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Note

Chemoenzymatic synthesis of the *Salmonella* group E_1 core trisaccharide using a recombinant β-(1→4)-mannosyltransferase

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Abstract

The chemical synthesis of the bacterial O-antigen from *Salmonella* serogroup E_1, 3-*O*-(4-*O*-β-D-mannopyranosyl-α-L-rhamnopyranosyl)-α-D-galactose, presents a particular challenge because it contains a β-(1→4) mannosidic linkage to L-rhamnose. We report a chemoenzymatic synthesis of this crucial antigenic material which culminates in the enzymatic formation of the critical β-mannosyl connection catalyzed by *Salmonella* GDP-α-D-Man:αRha1→3αGal-*PP*-Und β-(1→4)-mannosyltransferase ($ManT^{\beta 4}$). In comparison with previous synthetic routes, this method is advantageous since it utilizes intermediates, available in significant yield, which can be readily derivatized from the reducing end to present flexibility for analog construction, while the enzymatic construction of the Man1→4Rha glycosidic bond is both rapid and occurs in high yield. Furthermore, the reported spectroscopic and enzymatic structural characterization of the trisaccharide product furnishes the first indisputable functional link between *wba*O and $ManT^{\beta 4}$ and clearly sets the stage for the future mechanistic study and exploitation of this fascinating glycocatalyst.

Keywords: β-(1→4)-Mannosyltransferase; (1→4)-β-Mannosidic linkage; *Salmonella* serogroup E_1; O-Antigen; 3-*O*-(4-*O*-β-D-Mannopyranosyl-α-L-rhamnopyranosyl)-α-D-galactose

1. Introduction

Oligosaccharide epitopes of bacterial and tumor cell surfaces are critical biological molecular recognition components, important disease markers, and potential targets for therapeutic vaccine development [1,2]. However, because of the heterogeneous nature and low abundance from natural sources, oligosaccharide structure and function studies are severely dependent upon synthetic routes to these important molecules. The chemical synthesis of the trisaccharide O-antigen repeat unit **4** (Scheme 1) presents a particular challenge because it contains a (1→4)-β-mannosidic linkage to L-rhamnose [3–7]. In general, several strategies have been utilized to form (1→*x*)-β-mannosidic linkages chemically [8,9]: (i)

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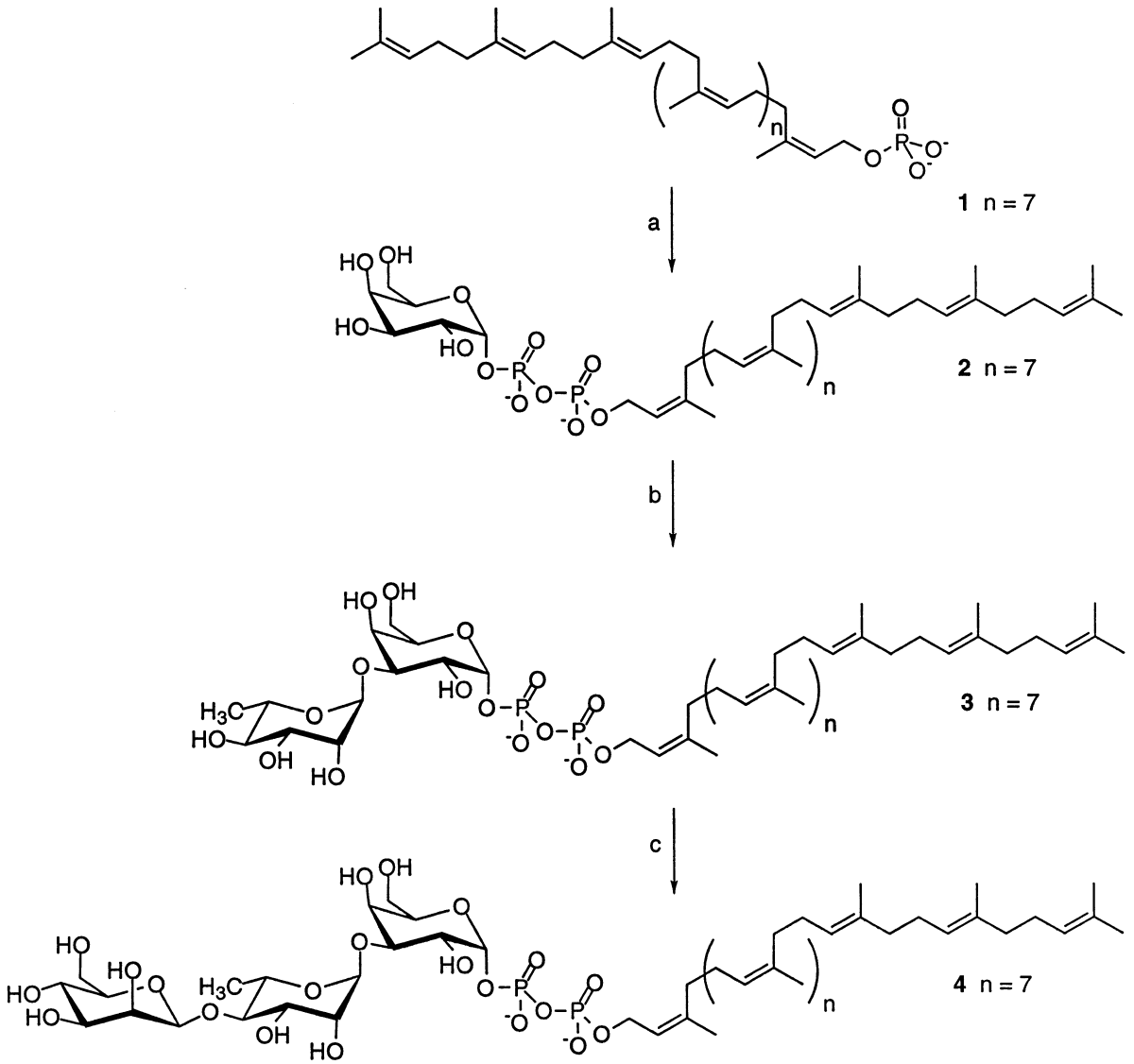
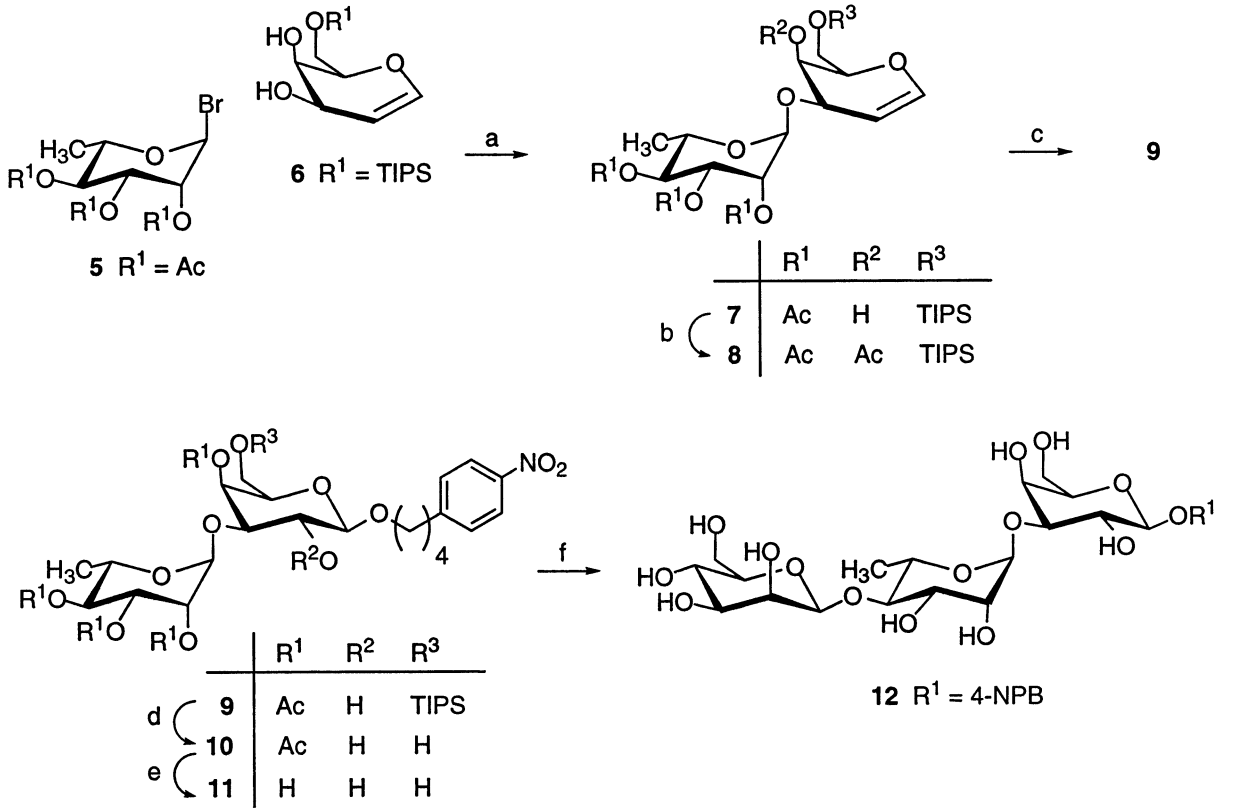
epimerization of β-D-glucosides via inversion at C-2 or sequential oxidation–reduction [10–13]; (ii) reaction of α-mannosyl donors in the presence of insoluble promoters [14]; (iii) intramolecular aglycone delivery [15–20]; (iv) via α-mannosyl sulfoxides [21,22]; (v) the use of 1,2-*O*-*cis*-stannylene mannose acetals [23,24]; and (vi) the selective reductive cleavage of mannose acetals [25,26]. Given the multistep protection and activation required for these routes, enzymatic methods are an attractive alternative, and both β-mannosidases [27–30] and β-mannosyltransferases [31,32] have been tested in this regard. However, preparative-scale enzymatic routes towards the bacterial antigen **4** have not been reported.

The gene (*wba*O) encoding the particular β-(1→4)-mannosyltransferase, GDP-α-D-Man: αRha1→3αGal-*PP*-Und β-(1→4)-mannosyltransferase (ManT$^{\beta4}$), involved in the biosynthesis of **4** was first identified by Reeves and colleagues [33,34] and subsequently overexpressed in *E. coli* to provide large amounts of the desired ManT$^{\beta4}$ to thereby aid in our mechanistic study of this intriguing enzyme [35]. Given the technical challenges of a ManT$^{\beta4}$ assay utilizing the native acceptor **3** [36], we presented a preliminary report that revealed a quantitative ManT$^{\beta4}$ assay system



Scheme 1. A schematic representation of the enzyme-catalyzed biosynthesis of the *Salmonella* group E_1 O-antigen precursor **4**: (a) Gal-1-*P*T/UDP-Gal; (b) RhaT$^{\alpha3}$/TDP-Rha; (c) ManT$^{\beta4}$/GDP-Man. The enzymatic step which requires ManT$^{\beta4}$ is highlighted.



Scheme 2. The chemoenzymatic synthesis of **12**: (a) AgOTf, 2,6-di-*tert*-butylpyridine, CH_2Cl_2, −40 °C; (b) Ac_2O, pyridine; (c) DMDO, 0 °C, CH_2Cl_2; 4-(4-nitrophenyl)-1-butanol, $ZnCl_2$, −78 °C→20 °C, THF; (d) TBAF, THF; (e) NaOMe, MeOH, 20 min; (f) $ManT^{\beta4}$/GDP-Man. The enzymatic step which requires $ManT^{\beta4}$ is highlighted.

using a synthetic analog of **3**, the chromophoric acceptor **11** (Scheme 2) [37]. While this work clearly demonstrated that $ManT^{\beta4}$ was able to transfer mannose from guanosine (α-D-mannosyl-5′-diphosphate) (GDP-α-D-Man) to the unnatural acceptor **11**, the regio- and stereochemistry of $ManT^{\beta4}$-catalyzed mannosyl transfer to **11** was not firmly established.

We now provide a full report of an improved chemical synthesis of **11** and the subsequent $ManT^{\beta4}$-catalyzed mannosylation of **11**, using a purified recombinant $ManT^{\beta4}$, to prepare preparative amounts of the *Salmonella* group E_1 O-antigen repeat unit **12**. In addition, we provide the structural characterization of **12** and thereby, the first irrefutable evidence that *Salmonella wba*O encodes for the inverting β-(1→4)-mannosyltransferase, $ManT^{\beta4}$. As a result, this work clearly sets the stage for future mechanistic study of this intriguing catalyst and the potential exploitation of $ManT^{\beta4}$ to construct β-linked carbohydrate-containing antigens.

2. Results and discussion

Chemical synthesis of acceptor **11**.—Our previously reported synthesis of the disaccharide analog **11** was initiated with Koenigs–Knorr coupling of bromide **5** and glycal **6** to give **7** (73% α, α/β = 10:1) [37]. In this coupling, the use of 6-*O*-triisopropylsilyl greatly influenced regioselectivity and the use of di-*tert*-butylpyridine gave slightly higher yields than either 2,4,6-collidine (50–55%) or 1,1,3,3-tetramethylurea (40%). The corresponding glycal **8** was epoxidized using 3,3-dimethyldioxirane to provide the 1,2-anhydro derivative, which furnished crystalline **9** (43% β, α/β = 1:9) in the presence of 1.0 equivalent of zinc chloride and 4-(4-nitrophenyl)-1-butanol. Originally, it was thought the poor yield of this reaction was the result of inhibition of epoxidation and/or coupling by the rhamnopyranosyl moiety, since galactal coupling in the absence of rhamnose reproducibly furnished [4-(nitrophenyl)-1-butyl] β-D-galac-

topyranoside in >90% yield. In fact, the dismal yield of this reaction results in part from hydrolysis of the rhamnosyl–galactal glycosidic linkage, as the major side product of this reaction is [4-(nitrophenyl)-1-butyl] β-D-galactopyranoside (15%). To compensate, a decrease of the Lewis acid (0.6 equivalent $ZnCl_2$) significantly increased the yield of the desired **9** (62% β, α/β = 1:9). Subsequent deprotection provided the desired ManT$^{\beta 4}$ substrate **11** (84%) as previously described [37].

Chemoenzymatic synthesis and characterization of **12**.—The incubation of the unnatural acceptor **11** with GDP-α-D-Man at 37 °C in the presence of purified ManT$^{\beta 4}$ gave the desired trisaccharide **12**. Under the conditions reported, integration of the analytical HPLC analysis of reactants and products revealed a turnover of ≥85% **11** in less than 5 h. Subsequent standard chromatography on silica gel followed by recrystallization provided purified **12** in 72% yield.

As previously reported, high-resolution mass spectrometry and the incorporation of [*U*-^{14}C]Man (from GDP-α-D-[*U*-^{14}C]Man) into **12** provided initial confirmation of mannosyl transfer [37]. Yet, the regio- and stereochemistry of ManT$^{\beta 4}$-catalyzed mannosyl transfer to **11** was not firmly established in the previous study. To do so, specific ^{1}H and ^{13}C resonances from **11** and **12** were assigned and are summarized in Tables 1 and 2. Determination of the regiochemistry of the ManT$^{\beta 4}$ product relied upon ROESY (rotating frame Overhauser effect spectroscopy). Consistent with a (1→4) linkage, a strong Man-H-1/α-Rha-H-4 correlation is apparent (Fig. 1). Furthermore, a weak Man-H-1/α-Rha-H-6 correlation is also evident (Fig. 1). Based on modeling, the average distance between these protons in Man-(1→4)-Rha is 4.7–5.1 Å, while the calculated analogous distances in Man-(1→3)-Rha and Man-(1→2)-Rha are predicted to be 5.5–6.7 and 6.4–7.1 Å, respectively. Given the maximum distance ROESY can be observed is ≤5 Å [38], the Man-H-1/α-Rha-H-6 ROE is also only consistent with a (1→4) linkage.

Table 1
^{1}H and ^{13}C NMR chemical shifts for compound **11** [a,b]

Residue	Proton	Chemical shift (ppm)	Coupling constants (Hz)	Carbon	Chemical shift (ppm)
β-D-Gal	H-1	4.42 (d)	7.8	C-1	103.39
	H-2	3.59 (dd)	7.8, 9.8	C-2	71.04
	H-3	3.68 (m)	– [c]	C-3	75.96
	H-4	3.99 (d)	2.7	C-4	69.31
	H-5	3.67 (m)	– [c]	C-5	81.47
	H-6, H-6′	3.66 (m), 3.71 (m)	– [c]	C-6	61.71
α-L-Rha	H-1	5.02 (broad s)		C-1	103.38
	H-2	4.07 (m)	– [c]	C-2	70.95
	H-3	3.84 (dd)	3.2, 9.6	C-3	70.95
	H-4	3.46 (dd)	9.6, 9.8	C-4	72.84
	H-5	3.80 (m)	– [c]	C-5	70.08
	H-6 (Me)	1.28 (d)	6.2	C-6	17.50
4-NPB [d]				C-1	146.72
	H-2, H-6	7.46 (d)	8.7	C-2, C-6	130.34
	H-3, H-5	8.17 (d)	8.7	C-3, C-5	124.51
				C-4	152.37
	H-α, H-α′	2.79 (t)	7.4	C-α	35.58
	H-β, H-β′	1.73 (m)	– [c]	C-β	29.08
	H-γ, H-γ′	1.65 (m)	– [c]	C-γ	27.45
	H-δ, H-δ′	3.75, 3.94 (m)	– [c]	C-δ	71.04

[a] ^{1}H and ^{13}C NMR spectra were acquired at 400 MHz in D_2O at 300 K. ^{1}H chemical shifts were referenced to D_2O at δ 4.82 ppm downfield from tetramethylsilane and ^{13}C chemical shifts were referenced to external acetone at δ 31.00 ppm.

[b] Peak assignments are based upon DQF-COSY, HSQC, DEPT and 2-D ROESY experiments. 2-D ROESY spectra were acquired at 400 MHz in D_2O at 300 K using a 250 ms mixing time and 180 of ni.

[c] Not determined.

[d] [4-(4-Nitrophenyl)-1-butyl], where C-α designates the benzylic carbon.

Table 2
^{1}H and ^{13}C NMR chemical shifts for compound **12** [a,b]

Residue	Proton	Chemical shift (ppm)	Coupling constants (Hz)	Carbon	Chemical shift (ppm)
β-D-Gal	H-1	4.41 (d)	7.8	C-1	103.38
	H-2	3.57 (dd)	7.8, 9.6	C-2	67.68
	H-3	3.68 (m)	– [c]	C-3	75.93
	H-4	3.99 (d)	– [c]	C-4	69.27
	H-5	3.69 (m)	– [c]	C-5	81.65
	H-6, H-6′	3.63 (m), 3.72 (m)	– [c]	C-6	61.89
α-L-Rha	H-1	5.01 (broad s)		C-1	103.29
	H-2	4.06 (m)	– [c]	C-2	71.26
	H-3	3.96 (dd)	– [c]	C-3	71.00
	H-4	3.36 (dd)	– [c]	C-4	77.10
	H-5	3.92 (m)	– [c]	C-5	71.00
	H-6 (Me)	1.31 (d)	6.2	C-6	17.85
β-D-Man	H-1	4.89 (broad s)		C-1	101.54
	H-2	4.07 (m)	– [c]	C-2	71.48
	H-3	3.59 (m)	– [c]	C-3	75.92
	H-4	3.87 (m)	– [c]	C-4	68.83
	H-5	3.70 (m)	– [c]	C-5	80.38
	H-6, H-6′	3.66 (m), 3.76 (m)	– [c]	C-6	61.70
4-NPB [d]				C-1	146.59
	H-2, H-6	7.47 (d)	8.7	C-2, C-6	130.34
	H-3, H-5	8.18 (d)	8.7	C-3, C-5	124.51
				C-4	152.34
	H-α, H-α′	2.80 (t)	7.4	C-α	35.57
	H-β, H-β′	1.74 (m)	– [c]	C-β	29.07
	H-γ, H-γ′	1.66 (m)	– [c]	C-γ	27.44
	H-δ, H-δ′	3.70 (m), 3.94 (m)	– [c]	C-δ	70.99

[a] ^{1}H and ^{13}C NMR spectra were acquired at 400 MHz in D_2O at 300 K. ^{1}H chemical shifts were referenced to D_2O at δ 4.82 ppm downfield from tetramethylsilane and ^{13}C chemical shifts were referenced to external acetone at δ 31.00 ppm.
[b] Peak assignments are based upon DQF-COSY, HSQC, DEPT and 2-D ROESY experiments. 2-D ROESY spectra were acquired at 400 MHz in D_2O at 300 K using a 250 ms mixing time and 180 of ni.
[c] Not determined.
[d] [4-(4-Nitrophenyl)-1-butyl], where C-α designates the benzylic carbon.

A combination of spectroscopic and enzymatic tools was employed for the stereochemical determination of the $ManT^{\beta 4}$ product. ^{1}H-coupling was not observed between the Man-H-1 and Man-H-2 (Table 2), consistent with the typical lack of scalar coupling (< 1 Hz) for β-Man residues. Furthermore, strong Man intra-residue H-1/H-3 and H-1/H-5 ROE correlations are observed (Fig. 1), also consistent with the β anomer. Finally, hydrolysis of the $ManT^{\beta 4}$ trisaccharide product to give **11** was observed only in the presence of β-mannosidase, while **11** was not observed in the presence of α-mannosidase or in the control (Fig. 2). These results, in conjunction with the observed ROE correlations in Fig. 1, are only consistent with product **12**.

3. Conclusions

It should be noted that a variety of pioneering *Salmonella* group E_1 O-antigen repeat unit syntheses have been reported [3–7] and, while much of the critical physical and spectral characteristics of these preliminary compounds were not provided, our results are consistent with the limited information reported. Several strategic advantages emerge upon comparing the chemoenzymatic preparation of **12** with previously reported methods. In particular, the chemoenzymatic method utilizes intermediates, available in significant yield, which can be readily derivatized from the reducing end to present flexibility for analog construction while the enzymatic con-

struction of the Man-(1→4)-Rha glycosidic bond is both rapid and occurs in high yield. Furthermore, the reported structural characterization of **12** furnishes an indisputable functional link between *wba*O and ManT$^{\beta 4}$, clearly setting the stage for future mechanistic study and exploitation of this fascinating and crucial glycocatalyst.

4. Experimental

General methods.—All enzymatic buffer and reaction components were purchased from Fisher (Springfield, NJ) and GDP-α-D-Man, jack bean α-mannosidase (EC 3.2.1.24) and snail β-mannosidase (EC 3.2.1.25) were purchased from Sigma (St. Louis, MO). All other chemicals used were reagent grade and used as supplied except where noted. Purified ManT$^{\beta 4}$ (specific activity $= 308$ U mg^{-1} where 1 U $= 1$ μM **12** min^{-1}) was obtained from pJB1070H/*E. coli* BL21 as previously described [35]. Infrared spectra were recorded on a Perkin–Elmer 1600 series FTIR spectrophotometer and ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AMX 400 (400 MHz) NMR spectrometer. High-resolution

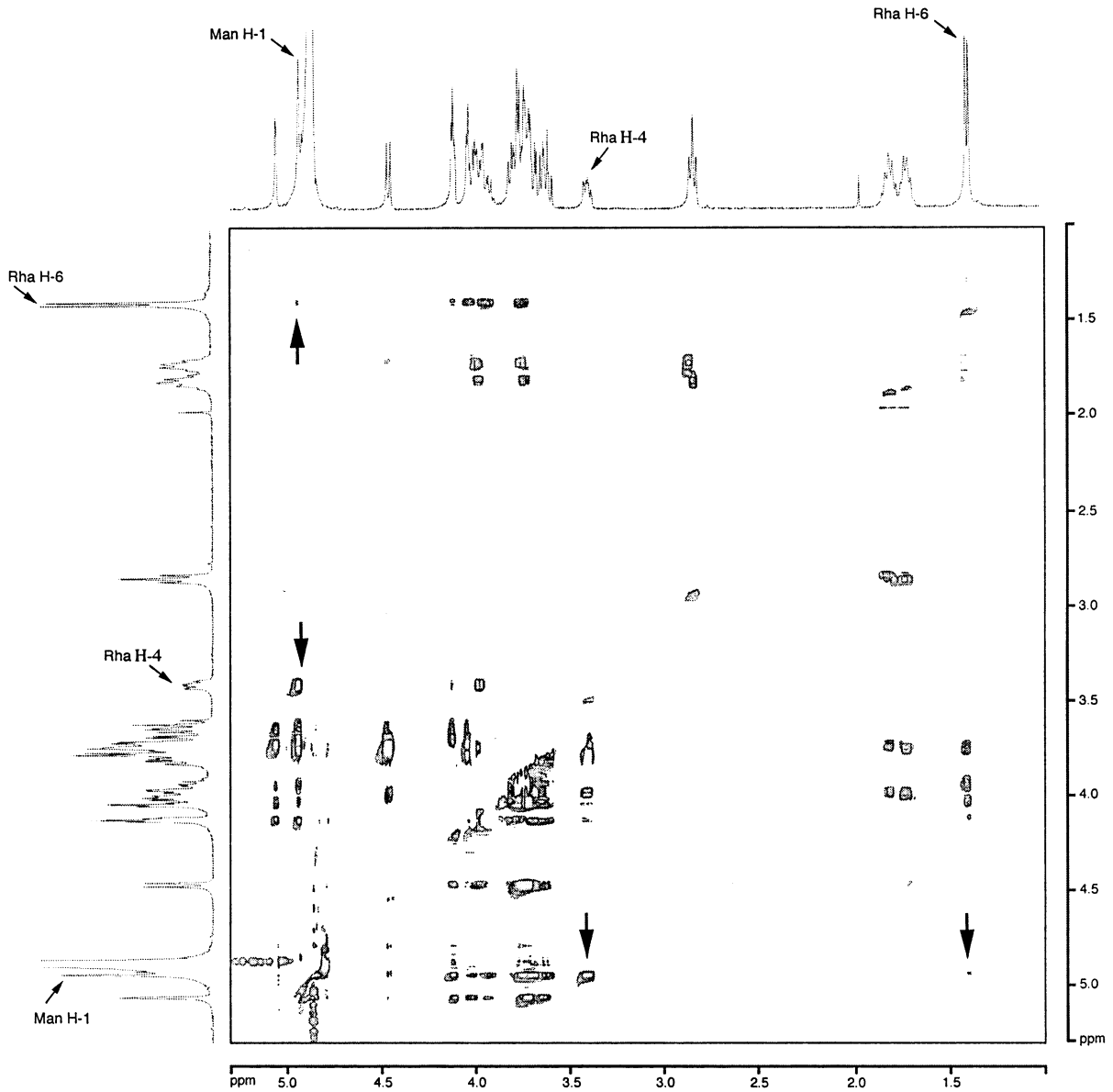


Fig. 1. ROESY spectra of **12** acquired at 400 mHz in D_2O at 300 K using a 250 ms mixing time and 180 of ni. The observed Man-H-1/α-Rha-H-6 and Man-H-1/α-Rha-H-4 ROEs are highlighted with arrows.

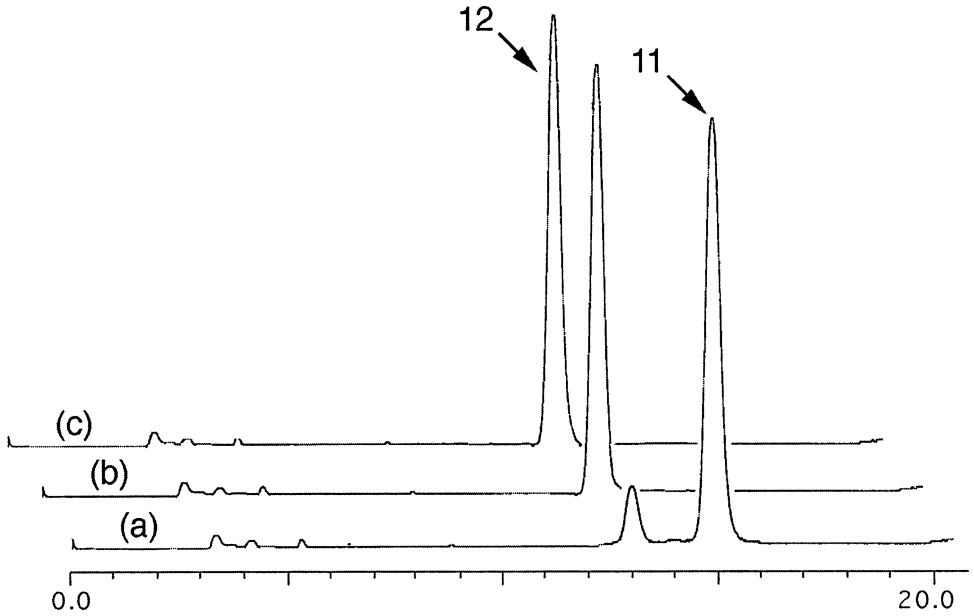


Fig. 2. Analytical reverse-phase chromatography of mannosidase-catalyzed hydrolysis of **12** monitored at 325 nm. Profiles: (a) 0.04 U of β-mannosidase; (b) 0.5 U of α-mannosidase; (c) buffer as the control.

mass spectra were determined by the University of California, Riverside, mass spectrometry facility. Optical rotations were recorded on a Jasco DIP-370 polarimeter using a 0.5 dm cell at 25 °C and the reported concentrations. Melting points were measured with an Electrothermal 1A-9100 digital melting point instrument. Analytical chromatography was performed on E. Merck Silica Gel 60 F254 plates. Compounds were visualized by spraying $I_2/KI/H_2SO_4$ or by dipping the plates in a cerium sulfate–ammonium molybdate solution followed by heating. HPLC chromatography was performed on an R Dynamax SD-200 controlled with Dynamax HPLC software and RP-18 analytical columns (0.46 × 250 cm Adsorbosil 5 micron, Alltech) equipped with guard column for analysis of reactions. The chemical modeling calculations were accomplished using the Chem3D program (CS ChemOffice, CambridgeSoft Corp.).

Chemoenzymatic synthesis of **12**.—Reaction mixtures contained 10 mM **11**, 10 mM of GDP-Man, 181 U $ManT^{\beta 4}$, 100 mM NaH_2PO_4, 1 mM EDTA, 0.02% NaN_3, 10 mM $MgCl_2$, pH 8.0 in a total volume of 750 μL (× 3) and were incubated at 37 °C for 5 h with gentle shaking. Upon completion, an equal volume of MeOH was added, denatured proteins removed by centrifugation (14,000*g*) and the supernatants were concentrated in vacuo. Silica gel chromatography (1:2 MeOH–$CHCl_3$) followed by crystallization from EtOH–EtOAc–hexanes gave 10.8 mg (72%) of the desired compound **12**. $R_f = 0.29$ (2:3 CH_3OH–$CHCl_3$); mp 236–237.4 °C, $[\alpha]_D - 52.2°$ (*c*, 1.3, H_2O); IR (KBr window) 3414 (s), 2928, 1578, 1079, 1036, 909; HRMS (FAB) Calcd for $C_{28}H_{43}NO_{17}Na$, 688.2416, found *m/z* 688.2432 [M + Na].

Mannosidase cleavage of **12**.—Purified **12** (10 mM) was dissolved in 180 μL of 200 mM NaH_2PO_4, 1 mM EDTA pH 5.5 buffer and separated into three equal aliquots. To the first was added 0.5 U of α-mannosidase, to the second 0.04 U of β-mannosidase and buffer was added to the third as a control. After incubation at 37 °C for 4 h, the reactions were analyzed by HPLC as previously described [37].

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References

[1] V. Kudryashov, G. Ragupathi, I.J. Kim, M.E. Breimer, S.J. Danishefsky, P.O. Livingston, K.O. Lloyd, *Glycoconjugate J.*, 15 (1998) 243–249.
[2] V. Kudryashov, H.M. Kim, G. Ragupathi, M.E. Breimer, S.J. Danishefsky, P.O. Livingston, K.O. Lloyd, *Cancer Immunol. Immunotherapy*, 45 (1998) 281–286.
[3] N.K. Kochetkov, B.A. Dmitriev, N.N. Malysheva, A.Y. Chernyak, E.M. Klimov, N.E. Bayramova, V.I. Torgov, *Carbohydr. Res.*, 45 (1975) 283–290.
[4] L.L. Danilov, M.F. Troitzky, N.S. Utkina, V.N. Shibaev, N.K. Kochetkov, *Carbohydr. Res.*, 87 (1980) 141–146.
[5] V.I. Betaneli, M.V. Ovchinnikov, L.V. Backinowsky, N.K. Kochetkov, *Carbohydr. Res.*, 84 (1980) 211–224.
[6] N.K. Kochetkov, B.A. Dmitriev, A.Y. Chernyak, A.B. Levinsky, *Carbohydr. Res.*, 110 (1982) C16–C20.
[7] A.Y. Chernyak, A.B. Levinsky, B.A. Dmitriev, N.K. Kochetkov, *Carbohydr. Res.*, 128 (1984) 269–282.
[8] F. Barresi, O. Hindsgaul, *Modern Methods in Carbohydrate Synthesis*, Harwood Academic Press, Amsterdam, 1996, pp 251–276.
[9] G.H. Veeneman, *Carbohydrate Chemistry*, Chapman and Hall, New York, 1998, pp 149–154.
[10] C. Augé, C.D. Warren, R.W. Jeanloz, M. Kiso, L. Anderson, *Carbohydr. Res.*, 82 (1980) 85–95.
[11] C. Warren, C. Augé, M.L. Laver, S. Suzuki, D. Power, R.W. Jeanloz, *Carbohydr. Res.*, 82 (1980) 71–83.
[12] H. Kunz, W. Gunther, *Angew. Chem., Int. Ed. Engl.*, 27 (1988) 1086–1087.
[13] S. David, A. Malleron, C. Dini, *Carbohydr. Res.*, 188 (1989) 193–200.
[14] H. Paulsen, *Angew. Chem., Int. Ed. Engl.*, 21 (1982) 155–173.
[15] F. Barresi, O. Hindsgaul, *J. Am. Chem. Soc.*, 113 (1991) 9376–9377.
[16] F. Barresi, O. Hindsgaul, *Synlett* (1992) 759–761.
[17] G. Stork, G. Kim, *J. Am. Chem. Soc.*, 114 (1992) 1087–1088.
[18] F. Barresi, O. Hindsgaul, *Can. J. Chem.*, 72 (1994) 1447–1465.
[19] M. Bols, H. Hansen, *Chem. Lett.* (1994) 1049–1052.
[20] Y. Ito, T. Ogawa, *Angew. Chem., Int. Ed. Engl.*, 33 (1994) 1765–1767.
[21] D. Crich, S. Sun, *J. Org. Chem.*, 61 (1996) 4506–4507.
[22] D. Crich, S. Sun, *J. Org. Chem.*, 62 (1997) 1198–1199.
[23] G. Hodosi, P. Kovac, *J. Am. Chem. Soc.*, 119 (1997) 2335–2336.
[24] G. Hodosi, P. Kovac, *Carbohydr. Res.*, 308 (1998) 63–75.
[25] T. Iimori, H. Ohtake, S. Ikegami, *Tetrahedron Lett.*, 38 (1997) 3415–3418.
[26] H. Ohtake, T. Iimori, T. Ikegami, S. Ikegami, *Tetrahedron Lett.*, 38 (1997) 3413–3414.
[27] N. Taubken, J. Thiem, *Synthesis*, 6 (1992) 517–518.
[28] N. Taubken, B. Sauerbrei, J. Thiem, *J. Carbohydr. Chem.*, 12 (1993) 651–667.
[29] S. Singh, M. Scigelova, D.H.G. Crout, *J. Chem. Soc., Chem. Commun.* (1996) 993–994.
[30] N. Taubken, J. Thiem, *Glycoconjugate J.*, 15 (1998) 757–767.
[31] V. Shibaev, T. Druzhinina, S. Maltsev, L. Danilov, V. Torgov, N. Kochetkov, S. Horzhnova, V. Kilesso, *Bioorganicheskaia Khimiia*, 9 (1983) 564–566.
[32] G.M. Watt, L. Revers, M.C. Webberley, I.B.H. Wilson, S.L. Flitsch, *Carbohydr. Res.*, 305 (1998) 533–541.
[33] L. Wang, L.R. Romana, P.R. Reeves, *Genetics*, 130 (1992) 429–443.
[34] D. Liu, A.M. Haase, L. Lindqvist, A.A. Lindberg, P.R. Reeves, *J. Bacteriol.*, 175 (1993) 3408–3413.
[35] Y. Zhao, J.B. Biggins, J.S. Thorson, *Biochemistry*, submitted.
[36] Y. Zhao, J.S. Thorson, *J. Org. Chem.*, 63 (1998) 7568–7572.
[37] Y. Zhao, J.B. Biggins, J.S. Thorson, *J. Am. Chem. Soc.*, 120 (1998) 12986–12987.
[38] A.E. Derome, *Modern NMR Techniques for Chemistry Research*, Pergamon Press, New York, 1987, p. 111.