical *provided* the products could be conveniently isolated in pure form and that their structures could be unequivocally established. The major difficulties anticipated in this work were therefore the chromatographic separation of the isomeric protected tetrasaccharide intermediates and their structural characterization, which would be complicated by the presence of four pyranose rings of identical D-manno configuration (Scheme I).

Results and Discussion

The key trisaccharide diol **14** was prepared in seven straightforward steps (29% overall yield) from the readily accessible methyl 2,4-di-*O*-benzyl-α-D-mannopyranoside **6**,[19] which was converted to the diacetate **7** (85%) and acetolyzed (85%) to provide the 1,3,6-tri-*O*-acetyl derivative **8**.[20] Reaction of **8** with HBr in dichloromethane gave the glycosyl bromide **9**, which was coupled with 8-(methoxycarbonyl)octanol, in the presence of silver trifluoromethanesulfonate and *sym*-collidine in dichloromethane, to produce the α-linked mannopyranoside **10** (73%). The α configuration of the glycosidic linkage in **10** was evident from its ^{1}H-coupled ^{13}C NMR spectrum, which showed the signal for the anomeric carbon at 97.7 ppm ($^1J_{C,H}$ = 168 Hz) in accord with the empirical rules formulated by Bock and Pedersen[21] for the dependence of the one-bond C–H coupling constants on the anomeric configuration of glycopyranosides and subsequently supported by a large number of observations on synthetic oligomannosides.[22] Deacetylation of **10** then gave the diol **11** (87%).

Reaction of **11** with an excess of the 2-*O*-acetylglycosyl chloride **12**, under standard glycosylation conditions (silver trifluoromethanesulfonate/*N*,*N*,*N*′,*N*′-tetramethylurea),[23] gave the fully protected trisaccharide **13** (73%). The α configuration of the two new glycosidic linkages in **13** was expected due to neighboring group participation of the acetoxy groups of **12** in the glycosylation reaction and were confirmed in the ^{13}C NMR spectrum, which showed all

(19) Ogawa, T.; Matsui, M. *Carbohydr. Res.* **1978**, *62*, C-1.
(20) Ogawa, T.; Sasajima, K. *Tetrahedron* **1981**, *37*, 2787.
(21) Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293.
(22) Ogawa, T.; Sasajima, K. *Carbohydr. Res.* **1981**, *97*, 205.
(23) Hanessian, S.; Banoub, J. *Carbohydr. Res.* **1977**, *53*, C-13.



6, R^1=OCH_3; R^2= H

7, R^1=OCH_3; R^2= Ac

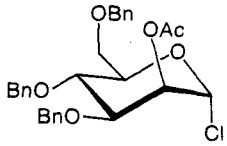
8, R^1= OAc; R^2= Ac

9, R^1=Br; R^2= Ac

10, R^1=$O(CH_2)_8COOCH_3$; R^2= Ac

11, R^1=$O(CH_2)_8COOCH_3$; R^2= H

three anomeric carbons with $^1J_{C,H}$ near 170 Hz (Table I). Deacetylation of **13** provided the key trisaccharide diol intermediate **14** (87%).



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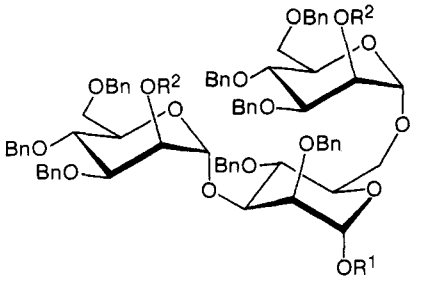
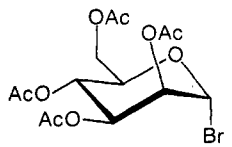


13, R^1=$O(CH_2)_8COOCH_3$; R^2=Ac

14, R^1=$O(CH_2)_8COOCH_3$; R^2=H

Glycosylation of **14** with an excess of acetobromomannose (**15**)[24] gave the expected pentasaccharide derivative **17** (60%) whose ^1H and ^{13}C NMR parameters (Table I) were in accord with the assigned structure. Reaction of **14** with an excess of **16**, which has previously been shown[9,18] to be a useful reagent for the introduction of terminal mannose 6-phosphate residues, gave the required diphosphorylated pentasaccharide derivative **18** in 63% yield. The structure of **18** is fully supported by its NMR parameters presented in Table I.



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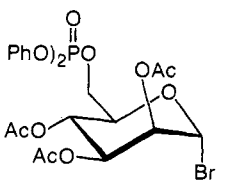

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In the above reaction of trisaccharide **14**, to ultimately produce pentasaccharide **17**, silica gel thin-layer chromatograms taken at earlier stages of the reaction suggested the formation of a resolvable tetrasaccharidic intermediate less polar than **14** along with a poorly resolved mixture of the other tetrasaccharidic intermediate and pentasaccharide **17**, the latter two essentially comigrating with the unreacted trisaccharide **14**. Interruption of the reaction when the concentration of this less polar compound appeared to reach a maximum produced a complex mixture from which it could be conveniently purified by chromatography. The structure of this tetrasaccharide, which was isolated in 22% yield, could not be assigned with certainty on the basis of its NMR parameters, presented in Table I. Its structure could, however, be unequivocally assigned as the product of glycosylation of the 6-*O*-mannopyranosyl

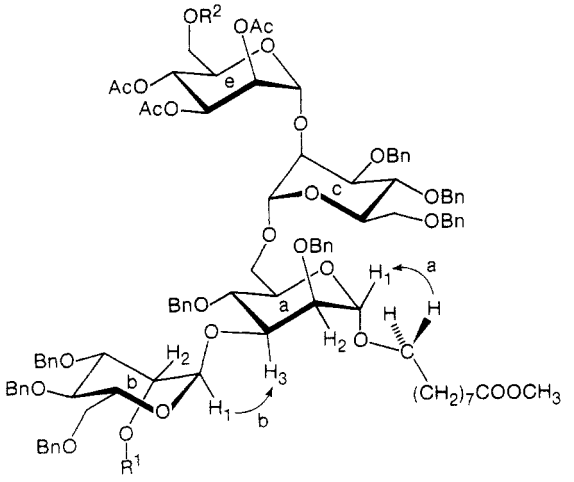
(24) Boomer, W. A. *J. Am. Chem. Soc.* **1958**, *80*, 3372.

Table I. Selected Chemical Shifts (ppm) and Coupling Constants (Hz) for Protected Oligosaccharide Intermediates[a]

nucleus[b]	13	14	17	18	19	20	21	22	23	24	25
H-1a ($J_{1,2}$)	4.760 (-)[c]	4.760 (1.5)	4.770 (-)[c]	4.807 (-)[c]	4.766 (1.6)	4.762 (1.7)	4.808 (-)[c]	4.748 (1.6)	4.713 (1.2)	4.763 (1.5)	4.837 (1.6)
H-1b ($J_{1,2}$)	5.192 (1.8)	5.228 (1.2)	5.233 (-)[c]	5.181 (1.7)	5.232 (1.5)	5.222 (1.6)	5.224 (1.6)	5.182 (1.9)	5.143 (1.5)	5.225 (-)[c]	5.335 (1.7)
H-1c ($J_{1,2}$)	4.969 (1.6)	5.088 (1.6)	5.015 (1.8)	5.024 (2.0)	5.048 (1.8)	5.068 (1.6)	5.067 (1.8)	5.052 (1.6)	4.958 (1.8)	5.033 (1.5)	5.082 (1.8)
H-1d ($J_{1,2}$)			4.770 (-)[c]	4.876 (1.7)		4.795 (-)[c]		4.911 (1.8)	4.870 (1.5)	4.815 (1.5)	4.878 (1.5)
H-1e ($J_{1,2}$)			4.923 (1.5)	4.854 (-)[c]	4.939 (1.7)		4.872 (1.6)		4.838 (1.6)	4.860 (-)[c]	
C-1a ($^1J_{C,H}$)	96.95 (168)	97.00 (167)	96.96 (167)	96.93 (168)	97.25 (168)		97.11 (167)		97.06 (167)	96.84 (167)	
C-1b ($^1J_{C,H}$)	99.87 (170)	101.49 (170)	101.49 (172)	100.94 (170)	101.46 (173)		101.42 (174)		100.95 (171)	101.08 (172)	
C-1c ($^1J_{C,H}$)	98.15 (172)	99.71 (170)	99.42 (172 ± 3)	99.29 (172)	99.43 (172)		99.27 (171)		99.43 (172)	99.05 (170)	
C-1d ($^1J_{C,H}$)			99.30 (172 ± 3)	99.04 (171)					99.09 (172)	99.29 (173)	
C-1e ($^1J_{C,H}$)			99.10 (171)	98.84 (173)	99.13 (172)		99.06 (173)		98.87 (173)	99.29 (173)	
C-6d ($^2J_{C,P}$)			62.54	67.18 (5)					66.78 (5)	62.55	
C-6e ($^2J_{C,P}$)			62.47	66.72 (5)	62.51		67.18 (5)		62.48	67.19 (5)	
P				-11.67			-11.73	-11.63	-11.68	-11.69	

[a] In $CDCl_3$; ^1H NMR at 300, 360, or 400 MHz; ^{13}C NMR at 75 MHz; ^{31}P NMR at 162 MHz. Other Experimental conditions and reference standards given in the Experimental Section. [b] Assignments are tentative. [c] Could not be determined due to spectral overlap.

Chart I.[a] **$^1H\{^1H\}$ Nuclear Overhauser Enhancements Used To Establish the Structures of 19 and 25**[a]



19, R^1=H, R^2=Ac

21, R^1=H, R^2=$P(OPh)_2O$

25, R^1=Benzoyl, R^2=$P(OPh)_2O$

[a] Enhancements were measured by difference spectroscopy as described by Richarz and Wüthrich.[26] Compound **19**: enhancement a = 1.9%, b = 4.2%. Compound **25**: enhancement a = 2.8%, b = 1.5%.

residue of **14**, namely **19**, following the $^1H\{^1H\}$ nuclear Overhauser enhancement (NOE) experiments summarized in Chart I. Thus, in $CDCl_3$ solution, the coupling with the D_2O-exchangeable proton allowed the assignment of H-2 (δ 4.03) of the nonglycosylated mannopyranosyl residue and, by further decoupling, the anomeric proton (H-1) of the same residue. Irradiation of this anomeric proton (δ 5.23) produced an intraresidue NOE of 2.9% on H-2 along with an interresidue enhancement of 4.2% (enhancement b, Chart I) on a doublet of doublets (δ 4.13) with coupling constants 3.2 and 9.5 Hz. These coupling constants require this signal to be H-3 of one of the sugar residues as expected for **19** but not for **20**. The definitive assignment of this enhanced signal as H-3a could be made following the observation of an NOE of 1.9% (enhancement a, Chart I) on H-1a following irradiation of one of the diastereotopic aglyconic protons (dt, ^2J = −9.5, ^3J = 6.5 Hz, δ 3.27) of the 8-(methoxycarbonyl)octyl chain. Decoupling of H-1a then identified the signal for H-2a (δ 3.85), which was in turn coupled to the signal that received the 4.2% NOE from H-1b.

A second tetrasaccharide was also produced in the same reaction, and sufficient material was isolated to allow its characterization only by ^1H NMR (Table I), which suggested it to have the structure **20**. Integration of the appropriate signals in the ^1H NMR of the mixed chromatographic fractions showed the distribution of products from the partial glycosylation reaction to be unreacted **14** (25%), pentasaccharide **17** (14%), and tetrasaccharides **19** (22%) and **20** (14%), after chromatography.

The partial glycosylation of **14** with the phosphorylated glycosyl bromide **16** gave a very similar distribution of products, i.e. unreacted **14** (31%), pentasaccharide **18** (9%), and tetrasaccharides **21** (21%) and **22** (11%). The product of the glycosylation of the 6-*O*-mannopyranosyl residue was again easily purified and its structure unequivocally established by ^1H NMR spectroscopy. The resonances for H-2b and H-2c in **21** fortuitously coincided at δ 4.03, but benzoylation provided the derivative **25** where the signal for H-2 of the nonglycosylated mannose residue moved downfield to δ 5.77. Repetition of the experiments described above in the proof of structure of **19**, including the NOE experiments that produce the intraresidue enhancements summarized in Chart I, left no doubt as to the structure of **25**, and hence **21**. The yields of **14**, **18**, and **22** were again determined by ^1H NMR analysis of the mixed chromatographic fractions, as were the data for **22** presented in Table I.

The condensation of diol **14** with either **15** or **16** produced, in each case, a major tetrasaccharidic product (**19**, **21**) derived from preferential glycosylation of the 6-*O*-mannopyranosyl unit c. The inherent reactivity of OH-2 in rings b and c should be the same, since the two sugar residues are identical, and the reason for preferential glycosylation of ring c is likely the result of steric factors imposed by the preferred conformation of **14**. Paulsen et al.[25] described a similar selectivity during the hydrolysis of the *O*-acetyl protecting groups of a related tetrasaccharide, containing the trisaccharide terminus of **13**, where the ester on O-2 of the α (1→6)-linked mannopyranose unit (ring c) was cleaved at twice the rate of the ring a ester. These authors proposed that the 4-*O*-benzyl ether of the reducing-end mannopyranose (ring a) sterically shielded the ring b 2-acetoxy group.

The comparative ease of isolation of **19** and **21** allowed ready access to pentasaccharides **23** and **24**. Reaction of **19** with **16** gave **23** in 71% yield, while glycosylation of **21** with **15** gave **24** in 68% yield. NMR data supporting the structures assigned to **23** and **24** are presented in Table I.

Deprotection of **17**, **18**, **23**, and **24** involved sequential hydrogenolysis of the benzyl ethers on 5% Pd/C, removal of the phosphate phenyl groups by hydrogenolysis over Adams' catalyst, and de-O-acetylation using methanolic sodium methoxide. The final products (**2–5**) were obtained as the lyophilized sodium salts, after chromatography on Bio-Gel P-2 and ion exchange, in yields of 70–80%. The ^1H, ^{13}C, and ^{31}P NMR data for **2–5** are presented in Table II.

In conclusion, direct and rapid syntheses of pentasaccharides **2–5** have been achieved that provide fully characterized products in quantities sufficient for biological studies. The results of these studies, which include the inhibition of binding of lysosomal enzymes with phosphomannosyl receptors and an investigation of the function of these receptors in the endocytosis of phosphorylated glycoconjugates, will be reported elsewhere.

Experimental Section

Optical rotations were measured with a Perkin-Elmer 241 polarimeter at ambient temperatures (22 ± 2 °C). Unless otherwise noted all reactions were carried out at ambient temperatures, and in the processing of reaction mixtures, solutions in organic solvents were washed with equal volumes of aqueous solutions. Thin-layer chromatography was performed on precoated plates of silica gel (60-F_{254}, E. Merck, Darmstadt) with detection by quencing of fluorescence or by charring, or both, after spraying with 5% H_2SO_4 in ethanol. Unless otherwise indicated, column chromatography was performed on silica gel Merck 60 (40–63 μm). The partisil column was from Waters Associates. ^1H NMR spectra were recorded at 300, 360, or 400 MHz, on Bruker instruments, with either tetramethylsilane (δ 0 in $CDCl_3$) or acetone (δ 2.225 in D_2O) as internal standards at ambient temperature. ^{13}C NMR spectra were recorded at 75 MHz with either internal tetramethylsilane (δ 0 in $CDCl_3$) or external 1%

(25) Paulsen, H.; Rauwald, W.; Lebuhn, R. *Carbohydr. Res.* **1985**, *138*, 29.

(26) Richarz, R.; Wüthrich, K. *J. Magn. Reson.* **1978**, *30*, 147.

Table II. Selected Chemical Shifts (ppm) and Coupling Constants (Hz) for Pentasaccharides 2–5[a]

nucleus[b]	2	3	4	5
H-1a ($J_{1,2}$)	4.818 (1.6)	4.832 (1.7)	4.821 (1.7)	4.826 (1.6)
H-1b ($J_{1,2}$)	5.356 (1.7)	5.295 (1.5)	5.328 (1.6)	5.359 (1.7)
H-1c ($J_{1,2}$)	5.148 (1.6)	5.078 (1.6)	5.150 (1.7)	5.099 (1.7)
H-1d ($J_{1,2}$)	5.048 (1.7)	5.072 (1.6)	5.066 (1.6)	5.052 (1.8)
H-1e ($J_{1,2}$)	5.028 (1.7)	5.060 (1.7)	5.032 (1.7)	5.045 (1.8)
H-2a ($J_{2,3}$)	4.080 (–)[c]	4.065 (–)[c]	4.050 (3.2)	4.068 (3.2)
H-2b ($J_{2,3}$)	4.098 (3.3)	4.107 (3.2)	4.107 (3.3)	4.103 (3.2)
H-2c ($J_{2,3}$)	4.005 (3.5)	4.057 (–)[c]	4.011 (3.4)	4.004 (3.5)
H-2d ($J_{2,3}$)	4.073 (3.3)	4.057 (–)[c]	4.063 (3.0)	4.068 (3.2)
H-2e ($J_{2,3}$)	4.073 (3.3)	4.036 (3.4)	4.073 (3.2)	4.053 (3.0)
OCH_3 (s)	3.690	3.689	3.690	3.689
CH_2COO (t, 7.5 Hz)	2.393	2.391	2.393	2.393
C-1a ($^1J_{C,H}$)	98.80 (172)	98.87 (173)	98.90 (172)	98.94 (172)
C-1b ($^1J_{C,H}$)	101.67 (173)	101.37 (171)	101.30 (174)	101.65 (173)
C-1c ($^1J_{C,H}$)	100.69 (171)	100.66 (171)	100.64 (171)	100.63 (171)
C-1d ($^1J_{C,H}$)	103.15 (171)	103.07 (171)	103.11 (171)	103.16 (171)
C-1e ($^1J_{C,H}$)	103.15 (171)	103.07 (171)	103.11 (171)	103.10 (171)
C-3a	79.75	79.28	79.51	79.71
C-6a	66.51	66.19	66.53	66.52
C-2b	79.55	78.86	78.72	79.40
C-6b	63.40	61.86	61.84	61.77
C-2c	79.31	78.74	78.30	79.28
C-6c	61.98	61.79	61.79	61.77
C-6d ($^2J_{C,P}$)	61.83	64.0 (≤5)	63.71 (5)	61.85
C-6e ($^2J_{C,P}$)	61.83	64.0 (≤5)	61.91	63.43 (4)
$COOCH_3$	178.75	178.80	178.78	178.77
$COOCH_3$	52.94	52.94	52.92	52.93
OCH_2CH_2–	68.90	69.01	69.03	68.87
CH_2COO	34.56	34.59	34.57	34.56
^{31}P		3.37	4.87	4.70

[a] In D_2O; ^1H NMR at 360 MHz; ^{13}C NMR at 75 MHz; ^{31}P NMR at 162 MHz. Other experimental conditions and reference standards are given in the Experimental Section. [b] Assignments are tentative, but ^1H connectivities were confirmed by homonuclear decoupling. [c] Could not be determined due to spectral overlap.

1,4-dioxane (δ 67.4 in D_2O) as reference standards. ^{31}P NMR spectra were recorded at 162 MHz with external 85% H_3PO_4 in D_2O as reference (δ 0) for both $CDCl_3$ and D_2O solutions. The microanalyses were carried out by the Analytical Services Laboratory of this department. 8-(Methoxycarbonyl)octanol was a generous gift from Chembiomed Ltd. (Edmonton, Alberta, Canada).

3,6-Di-*O*-acetyl-2,4-di-*O*-benzyl-α-D-mannopyranosyl Bromide (9). Dichloromethane (15 mL) was saturated with HBr at 0 °C and added to a solution of triacetate **8** (842 mg, 1.73 mmol) stirring in dichloromethane (2 mL) at 0 °C. After 2 h at 0 °C the solution was concentrated to near dryness and dry toluene (25 mL) was added and evaporated three times to remove acetic acid. The residual syrup appeared homogeneous by both TLC (R_f 0.51 in 1:2 ethyl acetate–hexane) and ^1H NMR and was used directly in the preparation of **10**: ^1H NMR ($CDCl_3$) δ 6.38 (d, 1 H, J = 1.5 Hz, H-1), 5.58 (dd, 1 H, J = 3.2, 9.2 Hz, H-3), 2.04 and 1.97 (each s, 3 H, $COCH_3$).

8-(Methoxycarbonyl)octyl 3,6-Di-*O*-acetyl-2,4-di-*O*-benzyl-α-D-mannopyranoside (10). The solution of **9** described above was added dropwise, over 0.5 h, to a mixture of 8-(methoxycarbonyl)octanol (217 mg, 1.15 mmol), *sym*-collidine, (230 μL, 1.73 mmol), silver trifluoromethanesulfonate (446 mg, 1.73 mmol), and pulverized 4-Å molecular sieves (3 g) stirring in 1,2-dichloromethane (5 mL) at room temperature. After 15 h, dichloromethane (25 mL) was added and the sieves were removed by filtration and washed with more dichloromethane (25 mL). *sym*-Collidine (230 μL), followed by silver trifluoromethanesulfonate (450 mg), was then added to the filtrate to destroy unreacted **9**, and after 0.5 h, tetraethylammonium bromide (360 mg) was added to precipitate excess silver. Solids were removed by filtration, and the resulting solution was washed twice with saturated aqueous $NaHCO_3$ and twice with water and taken to dryness. The residual syrup was purified by chromatography using 1:2 ethyl acetate–hexane as eluent, providing the title compound: 520 mg (73%); R_f 0.30 (1:2 ethyl acetate–hexane); $[\alpha]_D$ +13.5° (*c* 0.65, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.25 (dd, 1 H, J = 3.2, 9.0 Hz, H-3), 4.81 (d, 1 H, J = 1.8 Hz, H-1), 3.38 (m, 1 H, $OCHHCH_2$), 2.30 (t, 2 H, J = 7.5 Hz, CH_2COO), 2.07 and 1.94 (each s, 3 H, $COCH_3$); ^{13}C NMR ($CDCl_3$) δ 97.7 ($^1J_{C,H}$ = 168 Hz, C-1), 51.3 (OCH_3). Anal. Calcd for $C_{34}H_{46}O_{10}$: C, 66.43; H, 7.54. Found: C, 66.17; H, 7.65.

8-(Methoxycarbonyl)octyl 2,4-Di-*O*-benzyl-α-D-mannopyranoside (11). A solution of **10** (480 mg, 0.78 mmol) in dry methanol (5 mL), containing a trace of sodium methoxide, was kept for 15 h, neutralized with Amberlite IRC-50 (H^+), filtered, and evaporated. Chromatography of the residue using 2:3 ethyl acetate–hexane as eluent gave diol **11** [360 mg (87%)] as a syrup: R_f 0.43 (1:1 ethyl acetate–hexane); $[\alpha]_D$ +22.8° (*c* 0.71, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.82 (d, 1 H, J = 1.2 Hz, H-1), 2.40 (d, 1 H, J = 9.0 Hz, O*H*-3), 2.29 (t, 2 H, J = 7.5 Hz, CH_2COO), 2.12 (dd, 1 H, J = 5.5, 7.5 Hz, O*H*-6); ^{13}C NMR ($CDCl_3$) δ 97.1 ($^1J_{C,H}$ = 167 Hz, C-1), 62.3 (C-6), 51.4 (OCH_3). Anal. Calcd for $C_{30}H_{42}O_8$: C, 67.90; H, 7.98. Found: C, 67.82; H, 8.00.

8-(Methoxycarbonyl)octyl 3,6-Bis-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-2,4-di-*O*-benzyl-α-D-mannopyranoside (13). A solution of **12** (1.0 g, 1.98 mmol) in 1,2-dichloroethane (2 mL) was added dropwise in stirring to a mixture of diol **11** (350 mg, 0.66 mmol), silver trifluoromethanesulfonate (1.0 g, 3.93 mmol), and *N,N,N′,N′*-tetramethylurea (355 μL, 2.97 mmol) in 1,2-dichloroethane (3 mL). After 1 h, additional **12** (1.0 g) and tetramethylurea (355 μL) were added and stirring was continued for an additional 15 h. *sym*-Collidine (520 μL) followed by silver trifluoromethanesulfonate (400 mg) were then added to destroy excess **12**, and after 0.5 h, excess silver was precipitated by the addition of tetraethylammonium bromide (300 mg). Solids were removed by filtration, and the dichloromethane solution was washed with saturated aqueous $NaHCO_3$ (2 × 50 mL) and water (50 mL) before concentration and chromatography using 1:2 ethyl acetate–hexane as eluent. Trisaccharide **13** [710 mg (73%)] was obtained as a syrup: R_f 0.26 (1:2 ethyl acetate–hexane); $[\alpha]_D$ +42.0° (*c* 0.20, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.51 (dd, 1 H, J = 1.8, 3.2 Hz, H-2b), 5.49 (dd, 1 H, J = 1.6, 3.2 Hz, H-2c), 3.65 (s, OCH_3), 3.27 (m, 1 H, $OCHHCH_2$), 2.27 (t, 2 H, J = 7.5 Hz, CH_2COO), 2.14 and 2.09 (each s, 3 H, $COCH_3$); ^{13}C NMR ($CDCl_3$) δ 174.2 (CH_2COO), 170.2 and 170.1 ($COCH_3$), 69.2, 68.1 and 66.5 (C-6a,b,c), 51.4 (OCH_3), 21.1 and 21.0 ($COCH_3$). Anal. Calcd for $C_{88}H_{102}O_{20}$: C, 71.42;

H, 6.95. Found: C, 71.25; H, 7.00.

8-(Methoxycarbonyl)octyl 2,4-Di-*O*-benzyl-3,6-bis-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (14). Deacetylation of **13** (680 mg, 0.46 mmol), as described for the preparation of **11**, gave diol **14**, which was purified by chromatography using 5:6 ethyl acetate–hexane as eluant to provide a syrup: 560 mg (87%); R_f 0.25 (5:6 ethyl acetate–hexane); $[\alpha]_D$ +47.2° (*c* 0.17, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 3.65 (s, OCH_3), 2.35 and 2.34 (each d, 1 H, *J* = 2.5 Hz, D_2O-exchangeable, OH-2b,c), 2.27 (t, 2 H, *J* = 7.5 Hz, CH_2COO); ^{13}C NMR ($CDCl_3$) δ 174.1 (CH_2COO), 51.4 (OCH_3). Anal. Calcd for $C_{84}H_{98}O_{18}$: C, 72.29; H, 7.08. Found: C, 72.18; H, 7.27.

8-(Methoxycarbonyl)octyl 3,6-Bis-*O*-[2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-2,4-di-*O*-benzyl-α-D-mannopyranoside (17). Bromide **15** (0.14 mmol) in 1,2-dichloroethane (1 mL) was added to a stirring mixture of diol **14** (102 mg, 0.073 mmol), silver trifluoromethanesulfonate (280 mg, 1.1 mmol) and *N,N,N′,N′*-tetramethylurea (25 μL, 0.21 mmol) in 1,2-dichloroethane (2 mL). After 5, 15, and 24 h further additions of **14** (0.14 mmol) and tetramethylurea (0.21 mmol) were made, and the reaction mixture was stirred for a further 5 days. The reaction mixture was then processed as described for the preparation of **13**. Chromatography using 5:6 ethyl acetate–hexane as eluent gave pentasaccharide **17** [90 mg (59%)] as a syrup: R_f 0.21 (5:6 ethyl acetate–hexane); $[\alpha]_D$ +46.1° (*c* 0.18, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.45–5.18 (7 H, H-2d,e, H-3d,e, H-4d,e, H-1b), 3.65 (s, OCH_3) 3.27 (m, 1 H, $OCHHCH_2$), 2.27 (t, 2 H, *J* = 7.5 Hz, CH_2COO), 2.10 (s, 6 H, 2 $COCH_3$), 2.03 and 2.02 (each s, 3 H, $COCH_3$), 1.99 (s, 6 H, 2 $COCH_3$), 1.98 and 1.96 (each s, 3 H, $COCH_3$); ^{13}C NMR ($CDCl_3$) δ 174.2 (CH_2COO), 51.4 (OCH_3). Anal. Calcd for $C_{112}H_{134}O_{36}$: C, 65.42; H, 6.57. Found: C, 65.51; H, 6.47.

8-(Methoxycarbonyl)octyl 3,6-Bis-*O*-[2-*O*-[2,3,4-tri-*O*-acetyl-6-*O*-(diphenylphosphoryl)-α-D-mannopyranosyl]-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-2,4-di-*O*-benzyl-α-D-mannopyranoside (18). Reaction of diol **14** (190 mg, 0.14 mmol) with silver trifluoromethanesulfonate (8 equiv), *N,N,-N′,N′*-tetramethylurea (12 equiv), and phosphorylated bromide **16** (8 equiv, added in four equal portions over 24 h) in 1,2-dichloroethane, exactly as described for the preparation of **17**, gave, after chromatographic purification using 5:6 ethyl acetate–hexane as eluent, pentasaccharide **18** [210 mg (63%)] as a syrup: R_f 0.14 (5:6 ethyl acetate–hexane); $[\alpha]_D$ +50.5° (*c* 0.20, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.47–5.36 (6 H, H-2d,e, H-3d,e, H-4d,e), 3.64 (s, OCH_3), 3.21 (m, 1 H, $OCHHCH_2$), 2.25 (t, 2 H, *J* = 7.5 Hz, CH_2COO), 2.01, 1.99, 1.98, 1.97, 1.94 and 1.91 (each s, 3 H, $COCH_3$); ^{13}C NMR ($CDCl_3$) δ 174.2 ($COOCH_3$), 51.4 (OCH_3). Anal. Calcd for $C_{132}H_{148}O_{40}P_2$: C, 64.80; H, 6.37. Found: C, 65.07; H, 6.12.

8-(Methoxycarbonyl)octyl 6-*O*-[2-*O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-2,4-di-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (19) and 8-(Methoxycarbonyl)octyl 3-*O*-[2-*O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-2,4-di-*O*-benzyl-6-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (20). Glycosylation of diol **14** (180 mg, 0.13 mmol) with bromide **15** (4 equiv, added in four equal portions over 24 h), silver trifluoromethanesulfonate (4 equiv), and tetramethylurea (6 equiv) as described for the preparation of **17** gave a crude mixture that was purified by chromatography on Partisil using 5:6 ethyl acetate–hexane as eluent. The early fractions (R_f 0.28 in 5:6 ethyl acetate–hexane) gave tetrasaccharide **19** as a syrup: 48.5 mg (22%); $[\alpha]_D$ +44.0° (*c* 0.10, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.46 (dd, 1 H, *J* = 1.7, 3.3 Hz, H-2d), 4.13 (dd, 1 H, *J* = 3.2, 9.5 Hz, H-3a), 4.03 (m, H-2b), 4.03 (H-2c), 3.85 (H-2a), 3.65 (s, OCH_3), 3.27 (m, $OCHHCH_2$), 2.35 (d, 1 H, *J* = 2.5 Hz, D_2O-exchangeable, O*H*-2b), 2.27 (t, 2 H, *J* = 7.5 Hz, CH_2COO), 2.11, 2.04, 2.00 and 1.975 (each s, 3 H, $COCH_3$); ^{13}C NMR ($CDCl_3$) δ 174.3 ($COOCH_3$), 5.14 (OCH_3). Anal. Calcd for $C_{98}H_{116}O_{27}$: C, 68.20, H, 6.78. Found: C, 68.26; H, 6.74.

The later fractions (R_f ca. 0.23, total 119 mg) contained poorly resolved **14** [46.5 mg (32%)], **17** [40.5 mg (14%)], and **20** [32.0 mg (14%)] whose yields were estimated by integration of ^1H NMR spectra. Compound **20**: ^1H NMR ($CDCl_3$) δ 5.42 (dd, 1 H, *J* = 1.8, 3.2 Hz, H-2d), 5.38 (dd, 1 H, *J* = 3.2, 9.5 Hz, H-3d), 5.21 (dd, 1 H, *J* = 9.5, 9.5 Hz, H-4d), 3.65 (s, OCH_3), 3.28 (m, 1 H, $OCHHCH_2$), 2.33 (d, 1 H, *J* = 2.8 Hz, D_2O-exchangeable, O*H*-2c), 2.27 (t, 2 H, *J* = 7.5 Hz, CH_2COO), 2.11, 2.03, 2.00 and 1.99 (each s, 3 H, $COCH_3$).

8-(Methoxycarbonyl)octyl 6-*O*-[2-*O*-[2,3,4-Tri-*O*-acetyl-6-*O*-(diphenylphosphoryl)-α-D-mannopyranosyl]-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-2,4-di-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (21) and 8-(Methoxycarbonyl)octyl 3-*O*-[2-*O*-[2,3,4-Tri-*O*-acetyl-6-*O*-(diphenylphosphoryl)-α-D-mannopyranosyl]-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-2,4-di-*O*-benzyl-6-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (22). Reaction of diol **14** (190 mg, 0.14 mmol) with 4 equiv of bromide **16**, added over 24 h as described for the preparation of **19** and **20**, gave a crude product that was fractionated with 5:6 ethyl acetate–hexane. The early fractions (R_f 0.26 in 5:6 ethyl acetate–hexane) gave tetrasaccharide **21** [56 mg (21%)] as a syrup: $[\alpha]_D$ +50.80° (*c* 0.13, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.44 (H-2d), 4.03 (2 H, H-2b and H-2c), 3.64 (s, OCH_3), 3.23 (m, 1 H, $OCHHCH_2$), 2.34 (d, 1 H, *J* = 2.7 Hz, D_2O-exchangeable, OH-2b), 2.26 (t, 2 H, J = 7.5 Hz, CH_2COO), 2.02, 1.99 and 1.91 (each s, 3 H, $COCH_3$); ^{13}C NMR ($CDCl_3$) δ 174.2 ($COOCH_3$), 51.4 (OCH_3). Anal. Calcd for $C_{108}H_{123}O_{29}P$: C, 67.70; H, 6.47. Found: C, 67.34; H, 6.32.

The later fractions (R_f ca. 0.21 in 5:6 ethyl acetate–hexane, total 115 mg) contained poorly resolved **14** [59 mg (31%)], **18** [27 mg (9%)], and **22** [29 mg (11%)] whose yields were estimated by integration of ^1H NMR spectra. Compound **22**: ^1H NMR ($CDCl_3$) δ 5.42 (dd, 1 H, *J* = 1.8, 3.4 Hz, H-2d), 3.65 (s, OCH_3), 3.26 (m, 1 H, $OCHHCH_2$), 2.30 (d, 1 H, *J* = 2.8 Hz, D_2O-exchangeable, OH-2c), 2.26 (t, 2 H, *J* = 7.5 Hz, CH_2COO), 1.99, 1.98 and 1.94 (each s, 3 H, $COCH_3$).

8-(Methoxycarbonyl)octyl 6-*O*-[2-*O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-3-*O*-[2-*O*-[2,3,4-tri-*O*-acetyl-6-*O*-(diphenylphosphoryl)-α-D-mannopyranosyl]-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-2,4-di-*O*-benzyl-α-D-mannopyranoside (23). Condensation of alcohol **19** (182 mg, 0.11 mmol) and bromide **16** (0.88 mmol total, added over 24 h) in the presence of silver trifluoromethanesulfonate (0.88 mmol) and *N,N,N′,N′*-tetramethylurea (1.32 mmol), as described for the preparation of **17**, gave pentasaccharide **23** [162 mg (68%)], which was obtained as a syrup after chromatography using 2:3 ethyl acetate–hexane as eluent: R_f 0.24 (5:6 ethyl acetate–hexane); $[\alpha]_D$ +37.8° (*c* 0.14, chloroform); ^1H NMR ($CDCl_3$) δ 5.40–5.20 (6 H, H-2d,e, H-3d,e, H-4d,e), 3.65 (s, OCH_3), 3.25 (m, 1 H, $OCHHCH_2$), 2.26 (t, 2 H, *J* = 7.5 Hz, CH_2COO), 2.11, 2.04, 2.00, 1.99, 1.98 and 1.94 (each s, 3 H, $COCH_3$); ^{13}C NMR ($CDCl_3$) δ 174 .2 ($COOCH_3$), 51.4 (OCH_3). Anal. Calcd for $C_{122}H_{141}O_{38}P$: C, 65.23; H, 6.33. Found: C, 65.32; H, 6.45.

8-(Methoxycarbonyl)octyl 3-*O*-[2-*O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-6-*O*-[2-*O*-[2,3,4-tri-*O*-acetyl-6-*O*-(diphenylphosphoryl)-α-D-mannopyranosyl]-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-2,4-di-*O*-benzyl-α-D-mannopyranoside (24). Condensation of alcohol **21** (79 mg, 0.041 mmol) with bromide **15** (0.32 mmol) in the presence of silver trifluoromethanesulfonate (0.32 mmol) and *N,N,N′,N′*-tetramethylurea (0.48 mmol) as described for the preparation of **17** gave pentasaccharide **24** [65 mg (71%)] as a syrup after chromatographic purification using 2:3 ethyl acetate–hexane as eluent: R_f 0.24 (5:6 ethyl acetate–hexane); $[\alpha]_D$ +42.0° (*c* 0.27, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.43–5.18 (7 H, H-2d,e, H-3d,e, H-4d,e, *H*-1b), 3.65 (s, OCH_3), 3.23 (m, 1 H, $OCHHCH_2$), 2.26 (t, 2 H, *J* = 7.5 Hz, CH_2COO), 2.11 (s, 3 H, $COCH_3$), 2.02 and 1.99 (each s, 6 H, 2 $COCH_3$), 1.98 and 1.91 (each s, 3 H, $COCH_3$); ^{13}C NMR ($CDCl_3$) δ 174.2 ($COOCH_3$), 51.4 (OCH_3). Anal. Calcd for $C_{122}H_{141}O_{38}P$: C, 65.23; H, 6.33. Found: C, 65.26; H, 6.50.

8-(Methoxycarbonyl)octyl 6-*O*-[2-*O*-[2,3,4-Tri-*O*-acetyl-6-*O*-(diphenylphosphoryl)-α-D-mannopyranosyl]-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl]-3-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-2,4-di-*O*-benzyl-α-D-mannopyranoside (25). Compound **21** (10 mg, 0.005 mmol) was benzoylated with benzoyl chloride (10 equiv) in pyridine (1 mL) for 15 h. After addition of water (1 mL) the reaction was taken to dryness and the residue dissolved in dichloromethane (20 mL) and washed with water (20 mL), 5% HCl (20 mL), saturated

$NaHCO_3$ (20 mL), and water (2 × 20 mL) before concentration to a syrup (9 mg) that was chromatographically homogeneous: R_f 0.28 (5:6 ethyl acetate–hexane); ^1H NMR ($CDCl_3$) δ 5.77 (dd, 1 H, J = 1.7 Hz, 3.3 Hz, H-2b), 5.45 (H-2d), 4.18 (dd, 1 H, J = 3.0, 9.0 Hz, H-3a), 4.16 (dd, 1 H, J = 3.0, 9.0 Hz, H-3b), 4.03 (H-2c), 3.82 (H-2a), 3.63 (s, OCH_3), 3.22 (dt, 1 H, ^2J = −9.5, ^3J = 6.5 Hz), 2.25 (t, 2 H, J = 7.5 Hz, CH_2COO), 2.02, 2.00 and 1.91 (each s, 3 H, $COCH_3$).

8-(Methoxycarbonyl)octyl 3,6-Bis-*O*-[2-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyl]-α-D-mannopyranoside (2). A mixture of **17** (38.5 mg, 0.019 mmol) and 5% palladium on carbon (40 mg) in 95% ethanol (2 mL) was stirred under hydrogen (1 atm) for 36 h to provide a product with R_f 0.54 in 4:1 dichloromethane–methanol that was devoid of ultraviolet absorption in TLC. The catalyst was removed by filtration and concentrated to a glass that was dried overnight over P_2O_5 and dissolved in methanol containing a trace of sodium methoxide at 0 °C. After 4 h, the mixture was neutralized with Amberlite IRC-50 (H^+), resin was removed by filtration, methanol and evaporated, and the residue was passed through a column of Bio-Gel P-2 (200–400 mesh, 50 × 2.5 cm) in 10% ethanol. The carbohydrate-containing fractions were combined, concentrated, and lyophilized to provide pentasaccharide **2** [14.6 mg (78%)] as a white powder: R_f 0.47 (4:1 2-propanol–water); $[\alpha]_D$ +66.0° (*c* 0.1, water). Anal. Calcd for $C_{40}H_{70}O_{28}\cdot 3H_2O$: C, 45.62; H, 7.28. Found: C, 45.29; H, 7.31.

8-(Methoxycarbonyl)octyl 3,6-Bis-*O*-[2-*O*-(α-D-mannopyranosyl 6-disodium phosphate)-α-D-mannopyranosyl]-α-D-mannopyranoside (3). Compound **18** (50.5 mg, 0.021 mmol) was hydrogenated over 5% palladium on carbon (50 mg) as described for the preparation of **2**. After catalyst removal and washing, the filtrate was concentrated to a gum that was redissolved in 95% ethanol (2 mL) and stirred under hydrogen (1 atm) in the presence of Adams catalyst (PtO_2, 10 mg) for 3 h. At this stage only a single non-ultraviolet-absorbing product could be detected in TLC, R_f 0.61, 2:1 2-propanol–water. The catalyst was removed by filtration, and the residue was concentrated to a glass that was dried, treated with methanolic sodium methoxide, and passed through a Biogel P-2 as described for **2**. The carbohydrate-containing fractions were concentrated, dissolved in water, and passed through Dowex 50X8 (Na^+) (10 mL), and the eluate was lyophilized to provide **3** [19.8 mg (76%)] as a white powder: R_f 0.42 in 2:1 2-propanol–water; $[\alpha]_D$ +65.7° (*c* 0.10, water). Anal. Calcd for $C_{40}H_{68}O_{34}Na_4P_2\cdot 3.5H_2O$: C, 36.67; H, 5.77. Found: C, 36.34; H, 5.53.

8-(Methoxycarbonyl)octyl 6-*O*-[2-*O*-(α-D-Mannopyranosyl)-α-D-mannopyranosyl]-3-*O*-[2-*O*-(α-D-mannopyranosyl 6-disodium phosphate)-α-D-mannopyranosyl]-α-D-mannopyranoside (4). Deprotection of **23** (45 mg, .021 mmol) as described for the preparation of **3** gave **23** [17.4 mg (71%)] as a white lyophilized powder: R_f 0.32 (4:1 2-propanol–water); $[\alpha]_D$ +81.3° (*c* 0.12, water). Anal. Calcd for $C_{40}H_{69}O_{31}PNa_2\cdot 3H_2O$: C, 40.82; H, 6.42. Found: C, 40.46; H, 6.09.

8-(Methoxycarbonyl)octyl 3-*O*-[2-*O*-(α-D-Mannopyranosyl)-α-D-mannopyranosyl]-6-*O*-[2-*O*-(α-D-mannopyranosyl 6-disodium phosphate)-α-D-mannopyranosyl]-α-D-mannopyranoside (5). Compound **24** (42 mg, 0.019 mmol) was deprotected as described for the preparation of **3** to provide the sodium salt **5** [15.5 mg (74%)] as a white lyophilized powder: R_f 0.36 (3:1 2-propanol–water); $[\alpha]_D$ +75.8° (*c* 0.12, water). Anal. Calcd for $C_{40}H_{69}O_{31}PNa_2\cdot 3H_2O$: C, 40.82; H, 6.42. Found: C, 40.41; H, 6.09.

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Registry No. **2**, 108167-16-4; **3**, 108167-17-5; **4**, 108167-18-6; **5**, 108189-44-2; **6**, 67381-29-7; **7**, 80738-49-4; **8**, 80738-50-7; **9**, 86861-46-3; **10**, 108167-19-7; **11**, 108167-20-0; **12**, 65827-59-0; **13**, 108167-21-1; **14**, 108167-22-2; **15**, 13242-53-0; **16**, 108266-23-5; **17**, 108167-23-3; **18**, 108167-24-4; **19**, 108167-25-5; **20**, 108189-45-3; **21**, 108167-26-6; **22**, 108167-27-7; **23**, 108167-28-8; **24**, 108167-29-9; **25**, 108167-30-2; 8-(methoxycarbonyl)octanol, 34957-73-8.

A General Stereocontrolled Approach to the 5–8 Fused Ring System. Application to the Total Synthesis of Marine Natural Product (±)-Precapnelladiene†

Goverdhan Mehta* and A. Narayana Murthy

School of Chemistry, University of Hyderabad, Hyderabad 500134, India

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A new and general approach to the *cis*- and *trans*-bicyclo[6.3.0]undecane system from readily available *cis,syn,cis*-triquinane bis(enones) is delineated. The key concept in our approach is the recognition of a bicyclo[3.3.0]oct-1(5)-ene moiety as a masked cyclooctane-1,5-dione equivalent. Thus, a 5–5–5 to 5–8 strategy emerges in which the stereochemical preferences of the former can be fully transcribed into the latter. A number of tricyclo[6.3.0.$0^{2,6}$]undec-1(8)-enes have been synthesized and transformed into *cis*-bicyclo[6.3.0]undecane-2,6-diones via ruthenium-catalyzed oxidation. The approach has been extended to the stereoselective synthesis of the biogenetically important marine natural product (±)-precapnelladiene (1).

The eight-membered ring has been the latest entrant into the diverse assemblage of carbocyclic rings present among terpenoid natural products.[1] Indeed, the number of terpene carbon skeletons in which a cyclooctane ring forms a part of condensed or bridged polycyclic system has proliferated rapidly.[1] Among the more interesting carbocyclic variations that have been encountered in recent years embodying an eight-membered ring are the uncommon 5–8 and 5–8–5 fused ring systems. While the closely related sesquiterpenoids of marine origin, precapnelladiene (1),[2] dactylol (2),[3] and poitediol (3)[4] are examples of the 5–8 fused cyclopentacyclooctane nucleus, the diterpenoids

† Dedicated to Dr. Sukh Dev on the occasion of his 60th birthday.

(1) (a) Devon, T. K.; Scott, A. I. *Handbook of Naturally Occurring Compounds*; Academic: New York, 1972, Vol. II. (b) Glasby, J. S. *Encyclopaedia of the Terpenoids*; Wiley-Interscience: New York, 1982.
(2) Ayanoglu, E.; Gebreyesus, T.; Beechan, C. M.; Djerassi, C. *Tetrahedron* **1979**, *35*, 1035.
(3) Schmidt, F. J.; Hollenbeak, K. H.; Vanderah, D. J. *Tetrahedron* **1978**, *34*, 2719.
(4) Fenical, W.; Schulte, G. R.; Finer, J.; Clardy, J. *J. Org. Chem.* **1978**, *43*, 3628.

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