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Carbohydrate Research 266 (1995) C1–C6

CARBOHYDRATE RESEARCH

Preliminary communication

Synthetic studies on the lipooligosaccharide Nod Bj-IV ($C_{18:1}$, Fuc, Gro) produced by *Bradyrhizobium japonicum* strain USDA61 ☆

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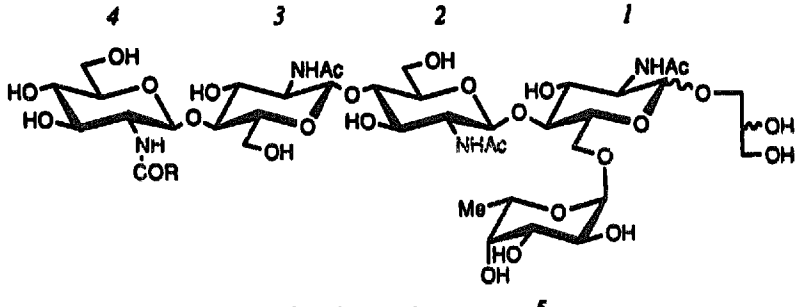
Received 4 October 1994; accepted 10 November 1994

Keywords: Lipooligosaccharide synthesis; Nod factors; *B*radyrhizobium japonicum

In 1993 Carlson et al. [1] reported the structures of lipooligosaccharide nodulation signals produced by Type II strain of *Bradyrhizobium japonicum* USDA61. As part of our project [2] on the synthesis of physiologically active plant glycoconjugates, we were interested in the synthetic study on lipooligosaccharide **1** among the reported structures for the nodulation signals, because of the lack of chemical evidence to fully characterize the stereochemistry both at C-2 of glycerol and C-1 of GlcNAc residue *1*, and the regiochemistry for the double bond of the fatty acid in structure **1**. We describe herein the unambiguous syntheses of stereochemically well-defined isomers **2**, **3**, **4**, and **5**.



1 RCO = Octadecenoyl **5**

☆ Part 14 in the series, Synthetic Studies on Plant and Microbial Glycoconjugates. For Part 13, see ref. [8].

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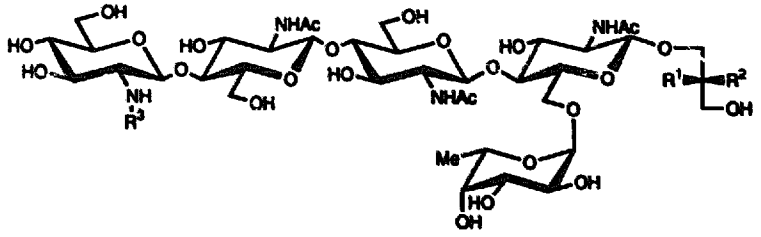
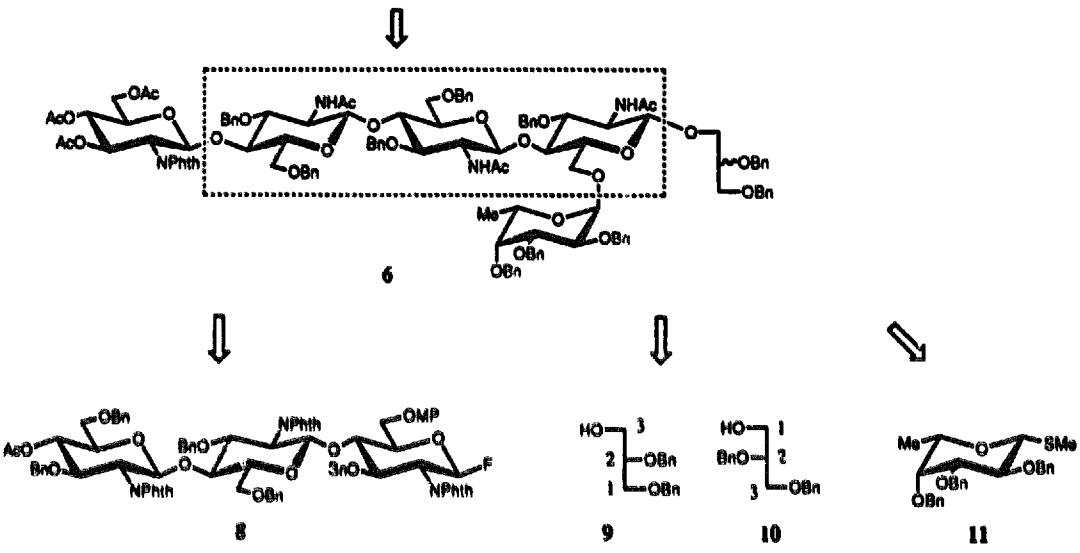
SSDI 0008-6215(94)00368-8

Synthetic Plan for Nod Bj-IV ($C_{18:1}$, Fuc, Gro)



	R^1	R^2	R^3
2	H	OH	$CO(CH_2)_6CH{=}CH(CH_2)_5CH_3$
3	OH	H	
4	H	OH	$CO(CH_2)_7CH{=}CH(CH_2)_7CH_3$
5	OH	H	

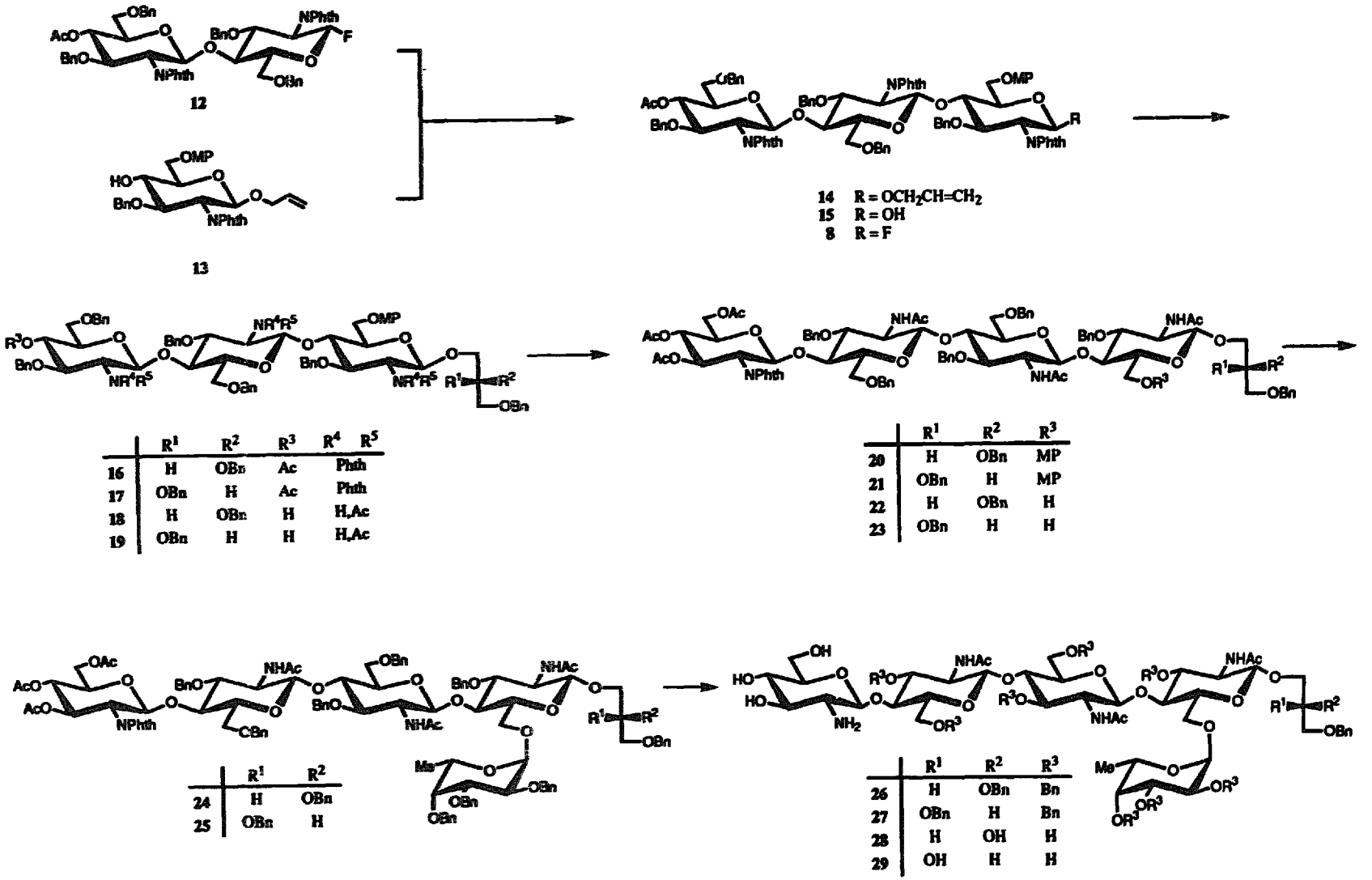


Scheme 1.

The retrosynthetic plan is shown in Scheme 1. Two key intermediates **6** with either the (2*S*) or (2*R*) configuration at C-2 of the glycerol moiety were designed so that a fatty acid with a definite stereochemistry at the double bond could be introduced at the last step of the synthesis. These intermediates were expected to be constructed from three glycosyl donors **7** [3], **8**, and **11** [4] and two glycosyl acceptors **9** [5] and **10** [6].

Synthesis of glycotriosyl fluoride **8** was investigated as follows. Glycosylation of alcohol **13** [7] with glycosyl fluoride **12** [8] in the presence of $Cp_2Hf(OTf)_2$ and powdered 4A molecular sieves (MS4A) in $(CH_2Cl)_2$ at −23°C according to Suzuki et al. [9] gave an 84% yield of trisaccharide **14**; $[\alpha]_D$ +52.8° (*c* 3.5) [1]; R_f 0.3 in 1:1

[1] The values of $[\alpha]_D$ and δ_H were determined for solutions in $CHCl_3$ and $CDCl_3$ at 25 ± 3°C, respectively, unless noted otherwise. Compounds with an assigned $[\alpha]_D$ value gave reasonable data for the elemental analysis.


12
13
14 R = OCH2CH=CH2
15 R = OH
8 R = F
16 R1 = H R2 = OBn R3 = Ac R4 R5 = Phth
17 R1 = OBn R2 = H R3 = Ac R4 R5 = Phth
18 R1 = H R2 = OBn R3 = H R4 R5 = H,Ac
19 R1 = OBn R2 = H R3 = H R4 R5 = H,Ac
20 R1 = H R2 = OBn R3 = MP
21 R1 = OBn R2 = H R3 = MP
22 R1 = H R2 = OBn R3 = H
23 R1 = OBn R2 = H R3 = H
24 R1 = H R2 = OBn
25 R1 = OBn R2 = H
26 R1 = H R2 = OBn R3 = Bn
27 R1 = OBn R2 = H R3 = Bn
28 R1 = H R2 = OH R3 = H
29 R1 = OH R2 = H R3 = H

Scheme 2.

hexane–EtOAc; δ_H 1.89 (s, Ac), 3.76 (s, OMe), 4.98 (d, 8.3 Hz), 5.12 (d, 9.3 Hz) and 5.30 (d, 8.3 Hz) for H-1^1, H-1^2 and H-1^3. Compound **14** was converted into fluoride **8** in two steps via hemiacetal **15**: (*i*) Ir-complex in THF then I_2–H_2O [10], (*i*) DAST in $(CH_2Cl)_2$ [11], 85% overall. Compound **8** had $[\alpha]_D$ +63.9° (*c* 1.1); R_f 0.4 in 2:3 hexane–EtOAc; δ_H 1.89 (s, Ac), 3.77 (s, OMe), 5.14 (d, 8.8 Hz, H-1^2), 5.30 (d, 8.3 Hz, H-1^3), 5.69 (dd, 53.6 and 7.3 Hz, H-1^1). Glycosylation of glycerol derivatives **9** and **10** with fluoride **8** was carried out in the presence of $Cp_2Hf(OTf)_2$ [9] as described above to give **16** and **17** in 71 and 88% yields, respectively. Compound **16** had $[\alpha]_D$ +49.7° *c* 0.9); R_f 0.3 in 1:1 hexane–EtOAc; δ_H 1.89 (s, Ac), 3.74 (s, OMe), 4.97 (d, 8.3 Hz, H-1^1), 5.12 (d, 7.3 Hz, H-1^2), 5.30 (d, 8.3 Hz, H-1^3). Compound **17** had $[\alpha]_D$ +41.5° (c 1.5); R_f 0.3 in 1:1 hexane–EtOAc; δ_H 1.89 (s, Ac), 3.74 (s, OMe), 4.98 (d, 8.3 Hz, H-1^1), 5.12 (d, 7.8 Hz, H-1^2), 5.30 (d, 8.3 Hz, H-1^3). Glycotriosyl glycerol derivatives **16** and **17** were then converted into glycotetraosyl glycerol derivatives **20** and **21** in three steps via compounds **18** and **19**, respectively, (*i*) $NH_2NH_2 \cdot H_2O$, in EtOH reflux, (*ii*) Ac_2O in MeOH, (*iii*) $Cp_2Hf(OTf)_2$, MS4A and **7** in $(CH_2Cl)_2$, in 68 and 66% overall yields, respectively. Compound **20** had $[\alpha]_D$ −25.8° (*c* 1.5); R_f 0.2 in 1:1 hexane–acetone; δ_H 1.73, 1.74, 1.86, 1.91, 1.95, and 2.00 (6 s, 6 × Ac), 3.72 (s, OMe), 5.45 (d, 8.3 Hz, H-1^4). Compound **21** had $[\alpha]_D$ −25.1° (*c* 4.0); R_f 0.2 in 1:1 hexane–Acetone; δ_H 1.73, 1.75, 1.86, 1.91, 1.94, and 2.00 (6 s, 6 × Ac), 3.72 (s, OMe), 5.45 (d, 8.3 Hz, H-1^4). Removal of the methoxyphenyl group at O-6^1 of compounds **20** and **21** by treatment with $(NH_4)_2Ce(NO_2)_6$ in 3:1 MeCN –H_2O [12] gave **22** and **23** in 75 and 72% yields, respectively, which were glycosylated with methyl thioglycoside **11** in the presence of 1:1 $CuBr_2$–Bu_4NBr [13] in CH_3NO_2 to give pentasaccharides **24** and **25** in 55 and 52% isolated yields, together with undesired β-linked products in 10 and 8% yields, respectively. Compound **24** had $[\alpha]_D$ −36.6° (*c* 0.4); R_f 0.59 in 1:1 toluene–THF; δ_H 0.97 (d, 6.3 Hz, H-6^5), 1.57, 1.78, 1.83, 1.85, 1.92, and 1.99 (6 s, 6 × Ac), 4.83 (d, 3.4 Hz, H-1^6), 5.13 (d, 7.3 Hz, H-1^4). Compound **25** had $[\alpha]_D$ −40.6° (*c* 0.4); R_f 0.57 in 1:1 toluene–THF; δ_H 0.95 (d, 6.3 Hz, H-6^5), 1.57, 1.79, 1.83, 1.85, 1.92 and 1.99 (6s, 6 × Ac), 4.81 (d, 3.9 Hz, H-1^5), 5.43 (d, 8.3 Hz, H-1^4).

Having prepared the complete backbone structures **24** and **25**, the remaining synthetic transformations were achieved as follows. Compounds **24** and **25** were treated first with $NH_2NH_2 \cdot H_2O$ in EtOH under reflux and then with $Pd(OH)_2/C$ and H_2 in aq MeOH to give free amino derivatives **28** and **29** via **26** and **27** in 83 and 86% yields, respectively. Compound **28** had $[\alpha]_D$ −48.1° (*c* 0.2 in 4:1 MeOH–H_2O); R_f 0.04 in 2:1:1 BuOH–EtOH–H_2O; δ_H (D_2O) 1.04 (d, 6.8 Hz, H-6^5), 1.85, 1.88, and 1.89 (3 s, 3 × Ac), 4.31 (d, 7.8 Hz), 4.33 (d, 8.3 Hz), 4.41 (d, 8.3 Hz) and 4.46 (d, 7.8 Hz) for H-1^1, H-1^2, H-1^3 and H-1^4, and 4.72 (d, 3.9 Hz, H-1^5). Compound **29** had $[\alpha]_D$ −50.0° (*c* 0.4 in 4:1 MeOH–H_2O); R_f 0.04 in 2:1:1 BuOH–EtOH–H_2O; δ_H (D_2O) 1.04 (d, 6.3 Hz, H-6^5), 1.85, 1.88 and 1.89 (3 s, 3 × Ac), 4.31 (d, 8.3 Hz), 4.32 (d, 8.3 Hz), 4.41 (d, 8.3 Hz) and 4.46 (d, 7.8 Hz) for H-1 H-1^2, H-1^3, and H-1^4, and 4.71 (d, 3.9 Hz, H-1^5). Finally, acylation of compounds **28** and **29** was carried out efficiently by employing either *cis*-vaccenic acid or *cis*-oleic acid and *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) [14] in aq MeOH to afford, after purification on a Bond Elute C_{18} column [15], the target compounds **2**, **3**, **4**, and **5** in 77, 88, 84, and 92% yields, respectively. Compound **2** had R_f 0.53 in 2:1:1 BuOH–EtOH–H_2O; δ_H (99:1

Me_2SO-d_6–D_2O) 1.07 (d, 6.8 Hz, H-6^5), 1.78, 1.80, and 1.81 (3 s, 3 × Ac), 4.27, 4.32, 4.35, and 4.43 (4d, 7.8–8.3 Hz, H-1^1, H-1^2, H-1^3 and H-1^4), 4.65 (b s, H-1^5). Compound **3** had R_f 0.53 in 2:1:1 BuOH–EtOH–H_2O; δ_H (99:1 Me_2SO-d_6–D_2O) 1.06 (d, 6.3 Hz, H-6^5), 1.78, 1.80, and 1.81 (3 s, 3 × Ac), 4.29, 4.32, 4.34, and 4.42 (4d, 7.3–8.3 Hz, H-1^1, H^2, H-1^3 and H-1^4), and 4.65 (b s, H-1^5). Compound **4** had R_f 0.51 in 2:1:1 BuOH–EtOH–H_2O; δ_H (99:1 Me_2SO-d_6-D_2O) 1.07 (d, 6.4 Hz, H-6^5), 1.77, 1.80, and 1.81 (3 s, 3 × Ac), 4.27, 4.32, 4.34, and 4.42 (4d, 6.8–8.3 Hz, H-1^1, H-1^2, H-1^3 and H-1^4) and 4.65 (b s, H-1^5). Compound **5** had R_f 0.51 in 2:1:1 BuOH–EtOH–H_2O; δ_H (99:1 Me_2SO-d_6–D_2O) 1.07 (d, 6.4 Hz, H-6^5), 1.78, 1.80 and 1.81 (3 s, 3 × Ac), 4.31, 4.32, 4.35, and 4.42 (4d, 7.8–8.3 Hz, H-1^1, H-1^2, H-1^3, H-1^4, and 4.65 (bs, H-1^5).

In summary, we have achieved for the first time an unambiguous synthesis of four stereo- and regio-isomers of Nod factor Nod Bj-IV ($C_{18:1}$, Fuc, Gro), which should serve as authentic samples to assign the stereochemistry of natural samples that are available in only minute amounts.

Acknowledgments

A part of this work was financially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, and also by the Special Coordination Funds of the Science and Technology Agency of the Japanese Government. We thank Dr. J. Uzawa and Mrs. T. Chijimatsu for NMR spectra, Mr. Y. Esumi for FABMS measurements, Ms. M. Yoshida and her staff for elemental analyses, and Ms. A. Takahashi for technical assistance.

References

[1] R.W. Carlson, J. Sanjuan, U.R. Bhat, J. Glushka, H.P. Spaink, A.H.M. Wijfjes, A.A.N. van Brussel, T.J.W. Stokkermans, N.K. Peters, and G. Stacey, *J. Biol. Chem.*, 268 (1993) 18372–18381.

[2] N. Hong, Y. Nakahara, and T. Ogawa, *Proc. Jpn. Acad.*, 69B (1993) 55–60.

[3] S. Ikeshita, A. Sakamoto, Yu. Nakahara, Y. Nakahara, and T. Ogawa,l *Tetrahedron Lett.*, 35 (1994) 3123–3126.

[4] S. Sato, M. Mori, Y. Ito, and T. Ogawa, *Carbohydr. Res.*, 155 (1986) C6–C10.

[5] C.A.A. van Boeckel, G.M. Visser, and J.H. van Boom, *Tetrahedron*, 41 (1985) 4557–4565; D.A. Mannock, R.N.A.H. Lewis, and R.N. Mcelhaney, *Chem. Phys. Lipids*, 43 (1987) 113–127.

[6] B. Wickberg, *Acta Chem. Scand.*, 12 (1958) 1187–1201; Y. Nishida, H. Uzawa, S. Hanada, H. Ohrui, and H. Meguro, *Agric. Biol. Chem.*, 53 (1989) 2319–2326.

[7] F. Yamazaki, T. Nukada, Y. Ito, S. Sato, and T. Ogawa, *Tetrahedron Lett.*, 30 (1989) 4417–4420; F. Yamazaki, T. Kitajima, T. Nukada, Y. Ito, and T. Ogawa, *Carbohydr. Res.*, 201 (1990) 15–30.

[8] S. Ikeshita, Y. Nakahara and T. Ogawa, *Glycoconj. J.*, 11 (1994) 257–261.

[9] K. Suzuki, H. Maeta, T. Mastumoto, and G. Tsuchihashi, *Tetrahedron Lett.*, 29 (1988) 3567–3574; K. Suzuki, H. Maeta, and T. Matsumoto, *Tetrahedron Lett.*, 30 (1989) 4853–4857.

[10] L.M. Haines and E. Singleton, *J. Chem. Soc., Dalton Trans.*, (1972) 1891–1896; J.J. Oltvoort, C.A.A. van Boeckel, J.H. De Koning, J.H. van Boom, *Synthesis*, (1981) 305–308.

[11] W. Rosenbrook, Jr., D.A. Riley, and P.A. Lartey, *Tetrahedron Lett.*, 26 (1985) 3-6; G.H. Posner and S.R. Haines, *Tetrahedron Lett.*, 26 (1985) 935–938.

[12] T. Fukuyama, A.A. Laird, and L.M. Hochkiss, *Tetrahedron Lett.*, 26 (1985) 6291–6294.
[13] S. Sato, M. Mori, Y. Ito, and T. Ogawa, *Carbohydr. Res.*, 155 (1986) C6–C10.
[14] B. Belleau and G. Malek, *J. Am. Chem. Soc.*, 90 (1968) 1651–1652; H. Yajima, H. Kawatani, *Chem. Pharm. Bull.* 19 (1971) 1905–1913.
[15] M.M. Palcic, L.D. Heerze, M. Pierce, and O. Hindsgaul, *Glycoconj. J.*, 5 (1988) 49–63.