ACES Asian Chemical Editorial Society

DOI: 10.1002/asia.201801157

CHEMISTRY AN ASIAN JOURNAL Full Paper

Chiral Spectroscopy

Recognition of Oligosaccharides by Chirality Induced in Europium (III) Compounds

Tao Wu,* Jiří Kessler, Jakub Kaminský, and Petr Bouř*[a]

Abstract: Identification of saccharides is difficult due to their similar chemical structure. However, they interact very selectively with lanthanide probes. To explore the potential for saccharide recognition, we compare circularly polarized luminescence induced by a variety of oligo- and polysaccharides in three europium compounds. Measurement on a standard Raman optical activity spectrometer made it possible to use high excitation powers and provided very distinct spectral patterns, which were sensitive both to the local structure and differences in molecular size. For example, α-, β- and γ-cyclodextrins provided unique spectroscopic responses. Titration data and molecular dynamics simulation confirmed that CPL spectra carry information about the binding mode and strength between the lanthanide probe and saccharide skeleton.

Introduction

Carbohydrates are essential parts of living matter, omnipresent also in food and pharmaceutical industry.[1] However, their identification and structural analysis is challenging due to their structural diversity and rather similar chemical properties.[2,3] Techniques suitable to study saccharide structure include chromatography,[4] colorimetry,[5] mass spectrometry,[6] precipitation with antisera,[7] optical rotation,[8] laser light scattering,[9] vibrational spectroscopy,[10] and NMR.[11]

A big obstacle in studying saccharides by spectroscopic methods using visible light is lack of suitable chromophores.[12] Vibrational techniques including vibrational optical activity (VOA) can be applied more generally, but the signal is often weak and spectral bands broadened by molecular flexibility and interaction of the OH groups with (usually aqueous) environment.[13]

Measurement of circularly polarized luminescence (CPL) induced in lanthanide(III) compounds can overcome many of these limitations. The signal is reasonably strong and the chiral spectroscopy is more sensitive than unpolarized technique, in this case total luminescence (TL). Previously, we obtained highly-specific spectral patterns for monosaccharides, when CPL of trivalent europium complexes was acquired on a Raman optical activity (ROA) spectrometer.[14] This technique enables one to explore also weak luminescence bands that may not be visible without the intense laser excitation source, although the spectral window is limited (≈532–612 nm).[14,15] Utilization of the Eu^{III} ion is particularly convenient, since total luminescence (TL) and CPL bands are narrow, well-resolved, and easy to recognize in the measured spectrum.

In the present study, we investigate oligosaccharides and some longer polymers (Scheme 1), which exhibit even grater variability in the spectra. An outstanding chirality induction (high CPL/TL ratio), for example, is observed for β-, and γ-cyclodextrins (CD) and starch. The nature of the saccharide-luminophore interaction is investigated on molecular dynamics models; obtained complexation energies roughly correlate with the CPL intensities.

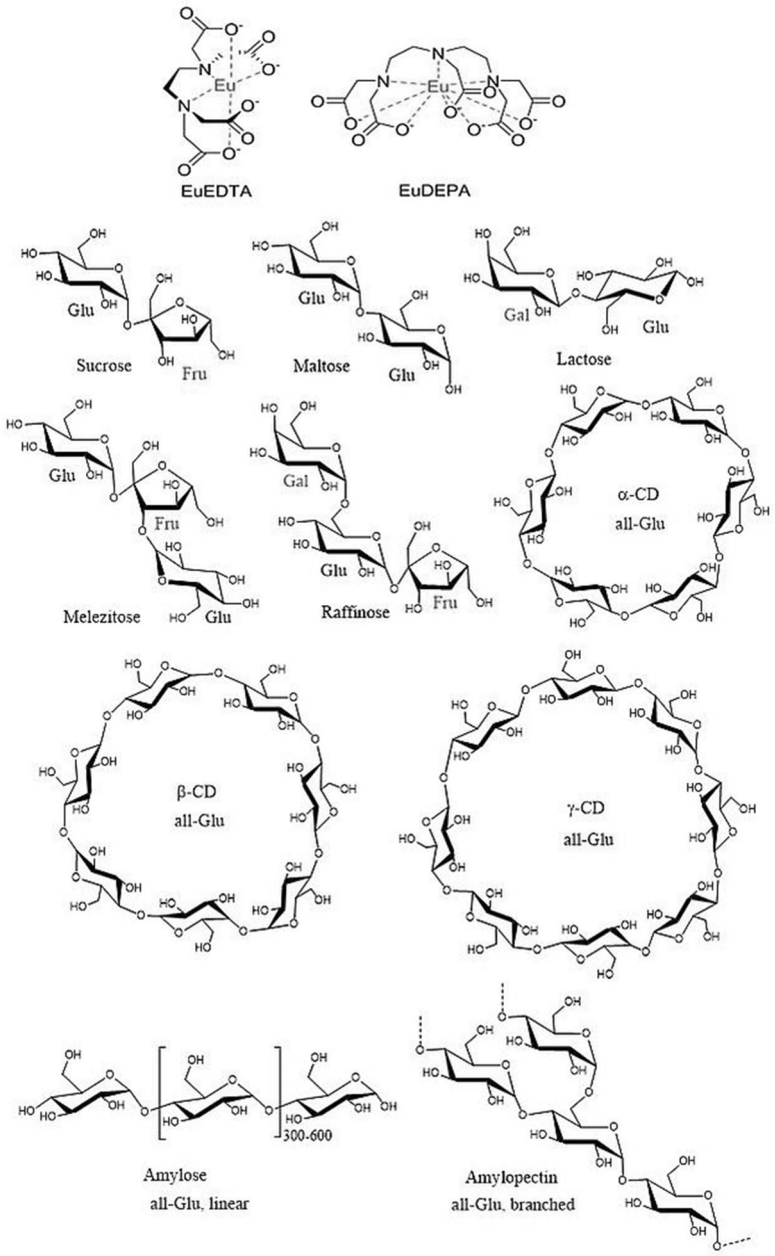
Results and Discussion

The whole spectra, including the vibrational Raman wavenumber region, can be found in SI. We concentrate on the main CPL signal induced in $EuCl_3$, EuEDTA and EuDEPA solutions within the 1500–2450 cm^{-1} shift from the 532 nm laser excitation line. For three disaccharides and two trisaccharides, the spectra are plotted in Figure 1. All CPL patterns are rather unique. Within the disaccharides, lactose provides the lowest signal; sucrose and maltose give similar magnitudes. Europium chloride provides smaller intensities than the EDTA and DEPA complexes. This can be attributed to lower symmetry of the complexes and the "antenna effect" of the organic ligands. For the chloride, the symmetry of the Eu^{3+} ion and its first hydration shell is roughly spherical and not much changed during the interaction with the sugars.[14]

For sucrose the DEPA complex provides an intense three-sign pattern ("+ + −" at 1825, 1950 and 2007 cm^{-1}), which is

[a] Dr. T. Wu, Dr. J. Kessler, Dr. J. Kaminský, Prof. P. Bouř
Department of Molecular Spectroscopy
Institute of Organic Chemistry and Biochemistry
The Czech Academy of Sciences
Flemingovo nám. 2, Prague 16610 (Czech Republic)
E-mail: wu@uochb.cas.cz
bour@uochb.cas.cz

Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under: https://doi.org/10.1002/asia.201801157.



Scheme 1. Structures of the EDTA and DEPA Europium(III) complexes, and investigated disaccharides (sucrose, maltose, lactose), trisaccharides (melezitose, raffinose), α-, β- and γ-cyclodextrins (α-, β- and γ-CD) and the amylose and amylopectin starch components.

somewhat distorted for lactose and approximately reversed (− − +) for maltose. This suggests that the saccharide components perturb equilibrium between two configurations of DEPA enantiomeric forms, analogous to the "Λ" and "Δ" forms in octahedral complexes.[15a,16] To some extent, this occurs also for EDTA exhibiting a w-shape "− + −" pattern. Nevertheless, the disaccharides modify also relative intensities, not only the sign, indicating that the interaction with the europium compounds is rather complex, potentially involving a direct coordination of the europium ion to the sugar OH groups.

The observed spectral patterns, even for the simplest europium chloride, are rather unique. Although all the disaccharides contain at least one glucose unit, only maltose exhibits CPL signal induced in $EuCl_3$ resembling that of monomeric glucose.[14,17] In sucrose, the OH group at C1(α1 orientation) of glucose and the C2 OH group of fructose are missing, and induced CPL is relatively weak, which may indicate the importance of these moieties for Eu^{III} binding.[14] The orientation of the OH group at C2 in fructose may also be important for the stereo-selective recognition of the EuEDTA complex solution as also the EuEDTA signal of monomeric fructose was larger than for sucrose.[14] The $^5D_0 \rightarrow {}^7F_0$ peak at 1551 cm^{-1} is invisible for $EuCl_3$, otherwise it has the same positive sign everywhere.

The melezitose and raffinose trisaccharides seem to exhibit particularly large affinity to the DEPA complex (cf. also the *CID* values in Table S1). A huge CPL signal (note the 0.2×multipli-

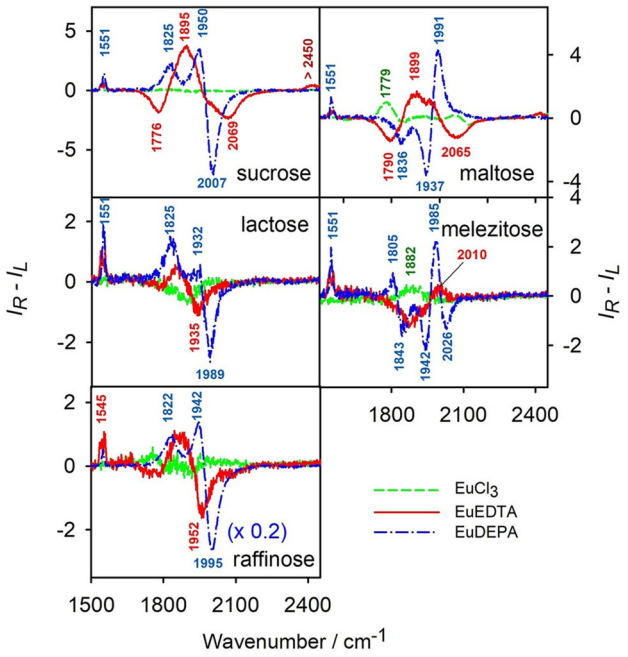


Figure 1. CPL spectra of the di- (sucrose, maltose, and lactose) and trisaccharides (melezitose and raffinose) mixed with the three Eu^{III} compounds, normalized to the water Raman band.

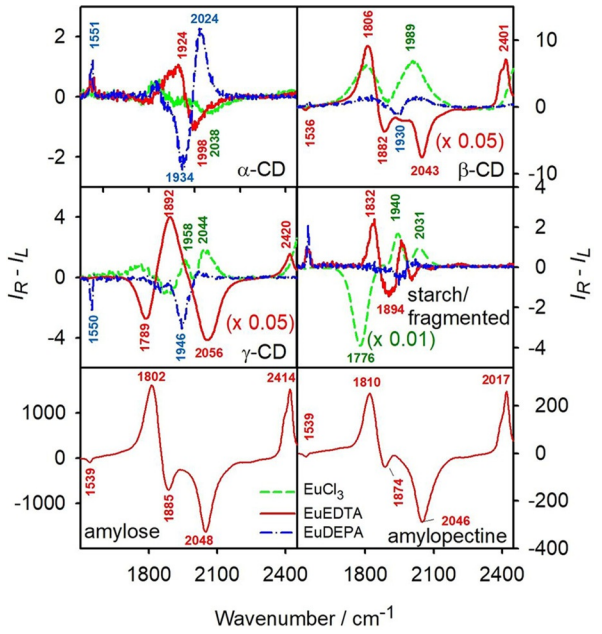


Figure 2. CPL spectra of α-, β- and γ-cyclodextrins, solubilized starch and its amylose and amylopectine components mixed with the europium compounds. The spectra were normalized to the water Raman band, the amylose spectrum was also divided by two to account for different Eu concentration (see Experimental Section).

cation factor in Figure 1) is induced by raffinose, shape of which is quite similar to three-sign pattern observed for the disaccharides. Melezitose induces more modest EuDEPA CPL, while five bands (1805, 1843, 1942, 1985 and 2026 cm^{-1}) instead of three appear in the $^5D_0 \rightarrow {}^7F_1$ region.

Chemically, melezitose can be considered as sucrose connected to glucose by a glycosidic bond (Scheme 1). Similarly, raffinose is galactose connected to sucrose or to more extent, fructose connected to lactose-like Gal-Glu disaccharide having contrary to lactose the α(1→6) linkage. This similarity is conveyed in the tiny differences between their vibrational Raman and ROA spectra (cf. Figures S4 and S5), but induced CPL is clearly more sensitive to the structural difference. As for the trisaccharides, weaker $EuCl_3$ CPL if compared with monosaccharides[14] suggests that the glucose C6 and fructose C3 OH groups are not important for the sugar-europium interaction.

The variability of CPL induced by the longer all glucose-based oligomers (Figure 2) is perhaps even more surprising than for the shorter ones. For example, for α-CD the EuDEPA gives a weak signal dominated by a negative (1934 cm^{-1}) and positive (2024 cm^{-1}) band, CPL of EuEDTA is roughly opposite, and $EuCl_3$ gives the weakest response. The bigger rings of β- and γ-CDs give larger CPL, where the EuEDTA probe is extreme, with a signal of about two orders (!) higher, still providing unique shapes for β- and γ-CD. For β-CD, we can see three $^5D_0 \rightarrow {}^7F_1$ bands, 1806, 1882 and 2043 cm^{-1}, and a band belonging to the $^5D_0 \rightarrow {}^7F_2$ transition at 2401 cm^{-1}. Note that bands higher than 2450 cm^{-1} (wavelengths longer than 612 nm) cannot be measured on our ROA spectrometer. In addition, the usually positive $^5D_0 \rightarrow {}^7F_0$ band (1550 cm^{-1}) is negative for β- and γ-CD. The 2043 cm^{-1} band also gives the largest *CID* (*CID* = $(I_R - I_L)/(I_R + I_L)$, where I_R and I_L are intensities of the right- and left circularly-polarized light, respectively) of -2.2×10^{-2} (Table S2), very close to a similar value (1.7×10^{-2}) previously found for an inherently chiral bipyridine-Eu^{III} complex.[18] This suggests a stable, rigid geometry of the resultant β-CD and EuEDTA associate. Outside the displayed region weaker bands at 619, 659, and 685 cm^{-1} assignable to the $^5D_1 \rightarrow {}^7F_2$ transition appear as well (Figure S7).

A huge "enhanced" CPL $^5D_0 \rightarrow {}^7F_1$ signal is observable for fragmented (solubilized) starch, but only for $EuCl_3$ (Figure 2) having the highest *CID* value of -8×10^{-3} coming from the 1776 cm^{-1} band (Table S3). Unlike for the cyclodextrins, the EuEDTA and EuDEPA complexes give weaker signals. The amylose and amylopectin starch components, however, seems to behave differently again, providing a strong CPL with the EuEDTA probe. The induced CPL is thus given both the shorter and longer (including arrangement of the polyglucose chains in the solution) range saccharide structure.

Compared to the solubilized starch (Figure 2), raw potato and corn starches provide also unique spectra when mixed with $EuCl_3$ and EuEDTA (Figure 3). The induced CPL is still rather high (*CID* ≈ 10^{-3}, cf. Table S3), but for $EuCl_3$, for example, about ten times smaller than for the solubilized starch. Interestingly, $EuCl_3$ provides rather similar CPL and TL spectral shapes, but different intensity magnitudes for the potato and corn products, whereas EuEDTA differs in CPL shape only. Although the results might be dependent on the production technology and starch purity, they document the potential of the method for discrimination of complex saccharide systems. The detailed binding mode remains to be determined in the

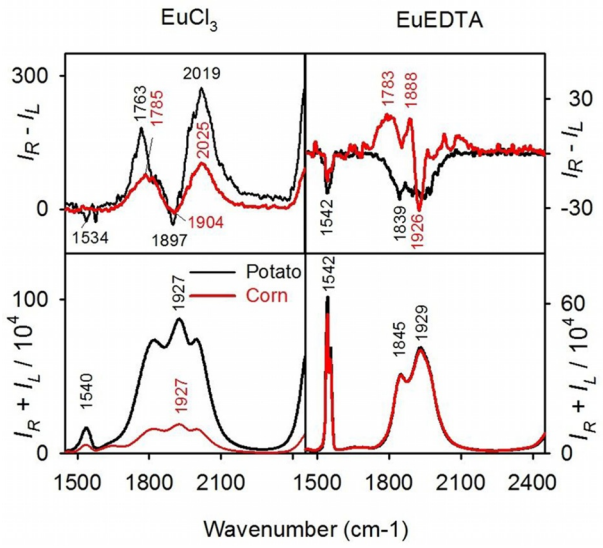


Figure 3. CPL and TL spectra of $EuCl_3$ and EuEDTA in presence of raw potato and corn starches. The spectra were normalized to the water Raman band.

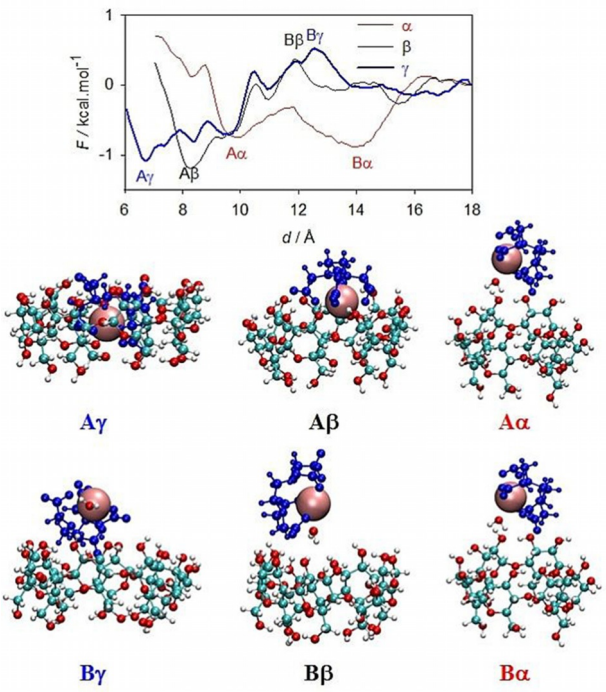


Figure 4. Free energies as dependent on the EuEDTA—cyclodextrin distance obtained from MD, for α-, β- and γ-CD, and typical geometries extracted along MD trajectories.

future. Most probably, an "optimal" polyglucose chain size exists (such as for γ-CD and fragmented starch including amylose and amylopectin) providing sufficient number of binding sites, flexibility and thus maximal CPL signal.

For EuEDTA the amylose CPL band shape and intensity is very similar to those for β-CD, indicating a similar binding mode. This shape is more or less conserved for the amylopectin, but the intensity is about five times smaller, and minor deviations appear, including shifts of peak positions.

Currently, no reliable computational tools allowing to relate lanthanide CPL with detailed structure are known to us. However, to obtain at least a partial insight, we selected the α-, β- and γ-cyclodextrins and EuEDTA, and using molecular dynamics calculated the free energies as dependent on the saccharide-europium distance (Figure 4, top). β- and γ-CD do provide the highest stabilization energy, $\Delta F \approx 1.2$ kcal mol^{-1} for $d \approx 6.5$–8.2 Å, which is in qualitative agreement with their exceptional CPL intensities. However, the stabilization energies related to α-CD (≈ 0.4 kcal mol^{-1}) are too small to explain the qualitatively different spectroscopic behavior. Most probably, the used force field (not supporting, for example, breaking and forming coordination bonds to the europium ion) is too simple to capture all aspects of involved interactions, and model systems do not account for larger-scale structural and conformational changes, involving two CD molecules.

The α- and β-CD free energy profiles are consistent with those previously obtained for monosaccharides;[15a] before forming an associate with the sugar, the water coordination sphere around the europium probe must rearrange (at $d \approx 12$–13 Å), which leads to a small increase of the free energy. This is not predicted for α-CD, providing a "pre-complex" at $d = 14$ Å, which is perhaps not chiral-specific and competes with the more rigid structure at $d = 10$ Å.

The MD snapshot structures (Figure 4, bottom) provide a comprehensive picture of the cyclodextrin-EuEDTA interaction. When within $d \approx 12$–14 Å the two components starts to react, EuEDTA needs to loose part of the water hydration sphere, which is connected with an increase (γ, β) or decrease (α) of the free energy. Only the β and γ cavities, however, can fully accommodate the complex. The sensitivity of EuEDTA binding towards different cyclodextrins might thus be useful for various applications including chiral separations,[19] MRI agents,[20] and asymmetrical catalysis.[21]

The predicted dependence of CPL intensity to the binding strength is confirmed by titration curves (Figure S10). The raffinose/EuDEPA provided low *CIDs* values and the signal did not saturate for experimentally achievable concentration ratios. For β-CD/EuEDTA a stronger binding was observed; estimated magnitude of the stabilization energy (≈ 2.9 kcal mol^{-1}, stability constant of $K = 10^2$ M) roughly corresponds to the MD simulations. For raffinose and other sugars providing weak CPL signal the stabilization energy is close to zero, which is consistent with NMR data obtained on similar systems.[14]

Conclusions

We performed a sensitive measuring of CPL spectra on the ROA spectrometer for a series of saccharides chelating with Eu compounds. The results document the potential of the method for characterization and detection of a broad range of chiral molecules including oligosaccharides and higher polymers. Unlike for simple sugars, significant variation of CPL magnitude was observed, with characteristic patterns for each saccharide type. Molecular dynamics simulation could qualitatively explain the observation, that is, the different behavior of

α-, β- and γ-cyclodextrins, and relatively weak sugar–europium interaction. We believe that the observations demonstrate the usefulness of this method for further structural and functional studies of saccharides.

Experimental Section

Spectra Measurement. All chemicals were purchased from Sigma–Aldrich. Aqueous solutions of $EuCl_3$ and NaEuEDTA and $Na_2EuDEPA$ complexes in 1 to 4 mM concentrations were prepared as described elsewhere.[14] They were added to sucrose, maltose, and lactose disaccharides (concentrations of 330–800 mM), melezitose and raffinose trisaccharides (concentrations of 400–500 mM), α- (α-CD, 118 mM), β- (β-CD, 44 mM in 1 M NaOH aqueous solution) and γ-cyclodextrin (γ-CD, 56 mM in 1 M NH_3 aqueous solution), and soluble starch (fragmented; potato starch treated with glycerol in 190°, 20 mg mL^{-1}). Starch components, amylose and amylopectin were also investigated: amylopectin (60 mg) was dissolved in 0.9 mL of H_2O, 0.1 mL 1 M KOH and 0.2 mL 4 mM EuEDTA; 10 mg amylose was dissolved in 0.5 mL 1 M KOH and 0.2 mL 4 mM EuEDTA. Raw potato and corn starches were obtained from local food store (sold as "Solamyl" and "Gustin" products, both from Dr. Oetker, s.r.o.), dissolved in elevated temperature (≈90 °C, 30 mg mL^{-1}) and the induced TL and CPL spectra with $EuCl_3$ and EuEDTA obtained as for the fragmented starch. The spectra were measured on the BioTools ROA spectrometer (range ≈535–612 nm, Raman shift 100–2450 cm^{-1}) using 532 nm laser excitation, resolution of 7 cm^{-1} and laser power at the sample of 120–900 mW. For luminescence bands the Raman shift (ν_R, in cm^{-1}) can thus be converted to absolute wavenumber scale (ν_A, in nm) as $\nu_A = 10^7/(10^7/532 - \nu_R)$. Collection times varied according to signal intensity from 1 to 16 hours. Water background was subtracted from the Raman spectra, 100–200 cm^{-1} low-frequency water Raman signal was used to normalize spectral intensities.

Molecular Dynamics Simulations. Initial geometries of the α-, β- and γ-cyclodextrins were taken from the Cambridge structural database (CCDC codes GAKPUG,[22] BCDEXD01-05,[23] and CIWMIE[24]). Starting geometry of the EuEDTA complex was constructed based on a DFT computation (B3LYP/6-31G**/ECP, using the Gaussian program[25]), including additional water molecule in the first europium coordination sphere. All MD simulations were performed using the Amber program.[26] One cyclodextrin and one EuEDTA molecule were placed in a cubic box (45 Å a side) filled with water molecules, using the Packmol script.[27] MD was run for an *NVT* ensemble at temperature of 300 K, using the GAFF[28] force field europium parameters from ref. [29], and 1 fs integration step. The simulations were done with fixed Eu-coordinating atoms distances, as the force field available to us do not allow for variable coordination during the dynamics. After an equilibration stage 13 subsequent simulations were performed, each for 1 ns, where the distances between the europium and three cyclodextrins oxygen atoms were constrained within 6–18 Å, using a harmonic force constant of 1 kcal mol^{-1} Å^{-2}. The weighted histogram analysis method (WHAM)[30] was used to obtain the dependence of free energy on the distance.

Acknowledgements

This work was supported by Czech Science Foundation (16-08764Y, 16-00270S and 18-05770S) and Ministry of Education (LTC17012 and CZ.02.1.01/0.0/0.0/16_019/0000729).

Conflict of interest

The authors declare no conflict of interest.

Keywords: circularly polarized luminescence · lanthanide optical activity · molecular dynamics · Raman optical activity spectroscopy · saccharide identification

[1] A. Varki, R. D. Cummings, J. D. Esko, H. H. Freeze, P. Stanley, C. R. Bertozzi, G. W. Hart, M. E. Etzler, *Essentials of Glycobiology*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor (NY), **2009**.
[2] a) J. W. H. Oldham, J. K. Rutherford, *J. Am. Chem. Soc.* **1932**, *54*, 366–378; b) P. Mischnick, B. Evers, J. Thiem, *Carbohydr. Res.* **1994**, *264*, 293–304.
[3] C. Kannicht, D. Grunow, L. Lucka, *Methods Mol. Biol.* **2008**, *446*, 255–266.
[4] a) S. G. You, S. T. Lim, *Cereal Chem.* **2000**, *77*, 303–308; b) R. A. Cave, S. A. Seabrook, M. J. Gidley, R. G. Gilbert, *Biomacromolecules* **2009**, *10*, 2245–2253.
[5] C. Martinez, *Starch/Staerke* **1996**, *48*, 86–89.
[6] a) W. Morelle, J. Lemoine, G. Strecker, *Anal. Biochem.* **1998**, *259*, 16–27; b) M. Suzuki, A. Suzuki, *Biol. Chem.* **2001**, *382*, 251–257.
[7] M. Heidelberger, *Annu. Rev. Biochem.* **1956**, *25*, 641–658.
[8] R. E. Reeves, F. A. Blouin, *J. Am. Chem. Soc.* **1957**, *79*, 2261–2264.
[9] P. Zhang, L. Zhang, S. Cheng, *Carbohydr. Res.* **2000**, *327*, 431–438.
[10] a) J. J. Cael, J. L. Koenig, J. Blackwell, *Biopolymers* **1975**, *14*, 1885–1903; b) L. D. Barron, A. R. Gargaro, Z. Q. Wen, *Carbohydr. Res.* **1991**, *210*, 39–49.
[11] a) K. Kimura, K. Matsumoto, C. Ishihara, K. Harada, A. Miyagi, *Carbohydr. Res.* **1995**, *270*, 33–42; b) U. R. Desai, H. Wang, T. R. Kelly, R. J. Linhardt, *Carbohydr. Res.* **1993**, *241*, 249–259; c) A. Isogai, M. Usuda, T. Kato, T. Uryu, R. H. Atalla, *Macromolecules* **1989**, *22*, 3168–3172.
[12] a) K. Matsuo, K. Gekko, *Carbohydr. Res.* **2004**, *339*, 591–597; b) W. C. Johnson, Jr., *Adv. Carbohydr. Chem.* **1987**, *45*, 73–124.
[13] a) N. A. Macleod, C. Johannessen, L. Hecht, L. D. Barron, J. P. Simons, *Int. J. Mass Spectrom.* **2006**, *253*, 193–200; b) M. Pecul, A. Rizzo, J. Leszczynski, *J. Phys. Chem. A* **2002**, *106*, 11008–11016.
[14] T. Wu, J. Průša, J. Kessler, M. Dračínský, J. Valenta, P. Bouř, *Anal. Chem.* **2016**, *88*, 8878–8885.
[15] a) T. Wu, J. Kessler, P. Bouř, *Phys. Chem. Chem. Phys.* **2016**, *18*, 23803–23811; b) T. Wu, J. Kapitán, V. Mašek, P. Bouř, *Angew. Chem. Int. Ed.* **2015**, *54*, 14933–14936; *Angew. Chem.* **2015**, *127*, 15146–15149; c) E. Brichtová, J. Hudecová, N. Vršková, J. Šebestík, P. Bouř, T. Wu, *Chem. Eur. J.* **2018**, *24*, 8664–8669.
[16] a) H. G. Brittain, *J. Coord. Chem.* **1989**, *20*, 331–347; b) E. Huskowska, J. P. Riehl, *Inorg. Chem.* **1995**, *34*, 5615–5621; c) G. Muller, J. P. Riehl, *J. Fluoresc.* **2005**, *15*, 553–558; d) P. Gawryszewska, J. Legendziewicz, Z. Ciunik, N. Esfandiari, G. Muller, C. Piguet, M. Cantuel, J. P. Riehl, *Chirality* **2006**, *18*, 406–412; e) A. Moussa, C. Pham, S. Bommireddy, G. Muller, *Chirality* **2009**, *21*, 497–506.
[17] A. F. Bell, L. Hecht, L. D. Barron, *J. Am. Chem. Soc.* **1994**, *116*, 5155–5161.
[18] T. Wu, J. Hudecová, X. Z. You, M. Urbanová, P. Bouř, *Chem. Eur. J.* **2015**, *21*, 5807–5813.
[19] Z. I. Szabó, L. Szocs, P. Horvath, B. Komjati, J. Nagy, A. Janoska, D. L. Muntean, B. Noszal, G. Toth, *J. Sep. Sci.* **2016**, *39*, 2941–2949.
[20] L. Di Bari, G. Pintacuda, P. Salvadori, *Chirality* **2010**, *22*, E11–E16.
[21] J. Nishikido, M. Nanbo, A. Yoshida, H. Nakajima, Y. Matsumoto, K. Mikami, *Synlett* **2002**, 1613–1616.
[22] B. Ivanova, M. Spiteller, *Int. J. Biol. Macromol.* **2014**, *64*, 383–391.
[23] J. M. MacLennan, J. J. Stezowski, *Biochem. Biophys. Res. Commun.* **1980**, *92*, 926–932.
[24] K. Harata, *Chem. Lett.* **1984**, *13*, 641–644.
[25] Gaussian 09, Revision D01 ed., M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, J. E. Peralta, F.

Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, Gaussian, Inc., Wallingford CT, USA, **2009**.

[26] D. A. Pearlman, D. A. Case, J. W. Caldwell, W. S. Ross, T. E. Cheatham, S. Debolt, D. M. Ferguson, G. Seibel, P. A. Kollman, *Comput. Phys. Commun.* **1995**, *91*, 1 – 41.

[27] L. Martínez, R. Andrade, E. G. Birgin, J. M. Martínez, *J. Comput. Chem.* **2009**, *30*, 2157 – 2164.

[28] J. Wang, R. M. Wolf, J. W. Caldwell, P. A. Kollman, D. A. Case, *J. Comput. Chem.* **2004**, *25*, 1157 – 1174.

[29] P. Li, L. F. Song, K. M. Merz, *J. Phys. Chem. B* **2015**, *119*, 883 – 895.

[30] S. Kumar, D. Bouzida, R. H. Swendsen, P. A. Kollman, J. M. Rosenberg, *J. Comput. Chem.* **1992**, *13*, 1011 – 1021.

Manuscript received: July 27, 2018
Revised manuscript received: October 3, 2018
Accepted manuscript online: October 18, 2018
Version of record online: November 9, 2018