Pergamon

Tetrahedron Letters, Vol. 38, No. 46, pp. 8041-8044, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)10104-6

Synthesis of Amphiphilic Chitoheptaose Derivative

Hiroshi Hinou, Atsushi Umino, Koji Matsuoka, Daiyo Terunuma, Shunya Takahashi,[#] Yasuaki Esumi,[#] and Hiroyoshi Kuzuhara*

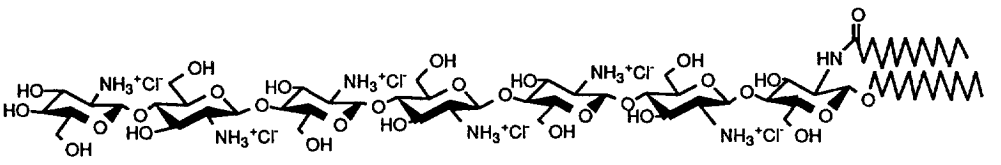
Department of Functional Materials Science, Faculty of Engineering, Saitama University, Urawa-shi 338, Japan
[#]The Institute of Physical and Chemical Research, Wako-shi, Saitama 351-01, Japan

Abstract: With interest in clustering of bioactive chitooligosaccharides of more than dp 6, a chitoheptaose derivative **1** carrying tetradecanoyl and tetradecyloxy groups at the NH and the C-1 of the reducing end, was prepared. The hydrophobic groups were introduced step by step after completion of the heptasaccharide skeleton using a chitobiose derivative as the elongation unit. Micelle formation of **1** in water was confirmed by dye solubilization check.

The recent papers that chitooligosaccharides of more than dp 6 or their per-*N*-acetyl derivatives show diverse biological activities for mammalians[1] and plants[2] stimulated us to prepare their amphiphilic derivative because clustering of such molecules was expected to improve their biological activities and the mode of clustering itself was quite interesting. Referring to the physicochemical data on glycolipids,[3] we designed a chitoheptaose derivative carrying binary hydrophobic chains in D-glucosamine moiety of the reducing end.

Here we describe the synthesis of the designed compound, tetradecyl 4-*O*-β-chitohexaosyl-2-deoxy-2-tetradecanamido-β-D-glucopyranoside hexahydrochloride (**1**), which includes such a series of reactions as synthesis of the heptasaccharide skeleton using a disaccharide synthon as the common unit for the chain elongation, introduction of a pair of hydrophobic groups into the 1,6-anhydro precursor of the reducing end, and complete deprotection followed by purification work-up.

Large-scale preparation of *N,N'*-diacetylchitobiose using chitinase[4] and the subsequent conversion into pentenyl β-glycoside[5] gave the starting compound for preparing the elongation synthon, which was benzylidenated to give **2**. Since the solubility of **2** was extremely low, **2** was, without purification, subjected



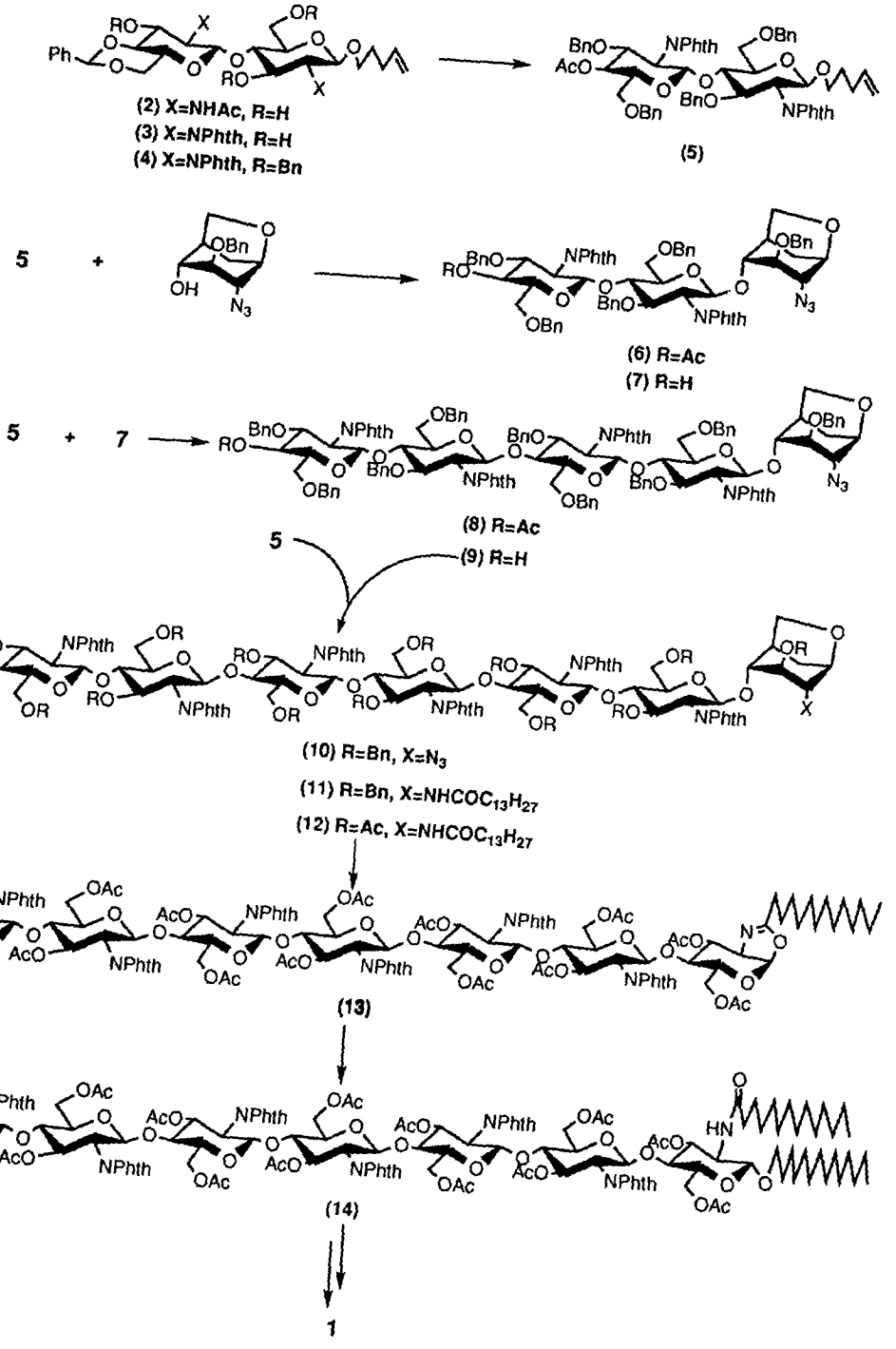
(1)

to replacement of the acetamido groups with the phthalimido groups in the usual way, giving **3**, mp 168 °C, $[\alpha]_D^{22}$ -36°,[6] which was then benzylated in conventional way to give the tri-*O*-benzyl derivative **4**, $[\alpha]_D^{24}$ +21°, as a glass. Compound **4** underwent reductive cleavage of the benzylidene group by treatment with $NaBH_3CN$-HCl[7] in THF followed by *O*-acetylation to give the disaccharidic elongation unit **5**, $[\alpha]_D^{26}$ +35°, ^{1}H NMR[8] δ 4.93 (d, 1H, J = 7.4 Hz, H-1), 5.15 (t, 1H, J = 9.0, H-4'), 5.33 (d, 1H, J = 8.2 Hz, H-1').

As a precursor of the reducing end of the heptasaccharide skeleton expected, we chose 1,6-anhydro-2-azido-3-*O*-benzyl-2-deoxy-β-D-glucopyranose,[9] which was glycosylated at 0 °C with **5** using *N*-iodosuccinimide (NIS) and triethylsilyl triflate (TESOTf)[10] as promoters to give the trisaccharide **6** in 83% yield, $[\alpha]_D^{21}$ +43°, ^{1}H NMR δ 5.16 (t, 1H, J = 9.2 Hz, H-4"), 5.20 (br.s, 1H, H-1), 5.25 (d, 1H, J = 8.4 Hz, H-1' or H-1"), 5.31 (d, 1H, J = 8.3 Hz, H-1' or H-1"). After de-*O*-acetylation of **6** with base, the resulting **7**, $[\alpha]_D^{21}$ +22°, was glycosylated again with **5** in the presence of NIS and TESOTf at -10 °C to give the pentasaccharide **8** in 64% yield, mp 177 °C, $[\alpha]_D^{21}$ +36°, ^{13}C NMR δ 96.55, 96.60, 96.71, 96.99, 100.50(anomeric carbons). Similarly, **8** was treated with base for deacylation and the resulting **9**, $[\alpha]_D^{19}$ +20°, was glycosylated once more with **5** using NIS and TESOTf at -40 °C, giving the heptasaccharide **10** in 57% yield, mp 196 °C, $[\alpha]_D^{20}$ +33°, ^{13}C NMR δ 96.40 (2C), 96.45, 96.50, 96.59, 96.87, 100.38 (anomeric carbons). The optimum temperatures for these glycosidation reactions using the common donor **5** and NIS-TESOTf as the promotor decreased from 0 °C to below -20 °C as the chain length of acceptors increased like mono- → tri- → penta-saccharides. When the glycosidation between **5** and **9** was carried out at 0 °C, **10** was obtainable in only 18% yield.

For introduction of a pair of linear hydrophobic groups into the terminal 1,6-anhydro-2-azido-2-deoxy-D-glucopyranose moiety, tetradecanoyl group was first to be introduced through formation of an amido bond. Thus, the azido group of **10** was reduced by treatment with H_2S gas in pyridine-triethylamine and the resulting amine was immediately acylated with tetradecanoyl chloride in the presence of triethylamine, giving **11** in 93% overall yield as amorphous powder, $[\alpha]_D^{25}$ +5.0°, ^{1}H NMR δ 5.70 (d, 1H, J = 9.9 Hz, NH). As a preparation for the next glycosidation process, we recently developed an efficient methodology for the one-pot synthesis of tetradecyl β-glycoside from 1,6-anhydro system carrying the tetradecanoylated amino group at C-2, using a disaccharide model compound.[11] Before applying the methodology to **11**, its trisaccharide homolog derived from **6** was tested, disclosing that the benzylated primary hydroxy groups suffered from partial cleavage under the reaction conditions employed. Therefore, the benzyl groups in **11** were to be replaced with acetyl groups like the model disaccharide derivative prior to application of the glycosidation methodology developed. Catalytic hydrogenation of **11** was conducted in *N,N*-dimethylformamide-acetic acid using $Pd(OH)_2$/C as catalyst. Perfect removal of all benzyl groups required a long period over 10 days and replacement of the catalyst with a fresh one during the reaction. The resulting debenzylated product was acetylated in usual way to give **12** as amorphous powder in 99% overall yield, $[\alpha]_D^{28}$ -13°, ^{1}H NMR δ 5.53-5.78 (m, 7H, H-3 of all sugar residues with $^4C_1$ conformation and NH), 4.75 (bs, 1H, H-3 of 1,6-anhydro sugar).

Similarly to the disaccharide model system,[11] **12** underwent acetolysis with acetic anhydride employing TESOTf as acidic catalyst. In this case, however, acetic anhydride had to be diluted with chloroform because of the poor solubility of **12** and the higher reaction temperature (30 °C) was also needed. In agreement with the results of the model experiment, this acetolysis gave the oxazoline derivative **13** as major


(2) X=NHAc, R=H
(3) X=NPhth, R=H
(4) X=NPhth, R=Bn
(5)
5
(6) R=Ac
(7) R=H
5 + 7
(8) R=Ac
5
(9) R=H
(10) R=Bn, X=N3
(11) R=Bn, X=NHCOC13H27
(12) R=Ac, X=NHCOC13H27
(13)
(14)
1

product accompanying the ordinary acetolysis product, α-glycosyl acetate, as sole side-product. Without separation of these products, the mixture was treated at 90 °C in 1,2-dichloroethane with 1-tetradecanol in the presence of (+)-10-camphorsulfonic acid. The resulted mixture was chromatographed on silica gel using chloroform-methanol (100:1 v/v) as eluent, giving the β-glucoside **14**, the remaining α-glycosyl acetate, and the starting **12**, in 53%, 19%, and 27% yields, respectively. Compound **14** (amorphous powder): $[\alpha]_D^{25}$ -4.2°, ^{1}H NMR δ 4.99 (t, 1H, J = 9.4 Hz, H-3), 5.23-5.27 (m, 5H, anomeric protons), 5.29 (d, 1H, J = 8.3 Hz, one of the anomeric protons), 5.40 (d, 1H, J = 8.4 Hz, one of the anomeric protons). The α-glycosyl acetate also converted to the oxazoline derivative **13** by treatment with TESOTf in 51% yield.

Removal of all protecting groups from **14** leaving the tetradecanamido group encountered difficulty since the solubilities of **14** in most solvents were very low. For de-*O*-acetylation, **14** was treated in methanol-chloroform with a large excess of sodium methoxide. After neutralization with aqueous HCl followed by evaporation, the mixture was heated with ethylenediamine[12] at 80 °C for 6 days in ethanol-methanol solution, resulting in the complete cleavage of the imido bonds. Since the deprotected product was insoluble in the basic media, the whole precipitate was filtered after cooling. A solution of the precipitate in water-ethanol-acetic acid was fractionated by passing through Sephadex G-15. Finally, 0.1 M aqueous HCl was added to the separated product and the mixture was lyophilized, giving **1** in 70% overall yield, $[\alpha]_D^{31}$ -13° (c 0.71, H_2O), IR (KBr) ν_{max} 3401 (OH,NH), 1644, 1559 (NHCO) cm^{-1}, ^{1}H NMR (D_2O, 348 °K) δ 4.52 (d, 1H, J = 7.9 Hz, H-1), 4.89-4.93(m, 6H, anomeric protons), FAB MS Calcd for $(M + 1)^+$:1552.9; Found: 1552.8.

The phenomenon that methyl yellow crystals with no solubility in water completely dissolved in 3% aqueous solution of **1** indicated the micelle formation due to **1.**

Acknowledgements: The authors express their thanks to Dr. Hiroyuki Minamikawa of National Institute of Materials and Chemical Research for suggestions and help in observing physical behavior of **1** in water.

References and Notes

1. Suzuki, K.; Mikami, T.; Okawa, Y.; Tokoro, A.; Suzuki, S.; Suzuki, M. Carbohydr. Res. **1986**, 151, 403-408.
2. Yamada, A.; Shibuya, N.; Kodama, O.; Akatsuka, T. Biosci. Biotech. Biochem. **1993**, 57, 405-409.
3. Hato, M.; Minamikawa, H. Langmuir **1996**, 12, 1658-1665; Tamada. K.; Minamikawa, H.; Hato,M. Langmuir **1996**, 12, 1666-1674.
4. Terayama, H.; Takahashi, S.; Kuzuhara, H. J. Carbohydr. Chem. **1993**, 12, 81-93.
5. Nishimura, S.; Matsuoka, K.; Furuike, T.; Ishii, S.; Kurita, K. Macromolecules **1991**, 24, 4236-4241.
6. All new compounds with the specific rotation data gave the satisfactory results of elemental analyses. Unless otherwise specified, the solvent for the specific rotation measurement was $CHCl_3$.
7. Garegg, P.J.; Hultberg, H. Carbohydr. Res. **1981**, 93, C10-C11.
8. Unless otherwise specified, the solvent for ^{1}H- and ^{13}C-NMR measurements was $CDCl_3$.
9. Sakairi, N.; Takahashi, S.; Wang, F.; Ueno, Y.; Kuzuhara, H. Bull. Chem. Soc. Jpn. **1994**, 67, 1756-1758.
10. Fraser-Reid, B.; Udodong, U.; Wu, Z.; Ottoson, H.; Merritt, J.R.; Rao, C.S.; Roberts, C.; Madsen, R. Synlett, **1992**, 927-942.
11. Umino, A.; Hinou, H.; Matsuoka, K.; Terunuma, D.; Takahashi, S.; Kuzuhara, H. Submitted to J. Carbohydr. Chem.
12. Kanie, O.; Cwawley, S.C.; Palcic, M. M.; Hindsgaul, O. Carbohydr. Res. **1993**, 243, 139-164.

(*Received in Japan* 4 *August* 1997; *revised* 11 *September* 1997; *accepted* 12 *September* 1997)