Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 23, pp. 2841-2846, 1996
Copyright © 1996 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00535-5

SOLID PHASE SYNTHESIS OF POLYLACTOSAMINE OLIGOSACCHARIDE

Hiroki Shimizu, Yukishige Ito*, Osamu Kanie, and Tomoya Ogawa*

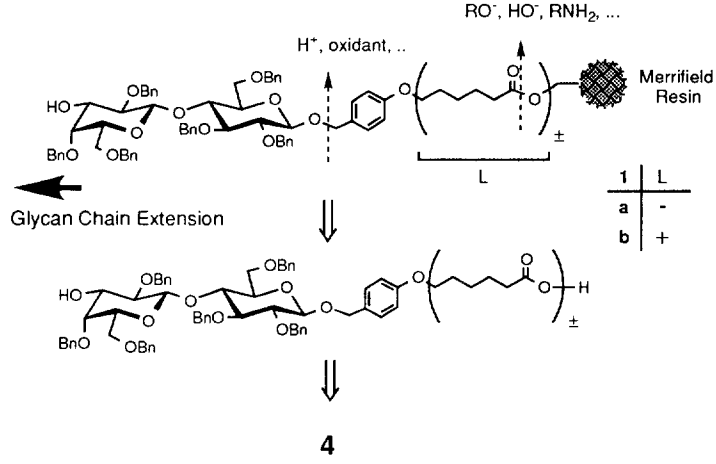
The Institute of Physical and Chemical Research (RIKEN)
Wako-shi, Saitama 351-01 Japan

Abstract: Solid phase synthesis of polylactosamine oligosaccharide was performed starting from resin supported lactose **1a**,**b**. Glycosylation of **1a** with the lactosamine unit **6** followed by delevulinoylation afforded tetrasaccharides, which were further converted into hexa- and octasaccharide and was cleaved from resin by $TrBF_4$ in CH_2Cl_2 to afford **7**. Ester linked **1b** was converted in a similar manner into hexasaccharide that was liberated under basic conditions to give **8**. Subsequent deprotection into **9** was performed in three steps. Copyright © 1996 Elsevier Science Ltd

In order to delineate the numerous functions of structurally diverse glycoconjugates[1] precisely, a convenient method for a facile supply of oligosaccharide with rigorously defined structure is highly desired. With this respect, use of chemically synthesized oligosaccharide is of obvious advantage over that of material isolated from natural source. In principle, one can make substantial amounts of homogeneous materials, including non-natural structures, by chemical means. Quite intense effort has been devoted in this area to develop novel methodologies useful for regio- and stereoselective glycoside bond formation[2]. As a result, it is now possible to synthesize quite complex oligosaccharide containing up to 25 sugar residues[3]. However, synthesis of oligosaccharide, in general, requires a large number of operations, including repeated protection-deprotection of hydroxy groups and glycoside bond forming transformations, and chromatographic purification is usually required after each step. Therefore, the overall sequence inevitably becomes highly time- and labour-consuming. On the other hand, the utility of solid phase synthesis technology has been convincingly demonstrated in the fields of oligopetide[4] and oligonucleotide[5], and very routine preparations of these biomolecules are now possible using fully automated synthesizers. Based on such a background, several intriguing approaches have been reported on polymer-support oligosaccharide synthesis[6]. Herein, we would like to report some results of our own effort to explore the methodology for solid phase synthesis of biologically relevant oligosaccharide.

Our basic strategy depicted in **Scheme 1** utilises the *p*-alkyloxybenzyl type functionality as a linker[7], based on the expectation that assembled oligosaccharide can be cleaved from solid support either under acidic, basic, or oxidative conditions. Polylactosamine type oligosaccharide was chosen as a synthetic target, because glycan chains classified in this group 1) occur both in glycoproteins and glycolipids and are related to a variety of biological events[8], 2) consist of repeating lactosamine

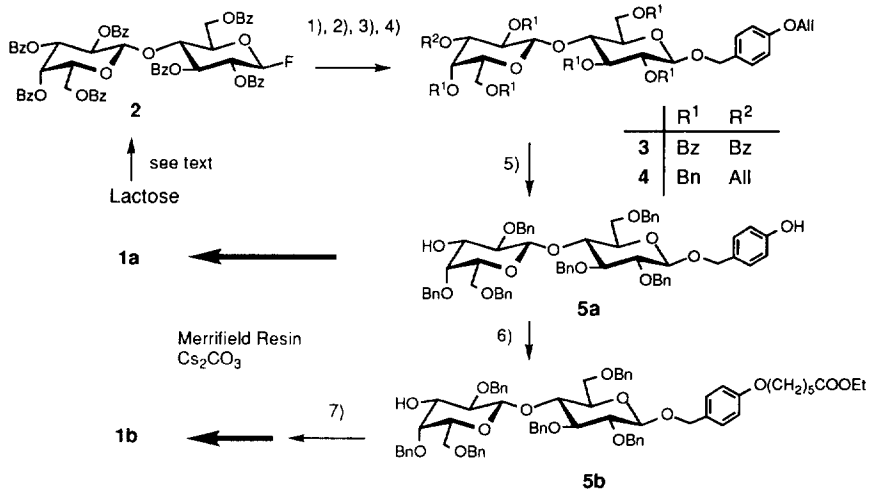
(→3βGal1→4βGlcNAc1) units and suitable for the initial demonstration of the strategy, and 3) have been a subject of extensive synthetic studies in solution phase[3]. The synthesis was started from the resin-bound lactose unit **1** as the "primer", so that the final product has structural similarity with neolacto-type glycosphingolipid.



Scheme 1

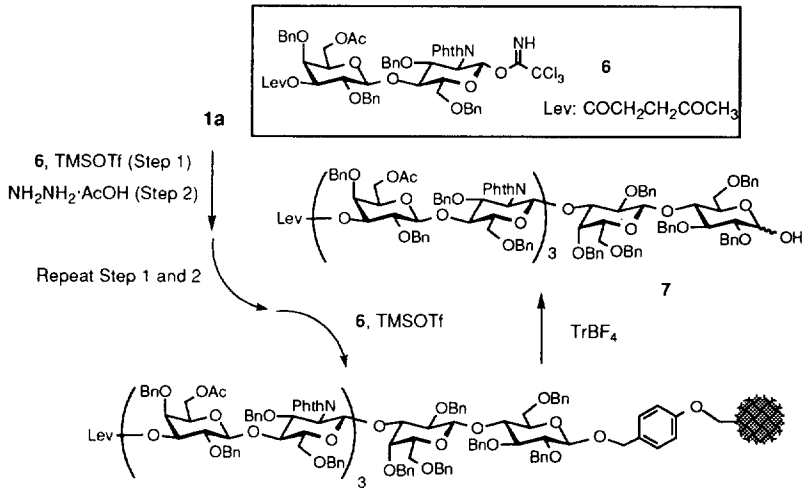
The disaccharide-resin conjugates **1a**,**b** were prepared as shown in **Scheme 2**. Thus *p*-allyloxybenzyl alcohol prepared from *p*-hydroxybenzaldehyde (1. allyl bromide, K_2CO_3 / acetone, reflux; 2. $LiAlH_4$ / ether) was coupled ($AgOTf\text{-}SnCl_2$[9], *sym*-collidine / $ClCH_2CH_2Cl$, 55°C, 86%) with the fluoride **2**, which was prepared from lactose (1. BzCl / pyridine, 90°C, 1d, 95%; 2. HBr-AcOH / $ClCH_2CH_2Cl\text{-}Ac_2O$, r.t. 3h, 99%; 3. ZnF_2-2,2-bipyridine[10] / MeCN, reflux., used without purification). Resultant β-glycoside **3**[11] was deacylated and subjected to dibutyltin oxide mediated regioselective allylation[12] followed by benzylation to afford **4**, which was further transformed into diol **5a**[11]. Subsequent coupling with Merrifield resin was performed in the presence of Cs_2CO_3 (DMF, 50°C, 18 h[13]) to afford **1a**. On the other hand, preparation of ester-linked **1b** was achieved in three steps [1. $Br(CH_2)_5COOEt$, Cs_2CO_3 / DMF, 92%; 2. NaOH / aq. THF, quantitative; 3. Merrifield resin, Cs_2CO_3 / DMF[13]] via ethyl ester **5b**.

Extension of the glycan chain was performed using previously reported trichloroacetimidate **6**[14] as a glycosyl donor. Thus, the lactose-resin conjugate **1a** was subjected to glycosylation (1.5 equiv. **6**, 0.2 equiv. TMSOTf / CH_2Cl_2, -78°C, 2h, repeated twice)-delevulinoylation[15] (NH_2NH_2·AcOH[14] / EtOH, r.t., 1d) cycle twice to afford what assumed to be resin-bound hexasaccharide. This material was subjected to additional glycosylation step with **6** and then cleaved off from the solid support with excess $TrBF_4$[16] in CH_2Cl_2 (r.t., 30min). Subsequent chromatographic purification afforded the octasaccharide **7** (42% overall yield from **5a**)[17].



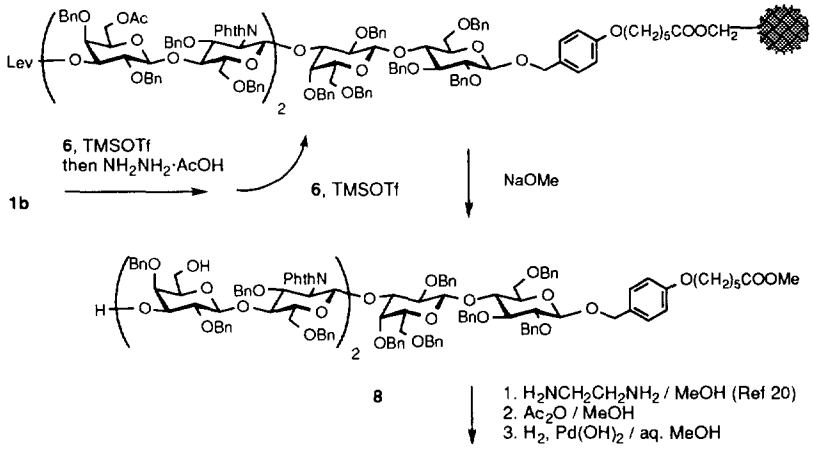
1) *p*-$AllOC_6H_4CH_2OH$, AgOTf, $SnCl_2$, *s*-collidine / $ClCH_2CH_2Cl$, 4A MS (86%). 2) NaOMe / MeOH. 3) *n*-Bu_2SnO / toluene, reflux, then AllBr, *n*-Bu_4NBr (63%). 4) BnBr, NaH / DMF (96%). 5) i) $Ir\{(COD)[PCH_3Ph_2]_2\}PF_6$ / THF. ii) $HgCl_2$, HgO / aq. acetone (88%). 6) $Br(CH_2)_5COOEt$, Cs_2CO_3 / DMF (92%). 7) aq. NaOH / THF-EtOH.

Scheme 2



Scheme 3

Above-mentioned experiments revealed that extension of polylactosamine-type glycan chain can be performed in a practically acceptable efficiency. However, starting from ether-linked primer **1a**, the immediate oligosaccharide product after cleavage from the resin ought to be the reducing sugar (i.e. **7**). Therefore, further deprotection of phthaloyl groups should be preceded by the reprotection of thus liberated anomeric hydroxy group. On the other hand, the use of ester-carrying **1b** as a primer should give us an additional option for the cleavage step, by which the oligosaccharide product can be liberated as a *p*-substituted benzyl glycoside. Thus, **1b** was subjected to the chain elongation process in a similar manner as described for **1a**. First cycle glycosylation[18]-delevulinoylation was followed by the second glycosylation to give hexasaccharide[19]. The product was cleaved under basic conditions (NaOMe / MeOH, 45°C, 7h) to afford corresponding methyl ester **8** which was isolated in 56% overall yield. Complete deprotection into **9** was achieved in three steps (56%) (**Scheme 4**).



Scheme 4

Our results demonstrated the ability of existing methodology established in solution-phase synthetic studies of polylactosamine-type glycans to be extended to solid-phase synthesis. There certainly exist a number of problems in oligosaccharide synthesis yet to be solved, and it is premature to say that solid phase synthesis can be applied to all types of glycoside. However, it may well be expected that continuous improvement in solution phase oligosaccharide synthesis with respect to selectivity and efficiency, coordinated with the progress in solid phase technology, will bear fruit into the development of a widely applicable method of solid phase oligosaccharide synthesis.

Acknowledgements

A part of this work was financially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture and also by the Special Coordination Funds of the Science and Technology Agency of the Japanese Government. We thank Ms. M. Yoshida and her staff for elemental analyses, and Ms. A. Takahashi for technical assistance.

References and Notes

1. A. Varki, *Glycobiology* **1993**, *3*, 97.
2. K. Toshima and K. Tatsuta, *Chem. Rev.* **1993**, *93*, 1503.
3. Y. Matsuzaki, Y. Ito, Y. Nakahara, and T. Ogawa, *Tetrahedron Lett.* **1993**, *34*, 1061.
4. M. Bodanszky, "Principles of Peptide Synthesis 2nd ed." Springer-Verlag, Berlin, 1993; P. Lloyd-Williams, F. Albericio, and E. Giralt, *Tetrahedron* **1993**, *48*, 11065.
5. M. J. Gait, "Oligonucleotide Synthesis, a Practical Approach" I R L Press, Oxford, Washington DC, 1984.
6. For recent examples, see; M. Adinolfi, G. Barone, L. De Napoli, A. Iadonisi and G. Piccialli, *Tetrahedron Lett.* **1996**, *37*, 5007; J. Rademann and R. R. Schmidt, *Tetrahedron Lett.* **1996**, *37*, 3989; J. T. Randolph, K. F. McClure, S. J. Danishefsky, *J. Am. Chem. Soc.* **1995**, *117*, 5712; S. P. Douglas, D. Whitfield, J. J. Krepinsky, *J. Am. Chem. Soc.* **1995**, 117, 2116; L. Yan, C. M. Taylor, R. Goodnow, Jr., D. Kahne, *J. Am. Chem. Soc.* **1994**, *116*, 6953; M. Schuster, P. Wang, J. C. Paulson, C.-H. Wong, *J. Am. Chem. Soc.* **1994**, *116*, 1135.
7. For the use of *p*-amidobenzyl-type linker for polymer-support enzymatic glycosylation, see; S.-I. Nishimura, K. Matsuoka, and Y. C. Lee, *Tetrahedron Lett.* **1994**, *35*, 5657.
8. M. Fukuda, *J. Biol. Chem.* **1991**, *266*, 21327.
9. T. Mukaiyama, Y. Murai, and S. Shoda, *Chem. Lett.* **1981**, 431.
10. K. D. Goggin, J. F. Lambert and S. W. Walinsky, *Synlett* **1994**, 162
11. Selected physical data are given for key compounds. **3**: mp 105-107°C; $[\alpha]_D$ +38.8° (c 1.2, $CHCl_3$); ^{1}H-NMR (270 MHz, $CDCl_3$) δ 6.1-6.0 (1 H, m, CH_2=*CH*), 5.73 (dd, J 9.6 and 9.2 Hz, H-3^1), 5.72 (d, J 3.3 Hz, H-4^2), 5.71 (dd, J 10.2 and 7.2 Hz, H-2^2), 5.51 (dd, J 9.6 and 7.9 Hz, H-2^1), 5.37 (dd, J 10.2 and 3.3 Hz, H-3^2), 4.86 (d, J 7.9 Hz, H-1^2), 4.68 (d, J 7.9 Hz, H-1^1), 4.26 (dd, J 9.6 and 9.2 Hz, H-4^1), z3.88 (t, J 6.6 Hz, H-5^2), 3.69 (m, H-6^2); ^{13}C-NMR (67.5 MHz, $CDCl_3$) δ 100.95, 98.71, 76.01, 72.96, 72.89, 71.74, 71.65, 71.34, 70.17, 69.85, 68.68, 67.46, 62.39, 61.03. **5a**: $[\alpha]_D$ -15.9° (c 0.7, $CHCl_3$); ^{1}H-NMR (270 MHz, $CDCl_3$) δ 5.16 (b, phenolic OH), 4.46 and 4.41 (d, J 7.3 Hz, H-1^1 and -1^2), 3.97 (dd, J 9.3 and 8.9 Hz, H-4^1), 3.83 (d, J 2.0 Hz, H-4^2), 3.55 (t, J 8.9 Hz, H-3^1), 2.19 (d, J 5.6 Hz, OH); ^{13}C-NMR (67.5 MHz, $CDCl_3$) δ 102.64, 102.14, 82.82, 81.71, 80.58, 76.70, 75.92, 75.33, 75.08, 74.97, 74.05, 73.35, 73.17, 70.69, 68.29, 67.94. **5b**: $[\alpha]_D$ -12.5° (c 1.1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 4.46 and 4.42 (d, J 7.6 and 7.3 Hz, respectively, H-1^1, -1^2), 4.13 (q, J 7.3 Hz, $COOCH_2CH_3$), 3.84 (d, J 2.1 Hz, H-4^2), 2.33 (t, J 7.6 Hz, CH_2COO), 1.9-1.4 (m, $OC_4H_8CH_2COO$), 1.25 (t, J 7.3 Hz, $COOCH_2CH_3$); ^{13}C-NMR ($CDCl_3$) δ 102.61, 102.10, 82.84, 81.69, 80.56, 76.66, 75.87, 75.29, 75.10, 75.02, 74.88, 74.04, 73.30, 73.14, 73.10, 70.62, 68.25,

67.89, 67.57, 60.18, 34.18, 28.90, 25.59, 24.66, 14.20. **7**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.41, 5.23 and 5.16 (d, J 8.4, 8.0 and 7.2 Hz, respectively, H-1^3 -1^5, -1^7), 2.16, 1.94, 1.81 and 1.79 (4s, $COCH_3$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 103.13, 103.00, 102.83, and 102.45 (C-1^2, -1^4, -1^6, -1^8), 99.82, 99.74, and 99.62 (C-1^3, -1^5, -1^7), 91.33 (C-1^1), 81.74, 78.28, 77.20, 75.60, 75.17, 74.81, 74.52, 74.04, 73.58, 73.17, 73.02, 71.65, 70.07, 68.49, 62.73, 61.81, 56.27, 37.71, 29.76, 27.87, 20.74, 20.64; FABMS (positive) m/z 3571.9 $[M+Na]^+$. **8**: $[\alpha]_D$ -3.30° (c 1.15, $CHCl_3$); ^{1}H-NMR (270 MHz, $CDCl_3$) δ 5.40 and 5.21 (d, J 8.6 and 7.6 Hz, respectively, H-1^3, -1^5), 3.66 (s, $COOCH_3$), 2.34 (t, CH_2COOCH_3); ^{13}C-NMR (67.5 MHz, $CDCl_3$) δ 103.22, 102.93, 102.32, 102.05, 99.93, and 99.55 (anomeric carbons), 82.88, 82.18, 81.83, 81.53, 80.32, 78.78, 78.64, 78.37, 77.93, 77.20, 75.94, 75.49, 75.29, 75.20, 75.02, 74.88, 74.81, 74.63, 74.54, 74.31, 73.89, 73.17, 73.12, 73.01, 72.89, 70.55, 68.36, 67.57, 62.09, 61.96, 56.28, 51.48, 33.95, 29.65, 28.90, 25.63, 24.64; FABMS (negative) m/z 2742.6 $[M-H]^-$. **9**: $[\alpha]_D$ +21.1° (c 0.7, H_2O); ^{1}H-NMR (400 MHz, D_2O) δ: 5.21 (d, J 3.9 Hz, H-1^1 α-anomer), 4.69 (d, J 8.3 Hz, H-1^3 and -1^5), 4.65 (d, J 7.8 Hz, H-1^1 β-anomer), 4.46, 4.45 and 4.42 (d, J 7.8 Hz, H-1^2, -1^4 and -1^6), ; ^{13}C-NMR (125 MHz, D_2O) δ: 104.58 and 104.47 (C-$1^{2\sim6}$), 97.44 (C-$1^1\beta$), 93.52 (C-$1^1\alpha$), 83.77, 79.95, 79.83, 77.06, 76.59, 76.51, 76.26, 76.05, 75.48, 74.20, 73.87, 73.10, 72.82, 72.67, 71.83, 71.68, 70.26, 70.05, 62.75, 62.67, 61.78, 61.55, 56.89, 23.88; FABMS (positive) m/z 1095.3 $[M+Na]^+$, (negative) m/z 1071.0 $[M-H]^-$.

12. S. David and S. Hanessian, *Tetrahedron* **1985**, *41*, 643.
13. Typical experimental procedure: To a stirred solution of **5a** (190 mg, 0.192 mmol) in DMF (7 ml) were added Merrifield resin (960 mg, 1% cross-linked, 100-200 mesh, PEPTIDE INSTITUTE. INC., 0.66 meq/g) and Cs_2CO_3 (116 mg, 0.356 mmol) at room temperature and the mixture was shaken for 2 d at 50°C. The resin was filtered, washed with DMF, MeOH, H_2O, MeOH and CH_2Cl_2 successively, and dried under vacuum to afford to **1a** (1.098 g, 96%, 0.132 mmol/g). Loading of **5b** on resin was performed in a nearly identical manner after converted into corresponding acid to afford **1b** (99% yield, 0.094 mmol/g). In this instance, the degree of substitution was confirmed to be >90% of the theoretical value based on the mass recovery of the cleavage experiment (NaOMe / MeOH, 45°C).
14. T. Nakano, Y. Ito, and T. Ogawa, *Carbohydr. Res.* **1993**, *243*, 43.
15. H. J. Koeners, J. Verhoeven, and J. H. van Boom, *Recl. Trav. Chim. Pays-bas* **1981**, *100*, 65.
16. H. Takaku and K. Kamaike, *Chem. Lett.* **1982**, 189.
17. Corresponding hexasaccharide was also isolated in 15% yield.
18. In this instance, the glycosylation step was followed by capping (Ac_2O/pyridine, r.t. overnight) of unreacted acceptor.
19. This material was subjected to HPLC analysis (60% *n*-hexane/AcOEt, 1.8 ml/min, Hibar LiChro CART®, Lichrospher®, Si 60 (5μm), 254 nm), after cleavage by $TrBF_4$. Higher oligosaccharide portion of this material revealed to consist of ~93% hexa- and ~7% tetrasaccharide.
20. O. Kanie, S. C. Crawley, M. M. Palcic, O. Hindsgaul, *Carbohydr. Res.* **1993**, *243*, 139.

(*Received in Japan* 26 *August* 1996; *accepted* 29 *October* 1996)