COMMUNICATION

ChemComm
www.rsc.org/chemcomm

A practical synthesis of amphiphilic cyclodextrins fully substituted with sugar residues on the primary face†

Florence Sallas,[a] Kenichi Niikura[b] and Shin-Ichiro Nishimura*[b]

[a] *Sapporo Laboratory for Glycocluster Project, Japan Bio-Industry Association, Sapporo, 001-0021, Japan. E-mail: florence@glyco.sci.hokudai.ac.jp*

[b] *Laboratory for Biomacromolecular Chemistry, Division of Biological Sciences, Graduate School of Science, Hokkaido University, Kita-Ku, Kita-21, Nishi-11, 001-0021, Sapporo, Japan. E-mail: shin@glyco.sci.hokudai.ac.jp*

Received (in Cambridge, UK) 15th December 2003, Accepted 16th January 2004
First published as an Advance Article on the web 3rd February 2004

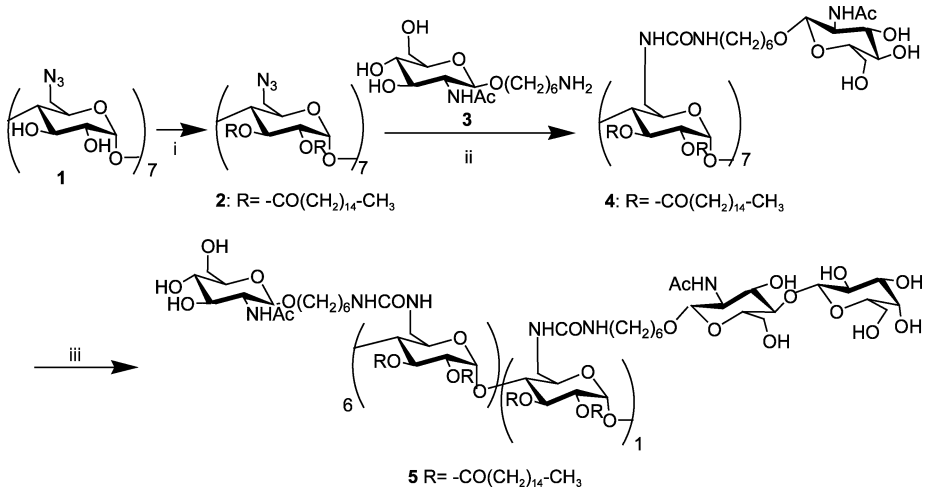
New amphiphilic cyclodextrins fully substituted with sugar residues on the primary face have been synthesised and enzymatically modified.

Over the past decade, many studies on cyclodextrins (CDs) have focused on their potential as drug carriers[1] due to their ability to host a variety of hydrophobic molecules while improving their solubilization and bioavailability. Nevertheless, the hydrophilicity of their outer surface prevents them from interacting properly with biological membranes. In order to minimize this inconvenience, one or both CD rims must be chemically modified to give the molecule amphiphilic properties. Thus, many derivatives of amphiphilic CDs have already been described[2] and some studied for their ability to form nanoparticles or liposomes that can be loaded with drugs.[3] Another challenging point is the targeting of the drug *via* its carrier. In this regard, numerous CD derivatives, mostly substituted with mono- or poly-saccharides, have already been described and proven recognizable by lectins.[4] In this field, we report here the synthesis of two new amphiphilic CD derivatives fully substituted with sugar residues on the primary rim.

Scheme 1 describes the synthetic strategy leading to the CD amphiphile **4** where the carbohydrate is linked to the CD by a urea moiety. Starting from the native β-CD, its perazido derivative **1** was prepared[5] then esterified using palmitoyl anhydride and 4-dimethylaminopyridine (DMAP) in dry pyridine according to Lesieur's conditions[6] that we have slightly modified. New intermediate **2**, with seven azido groups on the primary rim and fourteen palmitoyl chains on the secondary rim, was thus obtained in 40% yield and fully characterized. The one-pot coupling reaction between intermediate **2** and an excess of the amino terminal unprotected glucosamine derivative **3** [7] was carried out in pyridine in the presence of a large excess of triphenylphosphine and constantly bubbling CO_2.[8] The first step is similar to a Staudinger reaction after which the reaction proceeds through an isocyanate intermediate which is formed *in situ* and reacts with the nucleophilic amino derivative. The expected CD derivative **4** was thus obtained, after purification, in 30% yield.

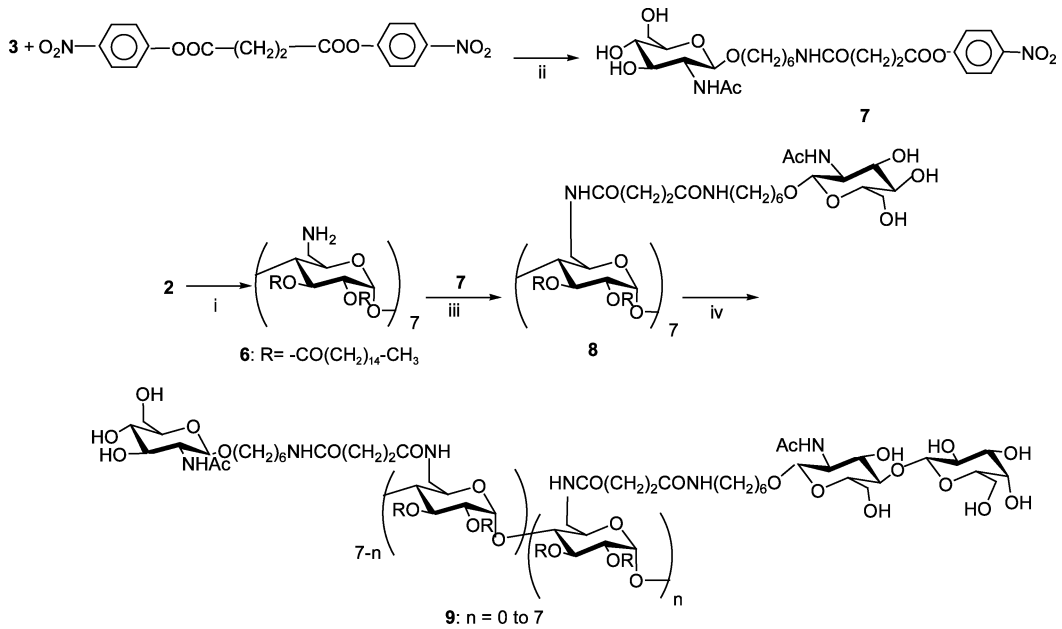
Another synthetic strategy was used for the synthesis of the CD amphiphile with an amide bond (**8**) (Scheme 2). First, the perazido groups of compound **2** were reduced to obtain the peramino esterified compound **6**. As the ester chains of this derivative are base-sensitive, the reduction was carried out at room temperature using PPh_3 and a large excess of water in THF. The peramino compound **6** was thus obtained in quantitative yield. On the other hand, the side chain of compound **3** was elongated using the readily prepared *p*-nitrophenyl succinate. The reaction occurred in dry DMF, using a six-fold excess of the diester to prevent dimerization. Compound **7** was obtained in 75% yield. The last step consisted of a simple coupling between an active ester and an amino group. The reaction was carried out in dry pyridine using a large excess of the active ester **7**. The new amphiphilic CD derivative **8**, with a slightly longer spacer arm than compound **4** between the CD and the glucosamine residue, was thus obtained in 28% yield. All newly synthesised compounds were characterized by NMR spectroscopy ($CDCl_3$ or pyridine-d_5), mass spectra (FAB and/or MALDI-TOF) and elemental analysis. These data were in agreement with their expected structure. In the final step, as compounds **4** and **8** are not water-soluble, liposomes were prepared using a mixture of **4** or **8** and dioleoyl phosphatidylcholine (DOPC) (1:9, respectively). These liposomes were suspended in HEPES buffer and submitted to enzymatic glycosylation using β-(1,4) galactosyl transferase and UDP-galactose. In the case of compound **4**, although several sets of conditions (*i.e.* buffer, pH, amount of UDP-gal) were used, only the

† Electronic supplementary information (ESI) available: selected analytical data for compounds **2**, **4**–**9**, preparation of liposomes, and MALDI-TOF spectra of **5** and **9**. See http://www.rsc.org/suppdata/cc/b3/b316365b/



Scheme 1 *Reagents and conditions*: (i) palmitoyl anhydride, DMAP, pyridine, 70 °C, 48 h; (ii) PPh_3, CO_2, pyridine, r.t., 24 h; (iii) UDP-galactose, (β-1,4) galactosyl transferase, HEPES buffer, 50 mM, pH 6, 37 °C, 5 d.

DOI: 10.1039/b316365b



Scheme 2 *Reagents and conditions*: (i) PPh_3, H_2O, THF, r.t., 7 d; (ii) DMF, r.t., 2 h; (iii) pyridine, r.t., 7 d; (iv) UDP-galactose, (β-1,4) galactosyl transferase, HEPES buffer, 50 mM, pH 6, 37 °C, 5 d.

monogalactosylated derivative **5** could be obtained (yield ≈ 95%; overall yield from **1**, 14%) as shown by the MALDI-TOF spectrum recorded from the liposome suspension. In contrast, in the case of **8**, although carried out under the same experimental conditions, the reaction led to a mixture of differently substituted compounds **9** including the seven-fold-substituted derivative. This may be due to the presence of a longer spacer arm between the CD and the sugar unit in the structure of **8** that decreases the steric hindrance.

Further studies are now in progress to optimize the enzymatic reaction, and to evaluate the physico-chemical properties as well as the capacities of these amphiphile CDs as a drug carrier system.

This work was supported by a grant for Research and Development on Glycocluster Controlling Biomolecules from New Energy and Industrial Technology Development (Japan).

Notes and references

1 D. Duchêne, D. Wouessidjewe and G. Ponchel, *J. Controlled Release*, 1999, **62**, 263–268; K. Uekama, F. Hirayama and T. Irie, *Chem. Rev.*, 1998, **98**, 2045–2076.

2 See, for example: T. Sukegawa, T. Furuike, K. Niikura, A. Yamagishi, K. Monde and S.-I. Nishimura, *Chem. Commun.*, 2002, 430–431; A. Dubes, D. Bouchu, R. Lamartine and H. Parrot-Lopez, *Tetrahedron Lett.*, 2001, **42**, 9147–9151; A. Mazzaglia, R. Donohue, B. J. Ravoo and R. Darcy, *Eur. J. Org. Chem.*, 2001, 1715–1721; A. Mazzaglia, R. Donohue, B. J. Ravoo and R. Darcy, *Chem. Commun.*, 2002, 2864–2865; C. E. Granger, C. P. Félix, H. P. Parrot-Lopez and B. R. Langlois, *Tetrahedron Lett.*, 2000, **41**, 9257–9260.

3 M. Skiba, C. Morvan, D. Duchêne, F. Puisieux and D. Wouessidjewe, *Int. J. Pharm.*, 1995, **126**, 275–279.

4 C. Ortiz Mellet, J. Defaye and J. M. García Fernández, *Chem. Eur. J.*, 2002, **8**, 1982–1990; T. Furuike, S. Aiba and S.-I. Nishimura, *Tetrahedron*, 2000, **56**, 9909–9915.

5 P. R. Ashton, R. Königer, J. F. Stoddart, D. Alker and V. D. Harding, *J. Org. Chem.*, 1996, **61**, 903–908.

6 S. Lesieur, D. Charon, P. Lesieur, C. Ringard-Lefevre, V. Muguet, D. Duchêne and D. Wouessidjewe, *Chem. Phys. Lipids*, 2000, **106**, 127–144.

7 F. Sallas and S.-I. Nishimura, *J. Chem. Soc., Perkin Trans. 1*, 2000, 2091–2103.

8 F. Sallas, A. Marsura, V. Petot, I. Pintér, J. Kovács and L. Jicsinszky, *Helv. Chim. Acta*, 1998, **81**, 632–645.