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4 **Preparation of S-Glycoside Surfactants and Cysteine Thioglycosides Using**
5 **Minimally Competent Lewis Acid Catalysis**
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16 Lajos Z. Szabó, Dillon J. Hanrahan, Evan M. Jones, Erin Martin,
17 Jeanne E. Pemberton and Robin Polt*
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26 Department of Chemistry and Biochemistry
27 BIO5
28

29 The University of Arizona
30 1306 E. University Blvd.
31 Tucson, AZ 85721
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34
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38

39 *Corresponding author
40 polt@u.arizona.edu
41
42 (520) 370-2654
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Table of Contents/Abstract Graphic.

Abstract.

Here we report a method for the preparation of anomerically pure β -S-glycopyranosides (1,2-*trans*-glycosides) from the corresponding peracetate donors. S-glycosylation was performed in CHCl_3 at reflux in the presence of a catalytic amount of InBr_3 . Deacylation of the intermediate peracetates were achieved under Zemplén conditions. Five pyranose examples, monosaccharides D-glucose and D-galactose and disaccharides cellobiose, maltose, and lactose, were used as donors, and five thiols including an α/ω dithiol and Fmoc-L-cysteine were used as acceptors. Melting points, high res MS, $[\alpha]_D$ and NMR data (^1H and ^{13}C , including COSY, HSQC and HMBC) are reported for compounds not previously described.

Keywords: thioglycoside, S-glycosidic linkage, glycolipid, InBr_3 , surfactant, glycosylated cysteine

Glycolipids represent a diverse class of compounds typically found in biological membranes¹ and are regarded as environmentally friendly bio-surfactants with broad use in cosmetics, food, environmental and other industrial applications,² as well as potential therapeutic drug delivery systems.^{3, 4} Despite high surface activity,⁵ high biodegradability, and low toxicity, their use is still hampered by a lack of knowledge regarding physical properties, including phase behavior. Even less is known about the S-linked thioglycosides. Thioglycosides (S-glycosides)⁶ may be regarded as relatively inert moieties *in vivo*,⁷ or as chemical intermediates that can be oxidatively activated⁸ to generate glycoside donors.⁹⁻¹¹ Thioglycosides were previously prepared from monosaccharide peracetates using a molar excess of BF₃•Et₂O or SnCl₄ as promoters by Kihlberg in the 1990's.¹² Here we disclose the *catalytic* use of a much weaker Lewis acid, InBr₃,¹³ which we dub a “*minimally competent*” Lewis acid.¹⁴ It should be noted that InBr₃ and other Lewis acids have been used in conjunction with the O-glycosylation of trichloroacetimidate donors.¹⁵ We have previously reported the catalytic use of InBr₃¹⁴ and other Lewis acids¹⁶ to generate O-glycosides from weakly electrophilic peracetate sugars.

The surfactant β-S-octyl-glucopyranoside and several of its analogues were previously prepared by S-alkylation of 2-(2,3,4,6-tetra-O-acetyl-β-glucopyranosyl) thiopseudourea•HBr by Saito and Tsuchiya.¹⁷ The present approach (**Figure 1**) seems to be superior, both in terms of the number of steps and in overall yield. β-D-Glucopyranose peracetate **1** was treated with two molar equivalents of 1-octanethiol in CHCl₃ at reflux for 1 hour in the presence of 1% InBr₃, to form the desired β-peracetate, which was purified by short column chromatography (SiO₂). No α anomer was

observed. We propose that the formation of the S-glycoside occurs *via* a similar route to the previously published O-glycoside example, resulting in only the β -S-glycoside.¹⁴ The peracetate product was deprotected using Zemplén deacylation¹⁸ to provide the desired surfactant.¹⁷ The glycosylation produced a similar yield in PhCH₃, but the reaction in CH₃CN decreased the yield.

Figure 1. Preparation of β -Thioglycosides.

Several mono- and disaccharide peracetates (**1-5**) and thiols (**6-11**, **Table 1**) were subjected to the reaction conditions to provide a variety of S-glycosides. Bolaamphiphile **15a** and mono-glycoside **16a** were prepared by adjusting the stoichiometry of the glycosyl donor and acceptor to favor either mono- or di-glycosylation of the α/ω dithiol.

Table 1. Results of InBr₃ Promoted Glycosylations

Thioglycosides from InBr₃ promoted glycosylations of various thiolipids (**6-10**) and Fmoc-L-cysteine (**11**) with five peracetylated mono- and disaccharides (**1-5**) to produce compounds **12-33**. After Zemplén deacylation of the lipids, compounds **12a-28a** were isolated and characterized. Compounds **12a**,^{19, 20} **13a**,²⁰ **14a**,²⁰ **17a**,^{19, 21} **18a**,²² **19a**,²² **20a**,²³ **22a**,²⁴ **23a**,²³ **24a**,²⁵ **25a**,²⁴ **26a**,²³ **28a**,²⁶ **29**,²⁷ and **30**^{28, 29} have been previously reported using alternate reaction conditions. Previously reported deacylated thioglycosides are noted in Lit. % indicates combined yields.

Donors	Sulfide Acceptors																							
	R'=	 6				 7				 8				 9				 10				 11		
	R=	Ac	H	Lit	%	Ac	H	Lit	%	Ac	H	Lit	%	Ac	H	Lit	%	Ac	H	Lit	%	Ac	Lit	%
 1		12	12a	^{19,20}	81	13	13a	²⁰	88	14	14a	²⁰	84	15	15a	—	86	16	16a	—	69	29	²⁷	72
 2		17	17a	^{19,21}	89	18	18a	²²	90	19	19a	²²	92									30	^{28, 29}	81
 3		20	20a	²³	88	21	21a	—	66	22	22a	²⁴	76									31	—	65
 4		23	23a	²³	85	24	24a	²⁵	87	25	25a	²⁴	88									32	—	85
 5		26	26a	²³	87	27	27a	—	82	28	28a	²⁶	80									33	—	83

Similar reaction conditions were applied to Fmoc-L-cysteine. Glycosylation of neurologically active endogenous peptides has been applied to a number of systems,³⁰ although these modifications have typically been performed by O-glycosylation. O-glycosyl linkages have the drawback of being susceptible to enzymatic degradation and hydrolysis. Therefore, S-glycosylated amino acid building blocks compatible with common solid-phase peptide synthesis conditions are an attractive target. Fmoc-L-Cys(S-D-Gal)-OH has been previously reported by Kamerling *et al*,²⁹ and Pieters *et al*,²⁸ although their methodology required the use of excess SnCl₄. We report similar yields using *catalytic quantities* of the minimally competent Lewis acid activator, and show the broad applicability of this approach through conjugation of five mono- and disaccharide glycosyl donors **29-33** to Fmoc-L-cysteine (**Figure 2**).

The importance of this study is twofold. First, only catalytic amounts of the Lewis acid promoter are required. Secondly, weakly electrophilic peracetates, generally regarded as synthetic intermediates rather than reactive donors, are suitable. This reduces the number of steps and greatly expands the application of this methodology. We have not observed the corresponding α -thioglycosides in any of the reactions.

Figure 2. Preparation of L-Cysteine β -thio-Glycosides.

1. Experimental Section

1.1 Materials and Instrumentation. ^1H and ^{13}C NMR spectra were recorded on a Bruker 400 (400 MHz) spectrometer using CDCl_3 and D_3COD as solvents with TMS as the internal standard. All NMR spectra were analyzed and interpreted using MestReNova® software. First-order chemical shifts and coupling constants were obtained from 1D spectra; proton and carbon resonances were assigned from COSY, HSQC and HMBC experiments. Mass spectrometry was achieved on a Thermo Finnigan LCQ Deca with positive and negative detection and a 1:1 $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ solvent system. Optical rotation measurements were performed using a JASCO P-1010 polarimeter using the Na D line (589 nm) in MeOH at room temperature, and specific rotation was determined using the JASCO software package. For flash chromatography Silia *Flash* P60 Silica Gel, 40-63 μm , 60 Å (Silicycle, R1200308) was employed and a Biotage, Inc. Horizon HPFC system was utilized.

Preparative HPLC separations were performed on a Gilson HPLC using a Phenomenex C-18 reverse phase column (1000 Å, 50 x 250 mm) with a flow rate of 25 mL/min and detection at 280 nm. Solvent systems A: 5% acetonitrile in 0.1% aqueous F_3CCOOH and B: 80% acetonitrile in 0.1% aqueous F_3CCOOH were used. A Dikma Technologies Inspire C-18 column (4.6 x 250 mm) and a flow rate of 1.0 mL/min was used for analytical HPLC separations. Thin layer chromatography (TLC) was performed on silica gel 60 F254 (Merck) with detection by UV light and charring with H_2SO_4 . Solvents were dried and distilled according to literature procedures or purchased as anhydrous solvents from a manufacturer. All chemicals were purchased from Aldrich Chemical Company, Acros Chemicals, Chem-Impex International, or EMD Chemicals.

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7 **1.2 Alkyl- β -1-thio-D-glycopyranoside (12a-14a, 16a-28a).** Peracetyl- β -D-
8 glycopyranose (**1-5**, 10 mmol), InBr_3 (106 mg, 0.3 mmol, 0.03 eq), and alkane-1-thiol
9 (20 mmol, 2 eq) were suspended in CHCl_3 (7 mL). The reaction mixture was heated to
10 reflux with magnetic stirring. The course of the reaction was followed by TLC
11 (hexane:EtOAc 1:1). After 1 hour the peracetate starting material could no longer be
12 detected by TLC. The reaction was cooled to RT, diluted with CH_2Cl_2 (20 mL) and this
13 solution was applied to a short column (4 cm x 12 cm) of SiO_2 . The HOAc that was
14 formed from the reaction and the excess alkane-1-thiol were eluted with hexanes. The
15 desired thio-glycoside peracetate was eluted with hexanes:EtOAc 7:3. The fractions
16 containing the desired material were combined, concentrated *in vacuo*, and the
17 intermediate peracetates (**12-14**, **16-28**) were characterized by 1D, 2D, ^1H -NMR and
18 ^{13}C NMR in CDCl_3 (in Tables).
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36 Zemplén's method was used to deprotect the peracetates.¹⁸ The thio-
37 glycopyranoside peracetates were dissolved in anhydrous CH_3OH (100 mL) and the
38 basicity was adjusted to pH = 9 by addition NaOCH_3 . After 16 hours the deacetylation
39 was deemed complete by TLC. Dowex® resin (H^+ form) was added until the pH was
40 slightly acidic, the resin was filtered off, and the solution was concentrated, to provide
41 **12a-14a**, **16a-28a**, which were characterized.
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53 **1.3 1,10 Dithio- β -D-glucopyranosyl-decane (15a)** Peracetyl- β -D-glucopyranose (25
54 mmol), InBr_3 (3 mmol, 0.03 eq), and decane-1,10-dithiol (10 mmol, 1 eq) were reacted
55 using the previous method to provide **15a**. M.p. 124-132 °C (crystallization from MeOH
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– Et₂O), [α]_D -36.5° (c 0.13, in MeOH), MS (ESI HRMS) for C₂₂H₄₂O₁₀S₂ (+Na) 553.21116, found 553.21195 [M+Na]⁺.

1-Thio-β-D-glucopyranosyl-10-thiol-decane (16a) M.p. 95-97°C (crystallization from MeOH – Et₂O), [α]_D -17.8° (c 0.20, in MeOH), MS (ESI HRMS) for C₁₆H₃₂O₅S₂ (+Na) 391.15834, found 391.15875 [M+Na]⁺.

Decyl-β-thio-cellobioside (21a) M.p. 200-214°C (crystallization from MeOH – Et₂O), [α]_D -22.1° (c 0.26, in MeOH), MS (ESI HRMS) for : C₂₂H₄₂O₁₀S (+Na) 521.23909, found 521.24009 [M+Na]⁺.

Decyl-β-thio-lactoside (27a) M.p. Decomp. ~200°C (crystallization from MeOH – Et₂O), [α]_D -18.3° (c 0.40, in MeOH), MS (ESI HRMS) for C₂₂H₄₂O₁₀S (+Na) 521.23909, found 521.24000 [M+Na]⁺.

1.4 N^α-Fluoren-9-ylmethoxycarbonyl-S-(peracetyl-β-D-glyco-pyranosyl)-L-cysteine (29-33). Peracetyl-β-D-glycopyranose (0.6 mmol, 2eq), InBr₃ (21 mg, 0.06 mmol, 0.2 eq), and N^α-fluoren-9-ylmethoxycarbonyl-L-cysteine (**11**, 103 mg, 0.3 mmol, 1 eq) were suspended in CHCl₃ (1.5 mL). The reaction mixture was heated to reflux with magnetic stirring. The course of the reaction was followed by analytical HPLC. After 1 hour HPLC indicated >80% conversion (**see Table 1**) of Fmoc-Cys(SH)-OH to the desired product. Additional reaction time yielded no further reaction progress that could be detected by HPLC. The reaction mixture was concentrated *in vacuo*, and the residue was purified by preparative HPLC on a C₁₈ reverse phase column with H₂O-CH₃CN gradient. The appropriate fractions were freeze-dried and the solid residue was characterized.

***N*^α-Fluoren-9-ylmethoxycarbonyl-S-(peracetyl-β-D-cellobiosyl)-L-cysteine (31)**

Phase transition point 104-105 °C (lyophilization from CH₃CN/H₂O), [α]_D -20.7° (c 0.15, in MeOH), MS (ESI HRMS) for C₄₄H₅₁NO₂₁S (+Na) 984.25665, found 984.25901 [M+Na]⁺.

***N*^α-Fluoren-9-ylmethoxycarbonyl-S-(peracetyl-β-D-maltobiosyl)-L-cysteine (32)**

Phase transition point 103-104 °C (lyophilization from CH₃CN/H₂O), [α]_D +21.5° (c 0.14, in MeOH), MS (ESI HRMS) for C₄₄H₅₁NO₂₁S (+Na) 984.25665, found 984.25866 [M+Na]⁺.

***N*^α-Fluoren-9-ylmethoxycarbonyl-S-(peracetyl-β-D-lactosyl)-L-cysteine (33)** Phase transition point 109-110 °C (lyophilization from CH₃CN/H₂O), [α]_D -20.6 (c 0.17, in MeOH), MS (ESI HRMS) for C₄₄H₅₁NO₂₁S (+Na) 984.25665, found 984.25817 [M+Na]⁺.

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