Cyclic Homooligomers from Sugar Amino Acids: Synthesis, Conformational Analysis, and Significance

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Abstract: Sugar amino acids (SAAs) were designed as new building blocks carrying an amino group and a carboxyl group on a carbohydrate scaffold. By exploiting standard solid- and solution-phase coupling procedures, linear and cyclic homooligomers containing glucosyluronic acid methylamine (Gum) were synthesized. We achieved a high yield and a very short coupling time for the oligomerization and cyclization of sequences encopassing two, three, four, and six Gum units. The synthesis of cyclic oligomers containing only SAAs as repetitive units has not been reported before. The conformational preferences in aqueous solution of the cyclic derivatives and their applications as potential host molecules are described herein. Benzoic acid and *p*-nitrophenol were chosen as model guest molecules to study the formation of cyclodextrin-like inclusion complexes. The complexation behavior of the cyclic hexamer was proved from three different points of view: chemical shifts, longitudinal relaxations (T_1), and diffusion coefficients. All of them showed different values for host and guest molecules measured independently and in the presence of each other.

Introduction

Structure activity relationship (SAR) studies on biopolymers have led to an increasing interest in the potentials of their synthetic analogues in order to modify pharmacokinetics and metabolic stability of the natural drugs. Peptides and oligosaccharides are attractive targets for mimicry due to their involvement in a complexity of biological processes.

Sugar amino acids (SAAs),[1] which are carbohydrates bearing both an amino group and a carboxyl group, are ideal peptidomimetic scaffolds[2] since they may function as structural pharmacophores,[3] and are attractive building blocks for the incorporation of a sugar moiety into combinatorial libraries using standard peptide coupling techniques.[4] They occur in nature as subunits of oligosaccharides (neuraminic acid), in cell walls of bacteria (muraminic acid), and in some antibiotics.[5]

As dipeptide isostere, we investigated the conformational and biological influence of a range of pyranose-based building blocks on peptide chains by replacing the original dipeptide sequences in a linear Leu-enkephalin analogue, in cyclic somatostatin analogues, and in cyclic α_v-selective RGD peptides.[3,6,7]

Oligomers of pyranose SAAs, first introduced by Fuchs and Lehmann,[8] were synthesized in solution[9] and on solid phase[10] and have been proposed to mimic oligosaccharide[11] and oligonucleotide[12] backbone structures via amide bond linkages. Fleet and co-workers developed tetrahydrofuran-based amino acids as dipeptide isosteres and described the potential for secondary structure predisposition of the correspondent oligomeric sequences.[13] However, cyclic SAA homooligomers have not been reported, to the best of our knowledge, until now.[14]

We present herein the synthesis and conformational analysis of linear and cyclic water-soluble oligomers containing glucosyluronic acid methylamine (Gum) as the repetitive unit. The Gum building block was designed in our laboratories as a

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(1) First SAAs: Heyns, K.; Paulsen, H. *Chem. Ber.* **1955**, *88*, 188−195.

(2) Reviews: Lohof, E.; Burkhart, F.; Born, M. A.; Planker, E.; Kessler, H. In *Advances in Amino Acid Mimetics and Peptidomimetics*; Abell, A., Ed.; JAI Press Inc.: Stanford, 1999; Vol. 2, p 263.

(3) Graf von Roedern, E.; Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 687−689. Graf von Roedern, E.; Kessler, H. *Angew. Chem.* **1994**, *106*, 684−686.

(4) McDevitt, J. P.; Lansbury, P. T. *J. Am. Chem. Soc.* **1996**, *118*, 3818−3828. Sofia, M. J.; Hunter, R.; Chan, T. Y.; Vaughn, A.; Dulina, R.; Wang, H.; Gange, D. *J. Org. Chem.* **1998**, *63*, 2802−2803. Ramamoorthy, P. S.; Gervay, J. *J. Org. Chem.* **1997**, *62*, 7801−7805. Fügedi, P.; Peto, C.; Wlasichuk, K. In *8th European Carbohydrate Symposium*, 1995; Sabesan, S., Ed. *Tetrahedron Lett.* **1997**, *38*, 3127.

(5) Fox, J. J.; Kuwada, Y.; Watanabe, K. A. *Tetrahedron Lett.* **1968**, *2*, 6029−6032. Isono, K.; Asahi, K.; Suzuki, S. *J. Am. Chem. Soc.* **1969**, *91*, 7490−7505. Lichtenthaler, F. W.; Morino, T.; Menzel, H. M. *Tetrahedron Lett.* **1975**, *9*, 665−668. Waltho, J. P.; Williams, D. H.; Selva, E.; Ferrari, P. *J. Chem. Soc., Perkin Trans. 1* **1987**, *9*, 2103−2107. Lindberg, B. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 279−318. Kotick, M. P.; Klein, R. S.; Watanabe, K. A.; Fox, J. J. *Carbohydr. Res.* **1969**, *11*, 369−377.

(6) Graf von Roedern, E.; Lohof, E.; Hessler, G.; Hoffmann, M.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 10156−10167. Lohof, E.; Born, M. A.; Kessler, H. In *Synthesis of Peptides and Peptidomimetics*; Goodman, M., Felix, A., Moroder, L., Toniolo, C., Eds.; Georg Thieme Verlag: Stuttgart, in press; Vol. E22b.

(7) Lohof, E.; Planker, E.; Mang, C.; Burkart, F.; Dechantsreiter, M. A.; Haubner, R.; Wester, H.-J.; Schwaiger, M.; Hölzemann, G.; Goodman, S. L.; Kessler, H. *Angew. Chem., Int. Ed.* **2000**, *39*, 2761−2764.

(8) Fuchs, E. F.; Lehmann, J. *Carbohydr. Res.* **1976**, *49*, 267−273. Fuchs, E. F.; Lehmann, J. *J. Chem. Ber.* **1975**, *108*, 2254−2260. Fuchs, E. F.; Lehmann, J. *J. Chem. Ber.* **1975**, *45*, 135−141. Fuchs, E. F.; Lehmann, J. *J. Chem. Ber.* **1976**, *49*, 267−273.

(9) Suhara, Y.; Hildreth, J. E. K.; Ichikawa, Y. *Tetrahedron Lett.* **1996**, *37*, 1575−1578. Suhara, Y.; Ichikawa, M.; Hildreth, J. E. K.; Ichikawa, Y. *Tetrahedron Lett.* **1996**, *37*, 2549−2552. Wessel, H. P.; Mitchell, C.; Lobato, C. M.; Schmid, G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2712−2713.

(10) Müller, C.; Kitas, E.; Wessel, H. P. *J. Chem. Soc., Chem. Commun.* **1995**, *23*, 2425−2426. Szabo, L.; Smith, B. L.; McReynolds, K. D.; Parrill, A. L.; Morris, E. R.; Gervay, J. *J. Org. Chem.* **1998**, *63*, 1074−1078.

(11) Nicolaou, K. C.; Flörke, H.; Egan, M. G.; Barth, T.; Estevez, V. A. *Tetrahedron Lett.* **1995**, *36*, 1775−1778.

(12) Goodnow, R. A. J.; Tam, S.; Pruess, D. L.; McComas, W. W. *Tetrahedron Lett.* **1997**, *38*, 3199−3202. Goodnow, R. A. J.; Richou, A.-R.; Tam, S. *Tetrahedron Lett.* **1997**, *38*, 3195−3198.

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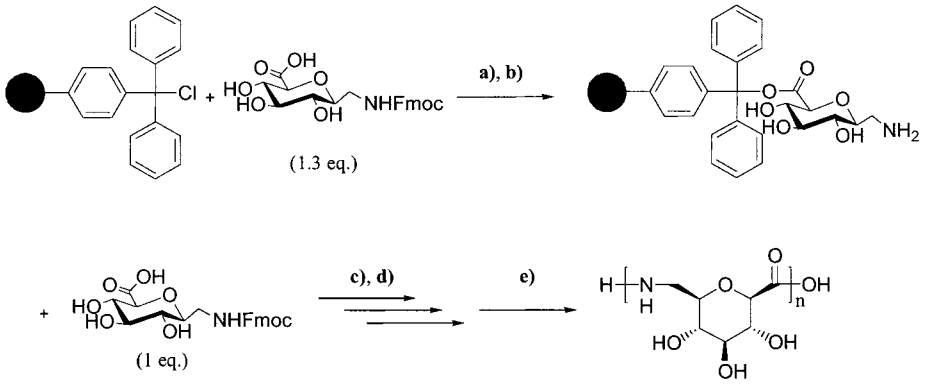
Scheme 1. Solid-Phase Synthesis of the Linear Oligomers Containing Gum[a]



[a] Conditions: (a) 1,3,5-collidine (10 equiv); (b) 20 vol % piperidine, DMF, twice 10 min; (c) 1,3,5-collidine (10 equiv), HATU (1 equiv), HOAt (1 equiv), DMF, 3 h; (d) 20 vol % piperidine, DMF, twice 10 min; (e) DCM, TFE, AcOH (3:1:1), twice 1 h.

dipeptide isostere of the Gly-Ser sequence held in a "flexible" β-turn conformation.[3]

We focused on the potentials of small oligomeric sequences with backbones of specific folding patterns and functionalities as novel artificial receptors. We assumed that a cyclic array of desired ring size and defined secondary structure of alternating carbohydrate moieties and amide groups might lead to exquisite specificity of recognition and catalysis. An application of this concept is the mimicry of cyclodextrin (CD) inclusion complexes.[15,16] Indeed, the complexation of the cyclic hexamer with two model guest molecules (*p*-nitrophenol, benzoic acid) was confirmed via NMR titration studies.[17]

Synthesis

The Fmoc-Gum-OH building block was derived in only four steps from very inexpensive α,β-D-glucose as previously described.[3] The synthesis was improved by selective oxidation of the primary hydroxyl group to carboxylic acid using the one-step oxyammonium (TEMPO)[18]-mediated reaction with sodium hypochlorite in H_2O/THF solution.[2]

The synthesis of the linear sequences H-(Gum)$_n$-OH ($n = 2$, 3, 4, 6) was performed using standard solid-phase peptide techniques with a tritylchloropolystyrene (TCP) resin applying the Fmoc strategy (Scheme 1). Our protocol did not require the orthogonal protection of the hydroxyl groups and, consequently, the final tedious step of deprotecting the oligomeric sequences. The first Fmoc-protected sugar amino acid was anchored to the tritylchloride resin with 1,3,5-collidine as base in dimethylformamide (DMF) as solvent. The use of diisopropylethylamine (DIPEA) as a stronger base was avoided since it led to epimerization at the carbon adjacent to the carboxyl moiety and consequent flipping of the pyranoid ring. Stepwise extensions of the sequence, i.e., Fmoc-deprotection with 20 vol % piperidine in DMF and subsequent coupling with Fmoc-Gum-OH under the influence of *N*-hydroxy-9-azabenzotriazole (HOAt), 2-(1*H*-9-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), and 1,3,5-collidine in DMF, followed by a final Fmoc deprotection step, afforded the immobilized dimer, trimer, tetramer, and hexamer. Due to the steric hindrance of the carboxyl group, the coupling of Gum required very efficient coupling reagents. The linear oligomers were cleaved from the resin using a mixture of dichloromethan (DCM), trifluorethanol (TFE), and acetic acid (AcOH), 3:1:1. A crucial step in the solid-phase synthesis of such hydrophilic compounds turned out to be making the right choice of the solvent for cleaning the oligomers from the solid support. Several treatments of the resin with DMF or DMSO afforded the target oligomers in 40−50% yields after purification using reversed-phase high-performance liquid chromatography (RP-HPLC). End-to-end cyclization was carried out under high dilution (0.1 mM) in DMF using an HATU/HOAt system and 1,3,5-collidine (Scheme 2).[19,20] Purification by RP-HPLC led to compounds which were >98% pure. The yields during cyclization were >90% for $n =$ 4, 6, while lower yields were achieved for the shorter sequences (35% for $n = 2$ and 60% for $n = 3$). During cyclization of the dimeric sequence, we also observed the formation of the cyclic tetramer as byproduct, which was easily separated by RP-HPLC. All compounds were characterized by ESI mass and NMR spectroscopy. The need for very strong C-terminal activation, the short coupling time, and the high yields achieved during cyclization indicate a folded conformation in solution of the linear precursors, bringing the two ends close to each other. Attempts to apply milder cyclization conditions via in situ activation using diphenyl phosphorazidate (DPPA) with sodium

(13) Smith, M. D.; Long, D. D.; Claridge, T. D. W.; Marquess, D. G.; Fleet, G. W. J. *J. Chem. Soc., Chem. Commun.* **1998**, *18*, 2039−2040. Long, D. D.; Smith, M. D.; Marquess, D. G.; Claridge, T. D. W.; Fleet, G. W. J. *Tetrahedron Lett.* **1998**, *39*, 9293−9296. Long, D. D.; Hungerford, N. L.; Smith, M. D.; Brittain, D. E. A.; Marquess, D. G.; Claridge, T. D. W.; Fleet, G. W. J. *Tetrahedron Lett.* **1999**, *40*, 2195−2198. Claridge, T. D. W.; Long, D. D.; Hungerford, N. L.; Smith, M. D.; Aplin, R. T.; Marquess, D. G.; Fleet, G. W. J. *Tetrahedron Lett.* **1999**, *40*, 2199−2203. Watterson, M. P.; Pickering, L.; Smith, M. D.; Hudson, S. H.; Marsh, P. R.; Mordaunt, J. E.; Watkin, D. J.; Newman, C. J.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **1999**, *10*, 1855−1859. Smith, M. D.; Fleet, G. W. J. *J. Pept. Sci.* **1999**, 425−441. Hungerford, N. L.; Claridge, T. D. W.; Watterson, M. P.; Aplin, R. T.; Moreno, A.; Fleet, G. W. J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3666−3679. Smith, M. D.; Long, D. D.; Martin, A.; Marquess, D. G.; Claridge, T. D. W.; Fleet, G. W. J. *Tetrahedron Lett.* **1999**, *40*, 2191−2194.

(14) Locardi, E.; Stöckle, M.; Gruner, S.; Kessler, H. In *20th International Carbohydrate Symposium*, 2000; p 51.

(15) Reviews: Schneider, H.-J.; Hacket, F.; Rüdiger, V. *Chem. Rev.* **1998**, *98*, 1755−1785. Rekharsky, M. V.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875−1917. Uekama, K.; Hirayama, F.; Irie, T. *Chem. Rev.* **1998**, *98*, 2045−2076. Loftsson, T.; Brewster, M. E. *J. Pharm. Sci.* **1996**, *85*, 1017−1169.

(16) Reviews: Szejtli, J. *Chem. Rev.* **1998**, *98*, 1743−1753.

(17) Fielding, L. *Tetrahedron* **2000**, *56*, 6151−6170.

(18) Davis, N. J.; Flitsch, S. L. *Tetrahedron Lett.* **1993**, *34*, 1181−1184. Anelli, P. L.; Biffi, C.; Montanari, F.; Quici, S. *J. Org. Chem.* **1987**, *52*, 2559−2562. de Nooy, A. E. J.; Besemer, A. C. *Tetrahedron* **1995**, *51*, 8023−8032. de Nooy, A. E. J.; Besemer, A. C.; van Bekkum, H. *Carbohydr. Res.* **1995**, *269*, 89−98.

(19) Ehrlich, A.; Rothemund, S.; Brudel, M.; Beyermann, M.; Carpino, L. A.; Bienert, M. *Tetrahedron Lett.* **1993**, *34*, 4781−4784. Ehrlich, A.; Heyne, H. U.; Winter, R.; Beyermann, M.; Haber, H.; Carpino, L. A.; Bienert, M. *J. Org. Chem.* **1996**, *61*, 8831−8838. Carpino, L. A.; El-Faham, A.; Minor, C. A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, 201−203.

(20) Carpino, L. A.; El-Faham, A.; Albericio, F. *Tetrahedron Lett.* **1994**, *35*, 2279−2282.

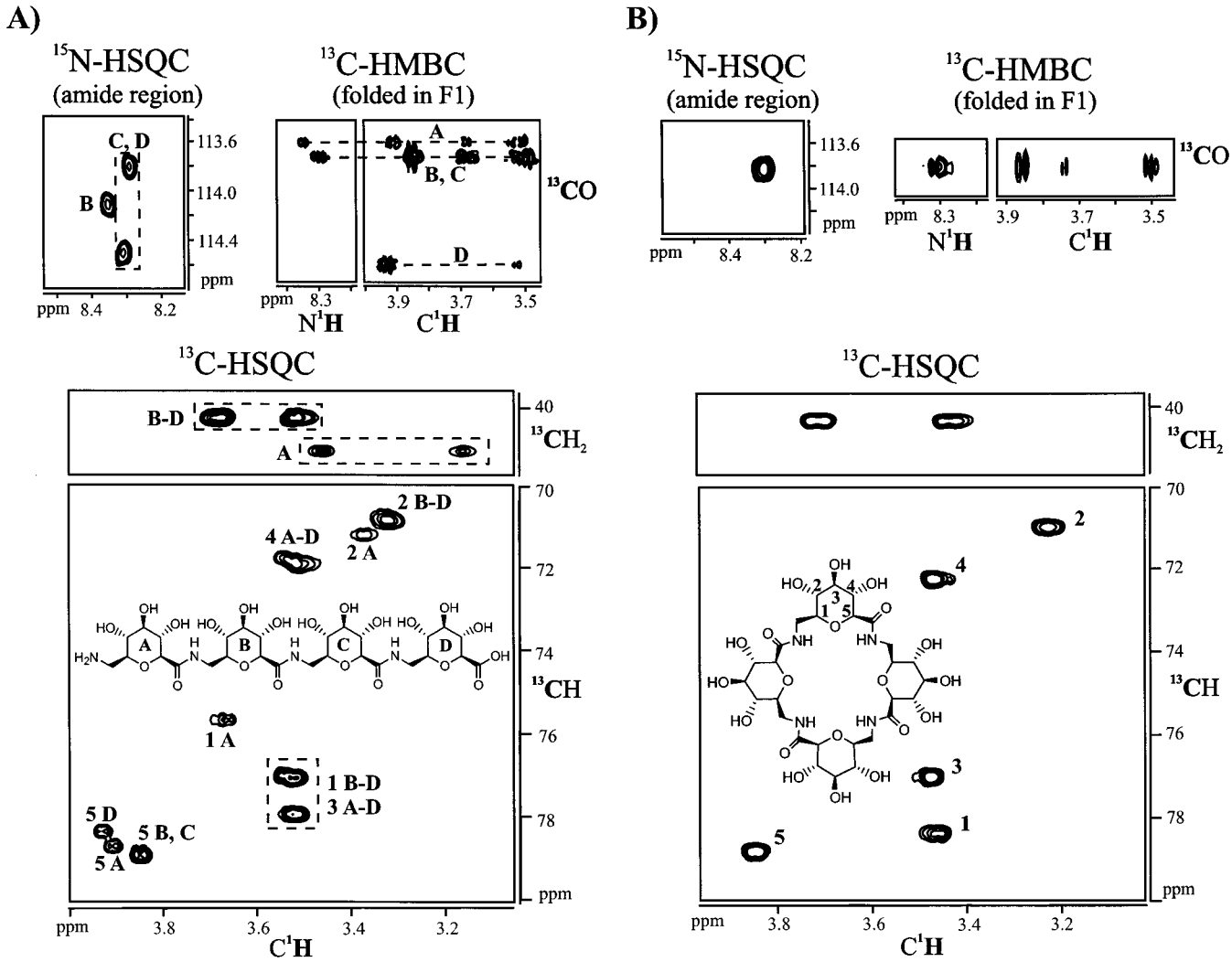
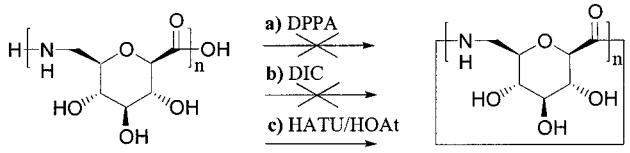


Figure 1. Comparison of the ^{1}H, ^{13}C, and ^{15}N spectral regions exhibited in water solution at 293 K by the linear (A) and cyclic tetramers (B).

Scheme 2. Cyclization in Solution of the Oligomeric Sequences Containing Gum[a]



[a] Conditions: (a) $NaHCO_3$ (5 equiv), DPPA (3 equiv), DMF (0.1 mM), 16 h, RT; (b) DIC (10 equiv), HOAt (1 equiv), NMM (3 equiv), DMF (0.1 mM), 16 h, 278 K; (c) 1,3,5-collidine (10 equiv), HATU (1 equiv), HOAt (1 equiv), DMF (0.1 mM), 6 h, RT.

bicarbonate as solid base[21,22] led only to very low yields of the cyclic products. Cyclization via the carbodiimide method using *N*,*N*′-diisopropylcarbodiimide (DIC), HOAt, and *N*-methylmorpholine (NMM) at 278 K[20,23] was also unsuccessful.

NMR Analysis

Since our target is the mimicking of water-soluble biopolymers, all spectra are acquired in aqueous solution.

Chemical Shifts. The spectra of the linear oligomers show severe overlap of the resonances: only the N- and C-terminal residues are assigned. After cyclization, the system collapses in a unique set of resonances for the Gum moieties, which are easily assigned using DQF-COSY, ^{13}C-HSQC, and ^{13}C-HMBC. A comparison between the linear tetrameric sequence and the corresponding cyclic derivative is shown in Figure 1. In general, cyclic trimer, tetramer, and hexamer show a pattern of the chemical shifts that is symmetrical on the NMR time scale, which does not change as the ring size increases. Interestingly, a more detailed analysis of the cyclic trimer in water shows a shift difference between the nonequivalent methylene protons as a function of temperature. Specifically, these resonances show a difference of 0.06 ppm at 293 K and completely overlap at lower temperature (273 K). The cyclic tetramer and hexamer show no relevant shift difference as the temperature increases. A difference of 0.22−0.23 and 0.20−0.21 ppm, respectively, is observed. The cyclic dimer is the only compound that shows slow exchange in solution, on the NMR time scale, between three different conformers. Two are populated for less than 10% (spin systems not assigned) and interconvert at high temperatures, as confirmed by exchange ROESY cross-peaks, which can easily be discriminated by the positive sign with the diagonal. This exchange peak is present in the ROESY spectrum only at 293 K but not at lower temperatures ($T = 273$ K). The third conformation, which is the most populated, shows a

(21) Brady, S. F.; Varga, S. L.; Freidinger, R. M.; Schwenk, D. A.; Mendlowski, M.; Holly, F. W.; Veber, D. F. *J. Org. Chem.* **1979**, *44*, 3101−3105. Zimmer, S.; Hoffmann, E.; Jung, G.; Kessler, H. *Liebigs Ann. Chem.* **1993**, 497−501. Zimmer, S.; Hoffmann, E.; Jung, G.; Kessler, H. In *Peptides 1992*; Schneider, C. H., Eberle, A. N., Eds.; ESCOM Science Publishers: Leiden, The Netherlands, 1993; pp 393−394.

(22) Shioiri, T.; Ninomiya, K.; Yamada, S.-I. *J. Am. Chem. Soc.* **1972**, *94*, 6203−6205.

(23) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398.

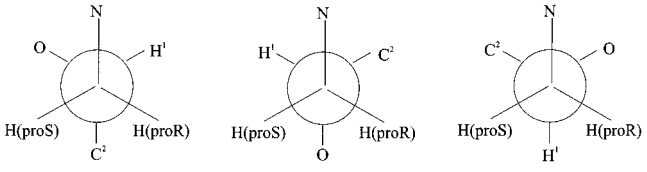


Figure 2. Rotamers around the CH_2-C^1 bond. The assignment $\pm sc$ ($\pm$synclinal) and *ap* (antiperiplanar) is based upon the Klyne and Prelog nomenclature.[43]

symmetrical pattern of the resonances as for the other cyclics. However, these do not resemble the one determined for the longer sequences. Once more, an invariable chemical shift difference between the nonequivalent methylene protons of 0.37−0.38 ppm is measured at low and high temperatures.

Coupling Constants. The 1D ^1H spectra of all the compounds show large vicinal ^3J(H−H) coupling constants (9−10 Hz) between the ring protons of the sugar moiety. This implies that no epimerization has occurred at the H^5 (see Figure 1) during the coupling step and that the pyranoic ring is held in the predicted $^4C_1$ chair conformation also after cyclization. The Gum sugar amino acid ϕ angle $CO-N-CH_2-C^1$ was determined directly from $^3J(CH_2-NH)$ coupling constants by means of the Karplus equation. Interestingly, for the most populated conformer, the amide proton of the cyclic dimer shows a large coupling ($^3J = 9.8$ Hz) with one of the methylene protons (lower field), which restricts the ϕ angle to $\pm 100.6°$ and $\pm 139.4°$. The same methylene proton exhibits also a large coupling with H^1 ($^3J \cong 10$ Hz), indicating a reciprocal (preferred) anti orientation (−*sc* or *ap* rotamers in Figure 2). The cyclic trimer exhibits for the methylene proton at lower field a large coupling with H^1 ($^3J \cong 10$ Hz), indicating the same rotamer populations around the CH_2-C^1 bond as for the cyclic dimer. For all the other compounds, an estimation of ^3J between the methylene proton at lower field and H^1 led to values smaller than the line width of the peak ($^3J \leq 2$ Hz). In all the spectra, the methylene proton at higher field exhibits spectral overlap. Moreover, the cyclic trimer and longer sequences reveal equivalent coupling constants between the NH group and the CH_2 protons ($^3J \cong 6$ Hz).

ROE Data. Only the cyclic derivatives were investigated in detail. Due to the symmetry of the spectra, it is not possible a priori to discriminate between intra- and interresidue ROEs. Therefore, we checked our models systematically for all possible combinations and considered only the unambiguous ROE connectivities. In this regard, the "fingerprint" region is very informative. All the compounds show strong ROEs between the H^5 and the amide proton (2.5−2.9 Å). Since the corresponding intraresidue connectivity would not cover such a short distance (3.9−4.6 Å), we assigned the ROE as sequential $H^5(i)-NH(i+1)$, which supports the trans configuration of the amide bonds. This is also confirmed by the symmetry of the chemical resonances, which would imply a cis conformation for all the amide bonds in the linear precursors, too. With the exception of the cyclic dimer, all the longer sequences show ROEs between the NH groups and H^2, H^4, which are positioned on the opposite side of the sugar ring with respect to H.[5] The intensity of these ROEs increases with increasing ring sizes.

Conformational Analysis

Molecular dynamic simulations were carried out on the cyclic oligomers in order to search for the whole conformational space accessible in solution (see Experimental Section). Molecular modeling studies run on the cyclic dimer show that indeed the predominant conformation possesses a two-fold symmetry, as confirmed by NMR by the magnetic equivalence of ^1H, ^{13}C, and ^{15}N nuclei. Taking into consideration the trans configuration of the amide bonds and the $^1C_4$ conformation of the pyranoid ring, two low-energy structures were found (Figure 3) which differ in the relative orientation (syn or anti) of the $C-H^5$ and C═O bonds. However, only one was in agreement with our experimental data. The anti conformation was confirmed by the $H^5(i)-NH(i+1)$ connectivity (2.3 Å measured and 2.4 Å calculated from the correspondent ROE), the ϕ dihedral angle (101.2° measured and 100.6° calculated from the Karplus equation), and the anti orientation of one of the methylene protons with respect to H^1 (−*sc* rotamer in Figure 2), as suggested by the $^3J(CH_2-H^1)$ value. The syn conformation was discarded due to the long $H^5(i)-NH(i+1)$ distance (3.6 Å) and the gauche position of both methylene protons relative to H^1 (+*sc* rotamer in Figure 2). Stereospecific assignments of the nonequivalent methylene protons were deduced by means of our model together with $^3J(CH_2-NH)$ and $^3J(CH_2-H^1)$ coupling constants, which identify the proton at lower field having the largest coupling constants with both NH and H^1 as *pro-S* (Figure 2). This is in agreement with the known deshielding property of the carbonyl group in syn position to the *pro-S* proton, considering the projection around $CO-N-CH_2$. The strain of the ring excludes the population of the *ap* rotamer, which would bring the *pro-R* proton in anti to H^1.

The syn and anti conformations of the Gum residue as described for the cyclic dimer were also encountered during molecular simulations run on the other cyclic compounds. The relative population of these structures is dependent on the ring size. Specifically, the syn contribution increases as the chain length increases, starting from $n = 3$ up to $n = 6$. This result is confirmed by the ROE analysis. As shown in Figure 4 and Table 1, proton−proton connectivities involving the amide group are unique for the syn and anti structures, due to the opposite orientations of the amide group with respect to the pyranoid ring. In fact, the NH bond is oriented to the $H^{2,4}$ side (syn) or to the $H^{1,5}$ side (anti). Hence, these characteristic ROEs are taken to evaluate the syn:anti populations as a function of chain length: We estimated 30:70 for $n = 3$ and 50:50 for $n = 4, 6$.

Based upon these results, the cyclic trimer has a strong tendency to occupy the anti conformation. Indeed, the structure reported in Figure 5B, bearing all the Gum in the anti position, is the most populated in aqueous solution. C_3 symmetry is achieved with an all-up and all-down arrangement of the carbonyl and the NH groups with respect to the macrocyclic ring. Once more, the −*sc* rotamer about the CH_2-C^1 bond is confirmed by the large $^3J(CH_2-H^1)$ coupling with one of the methylene proton (*pro-S*), which rules out the possibility of a structure bearing all the Gum in the syn position, since this would require a +*sc* rotamer (Figure 5A).

Binding Studies

As stated in the Introduction, we focused on the potentials of cyclic arrangements comprising carbohydrate moieties and amide groups as molecular carriers with enhanced solubility and improved complexation behavior. This concept has also been recently considered by van Boom and co-workers using cyclic sugar amino acid/amino acid hybrid molecules.[24]

Cyclodextrins (CD) have been extensively studied as water-soluble receptors due to their ability to include a variety of guest

(24) van Well, R. M.; Overkleeft, H. S.; Overhand, M.; Vang Carstenen, E.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **2000**, *41*, 9331−9335.

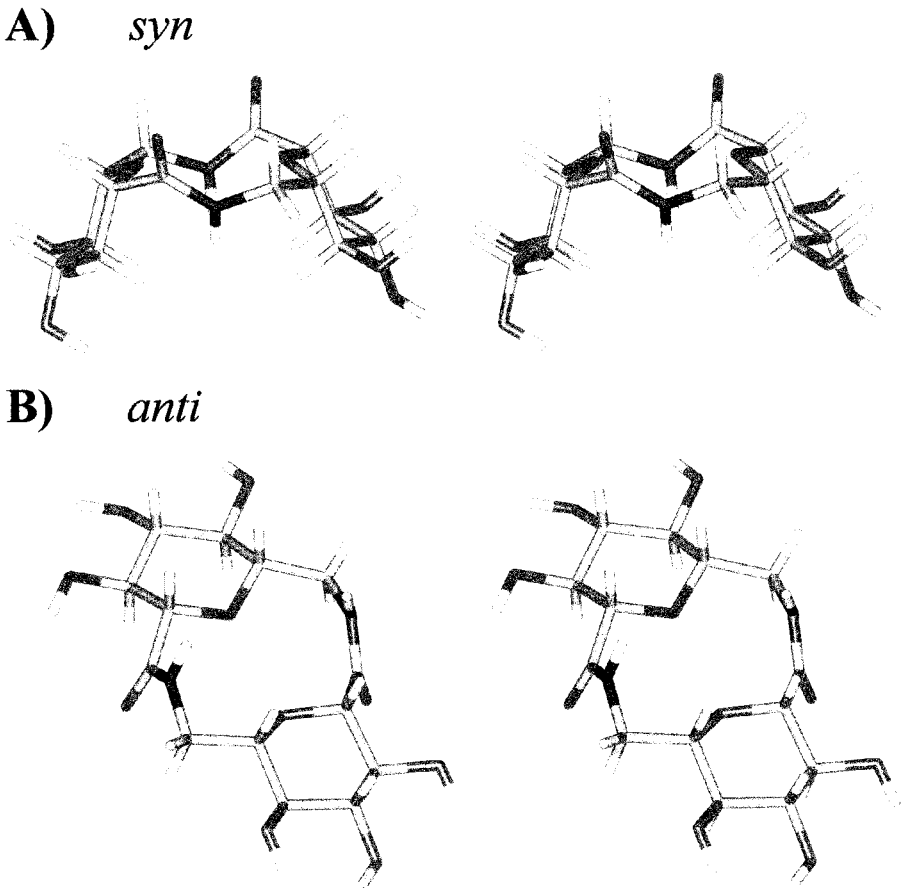


Figure 3. Stereoviews of the syn and anti conformations of the cyclic dimer.

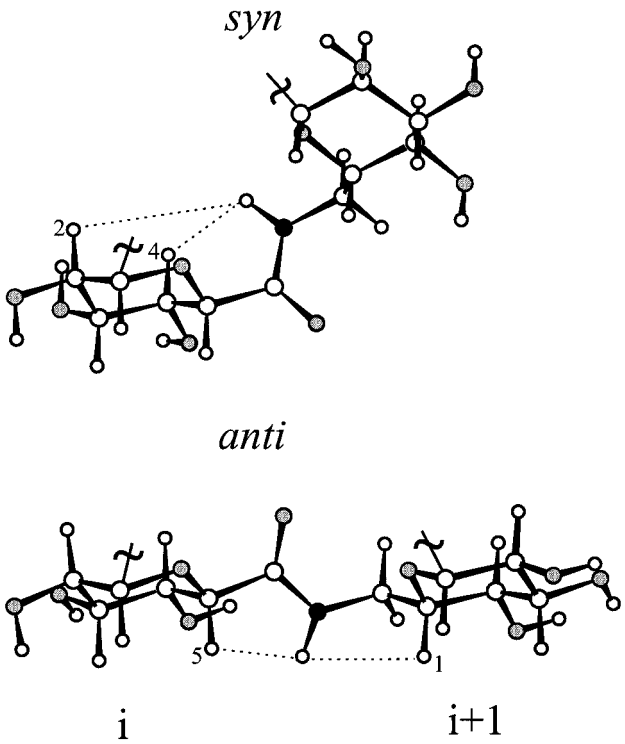


Figure 4. Proton−proton connectivities relevant for the conformational analysis. Hydrogen and carbon atoms are displayed as empty circles, nitrogens are filled in black, and oxygens in gray.

molecules in their hydrophobic cavities.[16] Furthermore, modified CDs with different functional groups showed additional specific interactions between host and guest molecules.[25]

Table 1. Comparison between Proton−Proton Distances (in Å) Characteristic of the Syn and Anti Conformations and ROE Data

			ROE[a]		
	syn	anti	$n=3$	$n=4$	$n=6$
$H^5(i)-NH(i+1)$	3.5−3.5	2.2−2.4	2.8	2.9	2.9
$H^4(i)-NH(i+1)$	2.1−2.8	4.0−4.5	3.6	nd[c]	nd
$H^2(i)-NH(i+1)$	3.8−4.0	4.5−4.9[b]	4.5	4.0	4.2

[a] ROEs were calibrated based upon the H^2-H^4 distance (2.6 Å). [b] Intraresidue connectivity. [c] Not determined due to spectral overlap.

On the basis of these results, the study of cyclic homooligomers containing SAAs as cyclodextrin mimetics was advisable. Furthermore, NMR and molecular modeling studies in water solution revealed a strong similarity between the conformation of the cyclic oligomers bearing all the Gum in the syn position and cyclodextrins which contain a relatively hydrophobic central cavity and hydrophilic outer surface.

Complexation behavior of the cyclic hexamer was investigated by NMR spectrometric titration with two guest molecules, *p*-nitrophenol and benzoic acid. We recorded ^1H NMR spectra at different host/guest ratios, keeping constant the host concentration and observed variation of the chemical shift of specific protons of the cyclic hexamer, as reported in Figure 6. This clearly identifies a fast-exchange regime, with respect to the NMR time scale, between the "free" and the "bound" states. The difference in chemical shift experienced by the H^5 and one of the methylene protons of the SAA is probably due to the ring-current effect of the aromatic ring of the guest molecule in the proximity of the cyclic oligomer.[26] The longitudinal relaxation time (T_1)[27] of the guest molecules was additionally

(25) Rauf Khan, A.; Forgo, P.; Stine, K. J.; D'Souza, V. T. *Chem. Rev.* **1998**, *98*, 1977.

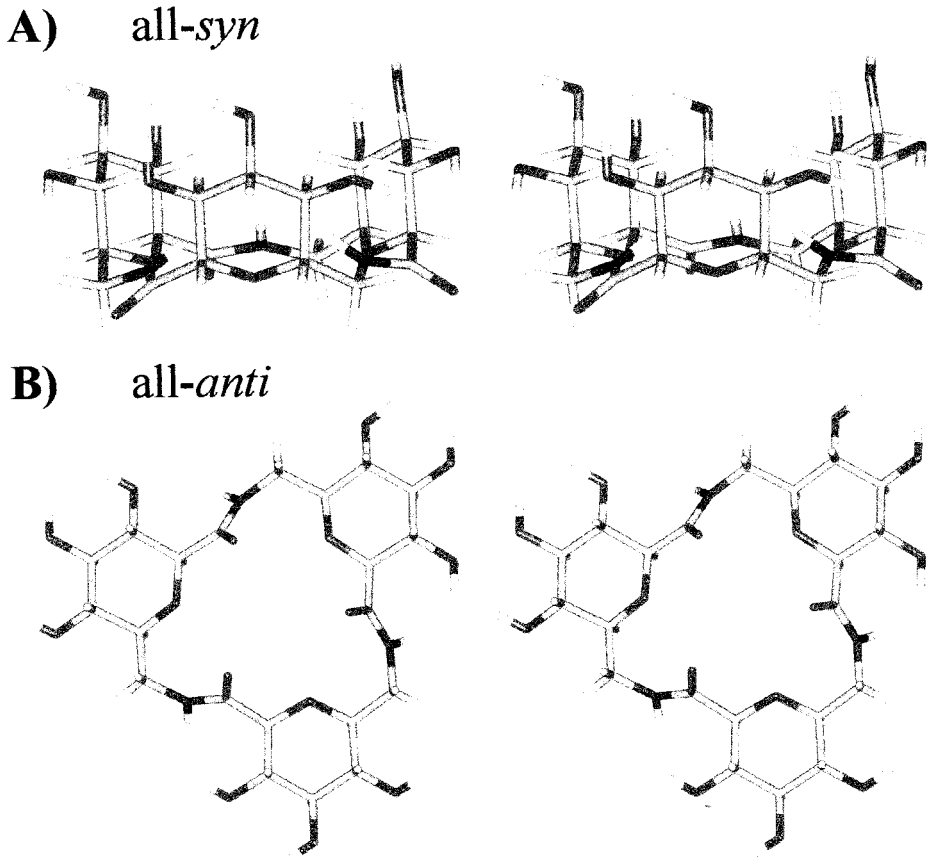


Figure 5. Stereoviews of the all-syn and all-anti conformations of the cyclic trimer.

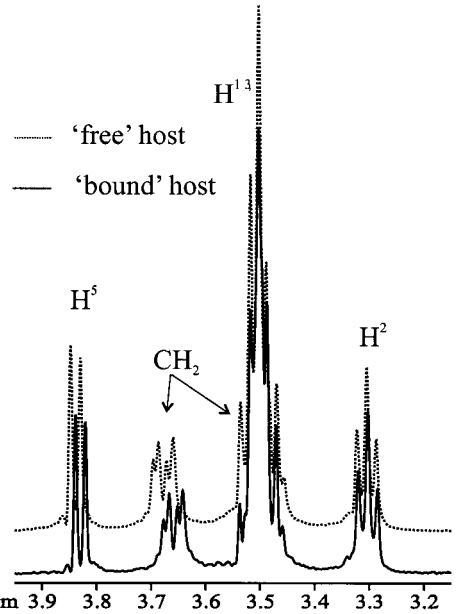


Figure 6. Comparison of the ^{1}H-1D spectra of the cyclic hexamer (host) in solution and in the presence of *p*-nitrophenol (guest) at a host/guest ratio of 4.

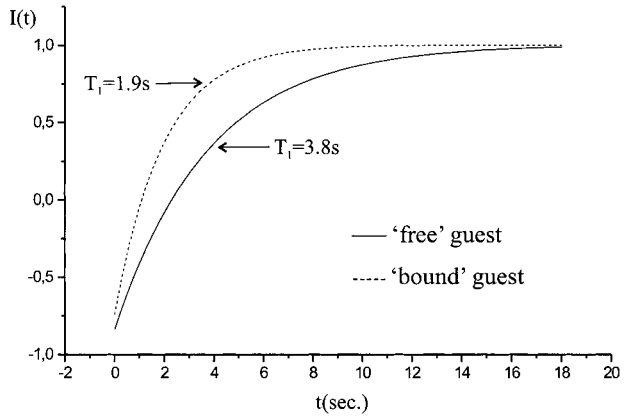


Figure 7. Comparison of the ^{1}H longitudinal relaxation times (T_1) of the benzoic acid (guest) in solution and in the presence of the cyclic hexamer (host) in a 1:1 ratio.

Table 2. Diffusion Coefficients (D) of Benzoic Acid (Guest) and the Cyclic Hexamer (Host) in a 1:1 Ratio

D_{guest} ($cm^2\ s^{-1}$), "free"	D_{host} ($cm^2\ s^{-1}$)	D_{guest} ($cm^2\ s^{-1}$), "bound"
7.4×10^{-6}	2.7×10^{-6}	5.7×10^{-6}

used to identify host−guest binding. Complexation by the cyclic hexamer caused a decrease in the correlation time of the guest molecules, and this in turn led to a decrease in the T_1 (Figure 7). Finally, the formation of a host−guest complex was studied by measurements of the diffusion rate (D).[28] By comparing the intensities of the signals of protons with the intensity of the magnetic field gradient, we obtained the mean values reported in Table 2. The change in the value of D of the benzoic acid in aqueous solution and in the presence of the cyclic hexamer supports the idea of the formation of an inclusion complex. In fact, the diffusion of a compound is proportional to the molecular size, and in the case of a host−guest complex, the diffusion

(26) Lehmann, J.; Kleinpeter, E.; Krechl, J. *J. Inclusion Phenom.* **1991**, *10*, 233. Salvatierra, D.; Diez, C.; Jaime, C. *J. Inclusion Phenom.* **1997**, *27*, 215. Butkus, E.; Jose, J. C.; Berg, U. *J. Inclusion Phenom.* **1996**, *26*, 209. Davies, D. M.; Savage, J. R. *J. Chem. Soc., Perkin Trans. 2* **1994**, *1994*, 1525. Inoue, Y.; Okuda, T.; Kuan, F. H.; Chujo, R. *Carbohydr. Res.* **1984**, *129*, 9. Inoue, Y.; Kitagawa, M.; Hoshi, H.; Sakurai, M.; Chujo, R. *J. Inclusion Phenom.* **1987**, *5*, 55−58.

behavior of a low-molecular-weight guest molecule increases in proportion to the molecular size of the host molecule and to the binding affinity. Similar behavior was found for cyclodextrins and their ligands.[28,29] Although the exact way of binding has not been defined yet, these results evidently identify this class of molecules as cyclodextrin-like artificial receptors.

Conclusions

In this paper we presented the straightforward synthesis of water-soluble materials possessing functional groups with well-defined secondary structure. Our approach involves the use of glucosyluronic acid methylamine (Gum) as the repetitive unit. The design and synthesis of this sugar amino acid was previously reported by workers in our laboratories.[3] We achieved a high yield and a very short coupling time for the oligomerization and cyclization of sequences encompassing two, three, four, and six Gum units using standard solid- and solution-phase techniques. To the best of our knowledge, the synthesis of cyclic oligomers containing only SAAs as repetitive units has not yet been reported.[14] Furthermore, our synthetic protocol did not require any orthogonal protection of the hydroxyl groups, which speeded up the synthesis of the building block and the oligomerization.

Conformational preferences in water solution were determined for the cyclic analogues based upon ^{1}H-^{1}H vicinal scalar correlations, ROE data, and molecular modeling. The molecular structure of the cyclic oligomers in the all-syn conformation, as reported in Figure 5A for the trimeric sequence, generates a hydrophilic exterior surface and a nonpolar interior cavity which resemble the cyclodextrin molecular shape. However, the all-anti conformation leads to a flat structure (Figure 5B). Here, the characteristic sequence of alternating ether and amide linkages arranged in a symmetrical array is clearly reminiscent of macrocyclic chelating agents. Through ROE analysis we were able to estimate the syn and anti population for the Gum residue. Specifically, an increase in the syn conformer and consequently in the tendency of forming a cyclodextrin-like shape, with increasing the size of the ring, was observed.

NMR titration studies revealed the cyclic hexamer as a mimetic of cyclodextrins in its ability to form inclusion complexes. Specifically, the decrease in the diffusion value of the benzoic acid in the presence of the cyclic hexamer is reminiscent of the host−guest chemistry.

The implications of linking sugar moieties in natural carbohydrates through amide bonds are various. Specifically, we can take advantage of a very well established peptide solid-phase chemistry which can lead to peptide−carbohydrate chimeras and to the extension to combinatorial chemistry. Cyclodextrins, in their native state, are rigid molecules and offer limitated utility in terms of size, shape, and availability of chemically useful functionalities. Our synthetic protocol offers an attractive alternative to the existing methods for selective modifications of cyclodextrins.[30] The synthesis and characterization of cyclic peptides containing sugar amino acids and natural amino acids is an ongoing project in our laboratories.[31] The replacement of the glycosidic linkage by the amide group allows also a more detailed structure elucidation via NMR through the NH−CH dipolar relaxation. The amide bond might itself participate in the binding as chelating group. Besides, these molecules as peptidomimetics overcome the limitations of peptidic drugs, generally associated with mediocre absorption and poor metabolic stability among other factors.[7]

In conclusion, the results of this study are encouraging from the perspective of preparing easily accessible materials for drug delivery. The possibility of choosing different ring sizes and consequently the number of chelation groups has led to the idea that this class of cyclic oligomers might represent versatile tools for ligand binding and molecular recognition.

Experimental Section

General Methods. All chemicals were used as supplied without further purification. All organic solvents were distilled before use. For solid-phase synthesis, TCP resin was bought from PepChem Goldammer & Clausen (Tübingen, Germany), and HATU and HOAT were bought from Perseptive Biosystems (Warrington, England). Pd/C was donated by Degussa (Frankfurt/M., Germany). RP-HPLC analysis and semiscale preparations were carried out on Waters (high-pressure pump 510, multiwavelength detector 490E, chromatography workstation Maxima 820), Beckman (high-pressure pump 110B, gradient mixer, controller 420, UV detector Uvicord from Knauer), and Amersham Pharmacia Biotech (Äkta Basic 10/100, autosampler A-900) instruments. RP-HPLC preparative separations were carried out on Beckman System Gold (high-pressure pump modul 126, UV detector 166). C_{18} columns were used. Solvents: (A) H_2O + 0.1% CF_3COOH and (B) CH_3CN + 0.1% CF_3COOH with UV detection at 220 and 254 nm. HPLC-ESI mass spectra were recorded on Finnigan NCQ-ESI with HPLC conjunction LCQ (HPLC system Hewlett-Packard HP 1100, Nucleosil 100 5C_{18}).

Oligomerization. The Gum oligomer assembly via Fmoc chemistry was performed manually (0.25 mmol scale, 2.5 mL wash volumes) starting from TCP resin (1 mmol/g, 250 mg). The first Fmoc-Gum-OH (1.3 equiv) was attached to the resin with 1,3,5-collidine (10 equiv) in 2.5 mL of DMF (1 × 4 h), followed by washing with DMF (6 × 1 min). Fmoc removal on resin was accomplished with 20 vol % piperidine/DMF (2 × 10 min), followed by washing with DMF (6 × 1 min). Successive Fmoc-Gum-OH building blocks (1 equiv) were coupled with HATU (1 equiv), HOAt (1 equiv) reagents, and 1,3,5-collidine (10 equiv) (1 × 3 h). Each coupling was followed by washing with DMF (6 × 1 min) and Fmoc removal as described above. Cleavage from the resin was done by using a mixture of DCM/TFE/AcOH (3:1:1, 2 × 1 h). The solid support was washed with DMF or DMSO (6 × 1 min) and the solvent vacuum-distilled at 303−308 K. The white-yellowish residue was dissolved in water and purified via RP-HPLC (40−50%).

Cyclization with DPPA. The linear oligomer was dissolved in DMF (0.1 mM) in the presence of DPPA[22] (3 equiv) and $NaHCO_3$ (5 equiv) and the solution stirred at room temperature for 16 h. Successively, $NaHCO_3$ was filtered off, excess DPPA was hydrolyzed by addition of a few drops of H_2O, and the solution was concentrated in vacuo. The residue, after RP-HPLC purification, was dissolved in *t*-BuOH (or dioxane)/H_2O and lyophilized (<10%).

Cyclization with DIC/HOAt. The linear compound was dissolved in DMF (0.1 mM), treated with HOAt (1 equiv), NMM (3 equiv), and DIC (10 equiv) at 278 K, and stirred at this temperature for 16 h. Excess DIC was hydrolyzed by addition of H_2O, and the solution was concentrated. The residue, after RP-HPLC purification, was dissolved in *t*-BuOH (or dioxane)/H_2O and lyophilized (<10%).

Cyclization with HATU/HOAt. The linear oligomer was dissolved in DMF (0.1 mM) and then treated with HATU (1 equiv), HOAt (1 equiv), and 1,3,5-collidine (10 equiv). The solution was stirred at room temperature for 6 h. The cyclization was monitored via analytical HPLC. Upon completion of the reaction, the solvent was removed. The residue, after RP-HPLC purification, was dissolved in *t*-BuOH (or dioxane)/H_2O and lyophilized (35% for $n = 2$, 60% for $n = 3$, and >90% for $n = 4$, 6).

(27) Behr, J. P.; Lehn, J. M. *J. Am. Chem. Soc.* **1976**, *98*, 1743−1747. Inoue, Y.; Kuan, F. H.; Chujo, R. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 2539−2545. Mock, W. M.; Shih, N.-Y. *J. Am. Chem. Soc.* **1989**, *111*, 2697−2699. Hilmersson, G.; Rebek, J. *Magn. Reson. Chem.* **1998**, *36*, 663−669.

(28) Rymden, R.; Carlfors, J.; Stilbs, P. *J. Inclusion Phenom.* **1983**, *1*, 159−167.

(29) Gafni, A.; Cohen, Y.; Kataky, R.; Palmer, S.; Parker, D. *J. Chem. Soc., Perkin Trans. 2* **1998**, 19−23.

(30) Khan, A. R.; Forgo, P.; Stine, K. J.; D'Souza, V. T. *Chem. Rev.* **1998**, *98*, 1977−1996.

(31) Stöckle, M.; Locardi, E.; Gruner, S.; Kessler, H. In *20th International Carbohydrate Symposium*, 2000; p 124.

NMR Spectroscopy. The spectra were acquired in H_2O (10% D_2O), pH ≅ 5.5, at $T = 293$ K (unless otherwise indicated) with Bruker DMX500 and DMX600 spectrometers and calibrated using 3-(trimethylsilyl)propionic acid-d_4 sodium salt as external reference.[32] Water suppression was achieved using WATERGATE.[33] The assignment of all proton and carbon resonances was carried out via standard procedures[34] using double-quantum-filtered (DQF) COSY[35] and heteronuclear ($^1H-^{13}C,^{15}N$) single-quantum coherence (HSQC)[36] with echo/antiecho coherence selection and decoupling during acquisition. Sequential assignment was accomplished by through-bond connectivities from heteronuclear multibond correlation (HMBC)[37] spectra with low-pass *J*-filters to suppress one-bond correlations, no decoupling during acquisition, and echo/antiecho coherence selection. Multiplicities are given (obtained from 1D spectra) as s (singlet), d (doublet), t (triplet), dd (doublet of doublets), m (multiplet), and br (broad). Data were processed on a Bruker X32 workstation using UXNMR software. Proton distances were calculated by using the two-spin approximation from rotating frame nuclear Overhauser enhancement (ROESY)[38] spectra with cw spinlock for mixing, $\tau_{mix} = 80-150$ ms, and phase-sensitive using the States-TPPI method. The offset correction, integration, and calibration of the ROE cross-peaks were achieved by using the program SYBYL (Version 6.6, Tripos, Inc., St. Louis, MO). ROE connectivities were calibrated on the basis of fixed geometric distances between protons within the pyranoid ring held in the $^1C_4$ conformation. Homonuclear coupling constants were determined from one-dimensional spectra and from exclusive COSY (E.COSY)[39] cross-peaks. In the case of $^3J(CH_2-NH)$, the use of the Karplus equation ($A = 9.4$ Hz, $B = -1.1$ Hz, $C = 0.4$ Hz, phase shift $\sigma = -60$) gave approximate values for the correspondent ϕ angle of the Gum sugar amino acid.

Computer Simulations. The structure calculations were performed on Silicon Graphics computers. Energy minimization (EM) and molecular dynamic (MD) calculations were carried out with the program DISCOVER using the CVFF force field[40] and a dielectric constant of 80. After EM using steepest descent and conjugate gradient, the system was heated gradually starting from 300 K up to 800 K and subsequently cooled to 300 K using at every temperature 5 ps steps, each by direct scaling of velocities. Configurations were saved every 25 ps for another 500 ps. All the structures coming from MD simulations were minimized using again steepest descent and conjugate gradient algorithms. During molecular modeling simulations, no restraints were taken into account. The structures were at the end evaluated on the basis of the NOE and the dihedral restraints derived from ^3J coupling constants.

Binding Studies. Complexation was monitored by recording ^1H NMR spectra for samples with different host/guest ratios which vary from 0.25 to 10. The host/guest ratio corresponding to each sample was deduced from direct integration of the NMR signal. The ^1H spin−lattice relaxation time (T_1) was measured by using an inversion recovery pulse sequence (180°−τ−90°). The data were analyzed by the UXNMR T_1/T_2 relaxation menu and fitted by using the equation I(t) = I(0)*-[1−2A*exp(−t/T_1)]. Diffusion coefficients were obtained from pseudo-2D diffusion experiments with first-order compensation of convection flux.[41,42] The diffusion measured on one proton was calculated by obtaining the intensity *I*(grad) of the signal of this nucleus at various gradient values. The data were fitted by the UXNMR T_1/T_2 relaxation menu by using the equation I(grad) = I(0)*exp[−1e−5*D*(BD−LD/3)*(2*π*g*LD*grad)∧2], where LD = 6 ms, BD = 42 ms, and the *g* factor (Hz/Gauss) was calibrated on the basis of the extrapolated diffusion coefficient of the water peak at 293 K.[42]

Selected Physical Data. H-(Gum)$_2$-OH: ^1H NMR (500 MHz, H_2O/D_2O, 293 K) $\delta = 8.36$ (t, $J = 6$ Hz, 1H), 7.83 (br s, 1H), 3.94−3.88 (m, 2H), 3.71−3.60 (m, 2H), 3.56−3.41 (m, 7H), 3.38−3.26 (m, 2H), 3.15 (br s, 1H); ESI-MS *m*/*z* 397.2 [M + H$^+$], MW calcd for $C_{14}H_{24}N_2O_{11}$, 396.1.

cyclo(-Gum$_2$-): ^1H NMR (500 MHz, H_2O/D_2O, 293 K) $\delta = 7.86$ (d, $J = 9.8$ Hz, 2H), 3.97−3.84 (m, 4H), 3.80 (d, $J = 9.5$ Hz, 2H), 3.65 (t, $J = 9.2$ Hz, 2H), 3.58−3.46 (m, 6H) [Assignment, $\delta = 7.86$ (NH), 3.93 (H^4), 3.88 (C*H*$_2$, *pro-S*), 3.80 (H^5), 3.65 (H^3), 3.53 (H^1), 3.51 (H^2), 3.50 (C*H*$_2$, *pro-R*)]; ^{13}C NMR (125 MHz, H_2O/D_2O, 293 K) $\delta = 80.25$ (C^5), 78.08 (C^3), 77.87 (C^1), 73.81 (C^2), 69.75 (C^4), 43.29 (*C*H$_2$); ESI-MS *m*/*z* 379.2 [M + H$^+$], MW calcd for $C_{14}H_{22}N_2O_{10}$, 378.1.

H-(Gum)$_3$-OH: ^1H NMR (500 MHz, H_2O/D_2O, 293 K) $\delta = 8.36$ (t, $J = 6$ Hz, 1H), 8.31 (t, $J = 6$ Hz, 1H), 7.83 (br s, 1H), 3.94−3.88 (m, 2H), 3.84 (d, $J = 9.3$ Hz, 1H), 3.71−3.60 (m, 3H), 3.56−3.41 (m, 11H), 3.38−3.26 (m, 3H), 3.15 (br s, 1H); ESI-MS *m*/*z* 586.4 [M + H$^+$], MW calcd for $C_{21}H_{35}N_3O_{16}$, 585.5.

cyclo(-Gum$_3$-): ^1H NMR (500 MHz, H_2O/D_2O, 293 K) $\delta = 8.17$ (t, $J = 6$ Hz, 3H), 3.84 (d, $J = 9.5$ Hz, 3H), 3.63 (m, 3H), 3.60−3.47 (m, 12H), 3.27 (t, $J = 9.0$ Hz, 3H) [Assignment, $\delta = 8.17$ (NH), 3.84 (H^5), 3.63 (C*H*$_2$), 3.57 (C*H*$_2$), 3.56 (H^1), 3.54 (H^3), 3.49 (H^4), 3.26 (H^2)]; ^{13}C NMR (125 MHz, H_2O/D_2O, 293 K) $\delta = 78.03$ (C^5), 77.60 (C^1), 77.05 (C^3), 72.53 (C^4), 71.00 (C^2), 39.95 (*C*H$_2$); ESI-MS *m*/*z* 568.3 [M + H$^+$], MW calcd for $C_{21}H_{33}N_3O_{15}$, 567.2.

H-(Gum)$_4$-OH: ^1H NMR (500 MHz, H_2O/D_2O, 293 K) $\delta = 8.36$ (t, $J = 6$ Hz, 1H), 8.34−8.26 (m, 2H), 7.83 (br s, 1H), 3.94−3.88 (m, 2H), 3.84 (d, $J = 9.3$ Hz, 2H), 3.71−3.60 (m, 4H), 3.56−3.41 (m, 15H), 3.38−3.26 (m, 4H), 3.15 (br s, 1H); ESI-MS *m*/*z* 775.5 [M + H$^+$], MW calcd for $C_{28}H_{46}N_4O_{21}$, 774.3.

cyclo(-Gum$_4$-): ^1H NMR (500 MHz, H_2O/D_2O, 293 K) $\delta = 8.27$ (t, $J = 6$ Hz, 4H), 3.84 (d, $J = 9.4$ Hz, 4H), 3.72 (dd, $J = 13.2$ and 6 Hz, 4H), 3.56−3.44 (m, 16H), 3.31 (t, $J = 9.2$ Hz, 4H) [Assignment, $\delta = 8.27$ (NH), 3.84 (H^5), 3.72 (C*H*$_2$), 3.51 (H^1), 3.52 (H^{3,4}), 3.49 (C*H*$_2$), 3.31 (H^2)]; ^{13}C NMR (125 MHz, H_2O/D_2O, 293 K) $\delta = 78.84$ (C^5), 78.38 (C^1), 77.01 (C^3), 72.23 (C^4), 70.96 (C^2), 40.29 (*C*H$_2$); ESI-MS *m*/*z* 757.4 [M + H$^+$], MW calcd for $C_{28}H_{44}N_4O_{20}$, 756.3.

H-(Gum)$_6$-OH: ^1H NMR (500 MHz, H_2O/D_2O, 293 K) $\delta = 8.36$ (t, $J = 6$ Hz, 1H), 8.34−8.26 (m, 4H), 7.83 (br s, 1H), 3.94−3.88 (m, 2H), 3.84 (d, $J = 9.3$ Hz, 4H), 3.71−3.60 (m, 6H), 3.56−3.41 (m, 23H), 3.38−3.26 (m, 6H), 3.15 (br s, 1H); ESI-MS *m*/*z* 1153.8 [M + H$^+$], MW calcd for $C_{42}H_{68}N_6O_{31}$, 1153.0.

cyclo(-Gum$_6$-): ^1H NMR (500 MHz, H_2O/D_2O, 293 K) $\delta = 8.30$ (t, $J = 6$ Hz, 6H), 3.84 (d, $J = 9.3$ Hz, 6H), 3.68 (dd, $J = 13.2$ and 6 Hz, 6H), 3.56−3.44 (m, 24H), 3.30 (t, $J = 9.2$ Hz, 6H) [Assignment, $\delta = 8.30$ (NH), 3.84 (H^5), 3.68 (C*H*$_2$), 3.52 (H^1), 3.51 (H^{3,4}), 3.49 (C*H*$_2$), 3.30 (H^2)]; ^{13}C NMR (125 MHz, H_2O/D_2O, 293 K) $\delta = 78.82$ (C^5), 78.11 (C^1), 77.08 (C^3), 72.19 (C^4), 70.90 (C^2), 40.34 (*C*H$_2$); ESI-MS *m*/*z* 1135.6 [M + H$^+$], MW calcd for $C_{42}H_{66}N_6O_{30}$, 1134.4.

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(32) Wishart, D. S.; Bigam, C. G.; Yao, J.; Abildgaard, F.; Dyson, H. J.; Oldfield, E.; Markley, J. L.; Sykes, B. D. *J. Biomol. NMR* **1995**, *6*, 135−140.

(33) Piotto, M.; Saudek, V.; Sklenar, V. *J. Biomol. NMR* **1992**, *2*, 661−666.

(34) Kessler, H.; Schmitt, W. In *Encyclopedia of Nuclear Magnetic Resonance*; Grant, D. M., Harris, R. K., Eds.; Wiley & Sons: New York, 1996; Vol. 6, pp 3527−3537. Kessler, H.; Seip, S. In *Two-Dimensional NMR−Spectroscopy, Applications for Chemists and Biochemists*; Croasmun, W. R., Carlson, M. K., Eds.; VCH: New York, 1994; pp 619−654.

(35) Piantini, U.; Sørensen, O. W.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 6800−6801. Rance, M.; Sørensen, O. W.; Bodenhausen, G.; Wagner, G.; Ernst, R. R.; Wüthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 479−485.

(36) Müller, L. *J. Am. Chem. Soc.* **1979**, *101*, 4481−4484. Bodenhausen, G.; Ruben, D. J. *Chem. Phys. Lett.* **1980**, *69*, 185−189. Bax, A.; Ikura, M.; Kay, L. E.; Torchia, D. A.; Tschudin, R. *J. Magn. Reson.* **1990**, *86*, 304−318. Norwood, T. J.; Boyd, J.; Heritage, J. E.; Soffe, N.; Campbell, I. D. *J. Magn. Reson.* **1990**, *87*, 488−501.

(37) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093−2094. Bermel, W.; Wagner, K.; Griesinger, C. *J. Magn. Reson.* **1989**, *83*, 223−232. Kessler, H.; Schmieder, P.; Köck, M.; Kurz, M. *J. Magn. Reson.* **1990**, *88*, 615−618.

(38) Bothner-By, A. A.; Stevens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811−813. Kessler, H.; Griesinger, C.; Kerssebaum, R.; Wagner, K.; Ernst, R. R. *J. Am. Chem. Soc.* **1987**, *109*, 607−609.

(39) Griesinger, C.; Sørensen, O. W.; Ernst, R. R. *J. Am. Chem. Soc.* **1985**, *107*, 6394−6396.

(40) Hagler, A. F.; Lifson, S.; Dauber, P. *J. Am. Chem. Soc.* **1979**, *101*, 5122−5130.

(41) Jerschow, A.; Müller, N. *J. Magn. Reson.* **1997**, *125*, 372−375.

(42) Diercks, T. Ph.D. Thesis, Institut für Organische Chemie und Biochemie, Technische Universität München, München, Germany, 1999; pp 1−266.

(43) Klyne, W.; Prelog, V. *Experientia* **1960**, *16*, 521.