ULTRASONIC AGITATION ACCELERATES *cis*-GLYCOSYLATION WITH HETEROGENEOUS PROMOTERS

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Dedicated to Professor Václav Horák on the occasion of his 70th birthday.

Ultrasonic agitation increases the yield of glycosylations with donors such as 3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl chloride using the heterogeneous promoters silver zeolite, cadmium zeolite or a mixture of silver perchlorate and silver carbonate on celite. The stereospecificity of the glycosylation depends on the nature of the alcohol to be glycosylated, the nature of the solid support and the solvent. Glycosylations catalyzed by silver zeolite in toluene solutions of the donor 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide, that usually produce *trans*-β-glycosides, yield *cis*-glycosides with unreactive alcohols. In these unreactive alcohols, e.g. methyl 2-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranoside and benzyl 3,4,6-tri-O-benzyl-α-D-mannopyranoside, the hydroxyls to be glycosylated are hydrogen-bonded to a *cis* vicinal oxygen. This feature was evident in their AM1 minimized conformations and was indicated by the solution $J(OH,CH)$ ^1H NMR coupling constants.

The demand for synthetic oligosaccharides has been rapidly growing due, in part, to the ever increasing realization of the differential biological activity of glycolipids and of the many glycoforms of glycoproteins. For example, even to a single glycosylation site on a glycoprotein may be attached more than 10 different oligosaccharides creating more than 10 glycoforms and each glycoform can exhibit a different biological activity[1]. In order to synthesize such an array of oligosaccharides, efficient and stereoselective methods for the formation of glycosidic linkages are required. Of particular difficulty are *cis*-glycosidic linkages since neighbouring group participation cannot be used to control the stereochemistry of their formation. It has been shown[2–6] that metal exchanged zeolites as promoters in the Koenigs–Knorr reaction can enhance the formation of *cis*-linkages. These promoters can be classified as heterogeneous catalysts[7].

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Another member of this group of promoters is silver silicate on alumina which has been used to directly form the Man(β1,4)GlcNAc linkage[8], probably the most difficult *cis*-glycosidic bond to create[9]. We observed a marked effect of the reactivity of the acceptor hydroxyls and of the solvent on the stereoselectivity (and the yields) of the glycosylation reactions catalyzed by these promoters. In this communication, we shall document this effect on the formation of allyl glycosides of 2-acetamido-2-deoxy-galactopyranoside, [GalNAc(αβ1,O)allyl], and glycosidic bonds of 2-acetamido-2-deoxy-glucopyranose with galactose [GlcNAc(αβ1,3)Gal] and with mannose [GlcNAc(αβ1,2)Man] and the influence of reaction conditions on the anomeric (α : β) ratios.

EXPERIMENTAL

Melting points were determined on a Reichert Thermovar melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer polarimeter (Model 243 B) at 26 ± 1 °C. Microanalyses were performed by the Microanalytical Laboratory Ltd., Markham, Ontario. ^{1}H and ^{13}C NMR spectra were recorded at 300.13 (75.47 ^{13}C) or 500.15 MHz with Bruker spectrometers. Some ^{13}C NMR spectra were recorded at 100.566 MHz with a Varian XL-400 spectrometer. Spectra were obtained at 19 ± 2 °C in $CDCl_3$ containing a trace of tetramethylsilane as the internal standard. Chemical shifts are given in ppm (δ-scale), coupling constants (J) in Hz. For ^{1}H NMR data of compounds *III*, *IVa*, *IVb*, *VI*, β-(*VII*), α-(*VIII*), *IX*, β-(*X*), and α-(*XI*) see Table I. Samples for the solvent study (cf. Table II) were prepared with dried alcohols trideuteriomethyl 2-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranoside (*VI*) 9 mg and benzyl 3,4,6-tri-O-benzyl-α-D-mannopyranoside (*IX*) 20 mg and 0.6 ml of solvent for each one as follows. To the 5 mm NMR tubes was added a small amount of 4 A molecular sieves and the spectra recorded sequentially in the order: CD_2Cl_2, CD_3CN and $C_6D_5CD_3$. The solvents were evaporated under a stream of argon and then the sample dried at high vacuum for 16 h to ensure the same concentration of the sample in each solvent. IR spectra were recorded on a Perkin–Elmer (Model 1430) spectrometer using thin film on NaCl plates, wavenumbers in cm^{-1}. Fast Atom Bombardment mass spectra (FAB-MS) were recorded with a VG Analytical ZAB-SE (for general conditions cf. ref.[10]). Thin-layer chromatography (TLC) was performed on silica gel $60F_{254}$ (Merck) plastic plates and visualized by spraying with 50% sulfuric acid, and/or 1% orcinol acidic ferric chloride[11], and heating at 200 °C. Silica gel (230 – 400 mesh; Toronto Research Chemicals) was used for flash chromatography. All starting materials were dried overnight under vacuum (0.13 Pa) prior to use and the solvents were distilled from appropriate drying agents. Solutions were concentrated at water aspirator pressure.

Materials. 3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (*V*) was purchased from Toronto Research Chemicals, Toronto, Canada. Trideuteriomethyl 2-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranoside (*VI*) was synthesized as described in ref.[6] and benzyl 3,4,6-tri-O-benzyl-α-D-mannopyranoside (*IX*) as described in ref.[12]. Trideuteriomethyl 2-O-benzoyl-4,6-O-benzylidene-3-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-α- and β-D-glucopyranosyl)-β-D-galactopyranoside, both α (*VII*) and β (*VIII*), were synthesized according to the procedures described in ref.[13]. A portion of the spectroscopic data for these compounds is cited below to allow a comparison with the analogous data for disaccharides *X* and *XI*. Compound *VII*: $[\alpha]_D$ +186.5° (c 1.15, $CHCl_3$). IR spectrum (neat): 1 780 sh, 1 750, 1 720 (CO). ^{13}C NMR spectrum ($CDCl_3$): 168.8 – 170.3 (3 × CH_3CO); 167.5 br (CONCO); 164.6 (PhCO); 101.5 (C1-Gal, ^2J(C,H) = 162); 99.9 ($PhCO_2$, ^2J(C,H) = 156); 93.7 (C1-Glc, ^2J(C,H) = 172). Compound *VIII*: $[\alpha]_D$ +53.7° (c 3.7, $CHCl_3$). IR spectrum (neat): 1 780, 1 760, 1 725, 1 715 (CO). ^{13}C NMR spectrum

TABLE I

^1H NMR spectra in $CDCl_3$, chemical shifts in ppm and coupling constants (in parentheses) in Hz

Moiety	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
	J(1,2)	*J*(2,3)	*J*(3,4)	*J*(4,5)	*J*(5,6a)	*J*(6a,6b)	*J*(5,6b)
			Compound *III*				
Gal-β-	4.418 d	3.714 dd	4.784 dd	5.331 dd	3.844 td	4.15 m	4.15 m
	(8.0)	(10.9)	(3.3)	(0.9)	(6.8)	–	–
Allyl	H-1'(CH_2) 4.42 ddd, H-2'(CH_2) 4.20 ddd, H-3'(CH=) 5.959 dddd, (5.3, 3.7)						
	H-4'(=CH_2 *cis*) 5.350 ddd,			H-5'(=CH_2 *trans*) 5.264 ddd			
	(15.5, 3.0, 0.4)			(10.4, 1.4, 1.2)			
Acetates	2.151 s, 2.054 s, 2.049 s						
			Compound *IVa*				
Gal-α-	5.057 d	3.678 dd	5.391 dd	4.456 dd	4.065 td	4.096 dd	4.096 dd
	(3.5)	(11.1)	(3.2)	(1.1)	(6.5)	–	(1.1)
Allyl	H-1'(CH_2) 4.2 m, H-2'(CH_2) 4.2 m, H-3'(CH=) 5.916 dddd, (5.3, 3.7)						
	H-4'(=CH_2 *cis*) 5.356 ddd,			H-5'(=CH_2 *trans*) 5.266 ddd			
	(15.5, 3.0, 1.4)			(10.4, 2.5, 1.2)			
Acetates	2.053 s (2 x Ac), 2.144 s						
			Compound *IVb*				
GalNAc	4.857 d	4.441 ddd	5.313 dd	5.408 dd	4.256 td	4.165 m	4.165 m
	(1.1)	(4.3)	(3.2)	(0.7)	(6.6)	–	–
Allyl	H-1'(CH_2) 4.16 ddd, H-2'(CH_2) 4.016 ddd, H-3'(CH=) 5.903 dddd, (12.7, 6.7, 2.5)						
	H-4'(=CH_2 *cis*) 5.356 dd			H-5'(=CH_2 *trans*) 5.266 dd			
	(15.5, 1.4)			(10.3, 1.1)			
Acetates	2.181 s, 2.061 s, 2.038 s, 2.001 s						
N–H	6.284 (9.6)						
			Compound *VI*^{a}				
Gal-β	4.507 d	5.227 dd	3.885 ddd	4.269 dd	3.556 brs	4.335 dd	4.269 dd
	(8.0)	(9.9)	(3.8)	(0.8)	(1.3)	(–12.5)	(1.8)
Aromatics	Bz(*o*) 8.153 brd(2 H),		Bz(*p*) 7.599 brt(1 H),		Bz(*m*) 7.501 brt(2 H),		
	+						
	(7.2)		(7.2)		(7.2)		
	Phth and Benzylidene		7.408 brs(4 H),	7.199 m(1 H), 7.115 brd(4 H)			
			(4.3)	(4.3)			
Benzylidene	$PhCHO_2$ 4.955 s						
Hydroxyl	2.488 (10.7)						

TABLE I
(*Continued*)

Moiety	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
	J(1,2)	*J*(2,3)	*J*(3,4)	*J*(4,5)	*J*(5,6a)	*J*(6a,6b)	*J*(5,6b)
				Compound β-(*VII*)			
GlcNPhth	5.488 d	4.31 dd	5.785 dd	5.147 dd	3.88 m	4.31 m	4.167 dd
	(8.5)	(10.6)	(9.2)	(9.9)	–	(–12.2)	(4.9)
Gal-β	4.460 d	5.651 dd	5.202 dd	4.240 brd	3.742 brt	3.88 m	4.070 dd
	(7.9)	(10.3)	(3.1)	(<0.5)	(6.1)	(–10.3)	(6.3)
Acetates	2.032 s, 2.026 s, 1.863 s						
Aromatics	Bz(*o*) 7.949, 7.929 2 × brdd(4 H), Bz(*p*) 7.48 m(2 H), Bz(*m*) 7.35 brt(4 H)						
	(2 × 7.0, 1.1) (7.5)						
	Phth(*o*) 7.852 brdd(2 H), Phth(*m*) 7.725 brdd(2 H)						
	(5.3, 3.0)						
				Compound α-(*VIII*)			
GlcNPhth	5.225 d	4.452 dd	6.384 dd	4.912 dd	3.9 m	4.787 dd	3.9 m
	(3.6)	(11.5)	(9.4)	(10.1)	(2.9)	(–12.2)	–
Gal-β	4.618 d	5.567 dd	4.037 dd	4.081 brd	3.425 brs	4.241 brd	3.9 m
	(7.9)	(10.0)	(2.9)	(2.9)	(<0.5)	(–12.2)	(<0.5)
Acetates	2.072 s, 1.776 s, 1.770 s						
Aromatics	Bz(*o*) 8.153. brd(2 H), Bz(*p*) 7.599 brt(1 H), Bz(*m*) 7.501 brt(2 H)						
	(7.2) (7.2) (7.2)						
	7.408 brs(4 H), 7.199 m(1 H), 7.115 brd(4 H)						
	(4.3) (4.3)						
Benzylidene	PhCHO$_2$ 4.955 s						
				Compound *IX*^{a}			
Man-α	4.943 brs	4.076 brs	3.888 dd	3.846 t	3.804 brdt	3.755 dd	3.691 brd
	(<0.5)	(3.0)	(8.6)	(9.4)	(1.6)	(–10.6)	(<0.5)
Benzyl	4.830 d(1 H), 4.552 d(1 H) (11.0) 4.723 d(1 H), 4.495 d(1 H) (11.8)						
CH$_2$	4.682 q(2 H) (8.1) 4.616 d(1 H), 4.552 d(1 H) (12.0)						
Hydroxyl	2.496 d (1.6)						
Aromatics	7.10 – 7.40 m(20 H)						
				Compound β-(*X*)			
GlcNPhth	5.497 d	4.500 dd	5.806 dd	5.187 dd	3.855 ddd	4.293 dd	4.201 dd
	(8.5)	(10.5)	(10.4)	(10.1)	(5.0)	(–12.2)	(2.2)
Man-α	4.711 d	4.155 dd	3.886 dd	3.635 dd	3.122 ddd	3.617 dd	3.433 dd
	(1.4)	(3.2)	(8.2)	(10.9)	(5.6)	(–10.7)	(<5)
Benzyl	4.800 d(1 H), 4.400 d(1 H) (10.8) 4.741 d(1 H) 4.507 d(1 H) (11.3)						
CH$_2$	4.554 d(1 H), 4.312 d(1 H) (11.6) 4.124 d(2 H) (3.3)						
Acetates	2.036 s, 2.031 s, 1.863 s						
Aromatics	Phth 7.75 – 7.55 m(4 H)						
	Benzyl 7.40 – 7.10 m (20 H)						

TABLE I
(*Continued*)

Moiety	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
	J(1,2)	*J*(2,3)	*J*(3,4)	*J*(4,5)	*J*(5,6a)	*J*(6a,6b)	*J*(5,6b)
			Compound α-(*XI*)				
GlcNPhth	5.104 d	4.554 dd	6.731 dd	5.049 dd	4.30 m	4.30 m	3.920 dd
	(3.9)	(11.6)	(9.1)	(9.6)	–	(–12.3)	(2.2)
Man-α	4.813 d	3.661 dd	3.728 m	3.89 m	3.80 m	3.80 m	3.80 m
	(1.8)	(2.1)	–	–	–	–	–
Benzyl	4.695 d(1 H), 4.429 d(1 H) (11.9)			4.146 d(1 H), 3.966 d(1 H) (11.8)			
CH_2	4.679 q(2 H)			4.303 d(2 H)			
Aromatics	Phth(*o*) 7.639 brdd(d), Phth(*m*) 7.546 brdd(2 H) (5.4, 3.1) Benzyl 7.445 brdd(1 H), 7.374 brdt(1 H), 7.322 brdt(1 H), 7.3 – 7.1 m(15 H) 7.034 brdd(1 H), 6.894 brdd						
Acetates	2.071 s, 2.025 s, 1.887 s						

[a] Recorded in CD_2Cl_2.

($CDCl_3$): 169.3 – 170.5 (3 × CH_3CO); 167.5 br (CONCO); 164.5 (PhCO); 101.7 (C1-Gal, ^2J(C,H) = 158); 100.8 ($PhCO_2$, ^2J(C,H) = 159); 99.3 (C1-Glc, ^2J(C,H) = 162).

Allyl 3,4,6-Tri-O-acetyl-2-azido-2-deoxy-α- and β-D-Galactopyranosides (*IVa*) and (*III*)

To the promoter under argon in a round bottom flask equipped with magnetic stirring bar were added sequentially the solvent, allyl alcohol and a solution of chloride *I* or bromide *II* (ref.[8]). Stirring was continued for the lengths of time indicated in Table III. The crude products were isolated by filtration, rinsing with the appropriate solvent, and evaporation. The allyl glycosides[14] *III* and *IVa* were purified by flash chromatography on silica gel using hexane–ethyl acetate (80 : 20). Compound *IVa*: $[\alpha]_D$ +97.6° (*c* 0.62, $CHCl_3$). IR spectrum (neat): 2 020 (N_3); 1 750 (CO). For $C_{15}H_{21}N_3O_8$ (371.3) calculated: 48.52% C, 5.70% H, 11.32% N; found: 48.54% C, 5.73% H, 11.36% N.

Allyl 2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranoside (*IVb*)

The azide *IVa* was reduced using H_2S in pyridine–Et_3N (2 : 1) according to ref.[14] and acetylated in pyridine–Ac_2O to yield *IVb* (60%), $[\alpha]_D$ +42.0° (*c* 0.92, $CHCl_3$). IR spectrum (neat): 3 450, 3 400 (NH); 1 750 (CO); 1 680, 1 520 (CON). For $C_{17}H_{25}NO_9$ (387.4) calculated: 52.71% C, 6.50% H, 3.62% N; found: 52.66% C, 6.42% H, 3.56% N.

Benzyl (3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2-O-3,4,6-tri-O-benzyl-α-D-mannopyranoside (*X*) and Benzyl (3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido-α-D-glucopyranosyl)-2-O-3,4,6-tri-O-benzyl-α-D-mannopyranoside (*XI*)

These glycosides were prepared by the procedure described[13] for disaccharides *VII* and *VIII* except that alcohol[12] *IX* was used. The disaccharides *X* and *XI* were separated by flash chromatography on silica gel using hexane–ethyl acetate (3 : 1). Compound *X*: $[\alpha]_D$ −0.01° (*c* 1.23, $CHCl_3$). ^{13}C NMR spectrum ($CDCl_3$): 170.6 – 169.4 (3 × CH_3CO); 167.6 br (CONCO); 138.4 – 137.0 (4 × benzyl C_{ipso}); 96.5 (C1-Man, $^2J(C,H)$ = 168); 96.8 (C1-Glc, $^2J(C,H)$ = 159). High resolution MS for $C_{54}H_{55}NO_{15}Na$ $(MNa)^+$ calculated 980.3480; found 980.3469. Compound *XI*: $[\alpha]_D$ +51.1° (*c* 1.51, $CHCl_3$). ^{13}C NMR spectrum ($CDCl_3$): 170.4 – 169.2 (3 × CH_3CO); 167.5 br (CONCO); 138.5 – 136.9 (4 × benzyl C_{ipso}); 99.6 (C1-Man, $^2J(C,H)$ = 173); 97.7 (C1-Glc, $^2J(C,H)$ = 169). High resolution MS for $C_{54}H_{55}NO_{15}Na$ $(MNa)^+$ calculated 980.3480, found: 980.3442.

Ultrasound or Heating

The procedure described above was used except that Erlenmeyer flasks fitted with reflux condensers were employed. Typically, the following ratios of reactants were utilized: 2 equivalents of donor to 1 equivalent of acceptor alcohol (0.5 mmol scale), 2 equivalents of a base, if added (2,4,6-trimethylpyridine, 1,1,3,3-tetramethylurea, silver carbonate or 2,6-di-tert-butyl-4-methylpyridine) together with 1 g of a promoter[5]. The reaction mixture was agitated in a propylene glycol or glycerol bath using a Bransonic 220 sonicator as a source. Alternatively, the reaction mixture was heated from room temperature to 55 – 65 °C in an oil bath. The reaction course was monitored by TLC and the ratio of reaction products in the reaction mixtures was determined by ^1H NMR.

Preparation of Catalysts

Silver zeolite (13X) or cadmium zeolite were prepared by vigorously stirring 0.1 M aqueous solutions of silver nitrate or suspensions of cadmium carbonate in water with the required amounts of Linde 13X molecular sieves overnight, filtering, washing well with water and drying in air at 300 °C overnight[2]. Immediately before use, the promoters were dried again at 300 °C overnight, except for Fétizon's reagent which was dried by azeotropic distillation with toluene[15] and the $Ag_2CO_3/AgClO_4$ mixtures which were dried at high vacuum. The products are slightly yellow powders which retain their color and activity for at least a year if stored in the dark in a desiccator. Such exchange procedures are known to lead to essentially complete exchange of sodium ions for silver ions[16,17]. Preparations resulting from treatment of the molecular sieves with $Cd(NO_3)_2$ were completely inactive, although comparable materials made by ion exchange of the zeolites with $Cd(CO_3)_2$ were active albeit less than the silver analogues. Metal exchange

TABLE II

Hydroxyl to vicinal methine ^1H NMR coupling constants (in Hz) in three different solvents

Compound	$C_6D_5CD_3$	CD_2Cl_2	CD_3CN	Calculated^a
VI	10.7	11.0	8.3	12.5
IX	2.3	1.6	3.6	1.5

^{a} $J(HC,OH) = 10.4\cos^2\theta - 1.5\cos\theta + 1.2$ (ref.[33]).

reactions with $Cd(NO_3)_2$ have been reported to proceed to completion[18]. Silver zeolite (13X) was found to be more reactive than the corresponding silver zeolite (4A) prepared according to ref.[2].

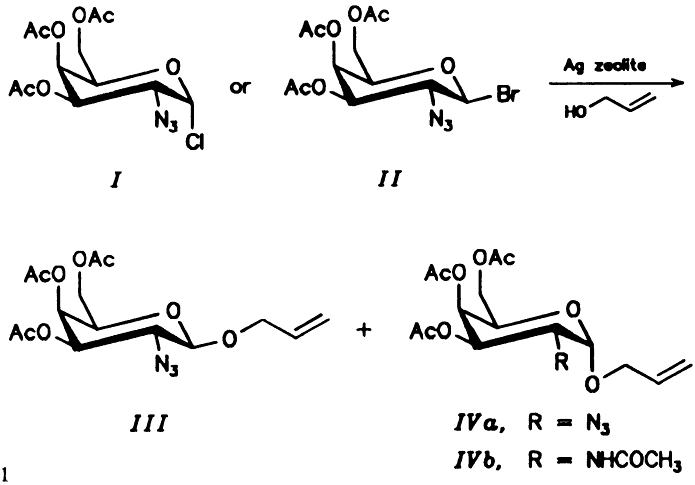
Semi-Empirical Calculations

All calculations were performed with the Hyperchem (Hypercube, Waterloo, Ontario, Canada; distributed by Autodesk, Sausalito, CA, U.S.A.) PC-based (using a 486 processor) package of programs. First, optimized structures of monosaccharides β-D-galactose and α-D-mannose were created and subsequently protecting groups methyl, benzyl, benzoyl or benzylidene were added, one at a time, with optimization until the final structures were obtained. For optimization, a first step of steepest descent optimization[19] with MM2 has been used to remove severe steric conflicts. It should be noted that the MM2 parameters for protecting groups are not available and so in this minimization only van der Waals forces are used. Finally, the structures were minimized (steepest descent followed by conjugated gradient) to a gradient of less then 0.1 Kcal using semi-empirical AMI calculations[20].

RESULTS AND DISCUSSION

Allyl 3,4,6-Tri-O-acetyl-2-azido-2-deoxy-α- and β-D-Galactopyranosides (III) and (IVa) – GalNAc(α1,0) and (β1,0)allyl

α-Linked 2-acetamido-2-deoxy-galactopyranosyl (GalNAc) is very common e.g. in glycolipids and glycoproteins and the search for reliable techniques to make this bond have continued for some time[21,22]. Starting with 3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl chloride[23] (*I*) or the 3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide (*II*), an acceptable even if variable α-stereoselectivity has been reported[24] using a combination of $Ag_2CO_3/AgClO_4$ (10 : 1) in dichloromethane (Scheme 1). For instance, this procedure has been used for glycosylation of protected serine or threonine. This α-stereoselectivity was increased when dichloromethane–



SCHEME 1

toluene mixtures were used. We could not achieve complete α-stereoselectivity under these conditions using a reactive alcohol, allyl alcohol (Table III, entry 1).

In our quest for better stereoselectivity we have therefore explored other heterogeneous promoters in order to find appropriate conditions for more stereospecific and more effective formation of this linkage. The yields and α : β ratios are compiled in Table I. For example, the results in toluene–dichloromethane mixtures did not differ from results in dichloromethane alone (Table III, entries 1 vs 2). Also the stereoselectivity was independent of the starting donor *I* or *II* (Table III, entries 2 vs 3). For the last two experiments Ag_2CO_3 on celite (Fétizons reagent) was used. The main advantage of this reagent is the ease with which it can be removed by filtration from the reaction medium whereas solid Ag_2CO_3 tends to clog filter media. Under magnetic stirring, Cd zeolite shows poor reactivity (yields 15 – 25%), however, agitated ultrasonically, it becomes reactive (yields 80 – 90%, Table III, entries 5 and 6 vs 7 and 8). These results demonstrate that ultrasonic agitation is able to increase the reactivity of reactants in glycosylation reactions with heterogeneous promoters.

In this way preparatively useful amounts of α-glycoside *IVa* could be obtained. The azide group in compound *IVa* was successfully reduced using H_2S, and acetylated, following the procedure of Lemieux et al.[14], to give the 2-acetamido derivative *IVb*.

TABLE III

Preparation of allyl 3,4,6-tri-O-acetyl-2-azido-2-deoxy-α- and β-D-galactopyranosides (*III*) and (*IVa*); for other conditions see Experimental

Entry	Donor	Mole ratio	Catalyst	Conditions	Solvent	Yield[a] %	Ratio α : β
1	β-Br(*II*)	1.5	$AgClO_4$/10 (Ag_2CO_3)	4A sieves stir 16 h	CH_2Cl_2	80	40 : 60
2	β-Br(*II*)	1.0	$AgClO_4$/10 (Ag_2CO_3 on celite)	4A sieves stir 16 h	CH_2Cl_2/ $C_6H_5CH_3$	90	45 : 55
3	α-Cl(*I*)	1.0	$AgClO_4$/10 (Ag_2CO_3 on celite)	4A sieves stir 16 h	CH_2Cl_2/ $C_6H_5CH_3$	90	40 : 60
4	α-Cl(*I*)	1.2	$HgBr_2$/$Hg(CN)_2$	4A sieves stir 16 h	CH3CN	85	25 : 75
5	β-Br(*II*)	2.0	Cd Zeolite	stir 140 h	$ClCH_2CH_2Cl$	25	35 : 65
6	β-Br(*II*)	2.5	Cd Zeolite	stir 16 h	$ClCH_2CH_2Cl$	15	30 : 70
7	β-Br(*II*)	2.5	Cd Zeolite	ultrasound 16 h	$ClCH_2CH_2Cl$	60	45 : 55
8	β-Br(*II*)	5.0	Cd Zeolite	ultrasound 16 h	$C_6H_5CH_3$	90	40 : 60
9	β-Br(*II*)	1.3	Ag Zeolite	ultrasound 16 h	$ClCH_2CH_2Cl$	80	10 : 90

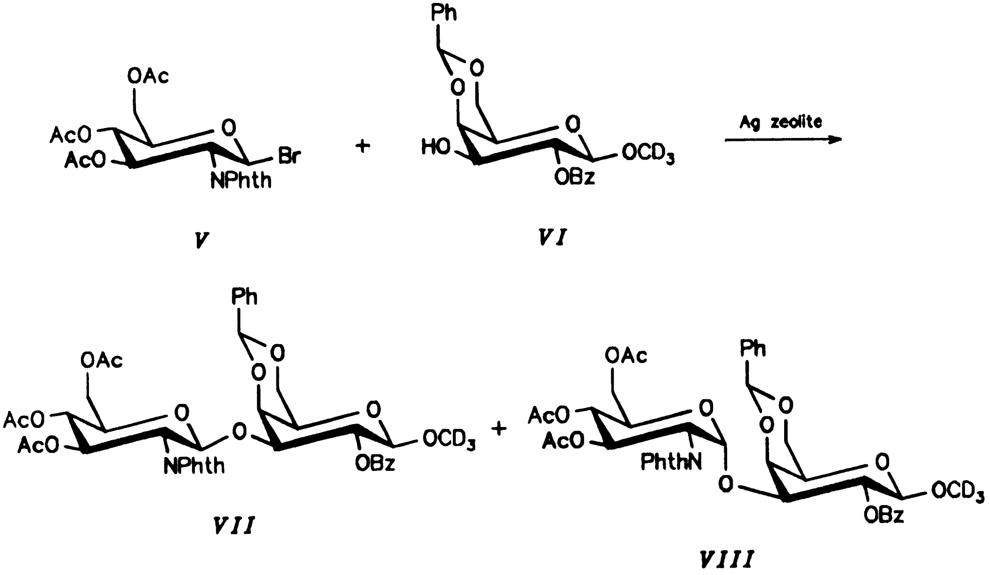
[a] Yields and α : β ratios are from ^1H NMR spectra of crude reaction products. Yields are calculated with respect to unreacted starting material and like the ratios are accurate to ±10%.

Both *IVa* and *IVb* are suitable intermediates for elaboration into larger oligosaccharides.

Trideuteriomethyl 2-O-Benzoyl-4,6-O-benzylidene-3-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-α- and β-D-Glucopyranosyl)-β-D-galactopyranoside (VIII) and (VII) – GlcNPhth(β1,3) and (α1,3)Gal

The formation of β-linkages using 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (*V*) is considered to be near to exclusive[25 - 27].

Surprisingly, under certain conditions with Ag zeolite as promoter, significant amounts of the α-1,3 linked disaccharide *VIII* are obtained in reaction of *V* with alcohol *VI* in dichloromethane[13] (Scheme 2). The ^{1}H and ^{13}C NMR data clearly show that compound *VIII* is the α-1,3-linked isomer of β-disaccharide *VII*, (e.g. H-1 GlcNPhth: $\delta = 5.225$, $^2J(1,2) = 3.6$ and C-1 GlcNPhth: $\delta = 93.7$, $J(\text{C-1,H-1}) = 172$). This unexpected observation prompted us to study this reaction in more detail. The results (cf. Table IV) are difficult to reconcile with the expected mechanism involving carbocation assisted by neighbouring group participation which predicts exclusive formation of β-isomers[9]. Moreover, the formation of β-glycosides with bromide *V* should be favoured because of steric hindrance created at the α-face of the monosaccharide by the bulky phthalimide group.



Phth = phthaloyl

SCHEME 2

The possible role of acid catalyzed isomerization of the β- to the α-glycoside was ruled out since the addition of neither the insoluble base Ag_2CO_3 (Table IV, entries 1 and 2) nor the soluble base 2,5-di-tert-butyl-4-methylpyridine, DTBMP, (Table IV, entry 8) appreciably affected the yield or the stereochemistry of the products. Weaker promoters like Cd zeolite or Ag_2CO_3 led to low yields of mixtures (Table IV, entries 9 and 3) suggesting that the yield is controlled by the reactivity of the donor, but the stereochemistry should be controlled by other factors. Isomerization of the β-disaccharide during the reaction is not likely since both pure α- and β-disaccharides can be subjected to prolonged ultrasonic agitation in the presence of Ag zeolite without decomposition or isomerization. It seems that the nature of the solid support directly

TABLE IV
Preparation of trideuteriomethyl 2-O-benzoyl-4,6-O-benzylidene-3-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-α- and β-D-glucopyranosyl)-β-D-galactopyranoside (*VII*) and (*VIII*)

Entry	Donor	Mole ratio	Catalyst[a]	Conditions	Solvent	Yield[b] %	Ratio α : β
1	Br(*V*)	2.0	Ag Zeolite/Ag_2CO_3	heat 16 h, 65 °C	$ClCH_2CH_2Cl$	80	45 : 55
2	Br(*V*)	2.0	Ag Zeolite/Ag_2CO_3	ultrasound 16 h	$ClCH_2CH_2Cl$	90	55 : 45
3	Br(*V*)	2.0	Ag_2CO_3	ultrasound sieves 16 h	$ClCH_2CH_2Cl$	5	5 : 95
4	Br(*V*)	4.0	Ag Zeolite	stir 5 days	CH_2Cl_2	70	40 : 60
5	Br(*V*)	2.0	Ag Zeolite	heat 16 h, 65 °C	$ClCH_2CH_2Cl$	85	50 : 50
6	Br(*V*)	2.0	Ag Zeolite	ultrasound 16 h	$ClCH_2CH_2Cl$	90	30 : 70
7	Br(*V*)	2.0	Ag Zeolite TMU	heat 16 h, 65 °C	$ClCH_2CH_2Cl$	5	1 : 99
8	Br(*V*)	2.0	Ag Zeolite DTBMP	ultrasound 16 h	$ClCH_2CH_2Cl$	80	65 : 35
9	Br(*V*)	2.0	Cd Zeolite	ultrasound 16 h	$ClCH_2CH_2Cl$	35	40 : 60
10	Br(*V*)	2.0	Tl Zeolite	ultrasound 16 h	$ClCH_2CH_2Cl$	trace[c]	–
11	Br(*V*)	2.0	$AgClO_4$/10 (Ag_2CO_3)	ultrasound 4A sieves 16 h	$ClCH_2CH_2Cl$	75	5 : 95
12	Br(*V*)	2.0	AgTfr DTBMP	stir 3 h	CH_2Cl_2	85	1 : 99
13	Br(*V*)	2.0	Ag Zeolite	ultrasound 14 h	$C_6H_5CH_3$	24	76 : 24
14	Br(*V*)	2.0	Ag Zeolite	ultrasound 14 h	$C_6H_5CH_3$/ $ClCH_2CH_2Cl$	34	50 : 50
15	Br(*V*)	2.0	Ag Zeolite	ultrasound 14 h	$ClCH_2CH_2Cl$	71	43 : 57
16	Br(*V*)	2.0	Ag Zeolite	ultrasound 14 h	$ClCH_2CH_2Cl$/ CH_3CN	35	16 : 84
17	Br(*V*)	2.0	Ag Zeolite	ultrasound 14 h	CH_3CN	16	1 : 99

[a] AgTfr silver triflate, TMU 1,1,3,3,-tetramethylurea, DTBMP 2,6-tert-butyl-4-methylpyridine; [b] yields and α : β ratios are from ^1H NMR spectra of crude reaction products. Yields are calculated with respect to unreacted starting material and like the ratios are accurate to ±10%; [c] turned black and all starting materials decomposed.

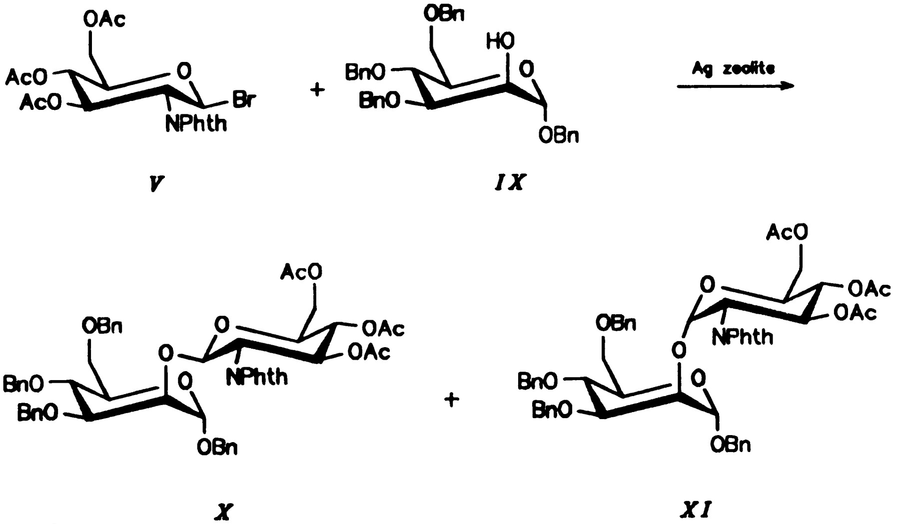
affects the observed stereochemical outcome. For example, this reaction using Ag zeolite with ultrasonic agitation with or without DTBMP (Table IV, entries 2, 6, and 8), or with added Ag_2CO_3, always led to high yields of similar mixtures. On the other hand, Ag_2CO_3 without Ag zeolite gave predominantly the β-isomer albeit in low yield (Table IV, entry 3).

Further information about this reaction was obtained from the solvent dependence data (Table IV, entries 13 – 17). Using toluene as solvent, the α-glycoside was formed predominantly, whereas in acetonitrile almost exclusive β-glycoside formation was observed. This result can be explained by solvent participation and the ensuing double inversion of configuration. It is postulated that acetonitrile acts as a participating solvent giving an "α-nitrilium adduct" (as described by Sinaÿ and coworkers[28]), that is a good leaving group for the nucleophilic attack by the hydroxyl. The low yields in acetonitrile could reflect the unreactivity of the secondary alcohol. Still another explanation may be that in a polar solvent, such as acetonitrile, the interaction is disrupted that occurs between the solid-supported promoter and a substrate in toluene. Furthermore, in the less ionizing solvents, the S_N2 like displacement of the bromide is favoured without invoking intermediates. The lower yields in toluene reflect the low solubility of alcohol *VI* in toluene. In dichloromethane, a better solvent for *VI*, the yields are higher but the ratio of anomers formed reflects a competition between S_N1 and S_N2-like reactions.

*Benzyl 3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido-*β*- and* α-D-*Glucopyranosyl-2-O-(3,4,6-tri-O-benzyl-*α-D-*mannopyranoside (X) and (XI) – GlcNPhth(*β*1,2) and (*α*1,2)Man*

2-O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl, [GlcNAc(β1,2)Man], is an important part of many N-linked oligosaccharides and a number of synthetic approaches have been applied to the formation of this linkage, e.g. refs[29] – [32]. This linkage is usually made using *V* and silver triflate (CF_3SO_3Ag) as a promoter. When we have used Ag zeolite as a promoter, the coupling of *V* with benzyl 3,4,6-tri-O-benzyl-α-D-mannopyranoside (*IX*) (Scheme 3) gave significant amounts of the *cis*-α-linked disaccharide *XI*, clearly identified as such by the ^1H and ^{13}C NMR data (e.g. H-1 GlcNPhth: $\delta = 5.112$, $J(1,2) = 3.9$; and C-1 GlcNPhth: $\delta = 97.7$, $J(\text{C-1,H-1}) = 172$). Also the doublet of doublets corresponding to H-3 of the α-linked GlcNPhth residue is shifted downfield to $\delta = 6.731$ for *XI* which compares to $\delta = 6.384$ for *VII*. This downfield multiplet is diagnostic for the presence of α-linked GlcNPhth residues. The solvent dependence of the reaction (cf. Table V) again shows that acetonitrile, an ionizing solvent, favours almost exclusive formation of β-disaccharide *X* and the poorly ionizing toluene favours the formation of α-linked disaccharide *XI*.

Since the reactions of the bromide *V* with both *VI* and *IX* showed striking similarity, we examined the alcohols *VI* and *IX* by NMR spectrometry and semiempirical calculations (AM1). Of particular interest was to determine the possible conformations of the hydroxyl group because of their putative effect on reactivity. The AM1 minimized model structures of *VI* and *XI* (cf. Fig. 1) showed that no large steric hindrance exists around either hydroxyl group. These model structures also show that the hydroxyl proton is hydrogen bonded to its *cis*-vicinal oxygen (OH to O4, 2.207 Å for *VI*; OH to



SCHEME 3

TABLE V
Preparation of benzyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-α- and β-D-glucopyranosyl-2-O-(3,4,6-tri-O-benzyl-α-D-mannopyranoside) (*X*) and (*XI*)

Entry	Donor	Mole ratio	Catalyst	Conditions	Solvent	Yield[a] %	Ratio α : β
1	Br(*V*)	2.0	Ag Zeolite	stir 16 h	CH_2Cl_2	80	40 : 60
2	Br(*V*)	2.0	Ag Zeolite	heat 16 h 65 °C	CH_3CN	55	10 : 90
3	Br(*V*)	2.0	Ag Zeolite	ultrasound 14 h	$C_6H_5CH_3$	80	60 : 40
4	Br(*V*)	2.0	Ag Zeolite	ultrasound 14 h	$C_6H_5CH_3$/ $ClCH_2CH_2Cl$	88	55 : 45
5	Br(*V*)	2.0	Ag Zeolite	ultrasound 14 h	$ClCH_2CH_2Cl$	81	39 : 61
6	Br(*V*)	2.0	Ag Zeolite	ultrasound 14 h	$ClCH_2CH_2Cl$/ CH_3CN	76	10 : 90
7	Br(*V*)	2.0	Ag Zeolite	ultrasound 14 h	CH_3CN	70	5 : 95

[a] Yields and α : β ratios are from ^1H NMR spectra of crude reaction products. Yields are calculated with respect to unreacted starting material and like the ratios are accurate to ±10%.

O3, 2.226 Å for *IX*; cf. Fig. 1). That these hydroxyl conformations are indeed present in solution was corroborated by the NMR coupling constants between the hydroxyl proton and the ring hydrogen (HO-H3 for *VI*; HO-H2 for *IX*) as presented in Table II. Using the Karplus equation of reference[33] these coupling constants were calculated to be 12.9 and 1.5 Hz at the calculated dihedral angles of –172.4° and –75.8°, in excellent agreement with experiment.

Since a non-polar toluene solution is more likely to resemble the gas phase then a polar acetonitrile solution, we believe that in toluene at least part of the reaction involves the hydrogen-bonded hydroxyl acting as a nucleophile, leading to the formation of the α-linked glycoside via an S_N2-like reaction. It should be noted that such hydrogen-bonded hydroxyl oxygens (hydrogen "donor" oxygens) are expected to be more strongly nucleophilic than hydroxyl oxygens not involved in hydrogen bonding[34]. Otherwise the usual S_N1 reaction prevails, with a possibility of neighbouring group or solvent participation determining the final stereochemical outcome. The ultrasonic agitation enhances the rate of glycosylation to such extent that the preparation of significant amounts of *cis*-linked oligosaccharides becomes possible.

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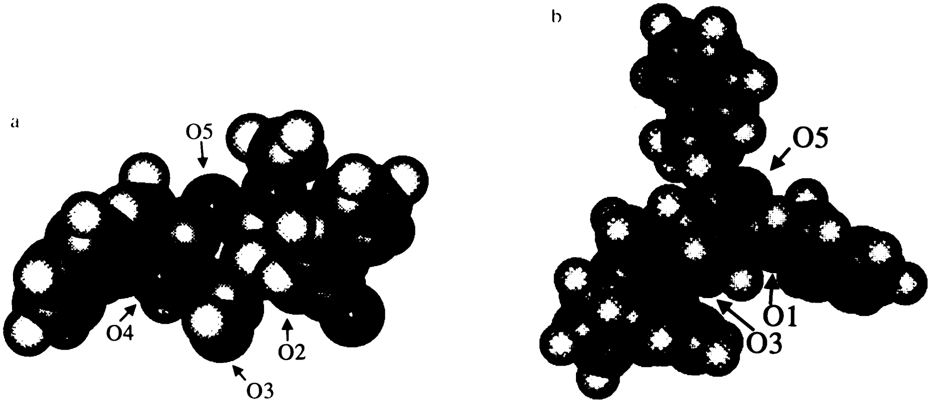

FIG. 1

Space-filling models of *a* trideuteriomethyl 2-O-benzyl-4,6-O-benzylidene-β-D-galactopyranoside (*VI*), and *b* benzyl 3,4,6-tri-O-benzyl-α-D-mannopyranoside (*IX*). Several oxygen atoms are identified by numbering. Short distances between O3 and O4 (*VI*) and O2 and O3 (*IX*) required by hydrogen bonding can be easily identified

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