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Mild Stereoselective Syntheses of Thioglycosides Under PTC Conditions and their Use as Active and Latent Glycosyl Donors

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Abstract: Mild and stereoselective arylthio glycoside syntheses were accomplished by inversion of configuration of glycosyl halides under phase transfer catalyzed conditions. Under such conditions, aryl α–thiosialosides having electron donating and withdrawing substituents were evaluated as active and latent thioglycosyl donors. A sialyl-α-(2→6)-galactoside was prepared in good yield using the above strategy.

Sialic acids constitute a family of about 30 derivatives of neuraminic acid, the most ubiquitous member of which being a nine carbon amino sugar called N-acetylneuraminic acid.[1] In most cases, sialic acids occupy the penultimate non-reducing end of glycolipids and glycoproteins. Their involvement in a wide number of biological phenomena and their receptor binding properties toward human influenza virus hemagglutinin (HA) have been well documented.[2] Recent interests for the synthesis of multivalent influenza virus HA inhibitors,[3,4] together with the rationale design of suitable α-stereoselective sialosyl donors in blockwise oligosaccharide synthesis prompted widespread research activities toward this family of carbohydrate derivatives.[5]

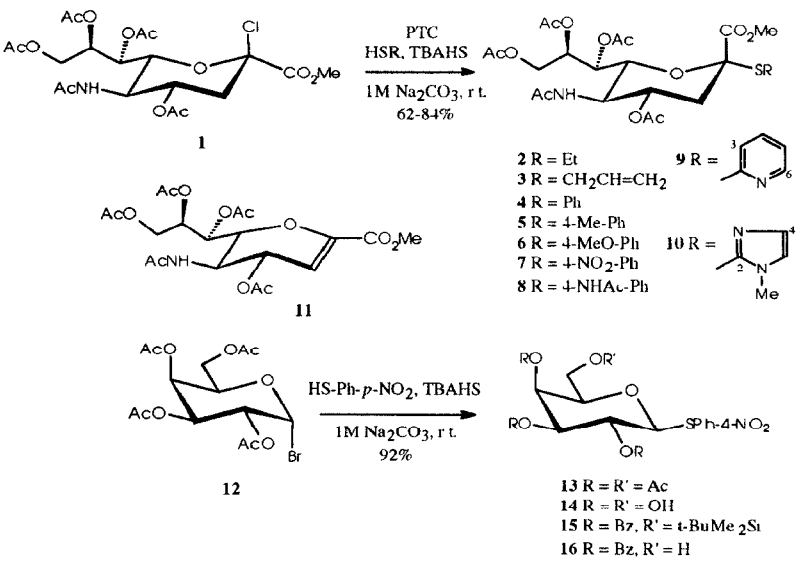
Hasegawa and his co-workers have previously demonstrated the usefulness of anomeric mixtures of peracetylated methyl and phenyl 2-thio-α/β-sialosides as stereoselective thioglycosyl donors in complex sialyloligosaccharide syntheses.[6] These observations and the fact that human influenza A and B viruses also possess, beside their HA properties, α-sialidase (neuraminidase) activities,[2] prompted us to prepare a number of other α-thiosialoside derivatives. These compounds were initially synthesized to serve as powerful glycosylating reagents and as potential sialidase inhibitors. Moreover, by a careful choice of the thiolated aglycon moieties, it became possible to design a new glycosylation strategy in which the thioglycosides could be used as "active and latent" thioglycosyl donors.[7]

This paper describes further applications of stereoselective phase transfer catalyzed (PTC) syntheses of glycosyl derivatives as applied to aryl thio-sialosides and galactosides. It will also illustrate an example of the "active and latent" principle in which an active 4-methoxyphenylthio sialoside was used as an efficient glycosyl donor while a latent (temporary inactive) 4-nitrophenylthio galactoside having a primary hydroxyl group was used as glycosyl acceptor.

RESULTS AND DISCUSSION

We have previously demonstrated that phase transfer catalysis (PTC) could be used as a general and stereoselective entry into a wide range of glycosyl derivatives including O-[8] and S-aryl[9] glycosides and disaccharides, glycosyl azides,[10] esters,[11] phosphates[12] and xanthates.[13] In all the above transformations, the phase transfer catalyzed nucleophilic displacements of glycosyl halides occurred with complete anomeric inversion. Thus, 1,2-*cis* acylated glycosyl halides provided 1,2-*trans* glycosyl derivatives. Hydrolysis and, in few cases dehydrohalogenations accounted for the only minor by-products obtained. In the case of the sialic acid family, the homologous β-glycosyl chloride (β-acetochloroneuraminic acid, **1**),[14] there is no substituents to participate in any anchimeric fashion which might help to provide nucleophilic displacement with inversion of configuration. In spite of this, previous applications of the PTC conditions to **1** have also afforded derivatives with inverted α-anomeric configurations.[8,10,11,13]

Therefore, treatment of freshly prepared β-acetochloroneuraminic acid (**1**) (Scheme 1) at room temperature with tetra-*n*-butylammonium hydrogen sulfate (TBAHS, 1 equivalent) as catalyst in either methylene chloride or ethyl acetate with arylthiols (1.5-3 eq.) and a 1M solution of Na_2CO_3 afforded the α-thiosialosides in 62-84% yields after purification by silica gel column chromatography (Table 1). Again, dehydrochlorination (**11**) and hydrolysis by-products were formed in minor quantities.



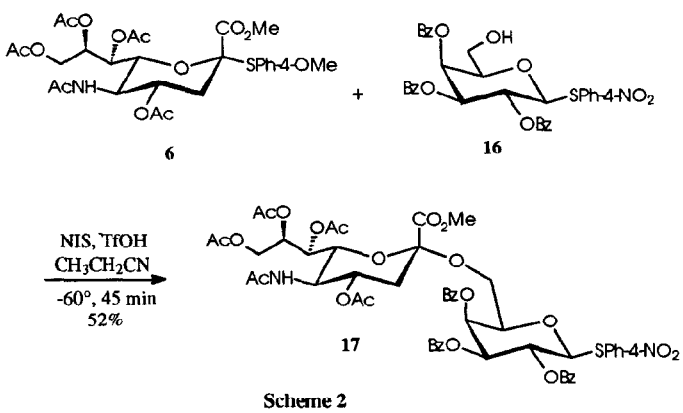
Scheme 1. Phase transfer catalyzed syntheses of thioglycosides

All the above reactions were essentially completed within <1 h. The reactions occurred with complete anomeric inversions as judged by thin layer chromatography (TLC), ^{1}H- and ^{13}C-NMR spectra of the crude reaction mixtures. The use of ethyl acetate had some advantages over methylene chloride since, in few control

experiments, products resulting from nucleophilic displacements of one or two of the chloride anions from methylene chloride resulted in the formation of chlorophenylthio- and bis(phenylthio)-methane ($PhSCH_2Cl$ and $(PhS)_2CH_2$) as shown by ^1H-, ^{13}C-NMR and mass spectrometry.

Some of the above α-sialosides are known compounds.[15] However, they were obtained under more drastic PTC conditions using refluxing chloroform and large excess of triethylbenzylammonium chloride used as catalyst. For those compounds, physical data (Table 1) were in good agreement with reported values. The fully assigned (HETCOR, COSY) ^1H- and ^{13}C-NMR spectroscopic data (Table 2 and 3) also confirmed their anomeric configurations. Although these sialic acid derivatives lack anomeric protons, the anomeric configurations were inferred on the basis of the characteristic downfield shifts of their H-3e signals relative to those of the β-anomers.[6] The H-3e signals in α-sialoside anomers are usually observed ~0.5 ppm downfield to those of the β-anomers. This assignment was also confirmed by the chemical shifts of H-4 which are shifted upfield in the α-anomers. Moreover, the coupling constants of $J_{7,8}$ (6.9-9.9 Hz) in α-sialosides are usually larger than those in β-sialosides (~2.4± 0.3 Hz).

For the synthesis of Neu5Ac-α-(2 →6)-β-D-Gal disaccharide in a form suitable for further conjugation to macromolecules,[3] the required "latent" thioglycosyl acceptor, 4-nitrophenyl 1-thio-β-D-galactopyranoside (**16**), was prepared under the same mild PTC conditions described above. Thus, α-acetobromogalactose (**12**) was transformed into peracetylated 4-nitrophenyl 1-thio-β-D-galactopyranoside (**13**) in 92% yield (Scheme 1) when EtOAc and 1M Na_2CO_3 were used. The ^1H-NMR spectra of **13** showed the anomeric proton at 4.83 ppm with a characteristic $^3J_{1,2}$ of 9.8 Hz. Zemplén de-O-acetylation (NaOMe/MeOH) of **13** provided **14** quantitatively. Selective protection of the primary hydroxyl group in **14** was achieved with t-butyldimethylsilyl chloride in pyridine followed by treatment with benzoyl chloride to afford **15** in 86% yield. Removal of the silyl ether was accomplished with 3% methanolic HCl to provide **16** in 91% yield after silica gel column chromatography.



Scheme 2

The usefulness of some of the α-thiosialosides **2-10** to act as efficient glycosyl donors was previously demonstrated.[7] Interestingly, thio-pyridyl (**9**) and -N-methylimidazolyl (**10**) sialosides failed to react with any glycosyl acceptors when either methyl iodide or N-bromosuccinimide were used as promotors. In the present situation, when the "active" (reactive) arylthio sialosides **4** (SPh), **5** (SPh-4-Me) and **6** (SPh-4-OMe) were used

as glycosyl donors and the "latent" (temporary inactive) 4-nitrophenylthio galactoside (**16**) as acceptor using N-iodosuccinimide (NIS) and triflic acid (TfOH) as promotors, the resulting disaccharide **17** was obtained in good yield (52%) and α-stereoselectivity (Scheme 2). The "latent" 4-nitrophenylthio sialoside **7** failed to react with **16** under the same conditions.

To further demonstrate the "active" and "latent" thioglycoside strategy, the previously inert sialosyl donor **7**, having a para-nitrophenyl electron withdrawing group in the aglycon moiety, was transformed into an "active" donor **8**, bearing a para-N-acetamidophenyl electron donating group. Thus, the nitro group in **7** was reduced with tin (II) chloride in refluxing ethanol[7] and the resulting para-aminophenylthio sialoside was immediately N-acetylated (pyridine, acetic anhydride) to provide the new sialosyl donor **8** in 87% overall yield. Preliminary results with **8** indicated its usefulness in oligosaccharide synthesis.

Table 1. Results from the synthesis of α-Thiosialosides **2-10** from ß-acetochloroneuraminic acid **1** under PTC conditions.

					Combustion analyses							
					Calcd (%)				Found (%)			
Compd	yield[a]	mp (°C)	$[\alpha]_D$[b]	Formula	C	H	N	S	C	H	N	S
2	62%	83-84	+31.4	$C_{22}H_{33}NO_{12}S$	49.34	6.21	2.62	5.99	49.54	6.19	2.57	5.93
3	84%	108.5-111	+38.9	$C_{23}H_{33}NO_{12}S$	50.45	6.07	2.56	-	50.65	5.97	2.38	-
4[c]	80%	141-142	+20.9	$C_{26}H_{31}NO_{12}S$	53.51	5.70	2.40	5.49	53.24	5.84	2.24	5.26
5	70%	114-115	+24.0	$C_{27}H_{35}NO_{12}S$	54.26	5.90	2.34	5.36	54.43	6.02	2.40	5.14
6	81%	136-137	+20.7	$C_{27}H_{35}NO_{13}S$	52.85	5.75	2.28	5.23	52.73	5.75	2.19	5.37
7[c]	81%	170-171	+18.2	$C_{26}H_{32}N_2O_{14}S$	49.68	5.13	4.46	5.10	49.66	5.38	4.46	5.24
9[c]	69%	150-152	+29.0	$C_{25}H_{32}N_2O_{12}S$	51.36	5.52	4.79	5.48	51.55	5.69	4.59	5.29
10	68%	140-142	+17.7	$C_{24}H_{33}N_3O_{12}S$	49.06	5.66	7.15	5.46	49.26	5.91	6.91	5.23

[a] Isolated, crystalline material.

[b] For c=1.0 in $CHCl_3$ at 23°C.

[c] Known compounds for which the reported values agreed with the literature.[15]

EXPERIMENTAL

General procedures

Melting points were determined on a Gallenkamp apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AMX 500 instrument. Optical rotations were measured on a Perkin Elmer 241 polarimeter and were run at 23°C for 1% solutions in chloroform unless stated otherwise. Elemental

Table 2. ^{1}H NMR (500 MHz) Chemical Shifts δ and Coupling Constant Data J (Hz) of α-Thiosialosides **2-10.**[a]

Compd	H-3a	H-3e	H-4	H-5	H-6	H-7	H-8	H-9a	H-9b	NH	MeO	NAc	Aglycon H's		
	($J_{3a,3e}$, $J_{3a,4}$)	($J_{3e,4}$)	($J_{4,5}$)	($J_{5,6}$)	($J_{6,7}$)	($J_{7,8}$)	($J_{8,9a}$)	($J_{9a,9b}$)	($J_{8,9b}$)	($J_{5,NH}$)			H-o	H-m	H-p[b]
2	1.96 (*12.8, 11.9*)	2.70 (*4.7*)	4.84 (*10.4*)	4.03 (*10.7*)	3.81 (*2.1*)	5.30 (*8.3*)	5.36 (*2.6*)	4.29 (*12.4*)	4.09 (*4.9*)	5.13 (*10.3*)	3.78	1.86	1.17 (CH_3)	2.76 (CH_2)	J_{vic} 7.5
3	1.96 (*12.7, 11.7*)	2.69 (*4.6*)	4.83 (*10.4*)	4.02 (*10.7*)	3.84 (*2.2*)	5.29 (*8.2*)	5.37 (*2.7*)	4.29 (*12.5*)	4.09 (*5.4*)	5.19 (*9.9*)	3.76	1.84	3.26, 3.35[c] (SCH_2)	5.73[d] (CH=)	5.06, 5.18[e] (=CH_2)
4	2.00 (*12.9, 11.7*)	2.80 (*4.7*)	4.81 (*10.1*)	3.96 (*10.7*)	3.83 (*1.6*)	5.27 (*7.4*)	5.22 (*2.4*)	4.36 (*12.5*)	4.18 (*5.0*)	5.08 (*9.8*)	3.55	1.85	7.49	7.32	7.37
5	2.02 (*12.9, 11.8*)	2.76 (*4.6*)	4.81 (*10.0*)	3.95 (*10.7*)	3.85 (*1.6*)	5.27 (*8.1*)	5.25 (*2.4*)	4.39 (*12.4*)	4.19 (*5.1*)	5.08 (*9.3*)	3.58	1.84	7.37	7.12	2.34 (CH_3)
6	1.97 (*12.7, 11.8*)	2.76 (*4.7*)	4.81 (*10.3*)	3.96 (*10.8*)	3.85 (*1.9*)	5.27 (*6.9*)	5.22 (*2.6*)	4.36 (*12.4*)	4.17 (*5.4*)	5.15 (*9.8*)	3.59	1.84	6.84	7.40	3.81 (CH_3)
7	2.08 (*12.8, 11.9*)	2.85 (*4.7*)	4.86 (*9.9*)	4.10 (*11.3*)	3.99 (---)	5.30 (---)	5.27 (*2.1*)	4.30 (*12.5*)	4.06 (*5.6*)	5.14 (*9.5*)	3.59	1.87	7.63	8.17	
9	2.18 (*12.8, 11.8*)	2.86 (*4.7*)	4.88 (*9.9*)	4.03 (*10.7*)	4.10 (*1.8*)	5.29 (*7.9*)	5.21 (*2.8*)	4.27 (*12.5*)	4.09 (*5.2*)	5.15 (*9.6*)	3.65	1.86	7.18, 7.54, 7.66, 8.46 Pyr. H-3/H-6		
10	2.17 (*13.0, 11.9*)	2.85 (*4.7*)	4.82 (*9.9*)	3.87 (*9.7*)	3.82 (*1.5*)	5.19 (*9.9*)	5.16 (*2.3*)	4.23 (*12.3*)	3.99 (*5.8*)	5.12 (*9.8*)	3.75	1.85	7.12 (H-4)	7.10 (H-5)	3.82 (CH_3)

[a] Chemical shifts in ppm from external TMS; coupling constants in Hz. Spectra in $CDCl_3$ at room temperature.

[b] H-ortho, meta and para respectively where applicable.

[c] J_{gem}=13.8 Hz; J_{vic}=6.3; J_{vic}=7.5

[d] J_{cis}=10.0 Hz; J_{trans}=17.0

[e] J_{gem}=1.3 Hz

Table 3. ^{13}C NMR Chemical Shifts (δ) of α-Thiosialosides **2-10**.[a]

Compd	C-1	C-2	C-3	C-4[b]	C-5	C-6	C-7	C-8	C-9	MeO	NAc	Aglycon C's				
												C-i	C-p	C-m	C-o[c]	
2	168.4	83.3	38.0	69.7	49.2	74.2	67.4	68.9	62.0	52.8	23.2	14.0 (CH_3)	49.2 (CH_2)			
3	168.3	82.9	37.8	69.5	49.3	74.1	67.3	68.6	62.2	52.8	23.1	31.6 (SCH_2)	132.9 (CH=)		118.0 (=CH_2)	
4	167.8	87.5	38.1	69.9	49.2	74.7	67.6	69.6	62.0	52.7	23.2	128.9	129.9	138.5	128.3	
5	168.1	87.4	37.9	70.1	49.1	74.7	67.6	69.6	61.9	52.6	22.9	140.3	125.0	136.6	129.7	
6	168.0	87.5	38.7	70.2	49.2	74.8	67.7	69.8	62.0	52.8	23.2	161.3	119.1	138.4	114.4	55.4 (CH_3)
7	167.5	86.8	38.1	69.1	48.8	74.4	67.2	68.6	62.0	52.9	22.8	148.3	137.8	135.1	123.8	
9	168.3	86.1	38.2	69.2	49.1	74.5	67.3	69.2	61.8	52.9	22.9	153.0	129.2	137.3	122.8	149.8 (C-6)
10	167.7	88.0	37.8	69.3	49.0	74.4	67.0	68.3	62.2	52.9	22.9	134.1 (C-2)	130.7 (C-4)		125.2 (C-5)	34.2 (CH_3)

[a] Chemical shifts in ppm from external TMS in $CDCl_3$.

[b] C-4 and C-8 chemical shifts may be interchanged.

[c] C-ipso, ortho, meta and para respectively where applicable.

analyses were performed by M-H-W Laboratories (Phoenix, AZ). Thin layer chromatography (TLC) were performed on pre-coated silica gel 60 F254 plates and column or radial chromatography on silica gel 60 (231-400 mesh, E. Merck No. 9385). All solvents and reagents were reagent grade and were used without further purification.

Typical PTC reactions

Allyl (methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulo-pyranosid) onate (3)

To a solution of ß-acetochloroneuraminic acid **1** (118 mg, 0.232 mmol) and tetrabutylammonium hydrogen sulfate (78.8 mg, 0.232 mmol) in ethyl acetate (1.2 mL) was added a solution of allyl mercaptan (75 µL, 0.928 mmol) in 1M sodium carbonate (1.2 mL). The reaction mixture was vigorously stirred at room temperature until TLC indicated complete transformation of the starting material (1h). Ethyl acetate (18 mL) was added to the reaction mixture and the organic phase was separated and washed three times with saturated sodium hydrogen carbonate (20 mL each), twice with water (20 mL) and once with saturated sodium chloride (10 mL). The organic extract was then dried using anhydrous sodium sulfate and evaporated near dryness. The oily residue was purified by silica gel column chromatography using ethyl acetate/hexanes containing 0.5% isopropanol as eluant, to obtain pure **3** (106.4 mg) in 84% yield; Rf (EtOAc) = 0.39, m.p. (Ether/Hexanes) 108.5-111.0 °C, $[\alpha]_D$ +38.9 (c 1.01, $CHCl_3$), M.S. for $C_{23}H_{33}NO_{12}S$ (C.I. ether, m/z): 547.9 ($[M+1]^+$, 68.4%), 487.9 ($[M\text{-}CO_2Me]^+$, and/or $[M\text{-}OAc]^+$, 55.8%), 474.0 ($[M\text{-aglycon}]^+$, 22.1%).

4-N-Acetamidophenyl (methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulopyranosid) onate (8)

Compound **7** (800 mg, 1.27 mmol) was dissolved in absolute ethanol (40 mL) to which was added tin (II) chloride dihydrate ($SnCl_2 \cdot H_2O$) (1.44 g, 6.37 mmol). The reaction mixture was stirred at 70°C for 3 h after which time TLC in dichloromethane containing 4% ethanol indicated the transformation to be completed. The reaction mixture was cooled and poured onto ice-water and the final pH of the solution adjusted to 7-8 with $NaHCO_3$. The resulting mixture was extracted with EtOAc (120 mL) which was successively washed with saturated $NaHCO_3$ and water. The recovered organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. The resulting crude 4-aminophenylthio glycoside was immediately treated overnight with pyridine (8 mL) and acetic anhydride (4 mL) at room temperature. The solution was thoroughly evaporated under reduced pressure using toluene co-evaporations. The crude residue was purified by silica gel column chromatography using first a mixture of CH_2Cl_2/MeOH (3%, v/v) then pure EtOAc. After pooling and evaporating the desired fractions, compound **8** was obtained in 87% overall yield (705 mg); m.p. 97-98 °C (EtOAc); $[\alpha]_D$ +33.9 (c=1.86, $CHCl_3$); M.S. (C.I. ether, m/z): 641($[M]^+$, 87.5%), 581($[M\text{-AcOH}]^+$, 100%), 521($[M\text{-2AcOH}]^+$, 3.1%), 476($[M\text{-HSPhNHAc}]^+$, 56.2%). I.R.(thin film) (υ cm^{-1}): 3302, 3099, 3015, 1741, 1667, 1590. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.81 (br, 1H, NH), 7.49 (d, 2H, $J_{o,m}$ 8.7 Hz, H-ortho), 7.39 (d, 2H, H-meta), 5.43 (d, 1H, $J_{5,NH}$ 9.3, NH), 5.27 (dd, 1H, $J_{7,8}$ 7.3 Hz, H-7), 5.22 (ddd, 1H, $J_{8,9a}$ 2.7 Hz, H-8), 4.81 (ddd, 1H, $J_{4,5}$ 10.4 Hz, H-4), 4.36 (dd, 1H, $J_{9a,9b}$ 12.5, H-9a), 4.16 (dd, 1H, $J_{8,9b}$ 5.4 Hz, H-9b), 3.95 (ddd, 1H, $J_{5,6}$ 10.1 Hz, H-5), 3.84 (dd, 1H, $J_{6,7}$ 1.9 Hz, H-6), 3.57 (s, 3H, OCH_3), 2.75 (dd, 1H, $J_{3e,4}$ 4.7 Hz, H-3e), 2.05 (dd, 1H, $J_{3a,4}$ 11.8, $J_{3a,3e}$ 12.8 Hz, H-3a), 2.14, 2.10, 2.03, 2.00 (4s, 12H, OAc), 1.98, 1.83 (2s, 6H, NAc). ^{13}C-NMR ($CDCl_3$) δ (ppm): 170.8, 170.7, 170.4, 170.2, 170.0, 169.0, 167.8 (C=O, C-1, NAc,

OAc), 140.0 (C-ipso), 137.3 (C-meta), 123.0 (C-para), 119.7 (C-ortho), 87.5 (C-2), 74.6 (C-6), 70.0 (C-8), 69.7 (C-4), 67.6 (C-7), 61.9 (C-9), 52.7 (CH_3O), 49.3 (C-5), 37.9 (C-3), 24.5, 23.0, 21.4, 20.8 (NAc, OAc). Anal. Calcd. for $C_{28}H_{36}N_2O_{13}S \cdot H_2O$: C, 51.06; H, 5.81; N, 4.25. Found: C, 51.41; H, 5.75; N, 4.29.

4-Nitrophenyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranoside (13)

To a solution of α-acetobromogalactose (**12**) (10.0 g, 24.3 mmol) and TBAHS (8.3 g, 24.3 mmol) in EtOAc (100 mL) was added 1M Na_2CO_3 (100 mL) and p-nitrothiophenol (5.7 g, 36.8 mmol, 1.5 eq). The two phase reaction mixture was vigorously stirred at room temperature and the progress of the reaction was monitored by TLC using EtOAc/Hexanes (1:1 v/v) as eluent. The reaction was completed within 55 min after which time, EtOAc was added and the organic phase was washed exhaustively with $NaHCO_3$, water, and brine. The organic extracts were then dried (Na_2SO_4), filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (EtOAc/Hex., 3:4 v/v) and the resulting residue was crystallized from ethanol. Compound **13** was obtained as yellowish needles (10.9 g, 92.4 %); m.p. 168.7-169.0 °C; $[\alpha]_D$ -8.3 (c = 1.0, $CHCl_3$), M.S. (C.I. ether, m/z): 486 ($[M+1]^+$, 1.9%), 331($[M+1\text{-}SPhNO_2]^+$, 100%). ^{1}H-NMR ($CDCl_3$) δ (ppm), 8.13 (d, 2H, $J_{m,o}$ 9.0 Hz, H-meta), 7.57 (d, 2H, H-ortho), 5.44 (dd, 1H, $J_{4,5}$ 0.9 Hz, H-4), 5.27 (dd, 1H, $J_{2,3}$ 9.9 Hz, H-2), 5.06 (dd, 1H, $J_{3,4}$ 3.3 Hz, H-3), 4.83 (d, 1H, $J_{1,2}$ 9.8 Hz, H-1), 4.19 (dd, 1H, $J_{6a,6b}$ 11.3 Hz, H-6a), 4.10 (dd, $J_{5,6b}$ 5.4 Hz, H-6b), 4.10 (ddd, 1H, $H_{5,6a}$ 7.2 Hz, H-5), 2.13, 2.06, 2.05, 1.96 (4s, 12H, OAc). ^{13}C-NMR ($CDCl_3$) δ (ppm), 170.2, 169.9, 169.8, 169.3 (C=O), 146.7 (C-para), 142.4 (C-ipso), 130.3 (C-meta), 123.7 (C-ortho), 84.7 (C-1), 74.7 (C-5), 71.6 (C-3), 67.0 (C-4), 66.6 (C-2), 61.6 (C-6), 20.7, 20.6, 20.6, 20.5 (OAc).
Anal. Calcd. for $C_{20}H_{23}NO_{11}S$: C, 49.50; H, 4.77; N, 2.89; S, 6.60. Found: C, 49.59; H, 4.89, N, 2.91; S, 6.53.

4-Nitrophenyl 1-thio-β-D-galactopyranoside (14)

To a suspension of **13** (10.0 g, 20.6 mmol) in 100 mL of methanol was added 1.5 mL of 1 M NaOMe (pH = 9-10). The mixture was stirred at room temperature for 30 min after which time, TLC ($MeOH/CHCl_3$, 1:10 v/v) indicated that complete de-O-acetylation occurred. The solution was neutralized with H^+ resin (Dowex 50w-X8) and evaporated under reduced pressure to afford **14** in essentially quantitative yield (6.85 g); m.p. 160.2-161.5 °C; $[\alpha]_D$ -100.8 (c = 1.0, CH_3OH); M.S. (C.I. ether, m/z): 318 ($[M+1]^+$, 1.1%), 163 ($[M+1\text{-}SPhNO_2]^+$, 38.3%). ^{1}H-NMR (D_2O) δ (ppm), 8.17 (d, $J_{m,o}$ 9.1 Hz, H-meta), 7.63 (d, H-ortho), 5.02 (d, 1H, $J_{1,2}$ 9.7 Hz, H-1), 4.06 (dd, 1H, $J_{3,4}$ 2.0 Hz, H-4), 3.92-3.69 (m, 5H, H-2, H-3, H-5, H-6a, & H-6b). ^{13}C-NMR (D_2O) δ (ppm), 148.2 (C-para), 146.5 (C-ipso), 130.9 (C-meta), 126.6 (C-ortho), 88.6 (C-1), 81.6 (C-5), 76.4 (C-3), 71.5 (C-4), 71.1 (C-2), 63.4 (C-6).

4-Nitrophenyl 2,3,4-tri-O-benzoyl-6-O-t-butyldimethylsilyl-1-thio-β-D-galactopyranoside (15)

To a solution of 4-nitrophenyl 1-thio-β-D-galactopyranoside (**14**) (2.0 g, 6.30 mmol) in 60 mL dried pyridine was added t-butyldimethylsilyl chloride (1.07 g, 7.10 mmol) at 0°C. The reaction mixture was stirred at room temperature during 3.5 h and then cooled to 0°C. Benzoyl chloride (2.7 mL) was added and the solution was stirred for a further 5 h at room temperature after which time, TLC (ethyl acetate-hexanes, 3:5 v/v) indicated complete transformation. The reaction mixture was poured onto ice and extracted with chloroform. The extracts were collected and successively washed with saturated $NaHCO_3$ and saturated NaCl.

The organic phase was then dried over sodium sulfate, filtered and evaporated under reduced pressure. The crude product was crystallized from ethanol to afford **15** (4.03 g, 86 %) as yellowish crystal; m.p. 159-160 °C; $[\alpha]_D$ +84.4 (c = 1.0, $CHCl_3$); M.S. (C.I. ether, m/z): 474 ($[M+1]^+$, 7.1%), 589 ($[M+1\text{-}SPhNO_2]^+$, 49.0%). ^{1}H-NMR ($CDCl_3$) δ (ppm), 8.20-7.18 (m, 19H, aryl), 5.98 (dd, 1H, $J_{4,5}$ 0.9 Hz, H-4), 5.76 (dd, 1H, $J_{2,3}$ 9.9 Hz, H-2), 5.63 (dd, 1H, $J_{3,4}$ 3.3 Hz, H-3), 5.23 (d, 1H, $J_{1,2}$ 9.8 Hz, H-1), 4.12 (ddd, 1H, $H_{5,6a}$ 6.8, $H_{5,6b}$ 6.2 Hz, H-5), 3.88 (dd, 1H, $J_{6a,6b}$ 10.2 Hz, H-6a), 3.77 (dd, 1H, H-6b), 0.84 (s, 9H, t-Butyl), -0.01, -0.05 (2s, 6H, $Si(Me)_2$). ^{13}C-NMR ($CDCl_3$) δ (ppm), 165.4, 165.2, 165.1 (C=O), 141.8-123.8 (aryl), 84.7 (C-1), 78.5 (C-5), 72.8 (C-3), 67.9 (C-2), 67.8 (C-4), 61.2 (C-6), 25.7 (3C, $C(CH_3)_3$), 18.2 ($C(CH_3)_3$), -5.5, -5.6 $[Si(Me)_2]$.

Anal. Calcd. for $C_{39}H_{41}NO_{10}SSi$: C, 62.96; H, 5.56; N, 1.88; S, 4.31. Found: C, 63.20; H, 5.54; N, 1.91; S, 4.52.

4-Nitrophenyl 2,3,4-tri-O-benzoyl-1-thio-β-D-galactopyranoside (16)

Acetyl chloride (3 mL) was added dropwise to methanol (60 mL) and the hydrogen chloride solution obtained was cooled to 20°C. A solution of **15** (3.7 g, 4.97 mmol) in diethyl ether (60 mL) was added and the mixture was stirred at room temperature for 30 min. TLC (EtOAc/hexanes, 3:5 v/v) indicated complete removal of the silyl protecting group. The reaction mixture was neutralized with Amberlite IR-45(OH^-), concentrated under reduced pressure and the crude residue was purified by silica gel column chromatography using ethyl acetate-hexanes (3:5 v/v) as eluent. Pure compound **16** (2.82g, 91%) was obtained as a white powder; m.p. 106.5-107.7°C; $[\alpha]_D$ +69.9 (c = 1.0, $CHCl_3$); M.S. (C.I. ether, m/z): 630 ($[M]^+$, 8.5%), 612 ($[M\text{-}H_2O]^+$, 4.1%), 504 ($[M\text{-}PhCO_2H]^+$, 4.8%), 475 ($[M\text{-}HSPhNO_2]^+$, 100%). ^{1}H-NMR ($CDCl_3$) δ (ppm), 8.17-7.18 (m, 19H, aryl), 5.87 (dd, 1H, $J_{4,5}$ < 1.0 Hz, H-4), 5.86 (dd, 1H, $J_{2,3}$ 9.9 Hz, H-2), 5.64 (dd, 1H, $J_{3,4}$ 3.3 Hz, H-3), 5.14 (d, 1H, $J_{1,2}$ 9.9 Hz, H-1), 4.17(ddd, 1H, $H_{5,6a}$ 6.8, $H_{5,6b}$ 6.2 Hz, H-5), 3.88 (dd, 1H, $J_{6a,6b}$ 12.0 Hz, H-6a), 3.65 (dd, 1H, H-6b), 2.05 (br, 1H, OH-6). ^{13}C-NMR ($CDCl_3$) δ (ppm), 166.5, 165.4, 165.1 (C=O), 147.1-123.8 (aryl), 84.5 (C-1), 78.2 (C-5), 72.7 (C-3), 68.8 (C-2), 67.6 (C-4), 60.6 (C-6).

4-Nitrophenyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-2,3,4-tri-O-benzoyl-1-thio-β-D-galactopyranoside (17)

To a solution of 4-methoxyphenyl (methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulopyranosid) onate (**6**) (58.5 mg, 95.4 μmol) and **16** (50 mg, 79.5 μmol) in dry propionitrile (4 mL) was added molecular sieves (4Å, 100 mg). The mixture was stirred for 4 h at room temperature and then cooled to -60°C. N-Iodosuccinimide (43 mg, 191 μmol) and trifluoromethanesulfonic acid (5.9 μL, 66.8 μmol) were then added. The solution was stirred for 45 min at -60°C. The progress of the reaction was monitored by TLC using a mixture of benzene/acetone (3:2 v/v) as eluent. The reaction mixture was diluted with CH_2Cl_2 and filtered through a pad of celite. The filtrate was successively washed with 10% aqueous sodium thiosulfate, saturated aqueous sodium bicarbonate and brine. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography using 3 % ethanol in dichloromethane as eluent. Compound **17** was obtained in 52% yield (44.8 mg); m.p: 117.4-118.5 °C (CH_2Cl_2); $[\alpha]_D$ +45.2 (c = 1.1, $CHCl_3$); M.S. (FAB^+, m/z): 1086.2 ($[M]^+$, 0.3%), 948.3 ($[M\text{-}SPhNO_2]^+$, 2.4%), 612.1 ($[M\text{-}Neu5Ac]^+$, 2.1%), 474.2 ($[M\text{-}Gal]^+$, 11.9%); IR (thin film), (υ), 3330, 3067, 2946, 1738, 1674, 1590, 1519 cm^{-1}. ^{1}NMR ($CDCl_3$) δ (ppm): 8.16 (d, 2H, $J_{o,m}$ 8.6 Hz, H-o

NO_2), 7.94 (d, 2H, H-m), 7.73-7.19 (m, 15H, aryl), 6.06 (dd, 1H, $J_{4,5} < 1.0$ Hz, H-4), 5.80 (dd, 1H, $J_{3,4}$ 3.2 Hz, H-3), 5.7 (dd, 1H, $J_{2,3}$ 10.7 Hz, H-2), 5.55 (ddd, 1H, $J_{8',9'a}$ 2.6 Hz, $J_{8',9'b}$ 4.9 Hz, H-8'), 5.41 (d, 1H, $J_{1,2}$ 9.7 Hz, H-1), 5.25 (dd, 1H, $J_{7',8'}$ 9.4 Hz, H-7'), 5.14 (d, 1H, $J_{NH,5'}$ 9.7 Hz, NH), 4.80 (ddd, 1H, $J_{4',5'}$ 10.2 Hz, H-4'), 4.50 (ddd, 1H, $J_{5,6a}$ 6.1, $J_{5,6b}$ 8.4 Hz, H-5), 4.44 (dd, 1H, $J_{9'a,9'b}$ 12.3 Hz, H-9'a), 4.12 (dd, 1H, $J_{6',7'}$ 1.9 Hz, H-6'), 4.07 (ddd, 1H, $J_{5',6'}$ 10.6 Hz, H-5'), 4.04 (dd, 1H, H-9'b), 3.85 (dd, 1H, $J_{6a,6b}$ 11.0 Hz, H-6a), 3.68 (dd, 1H, H-6b), 3.57 (s, 3H, OMe), 2.46 (dd, 1H, $J_{3'a,3'e}$ 12.9, $J_{3'e,4'}$ 4.6 Hz, H-3'e), 1.96 (dd, 1H, $J_{3'a,4'}$ 11.0 Hz, H-3'a), 2.24, 2.17, 1.99, 1.91 (4s, 12H, OAc), 1.87 (s, 3H, NAc). ^{13}C-NMR ($CDCl_3$) δ (ppm): 171.0, 170.9, 170.8, 170.2, 170.1, 167.8, 165.3, 165.2, 165.1 (C=O), 146.6 (C-para), 142.6 (C-ipso), 133.5-123.8 (aryl), 99.5 (C-2'), 83.9 (C-1), 75.8 (C-5), 72.8 (C-6'), 72.6 (C-3), 68.6 (C-4'), 67.9 (C-2, C-4), 67.8 (C-8'), 67.3 (C-7'), 63.4 (C-9'), 63.0 (C-6), 52.9 (OCH_3), 49.3 (C-5'), 37.8 (C-3'), 23.2 (NAc), 21.2, 20.8 (OAc).

Anal Calcd. for $C_{53}H_{34}N_2O_{21}S$: C, 58.56; H, 5.01; N, 2.58. Found: C, 58.15; H, 4.96; N, 2.42.

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