JOC*Note*

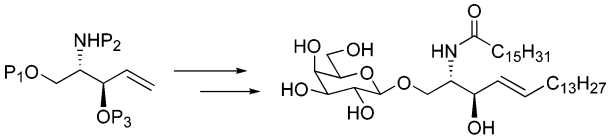
Synthesis of the Glycosphingolipid β-Galactosyl Ceramide and Analogues via Olefin Cross Metathesis

Anand Narain Rai and Amit Basu*

Department of Chemistry, Brown University, Box H, Providence, Rhode Island 02912

abasu@brown.edu

Received May 28, 2005

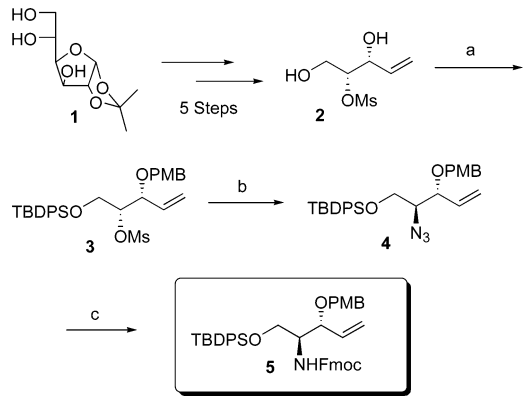


The preparation of the glycosphingolipid galactosyl ceramide from an orthogonally protected five-carbon building block is described. The main chain of the lipid is installed via a highly stereoselective olefin cross metathesis reaction. The methodology permits the facile preparation of glycolipids which vary in the length of the main carbon chain.

The glycosphingolipid β-galactosyl ceramide (β-GalCer) is abundant in the myelin sheath of both the central and peripheral nervous system. β-GalCer isolated from myelin consists of a variety of lipids, which vary in chain length, olefination, and hydroxylation state of the hydrocarbons in the main and acyl chains.[1] These lipids are also collectively referred to as cerebroside. A carbohydrate−carbohydrate interaction between cerebroside and cerebroside sulfate, the 3-sulfate derivatives of cerebroside, mediates the compaction of the myelin sheath.[2] Defective formation and/or compaction of myelin is implicated in a variety of pathologies, including multiple sclerosis, Krabbe's and metachromatic leukodystrophies (MLD), and congenital hypomyelination.[3] Additional roles for β-GalCer include its function as a ligand for the HIV-1 viral glycoprotein gp120, mediating viral entry into epithelial cells.[4] β-GalCer has been suggested as a possible ligand for the adhesion of *Helicobacter pylori* to cells in the gastric system.[5]

Our interest in β-GalCer stems from our ongoing studies of glycolipid carbohydrate−carbohydrate interactions.[6] As part of this work, we required a modular synthetic approach toward β-GalCer and related glycosphingolipids. We recently reported a cross metathesis route for the preparation of the sphingolipid 4,5-unsaturated lipid chain and demonstrated an application of this methodology for the synthesis of ceramide.[7,8] In this paper we present a cross metathesis approach for the synthesis of β-GalCer and its analogues.[9]

SCHEME 1[a]



[a] Conditions: (a) (i) tBuPh$_2$SiCl, imidazole, 61%; (ii) p-MeO-benzyl trichloroacetimidate, La(OTf)$_3$, 81%. (b) (i) NaN$_3$, Bu$_4$NCl, DMF, 90 °C; (ii) tBuPh$_2$SiCl, imidazole, 67% over two steps. (c) (i) Zn, NH$_4$Cl, MeOH, 83%; (ii) Fmoc-Cl, Hunig's base, CH$_2$Cl$_2$, 76%.

The centerpiece of the cross metathesis synthetic route is the orthogonally protected building block **5** (Scheme 1). Our previously reported preparation of **5** used diethyl tartrate as the starting material.[7] While the use of tartrate permits access to both enantiomers of sphingolipids, we now report a significantly improved method for the preparation of **5** starting from the mesylate diol **2**. The synthesis of **2** has been reported by Bundle and co-workers in a patent application.[10] Following these procedures, we prepared **2** on a multigram scale without

(1) (a) Stoffel, W.; Bosio, A. *Curr. Opin. Neurobiol.* **1997**, *7*, 654−661. (b) Curatolo, W. *Biochim. Biophys. Acta* **1987**, *906*, 137−160.

(2) Boggs, J. M.; Wang, H. M.; Gao, W.; Arvantis, D. N.; Gong, Y. P.; Min, W. X. *Glycoconjugate J.* **2004**, *21*, 97−110.

(3) (a) Kolter, T.; Sandhoff, K. *Angew. Chem., Int. Ed.* **1999**, *38*, 1532−1568. (b) Ohler, B.; Revenko, I.; Husted, C. *J. Struct. Biol.* **2001**, *133*, 1−9.

(4) (a) Villard, R.; Hammache, D.; Delapierre, G.; Fotiadu, F.; Buono, G.; Fantini, J. *ChemBioChem* **2002**, *3*, 517−525. (b) Nolting, B.; Yu, J. J.; Liu, G.; Cho, S. J.; Kauzlarich, S.; Gervay-Hague, J. *Langmuir* **2003**, *19*, 6465−6473.

(5) Tang, W.; Seino, K.; Ito, M.; Konishi, T.; Senda, H.; Makuuchi, M.; Kojima, N.; Mizuochi, T. *FEBS Lett.* **2001**, *504*, 31−35.

(6) (a) Santacroce, P. V.; Basu, A. *Angew. Chem., Int Ed.* **2003**, *42*, 95−98. (b) Santacroce, P. V.; Basu, A. *Glycoconjugate J.* **2004**, *21*, 89−95.

(7) Rai, A. N.; Basu, A. *Org. Lett.* **2004**, *6*, 2861−2863.

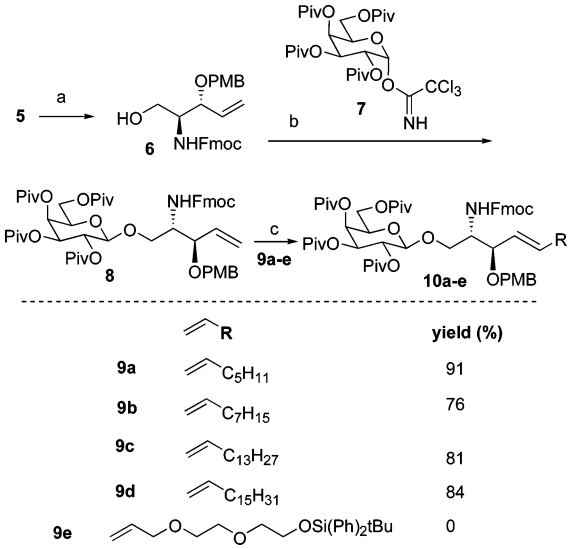
(8) For other approaches to sphingolipids involving olefin cross metathesis, see: (a) Hasegawa, H.; Yamamoto, T.; Hatano, S.; Hakogi, T.; Katsumura, S. *Chem. Lett*. **2004**, *33*, 1592−1593. (b) Singh, O. V.; Kampf, D. J.; Han, H. *Tetrahedron Lett*. **2004**, *45*, 7239−7242. (c) Torssell, S.; Somfai, P. *Org. Biomol. Chem*. **2004**, *2*, 1643−1646.

(9) For other applications of olefin cross metathesis for the preparation of O-linked as well as C-linked glycoconjugates, see the following. O-Linked: (a) Biswas, K.; Coltart, D. M.; Danishefsky, S. J. *Tetrahedron Lett*. **2002**, *43*, 6107−6110. (b) Kirschning, A.; Chen, G.-W. *Tetrahedron Lett*. **1999**, *40*, 4665−4668. (c) Berkowitz, D. B.; Maiti, G.; Charette, B. D.; Dreis, C. D.; MacDonald, R. G. *Org. Lett*. **2004**, *6*, 4921−4924. (d) Roy, R.; Dominique, R.; Das, S. K. *J. Org. Chem.* **1999**, *64*, 5408−5412. (e) Plettenburg, O.; Mui, C.; Bodmer-Narkevitch, V.; Wong, C.-H. *Adv. Synth. Catal*. **2002**, *344*, 622−626. (f) Barrett, A. G. M.; Beall, J. C.; Braddock, D. C.; Flack, K.; Gibson, V. C.; Salter, M. M. *J. Org. Chem*. **2000**, *65*, 6508−6514. C-Linked: (g) Huwe, C. M.; Woltering, T. J.; Jiricek, J.; Weitz-Schmidt, G.; Wong, C.-H. *Bioorg. Med. Chem.* **1999**, *7*, 773−788. (h) Chen, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. *Org. Lett*. **2004**, *6*, 4077−4080. (i) Vernall, A. J.; Abell, A. D. *Org. Biomol. Chem*. **2004**, *2*, 2555−2557. (j) Godin, G.; Compain, P.; Martin, O. R. *Org. Lett.* **2003**, *5*, 3269−3272. (k) Nolen, E. G.; Kurish, A. J.; Wong, K. A.; Orlando, M. D. *Tetrahedron Lett*. **2003**, *44*, 2449−2453. (l) Postema, M. H. D.; Piper, J. L. *Tetrahedron Lett.* **2002**, *43*, 7095−7099. (m) McGarvey, G. J.; Benedum, T. E.; Schmidtmann, F. W. *Org. Lett.* **2002**, *4*, 3591−3594. (n) Dondoni, A.; Giovannini, P. P.; Marra, A. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2380−2388. (o) Liu, S.; Ben, R. N. *Org. Lett.* **2005**, *7*, 2385−2388.

(10) Bundle, D. R.; Ling, C. C.; Zhang, P. In WO 03/101937 A1, 2003.

10.1021/jo051069y CCC: $30.25
Published on Web 08/25/2005

SCHEME 2[a]



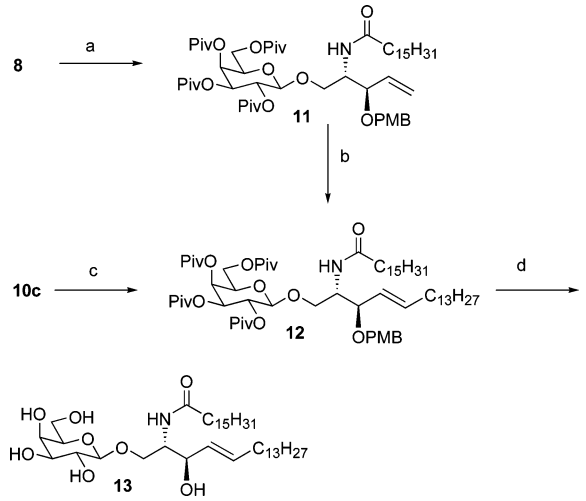
[a] Conditions: (a) HF·pyridine, 88%. (b) $BF_3{\cdot}Et_2O$, 4 Å MS, 71%. (c) Alkene, Grubbs second-generation catalyst.

chromatographic purification. The primary alcohol of the diol was selectively protected as a bulky silyl ether, followed by lanthanum triflate-promoted etherification of the secondary alcohol to provide **3**.[11] Subsequent azide displacement of the sulfonate proceeded efficiently, but the desired product **4** was always accompanied by some material that had lost the silyl ether under the reaction conditions. The crude reaction mixture was resubjected to silylation conditions to provide **4** in 67% overall yield. We had found that olefin cross metathesis reactions of the azide-containing building block **4** suffered from poor yields and undesirable side reactions,[7] so the azide was reduced and the resulting amine was protected as the Fmoc carbamate. The orthogonally protected alkene **5** was obtained in 20% overall yield from the diol **2** and could be prepared in multigram quantities.

The silyl ether was removed from **5** by treatment with HF·pyridine to afford the alcohol **6** in 88% yield. This alcohol was efficiently glycosylated with the tetrapivaloyl trichloroacetimidate **7** derived from galactose using $BF_3{\cdot}Et_2O$ as the promoter to provide the desired β-linked glycoside **8** in 71% yield. It is worth commenting that the glycosylation yield and anomeric selectivity obtained with the N-Fmoc-protected acceptor **5** compare favorably with that obtained when β-azido glycosyl acceptors were used.[12]

With the glycoside in hand, we evaluated its competence as a coupling partner in the olefin cross metathesis reaction (Scheme 2). The cross coupling could be effected with a variety of alkenes in high yields using the commercially available "Grubbs second-generation" ruthenium carbene catalyst.[13,14] As we had previously

SCHEME 3[a]



[a] Conditions: (a) (i) Piperidine/DMF; (ii) palmitoyl chloride, Et_3N, DMAP, CH_2Cl_2, 66% over two steps. (b) 1-Pentadecene, Grubbs second-generation catalyst, 72%. (c) (i) Piperidine/DMF; (ii) palmitoyl chloride, Et_3N, DMAP, CH_2Cl_2, 70% over two steps. (d) (i) $CF_3CO_2H/CH_2Cl_2/H_2O$; (ii) NaOMe/MeOH, 77% over two steps.

observed in the cross metathesis reactions of **5**, the *trans*-alkene was formed with very high selectivity. In most cases, only the *trans* isomer was observed by ^{1}H NMR. While α-olefins underwent facile cross metathesis to afford the protected glycolipids in good yield, the presence of adjacent heteroatoms was problematic. The ethylene glycol derivative **9e** failed to provide any cross-coupled product, despite variations in solvent, temperature, stoichiometry, and ruthenium carbene catalyst. One possible reason for the failure of **9e** to couple may be the formation of nonproductive chelated metallacarbene intermediates.[15]

Completion of the synthesis of β-GalCer was achieved by deprotecting the Fmoc group from **10c**, followed by acylation with palmitoyl chloride to provide **12**. Alternatively, **12** could be obtained from **8** by initial Fmoc group removal and palmitoylation, followed by metathesis as the last step in the sequence. Subsequent global deprotection of the PMB ether and pivaloyl esters provided β-galactosyl ceramide **13**. The ^{1}H NMR spectrum of the final product was in good agreement with that reported by Schmidt.[12b]

Alkene **5** is a versatile building block for (glyco)-sphingolipid synthesis. The majority of glycosphingolipid syntheses install the alkene-containing chain first, followed by glycosylation and acylation.[12c] Using building block **5**, one can install the three functionalities (sugar, acyl group, main chain) in a wider variety of sequences such as those shown in Scheme 3. Additionally, the terminal alkene in **5** provides a useful chemical handle for alternative functionalization chemistries and biocon-

(11) Rai, A. N.; Basu, A. *Tetrahedron Lett.* **2003**, *44*, 2267−2269.

(12) (a) Rich, J. R.; Bundle, D. R. *Org. Lett.* **2004**, *6*, 897−900. (b) Zimmermann, P.; Bommer, R.; Bär, T.; Schmidt, R. R. *J. Carbohydr. Chem.* **1988**, *7*, 435−452. (c) Vankar, Y. D.; Schmidt, R. R. *Chem. Soc. Rev*. **2000**, *29*, 201−216. (d) Compostella, F.; Franchini, L.; De Libero, G.; Palmisano, G.; Ronchetti, F.; Panza, L. *Tetrahedron* **2002**, *58*, 8703−8708.

(13) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953−956.

(14) Excess lipid alkene was used in these experiments to obtain products in reasonable yields and time frames. Although these reactions generate mixtures of the lipid alkene homodimer and monomer, we have not observed dimerization of the unreacted sugar alkene, which can be recovered.

(15) See: Hoveyda, A. H.; Vézina, M. *Org. Lett*. **2005**, *7*, 2113. McNaughton, B. R.; Buchholz, K. M.; Camaaño-Moure, A.; Miller, B. L. *Org. Lett.* **2005**, *7*, 733 and references contained therein.

jugation.[12a] This flexibility will facilitate the synthesis of glycolipid derivatives and provide a powerful tool for the elucidation of structure−function relationships in this class of biomolecules.[16]

Experimental Section

8: To a cold (−10 °C) solution of **6** (50 mg, 0.11 mmol) and **7**[4] (181 mg, 0.28 mmol) in dichloromethane (5 mL) was added BF_3·OEt (0.28 mL, 0.022 mmol, 0.78 M solution in CH_2Cl_2). The reaction mixture was stirred for 2 h. To the reaction mixture was added saturated sodium bicarbonate (aq). The mixture was stirred for 5 min and diluted with dichloromethane (20 mL). The dichloromethane layer was dried (Na_2SO_4) and concentrated to give the crude, which was purified by by column chromatography (SiO_2, ethyl acetate/hexanes, 1/12) to provide 80 mg (71%, 0.083 mmol) of **8** as a white solid. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 1.12 (s, 9H), 1.13 (s, 9H), 1.17 (s, 9H), 1.24 (s, 9H), 3.59 (d, J = 7.9 Hz, 1H), 3.77 (s, 3H), 3.89−3.90 (m, 2H), 3.96 (t, J = 6.9 Hz, 1H), 4.05 (dd, J = 7.2, 10.9 Hz, 1H), 4.14 (dd, J = 5.7, 11 Hz, 1H), 4.18−4.23 (m, 2H), 4.25−4.28 (m, 1H), 4.39 (ab, J = 10.9 Hz, 2H), 4.49 (d, J = 10 Hz, 1H), 4.51 (d, J = 10 Hz, 1H), 4.97 (d, J = 8 Hz, 1H), 5.12 (dd, J = 3.2, 10.4 Hz, 1H), 5.23 (dd, J = 7.8, 10.3 Hz, 1H), 5.30 (d, J = 9.8 Hz, 1H), 5.31 (d, J = 17.9 Hz, 1H), 5.415 (d, J = 2.8 Hz, 1H), 5.78 (ddd, J = 7.2, 9.9, 17 Hz, 1H), 6.86 (d, J = 8.6 Hz, 2H), 7.26 (m, 2H), 7.30 (td, J = 1.1, 7.4 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.55 (d, J = 7.6, 1H), 7.58 (d, J = 11 Hz, 1H), 7.76 (d, J = 7.6 Hz, 2H). **^{13}C NMR** (100 MHz, $CDCl_3$): δ 27.1, 27.14, 38.7, 38.76, 38.8, 39.1, 47.2, 53.6, 55.2, 61.1, 66.6, 67.8, 69.0, 70.85, 70.9, 71.0, 79.7, 101.1, 113.8, 119.6, 119.9, 125.0, 127.0, 127.6, 129.4, 130.2, 135.7, 141.3, 143.9, 155.9, 159.2, 176.8, 176.9, 177.3, 177.8. **HRMS** (FAB): calcd for $C_{54}H_{71}NaNO_{14}$ 980.4772, found 980.4791.

10c: To a solution of **8** (55 mg, 0.057 mmol) and pentadecene-1 (0.078 mL, 0.29 mmol) in dichloromethane (3 mL) was added Grubbs second-generation catalyst (13.6 mg, 0.016 mmol). The reaction mixture was heated to reflux for 24 h. To the reaction mixture was added pentadecene-1 (0.078 mL, 0.29 mmol), and the reaction mixture was refluxed for an additional 12 h. The reaction mixture was concentrated and purified by column chromatography (SiO_2, ethyl acetate/hexanes, 1/12) to provide 53 mg (81%, 0.046 mmol) of **10c** as white waxy solid. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 0.88 (t, J_{ave} = 6.8 Hz, 3H), 1.12 (s, 9H), 1.14 (s, 9H), 1.17(s, 9H), 1.24 (s, 9H), 1.25−1.33 (m, 22H), 2.0 (m, 2H), 3.61 (dd, J = 1.4, 8.6 Hz, 1H), 3.78 (s, 3H), 3.82−3.90 (m, 2H), 3.96 (m, 1H), 4.07 (dd, J = 7.4, 10.9 Hz, 1H), 4.15 (dd, J = 6.6, 10.8 Hz, 1H), 4.17−4.20 (m, 1H), 4.20−4.24 (m, 1H), 4.25 (d, J = 9 Hz, 1H), 4.39 (ab, J = 11 Hz, 2H), 4.4 (dd, J = 6.7, 6.8 Hz, 1H), 4.96 (d, J = 8.6 Hz, 1H), 5.12 (dd, J = 3.2, 10.4 Hz, 1H), 5.23 (dd, J = 7.8, 10.3 Hz, 1H), 5.37 (dd, J = 8, 15 Hz, 1H), 5.415 (d, J = 3.1 Hz, 1H), 5.73 (dt, J = 6.6, 15.4 Hz, 1H), 6.86 (d, J = 8.5 Hz, 2H), 7.24 (d, J = 8.5 Hz, 2H), 7.30 (t, J = 7.4 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.55 (d, J = 7.5 Hz, 1H), 7.59 (d, J = 7.5 Hz, 1H), 7.76 (d, J = 7.6 Hz, 2H). **^{13}C NMR** (100 MHz, $CDCl_3$): δ 14.1, 22.7, 27.07, 27.14, 27.2, 29.2, 29.4, 29.5, 29.7, 31.9, 32.3, 38.7, 38.75, 38.8, 39.1, 47.2, 53.8, 55.2, 61.0, 66.6, 66.8, 68.0, 69.1, 70.5, 70.9, 71.0, 79.4, 101.2, 113.7, 119.9, 125.05, 125.1, 127.0, 127.3, 127.6, 129.3, 130.4, 137.1, 141.2, 141.3, 143.9, 144.0, 155.8, 159.1, 176.8, 176.9, 177.2, 177.8. **HRMS** (FAB): calcd for $C_{67}H_{97}NaNO_{14}$ 1162.6806, found 1162.6830

12: A solution of **10c** (40 mg, 0.035 mmol) and 20% piperidine in DMF (3 mL) was stirred for 1 h. The excess piperidine and DMF was removed by flowing N_2 through the reaction mixture for 3 h to provide the crude amine. The crude amine was taken up in dichloromethane (3 mL), and triethylamine (0.03 mL, 0.2 mmol), DMAP (2 mg, 0.016 mmol), and palmitoyl chloride (0.2 mL, 0.07 mmol) were added. The reaction mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with dichloromethane (10 mL) and washed successively with 1 N HCl (5 mL), water (5 mL), and brine (5 mL). The dichloromethane layer was dried (Na_2SO_4) and concentrated to give the crude, which was purified by two successive column chromatography runs (SiO_2, ethyl acetate/hexanes, 1/10) to provide 28 mg (70%, 0.024 mmol) of **12** as a waxy solid. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 0.88 (t, J_{ave} = 6.8 Hz, 6H), 1.12 (s, 9H), 1.159 (s, 9H), 1.16 (s, 9H), 1.23−1.38 (m, 48 H), 1.25 (s, 9H), 1.99−2.13 (m, 4H), 3.59 (dd, J = 3.4, 9.1 Hz, 1H), 3.79 (s, 3H), 3.82 (t, J = 8 Hz, 1H), 3.94 (t, J = 7.1 Hz, 1H), 4.04 (dd, J = 7.4, 10.9 Hz, 1H), 4.12 (dd, J = 6.7, 10.9 Hz, 1H), 4.14−4.18 (m, 1H), 4.20 (dd, J = 4.0, 8.9 Hz, 1H), 4.36 (ab, J = 11 Hz, 2H), 4.50 (d, J = 7.6 Hz, 1H), 5.11 (dd, J = 3.2, 10.4 Hz, 1H), 5.19 (dd, J = 7.6, 10.4 Hz, 1H), 5.35 (dd, J = 8.4, 15.5 Hz, 1H), 5.405 (d, J = 2.9 Hz, 1H), 5.57 (d, J = 8.8 Hz, 1H), 5.67 (dt, J = 6.9, 15.3 Hz, 1H), 6.87 (d, J = 8.6 Hz, 2H), 7.22 (d, J = 8.6 Hz, 2H). **^{13}C NMR** (100 MHz, $CDCl_3$): δ 14.5, 23.1, 26.1, 27.5, 27.6, 29.7, 29.8, 30.0, 30.1, 32.4, 32.7, 37.4, 39.1, 39.2, 39.5, 52.0, 55.7, 61.4, 67.0, 68.4, 69.6, 70.7, 71.3, 71.4, 79.8, 101.6, 114.2, 127.9, 129.7, 131.0, 137.2, 159.5, 172.8, 177.2, 177.3, 177.6, 178.2. **HRMS** (FAB) calcd for $C_{68}H_{118}NO_{13}$ 1156.8603, found 1156.8570.

13: To a cold (0 °C) solution of **12** (22 mg, 0.019 mmol) in dichloromethane was added 20% TFA (2 mL, solution in dichloromethane). The reaction mixture was stirred for 12 h. To the reaction mixture was added solid sodium bicarbonate with vigorous stirring. The resulting mixture was filtered and concentrated. The crude reaction mixture was directly used in next step without any further purification. The crude mixture was taken in methanol (3 mL) and added to a solution of sodium methoxide (0.19 mmol) in methanol. The reaction mixture was stirred for 12 h. To the reaction mixture was added Amberlite (IR-120, H^+), and the reaction mixture was stirred until the solution was neutral. The reaction mixture was filtered and concentrated to give the crude, which was purified by column chromatography (SiO_2, hexanes/ethyl acetate, 9/1) to generate 10.3 mg (77%, 0.015 mmol) of **13**[4] as a waxy solid.

Acknowledgment. This work was supported by an NSF-CAREER award (CHE-0134908) to A.B.

Supporting Information Available: Experimental protocols for compounds **3**−**5**, **10a**,**b**,**d**, and **11** and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO051069Y

(16) (a) Chang, Y. T.; Choi, J.; Ding, S.; Prieschl, E. E.; Baumruker, T.; Lee, J.-M.; Chung, S.-K.; Schultz, P. G. *J. Am. Chem. Soc.* **2002**, *124*, 1856−1857. (b) Lingwood, C. A.; Mylvaganam, M. *Methods Enzymol*. **2003**, *363*, 264−283.