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Preliminary communication

Regio- and stereo-controlled synthesis of core oligosaccharides of glycopeptides

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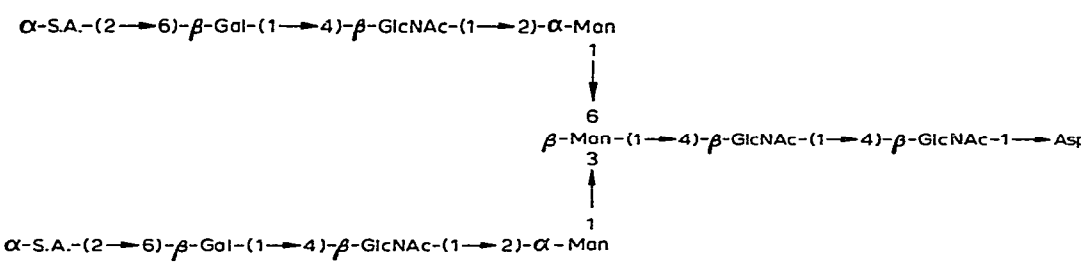
As a result of recent studies on the biochemistry of oligosaccharides present at cell surfaces, many of them have been found to have branched-chain structures[1] and to be linked to asparagine; and these may be classified into two types. For example, oligosaccharide 1 (which was isolated from immunoglobulin glycopeptide[2]) and oligosaccharide 2 (which was isolated from calf thyroglobulin[3]) may respectively be classified as an *N*-acetyllactosamine type, or type A, and an oligo-D-mannosidic type, or type B, of oligosaccha-



S.A. = sialic acid

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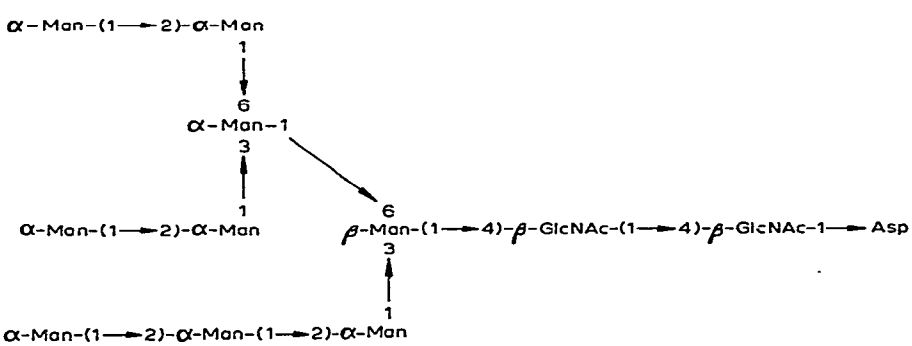

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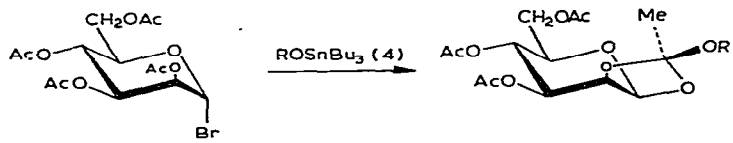
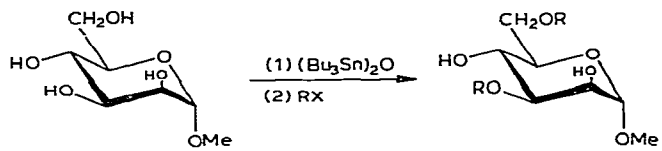
ride[4]. Not only their biological functions[5] but also their unique, branched-chain structures have stimulated efforts directed toward their chemical synthesis[6].

As part of a project on the chemical transformation of carbohydrates through trialkylstannylation, we report here a synthesis of core pentasaccharides, both of type A (22) and type B (17).

The reaction of tributyltin alkoxide[7] (4) with 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (3) was reported[8] to afford a high yield of orthoester 5. Moreover, a regioselective enhancement of the nucleophilicity of the hydroxyl groups of methyl α-D-mannopyranoside (6) by tributylstannylation and subsequent treatment with such electrophilic reagents as an acyl halide[9] or an alkyl halide[10] was reported to give a high yield of methyl 3,6-di-*O*-substituted α-D-mannopyranoside (7). Consequently, if a glycosyl halide such as 9 is used as an electrophilic reagent, instead of a simple alkyl halide, for the reaction with 6, a regio-controlled introduction of two D-mannosyl residues might be expected to occur at 0-3 and 0-6, *via* orthoesters.



3 5



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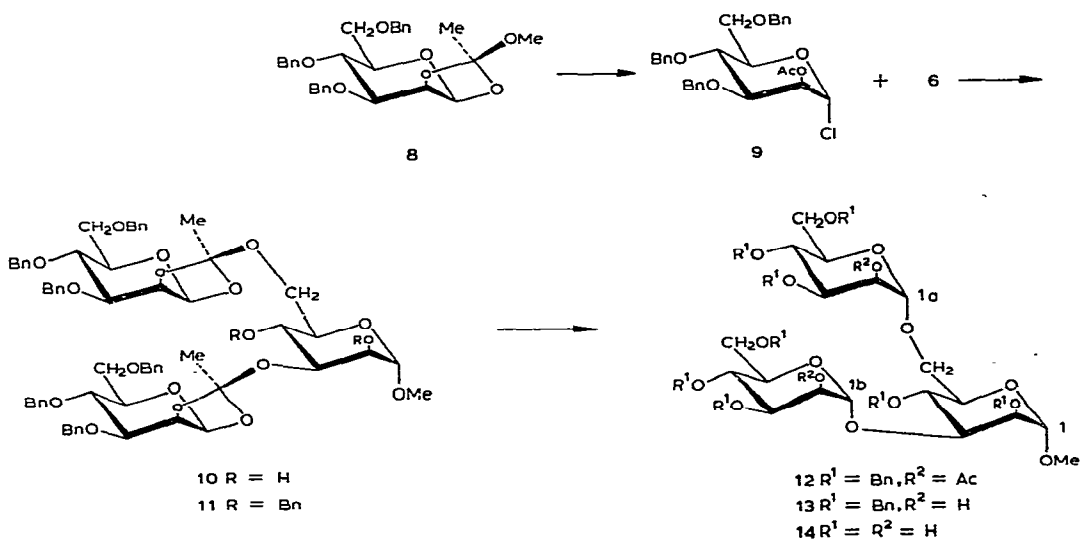
In fact, the reaction of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride [R_F 0.76 in 1:4 toluene–ethyl acetate; ^{1}H-n.m.r. data (in $CDCl_3$): δ 2.12 (s, 3 H, $COCH_3$), 5.44 (t, 1 H, *J* 2 Hz, H-2), and 6.03 (d, 1 H, *J* 2 Hz, H-1), which was prepared by the reaction of orthoester 8 with chlorotrimethylsilane[11]] with partially stannylated 6, prepared in the usual way[9,10] through reaction with 1.5 molar proportions of bis(tributyltin) oxide, afforded a 34% yield*** of 10, a molecule containing an orthoester form at 0-3 and 0-6 of methyl α-D-mannopyranoside, {[α]$_D$ +40.0° (*c* 0.52, $CHCl_3$)****; R_F† 0.48 (4:1 $CHCl_3$–acetone); ^{1}H-n.m.r. data (in $CDCl_3$): δ 1.76 (s, 3 H, C-CH_3) and 1.83 (s, 3 H, C-CH_3)} and a 2.2% yield of a molecule containing an orthoester form at 0-4 and 0-6 of methyl α-D-mannopyranoside, {[α]$_D$ +38.5° (*c* 0.46, $CHCl_3$); R_F 0.33 (4:1 $CHCl_3$–acetone); ^{1}H-n.m.r. data (in $CDCl_3$): δ 1.73 (s, 3 H, C-CH_3) and 1.82 (s, 3 H, C-CH_3)}.

***Yields are not optimized.

****All compounds for which [α]$_D$ is recorded gave an acceptable, elemental analysis.

†Thin-layer chromatography was performed on precoated plates of Silica Gel 60 F_{254}.

Treatment of **10** with sodium hydride and α-bromotoluene in *N,N*-dimethylformamide[12] afforded a 77.3% yield of per-*O*-benzylated **10** (**11**) {$[\alpha]_D$ +45.5° (*c* 0.44, $CHCl_3$); R_F 0.86 (2:1 toluene–ethyl acetate); ^{1}H-n.m.r. data ($CDCl_3$): δ 1.74 (s, 3 H, C-CH_3) and 1.86 (s, 3 H, C-CH_3)}. Rearrangement of **11** into the protected trimannoside **12** {$[\alpha]_D$ +41.7° (*c* 0.59, $CHCl_3$); R_F 0.36 (4:1 toluene–ethyl acetate); ^{1}H-n.m.r. data ($CDCl_3$): δ 2.06 (s, 3 H, $COCH_3$) and 2.13 (s, 3 H, $COCH_3$)} was achieved in 27.3% yield by heating at 120° in the presence of $HgBr_2$ under N_2. Deacetylation of **12** with sodium methoxide in methanol–tetrahydrofuran afforded an 83.8% yield of diol **13**, the key intermediate for further transformation {$[\alpha]_D$ +53.8° (*c* 0.47, $CHCl_3$); R_F 0.37 (2:1 toluene–ethyl acetate)}. The α stereochemistry was assigned to the two newly introduced D-mannosidic linkages in **13** by measuring the ^{13}C-n.m.r. data (D_2O) for methyl 3,6-di-*O*-α-D-mannopyranosyl-α-D-mannopyranoside (**14**) {$[\alpha]_D$ +96.7° (*c* 0.45, MeOH); R_F 0.2 (1:3:1 $CHCl_3$–MeOH–conc. NH_4OH); ^{1}H-n.m.r. data (CD_3OD): δ 3.36 (s, 3 H, OCH_3), 4.60 (d, 1 H, *J* 1.5 Hz), 4.83 (d, 1 H, *J* 1.5 Hz), and 5.06 (d, 1 H, *J* 2.0 Hz) for three anomeric protons}, obtained by hydrogenolysis of **13** in ethanol in the presence of 10% Pd–C. Three anomeric carbon atoms were observed, at δ 100.1 ($^1J_{CH}$ 170.9 Hz, C-1a), 101.8 ($^1J_{CH}$ 171.9 Hz, C-1), and 103.2 ($^1J_{CH}$ 169.9 Hz, C-1b), in accordance with the $^1J_{CH}$ value for the α-D-glycosyl stereochemistry[13] (see Fig. 1).



Reaction of **13** with **9** in the presence of silver triflate and tetramethylurea[14] in dichloromethane under N_2 afforded a 78.8% yield of the protected penta-D-mannoside **15** {$[\alpha]_D$ +34.7° (*c* 0.51, $CHCl_3$); R_F 0.67 (4:1 toluene–ethyl acetate)}. Deacetylation of **15** afforded a 78.1% yield of **16** {$[\alpha]_D$ +45.9° (*c* 0.7, $CHCl_3$); R_F 0.26 (4:1 toluene–ethyl acetate)}, which was hydrogenolyzed in aq. ethanol in the presence of 10% Pd–C to afford methyl 3,6-di-*O*-(2-*O*-α-D-mannopyranosyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**17**) {$[\alpha]_D$ +89.6° (*c* 0.51, MeOH); R_F 0.18 (3:1 MeOH–conc. NH_4OH)}. The ^{13}C-n.m.r. spectrum of **17** in D_2O showed five anomeric carbon atoms: δ 98.7 ($^1J_{CH}$ 170.0 Hz,

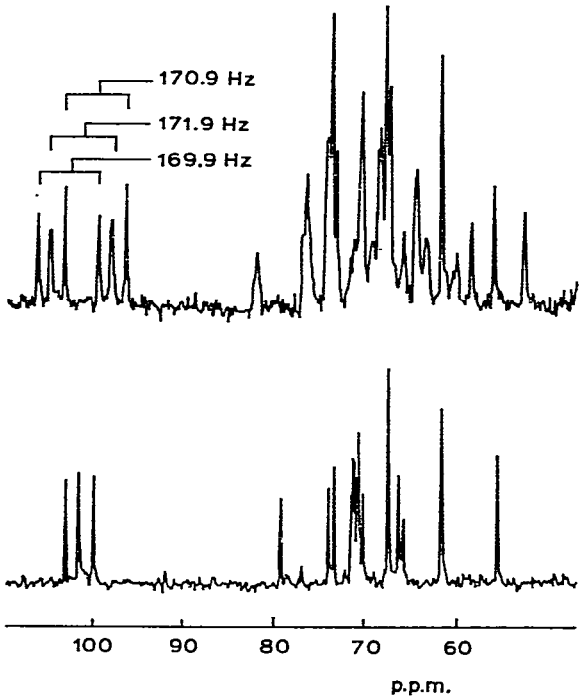
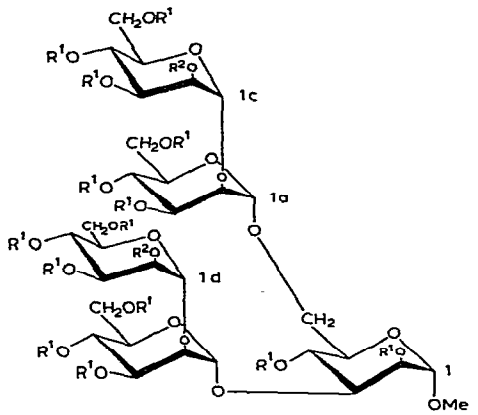


Fig. 1. 25.05-MHz, Fourier-transform, ^{13}C-n.m.r. spectrum of compound **14** in D_2O. (Top: proton-coupled spectrum by gated-decoupling technique; bottom: proton noise-decoupled spectrum. Chemical shifts from Me_4Si).

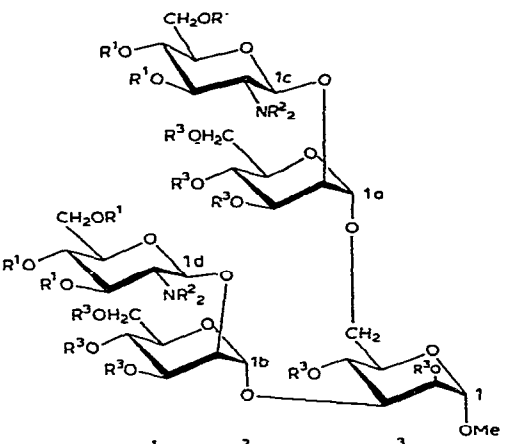
C-1a), 101.6 ($^1J_{CH}$ 170.0 Hz, C-1b), 101.7 ($^1J_{CH}$ 170.0 Hz, C-1), and 103.0 ($^1J_{CH}$ 170.0 Hz, C-1c and C-1d), in accordance with the assigned stereochemistry (see Fig. 2).

The key intermediate **13** could also be transformed into the core saccharide **22** (a type A oligosaccharide). Thus, the reaction of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl chloride[15] (**18**) with **13** in the presence of silver triflate and *s*-collidine in nitromethane afforded a 43.7% yield of protected pentasaccharide **19** {$[\alpha]_D$ +12.0° (*c* 0.59, $CHCl_3$); R_F 0.4 (2:1 toluene–ethyl acetate)}. Treatment of **19** with boiling ethanolic hydrazine hydrate[15] under reflux in an atmosphere of argon afforded **20**. Acetylation of **20** with acetic anhydride in methanol gave a 45.8% yield of the *N*-acetylated pentasaccharide **21** {$[\alpha]_D$ +21.3° (*c* 0.47, $CHCl_3$); R_F 0.43 (4:1) $CHCl_3$–MeOH)} . Hydrogenolysis of **21** in the presence of 10% Pd–C in aq. ethanol afforded methyl 3,6-di-*O*-[2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl]-α-D-mannopyranoside (**22**) {$[\alpha]_D$ +44.2° (*c* 0.51, MeOH); R_F 0.27 (4:1 MeOH-conc. NH_4OH)}. The assigned stereochemistry was confirmed by the following ^{13}C-n.m.r. data (D_2O): δ 97.5 ($^1J_{CH}$ 169.9 Hz, C-1a), 100.4 ($^1J_{CH}$ 160.0 Hz, C-1c and C-1d), 100.4 ($^1J_{CH}$ 168.0 Hz, C-1b), and 101.8 ($^1J_{CH}$ 172.9 Hz, C-1) (see Fig. 3).

In conclusion, regio-controlled activation of the hydroxyl groups of methyl α-D-mannopyranoside through tributylstannylation was successfully applied to the synthesis of the core pentasaccharides of glycoproteins.



15 R^1 = Bn, R^2 = Ac
16 R^1 = Bn, R^2 = H
17 R^1 = R^2 = H



19 R^1 = Ac, R^2_2 = phthaloyl, R^3 = Bn
20 R^1 = R^2 = H, R^3 = Bn
21 R^1 = H, R^2_2 = Ac, H, R^3 = Bn
22 R^1 = R^3 = H, R^2_2 = Ac, H

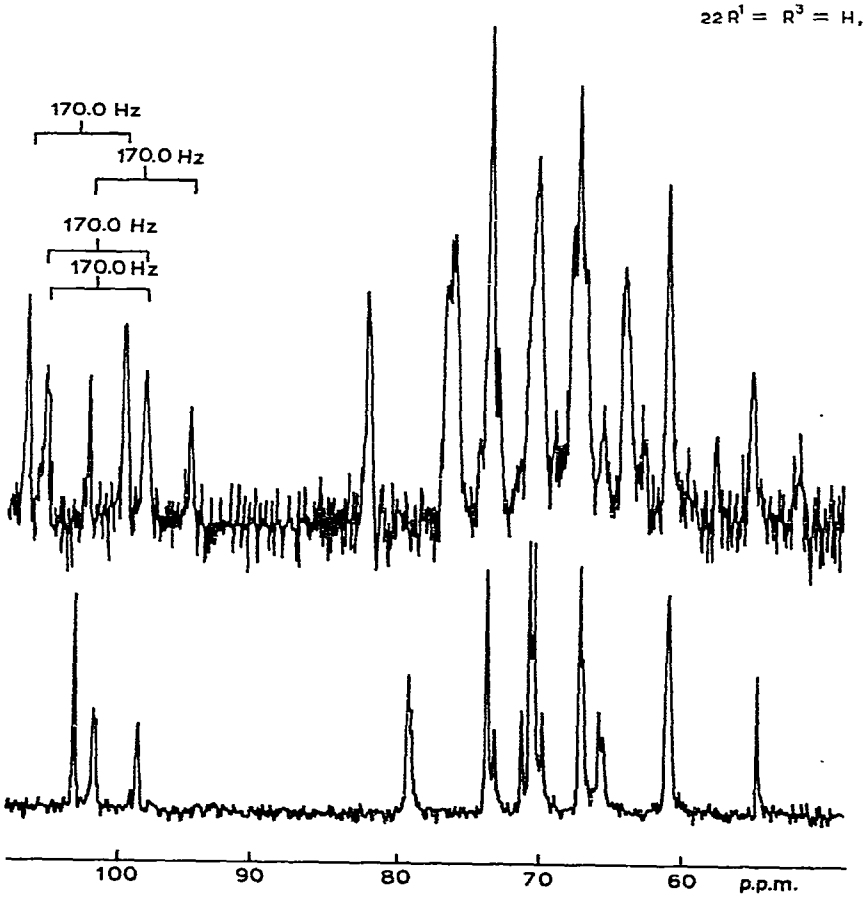


Fig. 2. 25.05-MHz, Fourier-transform, ^{13}C-n.m.r. spectrum of compound 17 in D_2O. (Top: proton-coupled spectrum by gated-decoupling technique; bottom: proton noise-decoupled spectrum. Chemical shifts from Me_4Si).

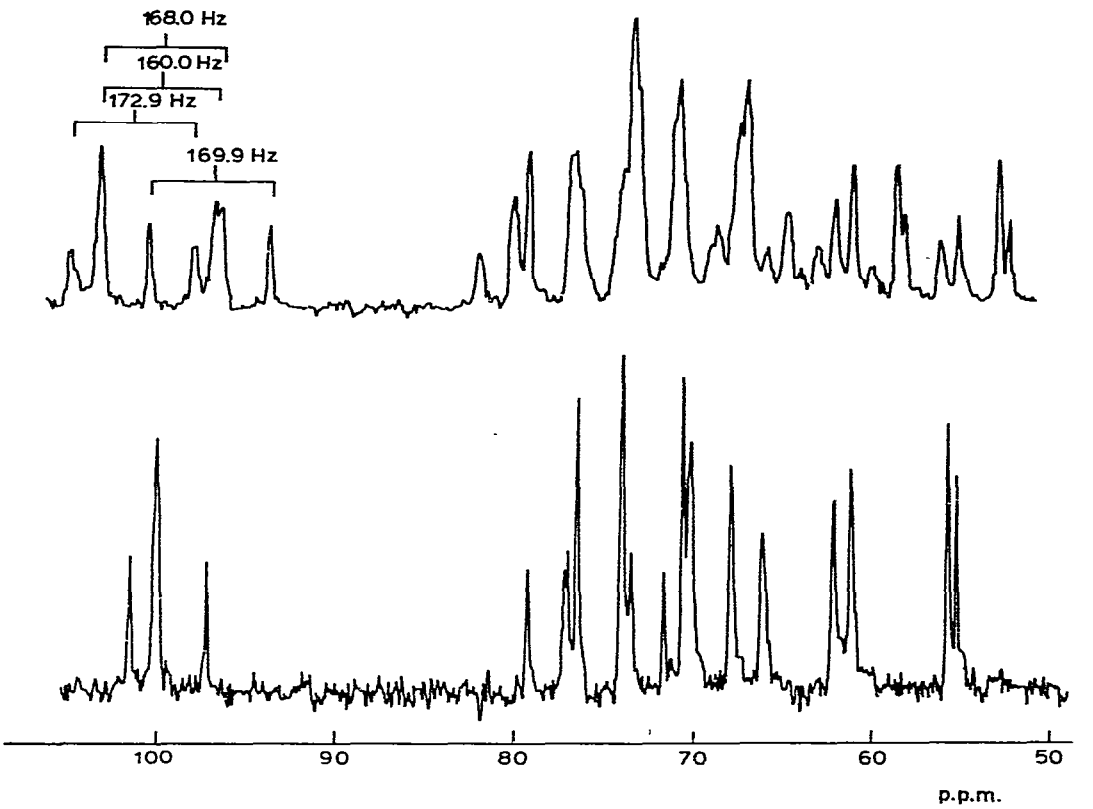


Fig. 3. 25.05-MHz, Fourier-transform, ^{13}C-n.m.r. spectrum of compound 22 in D_2O. (Top: proton-coupled spectrum by gated-decoupling technique; bottom: proton noise-decoupled spectrum. Chemical shifts from Me_4Si).

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REFERENCES

1 R. Kornfeld and S. Kornfeld, *Annu. Rev. Biochem.*, 45 (1976) 217–237.
2 J. Baenziger, S. Kornfeld, and S. Kochwa, *J. Biol. Chem.*, 249 (1974) 1897–1903; J. Baenziger and S. Kornfeld, *ibid.*, 249 (1974) 7260–7269.
3 S. Ito, K. Yamashita, R. G. Spiro, and A. Kobata, *J. Biochem. (Tokyo)*, 81 (1977) 1621–1631.
4 J. Montreuil, *Pure Appl. Chem.*, 42 (1975) 431–477.
5 K. W. Talmadge and M. M. Burger, *MTP Int. Rev. Sci. Biochem. Ser. One*, 5 (1975) 43–94.
6 C. Augé, S. David, and A. Veyrières, *J. Chem. Soc. Chem. Commun.*, (1977) 449–450.
7 A. G. Davies, *Synthesis*, (1969) 56–64.
8 T. Ogawa and M. Matsui, *Carbohydr. Res.*, 51 (1976) C13–C18.
9 T. Ogawa and M. Matsui, *Carbohydr. Res.*, 56 (1977) C1–C6.
10 T. Ogawa and M. Matsui, *Carbohydr. Res.*, 62 (1978) C1–C4.

11 M. S. Newman and D. R. Olson, *J. Org. Chem.*, 38 (1973) 4203–4204.
12 J. S. Brimacombe, *Methods Carbohydr. Chem.*, 6 (1972) 376–378.
13 K. Bock, I. Lundt, and C. Pedersen, *Tetrahedron Lett.*, (1973) 1037–1040; K. Bock and C. Pedersen, *J. Chem. Soc. Perkin Trans. 2*, (1974) 293–297; *Acta Chem. Scand. Ser. B*, 29 (1975) 258–264.
14 S. Hanessian and J. Banoub, *Carbohydr. Res.*, 53 (1977) C13–C16.
15 S. Akiya and T. Osawa, *Chem. Pharm. Bull.*, 8 (1960) 583–587; R. U. Lemieux, T. Takeda, and B. Y. Chung, *ACS Symp. Ser.*, 39 (1976) 90–115.