o-Methoxycarbonylphenyl 1-Thio-β-D-Galactopyranoside, A Non-malodorous Thio Glycosylation Donor for the Synthesis of Globosyl α (1-4)-Linkages

Hirofumi Dohi, Yoshihiro Nishida,* Hidehiko Tanaka, Kazukiyo Kobayashi*

Department of Molecular Design and Engineering, Graduate School of Engineering, Nagoya University, Chikusa-ku, Nagoya 464-8603, Japan

Received 7 June 2001

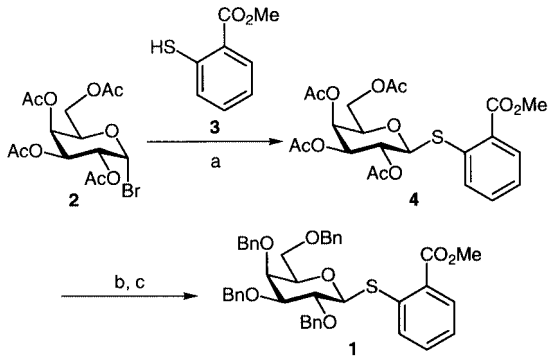
Abstract: *o*-Methoxycarbonylphenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactoside **1** was designed and demonstrated to serve as a non-malodorous thioglycosyl donor in α-selective glycosylation reactions with 4-OH galactoside and 4'-OH lactoside to give globosyl Gb_2 and Gb_3 saccharides, respectively.

Key words: stereoselective glycosylations, thioglycosides, globosyl oligosaccharides, glycoconjugate polymers, carbohydrates

Globosyl ceramides (Gb_2- and Gb_3-ceramide) are natural ligands of Shiga-like toxins (Stx-I and Stx-II) produced by *Escherichia coli* O-157: H-7 and other pathogenic *E. coli* species.[1] Intensive efforts have been directed to the development of artificial Stx ligands such as Gb_3-embedded glycoconjugate polymers,[2] dendrimers,[3] starfish-like models,[4] liposomes,[5] and so on.[6] In these and other synthetic studies on globosyl oligosaccharides, stereoselective and high-yield construction of the Galα1→4Gal linkage was the key investigative step.[7] Among many synthetic protocols for α-galactosylation, thioglycosylation methods seem to be the most preferentially employed.[8,9] This is mainly because thioglycoside donors are highly stable for storage and are chemo-selective under glycosylation conditions. These favorable properties have provided a key basis to the recent methodologies termed "orthogonal syntheses"[10] and "armed/disarmed glycosylations"[11], which have significantly extended the utility of thioglycosylation methods[12] including the syntheses of globosyl Gb_2 and Gb_3 oligosaccharides.[13,14]

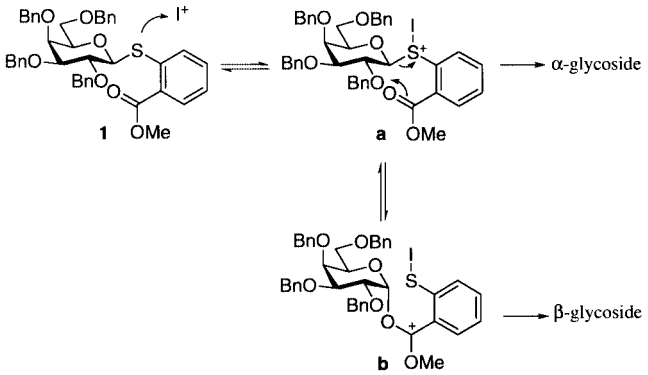
In our continuous efforts to develop glycoconjugate polymers carrying Gb_3 and *iso*-Gb_3 trisaccharides[2] and other biologically active oligosaccharides,[15] we have hesitated to use the thioglycosylation method because it evolves unpleasant odor in the process of preparing the thioglycosyl donors and isolating the glycosyl products. The malodor of mercaptanes should be unbearable even if the reaction achieves an excellent outcome. This communication reports the convenient use of a new thiogalactosyl donor **1**[16] that enables us to prepare both Gb_2 and Gb_3 linkages and their cluster models in high yields without suffering from the malodor in the laboratory.

The donor **1**[17] was synthesized as a colorless crystal in 90% yield via the S_N2 reaction of penta-*O*-acetyl-α-D-galactopyranosyl bromide **2** with methyl thiosalicylate **3** that smells faintly like a blend of methyl salicylate and thiophenol (Scheme 1). Compound **1** evolved little unpleasant odor during its preparation and treatment and could be kept as an active glycosyl donor for months without decomposition. Reactivity and stereoselectivity of **1** were examined preliminarily using cetyl alcohol as an acceptor (1.5 mol) and NIS/trifluorosulfonic acid (TfOH) as an activator[18] in CH_2Cl_2. The reaction gave a mixture of α- and β-galactosides (α/β = 45:55) at 0 °C, and β-galactoside predominantly (α/β = 30:70) at −40 °C. It may be noted that the higher β-selectivity of the glycosylation at lower temperature is a unique phenomenon. We assume that this unique tendency is attributable to the *o*-carboxylate function in the leaving group as shown in Scheme 2. The sulfur atom of **1** is activated by the iodonium cation to generate the intermediate **a** which affords the α-glycoside. The carbonyl oxygen atom of the *o*-carboxylate group interacts with the anomeric carbon in an intramolecular fashion to generate the ortho ester-type intermediate **b** which affords the β-glycoside. The presence of the intermediate **b** was suggested by the finding that cetyl thiosalicylate was obtained as a byproduct which may be produced via transesterification of **b** with cetyl alcohol. Since the electron-withdrawing *o*-carboxylate group reduces the leaving activity of the phenylthio group, the intermediate **a** is less reactive at low temperature, and the contribution of the intermediate **b** becomes predominant, which results in favored formation of β-galactoside.



Scheme 1 Preparation of *o*-methoxycarbonylphenyl thiogalactosyl donor **1**
Reagents and Conditions: (a) **3**, K_2CO_3, DMF, r.t., 2 h, 90%; (b) NaOMe, MeOH, r.t., 3 h, 99%; (c) NaH, BnBr, DMF, 16 h, 91%.

Though α-selective galactosylation was unsuccessful with cetyl alcohol, we attempted to apply the donor **1** to the more hindered and less reactive *p*-nitrophenyl (*p*NP) 6-*O*-benzyl-2,3-di-*O*-acetyl-β-D-galactopyranoside **5**[20] which



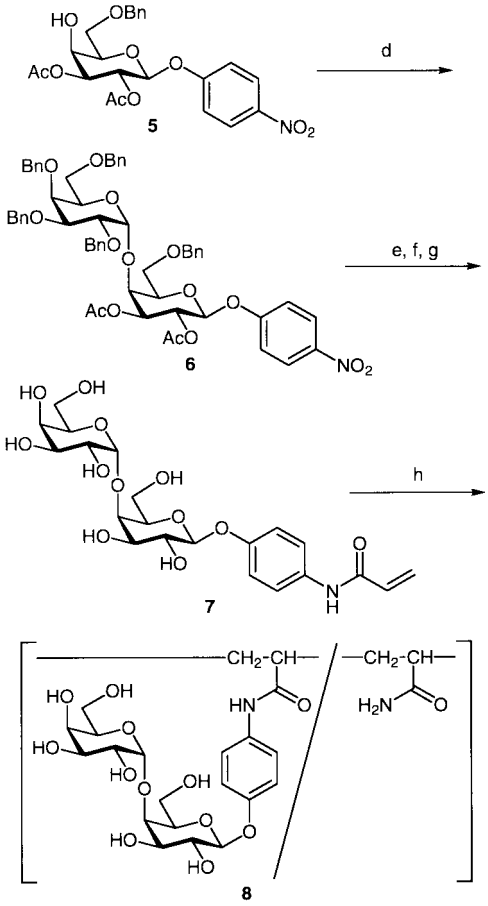
Scheme 2 Proposed mechanism of glycosylation of **1** with cetyl alcohol.

was prepared in a conventional way from *p*NP β-D-galactopyranoside via benzylidenation, acetylation, and regioselective cleavage of the benzylidene acetal[21] (Scheme 3). Although the glycosylation was conducted under the same conditions as for cetyl alcohol (CH_2Cl_2 at 0 °C), much higher α-selectively (α/β = 83/17, 89% yield) was achieved. Use of the Et_2O - CH_2Cl_2 mixture as the solvent at −10 °C could improve the α-selectivity up to 99% to give the desired Galα1→4Gal linkage **6** in 83% yield (Table 1). The high α-selectivity may be explained similarly as that of the other glycosylations on the basis of the anomeric effect[22] and also by solvent effect of Et_2O.[23] Deacetylation and the subsequent catalytic hydrogenolysis gave *p*-aminophenyl galabioside **7**, which was converted to a Gb_2 glycoconjugate polymer **8** via radical copolymerization with acrylamide (Mn = 4.4×10^5 (SEC analysis, pullulan standard), the molar ratio of Gb_2 unit:acrylamide = 0.20:0.80). Thus, the novel galactosyl donor **1** enabled us to prepare **8** from *p*NP β-D-galactoside totally in 40% yield (8 steps). The same pathway was extended to the synthesis of a Gb_3 derivative **10** via α-galactosylation (α:β = >99:1, 79%) using **1** of *p*NP lactoside derivative **9**[24] (Scheme 4).

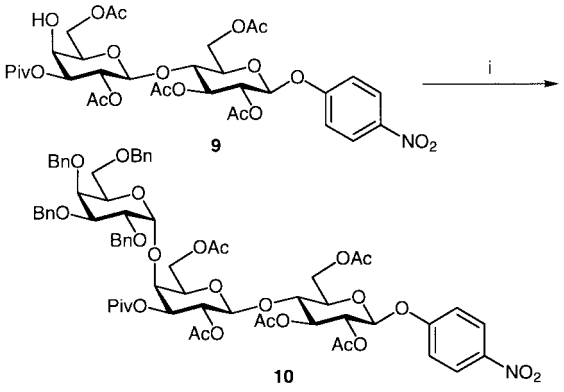
Table Coupling reaction between **1** and **5**[a]

Run	Solv. mL	Temp. °C	Yield %	α / β
1	CH_2Cl_2, 15	0	89	83 / 17
2	CH_2Cl_2= 5, Et_2O= 10	0	83	92 / 8
3	CH_2Cl_2, 15	-10	88	88 / 12
4	CH_2Cl_2= 5, Et_2O= 10	-10	86	α-only

[a]The glycosylations of **1** and **5** (1.5 eq) were carried out in the presence of *N*-iodosuccinimide (NIS, 2 eq.) and cat. TfOH for 1 h.



Scheme 3 Synthesis of glycoconjugate polymer carrying Gb_2 bioside **8**
Reagents and Conditions: (d) **1**, NIS, TfOH, CH_2Cl_2, Et_2O, -10°C, 30 min, 86%; (e) NaOMe, MeOH, r.t., 3 h, 99%; (f) $Pd(OH)_2$/C, H_2, MeOH, r.t., 2 h, 95%; (g) acryloyl chloride, Et_3N, CH_2Cl_2, MeOH, 0°C→r.t., 2 h, 91%; (h) acrylamide (4 eq.), 2, 2'-azobis (2-amidinopropane) dihydrochloride (1% mol against all monomers), H_2O, 60°C, 8 h, 72%.



Scheme 4 Synthesis of Gb_3 trioside derivative **10**
Reagents and Conditions: (i) **1**, NIS, TfOH, CH_2Cl_2, Et_2O, -10°C, 3 h, 79%.

In conclusion, we have designed a thiogalactosyl donor **1** with an *o*-methoxycarbonylphenyl thio leaving group and demonstrated its highly synthetic potential as a non-malodorous thioglycosyl agent to construct the α(1-4) linkage in Gb_2 and Gb_3 derivatives. Extension of the *o*-methoxycarbonylphenyl thio leaving group to other glycosylations, elucidation of its reaction mechanism, and biological applications[25] of the resulting substances are now in progress.

Acknowledgement

This work was carried out with support of the Japan Society for the Promotion Science Research Fellowship (DC) to H. D.

References and Notes

(1) (a) Lingwood, C. A.; Law, H.; Richardson, S.; Petric, M.; Brunton, J. L.; Grandis, S. D.; Karamali, M. *J. Biol. Chem.* **1987**, *262*, 8834. (b) Lindberg, A. A.; Brown, J. E.; Strömberg, N.; Westling-Ryd, M.; Schultz, J. E.; Karlsson, K. A. *J. Biol. Chem.* **1987**, *262*, 1779. (c) Arab, S.; Lingwood, C. A. *Glycoconjugate J.* **1996**, *13*, 159. (d) Karlsson, K. A. *Annu. Rev. Biochem.* **1989**, *58*, 309.

(2) Nishida, Y.; Dohi, H.; Uzawa, H.; Kobayashi, K. *Tetrahedron Lett.* **1998**, *39*, 8681.

(3) Matsuoka, K.; Terabatake, M.; Esumi, Y.; Terunuma, D.; Kuzuhara, H. *Tetrahedron Lett.* **1999**, *40*, 7839.

(4) Kitov, P. I.; Sadowska, J. M.; Mulvey, G.; Armstrong, G. D.; Ling, H.; Pannu, N. S.; Read, R. J.; Bundle, D. R. *Nature* **2000**, *403*, 669.

(5) (5) Arab, S.; Lingwood, C. A. *Glycoconjugate J.* **1996**, *2*, 159.

(6) (6) Lundquist, J. J.; Debenham, S. D.; Toone, E. J. *J. Org. Chem.* **2000**, *65*, 8245.

(7) For the recent synthesis Gb_2 derivative, see Ohlsson, J.; Magnusson, G. *Carbohydr. Res.* **2000**, *329*, 49.

(8) (8) Barresi, F.; Hindsgaul, O. *J. Carbohydr. Chem.* **1995**, *14*, 1043.

(9) (9) Medgyes, A.; Bajza, I.; Farkas, E.; Pozsgay, V.; Liptàk, A. *J. Carbohydr. Chem.* **2000**, *19*, 285.

(10) Kanie, O.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1996,** *37*, 4551.

(11) Mootoo, D. R.; Konradsson, P.; Uododong, U.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1988**, *110*, 5583.

(12) Fraser-Reid, B.; Wu, Z.; Uododong, U.; Ottoson, H. *J. Org. Chem.* **1990**, *55*, 6068.

(13) Qiu, D.; Schmidt, R. R. *Liebigs Ann. Chem.* **1992**, 217.

(14) Hashimoto, S.; Sakamoto, H.; Honda, T.; Abe, H.; Nakamura, S.; Ikegami, S. *Tetrahedron Lett.* **1997**, *52*, 8969.

(15) Dohi, H.; Nishida, Y.; Mizuno, M.; Shinkai, M.; Kobayashi, T.; Takeda, T.; Uzawa, H.; Kobayashi, K. *Bioorg. Med. Chem.* **1999**, *7*, 2053.

(16) Some groups already synthesized *o*-methoxycarbonylphenyl thioglycosides, but the glycosides were not employed as glycosyl donors. See: Li, P.; Sun, L.; Landry, D. W.; Zhao, K. *Carbohydr. Res.* **1995**, *275*, 179. Per-*O*-benzylated *o*-methoxycarbonylphenyl thiogalactoside **1** is a new compound,.

(17) Compound **1**: $[\alpha]_D$ -3.0 (c 0.05, $CHCl_3$, 24 °C). mp = 93-94°C. IR (cm^{-1}): 1720, 1249 (CO_2Me), 1290, 1128 (OBn). ^1H NMR (δ ppm, 500 MHz, $CDCl_3$, 22 °C): 7.26-7.37 (m, 20H, aromatic-H of Bn), 7.13, 7.17, 7.78, 7.86 (ddd×2 and dd×2, 1H×4, aromatic-H of *o*-methoxycarbonylphenyl group), 4.99 (d, 1H, *J* = 8.0 Hz, H-1), 4.42, 4.49, 4.62, and 4.72-4.80 (d×3 and m, 1H×3 and 5H, methylene of Bn), 4.04 (dd, 1H, *J* = 9.0 Hz, H-2), 4.00 (d, 1H, *J* = 2.6 Hz, H-4), 3.86 (s, 3H, H of Me), 3.61-3.69 (m, 4H, H-3, H-5, and H-6). *Anal. calcd.* for $C_{42}H_{42}O_7S$: C, 73.02; H, 6.13%; *found* C, 73.15; H, 6.11%.

(18) Sasaki, M.; Tachibana, K.; Nakanishi, H. *Tetrahedron Lett.* **1991**, *32*, 6873.

(19) Leaving group participation similar to that described here was previously reported. See: Shimizu, H.; Ito, Y.; Ogawa, T. *Synlett* **1994**, *7*, 535.

(20) Compound **5**: $[\alpha]_D$ = -16.4 (c 0.05, $CHCl_3$, 24 °C). mp = 144-146 °C. IR (cm^{-1}): 3513 (OH), 1745, 1241 (Ac), 1513 (NO_2), 1338, 1220 (OBn). ^1H NMR (δ ppm, 500MHz, $CDCl_3$, 22°C): 8.18 (d, 2H, *J* = 9.3 Hz, H-meta of *p*NP), 7.28-7.37 (m, 5H, aromatic-H of Bn), 7.10 (d, 2H, *J* = 9.3 Hz, H-ortho of *p*NP), 5.60 (dd, 1H, *J* = 8.6 and 11.4 Hz, H-2), 5.16 (d, 1H, *J* = 8.6 Hz, H-1), 5.06 (dd, 1H, *J* = 2.9 and 11.4 Hz, H-3), 4.56 and 4.58 (s×2, 1H×2, methylene of Bn), 4.24 (s, 1H, H-4), 3.90 (dd, 1H, H-5), 3.82 (m, 2H, H-6), 2.06 and 2.14 (s×2, 3H×2, H of Ac). *Anal. calcd.* for $C_{23}H_{25}NO_{10}$: C, 58.10; H, 5.30; N, 2.95%; *found* C, 57.46; H, 5.22; N, 2.85%.

(21) DeNinno, M. P.; Etienne, J. B.; Duplantier, K. C. *Tetrahedron Lett.* **1995**, *36*, 669.

(22) (a) Osborn, H.; Khan, T., In *Oligosaccharides*, Oxford University Press, 2000, p 58. (b) Oscarson, S., In *Carbohydrates in Chemistry and Biology*, Part 1: Chemistry of Saccharides; Ernst, B.; Hart, G. W.; Sinay, P., Ed.; Wiley-VCH: Weinheim, 1999, p 93.

(23) Garegg, P. J.; Helland, A. C. *J. Carbohydr. Chem.* **1993**, *12*, 105.

(24) Glycosylation of **9** with 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-D-galactopyranosyl fluoride and the subsequent preparation of the glycoconjugate polymers were previously reported.[2, 15]

(25) Dohi, H.; Nishida, Y.; Furuta, Y.; Uzawa, T.; Yokoyama, S.; Mori, H.; Kobayashi, K. to be submitted.

Article Identifier:
1437-2096,E;2001,0,09,1446,1448,ftx,en;Y12001ST.pdf