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Noncovalent Saccharide Recognition by Means of a Tetrakis(bile acid)–Porphyrin Conjugate: Selectivity, Cooperation, and Stability

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Molecular recognition of Glu, Glc_2-Glc_6 and Mal_3 by a tetrakis(bile acid)–porphyrin conjugate has been studied by using ESI-FTICR mass spectrometry. The bile acid conjugate was observed to form 1:1 noncovalent complexes with saccharides. The conjugate was found to have size-selectivity towards saccharides with three or more glucose residues. The Glc_3 and Glc_4 also formed kinetically the most stable complexes. The electron capture dissociation (ECD) experiments revealed that in complexation of an oligosaccharide three glucose residues interact with the bile acid conjugate, whereas additional glucose residues are susceptible to fragmentation. The ECD results also showed the significance of the porphyrin centre for complexation of an intact oligosaccharide. It is obvious that the complexation of an intact sugar requires at least one bile acid side arm and the porphyrin centre.

Introduction

Saccharides constitute a biologically unquestionably significant group of compounds, which possess functions ranging from recognition processes and membrane transport to immune response and storage of energy.[1] Oligosaccharides are particularly attractive due to their operation as biomarkers, and they can be considered as important molecular signatures of cell phenotype. Whether the objective is the development of a targeted site-specific drug or early diagnostics of cancerous modifications, the recognition of saccharides plays a vital role. Saccharides are, however, an extremely challenging class of compounds for selective detection, because they are structurally relatively similar, but still have small yet significant differences in their stereochemistry.[2] An effective and selective chemical probe for saccharide recognition is under continuous development. Supramolecular probes have attracted interest recently, and various chemical structures have been studied.[3] The utilization of bile acid and other steroid derivatives for molecular recognition is one of the approaches, and variety of different conjugates have been prepared.[4] By attachment of bile acid side-arms to a porphyrin core, an elaborated supramolecular receptor molecule capable of hydrogen bonding, hydrophobic/hydrophilic interactions and π-stacking is accomplished.[5] Previously, similar receptors have reportedly been able to recognize saccharides in protic media.[5b,5c,6] However, detailed information related to recognition complex formation is still lacking. Here we report a mass-spectrometric investigation on the recognition of oligosaccharides (Glu, Glc_2-Glc_6 and Mal_3) by deoxycholic acid porphyrin conjugate **1** (Scheme 1).

Our goal was to determine the selectivity of the conjugate towards oligosaccharides with increasing length as well as to get more detailed information about the complexation. Saccharide recognition by receptor **1** has previously been studied, but in this current study, especially the origin of selectivity and operation of the four bile acid side arms and porphyrin centre was the object of our interest. Although the earlier UV/Vis spectroscopic studies on complexation of oligosaccharides by bile acid porphyrin conjugates have suggested strong binding, the results have been somewhat unclear, and the stoichiometry of the complexation has not unquestionably been revealed.[5c] The bile acid conjugates have a tendency for self-aggregation, which complicates their analysis in high concentrations.[5c] The concentrations used in ESI-MS are only a fraction of concentrations used in, for example, NMR or UV/Vis spectroscopic experiments, which was hoped to simplify the analysis. Additionally, sophisticated mass spectrometry (such as Fourier transform ion cyclotron resonance, FTICR) has been recently successfully used in analysis of various supramolecular complexes, and it has been observed to produce information that is difficult to obtain by other analytical techniques.[7,8] Electron capture dissociation (ECD)[9,10] is one of the unique tools available in modern FTICR mass spectrometers. Previously, it has been mainly used for analysis of binding sites or fragmentation patterns of peptides, proteins, polymers and some oligosaccharides.[11] For the

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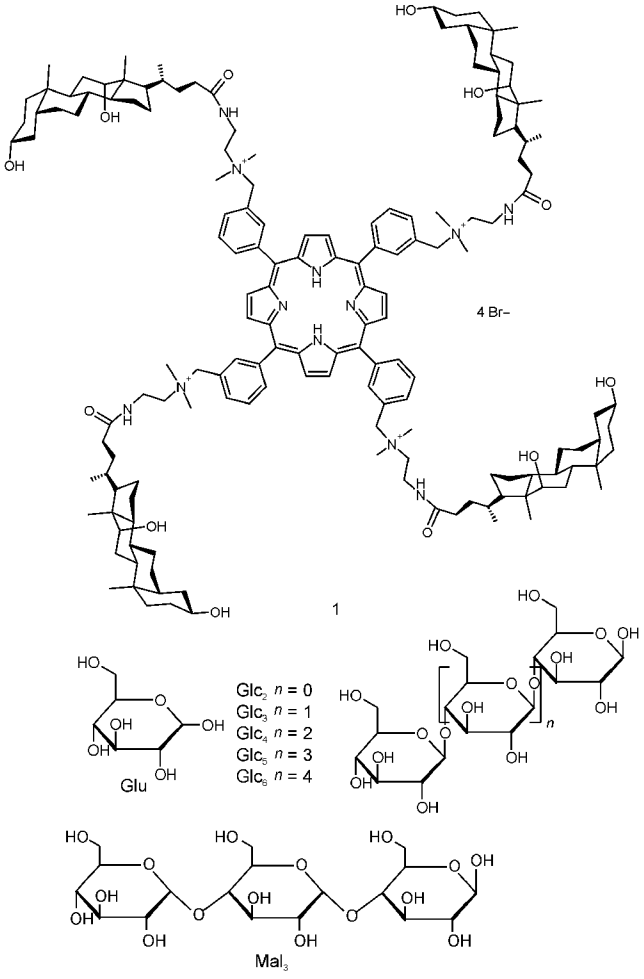

Scheme 1. Tetrakis(bile acid)–porphyrin conjugate **1** and the saccharides studied.

analysis of synthetic supramolecules, ECD has been still been quite limitedly applied.[12] Therefore, from the analytical aspect, it was also interesting to test ECD in the case of supramolecular radial shaped noncovalent complexes.

Results and Discussion

Complex Formation

In the profile spectra measured from MeOH or H_2O/MeOH solutions of conjugate **1** mainly 4^+ and 3^+ ions, $[\mathbf{1} - 4Br]^{4+}$, $[\mathbf{1} - 4Br - H]^{3+}$, and $[\mathbf{1} - 3Br]^{3+}$ (Figure 1, a) were observed. The most abundant ion observed had charge 4+. The sample was not totally homogenous, and 3+ charged fragments of **1**, slight oxidation (ca. 5–20% from the monoisotopic peak of $[\mathbf{1} - 4Br]^{4+}$) as well as partial methylation (ca. 2–10% from the monoisotopic peak of $[\mathbf{1} - 4Br]^{4+}$) were also observed as impurities in the spectra. The amount of oxidation varied in different spectra, so it might be possible that this is not an actual impurity, but is instead caused by oxidation during the electrospray process.

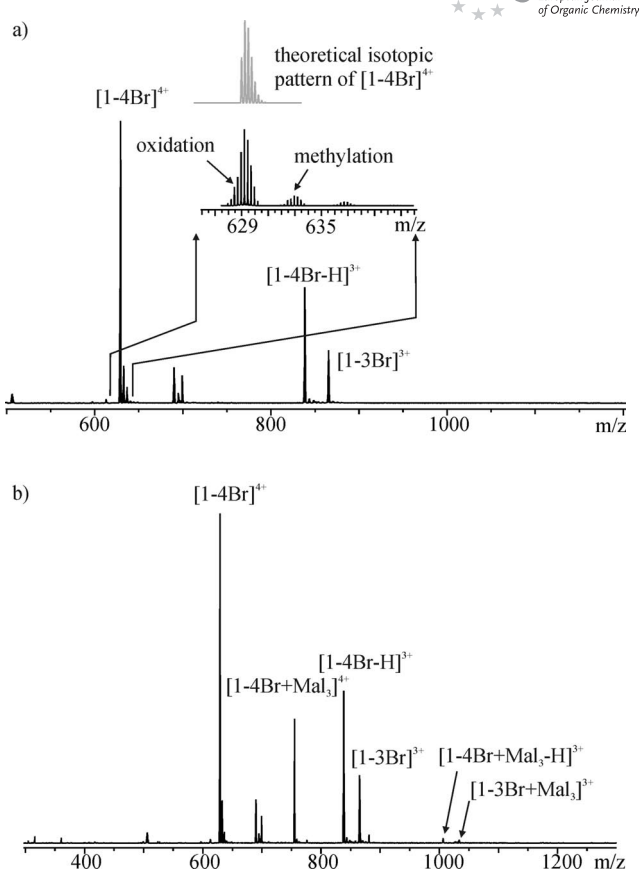

Figure 1. Profile spectra measured from solutions containing (a) **1** and (b) **1** and Mal_3 1:3. Concentration 4 μM and solvent H_2O/MeOH, 2:1.

Noncovalent 1:1 complex formation was observed between conjugate **1** and di- and oligosaccharides when only a threefold amount of saccharide was added to MeOH or H_2O/MeOH solutions of **1**. The complexes were observed at charges of 3^+ and 4^+, although the abundances of the 3^+ complexes constituted only a small fraction (ca. 1–4%) of the abundance of the 4^+ complex (Figure 1, b).

It has been previously suggested that Glu might form 2:1 and 1:1 complexes with conjugates similar to **1**, although the binding constants for formation of Glu complexes were much lower as compared to complexes formed with oligosaccharides.[6] The formation of 1:1 or 2:1 complexes with Glu (or with any other monosaccharide) was not, however, observed in our MS spectra. The relative selectivity of conjugate **1** towards oligosaccharides with increasing length was studied by bilateral competition experiments. According to the results (Figure 2), conjugate **1** shows size-selectivity towards oligosaccharides with at least three glucose residues. The complexation is clearly intensified upon moving from biose to triose, and since Glu also failed to form a complex, it is clear that at least three glucose residues are required for abundant complexation. The most abundant complex was formed with Glc_4, although the differences between the oligosaccharides were small. Consequently, the affinity of **1** towards di- and oligosaccharides follows the order: $Glc_2 << Mal_3 < Glc_3 < Glc_4 > Glc_5 \approx Glc_6$.

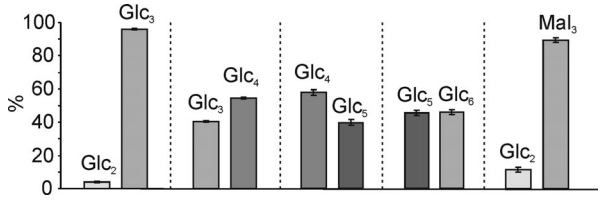

Figure 2. The results of di- and oligosaccharide competition experiments in the presence of **1**, relative intensities (%) presented.

In a competition experiment performed by using ESI-MS, it must nevertheless be emphasized that the abundances of the ions deduced from the mass spectrum only reflect the situation in solution, and the absolute abundance of the ions in a mass spectrum is always affected also by their chemical and physical properties (solvation energies, mass, surface activity etc.), which further affect the ESI response of an individual complex. In here, the selection of the competition pairs was performed so as to maintain the mass and chemical nature of the competitive saccharides as similar as possible. Consequently, enhanced formation of the complexes with the saccharides with at least three glucose residues is likely to result from increased concentration of these complexes in solution and not be caused by differences in the ESI response of the complexes.

Relative Stability of the Complexes

The kinetic stability of the saccharide complexes of **1** was studied by energy-resolved collision-induced dissociation (CID) experiments. As a tentative experiment the dissociation route of conjugate **1** (Figure 3, a) was determined. The dissociation of **1** was quite expected, and it was found to initiate from the quaternary nitrogen atoms. Four bile acid side arms were cleaved at the first stage. Subsequently, loss of four dimethylamine molecules is observed followed by the cleavage of two methyl radicals.

CID is one of the dissociation techniques commonly available in mass spectrometers, and it results in activation of the precursor (isolated) ion and subsequent formation of fragments by the lowest energy pathway. In host–guest complexes, the noncovalent interaction has usually the lowest "bond" energy and it is therefore expected to be ruptured resulting in dissociation of the complex to its components (a host and a guest). That was also the case in the CID experiment performed for saccharide complexes of **1**, and their dissociation was observed, which can therefore be considered as further evidence for their noncovalent nature (see Figure 3, b). Besides the dissociation of the complex, further dissociation of $[\mathbf{1} - 4Br]^{4+}$ was observed.

The energy-resolved investigation of the complex stability revealed (Figure 4) that the most stable complexes were formed with $\mathbf{Glc_3}$ and $\mathbf{Glc_4}$, and that the decreasing stability order for saccharide complexation can be written as $\mathbf{Glc_3} \approx \mathbf{Glc_4} > \mathbf{Mal_3} > \mathbf{Glc_5} \approx \mathbf{Glc_6}$, which is in line with the results obtained from bilateral competition experiments.

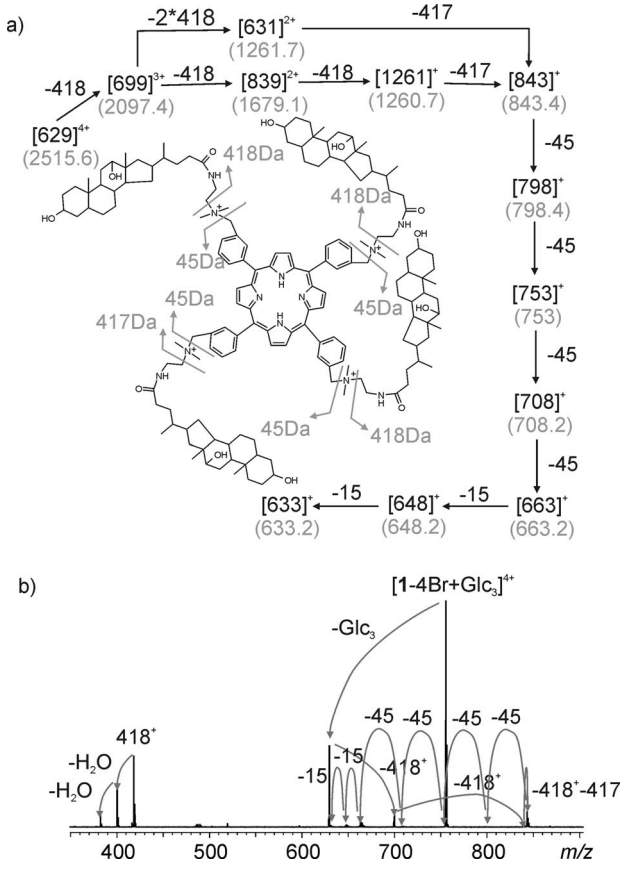

Figure 3. (a) Dissociation route for $[\mathbf{1} - 4Br]^{4+}$ and (b) CID spectrum of $[\mathbf{1} - 4Br + Glc_3]^+$ (the peak for fragment at *m*/*z* 1261 is not shown).

The properties of the complexes, which could affect their ESI response, does not affect their dissociation rate the in the CID experiments. Therefore, these results can be considered as a further confirmation on the selectivity trend observed in the bilateral competitions.

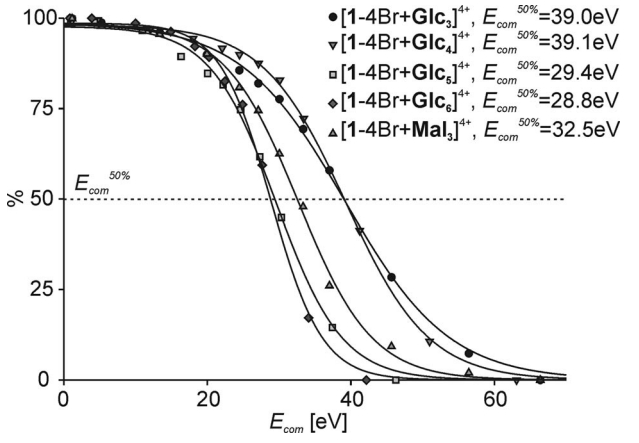

Figure 4. Dissociation curves for the complexes of **1**. Relative intensities of the isolated complexes are shown as a function of energy.

Interaction Site

In ECD, the dissociation of an isolated ion is initiated by a capture of electron(s). ECD is a nonergodic dissociation method, and noncovalent interactions often remain intact even if the covalent bonds in the interacting individual molecules dissociate.[13] Therefore, ECD can be considered complementary to other mass-spectrometric dissociative techniques (such as CID), and it provides information on noncovalent complexes (e.g. binding site information) not accessible by other tandem mass techniques. Consequently, it is rather surprising how limitedly this technique has been utilized by supramolecular researchers. We performed ECD experiments on free conjugate **1** and on its complexes formed with Glc_3–Glc_6 and Mal_3 to get more detailed information about the location of noncovalent interactions and the involvement of the bile acid side arms and the porphyrin core in the complexation. The dissociation of $[\mathbf{1} - 4Br]^{4+}$ in an ECD experiment initiates from quaternary nitrogen atoms, where electron capture takes place. This results in a charge-reduced hypervalent (Rydberg) quaternary ammonium radical.[14] Possibly because of its instability, the hypervalent radical is not observed in the spectra. Instead, subsequent eliminations of a hydrogen and methyl radicals occur. Further dissociation produces mainly neutral, cationic and radical cleavages from both sides of the quaternary nitrogen atom (Figures 5 and 6, a), and the peaks corresponding to cationic fragments (*m*/*z* 418, 463 and 461) are clearly detected. Eliminations of neutral *N*-methyl methanimine, as well as eliminations of methyl and dimethylamine are also observed.

Even if the dissociation of **1** initiates from the side-arms, it clearly does not proceed to the porphyrin core, which is not observed to open or dissociate. Consequently, the stability of the porphyrin core prevents the progress of the fragmentation along the backbone of the molecule, which is usually the main fragmentation pattern observed with biopolymers. Instead, dissociation of multiple side-arms due to multiple electron captures is observed.

In the ECD spectra of the Glc_3 and Mal_3 complexes (Figure 6, b), the main peaks correspond to dissociated **1**, but in addition some peaks corresponding to fragments from the complexes are also observed. The fragments from the complexes consist of the intact saccharide and the dissociated **1**, which has lost 1–3 bile acid side arms. Fragments that consist of an intact saccharide and the porphyrin centre are not observed, neither are ions which consist only of a side-arm and an intact saccharide. Instead, fragments that consist of $^{1,4}A$ or $^{1,4}X$ fragments (for nomenclature see[15]) of the saccharide and the bile acid side arm (*m*/*z* 463) are observed. Based on these limited experiments alone, however, it is difficult to tell whether the absence of certain fragments can be used to make further conclusions about the interaction site, although negative information is generally used in the interpretation of mass spectra. Hopefully, as this technique will be applied more for this kind of rather small

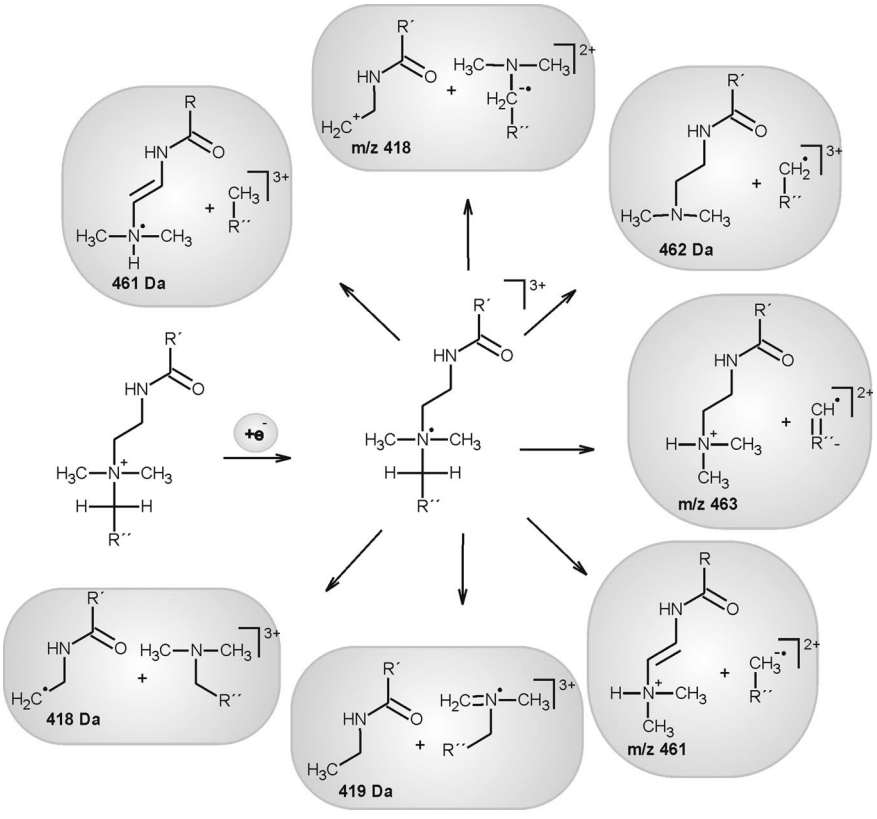

Figure 5. Schematic presentation of the possible dissociation of **1** in an ECD experiment.

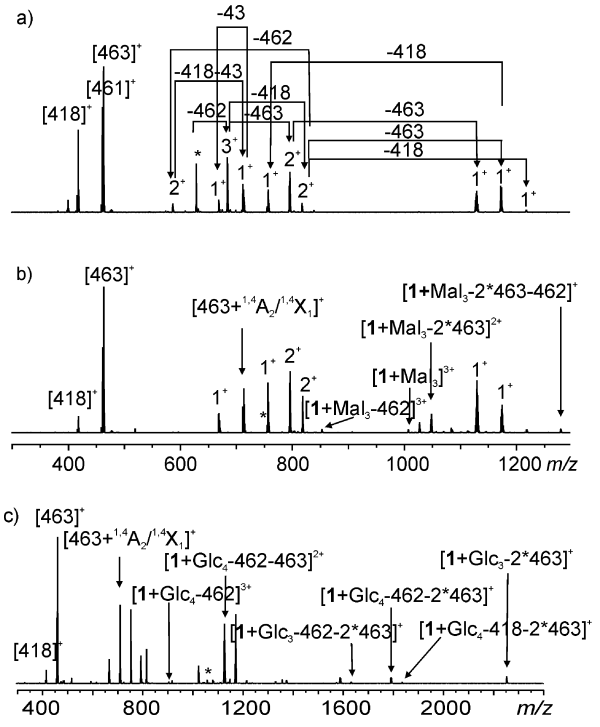

Figure 6. ECD spectra from (a) [**1** – 4Br]$^{4+}$, (b) [**1** – 4Br + Mal_3]$^{4+}$and (c) [**1** – 4Br + Glc_4]$^{4+}$. The isolated ion marked with an asterisk.

supramolecular complexes, the extent of justified conclusions will also be specified. However, it seems evident from this data that the porphyrin centre is significant for complexation of an intact oligosaccharide, and it seems that complexation of an intact sugar requires at least one bile acid side arm and the porphyrin centre.

Accordingly, the peaks corresponding to fragment ions consisting of the intact saccharide and dissociated conjugate **1**, which has at least one intact bile acid side-arm, are observed in the ECD spectra measured from the complexes formed with longer oligosaccharides (Glc_4–Glc_6) (Figure 6, c). In addition, dissociation of glucose residues from Glc_4, Glc_5 and Glc_6 complexes was observed. Elimination of glucose residues were, however, observed only up to triose. It should be noted that the corresponding eliminations from Mal_3 to Mal_2 or Glc_3 to Glc_2 were not observed. It therefore seems that three glucose residues have a closer interaction with the bile acid side-arm and the porphyrin core of the conjugate than the additional residues. As a consequence, the additional glucose residues are susceptible to fragmentation.

Conclusions

Previous studies on saccharide complexation by using UV/Vis spectroscopy showed very high apparent binding constants. However, these experiments suffered from clustering of conjugates, and stoichiometry or selectivity of complexation could not be revealed. Our MS experiments showed 1:1 complex formation and size-selectivity of **1** for oligosaccharides containing at least three glucose residues. The affinity of **1** is highest for cellotriose and cellotetraose, which also form the kinetically most stable complexes. A remarkably clear selectivity difference is observed between the cellobiose and higher oligosaccharides. This is rationalized by the ECD results, which show that in complexation of an oligosaccharide three glucose units interact with the bile acid conjugate, whereas additional glucose residues are susceptible to fragmentation. The ECD results also showed the significance of the porphyrin centre for complexation of an intact oligosaccharide. It is obvious that the complexation of an intact sugar requires at least one bile acid side arm and the porphyrin centre.

ECD has so far been rarely used to study binding sites in supramolecular recognition complexes. However, our first experience of the applicability of this technique is promising. Though ECD has its limitations (e.g. the requirement of highly, preferably positively, charged ions), in our opinion, it also bears real potential in revealing such information on complexation that is hard to obtain by other analytical techniques. Unfortunately the knowledge on this technique in the case of rather small supramolecular assemblies is still in its infancy, and the extent of conclusions justified on basis of ECD data will hopefully be specified as this technique is applied more for various supramolecular complexes.

Experimental Section

Materials: The synthesis and characterization of tetrakis(bile acid)–porphyrin conjugate **1** have been reported earlier.[5c] Conjugate **1** was dissolved in methanol, the final samples were prepared in methanol or MeOH/H_2O, 1:2 with a conjugate concentration of 4.0 μM. The saccharides are commercially available. All saccharides were dissolved in H_2O and 1:3 conjugate/saccharide molar ratios were generally used.

Mass Spectrometry: The MS experiments, except for the determination of the dissociation route, were performed by using an APEX Qe Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with 4.7 T superconducting magnet, Infinity® ICR cell, AP2 electrospray ion source and pre-cell quadrupole interface. The required 1×10^{-9} Torr vacuum was maintained by rotary vacuum pumps and turbomolecular pumps supplied by Edwards (Edwards High Vacuum International, Crawley, UK). The sample was introduced to a 70° off-axis sprayer (stainless steel metal capillary) through a syringe infusion pump (Cole–Parmer 74900 series, Cole–Parmer Instrument Company, Vernon Hills, IL) at a flow rate of 90 μL h^{-1}. N_2 was used as a nebulizing (room temperature, 0.3 to 1.0 psi) and counter-current drying gas (1.5 to 3.0 psi, 150 to 225 °C). The generated ions were collected at the end-plate electrode (3.2 to 3.5 kV) and passed through a dielectric glass capillary (entrance and exit potentials 4.2 to 4.5 kV and 350 V, respectively). After this, ions were focused with a skimmer-1 (20 to 24 V), pre-hexapole, skimmer-2 (7.5 to 12 V) to a (rf) hexapole (5.2 MHz, 300 Vp-p), which was used to accumulate the ions for a predefined time (0.2 to 1.0 s). From the hexapole, ions were extracted from the ion source and transferred to an ICR cell by electrostatic focusing of transfer optics. In the ICR cell, ions were trapped with the use of the Sidekick™ technique before conventional frequency sweep excitation and broadband detection. Data

sets of 256 k, consisting of 16 summed scans, were used. The measurements and data handling were accomplished with Bruker XMASS software, version 6.0.2. The mass spectra were externally calibrated with an ES tuning mix (Hewlett–Packard, Palo Alto, CA). The composition of the complexes was confirmed by comparing the experimental monoisotopic *m*/*z* values and isotopic patterns with the theoretical values calculated on the basis of natural abundances. Competition experiments were performed with a conjugate/saccharide$_1$/saccharide$_2$ ratio of 1:1:1. Each experiment was carried out on five different samples and each sample was measured five times. The overall variance was calculated from the standard deviation of sampling and the standard deviation of the measurement ($s_{tot}^2 = s_1^2 + s_2^2$). Measurements or samples were rejected if the average deviation of a suspect value from the mean was four or more times the average deviation of the retained values.

The MS^n CID for dissociation route determination was performed by using Bruker Esquire 3000 plus QIT mass spectrometer (Bruker Daltonik, Bremen, Germany) equipped with an ESI source. The sample was introduced to the ion source at a flow rate of 150 μL h^{-1}. N_2 (LC-MS-NGM 11 nitrogen generator, Bruker Daltonik) was used as a nebulizing (3 psi) and counter-current (2 L min^{-1}) drying gas. Helium (grade 5.6, AGA Espoo, Finland) was used as the buffer/collision gas. The high vacuum varied in the range of 10^{-6} to 10^{-5} mbar. The following voltages were generally adjusted: capillary –5000 V, capillary exit 170 V, skimmer 40 V, octopole-1 13.50, octopole-2 1.00 V, lens-1 –12 V, and lens-2 –50 V. However, the ion charge control target was 20,000, maximum allowed acquisition time 200 ms, and the value for the trap drive 70. In the CID the fragmentation amplitudes from 0.30 to 0.70 were used. The Instrument was controlled and the data were processed by using a Bruker Daltonics Compass 1.1 for Esquire/HCT. Each spectrum was an average of spectra collected within 1 min, each of these containing 24 individual scans that were averaged before being sent from the instrument to data system.

For energy-resolved collision-induced dissociation (CID) experiments the precursor ions were isolated twice in order to achieve a clean isolation, using the CHEF procedure.[16] Isolated ions were thermalized during a 3.0 s delay, translationally activated by an on-resonance radio frequency (RF) pulse and allowed to collide with a pulsed argon background gas. Each spectrum was a collection of 16 scans. Comparable conditions were maintained by keeping the parameters of the pulse program constant.

The isolation for ECD experiments was performed in quadrupole and the precursor ion was subsequently irradiated with low-energy electrons provided by the indirectly heated hollow dispenser cathode (irradiation time 200–400 ms, cathode bias 0.2–0.4 V, heating current 1.7 A, and lens voltage 10 V) mounted at the back end of the ICR cell on axis.

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[1] R. A. Dwek, *Chem. Rev.* **1996**, *96*, 683.
[2] J. F. Stoddart, *Stereochemistry of Carbohydrates*, Wiley, New York, **1971**; S. J. Angyal, *Angew. Chem. Int. Ed. Engl.* **1969**, *8*, 157.
[3] *Reviews*: a) A. P. Davis, R. S. Wareham, *Angew. Chem. Int. Ed.* **1999**, *38*, 2979; b) A. P. Davis, *Org. Biomol. Chem.* **2009**, *7*, 3629; c) S. Kubik, *Angew. Chem. Int. Ed.* **2009**, *48*, 1722; d) M. Mazik, *Chem. Soc. Rev.* **2009**, *38*, 935; *resorcinarenes*: e) K. Kobayashi, Y. Asakawa, Y. Kikuchi, H. Toi, Y. Aoyama, *J. Am. Chem. Soc.* **1993**, *115*, 2648; f) Y. Kikuchi, Y. Tanaka, S. Sutarto, K. Kobayashi, H. Toi, Y. Aoyama, *J. Am. Chem. Soc.* **1992**, *114*, 10302; g) E. Kalenius, T. Kekäläinen, R. Neitola, K. Beyeh, K. Rissanen, P. Vainiotalo, *Chem. Eur. J.* **2008**, *14*, 5220; *boronic acids*: h) C. J. Davis, P. T. Lewis, M. E. McCarroll, M. W. Read, R. Cueto, R. M. Strongin, *Org. Lett.* **1999**, *1*, 331; i) J. T. Bien, M. Shang, B. D. Smith, *J. Org. Chem.* **1995**, *60*, 2147; j) S. Stiegler, *Curr. Org. Chem.* **2003**, *7*, 81; k) P. J. Duggan, D. A. Offermann, *Tetrahedron* **2009**, *65*, 109; *pyridines*: l) S. Tamaru, S. Shinkai, A. B. Khasanov, T. W. Bell, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4972; m) M. Mazik, M. Kuschel, *Eur. J. Org. Chem.* **2008**, 1517; n) M. Mazik, H. Cavga, *J. Org. Chem.* **2006**, *71*, 2957; o) M. Mazik, M. Kuschel, *Chem. Eur. J.* **2008**, *14*, 2405; *porphyrins*: p) V. Král, O. Rusin, F. P. Schmidtchen, *Org. Lett.* **2001**, *3*, 873; q) O. Rusin, K. Lang, V. Král, *Chem. Eur. J.* **2002**, *8*, 655; *rosettes*: r) T. Ishi-i, M. A. Mateos-Timoneda, P. Timmerman, M. Crego-Calama, D. N. Reinhoudt, S. Shinkai, *Angew. Chem. Int. Ed.* **2003**, *42*, 2300; *lectin analogs*: s) Y. Ferrand, M. P. Crump, A. P. Davis, *Science* **2007**, *318*, 619.
[4] a) E. Virtanen, E. Kolehmainen, *Eur. J. Org. Chem.* **2004**, 3385–3399; b) J. Tamminen, E. Kolehmainen, *Molecules* **2001**, *6*, 21–46; c) A. P. Davis, J.-P. Joos, *Coord. Chem. Rev.* **2003**, *240*, 143–156; d) A. P. Davis, *Coord. Chem. Rev.* **2006**, *250*, 2939–2951; e) A. P. Davis, *Molecules* **2007**, *12*, 2106–2122; f) P. Wallimann, T. Marti, A. Fürer, F. Dietrich, *Chem. Rev.* **1997**, *97*, 1567–1608.
[5] a) E. Kolehmainen, J. Koivukorpi, E. Sievänen, V. Král, *Supramol. Chem.* **2005**, *17*, 437; b) J. Králová, J. Koivukorpi, Z. Kejík, P. Poučková, E. Sievänen, E. Kolehmainen, V. Král, *Org. Biomol. Chem.* **2008**, *6*, 1548; c) J. Koivukorpi, E. Sievänen, E. Kolehmainen, V. Král, *Molecules* **2007**, *12*, 13–24.
[6] M. Dukh, D. Šaman, K. Lang, V. Pouzar, I. Černý, P. Drašar, V. Král, *Org. Biomol. Chem.* **2003**, *1*, 3458.
[7] a) J. S. Brodbelt, *Int. J. Mass Spectrom.* **2000**, *200*, 57–69; b) C. A. Schalley, *Mass Spectrom. Rev.* **2001**, *20*, 253–309; c) A. Di Tullio, S. Reale, F. De Angelis, *J. Mass Spectrom.* **2005**, *40*, 845–865; d) B. Baytekin, T. Baytekin, C. A. Schalley, *Org. Biomol. Chem.* **2006**, *4*, 2825–2841.
[8] e.g. a) M. Kogej, C. A. Schalley, in: *Analytical Methods in Supramolecular Chemistry* (Ed.: C. A. Schalley), Wiley-VCH, Weinheim, **2007**, pp. 104–162; b) E. Ventola, K. Rissanen, P. Vainiotalo, *Chem. Eur. J.* **2004**, *10*, 6152; c) H. D. F. Winkler, D. P. Weinmann, A. Springerm, C. A. Schalley, *Angew. Chem. Int. Ed.* **2009**, *48*, 7246–7250; d) E. Kalenius, D. Moiani, E. Dalcanale, P. Vainiotalo, *Chem. Commun.* **2007**, 3865; e) E. Dalcanale, M. Melegari, M. Suman, L. Pirondini, D. Moiani, C. Massera, F. Ugozzoli, E. Kalenius, P. Vainiotalo, J.-C. Mulatier, J.-P. Dutasta, *Chem. Eur. J.* **2008**, *14*/*19*, 5772; f) D. P. Weimann, H. D. F. Winkler, J. A. Falenski, B. Koksch, C. A. Schalley, *Nature Chem.* **2009**, *1*, 573–577.
[9] a) R. A. Zubarev, N. L. Kelleher, F. W. McLafferty, *J. Am. Chem. Soc.* **1998**, *120*, 3265; b) F. W. McLafferty, D. M. Horn, K. Breuker, Y. Ge, M. A. Lewis, B. Cerda, R. A. Zubarev, B. K. Carpenter, *J. Am. Soc. Mass Spectrom.* **2001**, *12*, 245.
[10] R. A. Zubarev, *Curr. Opin. Biotechnol.* **2004**, *15*, 12–16.
[11] a) H. J. Cooper, K. Håkansson, A. G. Marshall, *Mass Spectrom. Rev.* **2005**, *24*, 201; b) J. T. Adamson, K. Håkansson, *Anal. Chem.* **2007**, *79*, 2901; c) C. Zhao, B. Xie, S.-Y. Chan, C. E. Costello, P. B. O'Connor, *J. Am. Soc. Mass Spectrom.* **2008**, *19*, 138.
[12] a) A. I. S. Holm, M. K. Larsen, S. Panja, P. Hvelplund, S. B. Nielsen, R. D. Leib, W. A. Donald, E. R. Williams, *Int. J. Mass Spectrom.* **2008**, *276*, 116–126; b) C. Hao, F. Tureček, *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 639–651; c) S. Lee, S. Ahn, S. Park, H. B. Oh, *Int. J. Mass Spectrom.* **2009**, *279*, 47–52.
[13] a) K. F. Haselmann, T. J. D. Jørgensen, B. A. Budnik, F. Jensen, R. A. Zubarev, *Rapid Commun. Mass Spectrom.* **2002**, *16*,

2260; b) R. B. J. Geels, S. M. van der Vies, A. J. R. Heck, R. M. A. Heeren, *Anal. Chem.* **2006**, *78*, 7191; c) Y. Xie, J. Zhang, S. Yin, J. A. Loo, *J. Am. Chem. Soc.* **2006**, *128*, 14432–14433.
[14] a) A. I. Boldyrev, J. Simmons, *J. Chem. Phys.* **1992**, *97*, 6621–6627; b) S. A. Shaffer, M. Sadílek, F. Tureček, *J. Org. Chem.* **1996**, *61*, 5234–5245.
[15] B. Domon, C. E. Costello, *Glycoconjugate J.* **1988**, *5*, 397–409.
[16] L. J. de Koning, N. M. M. Nibbering, S. L. Van Orden, F. H. Laukien, *Int. J. Mass Spectrom. Ion Processes* **1997**, *165*/*166*, 209–219.

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