Tetrahedron Vol. 46, No. 16, pp. 5533-5542, 1990
Printed in Great Britain

0040-4020/90 $3.00+.00

ADDITIVITY IN CD AMPLITUDES OF p-PHENYLBENZYL ETHERS AND p-PHENYLBENZOATES OF 2-AMINOSUGARs

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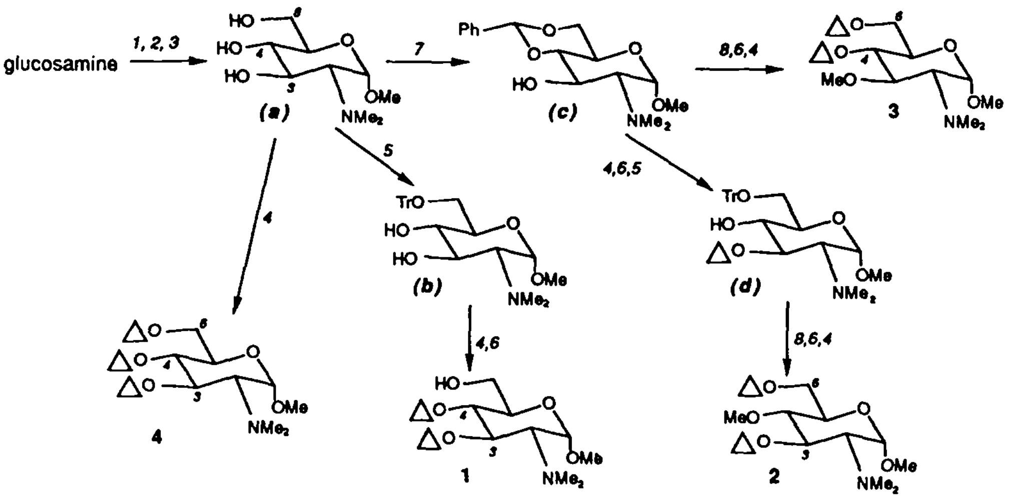
(*Received in USA* 8 *January* 1990)

Abstract. The additivity relation in amplitudes (A values) of split CD curves holds for p-phenylbenzyl ethers **1-12** and phenylbenzoates **13-28** of amino sugars. Thus the glycosidic linkage determination methods based on CD amplitudes are applicable to oligosaccharides containing amino sugars as well.

We have been investigating microscale methods to determine the site of oligosaccharide linkages[1,2] by application of the circular dichroic (CD) exciton chirality method,[3,4] which offers an alternative to conventional methylation analysis.[5] Two approaches have been developed, both based on the pairwise additivity relations found in the exciton-split CD curves. The more general one[1,2,6] employs two different chromophores to tag separately the free and bonded hydroxyls of the oligosaccharide; the tagged sugars are then identified by comparing the entire CD curve with standard reference curves (total 150 to cover all hexopyranosides).[7] The other method, the subject of this paper, is based on the pairwise additivity relation found[8] in the monochromatic amplitudes of split CD curves at their extrema; e.g., the CD amplitudes at 244 nm of hexopyranoside tri- and tetra-p-bromobenzoates represent the pairwise sum of A values of the constituent three and six dibenzoate interactions that are present in the tri- and tetra-benzoates, respectively.[8] This additivity relationship also holds for hexopyranosides p-phenybenzyl ethers which are resistant to conditions used for glycosidic bond cleavages.[9] Thus, an oligosaccharide is perphenylbenzylated to tag the free hydroxyls, cleaved, and the CD (and UV/MS) of HPLC-separated monosaccharide benzyl ethers are measured to identify the site of glycosidic linkages; furthermore, the benzyl ethers can be oxidized directly with ruthenium tetroxide to give, in ca. 60% yield, the corresponding p-phenylbenzoates that have split CD curves with ca. 5-fold intensity.[9] It is shown in the following that the method can be extended in a straightforward manner to aminosugars as well.

For preparation of the standard di- and tri-phenylbenzyl ethers or phenylbenzoates, the aminosugar was first derivatized to methyl 2-deoxy-2-(N,N-dimethylamino)-α-D-hexopyranoside **a** (Scheme 1) in three steps: peracetylation, methyl glycosylation, and N,N-dimethylation/deacetylation with paraformaldehyde and sodium cyanoborohydride.[10] The three hydroxyl groups of **a** were dibenzylated to the three different diphenybenzyl ethers **1-3**. 3,4-Diphenylbenzyl ether **1** was derived from **a** via three steps: 6-tritylation to **b**, phenylbenzylation and deprotection; the 3,6-diphenylbenzyl ether **2** was derived from **c** by 3-phenylbenzylation, removal of 4,6-dibenzylidene, 6-tritylation to **d**, α-methyl glycosidation and 4-methoxylation, detritylation, and finally phenylbenzylation. The remaining 3,6-dibenzyl ether **3** was prepared by similar routes, namely, 3-methoxylation of **c**, removal of 4,6-dibenzylidene, and phenylbenzylation. Other diphenylbenzyl ethers of galactosamine **5**-**7** and

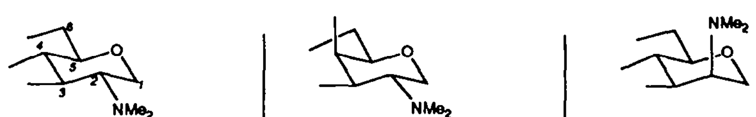


Scheme 1 1: Ac_2O / pyridine. 2: 5% HCl / MeOH. 3: $(CH_2O)_n$ $NaBH_3CN$. 4: PhBnBr / NaH. 5: TrBr / pyridine. 6: 1 N HCl. 7: $PhCH(OCH_3)_2$ TsOH. 8: CH_3I / NaH △ = PhBn

mannosamine **9**-**11** (Table 1) were prepared similarly from galactosamine and mannosamine, respectively. All synthetic phenylbenzyl ethers absorb at 235 nm and give split CD curves with extrema at 238/260 nm in MeCN (Fig. 1).

The additivity relation was tested by comparing the observed A values of triphenylbenzyl ethers, **4**, **8** and **12**, with the calculated values. Good agreement was observed between A_{obsd} and A_{cald} in all three cases,

Table 1 A values of Di- and Triphenylbenzyl Ethers of *Methyl 2-(N,N-dimethylamino) -α-D-hexapyranoside*



	GluN		GalN		ManN	
	entry	*A*	entry	*A*	entry	*A*
3,4-	1	- 29.5	5	+ 8.8	9	- 14.2
3,6-	2	+ 2.6	6	- 6.8	10	- 2.3
4,6-	3	+ 15.2	7	- 14.3	11	+ 12.6
3,4,6-	4	- 15.5 (- 11.7)	8	- 13.5 (- 12.3)	12	- 2.3 (- 3.9) *

* Values in parenthesis are A_{cald}

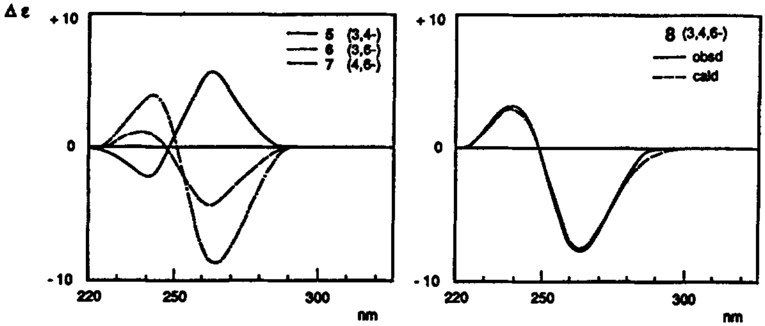
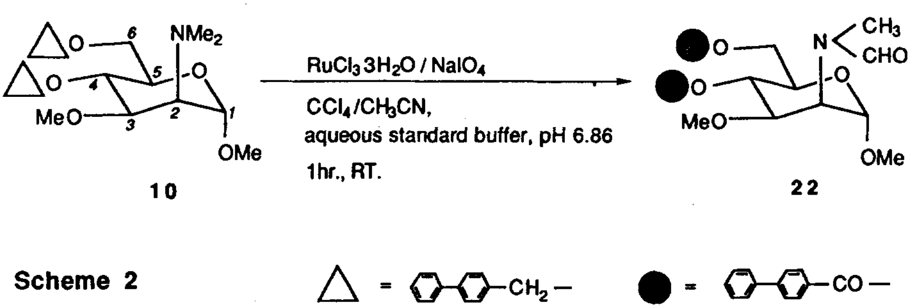


Fig.1. CD of diphenylbenzylates 5-7 and triphenylbenzylate 8 (observed and calculated): in MeCN.

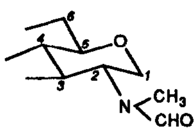
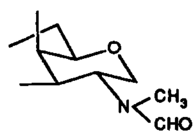
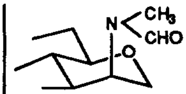
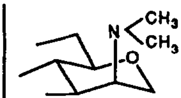
e.g., for galactosamine tribenzylate **8**, A_{obsd} is -13.5 (Table 1), whereas A_{calcd} is +8.8 (for 3,4-, **5**) - 6.8 (for 3,6-, **6**) -14.3 (for 4,6-, **7**) = -12.3 (Fig.1). Within each sugar class, the A values are sufficiently different to be characteristic of the substitution pattern. Not surprisingly, the A value of each aminosugar phenylbenzyl ether is similar to that of the corresponding hexopyranoside phenylbenzyl ether.[9]

The PhBn ethers **1-12** were easily oxidized with ruthenium tetroxide under conditions previously mentioned,[9,11] i.e., $RuCl_3 \cdot 3H_2O/NaIO_4$ in CCl_4/MECN, pH 6.86 aqueous buffer, 1 hr, at room temperature, to afford the corresponding phenylbenzoates, **13-24** (Scheme 2). All show maximum absorption at 273 nm and give split CD curves with enhanced extrema at 258/285 nm (Fig. 2). During the oxidation (Scheme 2), in addition to the PhBn → PhBz oxidations, one of the N-methyl groups in **13-24** was oxidized to N-formyl groups, the latter being readily deformylated upon NaOMe treatment to yield N-monomethyl derivatives (see Experimental). This is in agreement with reports[12-14] that the N,N-dimethylamino group, especially when attached to tertiary or quaternary carbons, is readily oxidized by RuO_4 to N-formyl-N-methyl, which upon treatment with base undergoes deacylation to NHMe . *All oxidized products 13-24 showed two interconvertible HPLC peaks* (see Experimental), due to the presence of two restricted rotamers around the N-CHO bond. Upon reinjection, the two separated HPLC peaks again gave two peaks, respectively ; the ^{1}H-NMR spectra of isolated HPLC peaks also showed each of them to be a ca. 1:1 mixture of the two rotamers.



Scheme 2

Table 2. *A* values of Di- and Triphenylbenzoates of *Methyl-α-D-hexopyranosaminide*

	 GluN		 GalN		 ManN		 ManN	
	entry	*A*	entry	*A*	entry	*A*	entry	*A*
3,4-	**13**	- 77.4	**17**	+ 43.8	**21**	- 76.4	**25**	- 75.1
3,6-	**14**	+ 19.0	**18**	- 16.4	**22**	+ 13.7	**26**	+ 14.6
4,6-	**15**	+ 28.3	**19**	- 22.2	**23**	+ 32.1	**27**	+ 28.4
3,4,6-	**16**	- 38.5 (- 30.1)	**20**	+ 8.6 (+ 5.3)	**24**	- 29.7 (-30.6)	**28**	- 29.2 (-32.1) *

* Values in parenthesis are A_{cald}.

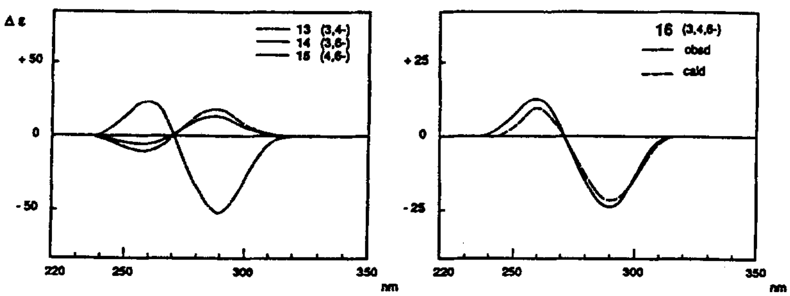


Fig.2. CD of diphenylbenzoates **13-15** and triphenylbenzoate **16** (observed and calculated): in MeCN.

The A values of phenylbenzoates **13-24** are enhanced 2-5 fold as compared with those of the corresponding PhBn ethers **1-12** (Table 2). Moreover, the additivity relation is valid here as well: for **24**, A_{obsd} is -29.7, whereas A_{cald} is -76.4 (for 3,4-, **21**) +13.7 (for 3,6-, **22**) + 32.1 (for 4,6-, **23**) = -30.6 (Fig. 2). The three diphenylbenzoates **25-27** and triphenylbenzoate **28** were directly prepared from methyl 2-deoxy-2-(N,N-dimethylamino)-α-D-mannopyranoside (see Experimental) and their A values compared with those of **21**, **22**, **23** and **24** (Table 2). Despite the fact that the two series have different N-substituents, *i.e.*, NMe_2 *vs.* MeNCHO, the corresponding pairs exhibit similar A values, suggesting that structural differences at the 2-amino group have little conformational affect on the chromophoric regions.

The additivity relation observed for neutral hexopyranosides thus also holds for phenylbenzyl ethers and phenylbenzoates of aminosugars. The findings described here should be useful in applying the monochromophoric approach of glycosidic linkage to oligosaccharides that contain aminosugars.

Experimental

Spectroscopic measurements of synthetic compounds were performed by ^{1}H-NMR spectroscopy on NICOLET NT-360(360 MHz) or JEOL JNM-FM100 (100 MHz), and LSI-MS on a HITACHI M-80B mass spectrometer, employing glycerol or 2,2'-dithiodiethanol as matrix. All compounds were purified by SiO_2(E. Merck., 230-400 mesh) flash column chromatography. Prior to measurements of UV/CD/MS, the samples were repurified by HPLC with Lichrosorb Si60 (Merck.), 10 mm (4.6 x150 mm) analytical column, monitoring peaks by UV detection at 254 nm for phenylbenzyl ethers and 273 nm for phenylbenzoates. For UV and CD measurements, all samples were prepared as MeCN solutions at concentrations between 1.0-3.0 x 10-5 M on the basis of experimentally determined average ε values at 254 nm for phenylbenzyl ethers: mono 20,300, di 40,300, tri 61,000; and at 273 nm for phenylbenzoates: mono 23,400, di 48,700, tri 68 000. UV measurements were performed on a Shimadzu double beam spectrophotometer, UV-210A. CD spectra were recorded on a JASCO J-20 or JASCO J-600 spectropolarimeter driven by a J-600/98 data processor.

Synthesis of phenybenzyl ethers 1-12.

D-glucosamine was acetylated by conventional methods using Ac_2O and pyridine, 24 h, rt, to produce glucosamine pentaacetate, α/β mixture, yield 90%. Four hundred mg (1.03 mmol) of the pentaacetate in 30 ml of 5% methanolic HCl was refluxed 12 h to give 140 mg of methyl 2-amino-2-deoxy-α-D-glucopyranoside, 70% yield. The glycoside (140 mg, 0.73 mmol) in 5 ml MeOH, was treated with 66 mg (2.19 mmol) of paraformaldehyde and 69 mg (1.10 mmol) of $NaBH_3CN$; stirred 48 h rt, to give 113 mg of methyl 2-deoxy-2-(N,N-dimethylamino)-α-D-glucopyranoside **a**, 70% yield. LSIMS : glycerol, m/z 222$(M+H)^+$. NMR (100 MHz, D_2O) : 4.90 (1H, d, J=4.0Hz, 1-H), 4.1-3.5 (5H, m, 3,4,5,6 and 6'-H), 3.44 (3H, s, -OMe), 2.78 (1H, dd, J=10.0, 4.0 Hz, 2-H) and 2.54 (6H, s, NMe_2).

Methyl 2-deoxy-2-(N,N-dimethylamino)-3,4-O-diphenylbenzyl-α-D-gluco-pyranoside ***1.***

Glycopyranoside **a** (20 mg, 0.09 mmol) was dissolved in 2 ml of pyridine, to which was added 87 mg (0.27 mmol) of triphenylmethyl bromide (TrBr); the solution was stirred for 12 h at 80°C to give 27.8 mg (0.06 mmol) of tritylate **b**, 66% yield. This was dissolved in 5 ml of THF/DMF(2:1), to which 14.4 mg (0.6 mmol) of NaH was added; the solution was stirred for 1 hr, under N_2 at rt, treated with 30 mg (0.12 mmol) of p-phenylbenzyl bromide (PhBnBr), and stirred for 12 h to give 29 mg (0.036 mmol) of benzylate, 61% yield. The tritylate benzylate was dissolved in 5 ml MeOH and treated with a few drops of 1N HCl for 10 min. at 70°C to give 18 mg of **1**, 98% yield. LSIMS: 2,2'-dithiodiethanol, m/z 554 $(M+H)^+$. NMR (360 MHz, $CDCl_3$): 7.6-7.2 (18H, m, aromatic-H), 5.00 (1H, d, J=4.0 Hz, 1-H), 5.0-4.7 (4H, 2xAB, Bn-CH_2), 4.2-3.6 (6H, m, 3,4,5,6,6'-H and -OH), 3.40 (3H, s, -OMe), 2.82 (1H, dd, J=10.0, 4.0 Hz, 2-H) and 2.64 (6H, s, NMe_2).

Methyl 2-deoxy-2-(N,N-dimethylamino)-3,6-O-diphenylbenzyl-4-O-methyl-α-D-glucopyranoside 2.

Methyl glycoside **d** (50 mg, 0.23 mmol) in 10 ml of DMF was treated with 65 mg (0.34 mmol) of p-toluenesulfonic acid and 87 mg (0.46 mmol) of benzaldehyde dimethylacetal, heated for 2 h at 80°C, and the MeOH removed to provide 45 mg of benzylidene **c**, 62% yield. This was converted into its 3-phenylbenzyl ether by dissolving 20 mg (0.065 mmol) in 6 ml of THF/DMF (2:1), adding 7.8 mg (0.33 mmol) of NaH, stirring for 1 h under N_2 at rt, adding 16 mg (0.065 mmol) of PhBnBr, stirring for additional 12 h to give 20 mg (0.042 mmol) of the benzyl ether, 65% yield. The 3-benzyl ether in 5 ml of MeOH was treated with a few drops of 1N HCl for 10 min at 70°C to give 15 mg (0.039 mmol) of the debenzylidene derivative, 92% yield. This was 6-tritylited by dissolving in 2 ml pyridine , adding 38 mg (0.12 mmol) TrBr , and stirring for 12 h at 80°C to produce 12.5 mg (0.02 mmol) of product **d**, 52%. Tritylate **d** was methoxylated by addition of 2.4 mg (0.1 mmol) NaH to **d** in 5 ml THF/DMF (2:1), stirring for 1 h under N_2 at rt, adding 2.8 mg (0.02 mmol) CH_3I and stirring for further 12 h, 9 mg (0.014 mmol) of product, 70% The 6-tritylate in 3 ml MeOH was readily 4-methoxylated by treatment with a few drops of 1N HCl for 10 min, at 70°C, 98% yield. The 4-methoxy-6-tritylate (5 mg) was dissolved in 3 ml THF/DMF(2:1), to which 2.4 mg (0.1 mmol) NaH was added; 3.5 mg (0.014 mmol) of PhBnBr was added after stirring the solution for 1 h under N_2, rt, and the stirring continued for a further 12 h to give 5.8 mg of **2**, 75% yield. LSIMS: 2,2'-dithiodiethanol, m/z $568(M+H)^+$. NMR (360 MHz, $CDCl_3$): 7.6-7.2 (18H, m, aromatic-H), 5.00 (1H, d, J=4.0 Hz, H-1), 5.0-4.6 (4H, 2xAB, Bn-CH_2), 4.0-3.5 (5H, m, 3,4,5,6 and 6'-H), 3.54 (3H, s, -OMe), 3.40 (3H, s, -OMe), 2.80 (1H, dd, J=10.0, 4.0 Hz, 2-H) and 2.60 (6H,s, NMe_2).

Methyl 2-deoxy-2-(N,N-dimethylamino)-4,6-O-diphenylbenzyl-3-O-methyl-α-D-glucopyranoside 3.

4,6-Dibenzylidene **c** (20 mg, 0.065 mmol) in 6 ml THF/DM(2:1) containing 7.8 mg (0.33 mmol) NaH was stirred for 1 h under N_2 , then treated with 10 mg (0.07 mmol) CH_3I and the solution was stirred further for 12 h to give 16.5 mg of the 3-methoxy derivative; 80% yield; the benzylidene group was removed by dissolving the 3-methoxy sugar in 3 ml MeOH, adding a few drops of 1N HCl and heating for 10 min,70°C. The deprotected sugar (12 mg, 0.05 mmol) was dissolved in 3 ml THF/DMF (2:1), to which 12.5 mg (0.52 mmol) of NaH was added; after stirring for 1 h, N_2, 24.7 mg (0.1 mmole) PhBnBr was added and stirring continued for 12 h to give 20.3 mg (0.036 mmol) of **3**, 70% yield. LSIMS: 2,2'-dithiodiethanol, m/z $568(M+H)^+$. NMR (360 MHz, $CDCl_3$); 7.6-7.2 (18H, m, aromatic-H), 4.82 (1H, d, J=4.0 Hz, 1-H), 4.9-4.5 (4H, 2xAB, Bn-CH_2), 3.9-3.6 (5H, m, 3,4,5,6 and 6'-H), 3.66 (3H, s, -OMe), 3.38 (3H, s, -OMe), 2.72 (1 and H, dd, J=10.0, 4.0 z, 2-H) and 2.56 6H, s, NMe_2).

Methyl-2-deoxy-2-(N,N-dimethylamino)-3,4,6-O-triphenylbenzyl-α-D-gluco pyranoside, 4.

Tribenzylate **4** (35 mg) was prepared in similarly starting from 20 mg (0.09 mmol) glycoside **a**, 22 mg (0.9 mmol) NaH, 6 ml THF/DMF (2:1) and 67 mg (0.27 mmol) PhBnBr, 55% yield. LSIMS: 2,2'-dithiodiethanol, m/z $720(M+H)^+$. NMR (360 MHz, $CDCl_3$): 7.6-7.2 (27H, m, aromatic-H), 4.95 (1H, d, J=4.0 Hz, 1-H), 5.0-4.5 (6H, 3xAB, Bn-CH_2), 4.2-3.6 (5H, m, 3,4,5,6 and 6'-H), 3.40 (3H, s, -OMe), 2.86 (1H, dd, J=10.0, 4.0 Hz, 2-H) and 2.60 (6H, s, NMe_2).

Compounds **5-8** and **9-12** were likewise prepared from D-galactosamine and D-mannosamine.

Methyl-2-deoxy-2-(N,N-dimethylamino)-α-D-galactopyranoside. LSIMS: glycerol, m/z 222$(M+H)^+$. NMR (100 MHz, D_2O): 5.05 (1H, d, J=4.0 Hz, 1-H), 4.24 (1H, dd, J=12.0, 4.0 Hz, 3-H), 4.1-3.6 (4H, m, 4,5,6 and 6'-H), 3.44 (3H, s, -OMe), 3.24 (1H, dd, J=12.0, 4.0 Hz, 2-H) and 2.74 (6H, s, NMe_2).

*Methyl 2-deoxy-2-(N,N-dimethylamino)-3,4-O-diphenylbenzyl-α-D-galacto pyranoside **5**.*
LSIMS: 2,2'-dithiodiethanol, m/z 554$(M+H)^+$. NMR (100 MHz, $CDCl_3$); 7.6-7.3 (18H, m, aromatic-H), 5.68 (1H, d, J=4.0 Hz, 1-H), 5.0-4.4 (4H, 2xAB, Bn-CH_2), 4.3-3.6 (7H, m, 2,3,4,5,6,6'-H and -OH), 3.52 (3H, s, -OMe), and 2.92 (6H, s, NMe_2).

*Methyl 2-deoxy-2-(N,N-dimethylamino)-3,6-O-diphenylbenzyl-4-O-methyl-α-D-galactopyranoside, **6**.*
LSIMS: 2,2'-dithiodiethanol, m/z 568 $(M+H)^+$. NMR (100 Hz, CDCl3); 7.6-7.3 (18H, m, aromatic-H), 5.28 1H, d, J=4.0 Hz, 1-H), 4.9-4.4 (4H, 2xAB, Bn-CH_2), 4.2-3.4 (6H, m, 2,3,4,5,6 and 6'-H), 3.58 (3H, s, -OMe), 3.46 (3H, s, -OMe) and 2.78 (6H, s, NMe_2).

*Methyl 2-deoxy-2-(N,N-dimethylamino)-4,6-O-diphenylbenzyl-3-O-methyl-α-D-galactopyranoside, **7**.*
LSIMS: 2,2'-dithiodiethanol, m/z 568$(M+H)^+$. NMR (100 MHz, $CDCl_3$); 7.6-7.3 (18H, m, aromatic-H), 5.60 H, d, J=4.0 Hz, 1-H), 4.9-4.5 (4H, 2xAB, Bn-CH_2), 4.2-3.4 (6H, m, 2,3,4,5,6 and 6'-H), 3.52 (3H, s, -OMe), 3.44 (3H, s, -OMe) and 2.86(6H, s, NMe_2).

*Methyl 2-deoxy-2-(N,N-dimethylamino)-3,4,6-O-triphenylbenzyl-α-D-galacto- pyranoside, **8**.*
LSIMS: 2,2'-dithiodiethanol, m/z 720$(M+H)^+$. NMR (100 MHz, $CDCl_3$); 7.6-7.3 (27H, m, aromatic-H), 5.04 (1H, d, J=4.0 Hz, 1-H), 5.0-4.4 (6H, 3xAB, Bn-CH_2), 4.3-3.6 (6H, m, 2,3,4,5,6 and 6'-H), 3.46 (3H, s, -OMe) and 2.72 (6H, s, NMe_2).

Methyl 2-deoxy-2-(N,N-dimethylamino)-α-D-mannopyranoside. LSIMS: glycerol, m/z 222 $M+H)^+$. NMR (100 MHz, D_2O); 4.96 (1H, d, J=3.0 Hz, 1-H), 4.1-3.5 (5H, m, 3,4,5 and 6-H), 3.42 (3H, s, -OMe), 2.96 (1H, dd, J= 4.0, 3.0 Hz, 2-H) and 2.54 (6H, s, NME_2).

*Methyl 2-deoxy-2-(N,N-dimethylamino)-3,4-O-diphenylbenzyl-α-D-manno-pyranoside **9**.*
LSIMS: 2,2'-dithiodiethanol, m/z 554$(M+H)^+$. NMR (360 MHz, $CDCl_3$); 7.6-7.3 (18H, m, aromatic-H), 4.86 , d, J=3.0 Hz, 1-H), 4.9-4.6 (4H, 2xAB, Bn-CH_2), 4.08 (1H, sbr; D_2O exchangeable, -OH), 4.0-3.6 (5H, m, 3,4,5,6 and 6'-H), 3.38 (3H, s, -OMe), 2.92 (1H, dd, J=4.0, 3.0 Hz, 2-H) and 2.54(6H, s, NME_2).

*Methyl-2-deoxy-2-(N,N-dimethylamino)-3,6-O-diphenylbenzyl-4-O-methyl-α-D-mannopyranoside **10**.*
LSIMS: 2,2'-dithiodiethanol, m/z 568 $(M+H)^+$. NMR (360 MHz, $CDCl_3$); 7.6-7.3 (18H, m, aromatic-H), 4.88 (1H, d, J=3.0 Hz, 1-H), 4.8-4.6 (4H, 2xAB, Bn-CH_2), 3.92 (1H, dd, J=6.2, 3.8 Hz, 3-H),

3.8-3.6 (4H, m, 4,5,6 and 6'-H), 3.45 (3H, s, OMe), 3.38 (3H, s, OMe), 2.90 (1H, dd, J=3.8, 3.0 Hz, 2-H) and 2.54 6H, s, NME_2).

*Methyl 2-deoxy-2-(N,N-dimethylamino)-4,6-O-diphenylbenzyl-3-O-methyl-α-D-mannopyranoside **11**.*

LSIMS: 2,2'-dithiodiethanol, m/z 568 $(M+H)^+$. NMR (360 MHz, $CDCl_3$); 7.6-7.3 (18H, m, aromatic-H), 4.86 (1H, d, J=3.0 Hz, 1-H), 4.8-4.5 (4H, 2xAB, Bn-CH_2), 3.9-3.7 (5H, m, 3,4,5,6 and 6'-H), 3.45 (3H, s, -OMe), 3.38 (3H, s, -OMe), 2.92 (1H, J=3.8, 3.0 Hz, 2-H) and 2.54 (6H, s, NME_2).

*Methyl 2-deoxy-2-(N,N-dimethylamino)-3,4,6-O-triphenylbenzyl-α-D-manno-pyranoside **12**.*

LSIMS: 2,2'-dithiodiethanol, m/z 720$(M+H)^+$. NMR (360 MHz, $CDCl_3$); 7.6-7.2 (27H, m, aromatic-H), 4.92 (1H, d, J≈3.0 Hz, 1-H), 4.9-4.5 (6H, 3xAB, Bn-CH_2), 4.1-3.7 (5H, m, 3,4,5,6 and 6'-H), 3.40 (3H, s, -OMe), 2.92 (1H, J=3.8, 3.0 Hz, 2-H) and 2.58(6H, s, NME_2).

Oxidation of phenylbenzylates 1-12 to phenylbenzoates 13-24.

Oxidation with RuO_4 converted compounds **1-12** to the corresponding phenylbenzoates **13-24**. The RuO_4 reagent was prepared from $RuCl_3 \cdot 3H_2O$ in the following manner: $RuCl_3 \cdot 3H_2O$ (64 mg) and $NaIO_4$ (760 mg) were stirred in a biphasic solution which contained 4 ml of CCl_4, 4 ml of MeCN and 6 ml of aqueous buffer (pH 6.86) for 18 h until the color of the organic phase turned yellow; the color was stable for over a month if stored at 5°C. The organic phase was used as the RuO_4 reagent. One mg each of the di- and tri-phenylbenzyl ethers **1-12** (**1**, **5** and **9** were acetylated before oxidation to protect the 6-OH group from RuO_4 oxidation) were dissolved in 2 ml MeCN/CCl_4 (1:1), to which 1 ml of the RuO_4 reagent, prepared as described above, was added; the reaction mixture was kept at rt for 1 h, then 2 ml of isopropanol was added and left for 10 min. The reaction mixture was evaporated to dryness, redissolved in CH_2Cl_2, and passed through a silica gel pad before HPLC purification. **13-24** showed two interconvertible peaks on HPLC , e.g., when 3:7 hexane/$CHCl_3$ was used as eluent, the retention times of two interconvertible peaks of **13** were 8 and 18 min, those of **14** were 4 and 7 min, and those of **15** were 10 and 14 min, etc.

*Methyl-2-deoxy-2-(N-formyl N-methyl)-α-D-gluco(galacto or manno)pyranoside 6-acetate 3,4-diphenylbenzoate, **13**, **17** and **21**.* LSIMS: 2,2'-dithiodiethanol, m/z 638$(M+H)^+$.

*Methyl-2-deoxy-2-(N-formyl N-methyl)-4-O-methyl-α-D-gluco(galacto or manno)pyranoside 3,6-diphenylbenzoate, **14**, **18** and **22**.* LSIMS: 2,2'-dithiodiethanol, m/z 610$(M+H)^+$.

*Methyl-2-deoxy-2-(N-formyl N-methyl)-3-O-methyl-α-D-gluco(galacto or manno)pyranoside 4,6-diphenylbenzoate, **15**,**19** and **23**.* LSIMS: 2,2'-dithiodiethanol, m/z 610$(M+H)^+$.

*Methyl-2-deoxy-2-(N-formyl N-methyl)-α-D-gluco(galacto or manno)pyranoside 3,4,6-triphenylbenzoate, **16**, **20** and **24**.* LSIMS: 2,2'-dithiodiethanol, m/z 776$(M+H)^+$.

*Deformylation of **21**, **22**, **23** and **24** to methyl 2-deoxy-2-methylamino-α-D-mannopyranoside **29**.*

The samples in 0.2% NaOMe/MeOH were left 10 min at rt. LSIMS: (tetraacetate), 2,2'-dithiodiethanol, m/z 376 $(M+H)^+$. NMR (100 MHz, D_2O); 4.96 (1H, d, J=2.0 Hz, 1-H), 4.1-3.6 (5H, m, 3,4,5,6 and 6'-H), 3.48 (3H, s, -OMe), 3.08 (1H, dd, J=6.0, 2.0 Hz, 2-H) and 2.52 (3H, s, -NMe).

Synthesis of phenybenzoates 25-28.

Methyl-2-deoxy-2-(N,N-dimethylamino)-α-D-mannopyranoside 6-acetate 3,4-diphenylbenzoate **25**.
Methyl 2-deoxy-2-(N,N-dimethylamino)-6-O-trityl-α-D-mannopyranoside (mannose equivalent of **b**, Scheme 1) (5 mg, 0.01 mmol) and 38 mg (0.1 mmol) of 4-phenylbenzoyl anhydride ($PhBz_2O$) in 2 ml of pyridine with a catalytic amount of N,N-dimethylaminopyridine (DMAP) was heated for 12 h at 80°C to give 6.5 mg of the 3,4-diphenylbenzoate, 81% yield. This was dissolved in 2 ml of MeOH, treated with 0.2 ml of 1N HCl, and heated at 70°C, 10 min, to give 4.2 mg methyl 2-deoxy-2-(N,N-dimethylamino)-α-D-mannopyranoside 3,4-diphenylbenzoate, which was converted to the 6-acetoxy derivative **25** with Ac_2O/pyridine, 24 h, rt. LSIMS: 2,2'-dithiodiethanol, m/z 624$(M+H)^+$. NMR (100 Hz, $CDCl_3$) ; 8.2-7.3 (18H, m, aromatic-H), 5.90 (1H, t, J=10.0 Hz, 4-H), 5.72 (1H, dd, J=10.0, 4.0 Hz, 3-H), 4.96 (1H, d, J=2.0 Hz, 1-H), 4.4-4.1(3H, m, 5,6 and 6'-H), 3.48 (3H, s, -OMe), 3.36 (1H, dd, J=4.0, 2.0 Hz, 2-H), 2.56 (6H, s, NME_2) and 2.06 (3H, s, $-COOCH_3$) .

Methyl-2-deoxy-2-(N,N-dimethylamino)-a-D-mannopyranoside 4-acetate 3,6- diphenylbenzoate **26**.
Methyl 4,6-O-benzylidene-2-deoxy-2-(N,N-dimethylamino)-α-D-mannopyranoside (mannose equivalent of **c**, Scheme 1) (5 mg, 0.016 mmol) and 30 mg (0.08 mmol) $PhBz_2O$ in 2 ml pyridine with catalytic DMAP were heated 12 h at 80°C to furnish 5.2 mg of the 3-phenylbenzoate, 67 %, which was deprotected by dissolving in 1 ml MeOH, adding 0.1 ml 1N HCl and heating for 15 min, 70°C, 3.8 mg of methyl-2-deoxy -2-(N,N-dimethylamino)-α-D-mannopyranoside 3-phenylbenzoate. The 4,6-dihydroxy 3-phenylbenzoate (3.8 mg, 0.009 mmol) and 6 mg (0.027 mmol) p-phenylbenzoyl chloride (PhBzCl) in 3 ml pyridine was heated for 12 h at 80°C to give 4.0 mg methyl 2-deoxy-2-(N,N-dimethylamino)-α-D-mannopyranoside 3,6-diphenylbenzoate, 76 % yield, which was acetylated with Ac_2O / pyridine to give **26**. LSIMS: 2,2'-dithiodiethanol, m/z 624$(M+H)^+$. NMR (100 MHz, $CDCl_3$) ; 8.2-7.3 (18H, m, aromatic-H), 5.78 (1H, t, J=10.0 Hz, 4-H), 5.58 (1H, dd, J=10.0, 4.0 Hz, 3-H), 4.94 (1H, d, J=2.0 Hz, 1-H), 4.6-4.4 (2H, m, 6,6'-H), 4.18 (1H, m, 5-H), 3.46 (3H, s, -OMe), 3.28 (1H, dd, J=4.0, 2.0 Hz, 2-H), 2.54 (6H, s, NME_2) and 2.02 (3H, s, $-COOCH_3$).

Methyl 2-deoxy-2-(N,N-dimethylamino)-3-O-methyl-α-D-mannopyranoside 4,6- diphenylbenzoate **27**.
Methyl 2-deoxy-2-(N,N-dimethylamino)-3-O-methyl-α-D-mannopyranoside (3 mg, 0.013 mmol) and 50 mg (0.13 mmol) $PhBz_2O$ in 2 ml pyridine with catalytic DMAP were heated 12 h at 80°C to yield 4.8 mg of the 4,6-diphenylbenzoate **27**, 67% LSIMS: 2,2'-dithiodiethanol, m/z 596$(M+H)^+$. NMR (100 MHz, $CDCl_3$); 8.2-7.3 (18H, m, aromatic-H), 5.56 (1H, dd, J=10.0, 6.0 Hz, 4-H), 4.90 (1H, d, J=4.0 Hz, 1-H), 4.6-4.4 (2H, m, 6,6'-H), 4.28 (1H, m, 5-H), 3.80 (1H, dd, J=6.0, 4.0 Hz, 3-H), 3.52 (3H, s, -OMe), 3.46 (3H, s, -OMe), 2.92 (1H, t, J=4.0 Hz, 2-H), 2.52 (6H, s, NME_2).

Methyl-2-deoxy-2-(N,N-dimethylamino)-α-D-mannopyranoside 3,4,6-triphenyl-benzoate **28**.
The mannose equivalent of **a** (2 mg, 0.009 mmol) and 50 mg (0.13 mmol) $PhBz_2O$ in 2 ml pyridine and catalytic DMAP were heated, 12 h, 80°C to give 4.8 mg **28**, 69% yield. LSIMS: 2,2'-dithiodiethanol, m/z 762$(M+H)^+$. NMR (100 MHz, $CDCl_3$): 8.2-7.3 (27H, m, aromatic-H), 6.04 (1H, t, J=9.0 Hz, 4-

H), 5.78 (1H, dd, J=9.0, 6.0 Hz, 3-H), 4.98 (1H, d, J=3.0 Hz, 1-H), 4.7-4.5 (2H, m, 6,6'-H), 4.36 (1H, m, 5-H), 3.50 (3H, s, -OMe), 3.36 (1H, dd, J=6.0, 3.0 Hz, H-2) and 2.58 (6H, s, NME_2).

References.

(1) Chang, M.; Meyers, H.V.; Nakanishi, K.; Ojika, M.; Park, J.H.; Park, M.H.; Takeda, R.; Vázquez, J.T.; Wiesler, W.T. *Pure & Appl. Chem.* **1989**, *61* (7), 1193.

(2) Ojika, M.; Meyers, H.V.; Chang, M.; Nakanishi, K. *J. Am. Chem. Soc.* **1989**, *111*, 8944.

(3) Nakanishi, K.; Kuroyanagi, M.; Nambu, H.; Oltz, E.M.; Takeda, R.; Verdine, G.L.; Zask, A. *Pure & Appl. Chem.* **1984**, *56*, 1031.

(4) Harada, N.; Nakanishi, K. "Circular Dichroic Spectroscopy-Exciton Coupling in Organic and Bioorganic Chemistry"; University Science Books: Mill Valley, CA, 1983.

(5) a) Lindberg, B. *Chem. Soc. Rev.* **1981**,*10*, 409. b) Biermann, C.J.; McGinnis, G.D. (Eds.), "Analysis of Carbohydrates by GLC and MS", CRC Press, Boca Raton, Florida, 1989.

(6) a) Wiesler, W.T.; Vázquez, J.T.; Nakanishi, K. *J. Am. Chem. Soc.* **1987**, *109*, 5586. b) Vázquez, J.T.; Wiesler, W.T.; Nakanishi, K. *Carbohydrate Res.* **1988**, *176*, 175.

(7) Wiesler, W.T.; Berova, B.; Ojika, M.; Meyers, H.V.; Chang, M.; Zhou, P.; Lo, L.-C.; Niwa, M.; Takeda, R.; Nakanishi, K. submitted.

(8) Liu, H.; Nakanishi, K. *J. Am. Chem. Soc.* **1982**, *104*, 1178

(9) Takeda, R.; Zask, A.; Nakanishi, K.; Park, M.H. *J. Am. Chem. Soc.* **1987**, *109*, 914.

(10) Ohfune, Y.; Kurokawa, N.; Higuchi, N.; Saito, M.; Hashimoto, M.; Tanaka, T. *Chemistry Letters* **1984**, *3*, 441

(11) Schuda, P.F.; Cichowicz, M.B.; Heimann, M.R. *Tetrahedron Lett.* **1983**, *24* (36), 3829

(12) Berkowitz, L.M.; Rylander, P.M. *J. Am. Chem. Soc.* **1958**, *80*, 6682.

(13) Endo, T.; Zemlica, J. *J. Org. Chem.* **1979**, *44* (21), 3652.

(14) Perrone, R.; Carbonara, G.; Tortorella, V. *Arch. Pharm. (Weinheim).* **1984**, 317, 635.