ORGANIC LETTERS

2008
Vol. 10, No. 8
1573−1576

De Novo Synthesis of a D-Galacturonic Acid Thioglycoside as Key to the Total Synthesis of a Glycosphingolipid from *Sphingomonas yanoikuyae*

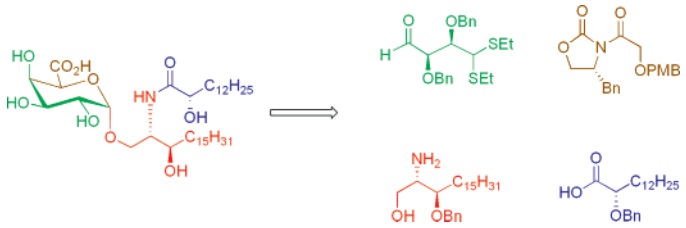
Pierre Stallforth, Alexander Adibekian, and Peter H. Seeberger*

Laboratory for Organic Chemistry, Swiss Federal Institute of Technology (ETH) Zurich, Wolfgang-Pauli-Strasse 10, HCI F312 CH-8093 Zurich, Switzerland

seeberger@org.chem.ethz.ch

Received February 4, 2008

ABSTRACT



A concise synthesis of a differentially protected D-galacturonic acid (D-GalA) thioglycoside and the construction of a potent immunomodulating glycosphingolipid are described. The key steps of the synthesis are an Evans aldol reaction between a C4 aldehyde and a PMB-protected glycolyloxazolidinone as well as a tandem-PMB-deprotection/cyclization to thioglycosides. The key glycosylation step is optimized by varying the anomeric leaving group, the activating agent, and the solvent system.

D-Galacturonic acid (D-GalA) constitutes the most abundant, naturally occurring uronic acid. As a α-(1−4)-linked homopolymer, homogalacturonane, it forms the major constituent of pectin,[1] and as a α-(1−4)-linked species it is also present in the immunogenic lipopolysaccharides of various pathogenic bacteria.[2] Furthermore, several bacterial glycosphingolipids contain D-GalA that forms an α-glycosidic linkage to a ceramide (e.g., **A**, Scheme 1).[3] These glycolipids exhibit an intriguing biological activity, since they are recognized by human natural killer T (NKT) cells, after binding to CD1d, and induce immune response.[4] NKT cell activation has been studied in the past decade in the context of autoimmune diseases and cancer research.[5] Efficient syntheses of α-(1−4)-linked D-GalA containing biologically active molecules as molecular probes for biomedical investigations are thus needed. Although oligosaccharide synthesis has been subjected to a large number of improvements, a typical monosaccharide building block synthesis still mainly relies on tedious protection and deprotection steps in order to access the required protecting group pattern.[6] De novo synthesis[7] circumvents these disadvantages by the use of preprotected linear fragments and not starting from the corresponding unprotected sugar.

Here, we report a short and high yielding synthesis of differentially protected D-galacturonic acid thioglycosides as part of our ongoing program directed at the de novo synthesis of selectively protected carbohydrate building blocks.[8] Our building block was then used to synthesize a highly im-

(1) Willats, W. G. T.; McCartney, L.; Mackie, W.; Knox, J. P. *Plant Mol. Biol.* **2001**, *47*, 9.

(2) (a) Katzenellenbogen, E.; Kocharova, N. A.; Zatonsky, G. V.; Korzeniowska-Kowal, A.; Shashkov, A. S.; Knirel, Y. A. *Carbohydr. Res.* **2003**, *338*, 2153. (b) Rozalski, A.; Sidorczyk, Z.; Kotelko, K. *Microbiol. Mol. Biol. Rev.* **1997**, *61*, 65.

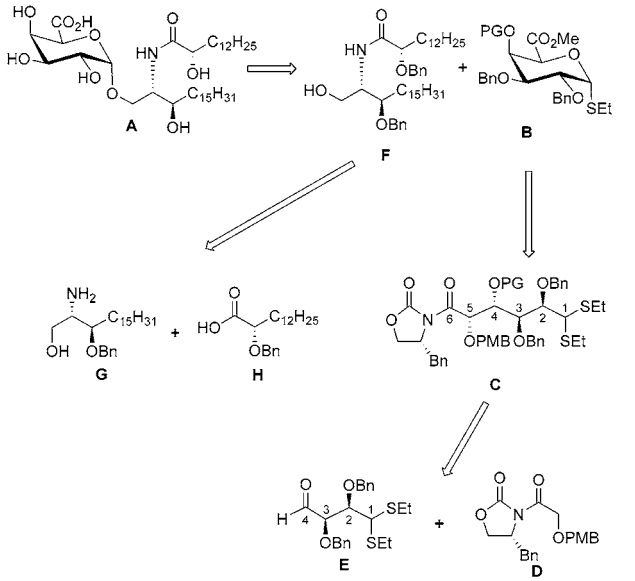
(3) (a) Hakomori, S. I. *Annu. Rev. Biochem.* **1981**, *50*, 733. (b) Wu, D.; Fujio, M.; Wong, C. H. *Bioorg. Med. Chem.* **2008**, *16*, 1073.

(4) (a) Kinjo, Y.; Wu, D.; Kim, G.; Xing, G.-W.; Poles, M. A.; Ho, D. D.; Tsuji, M.; Kawahara, K.; Wong, C.-H.; Kronenberg, M. *Nature* **2005**, *434*, 520. (b) Wu, D.; Xing, G. W.; Poles, M. A.; Horowitz, A.; Kinjo, Y.; Sullivan, B.; Bodmer-Narkevitch, V.; Plettenburg, O.; Kronenberg, M.; Tsuji, M.; Ho, D. D.; Wong, C. H. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 1351.

(5) Kronenberg, M. *Annu. Rev. Immunol.* **2005**, *23*, 877.

10.1021/ol800227b CCC: $40.75
Published on Web 03/26/2008

Scheme 1. Retrosynthesis of Glycosphingolipid **A**



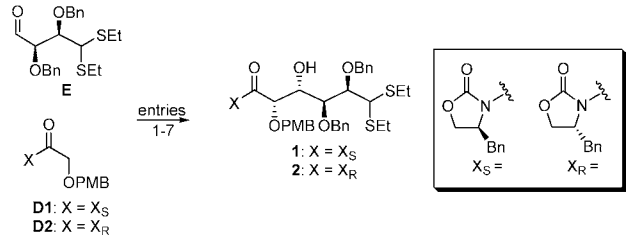
munogenic galacturonosylceramide (**A**) from *Sphingomonas yanoikuyae*.[9]

The retrosynthetic plan is presented in Scheme 1. Glycoconjugate **A** can be dissected into benzyl-protected ceramide **F** and D-GalA building block **B**. For the synthesis of the ceramide part, sphingosine **G** and fatty acid **H** are connected by amide coupling. Acid **H** is derived from 1-tetradecene via a dihydroxylation/oxidation sequence, whereas amino alcohol **G** is prepared according to Howell et al.[10] starting from the Weinreb amide of *N*-Boc-L-serine.[11] Key thioglycoside **B** will be formed by cyclization of thioacetal **C**, following two protection−deprotection steps. The linear C6-acetal **C** can be dissected into monoprotected C4-aldehyde **E** and known glycolate oxazolidinone **D**[12] by a retroaldol reaction. In order to establish the desired configuration on C-4 and C-5, a 2,3-syn-3,4-anti-selective Evans aldol reaction[13−15] is planned.

The doubly benzylated aldehyde **E** can be readily obtained from commercially available L-arabinose ethyl dithioacetal in four steps.[8a]

Our synthetic efforts started with trials focusing on the 2,3-syn-3,4-anti-selective Evans aldol reaction as the key step in the building block synthesis (Table 1). In principle, both

Table 1. Evans Aldol Reaction



entry	donor	conditions	dr	yield (%)
1	**D1**	Bu_2BOTf, NEt_3, toluene[a,14]		np
2	**D1**	$TiCl_4$, DIPEA, CH_2Cl_2, 0 °C[15]		np
3	**D1**	$TiCl_4$, DIPEA, CH_2Cl_2[b,14]		np
4	**D2**	LDA, CH_2Cl_2[b]		np
5	**D2**	LDA, toluene[b]	2.3:1	49
6	**D2**	LDA, Et_2O[b]	4.6:1	50
7	**D2**	LDA, THF[b]	4.9:1	90

[a] Temperature: −50 to −30 °C. [b] −78 °C.

enantiomeric forms of the oxazolidinone auxiliary can be employed to construct the desired diastereomer.[13] The desired aldol product was obtained after considerable trials by an LDA-promoted Evans aldol reaction at −78 °C with auxiliary **D2**[11] in toluene (Table 1, entry 7). Aldol **2** was obtained in a 90% yield and 4.9:1 diastereoselectivity, when THF as solvent was employed (entry 7). This transformation proceeds on a gram scale without noticeable loss of yield or selectivity.

With aldol product **2** in hand, further steps in the building block synthesis were conducted (Scheme 2). Both acetylation and levulination[16] of the free hydroxyl group proceeded

(6) (a) Paulsen, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 155. (b) Danishefsky, S. J.; McClure, K. F.; Randolph, J. T.; Ruggeri, R. B. *Science* **1993**, *260*, 1307. (c) Sears, P.; Wong, C. H. *Science* **2001**, *291*, 2344. (d) Plante, O. J.; Palmacci, E. R.; Seeberger, P. H. *Science* **2001**, *291*, 1523. (e) Davis, B. G. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2137. (f) Werz, D. B.; Ranzinger, R.; Herget, S.; Adibekian, A.; von der Lieth, C.-W.; Seeberger, P. H. *ACS Chem. Biol.* **2007**, *2*, 685. (g) Varki, A. *Glycobiology* **1993**, *3*, 97. (h) Roy, R. *Drug Discovery Today*, **2004**, *1*, 327. (i) Dube, D. H.; Bertozzi, C. R. *Nat. Rev. Drug Discovery* **2005**, *4*, 477. (j) Garegg, P. J. *Adv. Carbohydr. Chem. Biochem.* **2004**, *59*, 69. (k) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683. (l) Babu, R. S.; Zhou, M.; O'Doherty, G. A. *J. Am. Chem. Soc.* **2004**, *126*, 3428. (m) Zhou, M.; O'Doherty, G. A. *Org. Lett.* **2006**, *8*, 4339. (n) van den Bos, L. J.; Codée, J. D. C.; Litjens, R. E. J. N.; Dinkelaar, J.; Overkleeft, H. S.; van der Marel, G. A. *Eur. J. Biochem.* **2007**, 3963.

(7) For recent organocatalytic de novo syntheses of partially protected monosaccharides, see: (a) Northrup, A. B.; MacMillan, D. W. C. *Science* **2004**, *305*, 1752. (b) Enders, D.; Grondal, C. *Angew. Chem.* **2005**, *117*, 1235; *Angew. Chem., Int. Ed.* **2005**, *44*, 1210. (c) Utsumi, N.; Imai, M.; Tanaka, F.; Ramasastry, S. S. V.; Barbas, C. F., III. *Org. Lett.* **2007**, *9*, 3445. For reviews on the de novo synthesis of carbohydrates, see: (d) Schmidt, R. R. *Pure Appl. Chem.* **1987**, *59*, 415. (e) Kirschning, A.; Jesberger, M.; Schoning, K.-U. *Synthesis* **2001**, 507. (f) Vogel, P. In *The Organic Chemistry of Sugars*; Levy, D. E., Fugedi, P., Eds.; Taylor and Francis Group/CRC Press: Boca Raton, 2006; Chapter 13, p629.

(8) (a) Timmer, M. S. M.; Adibekian, A.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2005**, *44*, 7605. (b) Adibekian, A.; Bindschadler, P.; Timmer, M. S. M.; Noti, C.; Schutzenmeister, N.; Seeberger, P. H. *Chem. Eur. J.* **2007**, *13*, 4510.

(9) Kawahara, K.; Sato, N.; Tsuge, K.; Seto, Y. *Microbiol. Immunol.* **2006**, *50*, 67.

(10) Ndonye, R. M.; Izmirian, D. P.; Dunn, M. F.; Yu, K. O. A.; Porcelli, S. A.; Khurana, A.; Kronenberg, M.; Richardson, S. K.; Howell, A. R. *J. Org. Chem.* **2005**, *70*, 10260.

(11) Collier, P. N.; Campbell, A. D.; Patel, I.; Raynham, T. M.; Taylor, R. J. K. *J. Org. Chem.* **2002**, *67*, 1802.

(12) Evans, D. A.; Gage, J. R.; Leighton, J. L.; Kim, A. S. *J. Org. Chem.* **1992**, *57*, 1961.

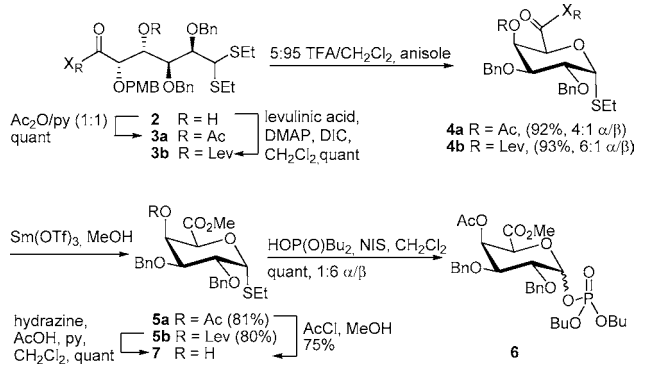
(13) (a) Mahrwald, R. *Modern Aldol Reactions*: Wiley-VCH: Weinheim, 2004. (b) Ager, D. J.; Prakash, I.; Schaad, D. R. *Aldrichim. Acta* **1997**, *30*, 3.

(14) (a) Evans, D. A.; Bartroli, J.; Shih, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 2127. (b) Jones, T. K.; Mills, S. G.; Reamer, R. A.; Askin, D.; Desmond, R.; Volante, R. P.; Shinkai, I. *J. Am. Chem. Soc.* **1989**, *111*, 1157.

(15) Crimmins, M. T.; King, B. W.; Tabet, E. A.; Chaudhary, K. *J. Org. Chem.* **2001**, *66*, 894.

(16) (a) Hassner, A.; Strand, G.; Rubinstein, M.; Patchornik, A. *J. Am. Chem. Soc.* **1975**, *97*, 1614. (b) van Boom, J. H.; Burgers, P. M. J. *Tetrahedron Lett.* **1976**, *17*, 4875.

Scheme 2. Synthesis of **5a**, **5b**, and **6**



smoothly to give the fully protected amides **3a** and **3b** in quantitative yield. Treatment of thioacetal **3a** with 1.8 equiv of anisole and 5% trifluoroacetic acid in dichloromethane resulted not only in the cleavage of the *p*-methoxybenzyl ether, but also in concomitant cyclization. The desired D-galacturonic acid thioglycoside **4a** was afforded after this transformation in 92% yield. Thioacetal **3b** was converted into **4b** in 93% yield employing the same procedure. In both cases, anomeric mixtures were separated by flash column chromatography and the α-product was used in further steps.

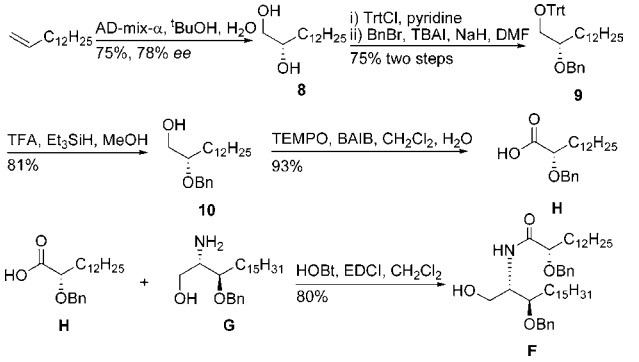
Methanolysis of the chiral auxiliary in the presence of an ester protecting group on C4 posed problems. Attempts to conduct this transformation with sodium methoxide in methanol[17] or catalytic amounts of DMAP in methanol[18] were not successful. In both cases partial *β*-elimination of the C4 ester groups occurred. The reaction proceeded smoothly to yield the methyl esters **5a** and **5b** in 80% and 81% yield, respectively, when samarium(III) triflate in methanol (30 mol %) was employed.[19] No competing cleavage of the ester groups was observed. Thus, both D-galacturonic acid thioglycosides were obtained in only four steps from known[8a] and readily accessible aldehyde **E**.

Thioethyl glycoside **5a** was easily converted to the alternative glycosylating agent, glycosyl phosphate **6**, upon NIS-promoted glycosidation with dibutyl phosphoric acid. Deprotection of the C4 protecting groups to yield thioglycoside **7** proved to be facile. Both levulinoate and acetate esters could be removed, with either hydrazine in acetic acid and pyridine[16b] or in a methanolic HCl solution respectively. Thus, this building block can be used for the assembly of structures containing (1−4)-linked D-GalA.

At this stage, the diagnostic coupling constants[20] served to confirm the D-galacto configuration on α-thioglycoside **5a**.[21]

The synthesis of fatty acid **H** starts from commercially available 1-tetradecene (Scheme 3). Treatment with AD-

Scheme 3. Synthesis of Lipid **F**



mix-α afforded diol **8** in 75% yield.[22] The enantiomeric excess[23] of 78% is in line with observations by Sharpless et al.[22b] for aliphatic alkenes with comparable chain length. Both hydroxyl groups were differentiated by protection of the primary alcohol with triphenylmethyl chloride. The secondary hydroxyl was then benzylated to furnish **9** in 75% yield, over two steps. Trityl cleavage with TFA and triethylsilane in methanol proceeded in 81% yield. Alcohol **10** was subsequently oxidized using catalytic amounts of TEMPO and bisacetoxyiodobenzene (BAIB) as cooxidant to give the corresponding carboxylic acid **H** in 93% yield.[24] Finally, EDCI/HOBt-promoted amide coupling between amine **G** and acid **H** yielded the desired diastereomer of ceramide **F** in 80% yield and at this stage the undesired diastereomer could be separated.

With galacturonic acids **5a** and **5b** and ceramide **F** in hand, glycosylation to yield conjugate **11** was studied (Table 2). The main challenges, when performing these reactions, could be attributed to the relatively poor solubility of ceramide **F** in many solvent systems at low temperature. During these studies, the well-known benefits of ether[25] and the remote anchimeric assistance[26] of C4 esters in galacto-configured systems in obtaining good α-selectivities became again apparent, as omission led to a dramatic increase in *β*-glycoside formation. A compromise between yield and selectivity was found by employing acetyl-protected thioglycoside

(17) Haigh, D.; Birrell, H. C.; Cantello, B. C. C.; Hindley, R. M.; Ramaswamy, A.; Rami, H. K.; Stevens, N. C. *Tetrahedron: Asymmetry* **1999**, *10*, 1335.

(18) Baek, S.; Jo, H.; Kim, H.; Kim, S.; Kim, D. *Org. Lett.* **2005**, *7*, 75.

(19) (a) Evans, D. A.; Coleman, P. J.; Dias, L. C. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2738. (b) Castagner, B.; Leighton, J. L. *Tetrahedron* **2007**, *63*, 5895.

(20) Altona, C.; Haasnoot, C. A. G. *Org. Magn. Reson.* **1980**, *13*, 417.

(21) Characteristic coupling constants between H-4 and H-5 (J = 1.3 Hz) as well as between H-3 and H-4 (J = 3.4 Hz) unambiguously reveal the desired galacto configuration of building block **5a**. NOE correlations between H-5 and H-4 as well as between H-4 and H-3 confirmed the assignment. NOE spectra can be found in the Supporting Information.

(22) (a) He, L.; Byun, H. S.; Bittman, R. *J. Org. Chem.* **2000**, *65*, 7618. (b) Kolb, H. C.; Vannieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483.

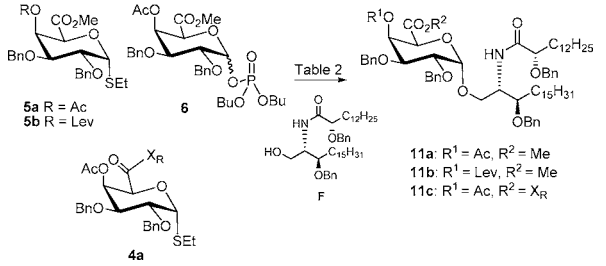
(23) The % ee was determined by conversion of the diol to the bis ester with (−)-*R*-Mosher's reagent and subsequent ^{1}H NMR analysis.

(24) (a) Friestad, G. K.; Marie, J. C.; Deveau, A. M. *Org. Lett.* **2004**, *6*, 3249. (b) Kloth, K.; Bruenjes, M.; Kunst, E.; Joege, T.; Gallier, F.; Adibekian, A.; Kirschning, A. *Adv. Synth. Catal.* **2005**, *347*, 1423.

(25) Wulff, G.; Röhle, G. *Angew. Chem., Int. Ed. Engl.* **1974**, *13*, 157.

(26) (a) Miljkovic, M.; Yeagley, D.; Deslongchamps, P.; Dory, Y. L. *J. Org. Chem.* **1997**, *62*, 7597. (b) Demchenko, A. V.; Rousson, E.; Boons, G.-J. *Tetrahedron Lett.* **1999**, *40*, 6523. (c) Cheng, Y.-P.; Chen, H.-T.; Lin, C.-C. *Tetrahedron Lett.* **2002**, *43*, 7721.

Table 2. Glycosylation Studies



entry	donor	conditions[a]	product	α/β[b]	yield (%)
1[d]	**4a** (α)	NIS,[f] TBSOTf[g]	**11c**		<10
2[d]	**4a** (α)	NIS,[f] TfOH[g]	**11c**	1.0:1	45
3[d]	**5b** (α)	NIS,[f] TfOH[g]	**11b**	2.0:1	49
4[d]	**5b** (α)	DMTST[h]	**11b**		<10
5[d]	**6** (α/β)	TMSOTf[e]	**11a**[c]	2.1:1	96
6[d]	**5a** (α)	NIS,[f] TfOH[g]	**11a**[c]	3.7:1	85
7[e]	**5a** (α)	NIS,[f] TfOH[g]	**11a**[c]	4.2:1	85

[a] Temperature −10 °C and 1.5 equiv of donor. [b] Ratio determined by ^{1}H NMR. [c] The α- and β-products could be separated by column chromatography. [d] Et_2O−dichloroethane (1:1). [e] Dioxane−toluene (3:1). [f] 1.5 equiv. [g] 0.15 equiv. [h] 6 equiv.

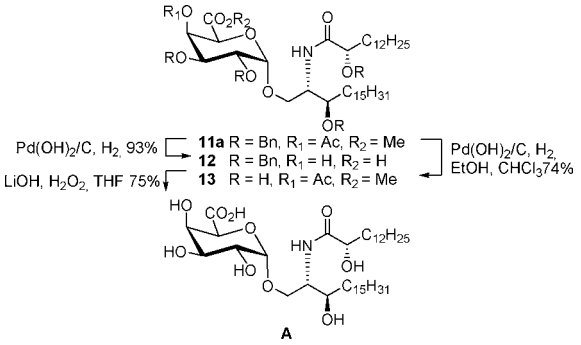
5a and the NIS/TfOH[27] activator system. Thus, the product **11a** was isolated in 85% yield and 4.2:1 selectivity with dioxane/toluene (3:1) as solvent. Both anomers were separated by flash column chromatography. To the best of our knowledge, this result represents the most α-selective glycosylation reaction between a galacturonic acid building block and a ceramide reported so far.[28]

Having optimized the glycosylation reaction, all protecting groups had to be removed. The initial plan was to cleave first the acetate and the methyl ester of glycoconjugate **11a** in one step using lithium hydroperoxide in THF, followed by removal of the benzyl groups with $Pd(OH)_2$/C. The first reaction proceeded in 93% yield, yet after hydrogenolysis with either Pd/C or $Pd(OH)_2$/C the desired alcohol **A** could not be isolated (Scheme 4). The order of the two deprotection steps was then inverted and debenzylation using Pearlman's catalyst in ethanol/chloroform was performed first to yield

(27) Veebham, G. H.; van Leeuven, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331.

(28) For α-selective glycosylations with galacturonic acid, see: Nolting, B.; Boye, H.; Vogel, C. *J. Carbohydr. Chem.* **2000**, *19*, 923.

Scheme 4. Global Deprotection of Glycosphingolipid **A**



ester **13** in 74% yield. Finally, the acetate and methyl ester of **13** were removed using lithium hydroperoxide in THF, to yield fully deprotected glycosphingolipid **A**.

The chemistry presented here offers a convenient and high-yielding route for the preparation of suitably protected building blocks of D-galacturonic acid. Our strategy for the de novo synthesis is based on the convergent connection of linear synthetic intermediates, thus regioselective protection steps as well as anomeric protection are no more necessary. Consequently, orthogonally protected D-GalA thioglycoside **5a** is obtained from known and easy available aldehyde **E** in only four steps and 56% overall yield.

These results should facilitate future syntheses of this important class of immunological probes.

Application of the de novo strategy for rapid and efficient preparation of other orthogonally protected carbohydrate building blocks is currently under investigation and will be reported in due course.

Acknowledgment. This research was supported by ETH Zurich (TH Grant 16 06-3), a Fellowship from Studienstiftung des deutschen Volkes (to P.S.), and a Kekulé Fellowship from the Fonds der Chemischen Industrie (to A.A.).

Supporting Information Available: Experimental procedures and spectral characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL800227B