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The Use of *O*-Glycosyl Trichloroacetimidates in The Synthesis of Unsymmetrical Trehalose Analogues

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Abstract: The coupling of *O*-glycosyl trichloroacetimidates with 2,3,4,6-tetra-*O*-benzylated monosaccharides (*gluco, manno, galacto)* promoted by TMSOTf is described, and the compositions of the crude reaction mixtures, determined by ^{13}C NMR spectroscopy, are presented. Unsymmetrical trehalose derivatives can be synthesized by such couplings. The versatility of the trichloroacetimidates for the synthesis of trehalose analogues has furthermore been demonstrated by the glycosylation of anomerically unprotected maltose heptabenzoylate affording two trehalose-containing trisaccharides, and by the synthesis of α-D-galactopyranosyl α-D-mannopyranoside.

INTRODUCTION

α,α-Trehalose (α-D-glucopyranosyl α-D-glucopyranoside) is a naturally occurring, non-reducing disaccharide with important biological functions. α,α-Trehalose stabilizes proteins, lipid membranes and other biomolecules in so-called anhydrobiotic organisms, which can survive almost complete desiccation for long periods of time, but the molecular basis for this phenomenon is not yet known in detail.[1-3] During studies of this property of α,α-trehalose we have recently tried to develop a general method for the synthesis of unsymmetrical trehalose analogues. Benzylated monosaccharides were glycosylated with benzoylated glycosyl bromides in the presence of silver trifluoromethanesulphonate as promotor. However, the method was not efficient, from a preperative point of view, due to the excessive, but inherent, formation of by-products.[4] We have therefore investigated the *O*-trichloroacetimidates[5] as alternative glycosyl donors and report herein on the successful glycosylation with imidates to obtain unsymmetrical trehalose analogues.

RESULTS AND DISCUSSION

O-(2,3,4,6-Tetra-*O*-benzyl-ß-D-glucopyranosyl)-trichloroacetimidate[6] (**1**, α/ß ratio 1:10) was reacted with each of the three hemiacetal acceptors **2-4** in dichloromethane in the presence of catalytic amounts of TMSOTf. The compositions of the crude product mixtures were determined by ^{13}C NMR spectroscopy.[4,7,8] The data are presented in Table 1. The reactions afforded trehalose type disaccharide products in high yields (typically higher than 90 %). Glycosylation of **2** with **1** afforded the α,α-configurated product **5** (39 %) in mixture with the α,ß-configurated product **6** (52 %) and **2** (9 %). By comparison, the use of silver triflate as promotor instead of TMSOTf afforded a 2:1 mixture of **5** and **6**. Attempts to perform the reaction under more polar conditions in either diethyl ether or acetonitrile were unsuccessful due to the low solubility of the reactants in these solvents.

Glycosylation of **3** with **1** afforded a complex product mixture containing the four theoretical products **7-10** in mixture with some minor impurities (Table 1). Since attempts to separate the products by HPLC were unsuccessful, an aliquot of the product mixture was *O*-deprotected by catalytic hydrogenolysis and the obtained mixture containing the free disaccharides **11-14** was characterized by ^{13}C NMR spectroscopy. The data, presented in Table 2, confirmed, with minor differences, the findings presented in Table 1.

Glycosylation of **4** with **1** afforded the α,α-configurated product **15** (54 %) in mixture with the α,ß-configurated product **16** (33 %) and some minor impurities (Table 1). *O*-Deprotection gave a mixture containing the free sugars **17** and **18**, which was characterized by ^{13}C NMR spectroscopy (Table 2). Again, the composition of the crude mixture presented in Table 1 was confirmed, within the accuracy (± 2.5 %) of the analytical method employed.

The two benzoylated hemiacetal acceptors **19** and **24** were glycosylated with **1**, and the compositions of the crude product mixtures were determined by ^{13}C NMR spectroscopy (Table 1). The maltose acceptor **19**, obtained by hydrolysis of the corresponding bromide[9], was glycosylated with **1** to afford the ß,α-configurated product **20** (42 %) in mixture with the ß,ß-configurated product **21** (22 %) and unreacted acceptor **19** (28 %, α-isomer). Separation by silica gel chromatography afforded compound **20** pure in 23 % yield, and compound **21** in 8 % yield (ca 80 % pure). The compounds were fully characterized by ^{1}H and ^{13}C NMR spectroscopy (Tables 3-5). For **20** the anomeric carbons resonated at δ 96.4, 100.7 and 98.7, respectively, whilst the anomeric protons resonated at δ 5.79 (d, *J* 3.9 Hz), 5.06 (d, *J* 7.7 Hz) and 5.05 (d, *J* 3.4 Hz), respectively. For **21** the anomeric carbons resonated at δ 96.3, 96.5 and 99.4, respectively, whilst the anomeric protons resonated at δ 5.85 (d, *J* 3.9 Hz), 5.34 (d, *J* 7.7 Hz) and 4.92 (d, *J* 7.7 Hz), respectively.

O-Deprotection of **20** afforded the free sugar **22** in 90 % yield. For **22**, the anomeric protons resonated at δ 5.40 (d, *J* 3.2 Hz), 4.62 (d, *J* 7.8 Hz), and 5.22 (d, *J* 3.1 Hz), respectively, whilst the anomeric carbons resonated at δ 100.4, 103.6, and 100.0, respectively (Tables 3-5).

Table 1. Coupling of *O*-glycosyl imidates (1.1 equiv) with anomerically unprotected glycosyl acceptors (1 equiv) in CH_2Cl_2 at 25 °C using TMSOTf as the catalyst. The compositions of the crude product mixtures, determined by ^{13}C NMR spectroscopy (accuracy ± 2.5 %).[a]

Synthesis D[b]	A[c]	Products	^{13}C NMR (δ anomeric C)	Dimerization by-products D	A	Others
1	2	**5** (39 %) **6** (52 %)	94.4(α) 99.5(α) 104.4(ß)			**2** (9 %)
1	3	**7** (36 %) **8** (19 %) **9** (23 %) **10** (12 %)	94.5(α) 93.5(α) 104.4(ß) 99.8(α) 99.1(α) 103.5(ß) 100.0(ß) 99.4(ß) 93.6(α)		6 %	**2** (2 %)[d] 2 %[e]
31	2	**7** (57 %) **8** (9 %) **9** (8 %) **10** (0 %)	94.5(α) 93.5(α) 104.4(ß) 99.8(α) 99.1(α) 103.5(ß) 100.0(ß) 99.4(ß) 93.6(α) 94.2(α)	3 %	3 %	**31** (1 %) **2** (7 %) **3** (7 %) 5 %[e]
1	4	**15** (54 %) **16** (33 %)	94.0(α) 92.8(α) 102.8(ß) 99.7(α) 93.3(α)		4 %	**2** (6 %)[d]
1	19	**20** (42 %) **21** (22 %)	98.7(α) 100.7(ß) 99.4(ß) 96.5(ß) 90.2(α)			8%[e] **19**(28%)
1	24	**25** (18 %) **26** (17 %) **27** (9 %) **28** (5 %)	96.5(α) 98.2(α) 94.5(α) 96.9(ß) 97.7(ß) 94.3(α) 101.1(ß) 103.0(ß) 92.2(α) 90.5(α) 96.1(ß)		3 %	6 %[e] **24**(34 %) **24**(8 %)
33	4	**34** (93 %)	93.7(α) 93.8(α) 93.3(α)			7 %[e]

[a] Figures denote percentage amount of the crude reaction mixtures as determined from the peak heights in the interval δ 90.0-105.0 ppm in the ^{13}C NMR spectra. The spectra were recorded at 125.76 MHz at 300 K in $CDCl_3$. [b] D denotes glycosyl donor. [c] A denotes glycosyl acceptor. [d] From hydrolysis of **1**. [e] Unidentified material.

Table 2. The compositions of three *O*-deprotected product mixtures, determined by ^{13}C NMR spectroscopy (accuracy ± 2.5 %).[a]

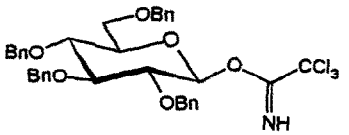
Synthesis D[b] A[c]	Deprotected products	^{13}C NMR (δ anomeric C)	Others
1 3	**11** (33 %) **12** (21 %) **13** (22 %) **14** (10 %)	94.1(α) 93.9(α) 104.3(ß) 100.2(α) 101.9(α) 103.5(ß) 100.6(ß) 100.0(ß)	Gal*p* (2 %) Glc*p* (2 %)[d] (5 %)[e] (5 %)[f]
1 4	**17** (45 %) **18** (28 %)	95.8(α) 94.2(α) 102.9(ß) 102.3(α) 101.3 100.2	Glc*p* (2 %)[d] Man*p* (3 %) (8 %)[f] (6 %)[e] α-Man α-Man (5 %) (3 %)[f]
1 24	**29** (33 %) **30,31**(39 %)[g] **32** (16 %)	101.2(α) 101.2(α) 95.7(α) 98.1(ß) 103.4(ß) 103.4(ß)	(2 %)[e] Glc*p* (5 %) (5 %)[f]

[a] Figures denote percentage amount of the crude reaction mixtures as determined from the peak heights in the interval δ 90.0 - 105 ppm in the ^{13}C NMR spectra. The spectra were recorded at 125.76 MHz at 300 K in D_2O. [b] D denotes glycosyl donor. [c] A denotes glycosyl acceptor. [d] From hydrolysis of **1**. [e] α,α-Trehalose. [f] Unidentified material. [g] **30** and **31** are enantiomers.

The unprotected sugar **23** was obtained pure by *O*-debenzoylation followed by VLC and subsequent *O*-debenzylation (5 % yield calc. from 19). For **23**, the anomeric protons resonated at δ 5.40 (d, *J* 3.4 Hz), 4.32 (d, 8.6 Hz), and 4.80 (d, *J* 8.5 Hz), respectively, whilst the anomeric carbons resonated at δ 100.3, 99.9, and 99.7, respectively. Compounds **22** and **23** are stereoisomers of the recently reported, naturally occurring trisaccharide bemisiose (the α,α,α isomer).[10] Other trehalose-containing trisaccharides have been reported previously,[11-14], but **22** and **23** have not been described earlier.

Glycosylation of 2,3,4,6-tetra-*O*-benzoyl-L-glucose (**24**), obtained from hydrolysis of the corresponding bromide, afforded a complex product mixture containing the four products **25-28** in mixture with unreacted material (Table 1).

To demonstrate the generality of the glycosylation reaction futher, acceptor **4** was glycosylated with *O*-(2,3,4,6-tetra-*O*-benzyl-ß-D-galactopyranosyl)-trichloroacetimidate[15] (**33**). This afforded the α,α-configurated product **34**, constituting 93 % of the crude product mixture (Table 1). Compound **34** was fully characterized by ^1H and ^{13}C NMR spectroscopy (Tables 3-5). The anomeric protons resonated at δ 5.31 (d, *J* 3.6 Hz) and 5.25 (d, *J* 1.4 Hz), respectively, whilst the anomeric carbons resonated at δ 93.7 and 93.8, respectively. Since **34** could not be obtained pure by application to silica gel chomatography, it was converted



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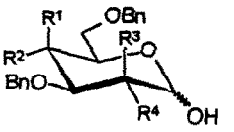
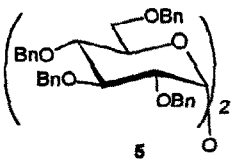


2 R^1, R^3 = H ; R^2, R^4 = OBn
3 R^1, R^4 = OBn ; R^2, R^3 = H
4 R^1, R^4 = H ; R^2, R^3 = OBn



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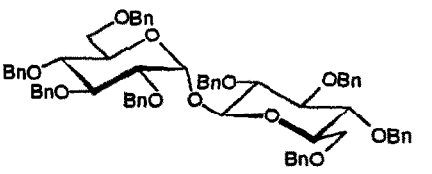

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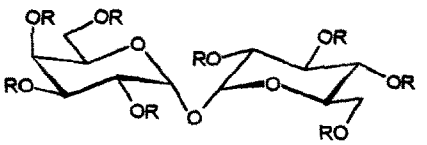

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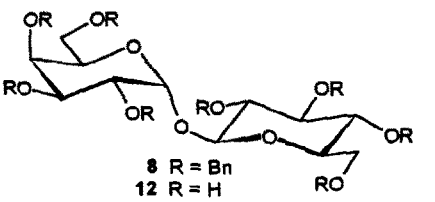
11 R = H



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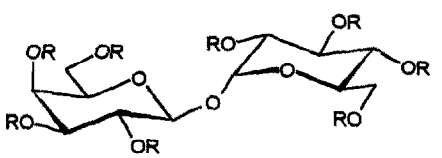

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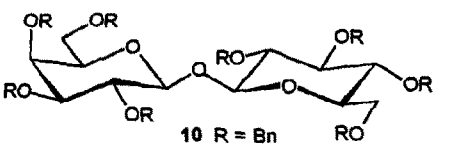
13 R = H



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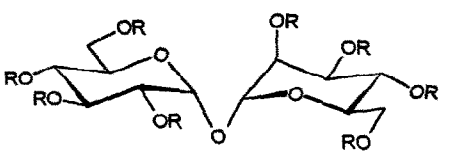
14 R = H



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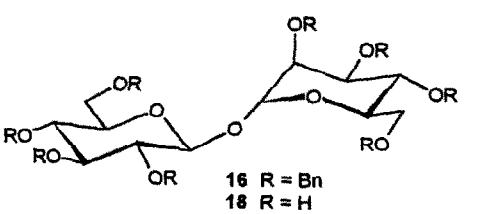

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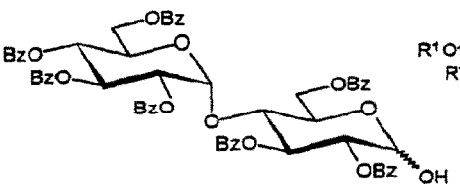
18 R = H



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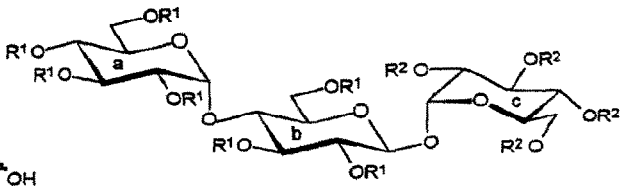
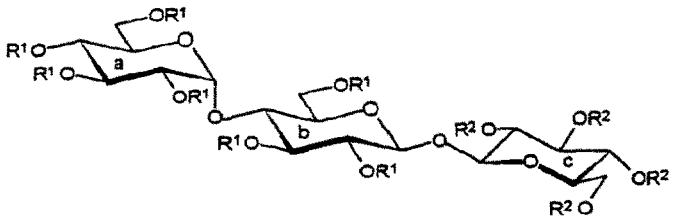
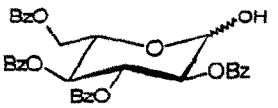


20 R^1 = Bz ; R^2 = Bn
22 R^1 = R^2 = H



21 R^1 = Bz ; R^2 = Bn
23 R^1 = R^2 = H



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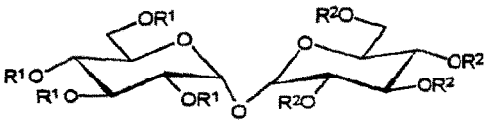
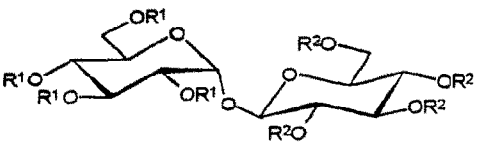
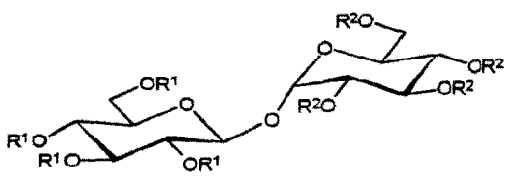


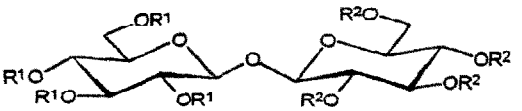
25 R^1 = Bn ; R^2 = Bz
29 R^1, R^2 = H



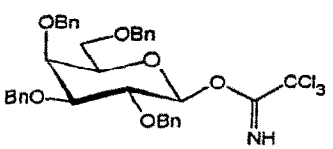
26 R^1 = Bn ; R^2 = Bz
30 R^1, R^2 = H



27 R^1 = Bn , R^2 = Bz
31 R^1, R^2 = H



28 R^1 = Bn ; R^2 = Bz
32 R^1, R^2 = H



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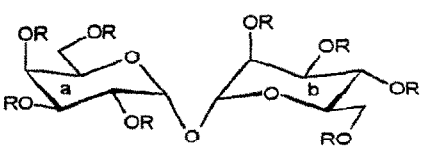

34 R = Bn
35 R = Ac
36 R = H

Table 3. ^{13}C NMR chemical shift data for compounds 20-23 and 34-36.

Compound	(unit)	C-1	C-2	C-3	C-4	C-5	C-6	$PhCH_2$-
20[a]	a	96.41	70.89	69.90	69.11	69.11	63.47	75.45, 74.80, 73.30, 72.30
	b	100.69	72.18	74.72	73.04	73.14	62.49	
	c	98.70	79.14	81.58	72.25	71.45	68.02	
21[a]	a	96.25	71.06	69.96	69.17	69.17	63.46	75.47, 74.87, 74.62, 73.47
	b	96.49	72.28	75.32	72.90	72.98	62.59	
	c	99.35	81.40	84.28	77.47	75.15	68.88	
22[b]	a	100.37	72.46	73.62	70.19	73.62	61.29	
	b	103.56	73.81	76.63	77.31	75.58	61.44	
	c	101.01	72.23	72.23	70.19	73.49	61.29	
23[b]	a	100.34	72.44	73.62	70.13	75.48	61.28	
	b	99.90	73.33	76.98	77.33	73.51	61.44	
	c	99.74	73.42	76.51	70.30	76.87	61.44	
34[a]	a	93.69	74.86	78.35	74.86	70.04	69.00	72.1-75.6 (8 C)
	b	93.78	74.86	79.35	75.63	72.30	69.00	
35[a]	a	92.05	66.99	68.47	67.70	67.48	61.42	
	b	93.00	69.14	67.20	65.98	69.36	62.26	
36[b]	a	94.47	68.63	71.04	70.09	72.42	61.73	
	b	95.79	70.81	69.86	67.53	74.01	62.06	

Spectra were recorded at 125.76 MHz at 300 K in [a] $CDCl_3$, [b] D_2O.

Table 4. ^{1}H NMR chemical shift data for compounds 20-23 and 34-36.

Compound	(unit)	H-1	H-2	H-3	H-4	H-5	H-6A	H-6B	CH_3CO-	Arom.	$PhCH_2$-
20[a]	a	5.79(d)	5.30(dd)	6.13(t)	5.70(t)	4.47(m)	4.89(dd)	4.70(dd)		7.0-8.13	
	b	5.06(d)	5.44(dd)	5.82(t)	4.50(t)	4.10(m)	4.32	4.47			
	c	5.05(d)	3.43(dd)	3.93(t)	3.64(t)	4.03(m)	3.64(d)	3.55(dd)			4.2-4.8
21[a]	a	5.85(d)	5.34(dd)	6.19(t)	5.75(t)	4.55(m)	4.87(dd)	4.99(dd)		7.27-8.20	
	b	5.34(d)	5.53(dd)	5.87(t)	4.78(m)	4.18(m)	4.33	4.4			
	c	4.92(d)	3.46(t)	3.66(t)	3.55(t)	3.50(m)	3.63	3.72			4.5-5.1
22[b]	a	5.40(d)	3.57	3.71	3.40(t)	3.72	3.7-3.95	3.7-3.95			
	b	4.62(d)	3.43(t)	3.80	3.64	3.76	3.7-3.95	3.7-3.95			
	c	5.22(d)	3.58	3.59	3.43(t)	3.43)	7.7-3.95	3.7-3.95			
23[b]	a	5.40(d)	3.57(dd)	3.65(t)	3.40(t)	3.72(m)	3.7-3.95	3.7-3.95			
	b	4.82(d)	3.38(t)	3.80	3.65(t)	3.59(m)	3.7-3.95	3.7-3.95			
	c	4.80(d)	3.36(t)	3.50(t)	3.40(t)	3.47(m)	3.76	3.84			
34[a]	a	5.31(d)	4.09(dd)	3.80(dd)	3.98(d)	3.76(t)	3.49(dd)	3.57(dd)		7.25-7.45	4.5-5.0
	b	5.25(d)	3.64	3.96(dd)	4.09(t)	4.14(m)	3.64(dd)	3.72(dd)			
35[a]	a	5.38(d)	5.25(dd)	5.36(dd)	5.52(d)	4.28(t)	4.13(dd)	4.05(m)	2.0-2.17		
	b	5.12(s)	5.24	5.32	5.22	3.9	4.20(dd)	4.10(dd)			
36[b]	a	5.19(d)	3.87	3.92	3.98	3.89	3.74	3.86			
	b	5.11(s)	3.98	3.94	3.66(t)	3.77	3.7-3.8	3.7-3.8			

Spectra were recorded at 500.14 MHz at 300 K in [a] $CDCl_3$, [b] D_2O.

Table 5. ^{1}H NMR coupling constants for compounds 20-23 and 34-36.

Compound	(unit)	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6A}$	$J_{5,6B}$	$J_{6A,6B}$
20[a]	a	3.9	10.5	10.1	9.8	2.1	4.2	12.3
	b	7.7	9.4	9.2	9.2	3.6	3.1	12.2
	c	3.4	9.6	9.4	9.9	-	1.6	10.8
21[a]	a	3.9	10.4	10.0	9.8	3.2	-	12.1
	b	7.7	8.5	9.0	10.6	3.6	-	12.4
	c	7.7	8.7	8.7	9.7	4.4	-	-
22[b]	a	3.2	-	9.1	9.1	-	-	-
	b	7.8	9.1	-	-	-	-	-
	c	3.1	-	9.1	9.1	-	-	-
23[b]	a	3.4	9.8	8.5	10.0	-	-	-
	b	8.6	8.8	8.5	8.5	-	-	-
	c	8.5	8.8	9.1	10.0	-	-	-
34[a]	a	3.6	9.8	2.7	-	6.0	7.1	9.2
	b	1.4	3.1	9.1	10.1	1.4	4.5	10.8
35[a]	a	3.7	10.0	2.9	-	6.4	6.4	11.4
	b	-	1.4	-	9.3	6.7	-	12.2
36[b]	a	3.0	-	-	-	-	-	-
	b	-	-	9.4	9.4	-	-	-

Spectra were recorded at 500.14 MHz at 300 K in [a] $CDCl_3$, [b] D_2O.

into the corresponding octa-acetate **35**, which was obtained pure in 49 % yield after chromatographic purification and crystallization. For **35**, the anomeric protons resonated at δ 5.38 (d, J 3.7 Hz) and 5.12 (s), respectively, whilst the anomeric carbons resonated at δ 92.05 and 93.0, respectively (Tables 3-5). *O*-Deacetylation afforded the unprotected disaccharide **36** in 88 % yield. The anomeric protons resonated at δ 5.19 (d, J 3.0 Hz) and 5.11 (s), respectively, whilst the anomeric carbons resonated at δ 94.5 and 95.8, respectively.

Finally, the *galacto*-imidate **33** was reacted with the *gluco*-acceptor **2**. This afforded (Table 1) the four theoretical products **7-10**, with, interestingly, the α,α-configurated **7** as the major product (57 %). Comparing this result with the coupling of **1** with **3**, it appears that imidate **33** (*galacto*) gives higher α/β ratio than imidate **1** (*gluco*). This tendency has been observed previously,[15] but is very convincingly demonstrated with the results presented here, since the products from the two glycosylation reactions are identical. Futhermore, the results suggest the product mixtures to have *kinetic* distributions, in general.

In conclusion, it has been demonstrated that trichloroacetimidates can be used successfully as glycosyl donors in reactions leading to unsymmetrical trehalose derivatives. The glycosylation reactions described here are less hampered by the formation of dimerization by-products than the Koenig-Knorr reactions previously described.[4] Thus, it should be possible to separate and isolate the individual product isomers in higher yields. Furthermore, it has been shown that by proper choice of reactants, it is possible to synthesize trehalose analogues stereoselectively to some extend. The synthesis of the two trisaccharides **22** and **23** has demonstrated that trichloroacetimidates can glycosylate even very unreactive glycosyl acceptors in reactions affording trehalose-containing oligosaccharide derivatives.

EXPERIMENTAL

General methods. Analytical grade solvents were dried over molecular sieves (pyridine was destilled from KOH and CH_2Cl_2 from P_2O_5). 2,3,4,6-Tetra-*O*-benzyl-D-glucose, 2,3,4,6-tetra-*O*-benzyl-D-galactose and 2,3,4,6-tetra-*O*-benzyl-D-mannose was purchased from Janssen. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter, TLC was performed on Merck coated aluminium foil plates F_{254}, vacuum liquid chromatography (VLC) was performed on silica Gel 60 H (E. Merck). ^{1}H NMR spectra were recorded on a Bruker AM500 instrument. ^{13}C NMR spectra were recorded at 300 K in $CDCl_3$ relative to $CDCl_3$ (δ 77.0) or in D_2O with external reference 1,4-dioxane (δ 67.4). ^{1}H NMR spectra were recorded at 300 K in $CDCl_3$ relative to Me_4Si (δ 0.000), or in D_2O relative to acetone (δ 2.225). Assignment of ^{1}H NMR spectra was achived by 2-D homonuclear correlation spectroscopy and of ^{13}C NMR spectra by heteronuclear 2-D correlation spectroscopy.

Glycosylation procedure using AgOTf as the promotor.[16] A mixture of 2,3,4,6-tetra-*O*-benzyl-D-glucose (**2**, 1.00 g, 1.85 mmol, α/ß ratio 5:1), *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-trichloroacetimidate[6] (**1**, 1.65 g, 2.41 mmol, α/ß ratio 1:10) and AgOTf (0.69 g, 2.41 mmol) was dried in a three-necked round-bottomed flask in darkness. The flask was opened to argon, and dry CH_2Cl_2 (20 ml) was added. The mixture was stirred at room temperature for 48 h, then filtered through celite, washed with satd. $NaHCO_3$, and water, dried ($MgSO_4$), and concentrated to afford a colourless syrup (2.65 g), which was characterized by ^{13}C NMR spectroscopy. Subjection to silica gel chromatography (eluent: pet. ether-EtOAc 8:2) afforded a 2:1 mixture (1.62 g, 82 %) of **5** and **6**.

General glycosylation procedure using TMSOTf as the catalyst. A mixture of TMSOTf (6.25 μl) and dry CH_2Cl_2 (0.5 ml) was quickly added to a mixture of 2,3,4,6-tetra-*O*-benzyl-D-glucose (**2**, 0.36 g, 0.67 mmol, α/ß ratio 5:1), *O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-trichloroacetimidate[6] (**1**, 0.50 g, 0.73 mmol, α/ß ratio 1:10), dry CH_2Cl_2 (5 ml) and molecular sieves (4 Å). The mixture was stirred under argon atmosphere at room temperature for 1.5 h. NEt_3 (one drop) was added and the mixture was filtered, concentrated to dryness, and concentrated twice from toluene under reduced pressure to afford a pale yellow syrup (0.86 g), which, estimated from a ^{13}C NMR spectrum, was **5** (39 %), **6** (52 %) and 2,3,4,6-tetra-*O*-benzyl-D-glucose (9 %). This procedure was used in all the glycosylation reactions described below.

Glycosylation of 2,3,4,6-tetra-*O*-benzyl-D-galactose (3) with 1. 2,3,4,6-Tetra-*O*-benzyl-D-galactose (**3**, 0.50 g, 0.93 mmol, α/ß ratio 3:1) was glycosylated with **1** (0.67 g, 1.02 mmol, α/ß ratio 1:10), and worked up as described above to afford a syrup (1.2 g), which was characterized by ^{13}C NMR spectroscopy (data are presented in Table 1). Attempts to separate the products by HPLC were unsuccessful. An aliqout of the syrup (0.5 g), MeOH (50 ml), AcOH (5 ml) and palladium-on-charcoal (10 %, 0.50 g) was hydrogenated at atmospheric pressure for 24 h. The mixture was filtered through celite, concentrated twice from toluene, and lyophilized from water to afford a yellow solid (0.15 g), which was characterized by ^{13}C NMR spectroscopy (data are presented in Table 2).

Glycosylation of 2,3,4,6-tetra-*O*-benzyl-D-mannose (4) with 1. 2,3,4,6-Tetra-*O*-benzyl-D-mannose (**4**, 0.95 g, 1.76 mmol, α/ß ratio 4:1) was glycosylated with **1** (1.32 g, 1.93 mmol, α/ß ratio 1:10), and worked up as above to afford a syrup (2.3 g), which was characterized by ^{13}C NMR spectroscopy (data are presented in Table 1). An aliqout of the syrup (0.5 g), MeOH (50 ml), AcOH (5 ml) and palladium-on-charcoal (10 %, 0.50 g) was hydrogenated at atmospheric pressure for 24 h. The mixture was filtered through celite, concentrated twice from toluene, and lyophilized from water to afford a yellow solid (0.20 g), which was characterized by ^{13}C NMR spectroscopy (data are presented in Table 2).

Glycosylation of 2,3,4,6-tetra-*O*-benzoyl-L-glucose (24) with 1. 2,3,4,6-Tetra-*O*-benzoyl-L-glucose

(**24**, 0.8 g, 1.48 mmol, α/ß ratio 4.4:1) was glycosylated with **1** (1.12 g, 1.63 mmol, α/ß ratio 1:10), and worked up as above to afford a syrup (1.91 g), which was characterized by ^{13}C NMR spectroscopy (data are presented in Table 1). An *O*-deprotected aliquot of the product mixture was characterized by ^{13}C NMR spectroscopy (data are presented in Table 2).

Glycosylation of 2,3,4,6-tetra-*O*-benzyl-D-mannose (4) with 33. 2,3,4,6-Tetra-*O*-benzyl-D-mannose (**4**, 0.33 g, 0.61 mmol, α/ß ratio 4:1) was glycosylated with *O*-(2,3,4,6-tetra-*O*-benzyl-ß-D-galactopyranosyl)-trichloroacetimidate[15] (**33**, 0.46 g, 0.67 mmol), and worked up as described above to afford a syrup (1.2 g), which was characterized by ^{13}C NMR spectroscopy (data presented in Table 1). The mixture was subjected to silica gel chromatography (eluent CH_2Cl_2 - EtOAc 24:1) to afford **34** (0.37 g, 57 %) ca. 95 % pure. Further attempts to purify **34** were unsuccessful.
^1H and ^{13}C NMR spectroscopy data for **34** are presented in Tables 3-5.

Glycosylation of 2,3,4,6-tetra-*O*-benzyl-D-glucose (2) with 33. 2,3,4,6-Tetra-*O*-benzyl-D-glucose (**2**, 0.25 g, 0.46 mmol, α/ß ratio 5:1) was glycosylated with *O*-(2,3,4,6-tetra-*O*-benzyl-ß-D-galactopyranosyl)-trichloroacetimidate[15] (**33**, 0.34 g, 0.49 mmol), and worked up as above to afford a syrup (0.6 g), which was characterized by ^{13}C NMR spectroscopy (data are presented in Table 1).

2,3,6-Tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl)-ß-D-glucopyranosyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (20). A mixture of TMSOTf (12.5 μl) and dry CH_2Cl_2 (1.0 ml) was quickly added to a mixture of **19** (1.36 g, 1.28 mmol), **1** (0.96 g, 1.4 mmol), dry CH_2Cl_2 (5 ml) and molecular sieves (4 Å) under argon at room temperature. The mixture was stirred for 1.5 h, then NEt_3 (two drops) was added. The mixture was filtered and concentrated twice with toluene to afford a syrup (2.1 g, 100 %), which was characterized by ^{13}C NMR spectroscopy (data are presented in Table 1). Separation by silica gel chromatography (eluent: petroleum ether-EtOAc-toluene 7:3:5) gave a fraction of impure 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl)-ß-D-glucopyranosyl 2,3,4,6-tetra-*O*-benzyl-ß-D-glucopyranoside (**21**, 0.18 g, 8 %), a fraction of unreacted α-**19** (0.42 g, ^{13}C NMR: δ 96.6, 90.2), a fraction containing a 1:1 mixture of **20** and **21** (0.18 g, 8 %), and the pure *title compound* **20** (0.17 g, 23 %), mp 55-57 °C, $[\alpha]_D^{22}$ +40.1 (*c* 0.6, chloroform).Anal. Calcd. for $C_{95}H_{84}O_{23}$: C 71.59; H 5.31. Found: C 71.62; H 5.46. Further attempts to purify **21**, containing 10 % of both **19** and **20**, by VLC were unsuccessful. ^1H and ^{13}C NMR spectroscopy data for compounds **20** and **21** are presented in Tables 3-5.

4-*O*-(α-D-Glucopyranosyl)-ß-D-glucopyranosyl α-D-glucopyranoside (22). Compound **20** (0.08 g, 0.05 mmol) was *O*-deproteced as previously described[4] and further purified on a Sephadex G-10 column (eluent: water) to afford the *title compound* (0.024 g, 90 %) as a hygroscopic material with mp 58-61 °C, $[\alpha]_D^{22}$ +111 (*c* 0.2, water). ^1H and ^{13}C NMR spectroscopy data for **22** are presented in Tables 3-5.

4-*O*-(α-D-Glucopyranosyl)-ß-D-glucopyranosyl ß-D-glucopyranoside (23). Impure **21** (0.18 g) was *O*-debenzoylated as previously described.[4] The resulting syrup was subjected to silica gel chromatography (eluent: MeOH-EtOAc 1:6). The relevant fractions were evaporated, and the resulting syrup was subsequently *O*-debenzylated and worked-up as previously described[4] to afford the *title compound* (0.034 g, 5 % from **19**), mp 220 °C (dec), $[\alpha]_D^{22}$ +34 (*c* 0.3, water). ^1H and ^{13}C NMR spectroscopy data for **23** are presented in Tables 3-5.

2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (35). Impure **34** (0.25 g) was *O*-deprotected as described previously[4] to afford a solid (0.07 g) which was acetylated with acetic anhydride in pyridine to afford, after work-up,[4] a syrup (0.14 g, 99 %). Separation by to silica gel chromatography followed by crystallization from Et_2O-pentane afforded the *title compound* (0.7 g, 49 %), mp 70-72 °C, $[\alpha]_D^{22}$ +98 (*c* 0.2, chloroform).
Anal. Calcd. for $C_{28}H_{38}O_{19}$: C 49.55; H 5.64. Found: C 49.13; H 5.69.
^1H and ^{13}C NMR spectroscopy data for **35** are presented in Tables 3-5.

α-D-Galactopyranosyl α-D-mannopyranoside (36). Compound **35** (0.05 g, 0.08 mmol) was *O*-deacetylated with sodium methoxide[4] to afford the *title compound* **36** (0.02 g) in 88 % yield, mp 143-145 °C, $[\alpha]_D^{22}$ +161 (*c* 0.2, water). ^1H and ^{13}C NMR spectroscopy data for **36** are presented in Tables 3-5.

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REFERENCES

1. B. Roser, *Trends Food Sci. & Technol.*, *2(7)*, 166 (1991).
2. J.H. Crowe, F.A. Hoekstra, and L.M. Crowe, *Ann. Rev. Physiol.*, *54*, 579 (1992).
3. J.H. Crowe, L.M. Crowe, and D. Chapman, *Science*, *223*, 701 (1984).
4. T.E.C.L. Rønnow, M. Meldal, and K. Bock, *J. Carbohydr. Chem.*, Submitted (1994).
5. R.R. Schmidt, *Angew. Chem.*, *98*, 213 (1986).
6. R.R. Schmidt, J. Michel, and M. Roos, *Liebigs Ann. Chem.*, 1343 (1984).

7. A.A. Pavia, J.-M. Rocheville, and S.N. Ung, *Carbohydr. Res.*, *79*, 79 (1980).
8. S. Koto, S. Inada, and S. Zen, *Chem. Lett.*, 403 (1980).
9. F.W. Lichtenthaler, E. Kaji, and S. Weprek, *J. Org. Chem.*, *50*, 3505 (1985).
10. D.L. Hendrix and Y.-a. Wei, *Carbohydr. Res.*, *253*, 329 (1994).
11. S. Iwahara, K. Takegawa, K. Kawaguchi, and G. Okamoto, *Biosci. Biotech. Biochem.*, *57*, 1220 (1993).
12. S. Koto, K. Yago, S. Zen, F. Tomonaga, and S. Shimada, *Bull. Chem. Soc. Jap.*, *59*, 411 (1986).
13. H.P. Wessel, *Tetrahedron Lett.*, *31*, 6863 (1990).
14. K. Ajisaka and H. Fujimoto, *Carbohydr. Res.*, *199*, 227 (1990).
15. B. Wegmann and R.R. Schmidt, *Carbohydr. Res.*, *6*, 357 (1987).
16. S.P. Douglas, D.M. Whitfield, and J.J. Krepinsky, *J. Carbohydr. Chem.*, *12*, 131 (1993).

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