Direct and Efficient Glycosylation Protocol for Synthesizing α-Glycolipids: Application to the Synthesis of KRN7000

Omar Boutureira,[a] José Antonio Morales-Serna,[a] Yolanda Díaz,[a] M. Isabel Matheu,[a] and Sergio Castillón*[a]

Keywords: Glycosylation / Glycolipids / Phytosphingosine / Tin

Herein we describe a simple and practical protocol for accessing the biologically active galactosyl ceramide KRN7000 and other α-glycolipids with excellent yield and stereoselectivity by using per-*O*-silylated galactosyl iodide and stannyl ethers as glycosylation partners. This direct glycosylation reaction reduces the overall number of steps and provides rapid access to biologically important α-galactosyl ceramide derivatives.

Introduction

Since the isolation of a group of marine galactosyl ceramides in the 1990s from *Agelas mauritianus*,[1] and the subsequent synthesis of various analogues,[2] this family of compounds has received much attention because of the potent antitumor activity of some of these compounds in vivo and because the galactosyl ceramides containing α-glycosidic bonds, for example, agelasphin-9b **1** and KRN7000 **2** (Figure 1), despite being naturally occurring compounds, are in general not found in higher organisms, that is, they are not the natural products of mammalian cells.

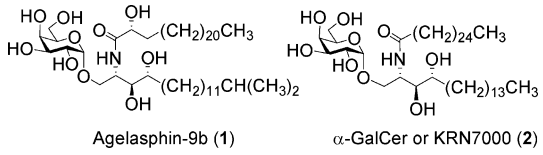


Figure 1. α-Glycosphingolipids.

At the molecular level, the glycolipid agelasphin-9b (**1**) has been shown to act as a connecting ligand presented by the CD1d molecule of antigen-presenting cells to the murine Vα14 receptor and the human Vα24 receptor of natural killer T (NKT) cells.[3] Upon recognition of the galactosyl ceramide in the context of CD1d, the NKT cell is stimulated to produce interferon-γ (IFN-γ), interleukin-4 (IL-4), and interleukin-2 (IL-2).[3] In addition, exploration of the biological effects of KRN7000 (**2**) has unveiled its promising activity against various diseases, including cancer,[4] malaria,[5] juvenile diabetes,[6] hepatitis B,[7] and autoimmune encephalomyelitis.[8] These results and other findings have stimulated research on the synthesis of α-glycosphingolipids as alternatives to the naturally occurring compounds. Retrosynthetic analysis reveals three important steps in the total synthesis of these compounds: the glycosylation reaction, *N*-acylation, and elimination of the protecting groups (Figure 2). The latter two steps usually proceed without problems, whereas the first step − the glycosylation reaction − poses the main synthetic challenge. The need for dependable methods of forming the glycosidic bond in the presence of sensitive functionalities with high stereoselectivity has led to the development of several glycosylation methods.[9] The most recognized glycosyl donors for this purpose are trichloroacetimidate[10] and fluoride;[11] however, the yields are always moderate. In this regard, the coupling of a ceramide unit with a glycoside generally gives the desired product in rather low yield. Good yields in the glycosylation step can be obtained by using an azidosphingosine, but this approach requires further reduction of the azido group and acylation. The difference in reactivity between azidosphingosines and ceramides has been attributed to the low nucleophilicity of ceramides,[12,13] which are highly ordered as a result of head-group hydrogen bonding. This driving force for molecular self-assembly allows ceramides to form hexagonal and orthorhombic phases. This

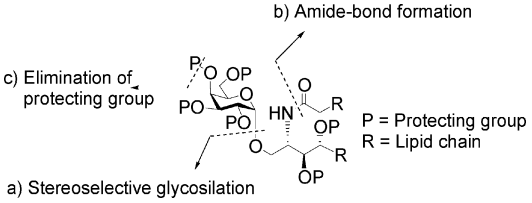


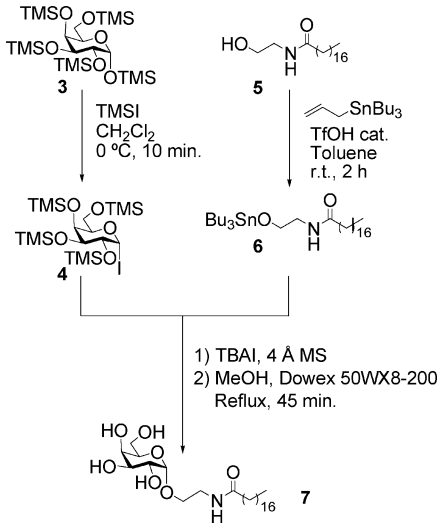
Figure 2. Key disconnections in the preparation of α-glycosphingolipids.

[a] Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili,
C/ Marcel·lí Domingo s/n, 43007, Tarragona Spain
Fax: +34-977-558446
E-mail: sergio.castillon@urv.cat

structural organization features of ceramides mean that they form highly stable structures in both crystalline and hydrated states.[14]

Results and Discussion

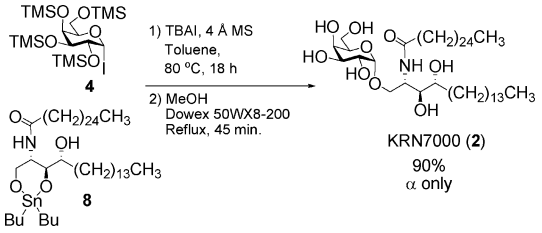
Gervay-Hague recently demonstrated the effectiveness of α-glycosyl iodides in the synthesis of α-galactosyl azidosphingolipids[15] and α-GalCer.[16,17] In the case of α-GalCer, per-*O*-silylated galactosyl iodide donor **4** and an unprotected ceramide acceptor were used under microwave conditions. The reaction proceeded with complete stereoselectivity and a remarkable 67% yield for the synthesis of KRN7000 (**2**). However, in our hands, this procedure (TBAI, DIPEA, CH_2Cl_2, 120 °C, 300 W, 2 h) proved ineffective for the preparation of simple α-GalCer analogues such as **7** starting from per-*O*-silylated galactosyl iodide **4** and acceptor **5** (Scheme 1). Under these conditions, the expected product was obtained in only 10% yield (α/β ratio 87:13).



Scheme 1. Glycosylation reaction of **6** with galactosyl iodide **4** in the presence of TBAI.

In an effort to improve the yield of the direct glycosylation reaction, we applied our experience with stannyl ethers[18] in the glycosylation of ceramides to give β-glycolipids recently reported by our group[19] in the synthesis of α-glycolipids. This procedure involves the reaction of tetra-*O*-acetyl-α-D-galactosyl iodide with stannyl ceramides in the presence of TBAI as activator to give β-GalCer analogues in high yield with complete stereoselectivity. TBAI inverts the configuration of the anomeric iodine to afford the highly reactive β-glycosyl iodide.

We show herein a new glycosylation protocol providing α-GalCer analogues, and particularly KRN7000 (**2**). This method activates classically reluctant acceptors such as amido alcohols and ceramides as stannyl derivatives in the TBAI-promoted glycosylation with glycosyl iodides (Schemes 1 and 2).



Scheme 2. Direct glycosylation of ceramide to obtain KRN7000 (**2**).

The glycosylation reaction conditions were optimized by starting from amido alcohol **5** (Scheme 1), which was converted into stannyl ether **6** to increase the nucleophilicity of the oxygen atom without significantly changing the basicity.[20] Initially, the reaction of **6** with α-iodogalactose **4**[21] in the presence of TBAI was carried out in CH_2Cl_2 at room temperature (Table 1, Entry 1). After hydrolysis by using Dowex 50WX8–200, isomer **7**α was obtained as the major product in a modest 25% yield. Next, we tried the reaction in toluene at 110 °C for 5 h in the presence of TBAI,[19] but unexpectedly, the yield remained moderate (Table 1, Entry 2). To our delight, however, we found that the order of addition of the reagents was important; specifically, we found that by adding the galactosyl iodide over the stannyl ether in toluene at 110 °C afforded the expected glycoside in an excellent overall yield of 95% and 88:12 α/β ratio (Table 1, Entry 3). Moreover, when the reaction was performed at 80 °C, the glycoside was obtained in almost quantitative yield with excellent selectivity (α/β ratio 97:3; Table 1, Entry 4).

Table 1. Synthesis of **7** by glycosylation of stannyl ether **6** with iodide **4** in the presence of TBAI.

Entry	Reaction conditions[a] (equiv.)	Yield [%]	α/β Ratio[b]
1	TBAI (2), 4 Å MS, CH_2Cl_2, room temp., 48 h	25	92:8
2[c]	TBAI (2), 4 Å MS, toluene, 110 °C, 5 h	47	89:11
3[d,e]	TBAI (2), 4 Å MS, toluene, 110 °C, 18 h	95	88:12
4	TBAI (2), 4 Å MS, toluene, 80 °C, 18 h	98	97:3

[a] 1) α-Galactosyl iodide: TMSO-Gal (1 equiv.), TMSI (1 equiv.), CH_2Cl_2, 0 °C, 10 min; stannyl ether/amide (1 equiv.), $Bu_3SnCH_2CH{=}CH_2$ (1.3 equiv.), TfOH (0.3 equiv.), r.t., 2 h. 2) Dowex 50WX8–200, MeOH, reflux, 1 h. [b] Determined by integration of the anomeric proton signals in the ^1H NMR spectrum of the crude reaction mixture. [c] Addition of the stannyl ether over the α-galactosyl iodide for 3 h. [d] Addition of the α-galactosyl iodide over the stannyl ether for 3 h. [e] Dowex 50WX8–200, MeOH, r.t., 4 h.

The highly stereoselective glycosylation method described above offers a very efficient and direct synthesis of unprotected α-glycolipids. To further demonstrate its synthetic value, we showed that the method can be used to rapidly prepare the biologically active compound KRN7000 **2** (Scheme 2).

The reaction of the stannyl derivative of ceramide **8** with donor **4** was performed under optimized reaction conditions. After hydrolysis of protecting groups by using acidic resin, final product **2** was obtained in 90% yield with com-

plete α-selectivity. The overall process takes place with complete chemoselectivity (differentiation of the primary and secondary OH at the lipid moiety) and stereoselectivity (α-anomer only).

Conclusions

We have developed a highly efficient and practical approach for synthesizing enantiomerically pure α-glycolipids by the TBAI-mediated glycosylation of stannyl ethers with per-*O*-silylated galactosyl iodides. This method afforded KRN7000 (**2**), a powerful immunostimulant in mammals, in excellent yield (90%) with complete α-selectivity. After treatment with acidic media to remove the silyl groups, the fully deprotected galactosyl ceramide was obtained in a one-pot fashion. This direct glycosylation procedure provides a solution to the long-standing problem of direct construction of a C–O bond between ceramides and sugars, reduces the overall number of steps, and provides rapid access to biologically important α-glycolipids and their derivatives. The application of the proposed scheme to branched oligosaccharides is currently under investigation.

Experimental Section

General Remarks: ^{1}H and ^{13}C NMR spectra were recorded by using a Varian Mercury 400 MHz spectrometer. In the ^{1}H NMR spectra, TMS was used as an internal reference. In the ^{13}C NMR spectra, the residual solvent signal was used as an internal reference ($CDCl_3$, triplet at δ = 77.23 ppm) unless otherwise stated. Elemental analysis (C, H, N, S) was performed by using a Carlo Erba EA 1108 Analyzer in the Servei de Recursos Científics (URV). Flash-column chromatography was performed with silica gel 60 (E. Merck, 40–63 μm). Solvents were purified by using standard procedures. Thin-layer chromatography (TLC) was performed on aluminum sheets coated with silica gel 60 F_{254} (E. Merck). Compounds were visualized under UV (254 nm) light and also by spraying the TLC plates with 6% H_2SO_4 in EtOH followed by charring at 150 °C for a few minutes. Starting material **5** was prepared as described in the literature.[19] All other reagents were used as received from commercial suppliers.

KRN7000 (2): To a solution of 1,2,3,4,6-penta-*O*-trimethylsilyl-D-galactopyranose **3** (38 mg, 0.071 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added TMSI (14 mg, 0.071 mmol), and the reaction mixture was stirred under an atmosphere of argon at 0 °C for 20 min. The reaction was stopped by adding anhydrous benzene (15 mL), after which the solvent was evaporated under reduced pressure. The resulting slightly yellow oil **4** was dissolved in toluene (3 mL) and kept under an atmosphere of argon. In a separate flask, molecular sieves (MS, 4 Å, 50 mg), TBAI (53 mg, 0.143 mmol), and **8** (66 mg, 0.071 mmol) were added into toluene (5 mL). The mixture was stirred under an atmosphere of argon and glycosyl iodide **4** was added dropwise. The reaction mixture was stirred at 80 °C for 18 h, and the solvent was then evaporated. MeOH (15 mL) and Dowex 50WX8–200 ion-exchange resin (0.5 g) were added and the reaction was stirred at room temperature for 4 h. The resin was then removed by filtration. The solvent was removed in vacuo, and the resulting residue was purified by silica gel chromatography (hexane/ethyl acetate/methanol = 6:3:1). The ^{1}H and ^{13}C NMR spectroscopic data of **2** were in good agreement with those in the literature.[22] ^{1}H NMR (400 MHz, [D$_5$]pyridine): δ = 8.43 (d, J = 8.6 Hz, 1 H), 5.55 (d, J = 3.5 Hz, 1 H), 5.25–5.21 (m, 1 H), 4.66–4.61 (m, 2 H), 4.53 (m, 1 H), 4.49 (t, J = 5.8 Hz, 1 H), 4.42–4.36 (m, 4 H), 4.31–4.29 (m, 1 H), 2.43 (t, J = 7.4 Hz, 2 H), 2.23 (m, 1 H), 1.92–1.86 (m, 2 H), 1.82–1.77 (m, 2 H), 1.63 (m, 1 H), 1.44–1.18 (m, 66 H), 0.88–0.83 (m, 6 H) ppm. ^{13}C NMR (100.6 MHz, [D$_5$]pyridine): δ = 174.5, 102.8, 77.9, 74.3, 72.8, 72.2, 71.5, 69.9, 63.9, 52.7, 38.0, 35.6, 33.4, 31.6, 31.4, 31.3, 31.2, 31.2, 31.1, 31.0, 30.9, 27.8, 27.6, 24.2, 15.5 ppm.

Glycolipid 7: The general procedure as reported for the preparation of **2** was followed. ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$, 4:1): δ = 7.52 (s, 1 H), 4.87 (d, J = 3.2 Hz, 1 H), 3.93 (m, 1 H), 3.80–3.72 (m, 6 H), 3.49 (m, 1 H), 3.32 (m, 2 H) 2.20 (t, J = 8.0 Hz, 2 H), 1.62 (m, 2 H), 1.27 (m, 28 H), 0.89 (t, J = 7.2 Hz, 3 H) ppm. ^{13}C NMR (100.6 MHz, 4:1 $CDCl_3/CD_3OD$): δ = 174.9, 98.8, 70.6, 69.9, 69.5, 68.7, 66.7, 61.4, 41.5, 36.0, 29.3–28.9, 25.6, 22.3, 13.5 ppm.

Stannyl Acetal 8: A mixture of ceramide (50 mg, 0.071 mmol) and dibutyltin oxide (0.176 mmol) in dry toluene (15 mL) was heated to reflux and subjected to azeotropic dehydration by using a Dean–Stark system or 4 Å MS overnight. Removal of the solvent under reduced pressure afforded stannyl acetal **8**, which was used for the glycosylation reaction without further purification.

Acknowledgments

Financial support from DGESIC (Ministerio de Educación y Ciencia, Spain) CTQ-2005–03124/BQU is acknowledged. We are also grateful to the Servei de Recursos Cientifics (URV) for its technical assistance. Fellowships from DURSI (Generalitat de Catalunya) and Fons Social Europeu to J. A. M. S. and O. B. are gratefully acknowledged.

[1] a) T. Natori, Y. Koezuka, T. Higa, *Tetrahedron Lett.* **1993**, *34*, 5591–5592; b) K. Akimoto, T. Natori, M. Morita, *Tetrahedron Lett.* **1993**, *34*, 5593–5596; c) T. Natori, M. Morita, K. Akimoto, Y. Koezuka, *Tetrahedron* **1994**, *50*, 2771–2784.

[2] a) R. W. Franck, M. Tsuji, *Acc. Chem. Res.* **2006**, *39*, 692–701; b) P. B. Savage, L. Teyton, A. Bendelac, *Chem. Soc. Rev.* **2006**, *35*, 771–779.

[3] a) M. Brigl, M. B. Brenner, *Annu. Rev. Immunol.* **2004**, *23*, 817–890; b) N. A. Borg, K. S. Wun, L. Kjer-Nielsen, M. C. J. Wilce, D. G. Pellicci, R. Koh, G. S. Besra, M. Bharadwaj, D. I. Godfrey, J. McCluskey, J. Rossjohn, *Nature* **2007**, *448*, 44–49.

[4] a) A. Kikuchi, M. Nieda, C. Schmidt, Y. Koezuka, S. Ishihara, Y. Ishikawa, K. Tadokoro, S. Durrant, A. Boyd, T. Juji, A. Nicol, *Br. J. Cancer* **2001**, *85*, 741–746; b) G. Matsumoto, S. Nagai, M. Toi, S. Ishiyama, K. Kuroi, K. Turuta, A. Okamoto, T. Takahashi, *Nippon Geka Gakkai Zasshi* **2001**, *102*, 421; c) R. Nakagawa, K. Motoki, H. Ueno, H. Iijima, H. Nakamura, E. Kobayashi, A. Shimosaka, Y. Koezuka, *Cancer Res.* **1998**, *58*, 1202–1207; d) Y. Hayakawa, S. Rovero, G. Forni, M. J. Smyth, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 9464–9469.

[5] G. Gonzalez-Aseguinolaza, C. de Oliveira, M. Tomaska, S. Hong, O. Bruna-Romero, T. Nakayama, M. Taniguchi, A. Bendelac, L. Van Kaer, Y. Koezuka, M. Tsuji, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 8461–8466.

[6] a) S. Hong, M. T. Wilson, I. Serizawa, L. Wu, N. Singh, O. V. Naidenko, T. Miura, T. Haba, D. C. Scherer, J. Wei, M. Kronenberg, Y. Koezuka, L. Van Kaer, *Nat. Med.* **2001**, *7*, 1052–1056; b) S. Sharif, G. A. Arreaza, P. Zucker, Q. S. Mi, J. Sondhi, O. V. Naidenko, M. Kronenberg, Y. Koezuka, T. L. Delovitch, J. M. Gombert, M. Leite-De-Moraes, C. Gouarin, R. Zhu, A. Hameg, T. Nakayama, M. Taniguchi, F. Lepault, A. Lehuen, J. F. Bach, A. Herbelin, *Nat. Med.* **2001**, *7*, 1057–1062.

[7] K. Kakimi, L. G. Guidotti, Y. Koezuka, F. V. Chisari, *J. Exp. Med.* **2000**, *192*, 921–930.
[8] K. Miyamoto, S. Miyake, T. Yamamura, *Nature* **2001**, *413*, 531–534.
[9] J. A. Morales-Serna, O. Boutureira, Y. Díaz, M. I. Matheu, S. Castillón, *Carbohydr. Res.* **2007**, *342*, 1595–1612.
[10] a) C. Xia, Q. Yao, J. Schümann, E. Rossy, W. Chen, L. Zhu, W. Zhang, G. De Libero, P. G. Wang, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2195–2199; b) S. Kim, S. Song, T. Lee, S. Jung, D. Kim, *Synthesis* **2004**, 847–850; c) O. Plettenburg, V. Bodmer-Narkevitch, C.-H. Wong, *J. Org. Chem.* **2002**, *67*, 4559–4564; d) S. Figueroa-Perez, R. R. Schmidt, *Carbohydr. Res.* **2000**, *328*, 95–102.
[11] a) M. Shimamura, N. Okamoto, Y.-Y. Huang, J. Yasuoka, K. Morita, A. Nishiyama, Y. Amano, T. Mishina, *Eur. J. Med. Chem.* **2006**, *41*, 569–576; b) R. M. Ndonye, D. P. Izmirian, M. F. Dunn, K. O. A. Yu, S. A. Porcelli, A. Khurana, M. Kronenberg, S. K. Richardson, A. R. Howell, *J. Org. Chem.* **2005**, *70*, 10260–10270; c) Y. Vo-Hoang, L. Micouin, C. Ronet, G. Gachelin, M. Bonin, *ChemBioChem* **2003**, *4*, 27–33; d) X.-T. Zhou, C. Forestier, R. D. Goff, C. Li, L. Teyton, A. Bendelac, P. B. Savage, *Org. Lett.* **2002**, *4*, 1267–1270.
[12] R. R. Schmidt, P. Zimmermann, *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 725–726.
[13] R. Polt, L. Szabo, J. Treiberg, Y. Li, V. J. Hruby, *J. Am. Chem. Soc.* **1992**, *114*, 10249–10258.
[14] M. E. Rerek, H.-C. Chen, B. Markovic, D. Van Wyck, P. Garidel, R. Mendelsohn, D. J. Moore, *J. Phys. Chem. B* **2001**, *105*, 9355–9362.
[15] W. Du, J. Gervay-Hague, *Org. Lett.* **2005**, *6*, 2063–2065.
[16] W. Du, S. S. Kulkarni, J. Gervay-Hague, *Chem. Commun.* **2007**, 2336–2338.
[17] For recent synthesis of α-GalCer, see: a) F. Facciotti, L. Mori, G. De Libero, G. Lombardi, S. Fallarini, L. Panza, F. Compostella, F. Ronchetti, *J. Org. Chem.* **2007**, *72*, 7757–7760; b) T. Tsujimoto, Y. Ito, *Tetrahedron Lett.* **2007**, *48*, 5513–5516.
[18] a) S. J. Danishefsky, J. Gervay, J. M. Peterson, F. E. McDonald, K. Koseki, D. A. Griffith, T. Oriyama, S. P. Marsden, *J. Am. Chem. Soc.* **1995**, *117*, 1940–1953; b) S. Yamago, T. Yamada, O. Hara, H. Ito, Y. Mino, J.-I. Yoshida, *Org. Lett.* **2001**, *3*, 3867–3870; c) S. Yamago, T. Yamada, H. Ito, O. Hara, Y. Mino, J.-I. Yoshida, *Chem. Eur. J.* **2005**, *11*, 6159–6174; d) P. J. Garegg, J.-L. Maloisel, S. Oscarson, *Synthesis* **1995**, 409–414; e) R. K. P. Kartha, M. Kiso, A. Hasegawa, H. J. Jennings, *J. Chem. Soc. Perkin Trans. 1* **1995**, 3023–3026; f) E. Kaji, K. Shibayama, K. In, *Tetrahedron Lett.* **2003**, *44*, 4881–4885; g) S. David, S. Hanessian, *Tetrahedron* **1985**, *41*, 643–663.
[19] J. A. Morales-Serna, O. Boutureira, Y. Díaz, M. I. Matheu, S. Castillón, *Org. Biomol. Chem.* **2008**, *6*, 443–446.
[20] S. Yamago, T. Yamada, R. Nishimura, H. Ito, Y. Mino, J.-I. Yoshida, *Chem. Lett.* **2002**, 152–154.
[21] A. S. Bhat, J. Gervay-Hague, *Org. Lett.* **2001**, *3*, 2081–2084.
[22] G. Xia, Q. Yao, J. Schümann, E. Rossy, W. Chen, L. Zhu, W. Zhang, G. De Libero, P. G. Wang, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2195–2199.