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Multivalent binding of galactosylated cyclodextrin vesicles to lectin†

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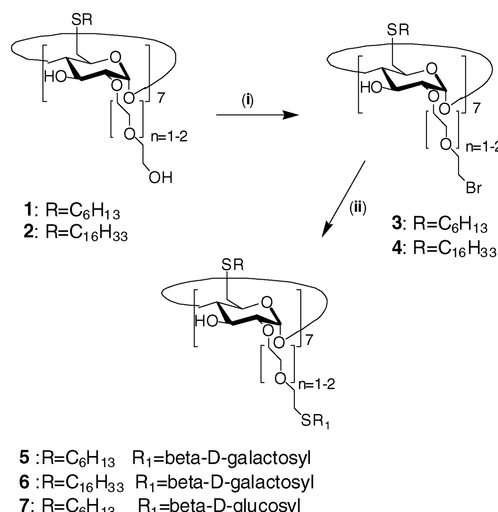
Amphiphilic β-cyclodextrins with alkylthio chains at the primary-hydroxyl side and galactosylthio-oligo-(ethylene glycol) units at the secondary-hydroxyl side, which form nanoparticles and vesicles, show multivalent effects in their binding to lectin.

Cyclodextrin oligosaccharides (CDs) contain six, seven or eight α(1–4)-linked glucopyranosyl units (α, β, or γ-CD) in their macrocyclic structures and can act as host molecules to form inclusion complexes with lipophilic guests.[1] Lipophilic modifications to CDs bestow cell-membrane permeation ability.[2] When further modified with polar groups, lipophilic CDs become amphiphilic and can form monolayers and nanoparticles,[3] thermotropic liquid crystals[4] or micelles.[5] More recent examples have been shown to form bilayer vesicles.[6]

Targeting to cell receptors for drug delivery and transfection are other main goals in adapting CDs for pharmaceutical applications. In line with this concept, a number of glycosylated CDs have been synthesised to demonstrate their binding properties towards saccharide-specific cell wall lectins.[7] They have included multi-glycosylated CDs intended to exploit glycosyl-cluster recognition effects. Such CDs however must attain atypically low dissociation constants, 10^{-7} M or less, for their molecular inclusion complexes if they are to be effective in pharmaceutical delivery.[7g] We have shown that oligo(ethylene glycol)-modified amphiphilic CDs such as **1** and **2** form colloidal assemblies, and that where these are vesicular they encapsulate both polar and hydrophobic guest molecules in complexes which are stable towards dilution.[6] We have now synthesised the first family of CDs that form nanoparticles modified with surface glycosyl groups for effective targeting to lectin proteins.

Scheme 1 shows the synthetic route to the glycosylated β-CD amphiphiles **5**, **6** and **7**. The alkylthio β-CDs **1** and **2** were prepared as already described, with seven identical alkylthio groups per CD (one per glucose) and 2–3 units of ethylene glycol on average per glucose.[8] The ω-bromo CD derivative **4** was synthesised as reported for **3**,[8] and isolation and purification were easier due to its high lipophilicity, resulting in



Scheme 1 *Reagents and conditions*: (i) DMF, PPh_3 (**1**: 20 equiv., **2**: 16 equiv.), NBS (**1**: 20 equiv., 80 °C, 3–4 h, 32%, **2**: 16 equiv., 65 °C, 4 h, 70%), (ii) DMF, R_1SNa (40 equiv.), 80 °C, 7 days, 40%.

a higher yield. Complete bromination in **3** and **4** was confirmed by ^{13}C NMR (replacement of terminal alcohol carbons at δ = 61.2 by terminal bromine carbons at δ = 29.8) and by microanalysis.

Targets **5** and **6** were prepared by treatment of **3** and **4** with the sodium salt of 1-thio-β-D-galactose. The corresponding glucosylated **7** was synthesised by reaction with the sodium salt 1-thio-β-D-glucose. All compounds were characterised by ^1H and ^{13}C NMR as well as ^1H–^{13}C HSQC NMR spectroscopy (500 MHz, $CDCl_3$ or d_6-DMSO), MALDI-TOF MS and elemental analysis. The electronic supplementary information includes a detailed description of the synthesis and characterisation of **4**–**7**. †

The ^{13}C NMR spectra of CDs **5**–**7** show replacement of the terminal brominated carbons, at 29.8 ppm, by CH_2–S–Gal for CDs **5** and **6** and by CH_2–S–Glc for CD **7** at 31.4 ppm. MALDI-MS spectra of **5**–**7** show envelopes of signals separated by 44 mass units (corresponding to one ethylene oxide group, EO) indicating a variable degree of substitution. The most abundant mass is 3722 for **5** which corresponds to seven thiogalactose units connected to seven oligo (ethylene oxide) chains averaging two EO groups each plus one sodium

† Electronic supplementary information (ESI) available: Synthesis and analytical data of CDs **4**–**7**. Spectroscopic and analytical data were consistent with the assigned structures. See http://www.rsc.org/suppdata/ob/b4/b400988f/

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ion. The polydisperse substitution causes loss of symmetry which is evident as significant line broadening in the NMR spectra for the anomeric proton and C1, C2, and C4.

In spite of the addition of seven sugar groups to their amphiphilic precursors, the new CDs **5**–**7** still aggregate in aqueous solution.

Dynamic light scattering (DLS) ‡ showed particles with average hydrodynamic diameter of *ca*. 100 nm for CDs **5** and **7** and of 150 nm for CD **6**. The critical aggregation concentration (cmc) was clearly evident at 8 μM for CD **7** and we assume that it is similar for CD **5**. On the other hand CD **6** formed aggregates at much lower concentration (0.2–0.5 μM) and, like its precursor **2**, did not show a detectable cmc.

With transmission electron microscopy (TEM) ‡ (Fig. 1) the particles displayed the same size ranges as those indicated by DLS, as well as distinctly different morphologies: disc-like images for **5**, and discs showing dark centres originating from aqueous cores for **6**. The aggregation properties therefore parallel those of the unglycosylated precursors. The ability of precursor **2** [6a] to form vesicles is preserved for glycosylated **6**. CDs **5** and **7** form nanoparticles as did their precursor **1**.[5d] The aggregation behaviour is determined by the balance between hydrophobic alkyl chains and hydrophilic ω-thioglycosyl-oligo (ethylene glycol) head groups as described for CDs **1** and **2**; [5d,6a] also by the size of the head group and its hydration shell relative to the hydrophobic alkyl chains,[9] as observed for cationic CD vesicles.[6c]

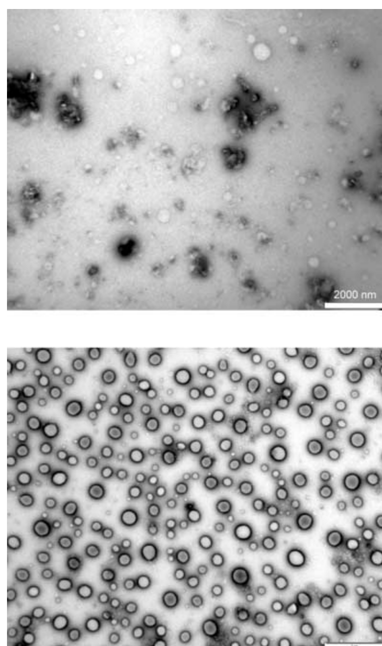

Fig. 1 Electron micrographs of ω-galactosyl CDs **5** (upper) and **6** (lower) in water (0.1 mg mL^{-1}). Negative staining with UO_2Ac. Scale bars = 2 μm.

Binding of the galactosylated CD colloids to galactose-specific lectins was investigated by MALDI-MS, fluorescence spectroscopy and surface plasmon resonance.

The MALDI mass spectrum of the galactose-binding lectin from *Pseudomonas Aeruginosa* § (PA-1) and the partial spectrum of the PA-1 complex with **5** are shown in Fig. 2. This lectin is specific for galactosyl epitopes and was also chosen for its molecular weight which is only three times that of the cyclodextrin, to provide a well resolved mass spectrum of the complex. The MALDI mass spectrum of an equimolar mixture of lectin and CD **5** shows groups of peaks corresponding to the CD plus sodium ion (main peak at 3722, calculated as 3719, not shown), PA-1 plus sodium ion (peak at 12777; calculated as 12768), PA-1·CD complex plus sodium complex (main peak

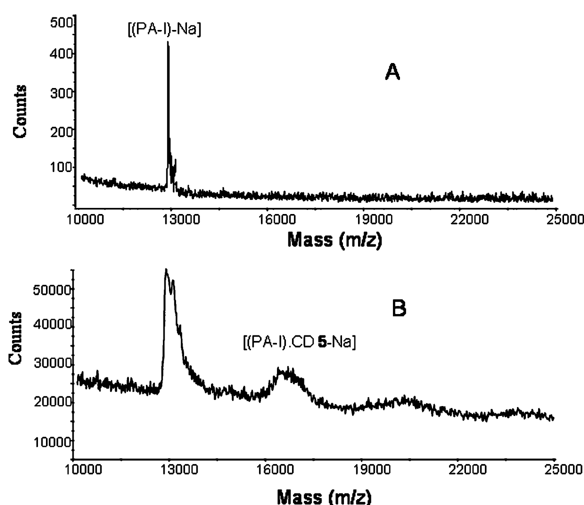

Fig. 2 MALDI mass spectrum of (**A**) lectin PA-1 and (**B**) an equimolar mixture of lectin PA-1 and galactosyl CD **5**.

at 16588; calculated as 16599, corresponding to PA-1 and CD with 17 EO plus sodium ion), PA-1·CD 1 : 2 complex. These results show that the binding of CD to lectin is relatively strong, and involves effects other than inclusion by the CD of lectin lipophilic side chains, since it is known that MALDI-MS does not detect the normal inclusion complexes of cyclodextrins, while it reveals protein–saccharide ligand complexes.[10] A MALDI mass spectrum under the same conditions for the glucosylated CD **7** showed a barely detectable peak corresponding to lectin–CD complex, and no evidence for a 1 : 2 complex. §

The fluorescence emission spectrum of PA-1 (3.5 μM) (Fig. 3¶), obtained by excitation at λ_{exc}= 290 nm, showed a maximum at 331 nm which decreased significantly on addition of an excess of CD **5** (21 μM – compare cmc of 8 μM). Despite a slight turbidity of the solution, the spectrum remained unchanged after 5 h.

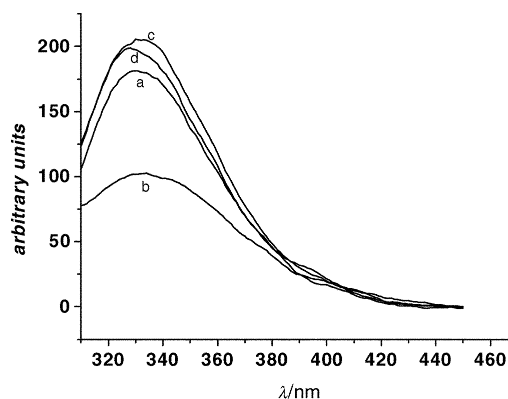

Fig. 3 Fluorescence spectra of lectin PA-1 at 3.5 μM (trace a), and after addition of CD **5** (trace b), of CD **7** (trace c) and of CD **1** (trace d) at the respective concentrations of 21 μM.

The study of fluorophores (tryptophans and tyrosines) emission is a useful criterion of carbohydrate–protein interactions.[11] The crystal structure of PA-1 in the presence of calcium has been solved as a dimer containing four tryptophans and three tyrosines for each subunit.[12] It has been recently shown that the lectin-galactose contact points participate in the coordination of the calcium ion.[13] Binding of CD **5** to PA-1 is shown by the reduced intensity of the emission band. This is ascribed to a conformationally modified, more polar, environment of fluorophores in proximity to the binding site and to their degree of exposure to solvent when galactose residues interact with the lectin binding clefts. In contrast, the addition

of unglycosylated precursor **1** or of glucosylated CD **7** to a PA-1 solution did not decrease the fluorescence, indicating an anomalous interaction[11] or no interaction. These results confirm the lectin binding by CD **5** evident from the MALDI-MS results.

The above spectroscopic methods did not reveal any effects which would serve to evaluate contributions by individual molecules to binding. For this purpose surface plasmon resonance (SPR) was employed. A second lectin, from *Arachis hypogaea* (peanut agglutinin, PNA), was immobilised on a sensor surface for surface plasmon resonance to measure its interaction with the galactosylated CDs **5** and **6**.‖ Response changes obtained at a CD concentration of 0.1 mM were: 1 arc second for glucosylated CD **7** as the negative control, 130 for CD **5** and 170 for CD **6**.

Analysis of results of binding at different concentrations of CDs (above that for nanoaggregate formation) shows apparent dissociation constants $K_d = 5.8 \times 10^{-4}$ M for CD **5** and $3.3.10^{-5}$ M for CD **6**. The difference in response for the two galactosylated CDs cannot be due merely to the different masses caused by their lipid chains (C6 and C16). At the concentrations used, the great majority of amphiphile is in the aggregate form, and the difference in response between **5** and **6** fits the concept of the larger (vesicular) aggregates being adsorbed for CD **6**. The smaller dissociation constant for the vesicle-forming CD probably indicates that it presents its galactosyl to the lectin more effectively. This may be expected for a unilamellar vesicle, since about half of the carbohydrate headgroups are at the surface, as compared with a nanoparticle, where the solid structure occludes a higher proportion of the groups.

Due to the aggregation at low concentrations, and lack of sensor sensitivity at lower levels than these, we used dilution of the targeted CDs within the assemblies in order to see if the effect of adsorption of individual molecules could be distinguished from the adsorption of aggregates. Colloidal preparations were used in which the targeted CDs were diluted with untargeted precursors: galactosyl-conjugated CD **5** was diluted by **1** at a total concentration 0.055 mM, and **6** by **2** at a total concentration 0.045 mM.

We anticipated that in a liquid-crystalline surface the targeted molecules might adapt a configuration which would self-optimise for interaction with the lectin. Fig. 4 shows the binding results for the mixed-molecule assemblies. A gradual increase in response is at first observed with increasing concentration of targeted CD in the aggregates. Eventually at a particular composition (approximately 30% targeted CD) a pronounced increase in binding activity appears. The effect then levels off as expected for saturation of the lectin sites.

Previously attempts have been made to create such a self-optimising mobile system by anchoring glycolipids, or neoglycoprotein in liposome membranes.[15a–c] Synthetic multivalent ligands anchored in liposomes have been studied,[15d,15e] also liposomes incorporating a sialoside lipid.[15f] However the effectiveness of the lectin binding was in each case measured by competitive haemagglutination effects, attributable both to binding and to accompanying steric effects preventing the lectin from crosslinking substrate cells. Saccharide-appended CD derivatives threaded onto hydrophobic polymers were recently prepared as dynamic multivalent systems for binding orientation-specific receptors.[15g] Polymer-attached sugars have also been assessed for lectin binding, but there the multivalent interactions can be due to statistical rather than chelate effects.[15h] The legume lectins Conconavalin A (Con A), Erythrina Corallodendron (EcorL) and PNA are similar in the tertiary structures of their four subunits, but differ in quaternary assembly. Whereas EcorL dissociates into dimers at physiological pH, both Con A and PNA are still tetrameric. In the tetramer of PNA, there are two pairs of carbohydrate-binding sites, each pair on opposite sides of the protein. As with Con A[16] therefore, each pair is amenable to the chelate effect, provided the ligand has two carbohydrate units separated, as are the binding sites, by tens of angstroms to effect a highly favoured interaction. Binding to this lectin will also be favoured by the statistical effect if there is a high concentration of neighbouring carbohydrate units on the ligand. A feature of chelation however is the sharp increase in binding at a concentration where the ligand presents the required 'cluster' to the lectin, and this is evident here for the two lyotropic liquid-crystalline, vesicle- and nanoparticle-forming CDs.

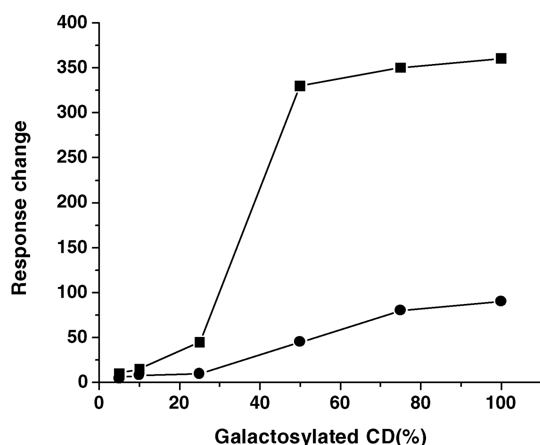

Fig. 4 Relationship between binding ability and aggregate composition for CDs **6** + **2** (■) and **5** + **1** (●).

CDs **5** and **6** are therefore the first examples of a new family of amphiphilic cyclodextrins labelled with glycosyl groups for the recognition of carbohydrate-binding proteins. The resulting nanoparticles and vesicles, besides being colloidally stable and probably less immunogenic due to their oligo(ethylene oxide) exterior, consist of macrocyclic oligosaccharides which can be modified for investigation of cellular interactions without affecting colloidal stability. Such amphiphilic CDs will serve to investigate and optimise factors such as carbohydrate–protein interactions at cell receptors, as well as providing more versatile artificial liposomes.

We dedicate this study to the memory of Dr Lawrence Penkler. We gratefully acknowledge inspiring discussions with Professor Luigi Monsù Scolaro (University of Messina) and Dr Norberto Micali (IPCF-CNR Messina). Dr Anna Rencurosi (CNR, Milan), and Mr Antonio Valerio (University of Milan) are acknowledged for their collaboration in synthetic work. A. M. thanks Enterprise Ireland, MURST-Cofin 2002 and CNR.

Notes and references

‡ CD solution (0.1 mg mL^{-1}) was prepared from stock solution (1 mg mL^{-1}) of CDs **5**, **6**, **7** in $CHCl_3$ which was slowly evaporated under a stream of N_2 to form a thin film. The CD film was hydrated and maintained at 60 °C for 1 h, then sonicated for 1.5 h at 60 °C and filtered through a 1.0 μm microfilter. The solution was investigated by DLS using Malvern instrumentation, and by TEM using a JEOL 2000 electron microscope after negative staining with uranyl acetate. DLS and TEM on a solution of CD **7** showed particles with the same size range as for CD **5**. DLS results are reported as ESI. Solutions of CDs **5** and **7** are still clear at 3% w/w. In contrast CD **6**, even at a concentration of 0.5 mg mL^{-1}, forms a hazy dispersion. Results of surface tensiometry of glucosylated CD **7** are also reported as ESI.

§ Lectin was PA-1 from *Pseudomonas Aeruginosa*, MW 13–13.7 × 10^3 g mol^{-1} (Sigma). All MALDI-MS spectra are given as ESI.

¶ Steady state fluorescence experiments were performed on a Jasco model FP-750. All experiments were run at least three times. Fluorescence is not corrected for the absorbance of the samples. Scattering effects were minimised by using a Helma cuvette with light path d = 3 mm.[11] The crystal structure of PA-1[12] has a sequence containing four tryptophans (Trp) and three tyrosines (Tyr) which adsorb in the UV region (260–295 nm). In particular the structure[13] shows that Tyr36 participates in the coordination of calcium ions and Tyr98, Tyr105 and Trp42 are located in neighbouring loops By exciting at 290 nm the

contribution of the Tyr component is minimal and does not obscure the tryptophan emission spectrum. From surface tension and a detailed DLS investigation (data not shown) **5** is a mixture of individual molecules (38%) and of aggregates (62%) at a concentration of 21 µM (fluorescence experiment).

|| Binding of the immobilised lectin ligand of *Arachis hypogaea* (Sigma) to CDs **5** and **6** was studied using an IAsys™ surface plasmon resonance (SPR) instrument according to the kinetic model reported in the literature.[14] The mixed vesicles were made up as varying percentage mixtures of galactosylated and non-galactosylated (100%, 75%, 25%, 10%, 5%) CDs. Each sample was dissolved in 1 mL of CH_3Cl which was evaporated to form a film. Deionised water was added, followed by sonication (15 min). Measurements were done within 24 h. Further details are given as ESI.

1 J. Szejtli, in *Cyclodextrins in Pharmacy*, ed. K.-H. Fromming, Kluwer Academic, Dordrecht, 1994.
2 Z. Shao, Y. Li, T. Chermak and A. K. Mitra, *Pharm. Res.*, 1994, **11**, 1174.
3 (*a*) Y. Kawabata, M. Matsumoto, M. Tanaka, H. Takahashi, Y. Irinatsu, S. Tamura, Y. Tagaki, H. Nakahara and K. Fukuda, *Chem. Lett.*, 1986, 1933; (*b*) H. Parrot-Lopez, C.-C. Ling, P. Zhang, A. Baszkin, G. Albrecht, C. de Rango and A. W. Coleman, *J. Am. Chem. Soc.*, 1992, **114**, 5479; (*c*) M. M. Greenhall, P. Lukes, K. Kataky, N. E. Agbor, J. P. S. Badyal, J. Yarwood, D. Parker and M. C. Petty, *Langmuir*, 1995, **11**, 3997; (*d*) A. Gulik, H. Delacroix, D. Wouessidjewe and M. Skiba, *Langmuir*, 1998, **14**, 1050; (*e*) K. Kobayashi, K. Kajikawa, H. Sasabe and W. Knoll, *Thin Solid Films*, 1999, **349**, 244; (*f*) M. Skiba, D. Duchêne, F. Puisieux and D. Wouessidjewe, *Int. J. Pharm.*, 1996, **129**, 113.
4 C.-C. Ling, R. Darcy and W. Risse, *J. Chem. Soc., Chem. Commun.*, 1993, 438.
5 (*a*) R. C. Petter, J. S. Salek, C. T. Sikorski, G. Kumaravel and F.-T. Lin, *J. Am. Chem. Soc.*, 1990, **112**, 3860; (*b*) F. Witte and H. Hoffmann, *J. Inclusion Phenom. Mol. Recogn. Chem.*, 1996, **25**, 25; (*c*) R. Auzély-Velty, F. Djedaïni-Pilard, S. Désert, B. Perly and T. Zemb, *Langmuir*, 2000, **16**, 3727; (*d*) A. Mazzaglia, B. J. Ravoo, R. Darcy, P. Gambadauro and F. Mallamace, *Langmuir*, 2002, **18**, 1845.
6 (*a*) B. J. Ravoo and R. Darcy, *Angew. Chem., Int. Ed.*, 2000, **39**, 4324; (*b*) T. Sukegawa, T. Furuike, K. Niikura, A. Yamagishi, K. Monde and S. Nishimura, *Chem. Commun.*, 2002, 430; (*c*) R. Donohue, A. Mazzaglia, B. J. Ravoo and R. Darcy, *Chem. Commun.*, 2002, 2864; (*d*) A. Mazzaglia, L. Monsù Scolaro, R. Darcy, R. Donohue and B. J. Ravoo, *J. Inclusion Phenom. Macr. Chem.*, 2002, **44**, 127; (*e*) S. Sortino, S. Petralia, R. Darcy, R. Donohue and A. Mazzaglia, *New. J. Chem.*, 2003, **27**, 602; (*f*) D. Nolan, R. Darcy and B. J. Ravoo, *Langmuir*, 2003, **19**, 4469; (*g*) B. J. Ravoo, J. C. Jacquier and G. Wenz, *Angew. Chem., Int. Ed.*, 2003, **42**, 2066; (*h*) A. Mazzaglia, N. Angelini, R. Darcy, R. Donohue, D. Lombardo, N. Micali, V. Villari, M. T. Sciortino and L. Monsù Scolaro, *Chem. Eur. J.*, 2003, **9**, 5762.
7 (*a*) L. de Robertis, C. Lancelon-Pin and H. Driguez, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 1127; (*b*) E. Leray, H. Parrot-Lopez, C. Augè, A. W. Coleman, C. Finance and R. Bonaly, *J. Chem. Soc., Chem. Commun.*, 1995, 1019; (*c*) R. Roy, F. Hernández- Mateo and F. Santoyo-González, *J. Org. Chem.*, 2000, **65**, 8743; (*d*) F. Ortega-Caballero, J. J. Giménez-Martínez, L. García-Fuentes, E. Ortiz-Salmerón, F. Santoyo-González and A. Vargas-Berenguel, *J. Org. Chem.*, 2001, **66**, 7786; (*e*) A. Vargas-Berenquel, F. Ortega-Caballero, F. Santoyo-González, J. J. García-López, J. J. Giménez-Martínez, L. García-Fuentes and E. Ortiz-Sálmerón, *Chem. Eur. J*, 2002, **8**, 812; (*f*) I. Bausanne, J. B. Benito, C. Ortiz Mellet, J. M. Garcia Fernández, H. Law and J. Defaye, *Chem. Commun.*, 2000, 1489; (*g*) D. A. Fulton and J. F. Stoddart, *Bioconjugate Chem.*, 2001, **12**, 655; (*h*) H. Abe, A. Kenmoku, N. Yamaguch and K. Hattori, *J. Inclusion Phenom. Macr. Chem.*, 2002, **44**, 39; (*i*) D. A. Fulton and J. F. Stoddart, *Org. Lett.*, 2000, **2**, 1113; (*j*) I. Bausanne, J. M. Benito, C. Ortiz Mellet, J. M. Garcia Fernández and J. Defaye, *ChemBioChem*, 2001, 777; (*k*) S. André, H. Kaltner, T. Furuike, S.-I. Nishimura and H.-J. Gabius, *Bioconjugate Chem.*, 2004, **15**, 87.
8 A. Mazzaglia, R. Donohue, B. J. Ravoo and R. Darcy, *Eur. J. Org. Chem*, 2001, 1715.
9 (*a*) J. Israelachvili, in *Intermolecular and Surface Forces*, Academic Press, London, 1991, ch. 16; (*b*) J.-H. Fuhrhop and J. Konig, in *Membranes and Molecular Assemblies: The Synkinetic Approach*, RSC, London, 1994, p. 50; (*c*) M. Corti and L. Cantu, in *Non Medical Application of Liposomes*, eds. Y. Barenholtz and D. Lasic, CRC Press, Boca Raton, FL, 1995, p. 219.
10 (*a*) J. Bundy and C. Fenselan, *Anal. Chem.*, 1999, **71**, 1460; (*b*) M. Guerrini, T. Aguelles, A. Bisio, M. Hricovini, L. Lay, A. Naggi, L. Poletti, L. Sturiale, G. Torri and B. Casu, *Biochem. Biophys. Res. Commun.*, 2002, **292**, 222.
11 A. S. Ladokhin, S. Jayasinghe and S. H. White, *Anal. Biochem.*, 2000, **285**, 235–245.
12 (*a*) Z. J. Liu, W. Tempel, D. Lin, K. Karaveg, R. J. Doyle, J. P. Rose and B. C. Wang, *American Crystallographic Association Abstract Papers*, 2002, **29**, 98 (PDB ref.1L7L); (*b*) K. Karaveg, Z. J. Liu, W. Tempel, R. J. Doyle, J. P. Rose and B. C. Wang, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 2002, **59**, 1241.
13 G. Cioci, E. P. Mitchell, C. Gautier, M. Wimmerová, D. Sudakevitz, S. Pérez, N. Gilboa-Garber and A. Imberty, *FEBS Lett.*, 2003, **555**, 297.
14 (*a*) I. D. Parsons, B. Persson, A. Mekhalfia, G. M. Blackburn and P. G. Stockley, *Nucleic Acids Res.*, 1995, **23**, 211; (*b*) H. Imata, K. Kubota, K. Hattori, M. Aoyagi and C. Jindoh, *Bioorg. Med. Chem. Lett.*, 1997, **2**, 109.
15 (*a*) R. T. Lee, Y. C. Lee, in *Glycosciences: Status and Perspectives*, eds. H.-J. Gabius and S. Gabius, Chapmann and Hall, London, 1997, p. 55; (*b*) N. Yamazaki, S. Gabius, S. Kojima and H.-J. Gabius, in *Lectins and Glycobiology*; Springer, Berlin, New York, 1993, p. 319; (*c*) N. Yamazaki, S. Kojima, N. V. Bovin, S. Andrè, S. Gabius and H.-J. Gabius, *Adv. Drug Delivery Rev.*, 2000, **43**, 225; (*d*) W. Spevak, J. O. Nagy, D. H. Charych, M. E. Schaefer, J. H. Gilbert and M. D. Bednarski, *J. Am. Chem. Soc*, 1993, **115**, 1146; (*e*) W. Spevak, C. Foxall, D. H. Charych, F. Dasgupta and J. O. Nagy, *J. Med. Chem.*, 1996, **39**, 1018; (*f*) J. E. Kingery-Wood, K. W. Williams, G. B. Sigal and G. M. Whitesides, *J. Am. Chem. Soc*, 1992, **114**, 7303; (*g*) A. Nelson and J. F. Stoddart, *Org. Lett.*, 2003, **5**, 3783; (*h*) G. B. Sigal, M. Mammen, G. Dahmann and G. M. Whitesides, *J. Am. Chem. Soc.*, 1996, **118**, 3789.
16 (*a*) E. K. Woller, E. D. Walter, J. R. Morgan, D. J. Singel and M. J. Cloninger, *J. Am. Chem. Soc*, 2003, **125**, 8820; (*b*) J. C. Sacchettini, L. G. Baum and C. F. Brewer, *Biochemistry*, 2001, **40**, 3009.