Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 47 (2006) 6647–6650

Stereoselective synthesis of 1,2-*cis* galactosides: synthesis of a glycolipid containing Galα1-6Gal component from *Zygomycetes* species

Noriyasu Hada, Junko Oka, Ayaka Nishiyama and Tadahiro Takeda*

Kyoritsu University of Pharmacy, 1-5-30 Shibakoen, Minato-ku, Tokyo 105-8512, Japan

Received 24 May 2006; revised 29 June 2006; accepted 30 June 2006
Available online 31 July 2006

Abstract—An α-selective galactosylation was demonstrated under various conditions. Among these α-galactoside approaches, high α-selectivity was achieved by the virtue of 4,6-*O*-di-*tert*-butylsilylene (DTBS) group. Yield was further improved by the influence of a 2-*O*-benzylated donor compared to 2-*O*-benzoylated donor. This method was then applied to the first highly stereoselective synthesis of a newly found trisaccharide glycosphingolipid in *Zygomycetes* species.

Chemical synthesis of oligosaccharides and glycoconjugates has great contribution in the elucidation of their biological functions. It is clearly evident that chemical synthesis of such complex structures in many laboratories requires access to reliable and high-yielding glycosylation methods. Moreover the stereoselective introduction of the glycosidic linkage is one of the most challenging aspects of oligosaccharide synthesis. The historical development of glycoside synthesis is discussed in many review articles and books.[1] However, until now stereoselective introduction of 1,2-*cis* glycosides has often imposed serious problems. Stereoselective formation of 1,2-*cis*-glycosides is generally a difficult issue where no assisting effect such as participation of the neighboring group is available. Construction of the α-glycosidic linkage has been developed by many carbohydrate chemists. Among them, Lemieux's in situ anomerization using glycosyl halides as donors and Bu_4NBr as a promoter has already been reported in 1975,[2] and the use of combinations of diethyl ether as a solvent and perchlorates as a source of counter anion against oxocarbenium ion has been frequently reported by other groups. Recently, regarding the selective α-glycosylation of the gluco- and/or galacto-, Boons et al. have reported α-orienting solvent effect of dioxane–toluene.[3] Similarly, Fukase et al. have performed extensive studies in this field.[4] They have reported that *N*-phenylselenophthalimide (N-PSP) promoted glycosylation with thioglycosides when used in combination with $Mg(ClO_4)_2$,[4a] and stereoselectivity was observed under their reaction condition from the acid promoter point of view. Furthermore, they also found that stereoselectivity can also be controlled by effect of the substitution group. The bulky protective groups (TBDMS, Trt, TBDPS, and Troc) introduced at the 6-position of glucosyl donors increase α-selectivity. However, examination of α-stereoselective Gal1-6Gal linkage has hardly been conducted, so we paid more attention on α-stereoselectivity.

In our continuing and systematic studies to elucidate the biological functions of glycosphingolipids, we have been synthesizing glycolipids from various lower animal species.[5] Thus this time, a trisaccharide glycolipid, Galα1-6Galβ1-6Galβ1-Cer was the target for the synthetic studies as described herein as part of our investigation on Galα1-6Gal construction. The constructed Galα1-6Galβ1-6Galβ1-Cer, a neutral glycosphingolipid was isolated by Aoki et al. along with Galα1-6Galα1-6Galβ1-6Galβ1-Cer and Galα1-6Galα1-6Galα1-6Galβ1-6Galβ1-Cer from *Mucor hiemails*, a typical *Zygomycetes* species.[6] Their structures were completely determined by compositional sugar, fatty acid, and sphingoid analyses, methylation analysis, MALDI-TOFMS spectrometry, and NMR spectroscopy. These three molecules constitute a novel family of neutral glycosphingolipids.

Keywords: α-Galactosylation; 1,2-*cis*-Glycoside; D-Galactose; Di-*tert*-butylsilylene group; *Zygomycetes* species.
* Corresponding author. Tel.: +81 (3) 54002696; fax: +81 (3) 54002556; e-mail: takeda-td@kyoritsu-ph.ac.jp

0040-4039/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2006.06.181

For construction of Galα1-6Galβ1-6Galβ1-Cer, we first carried out the glycosylation under Ar atmosphere in a mixture of 1:3 CH_2Cl_2–THF by the use of 1.5–2.0 equiv of *N*-iodosuccinimide (NIS) and 0.2 equiv of trifluoromethanesulfonic acid (TfOH) against a donor as a common method. An excess (1.2 equiv) of a donor was used against the acceptor. As summarized in Chart 1 and Table 1, desired α-galactosides were not obtained (α:β = 1.5:1∼3:1) stereoselectively although yield was satisfactory in all case (entries 1–3). Even by using the favorable effect of ether type, cyclopentyl methyl ether, there was no stereoselectivity (entries 4 and 5). In addition, more expectable α-selective glycosylation was carried out by using the combination of N-PSP and $Mg(ClO_4)_2$ as a effective promotor. The results of Galα1-6Gal linkage increased α-selectivity compared to entries 1–5 but the α-selectivity did not improve as high as in the case of Glcα1-6Glc by Fukase.[4a] In the entry 8, the use of 6-*O*-TBDMS protecting glycosyl donor caused many undesired side reactions and low yield. In their letter, they have also mentioned that more reliable α-selective glycosylation was found by using 6-*O*-Troc thioglycosyl donor. Thus we also examined the α-glycosylation with **1** and **6** by using some conditions (entries 9–11), out of which entry 11 showed much better selectivity compared to entry 1. After coming up to this point, we found that stereoselectivity of α-glycosylation using galactosyl donor with 6-OH galactosyl acceptor is difficult in comparison to Glcα1-6Glc.

On the other hand, Kiso et al. have reported that 4,6-*O*-di-*tert*-butylsilylene (DTBS) group on the galacto-type donors is responsible for α-selective galactosylation compatible with the neighboring functionality on the C-2 position, for example, NTroc and OBz.[7] Thus we carried out the glycosylation using this method also, and found that condensation of galactosyl acceptor **2** with galactosyl donor **7** (OBz group on the C-2 position) in the presence of NIS/TfOH gave desired α1-6 disaccharide **15** in the 63% yield (entry 12). Obviously, this time also it was only α. In addition, coupling of **1** or **2** with donor **8**[8] (OBn group on the C-2 position), which was prepared by silylation with di-*tert*-butylsilyl bis(trifluoromethanesulfonate) of phenyl 2,3-di-*O*-benzyl-1-

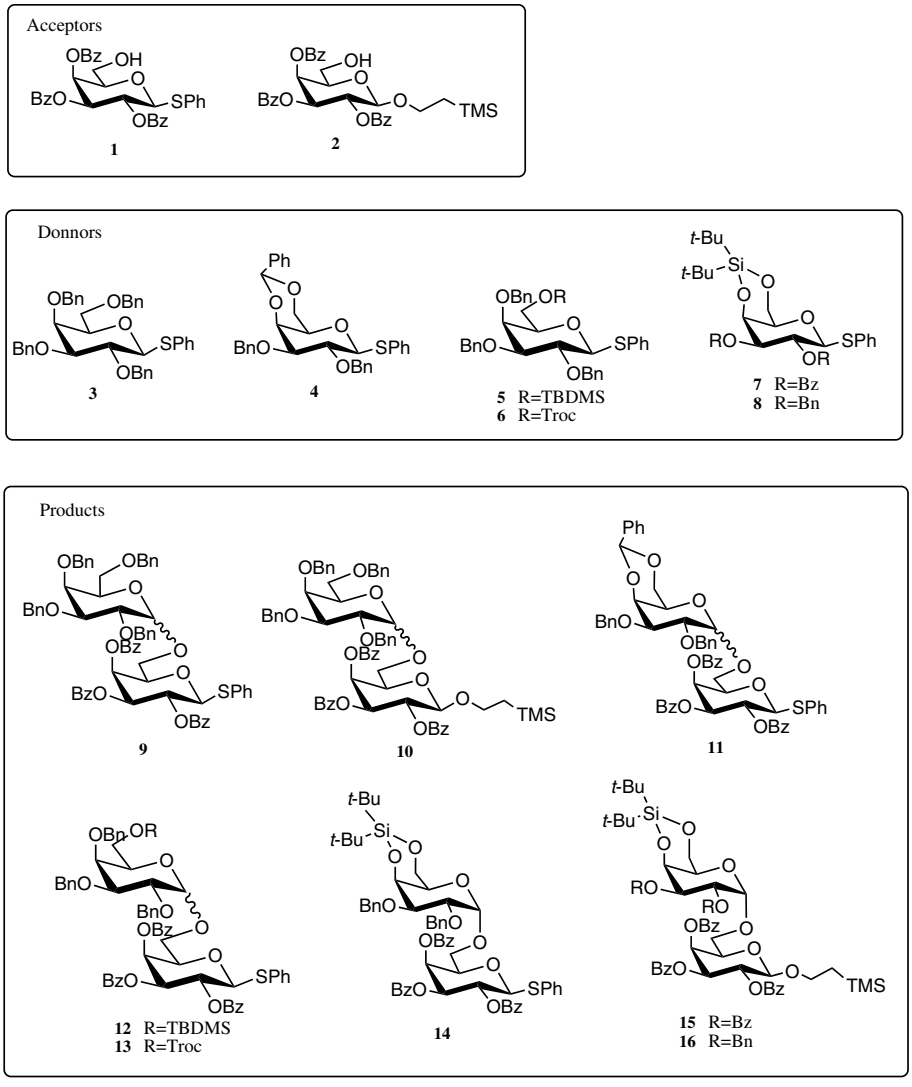


Chart 1.

Table 1. Galactosylation of various conditions

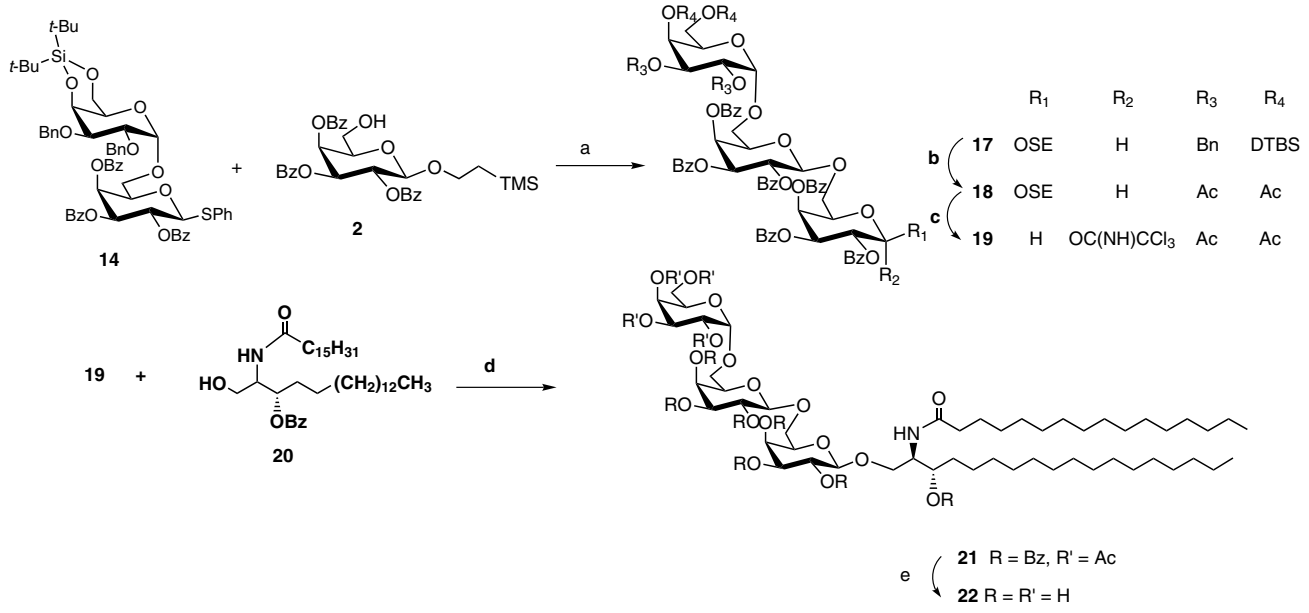
Entry	Donor	Acceptor	Time	Product	Promoter	Sol.	Temperature	α:β[a]	Yield (%)
1	**3**	**1**	4 h	**9**	NIS (1.5)–TfOH (0.2)	1:3 CH_2Cl_2–THF	−60 °C	2:1	92
2	**4**	**1**	12 h	**11**	NIS (2.0)–TfOH (0.2)	1:3 CH_2Cl_2–THF	−20 °C	3:1	92
3	**5**	**1**	12 h	**12**	NIS (2.0)–TfOH (0.2)	1:3 CH_2Cl_2–THF	−20 °C	1.5:1	75
4	**3**	**1**	4 h	**9**	NIS (1.5)–TfOH (0.2)	Cyclopentyl methyl ether	−60 °C → −10 °C	1:1	90
5	**4**	**1**	12 h	**11**	NIS (2.0)–TfOH (0.2)	Cyclopentyl methyl ether	−20 °C	1:1	77
6	**3**	**1**	48 h	**9**	N-PSP (1.5)–$Mg(ClO_4)_2$ (0.5)	Diethyl ether	rt	3.7:1	78
7	**3**	**2**	44 h	**10**	N-PSP (1.5)–$Mg(ClO_4)_2$ (0.5)	Diethyl ether	rt	3.8:1	87
8	**5**	**1**	22 h	**12**	N-PSP (1.5)–$Mg(ClO_4)_2$ (0.5)	Diethyl ether	rt	3.7:1	14
9	**6**	**1**	66 h	**13**	N-PSP (1.5)–$Mg(ClO_4)_2$ (0.5)	Diethyl ether	rt	2.5:1	88
10	**6**	**1**	3 h	**13**	NIS (2.0)–TfOH (0.2)	Cyclopentyl methyl ether	−40 °C → −20 °C	2.7:1	74
11	**6**	**1**	6 h	**13**	NIS (2.0)–TfOH (0.2)	1:3 CH_2Cl_2–THF	−40 °C → −20 °C	3.7:1	93
12	**7**	**2**	4 h	**15**	NIS (2.0)–TfOH (0.2)	CH_2Cl_2	0 °C	α only	63
13	**8**	**1**	30 min	**14**	NIS (2.0)–TfOH (0.2)	CH_2Cl_2	0 °C	α only	98
14	**8**	**2**	30 min	**16**	NIS (2.0)–TfOH (0.2)	CH_2Cl_2	0 °C	α only	97

[a] The anomer ratios were determined by comparison of the intensities of the H-1′ signal of the disaccharides in ^1H NMR.

thio-β-D-galactopyranoside, gave disaccharide **14**[9] or **16** in 98% or 97% yield (entries 13 and 14). As described above, the combinations of DTBS group and 2-*O*-benzyl donors effectively promotes glycosylation with thioglycoside in excellent yield.

Next, we applied this method to the synthesis of the glycolipid, and this is the first report on the chemical synthesis of Galα1-6Galβ1-6Galβ1-Cer from the natural products. Glycosylation of acceptor **2** with **14** in the presence of NIS, TfOH and 4 Å molecular sieves in dichloromethane gave the desired trisaccharide (**17**) in 85% yield after purification.[10] The stereochemistry of the newly formed glycosidic linkage could be determined by ^1H NMR spectroscopy (H-1′, 4.79 ppm, *J* 7.9 Hz). Selective removal of the DTBS group in **17** with TBAF, benzyl group by catalytic hydrogenolysis over 10% Pd–C in MeOH–AcOH (5:1) and subsequent acetylation gave **18**. Selective removal of the 2-(trimethylsilyl)ethyl (SE) group with trifluoroacetic acid in dichloromethane, and treatment with trichloroacetonitrile in the presence of DBU gave the corresponding α-trichloroacetimidate **19**. Glycosylation of (2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-hexadecanamido-4-octadecane-1,3-diol **20**[5f] with **19** was carried out in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) and 4 Å molecular sieves to afford the desired β-glycoside **21** in 64% yield. Finally, removal of acyl groups in **21** under Zemplen conditions and column chromatography on Sephadex LH-20 furnished a target glycolipid **22** (Scheme 1). The structure and purity of **22** were demonstrated by the ^1H NMR and HR-FABMS.[11]

In conclusion, a highly stereoselective efficient synthesis of a newly found trisaccharide glycosphingolipid from *Zygomycetes* species has been achieved.



Scheme 1. Reagents: (a) NIS, TfOH, MS 4 Å CH_2Cl_2 85%; (b) (i) 1 M TBAF AcOH–THF; (ii) H_2, Pd–C, MeOH–AcOH; (iii) Ac_2O–Pyr., 65% (three steps); (c) (i) TFA, CH_2Cl_2; (ii) CCl_3CN, DBU, CH_2Cl_2, 87%; (d) TMSOTf, MS 4 Å CH_2Cl_2 64%; (e) NaOMe, 1,4-dioxane–MeOH, 89%.

Acknowledgements

This work was supported under the High-Tech Research Center project of the Ministry of Education, Culture, Sports, Science and Technology of Japan. The authors are grateful to Ms. J. Hada for providing NMR and MS data.

References and notes

1. (a) Fraser-Reid, B.; Tatsuta, K.; Thiem, J. *Glycoscience: Chemistry and Chemical Biology*; Springer, 2001; (b) Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1503–1531; (c) Boons, G. J. *Tetrahedron* **1996**, *52*, 1095–1121.
2. Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056–4062.
3. Demchenko, A.; Stauch, T.; Boons, G. J. *Synlett* **1997**, 818–819.
4. (a) Fukase, K.; Nakai, Y.; Kanoh, T.; Kusumoto, S. *Synlett* **1998**, 84–86; (b) Fukase, K.; Hasuoka, A.; Kinoshita, I.; Kusumoto, S. *Tetrahedron* **1992**, *33*, 7165–7168; (c) Fukase, K.; Kinoshita, I.; Kanoh, T.; Nakai, Y.; Hasuoka, A.; Kusumoto, S. *Tetrahedron* **1996**, *52*, 3897–3904; (d) Fukase, K.; Nakai, Y.; Egusa, K.; Porco, J. A., Jr.; Kusumoto, S. *Synlett* **1999**, 1074–1078; (e) Tokimoto, H.; Fujimoto, Y.; Fukase, K.; Kusumoto, S. *Tetrahedron: Asymmetry* **2005**, *16*, 441–447.
5. (a) Hada, N.; Kuroda, M.; Takeda, T. *Chem. Pharm. Bull.* **2000**, *48*, 1160–1165; (b) Hada, N.; Ohtsuka, I.; Sugita, M.; Takeda, T. *Tetrahedron Lett.* **2000**, *41*, 9065–9068; (c) Hada, N.; Sato, K.; Takeda, T. *Chem. Pharm. Bull.* **2001**, *49*, 1464–1467; (d) Ohtsuka, I.; Hada, N.; Ohtaka, H.; Sugita, M.; Takeda, T. *Chem. Pharm. Bull.* **2002**, *50*, 600–604; (e) Ohtsuka, I.; Hada, N.; Sugita, M.; Takeda, T. *Carbohydr. Res.* **2003**, *337*, 2037–2047; (f) Yamamura, T.; Hada, N.; Kaburaki, A.; Yamano, K.; Takeda, T. *Carbohydr. Res.* **2004**, *339*, 2749–2759.
6. Aoki, K.; Uchiyama, R.; Yamauchi, S.; Katayama, T.; Itonori, S.; Sugita, M.; Hada, N.; Hada, J.; Takeda, T.; Kumagai, H.; Yamamoto, K. *J. Biol. Chem.* **2004**, *279*, 32028–32034.
7. Imamura, A.; Ando, H.; Korogi, S.; Tanabe, G.; Muraoka, O.; Ishida, H.; Kiso, M. *Tetrahedron Lett.* **2003**, *44*, 6725–6728.
8. An experimental procedure of compound **8**: To a solution of phenyl 2,3-di-*O*-benzyl-1-thio-β-D-galactopyranoside (319 mg, 0.70 mmol) in dry DMF was added di-*tert*-butylsilyl bis(trifluoromethanesulfonate) (334 μl, 0.92 mmol) at 0 °C, and the mixture was stirred for 15 min, then neutralized with Et_3N. Toluene was added and concentrated. Column chromatography of the residue on silica gel (10:1 hexane–ethyl acetate) gave **8** (358 mg, 85.7%). $[\alpha]_D^{25}$ +9.8 (*c* 0.8, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.54–7.24 (m, 15H, 3Ph), 4.90 (br s, 2H, benzylmethylene), 4.77 and 4.69 (each d, 2H, J_{gem} = 11.6 Hz, benzylmethylene), 4.65 (d, 1H, $J_{1,2} = 9.8$ Hz, H-1), 4.49 (d, 1H, $J_{3,4} = 2.4$ Hz, H-4), 4.18 (br dd, 2H, H-6a, 6b), 3.85 (t, 1H, $J_{2,3} = 9.2$ Hz, H-2), 3.47 (dd, 1H, H-3), 3.27 (s, 1H, H-5), 1.14 and 1.08 (each s, 18H, 2*t*-Bu); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4, 134.8, 128.7, 128.4, 128.3, 127.8, 127.7, 127.2, 88.7 (C-1), 82.8, 77.2, 77.0, 76.8, 75.9, 74.8, 71.0, 70.0, 67.4, 27.7, 27.6, 23.4, 20.7, 20.2. HR-FABMS: calcd for $C_{34}H_{44}O_5SSi$+Na $[M+Na]^+$: *m*/*z* 615.2577. Found: *m*/*z* 615.2570.
9. NMR data of compound **14**: ^1H NMR (500 MHz, $CDCl_3$) δ 7.97–7.20 (m, 30H, 6 Ph), 5.86 (d, 1H, $J_{3,4} = 3.1$ Hz, H-4), 5.71 (t, 1H, $J_{1,2} = 9.8$ Hz, $J_{2,3} = 9.8$ Hz, H-2), 5.54 (dd, 1H, H-3), 5.04 (d, 1H, H-1), 4.82–4.62 (m, 4H, 2 benzylmethylene), 4.65 (d, 1H, $J_{1',2'} = 3.7$ Hz, H-1′), 4.41 (d, 1H, $J_{3',4'} = 3.1$ Hz, H-4′), 4.28 (dd, 1H, $J_{5,6a} = 7.3$ Hz, $J_{5,6b} = 4.3$ Hz, H-5), 4.11 (s, 2H, H-6′), 3.96 (dd, 1H, $J_{2',3'} = 9.8$, H-2′), 3.85 (dd, 1H, $J_{6a,6b} = 10.4$ Hz, H-6a), 3.79 (dd, 1H, H-3′), 3.75 (s, 1H, H-5′), 3.66 (dd, 1H, H-6b), 1.04 and 0.95 (each s, 18H, 2*t*-Bu); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.4, 165.1, 139.1, 138.4, 133.6, 133.3, 133.2, 132.6, 131.5, 130.0, 129.8, 129.7, 129.3, 129.0, 128.9, 128.8, 128.6, 128.4, 128.4, 128.3, 127.9, 127.8, 127.5, 98.5 (C-1′), 84.7 (C-1), 77.7, 76.6, 74.2, 73.9, 73.1, 71.0, 68.8, 67.8, 67.5, 67.3, 67.2, 27.7, 27.3, 23.4, 20.6.
10. NMR data of compound **17**: ^1H NMR (500 MHz, $CDCl_3$) δ 8.08–7.19 (m, 40H, 8 Ph), 5.87–5.85 (m, 2H, H-4, 4′), 5.75–5.69 (m, 2H, H-2, 2′), 5.51–5.46 (m, 2H, H-3, 3′), 4.79 (d, 1H, $J_{1',2'} = 7.9$ Hz, H-1′), 4.77–4.58 (m, 4H, 2 benzylmethylene), 4.69 (d, 1H, $J_{1,2} = 7.9$ Hz, H-1), 4.62 (d, 1H, $J_{1'',2''} = 2.4$ Hz, H-1″), 4.54 (d, 1H, $J_{3'',4''} = 2.4$ Hz, H-4″), 4.26–4.08 (m, 5H, H-5′, 6a, 6b, 6″a, 6″b), 3.93 (dd, 1H, $J_{2'',3''} = 9.8$ Hz, H-2″), 3.86–3.83 (m, 2H, H-6′a, CH_2CH_2O), 3.73 (dd, 1H, H-3″), 3.67 (s, 1H, H-5″), 3.66–3.59 (m, 2H, H-5, 6′b), 3.44 (dd, 1H, CH_2CH_2O), 1.04 and 0.96 (each s, 18H, 2*t*-Bu), 0.81–0.63 (m, 2H, CH_2CH_2O), −0.13 (s, 9H, Si $(CH_2)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.5, 165.4, 165.1, 139.0, 138.4, 133.4, 133.1, 133.0, 129.9, 129.7, 129.5, 129.4, 129.1, 128.9, 128.5, 128.5, 128.3, 128.2, 127.6, 127.3, 101.1 (C-1′), 100.8 (C-1), 99.3 (C-1″), 77.6, 73.9, 73.5, 73.1, 72.2, 72.0, 71.8, 70.9, 69.8, 69.8, 68.7, 68.1, 67.9, 67.8, 67.4, 67.1, 27.6, 27.3, 23.3, 20.6, 17.6.
11. NMR and HR-FABMS data of compound **22**: ^1H NMR (500 MHz, DMSO-d_6–D_2O 49:1) 4.69 (d, 1H, $J_{1'',2''}$ = 3.7 Hz, H-1″), 4.17 (d, 1H, $J_{1',2'} = 8.0$ Hz, H-1′), 4.07 (d, 1H, $J_{1,2} = 7.3$ Hz, H-1), HR-FABMS: calcd for $C_{52}H_{99}NO_{18}$+Na $[M+Na]^+$: *m*/*z* 1048.6760. Found: *m*/*z* 1048.6793.