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Stereoselective Phase Transfer Catalyzed Syntheses of Glycosyloxysuccinimides and their Transformations into Glycoprobes

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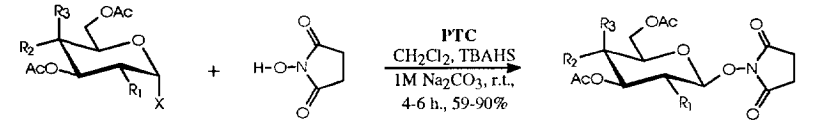
Abstract: Glycosyloxysuccinimides of D-galactose, D-glucose, N-acetyl-D-glucosamine, lactose, maltose (**7-11**) and sialic acid (**13**) were prepared from their glycosyl halides under PTC conditions. Ring opening of the succinimide moieties occurred with sodium hydroxide or methoxide to provide extended aglycons, while treatment of **8** with hydrazine afforded O-galactopyranosylhydroxylamine **17**. Treatment of **9** with tris(2-aminoethyl)amine gave divalent cluster **20** which upon further treatment with fluorescein isothiocyanate provided divalent glycoprobe **21**.

The occurrence[1] of glycosidic N-O linkages in the oligosaccharide moieties of the potent antitumor antibiotics calicheamicin γ_1 and esperamycin A_1 has triggered novel synthetic approaches for the construction of such linkages. To this end, the Mitsunobu reaction of N-hydroxyphtalimide on reducing sugars has been used[2-4] as well as Lewis acid catalyzed treatment of per-O-acetylated sugars.[5] These methods are however poorly stereoselective. Another elegant and stereoselective alternative makes use of 2,2-dimethyldioxirane oxidation of pre-installed amino glycosides to afford, in most cases, hydroxylamino sugar derivatives.[6] More recently, nitrone glycosylation has been used, however the key step still requires stereoselective oxime reductions.[7]

The fair acidity of N-hydroxysuccinimide **6** (pKa = 6.0)[8] and previous experience in phase transfer catalyzed glycosylations of a wide number of nucleophiles[9-11] prompted us to investigate the glycosylation of N-hydroxysuccinimide with a large number of glycosyl halides (**1-5**) including sialosyl chloride (**12**) (Scheme 1). This paper describes the successful stereoselective syntheses of O-glycosyl N-hydroxysuccinimide.



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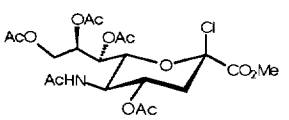
2 X = Br, R_1= OAc, R_2= H, R_3= OAc
3 X = Cl, R_1= NHAc, R_2= OAc, R_3= H
4 X = Br, R_1= OAc, R_2= β-D-Gal(OAc)$_4$, R_3= H
5 X = Br, R_1= OAc, R_2= α-D-Glc(OAc)$_4$, R_3= H

6

7 R_1= OAc, R_2= OAc, R_3= H
8 R_1= OAc, R_2= H, R_3= OAc
9 R_1= NHAc, R_2= OAc, R_3= H
10 R_1= OAc, R_2= β-D-Gal(OAc)$_4$, R_3= H
11 R_1= OAc, R_2= α-D-(Glc(OAc)$_4$, R_3= H



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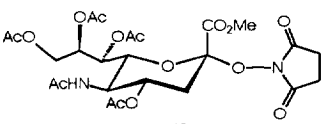

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Scheme 1. PTC glycosylation of N-hydroxysuccinimide.

Moreover, O-Glycosyl N-hydroxysuccinimides can be easily transformed by mild hydrazinolysis into O-oxamino glycosides useful in antibiotic syntheses[3,4] or converted into glycosyl hydroxamic esters with a terminally functionalized aglycon spacer suitable for glycoconjugate syntheses.[12] The latter derivatives could also prove useful in determining the unknown effects of anomeric O-oxamino linkages toward glycohydrolases.

RESULTS AND DISCUSSIONS

Trans-1,2 β-D-glycosides and disaccharides (**7-11**) together with an N-O linked sialoside (**13**) of N-hydroxysuccinimide **6** were readily prepared under mild PTC conditions (Table 1). Glycosylations were performed at room temperature using a slight excess of N-hydroxysuccinimide and one equivalent of tetrabutylammonium hydrogen sulfate (TBAHS) as catalyst. The best results were obtained with methylene chloride as the organic phase and 1M Na_2CO_3 as the aqueous phase. Under these conditions, good to excellent yields (59-90%, not all optimized) of O-glycosyl N-hydroxysuccinimides could be obtained in 2-4 hours from glycosyl halides **1-5** and **12**. As opposed to most previous PTC reactions of glycosyl halides, the use of ethyl acetate instead of methylene chloride greatly reduced the reaction rates and yields.[9-11] The reactions could only be completed in two days when ethyl acetate was used. In most cases, the major side reaction of the PTC synthesis of glycosyloxysuccinimides was the dehydrohalogenation of the starting glycosyl halides. The extent of this side reaction was however less pronounced than with phenoxide nucleophiles.[10,13]

As previously observed, the glycosylations were deemed to be stereospecific as judged by the TLC and the ^{1}H-NMR spectra of the crude reaction mixtures. All the reactions occured with complete anomeric inversions irrespective of the initial anomeric configurations of the glycosyl halides and the existence or absence of possible anchimeric participation as seen in the case of the deoxy sugar **12**. It is also worth mentioning that in all cases (**7-11**) but **13**, the methylene signals of the N-hydroxysuccinimide moieties appeared as singlets in the ^{1}H-NMR spectra: 2.72 ppm for the monosaccharides **7-9** and 2.67 ppm for the disaccharides **10-11**. In the case of the sialoside **13**, presumably through restricted rotation, the methylene protons are diastereotopics and appeared as multiplet with an AA'BB' pattern between 2.61-2.80 ppm.

Table 1. Results from the PTC Glycosylation of N-Hydroxysuccinimide 6.

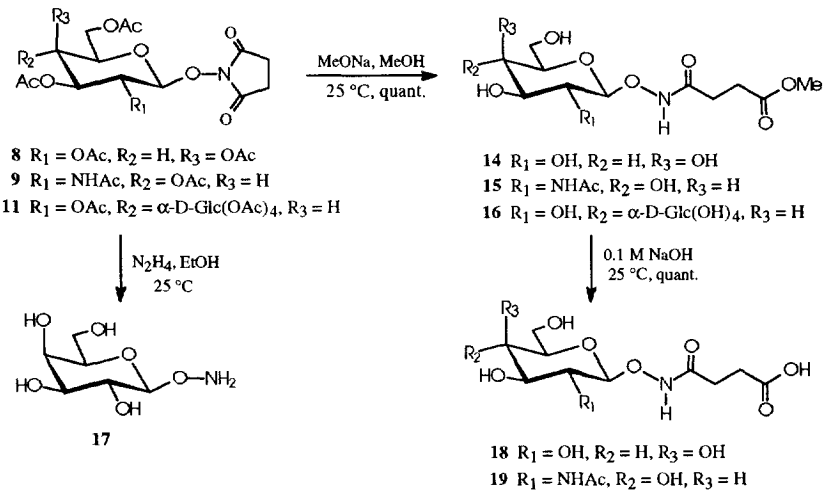
Glycosyloxy-succinimides	Yield (%)[a]	M.p. (°C)	$[\alpha]_D$[b]	^{1}H-NMR[c] (H-1 ppm) ($J_{1,2}$ Hz)	^{13}C-NMR[c] (C-1 ppm)
7	59	182.4-183.7	-43.0	5.04 (6.3)	103.7
8	78	190.1-191.4	-52.1	4.84 (8.2)	105.2
9	76	201.7-203.2	-44.7	5.13 (8.7)	104.3
10	90	92.5-93.7	-23.6	5.09 (6.3)	102.2
11	60	219.0-219.6	+36.5	5.16 (6.3)	102.3
13	71	amorphous	+7.3	2.86 (H_{3e})	101.5 (C-2)

[a] Isolated crystalline materials. [b] In $CHCl_3$ at room temperature (c = 1.0). [c] Chemical shifts are in ppm relative to internal $CHCl_3$ (7.23 and 77.0 ppm) for solutions in $CDCl_3$. Assignments are based on 2D ^{1}H-^{1}H COSY, HMQC and ^{1}H-^{13}C HETCOR experiments.

When the O-Glycosyl N-hydroxysuccinimides **8-11** were treated under mild conditions of de-O-acetylation (NaOMe, MeOH), concomitant ring opening of the succinimidyl residues also occurred to afford O-glycosyl hydroxamic esters **14-16** in quantitative yields (Scheme 2). All attempts to achieve chemoselective ester hydrolysis and to prevent ring openings were unsuccessful. Such process would have been valuable to provide anomerically protected glycosides suitable for further transformations into different

glycosyl acceptors. In addition, saponification of the methyl esters **14** and **15** with 0.1M NaOH gave the corresponding terminal carboxylic acids **18** and **19** respectively also in quantitative yields.

The de-O-acetylation with concomitant ring opening of the succinimidyl aglycon under Zemplén conditions is conceptually similar to reactions performed very recently by Andersson et *al.*[12] where N-oxysuccinimidyl glycosides were treated with amines to provide the corresponding N-O linked prespacer glycosides. Alternatively, hydrazinolysis of N-oxysuccinimidyl glycosides with an excess of hydrazine hydrate was performed to determine if O-glycosylhydroxylamine, useful in antibiotic syntheses, could be made available. Such new N-O linked glycosides also have potential in glycoconjugate syntheses. Indeed, as anticipated, O-glycosylhydroxylamine of the galactose derivative **17** was produced smoothly when succinimide **8** was treated with an excess of hydrazine in ethanol at room temperature overnight. However, the polar and hygroscopic nature of **17** together with the simultaneous production of acetic acid hydrazide and butanedioic acid dihydrazide complicated further purification steps on large scales.

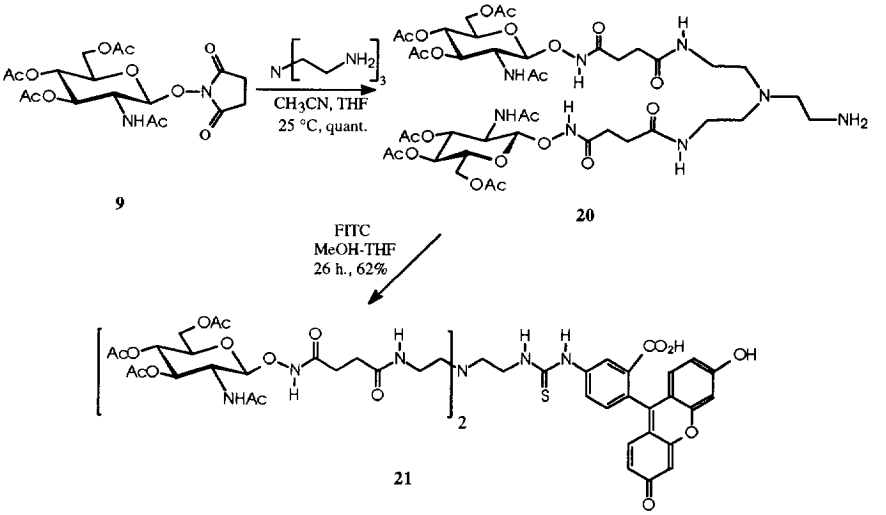


Scheme 2

Although glycosides containing acid functionality such as **18,19** above represent suitable candidates for glycoconjugate syntheses,[12] including glycopeptide syntheses,[14] we were interested to get direct access to glycoconjugates in the form of carbohydrate clusters. Clustered ligands have been shown to have greater affinities toward carbohydrate binding receptors such as those found on hepatic asialoglycoproteins,[15] influenza virus hemagglutinins[16] and endothelial leukocyte adhesion molecules.[17] Moreover, the possibility of making carbohydrate clusters simultaneously incorporating biochemical probes is also very attracting.[9, 18,19] To this end, O-glycopyranosyl N-hydroxysuccinimide **9**, used as model, was treated with tris(2-aminoethyl)amine at room temperature to provide amino-terminated divalent cluster **20** in essentially quantitative yield. Interestingly, even with a large excess of succinimide **9**, the three amine groups could not be all substituted with the sugar moiety (Scheme 3). Under forcing conditions, exhaustive degradation of products occurred. Finally, treatment of amine **20** with fluorescein isothiocyanate in a mixture of methanol-THF under slightly alkaline conditions afforded glycoprobe **21** in 62% yield after purification by silica gel column chromatography.

In conclusion, glycosyl halides with or without anchimeric participating groups can be stereoselectively transformed into O-glycosyl N-hydroxysuccinimides with complete inversion of anomeric configurations under PTC conditions. Ongoing PTC transformations with both α- and β-glycosyl halides having acetates as potential participating groups have also revealed complete anomeric inversions, thus demonstrating the

stereospecificity of the process.[11] The resulting glycosyl oxysuccinimides can be transformed into prespacer glycosides having acid functionality. Alternatively, the succinimide moieties can be ring-opened with hydrazine with concomitant transformation into O-glycosylhydroxylamines such as those found in natural antibiotic calicheamicin γ_1 and esperamycin A_1. More interestingly, the possibility of direct transformation of glycosyl oxysuccinimides into clusters represents a good opportunity for glycodendrimer syntheses.[9, 20-21]



Scheme 3

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 or a Varian Gemini 200 MHz. IR spectra were run on a Bomen Michelson FT-IR 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 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 Glycosylation of N-Hydroxysuccinimide 6.

Some of the yields reported in Table 1 were not optimized. The procedure described below for the lactose derivative has been optimized. Acetobromolactose (**4**) (350 mg, 0.499 mmol), N-hydroxysuccinimide (**6**) (575mg, 5 equiv.) and tetrabutylammonium hydrogen sulfate (TBAHS, 170 mg, 1 equiv.) were vigorously stirred at room temperature in CH_2Cl_2 (4 mL) and 1M Na_2CO_3 (4 mL). After 4 hours, TLC (2/1, EtOAc/Hexanes, v/v) showed complete disappearance of acetobromolactose (**4**) (Rf = 0.53) to give **10** (Rf = 0.11) as the major product. The reaction mixture was worked up by adding dichloromethane (20 mL). The organic phase was washed with distilled water (2x) and sat. NaCl. The organic extracts were dried ($NaSO_4$), filtered and evaporated under reduced pressure to give a white solid which was purified on silica gel column chromatography (EtOAc/Hexanes, 5/3) and recrystallised from ethanol. Compound **10** was obtained as white needles (329 mg, 90%).

O-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-N-hydroxysuccinimide (7): Obtained as above from acetobromoglucopyranose (**1**) (Rf = 0.64 in $CHCl_3$-i-PrOH, 35:1). The crude product was crystallised from CH_2Cl_2/ether to give **7** (Rf = 0.26) in 59% yield; m.p.: 182.4-183.7 °C (EtOAc/hexanes); $[\alpha]_D$ - 43.0° (c = 1.0, $CHCl_3$); ^{1}H-NMR δ ppm ($CDCl_3$): 5.27-5.18 (m, 3H, H-2-H-4), 5.04 (d, 1H, $J_{1,2}$ = 6.3 Hz, H-1), 4.27 (ddd, 1H, $J_{6a,6b}$ = 12.5 Hz, H-6a), 4.13 (ddd, 1H, $J_{5,6b}$ = 2.5 Hz H-6b), 3.73 (ddd, 1H, $J_{5,6a}$ = 4.9 Hz, H-5), 2.72 (s, 4H, CH_2), 1.92-2.14 (4s, 12H, OAc); ^{13}C-NMR δ ppm ($CHCl_3$): 170.6-169.2 (C=O), 103.7 (C-1), 72.2 (2xC, C-3, C-5), 69.6 (C-2), 68.1 (C-4), 61.7 (C-6), 25.3 (CH_2), 20.8- 20.6 (OAc). *Anal.* Calcd. for $C_{18}H_{23}NO_{12}$: C, 48.54; H, 5.21; N, 3.14. Found: C, 48.51; H, 5.26; N, 3.09.

O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-N-hydroxysuccinimide (8): Obtained as above from acetobromogalactopyranose (**2**) after silica gel column chromatography using a gradient of EtOAc/hexanes (1:1 to 3:1), Rf = 0.36 in $CHCl_3$-MeOH (35:1), yield 78%: m.p.: 190.1-191.4 °C (EtOAc/hexanes); $[\alpha]_D$ - 52.1° (c = 1.0, $CHCl_3$); ^{1}H-NMR δ ppm ($CDCl_3$): 5.38 (dd, 1H, $J_{2,3}$ = 10.4 Hz, H-2), 5.36 (dd, 1H, $J_{4,5}$ = 1.0 Hz, H-4), 4.84 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1), 5.04 (dd, 1H, $J_{3,4}$ = 3.4 Hz, H-3), 4.22 (ddd, 1H, $J_{5,6a}$ = 6.2, $J_{6a,6b}$ = 11.3 Hz, H-6a), 4.11 (ddd, 1H, $J_{5,6b}$ = 7.4 Hz, H-6b), 3.88 (ddd, 1H, H-5), 2.72 (s, 4H, CH_2), 1.92-2.14 (4s, 12H, OAc); ^{13}C-NMR δ ppm ($CHCl_3$): 170.6-169.2 (C=O), 105.2 (C-1), 71.2 (C-5), 70.4 (C-3), 66.8 (C-2), 66.1 (C-4), 60.6 (C-6), 25.3 (CH_2), 20.8- 20.6 (OAc). *Anal.* Calcd. for $C_{18}H_{23}NO_{12}$: C, 48.54; H, 5.21; N, 3.14. Found: C, 48.53; H, 5.41; N, 3.16.

O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-N-hydroxysuccinimide (9): Obtained as above from 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl chloride (**3**): the crude product was purified on silica gel (EtOAc/Hexanes, 7/3) and recrystallised from CH_2Cl_2/ether to give **9** as fine crystal (76%); m.p.: 201.7-203.2 °C; $[\alpha]_D$ - 44.7° (c = 0.92, $CHCl_3$). IR (thin film, $\upsilon_{cm^{-1}}$): 3392, 3312, 2942, 1788, 1737, 1665, 1534. CI-MS (ether) gave *m/z* (ion, relative intensity): 445 ($[M+1]^+$, 2.4%), 330 ([M+1-aglycon]$^+$, 100%); ^{1}H-NMR δ ppm ($CDCl_3$): 6.22 (d, 1H, $J_{2,NH}$ = 8.6 Hz, NH), 5.32 (dd, 1H, $J_{3,4}$ = 9.4 Hz, H-3), 5.13 (d, 1H, $J_{1,2}$ = 8.7 Hz, H-1), 5.10 (dd, 1H, $J_{4,5}$ = 9.7 Hz, H-4), 4.28 (ddd, 1H, $J_{6a,6b}$ = 12.2 Hz, H-6a), 4.12 (ddd, 1H, H-6b), 4.09 (dd, 1H, $J_{2,3}$ = 10.2 Hz, H-2), 3.72 (ddd, 1H, $J_{5,6a}$ = 2.7, $J_{5,6b}$ = 4.6 Hz, H-5), 2.72 (s, 4H, CH_2), 2.14 (s, 3H, NHAc), 2.06, 2.00, 1.99 (3s, 9H, OAc); ^{13}C-NMR δ ppm ($CHCl_3$): 171.1, 170.7 (2xC), 170.4 (2xC) 169.2 (6 C=O), 1043 (C-1), 72.3 (C-5), 71.9 (C-3), 68.4 (C-4), 61.7 (C-6), 53.0 (C-2), 25.4 (CH_2), 23.2, 20.7, 20.6 (OAc). *Anal.* Calcd. for $C_{18}H_{24}N_2O_{11}$: C, 48.63; H, 5.45; N, 6.31. Found: C, 48.52; H, 5.48; N, 6.24.

O-(2,2',3,3',4',6,6'-Hepta-O-acetyl-β-D-lactopyranosyl)-N-hydroxysuccinimide (10): Obtained as above from acetobromolactose (**4**): m.p.: 92.5-93.7 °C; $[\alpha]_D$ -23.6° (c =1.0, $CHCl_3$); IR (thin film, $\upsilon_{cm^{-1}}$): 2962, 1745, 1645, 1534. FAB-MS (glycerol) gave *m/z* (ion, relative intensity): 734.3 ($[M+1]^+$, 2.9%), 619.3 ([M+1- aglycon]$^+$, 22.9%); ^{1}H-NMR δ ppm ($CDCl_3$): 5.27 (dd, 1H, $J_{4,5}$ = 1.0 Hz, H-4'), 5.14 (dd, 1H, $J_{3,4}$ = 8.4 Hz, H-3), 5.09 (d, 1H, $J_{1,2}$ = 6.3 Hz, H-1), 5.07 (dd, 1H, $J_{2,3}$ = 7.4 Hz, H-2), 5.05 (dd, 1H, $J_{2',3'}$ =10.4 Hz, H-2'), 4.92 (dd, 1H, $J_{3',4'}$ = 3.4 Hz, H-3'), 4.50 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1'), 4.37 (dd, 1H, $J_{6a,6b}$ = 12.0 Hz, H-6a), 4.13 (dd, 1H, $J_{4,5}$ = 10.1 Hz, H-4), 4.08 (dd, 1H, $J_{5,6b}$ = 6.1 Hz, H-6b), 4.05 (dd, 1H, $J_{6a',6b'}$ =11.3 Hz, H-6a'), 4.01 (dd, 1H, $J_{5',6b'}$ = 7.1 Hz, H-6b'), 2.67 (s, 4H, CH_2), 3.84 (ddd, 1H, $J_{5',6a'}$ = 6.7 Hz, H-5'), 3.70 (ddd, 1H, $J_{5,6a}$ = 2.4 Hz, H-5), 2.09, 2.06, 2.04, 2.02, 2.00, 1.99, 1.90 (7s, 21H, OAc); ^{13}C-NMR δ ppm ($CHCl_3$): 170.3, 170.3, 170.1, 170.0, 169.5, 169.3, 169.1 (7x C=O), 102.2 (C-1), 101.1 (C-1'), 75.7 (C-4), 72.8 (C-3), 72.7 (C-5), 70.9 (C-5'), 70.8 (C-3'), 70.1 (C-2'), 69.1 (C-2), 66.7 (C-4'), 61.8 (C-6), 60.9 (C-6'), 25.4 (CH_2), 20.8, 20.7, 20.6, 20.6, 20.5 (OAc). *Anal.* Calcd. for $C_{30}H_{39}NO_{20}$: C,49.12; H, 5.36; N, 1.91. Found: C, 48.94; H, 5.43; N, 2.09.

O-(2,2',3,3',4',6,6'-Hepta-O-acetyl-β-D-maltosyl)-N-hydroxysuccinimide (11): Obtained as above in 60% yield from acetobromomaltose (**5**): m.p.: 219.0-219.6 °C; $[\alpha]_D$ +36.5° (c =1.0, $CHCl_3$); ^{1}H-NMR δ ppm ($CDCl_3$): 5.41 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1'), 5.35 (dd, 1H, $J_{3',4'}$ = 9.5 Hz, H-3'), 5.19 (dd, 1H, $J_{3,4}$ = 8.3 Hz, H-3), 5.16 (d, 1H, $J_{1',2'}$ = 6.3 Hz, H-1), 5.08 (dd, 1H, $J_{2,3}$ = 7.1 Hz, H-2), 5.03 (dd, 1H, $J_{4,5}$ = 10.0 Hz, H-4'), 4.82 (dd, 1H, $J_{2',3'}$ =10.5 Hz, H-2'), 4.43 (ddd, 1H, $J_{6a,6b}$ = 12.2 Hz, H-6a), 4.34 (dd, 1H, $J_{4,5}$ = 9.6 Hz, H-4), 4.28 (ddd, 1H, $J_{5,6b}$ = 4.6 Hz, H-6b), 4.26 (ddd, 1H, $J_{6a',6b'}$ =12.4 Hz, H-6a'), 4.01 (ddd, 1H,

$J_{5',6b'}$ = 7.1 Hz, H-6b'), 2.67 (s, 4H, CH_2), 3.84 (ddd, 1H, $J_{5',6a'}$ = 6.7 Hz, H-5'), 3.70 (ddd, 1H, $J_{5,6a}$ = 2.4 Hz, H-5), 2.09, 2.06, 2.04, 2.02, 2.00, 1.99, 1.90 (7s, 21H, OAc); ^{13}C-NMR δ ($CHCl_3$): 170.3, 170.3, 170.1, 170.0, 169.5, 169.3, 169.1 (7x C=O), 102.2 (C-1), 101.1 (C-1'), 75.7 (C-4), 72.8 (C-3), 72.7 (C-5), 70.9 (C-5'), 70.8 (C-3'), 70.1 (C-2'), 69.1 (C-2), 66.7 (C-4'), 61.8 (C-6), 60.9 (C-6'), 25.4 (CH_2), 20.8, 20.7, 20.6, 20.6, 20.5 (OAc). *Anal.* Calcd. for $C_{30}H_{39}NO_{20}$: C,49.12, H, 5.36; N, 1.91. Found: C, 49.36; H, 5.50; N, 1.86.

O-[Methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl) onate] N-hydroxysuccinimide (13): Obtained as above from methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosyl chloride)onate (**12**). The crude product was purified on silica gel column (EtOAc/Hexanes, 7/3) to provide pure **13** as an amorphous solid in 71% yield; $[\alpha]_D$ +7.3° (c = 0.10, $CHCl_3$); IR (thin film, $\upsilon_{CM^{-1}}$): 3329, 3017, 2960, 1743, 1671, 1542. CI-MS (ether) gave *m/z* (ion, relative intensity): 589 ($[M+1]^+$, 100%), 529 ($[M\text{-}AcOH]^+$, 21.6%), 476 ($[M\text{- aglycon}]^+$, 17.8%); ^{1}H-NMR (200 MHz) δ ppm ($CDCl_3$): 5.36 (d, 1H, $J_{5,NH}$ = 9.8 Hz, NH), 5.26 (dd, 1H, $J_{7,8}$ = 9.8 Hz, H-7), 5.15 (ddd, 1H, $J_{8,9a}$ = 2.7 Hz, H-8), 4.87 (ddd, 1H, $J_{3a,4}$ = 12.1, $J_{4,5}$ = 10.3 Hz, H-4), 4.13 (dd, 1H, $J_{9a,9b}$ = 12.5 Hz, H-9a), 4.11 (ddd, 1H, $J_{5,6}$ = 10.8 Hz, H-5), 3.97 (dd, 1H, $J_{8.9b}$ = 4.6 Hz, H-9b), 3.84 (dd, 1H, $J_{6,7}$ = 2.2 Hz, H-6), 2.86 (dd, 1H, $J_{3a,3e}$ = 12.2, $J_{3e,4'}$ = 4.5 Hz, H-3e), 2.80-2.61 (m, AA'BB' pattern, 4H, CH_2), 2.02 (dd, 1H, $J_{3a,4}$ = 10.3 Hz, H-3a), 2.12, 2.06, 2.00, 1.98, 1.83 (5s, 15H, Ac); ^{13}C-NMR δ ppm ($CDCl_3$): 171.2, 170.7, 170.5, 170.2, 169.9 (7xC=O, Ac), 165.0 (C-1), 101.5 (C-2), 72.6, 68.4, 67.1, 66.3, 62.1 (C-4, C-6, C-7, C-8), 60.3 (C-9), 53.6 (OMe), 48.8 (C-5), 35.2 (C-3), 26.4 (2C, CH_2), 23.0 (NAc), 21.1, 20.8, 20.7, 20.6 (OAc). *Anal.* Calcd. for $C_{24}H_{32}N_2O_{15}$ (588.180): C, 48.96; H, 5.48; N, 4.76. Found: C, 48.45; H, 5.50; N, 5.02.

N-(Succinylmethylester)-β-D-galactopyranosylhydroxylamine (14): The glycosyloxysuccinimide **8** (20 mg, 0.045 mmol) in methanol (1 mL) was treated with 1M sodium methoxide in methanol until the pH reach 8.5-9. The reaction mixture was maintained at that pH with additional sodium methoxide. The transformation was completed after 24h as shown by TLC in a mixture of $CHCl_3$-MeOH-H_2O (30:10:1) in which compound **14** has a Rf = 0.2. The reaction mixture was neutralized with Dowex 50WX2 resin (H^+), filtered and concentrated to give pure amorphous **14** (as seen by TLC and ^{1}H-NMR) in quantitative yield. $[\alpha]_D$ -41.85° (c = 1.0, CH_3OH); FAB-MS (glycerol) gave *m/z* (ion, relative intensity) for $C_{11}H_{19}NO_9$: 928.2 ($[3M+1]^+$, 2.1 %), 619.1 ($[2M+1]^+$, 10.6%), 310.1 ($[M+1]^+$, 98.8%); ^{1}H-NMR δ ppm (D_2O): 4.66 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1), 3.94 (dd, 1H, $J_{4,5}$ = 2.4 Hz, H-4), 3.86-3.62 (m, 5H, H-2, H-3, H-5, H-6), 3.71(s, 3H, OMe), 2.73 (t, 2H, J_{gem} = 6.6 Hz, CH_2CO_2), 2.52 (t, 2H, J_{gem} = 6.5 Hz, CH_2CON); ^{13}C-NMR δ ppm (D_2O): 177.5, 173.9 (C=O), 108.3 (C-1), 77.9 (C-5), 74.9 (C-3), 71.3 (C-4), 63.3 (C-6), 70.7 (C-2), 54.7 (OMe), 31.1, 29.5 (2xC, CH_2).

N-(Succinylmethylester)-2-acetamido-2-deoxy-β-D-glucopyranosylhydroxylamine (15): Compound **9** (60 mg, 0.135 mmol) was dissolved in methanol (4 mL) to which 1.0M sodium methoxide (100 µL) was added. The solution was stirred at room temperature for 18h. The reaction was monitored by TLC using CH_3CN-MeOH (2:3). The reaction mixture was neutralized with Dowex 50WX2 resin (H^+), filtered and concentrated to give **15** (43 mg). The product was further purified by silica gel column chromatography using a gradient of CH_2Cl_2-MeOH (9:1 to 2:1). The overall purified yield was 96%. $[\alpha]_D$ -58.5° (c = 0.6, CH_3OH); FAB-MS (glycerol) gave *m/z* (ion, relative intensity): 1051.4 ($[3M+1]^+$, 0.7 %), 701.3 ($[2M+1]^+$, 5.3%), 351.2 ($[M+1]^+$, 100%), 204.1 ($[M - aglycon]^+$, 97.7%), 185.1 ($[M\text{-aglycon-}H_2O]^+$, 41.2%); ^{1}H-NMR δ ppm (D_2O): 4.85 (d, 1H, $J_{1,2}$ = 8.7 Hz, H-1), 3.81-3.32 (m, 7H, H-2-H-6, NH), 3.55(s, 3H, OMe), 2.54 (t, 2H, J_{gem} = 6.5 Hz, CH_2CO_2), 2.32 (t, 2H, J_{gem} = 6.5 Hz, CH_2CON), 1.90 (s, 3H, NHAc); ^{13}C-NMR δ ppm (D_2O): 176.7, 176.2, 173.0 (3 C=O), 105.4 (C-1), 77.7 (C-5), 75.0 (C-3), 70.9 (C-4), 62.0 (C-6), 54.8 (C-2), 53.8 (OMe), 30.2, 28.7 (2xC, CH_2), 23.7 (NAc).

N-(Succinylmethylester)-β-D-maltosylhydroxylamine (16): Compound **11** was treated with a solution of sodium methoxide as described for **9** above to provide **16** as a white amorphous solid in quantitative yield. $[\alpha]_D$- 42.6° (c =1.0,MeOH); FAB-MS (glycerol) gave *m/z* (ion, relative intensity) for $C_{17}H_{29}NO_{14}$: 472.3

($[M+1]^+$, 70.1%); ^{1}H-NMR δ ppm (D_2O): 5.42 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1'), 4.73 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 3.97-3.39 (m, 12 H, ring C-H), 3.71 (s, 3H, OMe), 2.72 (s, 2H, J_{gem} = 6.6 Hz, CH_2), 2.50 (s, 2H, J_{gem} = 6.6 Hz, CH_2); ^{13}C-NMR δ ppm (D_2O): 177.5, 174.0 (C=O), 107.5 (C-1), 102.1 (C-1'), 78.6 (C-5), 78.2 (C-3), 77.2 (C-4), 75.2 (C-5'), 75.0 (C-3'), 74.0 (C-2), 73.5 (C-4'), 71.6 (C-2'), 62.8 (C-6, C-6'), 54.7 (OMe), 31.1, 29.5 (2x CH_2).

O-β-D-Galactopyranosylhydroxylamine (17): O-Galactopyranosyloxy succinimide **8** (30 mg, 0.067 mmol) was treated with hydrazine hydrate (50 μL, 7 equiv.) in ethanol (5 mL) at room temperature overnight. The reaction was monitored by TLC using a mixture of $CHCl_3$ /MeOH (4:1) as eluent. The solvent and excess hydrazine were removed by evaporation under reduced pressure. CI-MS gave *m/z* for $C_6H_{13}NO_6$: 391.1 ($[2M+1]^+$, 8%), 196.0 ($[M+ 1]^+$, 58%); ^{1}H-NMR δ ppm (D_2O): 4.49 (d, 1H, $J_{1,2}$ = 8.0 HZ, H-1), 3.93 (dd, 1H, $J_{2,3}$ = 9.0, $J_{3,4}$ = 3.3 Hz, H-3), 3.80-3.51 (m, 5H, H-2, H-4-H-6); ^{13}C-NMR δ ppm (D_2O): 105.2 (C-1), 74.1 (C-5), 72.4 (C-3), 69.0 (C-4), 68.2 (C-2), 60.6 (C-6).

N-(Succinyl)-O-β-D-galactopyranosylhydroxylamine (18): The methyl ester **14** (120 mg, 0.407 mmol) was stirred in 0.05M NaOH (10 mL) at room temperature overnight. The solution was then neutralized with cation exchange resin Dowex (H^+), filtered and lyophilized to give **18** as a white amorphous solid. A quantitative yield of pure **18** was obtained after drying over P_2O_5 under high vacuum for 3 days. The product failed all attempts of crystallization. $[\alpha]_D$ -35.3° (c =1.0, MeOH); FAB-MS (glycerol) gave *m/z* (ion, relative intensity) for $C_{10}H_{17}NO_9$: 886.3 ($[3M+1]^+$, 1.1%), 591.2 ($[2M+1]^+$, 8.2%), 296.1 ($[M+1]^+$, 83.7%). ^{1}H-NMR δ ppm (D_2O): 4.67 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1), 3.85-3.63 (m, 6H, H-2-H-6), 2.71 (t, 2H, J_{gem} = 6.6 Hz, CH_2CO_2), 2.50 (t, 2H, J_{gem} = 6.7 Hz, CH_2CON); ^{13}C-NMR δ ppm (D_2O): 178.8, 174.1 (C=O), 108.2 (C-1), 77.8 (C-5), 74.8 (C-3), 71.2 (C-4), 70.7 (C-2), 63.3 (C-6), 31.1, 29.5 (2xC, CH_2).

N-(Succinyl)-2-acetamido-2-deoxy-β-D-glucopyranosylhydroxylamine (19): Compound (**15**) (200 mg, 0.425 mmol) was dissolved in 0.25 M aqueous sodium hydroxide-ethanol (1:1) (8 mL). The reaction mixture was stirred at room temperature for 3h after which time TLC (CH_3OH-CH_2Cl_2, 2:1) showed complete conversion of the starting material (Rf = 0.87) to product (Rf = 0.13). The solution was neutralized with Dowex resin (H^+), filtered and concentrated under reduced pressure to give pure **19** (150 mg) quantitatively. $[\alpha]_D$ -53.2° (c =1.0, MeOH); FAB-MS (glycerol) gave *m/z* (ion, relative intensity): 1009.3 ($[3M+1]^+$, 0.4 %), 673.3 ($[2M+1]^+$, 4.2%), 337.1 ($[M+1]^+$, 72.7%), 204.1 ($[M$ - aglycon$]^+$, 53.1%), 185.1 ($[M$-aglycon-$H_2O]^+$, 81.5%). ^{1}H-NMR δ ppm (D_2O): 4.65 (d, 1H, $J_{1,2}$ = 8.8 Hz, H-1), 3.93 -3.44 (m, 7H, H-2-H-6, NH), 3.71 (s, 3H, OMe), 2.67 (t, 2H, J_{gem} = 6.4 Hz, CH_2CO_2), 2.42 (t, 2H, J_{gem} = 6.4 Hz, CH_2CON), 1.89 (s, 3H, NHAc); ^{13}C-NMR δ ppm (D_2O): 175.9, 174.2, 171.0 (3 C=O), 105.3 (C-1), 75.6 (C-5), 73.1 (C-3), 68.9 (C-4), 59.9 (C-6), 52.8 (C-2), 28.2, 26.7 (2xC, CH_2), 21.7 (NAc).

Divalent compound (20): To compound **9** (200 mg, 0.425 mmol) in 8 mL of CH_3CN-THF (2:1) containing 4Å powdered molecular sieves was added tris(2-aminoethyl)amine (15.6 μL, 0.102 mmol) at room temperature. The reaction mixture was kept stirring at room temperature for 14 h. The reaction mixture was then filtered through Celite and concentrated to dryness under reduced pressure. The crude residue was purified by silica gel column chromatography (CH_3OH-CH_3CN-HOAc, 1:1:0.05) which gave compound **20** (101 mg, 97% based on tris(2-aminoethyl)amine and recovered **9** (67.4 mg)). $[\alpha]_D$ -14.6° (c = 1.25, H_2O). FAB-MS (glycerol) gave *m/z* (ion, relative intensity): 2069.8 ($[2M+1]^+$, 1.2%), 1035.4 ($[M+1]^+$, 100%); ^{1}H-NMR δ ppm (D_2O): 5.35 (dd, 2H, $J_{3,4}$ = 9.4 Hz, H-3), 5.16 (dd, 2H, $J_{4,5}$ = 10.1 Hz, H-4), 5.09 (d, 2H, $J_{1,2}$ = 8.8 Hz, H-1), 4.45 (ddd, 2H, $J_{6a,6b}$ = 12.4 Hz, H-6a), 4.29 (ddd, 2H, H-6b), 4.19 (dd, 2H, $J_{2,3}$ = 10.4 Hz, H-2), 4.09 (ddd, 2H, $J_{5,6a}$ =1.8, $J_{5,6b}$ = 4.0 Hz, H-5), 3.34 (t, 4H, J = 6.5 Hz, CH_2 of tris(2-aminoethyl)amine), 3.12 (t, 2H, J_{gem} = 6.1 Hz, CH_2 of tris(2-aminoethyl)amine), 2.86 (t, 2H, J_{gem} = 6.1 Hz, CH_2 of tris(2-aminoethyl)amine), 2.73 (t, 4H, J_{gem} = 6.5 Hz, CH_2 of tris(2-aminoethyl)amine), 2.58 (t, 4H, J_{gem} = 6.3 Hz, CH_2 of succinyl), 2.51 (t, 4H, J_{gem} = 6.3 Hz,CH_2 of succinyl), 2.19, 2.14, 2.11, 2.04 (s, 24H, OAc, NHAc). ^{13}C-NMR δ ppm (D_2O): 174.0, 173.2, 172.7, 172.3 (C=O of AcO, NHAc), 102.3 (C-1), 72.4 (C-3), 71.0 (C-5), 67.9 (C-4), 61.5 (C-6), 51.9 (CH_2 of tris(2-aminoethyl)amine), 51.0 (C-2), 50.2 (CH_2 of tris(2-

aminoethyl)amine), 36.66 (CH_2 of tris(2-aminoethyl)amine), 30.2 (CH_2 of succinyl), 27.6 (CH_2 of succinyl), 22.8, 21.6, 19.7, 19.6 (OAc, NHAc).

Treatment of 20 with fluorescein isothiocyanate (21): Compound **20** (104 mg, 0.1 mmol) in 20 mL of a mixture of methanol and tetrahydrofuran (1/1) was added fluorescein isothiocyanate (116 mg, 0.3 mmol) and 1M aqueous sodium hydroxide (150 µL). The reaction mixture was kept at room temperature for 26 h. Then the reaction mixture was concentrated to dryness under reduced pressure. The crude residue was purified by silica gel column chromatography using dichloromethane/methanol/acetic acid (4/1/0.05) as eluent to give fluorescein-labeled divalent compound **21** (88 mg, 62%) having Rf = 0.32 (CH_2Cl_2/MeOH/AcOH, 7.7: 2.5: 0.2). $[\alpha]_D$ -46.6° (c = 0.42, MeOH). UV (λmax, MeOH, nm) 483; FAB-MS (glycerol) gave *m/z* (ion, relative intensity) for $C_{63}H_{77}N_9O_{27}S$: 1425.4 ($[M+1]^+$, 0.7 %). ^{1}H-NMR δ ppm (DMSO-d_6): 8.33-6.54 (m, 9H, Ar), 5.13 (dd, 2H, $J_{3,4}$ = 9.5 Hz, H-3), 4.85 (dd, 2H, $J_{4,5}$ = 9.6 Hz, H-4), 4.82 (d, 2H, $J_{1,2}$ = 9.0 Hz, H-1), 4.21 (ddd, 2H, H-6a), 4.19-3.72 (m, 6H, H-6b, H-5, H-2), 3.55 (t, 2H, J_{gem} = 6.5 Hz, CH_2 of tris(2-aminoethyl)amine), 3.12 (t, 4H, CH_2 of tris(2-aminoethyl)amine), 2.61 (t, 2H, CH_2 of tris(2-aminoethyl)amine), 2.50 (t, 4H, CH_2 of tris(2-aminoethyl)amine), 2.45 (t, 4H, CH_2 of succinyl), 2.30 (t, 4H, CH_2 of succinyl), 2.01, 1.99, 1.95, 1.94, (4s, 12H, OAc, NHAc). ^{13}C-NMR δ ppm (DMSO-d_6): 180.3, 177.9, 170.1, 169.7, 169.5, 169.3 (C=O), 168.6-102.2 (17xC, Ar), 103.5 (C-1), 72.4 (C-3), 70.8 (C-5), 68.3 (C-4), 61.7 (C-6), 53.4 (CH_2 of tris(2-aminoethyl)amine), 52.4 (CH_2 of tris(2-aminoethyl)amine), 51.7 (C-2), 37.0 (CH_2 of tris(2-aminoethyl)amine), 37.0 (CH_2 of tris(2-aminoethyl)amine), 36.4 (CH_2 of succinyl), 27.6 (CH_2 of succinyl), 22.8, 20.5, 20.4, 20.3 (OAc, NHAc).

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