Interaction of metallotexaphyrins with mono- and polysaccharides

Alla Synytsya,[a] Andriy Synytsya,[b] Vladimír Král,*[a] Karel Volka,[a] Jana Čopíková[b] and Jonathan L. Sessler*[c]

[a] *Department of Analytical Chemistry,* [b] *Department of Carbohydrate Chemistry and Technology, Institute of Chemical Technology, Technická 5, 166 28 Prague, Czech Republic*
[c] *Department of Chemistry and Biochemistry and Institute for Cellular and Molecular Biology, The University of Texas at Austin, Austin, Texas, 78712-1167, USA*

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The interactions of two water-soluble metallotexaphyrins, containing coordinated lutetium(III) and gadolinium(III) cations, with uronic acids (D-galacturonic and D-glucuronic acids), neutral (amylose, galactan) and anionic (pectate, alginate) polysaccharides were studied using UV–VIS titrations. In the case of polyuronides, strong red shifts in the Soret (9–12 nm) and Q-like (4–9 nm) transitions were observed when solutions of the metallotexaphyrin were subject to titration with increasing ligand concentrations. The interaction with neutral polysaccharides leads to significantly smaller bathochromic shifts in these same bands (1–2 nm). No shift of these bands was observed in the case of interaction with uronic acids. If pectate is replaced by partially C-6 methylated pectinates, the extent of the bathochromic shift was seen to decrease as the extent of methylation increased, becoming minimal in the case of methyl pectate (0.5–1 nm). The origin and magnitude of the observed red shifts is rationalised in terms of the conversion of aggregated forms of the metallotexaphyrin into less aggregated (*e.g.*, monomeric) texaphyrin–polysaccharide species. UV–VIS titrations support the conclusion that polyuronides interact with metallotexaphyrins, presumably by acting as polydentate carboxylic ligands for the Lewis acidic lanthanide(III) metallotexaphyrin centres. In the case of the polyuronides, where near complete conversion to monomers is observed, this decrease in aggregation is thought to reflect binding to carboxylate sites. Such binding interactions are not possible in the case of neutral polysaccharides and methyl pectate and the macrocycles remain highly aggregated. Interaction with uronic acids, however, also does not lead to deaggregation.

Metallotexaphyrins are water-soluble complexes of tripyrrolic pentaaza expanded porphyrins.[1] These macrocyclic compounds exhibit strong, low energy optical absorptions in the physiologically transparent 730–770 nm range[2] and are, compared to porphyrins, relatively easy to reduce.[1] Certain water soluble metallotexaphyrins have been shown to localise effectively in tumours, and atheromatous plaque.[1,3] Diamagnetic texaphyrins are also known to produce singlet oxygen in high quantum yield.[4] Currently, two texaphyrin complexes, known by their generic names motexafin gadolinium and motexafin lutetium, are being tested clinically as sensitisers for X-ray radiation tumour therapy and as photosensitisers for photodynamic tumour therapy, the light-based treatment of age-related macular degeneration, and photoangioplastic treatment of atheromatous plaque.[1,3] While considerable work continues to be devoted to understanding the chemical and biochemical basis for these various medical applications, it is now appreciated that metallotexaphyrins can form adducts with a range of biologically important macromolecules. For instance, interactions of lanthanide(III) texaphyrins and related complexes with oligonucleotides, RNA and DNA,[5–7] and oligopeptides[8] have been reported. On the other hand, interactions between texaphyrins and saccharides have yet to be explored in a systematic fashion, even though mannitol is added to aqueous formulations of motexafin gadolinium and motexafin lutetium and sugars and their derivatives have also been used to solubilise[9] generally insoluble[10] lanthanide(III) species that, like certain lanthanide complexes of texaphyrin,[11] can be used to hydrolyse oligonucleotides.[9,10] It was thus to explore the potentially important interactions between lanthanide(III) texaphyrins and saccharides that the present study was undertaken.

Sugars are very important targets for drug development due to their role in, among other things, carcinogenesis. The saccharide portion of glycoproteins and glycolipids, for instance, make up the glycocalix, or "sugar coat" of the cell. In the case of tumour cells, glycocalix components such as galectins, galactose-specific lectins, are responsible for adhesion to target organs, a step necessary for metastasis.[12] Destruction or blocking of galectins by specific drugs can thus lead to inhibition of metastatic growth.[13] Anionic polysaccharides are also the main structural components of proteoglycans (constituting about 95%), species that form the basis for conjunctive tissue. Conjunctive tissue, in turn, plays a critical role in the establishment of tumour vasculature. This makes these anionic polysaccharides directly relevant to photodynamic therapy (PDT). Animal model studies have shown that light-induced damage to the tumour vasculature could be an important determinant of PDT.[2,14] While the nature of these effects remains the subject of ongoing study, they could be explained in terms of possible degradation of the proteoglycan. To the extent this is true, the formation of photosensitiser-sugar conjugates could prove to be an important intermediate step in photodynamic therapy. It was an appreciation of this possibility, coupled with the fact that motexafin lutetium is being used as a PDT sensitiser, that prompted the present study. For this study, two prototypical metallotexaphyrin complexes, the gadolinium(III) and lutetium(III) species **1** and **2** (Fig. 1), were employed as were various neutral (amylose, galactan) and anionic (pectate, alginate) polysaccharides of plant origin. The choice of plant polysaccharides reflects their relatively simple structure and greater availability as compared to more complex polysaccharide contained species of animal origin such as, *e.g.*, proteoglycans.

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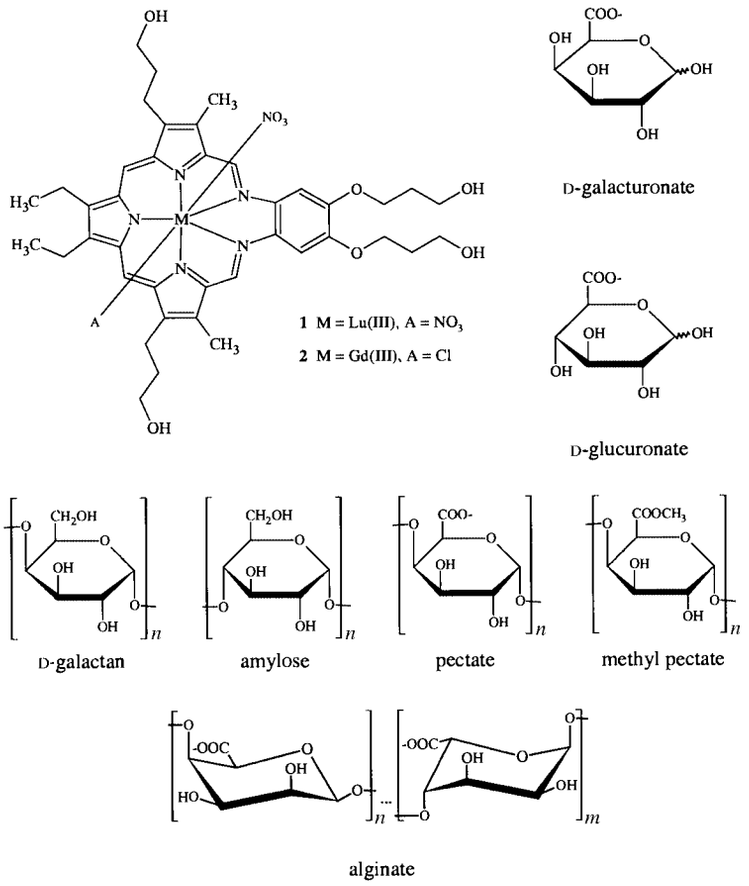

Fig. 1 Structure of metallotexaphyrins **1**, **2** and carbohydrate ligands used in this study.

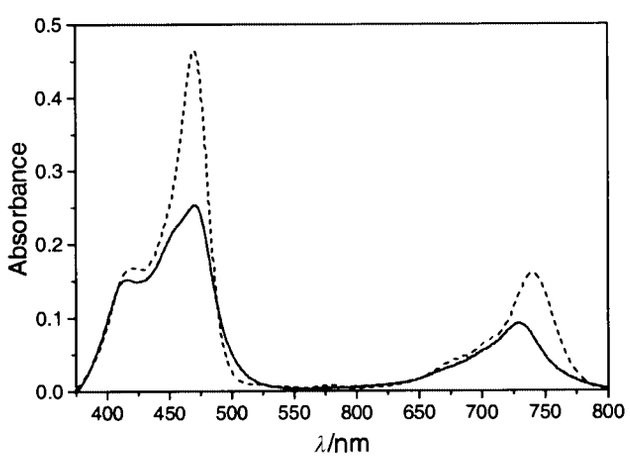

Fig. 2 Absorption spectra of metallotexaphyrins **1** (——) and **2** (---) (5×10^{-6} mol dm^{-3}) at 25 °C in water, pH = 7.

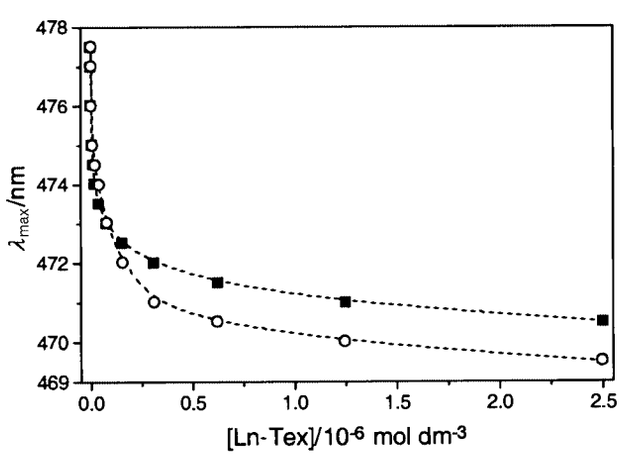

Fig. 3 Change in the λ_{max} of metallotexaphyrins **1** (■) and **2** (○) in aqueous solutions (pH = 7) as a function of their concentration [Ln–Tex] in the range of 1.5×10^{-10}–2.5×10^{-6} mol dm^{-3}.

Results and discussion

The UV–VIS spectra of **1** and **2**, recorded as 5×10^{-6} mol dm^{-3} aqueous solutions, are reproduced in Fig. 2. As can be seen from an inspection of this figure, aqueous solutions of metallotexaphyrins exhibit an intense Soret band at 470 nm and a relatively weaker Q band in the 730–740 nm spectral region.

Aggregation (self-association) of macrocyclic polypyrrole compounds in water solution is a common phenomenon as the result of strong intermolecular van der Waals attractions between these generally flat systems.[15] Such interactions have been demonstrated for both porphyrins[16] and sapphyrins[5c–d,17] and it is well appreciated that in both cases aggregation significantly alters the photochemical characteristics of the macrocycles in solution. It is also reflected in large changes in the observable spectral features. For instance, a stacked face-to-face, or parallel orientation (H-aggregation) leads to a blue shift in the $\pi \rightarrow \pi^*$ absorption band as compared to the monomer. By contrast, a tilted interaction (J-aggregation) leads to a red shift of corresponding bands. Further, H-aggregates generally display broader bands whereas J-aggregates are characterised by bands that are more narrow and more intense than those of corresponding monomers. The extent of these changes strongly depends on the degree of aggregation.[15,16a,d–h,17,18] At appropriately low concentrations, polypyrrole macrocycles will exist as monomers in solution.[16e,17] Just what the upper limit of this concentration is, however, remains something that needs to be determined by experiment on a case-by-case basis.

In the case of the metallotexaphyrins **1** and **2**, where studies of interactions with polysaccharides were to be carried out in water, dilution studies were carried out over a concentration range of 1.53×10^{-10}–2.5×10^{-6} mol dm^{-3}. As illustrated in Fig. 3, the absorption maximum (λ_{max}) of the Soret band for

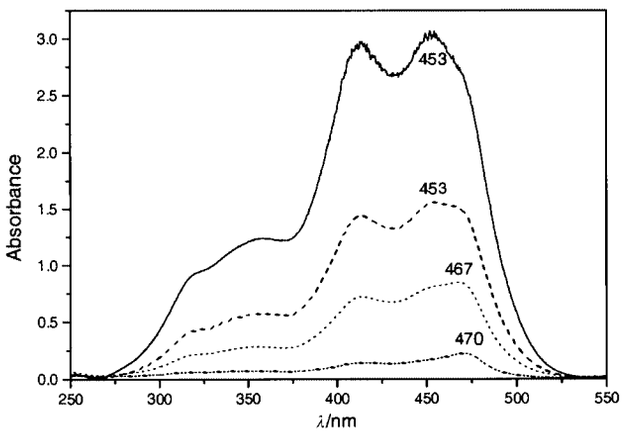

Fig. 4 Absorption spectra of metallotexaphyrin **1** in aqueous solutions at different concentrations: 10^{-4} mol dm^{-3} (——), 5×10^{-5} mol dm^{-3} (---), 2.5×10^{-5} mol dm^{-3} (···), 5×10^{-6} mol dm^{-3} (-·-).

both **1** and **2** was observed to change significantly as a function of metallotexaphyrin concentration. In the case of these complexes, a decrease in concentration leads to a red shift in the observed λ_{max}. This behaviour is rationalised in terms of the decreased aggregation, including the ultimate formation of monomers that occurs upon dilution. By contrast, the absorbance band at 453 nm was seen to increase in intensity when more concentrated solutions of **1** and **2** (2.5×10^{-5}–10^{-4} mol dm^{-3}) were subject to further increases in concentration. At lower concentrations, this latter band appears in the form of a shoulder off the main Soret band near 470 nm (Fig. 4). On the basis of these spectral features, the aggregates of **1** and **2** are considered to be predominantly of the H-type (*i.e.*, aggregates that involve face-to-face stacking between the macrocycles). Still, more complicated aggregations are conceivable and could well be present in aqueous media, reflecting perhaps less-than-straightforward interactions between the coordinated lanthanide cations and various apical ligands such as, *e.g.*, nitrate, acetate, or chloride anions.

Comparisons of the absorption spectral characteristics for complexes **1** and **2** in various solvents (*e.g.*, water, methanol, propan-2-ol and DMSO) serve to demonstrate that the position of the Soret band shifts to the red by 5–10 nm when the solvent becomes less polar. These red shifts (Fig. 5) reflect the conversion from aggregates ($\lambda_{max} = 470$ nm) to free monomers (λ_{max} ~480 nm) that occurs as the extent of macrocycle–solvent interaction increases.

Similar red shifts in the Soret band λ_{max} were observed when sodium dodecyl sulfate (SDS) was added to water solutions of these photosensitisers (Fig. 5). At concentrations above the critical micellar concentration (8.2×10^{-3} mol dm^{-3} or 0.19%),[4b] SDS interacts with lipophilic compounds by its aliphatic group and supports their solubilisation in the micellar phase. In the case of metallotexaphyrins, SDS treating can lead to monomerization as has been noted previously.[3a,4c–e] The result of this complete deaggregation is a Soret band at 478 nm. The position of this band is thus very different in the presence of SDS than in simple aqueous solution at the 5 μmol l^{-1} concentrations used in our titration experiments. We thus conclude that in these latter studies, texaphyrins **1** and **2** are initially present in their aggregated forms.

As implied above, interactions between metallotexaphyrins and various apical ligands can influence the state of aggregation in aqueous solution.[5a,d,16h,19] Such apical ligands, if they bind to the coordinated metal centres strongly, could overcome the normal van der Waals attractions between macrocycles leading, in certain instances, to complete conversion to monomers. Such a complete deaggregation is inferred when a red shift of about 8–9 nm is seen in the position of the Soret band. Weaker shifts, on the other hand, are taken as reflecting a lesser degree of

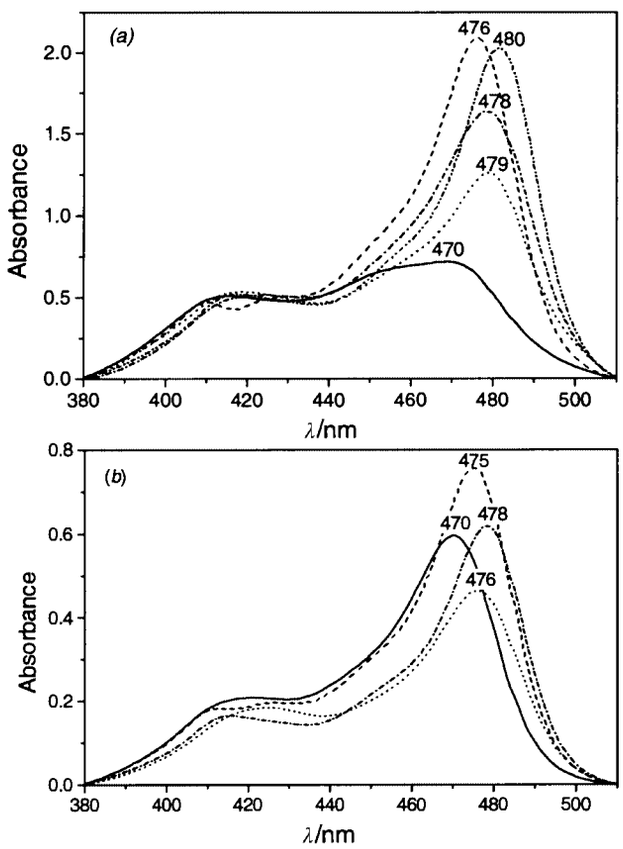

Fig. 5 Absorption spectra of metallotexaphyrins **1** (*a*) and **2** (*b*) (5×10^{-6} mol dm^{-3}) at 25 °C in water (——), methanol (---), propan-2-ol (····), aqueous SDS (5% m/m) (-·-) and in DMSO (-···-).

deaggregation. Thus, by looking at the extent of Soret band shift as a function of added saccharide (ligand) concentration, information can be obtained not only about the extent of deaggregation but also about fundamental texaphyrin–ligand interaction.

On the basis of previous studies involving phosphate anion,[20] it is inferred that treatment of the nitrate or acetate forms of lanthanide(III) texaphyrins with carboxylate ligands would give rise to axial ligand exchange.[21] In this work, acetate anion (sodium counter cation) was used as a model for the carboxylate ligands that could be provided by certain saccharides. The diacetate forms of **1** and **2** are rather insoluble in water at neutral pH. However, they are freely soluble in methanol and, in this solvent, give rise to UV–VIS absorption spectra (**1**: λ_{max}/nm 477 and 733; **2**: λ_{max}/nm 475 and 740) that are identical to those of the nitrate (**1**) or nitrate–chloride (**2**) forms. This leads us to suggest that axial ligand exchange between nitrate and acetate takes place without leading to appreciable deaggregation. The relative insolubility of the acetate complexes in water can be explained in terms of a net decrease in overall complex ([Ln–Tex]·2X) polarity due to the presence of a more hydrophobic counter anion ($X = CH_3CO_2^-$ *vs.* Cl^-).

The results of UV–VIS titrations of metallotexaphyrins with mono- and polysaccharides are presented in Table 1 and Figs. 6–8.

Titration of **1** and **2** with increasing quantities of sodium D-galacturonate, as well as sodium D-glucuronate, did not lead to any shift in the position of Soret or Q bands. Only an increase in absorption intensity was observed during these titration experiments (Fig. 6, Table 1). Therefore, to the extent it occurs, complexation takes place without causing appreciable deaggregation. We suggest that, as is true for acetate replacing nitrate, both D-galacturonate and D-glucuronate act as axial bidentate ligands, with the uronic carboxy group, in particular, interacting with the lanthanide(III) cation. Further, because

Table 1 Absorption maxima in spectra of aqueous solutions of metallotexaphyrins **1**, **2** (5×10^{-6} mol dm^{-3}, pH = 7) alone and in the presence of mono- and polysaccharides

	1		**2**	
Ligand	Soret band	Q band	Soret band	Q band
—	469 (469.2 ± 0.4)	730	470 (469.9 ± 0.3)	740
D-galacturonate (4×10^{-5} mol dm^{-3})	470 (470.3 ± 0.5)	730	470 (469.9 ± 0.3)	740
D-glucuronate (4×10^{-5} mol dm^{-3})	470 (470.2 ± 0.6)	730	470 (470.0 ± 0.4)	740
Galactan[a]	471 (471.3 ± 0.4)	732	472 (472.1 ± 0.3)	741
Amylose[a]	472	733	472	743
Sodium alginate[a]	478	738	475	746
Sodium pectate[a]	479	744	478	745
Pectinate, DM = 20%[a]	479	740	475	743
Pectinate, DM = 60%[a]	478	741	474	743
Pectinate, DM = 75%[a]	478	742	472	742
Methyl pectate[a]	470	730	471	741

[a] 6.75×10^{-3} mol dm^{-3}.

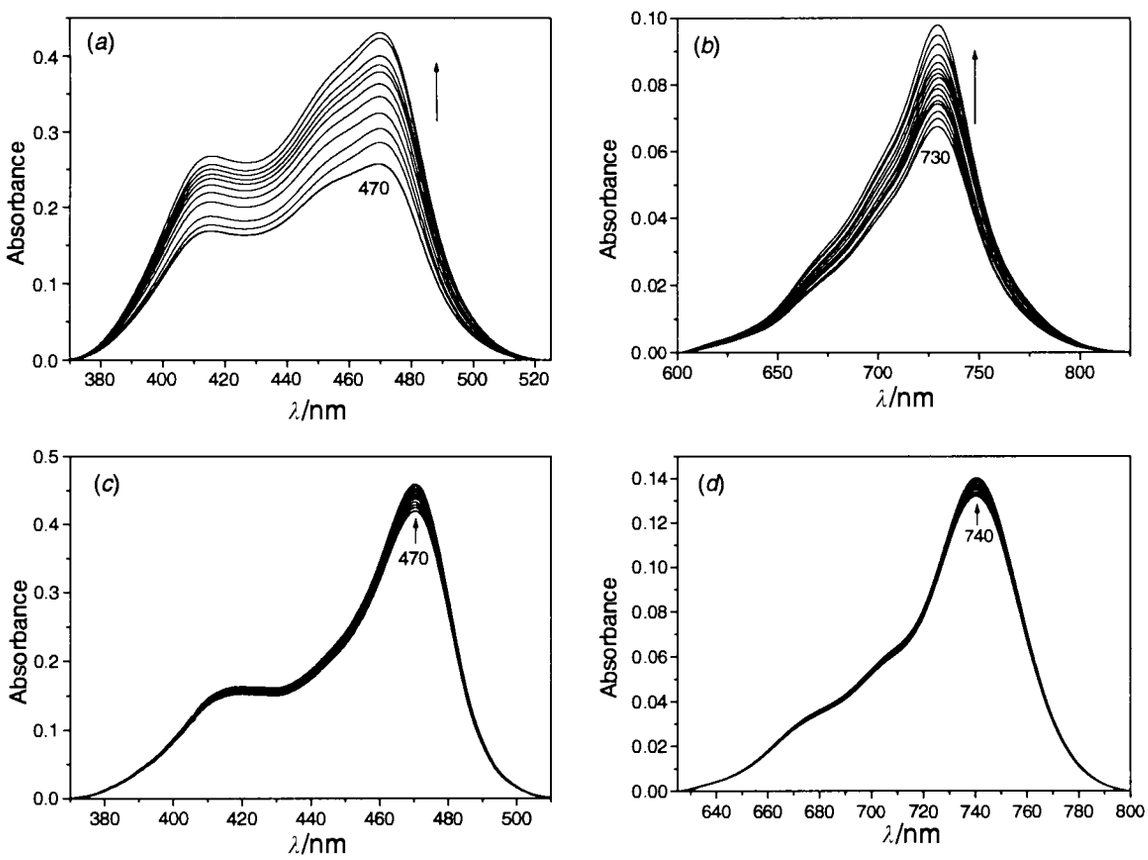

Fig. 6 Absorption spectra of 5×10^{-6} mol dm^{-3} metallotexaphyrins **1** (*a*, *b*) and **2** (*c*, *d*) in the presence of sodium D-galacturonate (2×10^{-6}–8×10^{-5} mol dm^{-3}) in aqueous solutions, pH 7.

they are small, both these anions react only with the outer macrocycles in the metallotexaphyrin aggregates and thus do not induce deaggregation.

Titration of **1** and **2** with neutral polysaccharides (galactan, amylose) gives rise to a rather modest (1–2 nm) red shift in the position of the Soret and Q bands that is accompanied by some hyperchromicity (Fig. 7, Table 1). On the basis of these observations, it is inferred that the interaction between these substrates and the metallotexaphyrin is weak. Chemically, this is considered as being reasonable since the hydroxy groups present on these polysaccharides, in contrast to the carboxylates of D-galacturonate, cannot form bidentate, electrostatically stabilised, complexes with the coordinated lanthanide cations. Any supramolecular conjugates formed between metallotexaphyrins **1** or **2** and these neutral polysaccharides would thus have to be stabilised by relatively weak polar interactions only. On the other hand, the large number of putative, albeit weak, binding sites present on these neutral polysaccharides and the ability to interact with several macrocycles at the same time could lead to limited deaggregation as is indeed observed by experiment.

Titration of **1** or **2** with polyuronides (pectate and alginate) leads to strong red shifts in the Soret (5–9 nm) and Q (5–14 nm) bands (Fig. 8, Table 1). In the case of pectate interacting with **2** (Fig. 8*c*), a decrease in the absorption at 470 nm (aggregates) and concomitant increase in the absorption at 478 nm (monomers) was observed. We thus suggest that, as was proposed for D-galacturonate, these polyuronide anions interact with the texaphyrin metal centres *via* their carboxylate functionality.

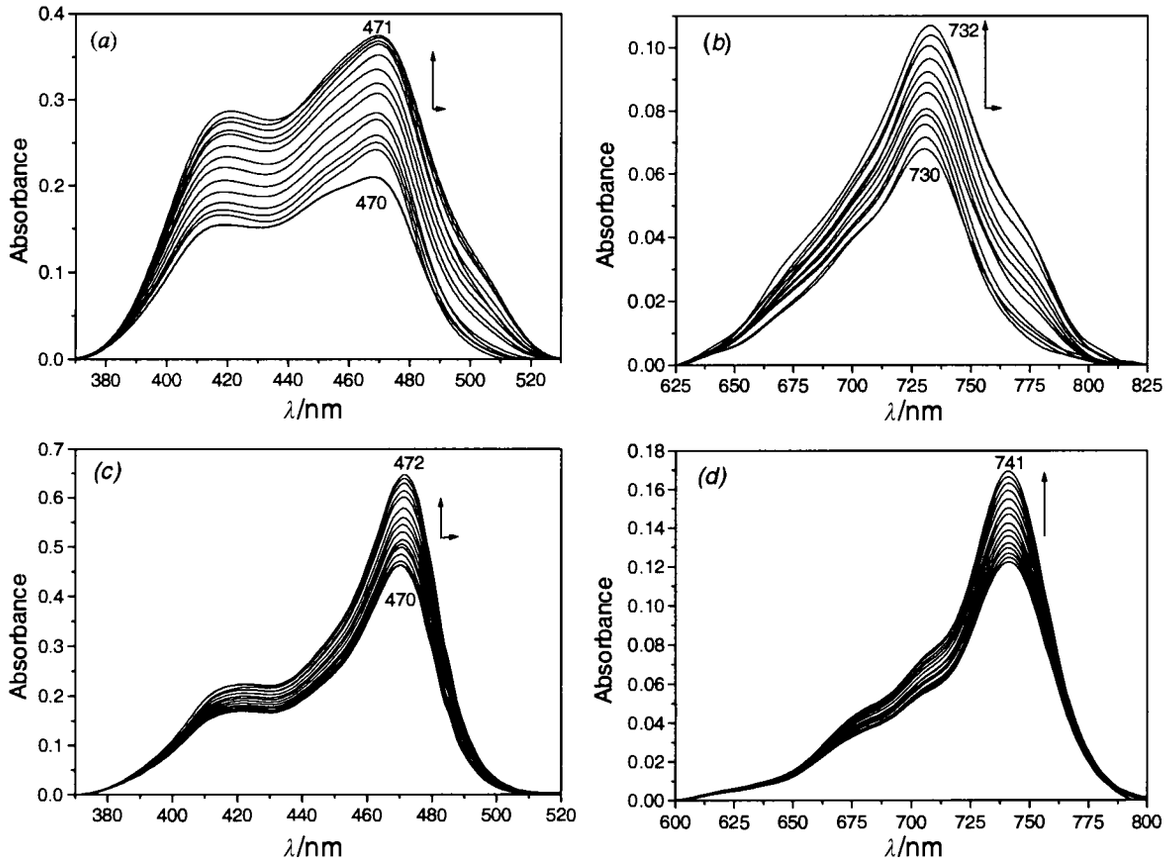

Fig. 7 Absorption spectra of 5×10^{-6} mol dm^{-3} metallotexaphyrins **1** (*a*, *b*) and **2** (*c*, *d*) in the presence of D-galactan (3×10^{-4}–1.35×10^{-2} mol dm^{-3}) in aqueous solution, pH 7.

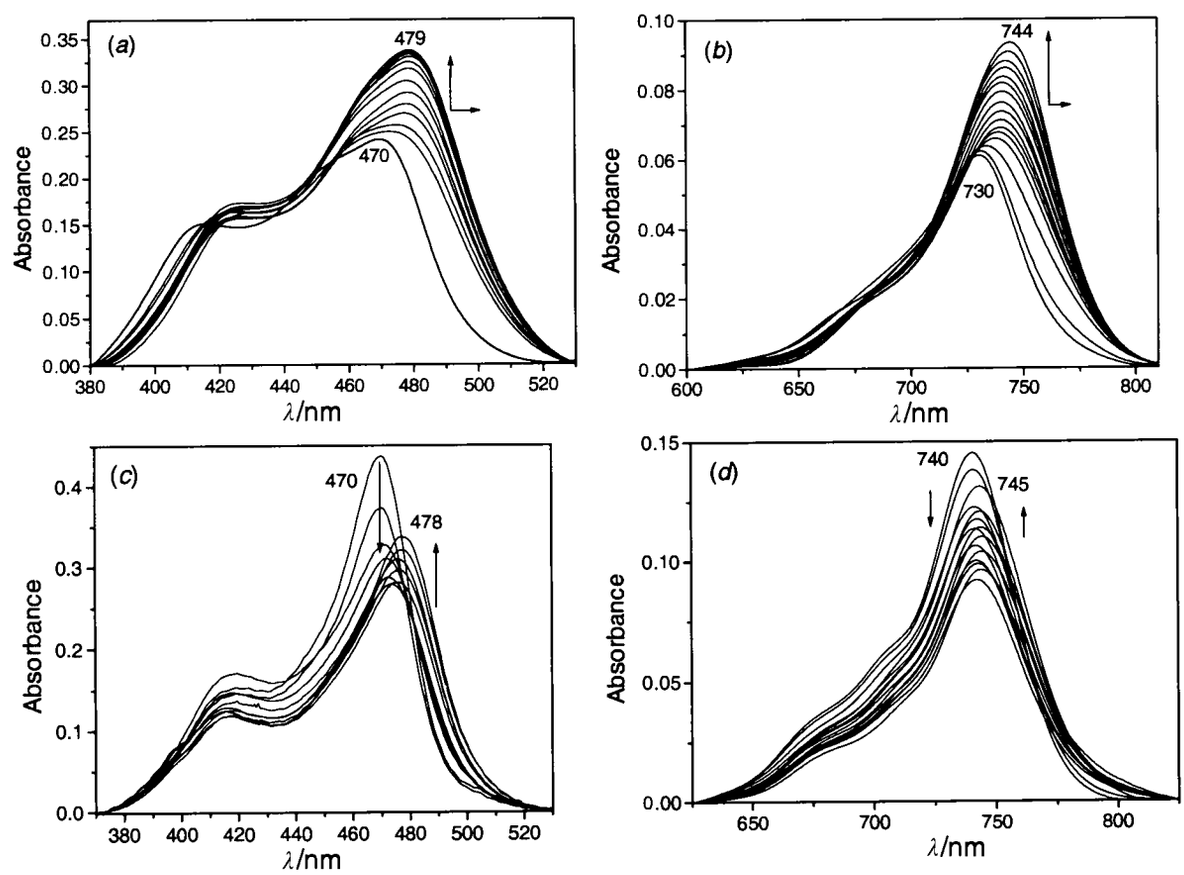

Fig. 8 Absorption spectra of 5×10^{-6} mol dm^{-3} metallotexaphyrins **1** (*a*, *b*) and **2** (*c*, *d*) in the presence of sodium pectate (3×10^{-4}–1.35×10^{-2} mol dm^{-3}) in aqueous solutions, pH 7.

In contrast to simpler carbohydrate ligands, however, these polyuronides (pectate, alginate) consist of polymeric entities richly adorned with carboxylate substituents. Therefore, they can interact with metallotexaphyrins by acting as polydentate carboxylate-containing ligands. As such, they can span two or more texaphyrins. Alternatively, if the concentration range is suitable, they can wrap around the top and bottom faces of a single metallotexaphyrin (Fig. 9) and can provide the same complete charge neutralisation that two acetate anions would otherwise provide. This, in turn, can lead to significant deaggregation and produce, ultimately, monomers.

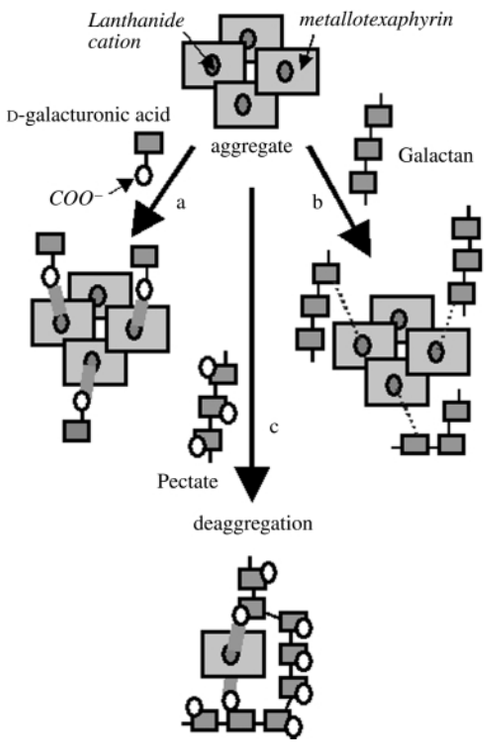

Fig. 9 Scheme illustrating the proposed modes of interaction that can occur when aqueous solutions of metallotexaphyrins **1** and **2** are treated with various carbohydrate ligands. See text for details.

The absorption spectra of metallotexaphyrin (**1**, **2**) solutions in the presence of sodium pectate, partially methyl esterified sodium pectinates (degree of esterification, DM = 20–75%) and the methyl ester of pectic acid (methyl pectate) at near-equal high saccharide concentrations of 1.1×10^{-2}–1.3×10^{-2} mol dm^{-3} per monomeric subunit (aGalA) are reproduced in Fig. 10. In the case of the pectinates, bathochromic shifts were observed in both the Soret and Q bands. These shifts were seen to decrease with increasing DM values becoming minimal for methyl pectate (~1 nm). These experimental observations are made easier to visualise by the use of second derivative plots (Fig. 10*b*,*d*). Using such plots, it is also easy to see that there is a difference between metallotexaphyrins **1** and **2** in terms of how they react when titrated with the various pectinates. In both cases, the greatest red shift for the Soret band is seen in the case of pectate anion itself. In the case of metallotexaphyrin **1**, large red shifts were also seen for the partially esterified pectinates with a drop off in the extent of ligand-induced red shifting being seen only in the case of methyl pectate itself. In other words, for **1**, the extent of the shift is not a sensitive function of the degree of esterification (DM). By contrast, for **2**, the extent of the shift gradually decreases with increasing DM.

To a first approximation, the above findings can be rationalised in terms of free pectinate being able to bind to a texaphyrin-bound lanthanide cation only through its free carboxylate moieties, with the latter acting as axial ligands for the metal centre. The methylation of uronic carboxy groups in pectins leads to an obvious decrease in the number of binding sites per overall macromolecular unit. Further, these same methyl ester groups can act as steric "buffers" that hinder the to-metal binding of the remaining free carboxylate groups. These same methyl ester substituents can also interact with the texaphyrin

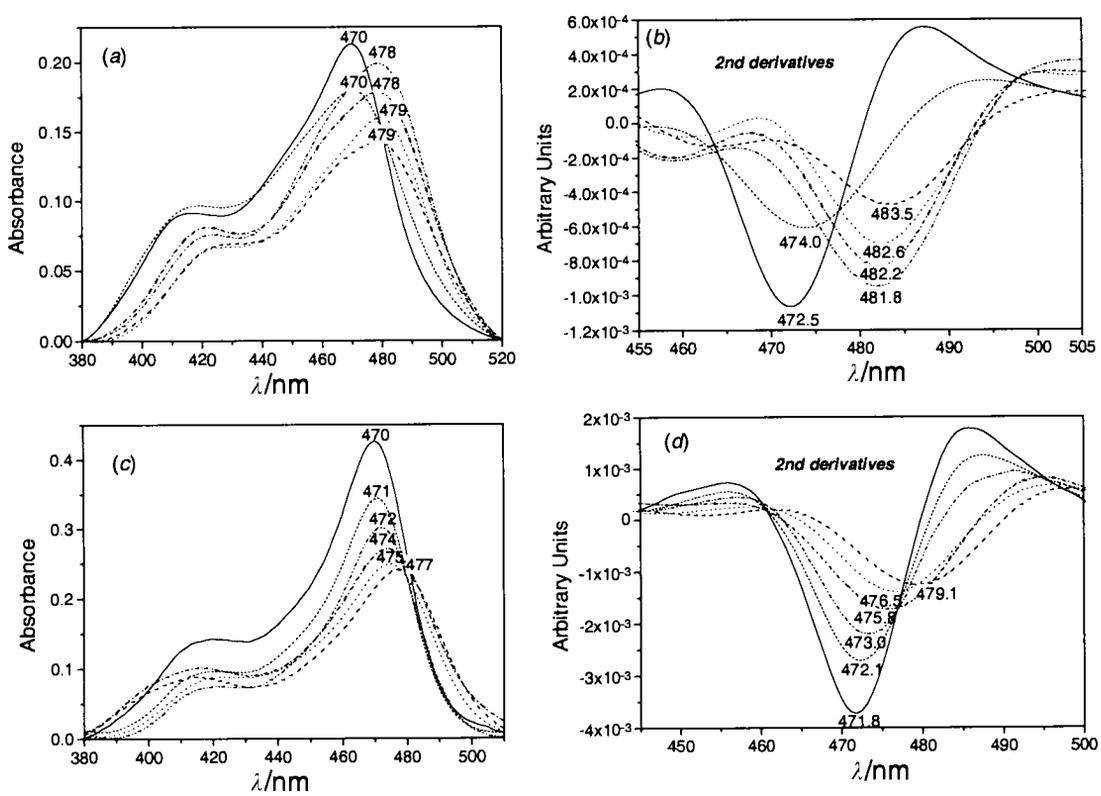

Fig. 10 Absorption spectra and corresponding second derivative plots of 5×10^{-6} mol dm^{-3} metallotexaphyrins **1** (*a*, *b*) and **2** (*c*, *d*) recorded without ligand (——) and in the presence of sodium pectate (---), sodium pectinate with DM = 20% (····), DM = 60% (-·-), DM = 75% (-···), and methyl pectate (----) in aqueous solution, pH 7.

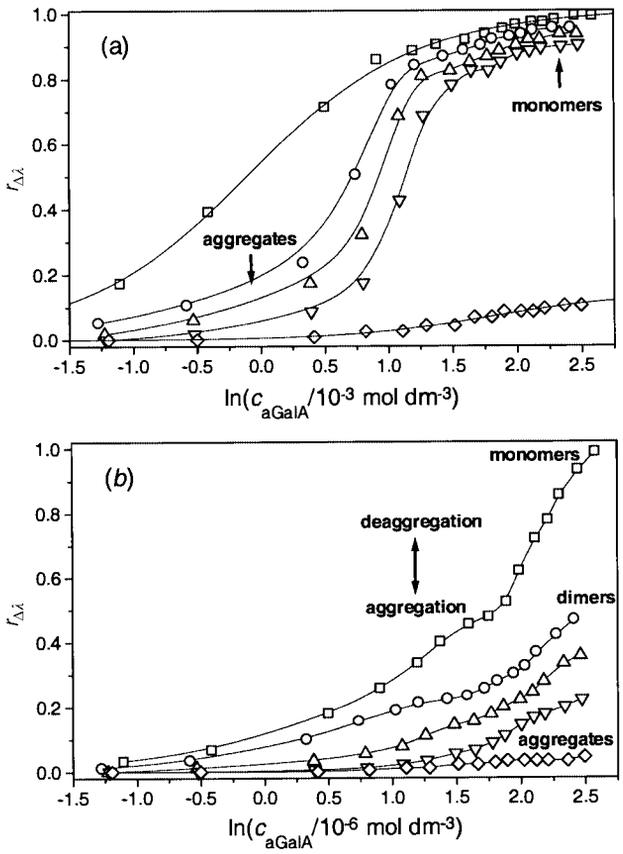

Fig. 11 Natural log plots of saturation curves obtained when aqueous solutions (pH = 7) of metallotexaphyrins **1** (*a*) and **2** (*b*) are treated with sodium pectate (□), sodium pectinate having a degree of methylation (DM) of 20% (○), a DM = 60% (△), a DM = 75% (▽), and methyl pectate (◇).

ring or its substituents *via* non-polar interactions, thereby influencing the binding process in ways that are rather complex. This complexity is expected to resolve somewhat in the case of the fully esterified system, methyl pectate since in this instance all binding interactions with the metal centre are precluded; this particular macromolecule is thus expected to react with lanthanide(III) texaphyrins in a way analogous to that seen for the other neutral polysaccharides included in this study.

As discussed above, the extent of red shift of the Soret band can be used to characterise the aggregation state of the supramolecular products formed from metallotexaphyrins and polysaccharides. For the purpose of comparison, therefore, it is instructive to define a new variable, $r_{\Delta\lambda} = \Delta\lambda/\Delta\lambda_{max}$, where $\Delta\lambda_{max}$ is the shift observed upon converting from aggregate to monomer and $\Delta\lambda$ is the shift observed under any given conditions. Defined in this way $r_{\Delta\lambda}$ will be 1 for pure monomeric forms and 0 for the initial aggregates obtained in the absence of added saccharide. Plots of $r_{\Delta\lambda}$ *vs.* ln [aGalA] (*i.e.*, per monomer sugar subunit concentration) for the interaction of metallotexaphyrins with pectate, pectinates, and methyl pectate are shown in Fig. 11. Like the derivative plots of Fig. 10, these plots help underscore how metallotexaphyrins **1** and **2** react quite differently. While the interaction of **1** with pectate gave rise to a curve that can be described in terms of a modified hyperbolic function similar to that seen for other macrocyclic species,[22] the corresponding curves for **2** were much more complicated. We suggest that, in case of pectate and **1**, break up to monomeric macrocyclic species occurs early on in the titration. By contrast, the interaction of pectate and **2** produces a curve that reflects a more gradual deaggregation process, as well as the formation of a partially aggregated intermediate. In fact, this curve shows a definitive break at $r_{\Delta\lambda} \sim 0.5$ that may reflect the formation of a dimeric texaphyrin species. While further experiments will be required to assess the merits of this latter hypothesis, it is nonetheless apparent that the aggregated forms of **2** are less prone to undergo deaggregaion in the presence of pectate than those of **1**.

For both metallotexaphyrins **1** and **2**, the curves obtained for the various pectinates lie between those of the corresponding pectate and pectic acid methyl ester titrations. In comparison to the $r_{\Delta\lambda}$ *vs.* ln [aGalA] curve obtained when **1** was titrated with pectate, the corresponding curves for titrations with pectinates showed a significant change at the beginning of the titration, although the $r_{\Delta\lambda}$ values at the end of titration proved similar. Further, in the case of the gadolinium(III) texaphyrin **1**, a significant decrease in $r_{\Delta\lambda}$ was detected throughout the titration for pectinates with high DM values.

The results of the titrations with pectinates lead to the conclusion that the presence of multiple methyl ester groups in a putative polysaccharide ligand serves to limit the extent to which deaggregation can be induced. By contrast, highly aggregated states of **1** are stable only at low concentration of free pectinate anions. In this case, increasing the pectinate: macrocycle ratio leads to a break up of the initial aggregated complex and the appearance of monomeric complexes. On the other hand, exposure of **2** to pectinates did not lead to the production of monomers. However, partial deaggregation was observed.

Conclusion

The results reported herein are consistent with the conclusion that simple monosaccharides (uronic acids), neutral polysaccharides, and polyuronides react with metallotexaphyrins in different ways. The three modes of proposed interaction, associated with these different kinds of interaction, are illustrated schematically in Fig. 9. Specifically, it is suggested that complexation between metallotexaphyrins **1** and **2** and uronic acid anion, *i.e.* D-galacturonate, takes place predominantly *via* complexation of the uronic carboxylate moieties to the coordinated lanthanide(III) cation (mode a, Fig. 9). This complexation, which involves straightforward axial ligation, does not induce appreciable deaggregation presumably because these small hydrophilic sugars react only with the outer layer of macrocycles present in the metallotexaphyrin aggregates.

The interaction between metallotexaphyrins **1** and **2** and neutral polysaccharides is very weak because the uncharged sugar hydroxy groups do not coordinate the texaphyrin-bound lanthanide(III) centres strongly. Acting thus as weak axial ligands, these saccharides stabilise supramolecular conjugates with the texaphyrins that are stabilised in large measure by hydrogen bonds. The net result is limited deaggragation as illustrated by mode b in Fig. 9.

In contrast to the above, polyuronides (pectate and alginate) can interact with metallotexaphyrins by acting as polydentate carboxylate ligands (mode c, Fig. 9). As in the case of mode a, this can lead to a direct coordinate link between the polysaccharide and the bound lanthanide(III) centre. However, in this instance the greater size and complexity of the saccharide skeleton (*i.e.*, presence of multiple uronic acid groups) can lead to cross-linking type interactions, either between pectin macromolecules or between two texaphyrins, as well as the complexation of more than one metallotexaphyrin entity to a given polysaccharide. Such interactions, in turn, are expected to lead to deaggregation, producing in the limit pure monomers. The presence of ester groups in the pectinates significantly decreases the extent and efficacy of the proposed metal–carboxylate interactions. Increasing esterification thus leads to an ever-decreasing degree of deaggregation, with this latter effect becoming minimal in the case of pectic acid methyl ester.

Looking forward, the present study offers the possibility of tuning the aggregation state in metalated macrocycles through the judicious choice of sugar entities. Such adjustments could

prove beneficial not only in the formulation of PDT sensitisers but also in the generation of other drug products. Further, the understanding that could come from the associated predicative analyses of the macrocycle–saccharide interactions might prove beneficial in understanding how various species get distributed *in vivo* while providing some important insights into possible biological targets.

Experimental

Materials

The lutetium(III) and gadolinium(III) texaphyrin complexes used in this work (structures **1** and **2**, Fig. 1) were prepared using procedures described previously.[1d] These complexes were converted to their less water soluble acetate forms by mixing equal volumes, but differing concentrations (2×10^{-3} *vs.* 10^{-3} mol dm^{-3}, respectively) of aqueous solutions of sodium acetate and the metallotexaphyrin in question. The resulting mixtures were allowed to stand for approximately 5 min at 25 °C. The precipitated solids that resulted were then washed twice with water, centrifuged at 1000*g* on a Hettich EBA-8S centrifuge, and dried at 35 °C. FTIR and FT Raman spectroscopic analysis of the largely water insoluble materials obtained in this way confirmed the presence of acetyl groups as a result of axial ligand exchange.[21]

Pectinates, derived from citrus pectin, with known degrees of methylation (DM) between 0–60%, as well as the methyl ester of pectic acid (methyl pectate) were purchased from the Institute of Chemistry, Slovak Academy of Science, Bratislava, Slovak Republic. Highly methylated (HM) citrus pectin (150 grade USA-SAG type B rapid set, DM = 73%) was purchased from Genu Pectin, Copenhagen Pectin Factory, Denmark. Sodium alginate, amylose, D-galacturonic acid and D-glucuronic acid were purchased from Sigma. Galactan was purchased from Aldrich Chemical Co. Aqueous solutions of polysaccharides (3×10^{-4}–1.35×10^{-2} mol dm^{-3} per monomeric unit) and uronic acids (2×10^{-6}–8×10^{-5} mol dm^{-3}) were prepared for use in the titration experiments (*vide infra*). Uronic acids and the various polysaccharides were dissolved or suspended in small volumes of water. The solutions and suspensions were adjusted to pH 7 by adding, as needed, several drops of 5×10^{-2} mol dm^{-3} NaOH. This led to complete dissolution of all solids. Stock solutions were then made up by adding sufficient distilled water to obtain the desired concentration. The resulting solutions were stored at 4 °C. Methanol, ethanol, propan-2-ol and sodium dodecyl sulfate (SDS) were purchased from Lachema, Brno. Dimethyl sulfoxide (DMSO) was purchased from Fluka Chemical Co.

Binding and deaggregation studies

Studies of saccharide binding and metallotexaphyrin deaggregation were made using UV–VIS titration methods. In general these titrations were carried out by first making up a 1 ml aqueous solution of either metallotexaphyrin **1** or **2** at a concentration of 10^{-5} mol dm^{-3} and then mixing it with a 1 ml solution of either aqueous sodium D-galacturonate or D-glucuronate (2×10^{-6}–8×10^{-5} mol dm^{-3}) or the polysaccharide in solution (3×10^{-4}–1.35×10^{-2} mol dm^{-3} per monomeric unit) in a spectrophotometric cuvette ($l = 1$ cm). After allowing to stand 15 minutes at 20–22 °C, the UV–VIS spectra of each of the resulting solutions were recorded and referenced to a blank solution containing pure water instead of the texaphyrin in question. Spectroscopic measurements were made on a Cary 4000 (Varian) UV–VIS spectrophotometer over a spectral range of 200–800 nm. An effective spectral bandwidth of 1 nm was used while a scan rate of 454–600 nm min^{-1} was employed. The average scan time was 0.066 s with data intervals of 0.5–0.66 nm being typical. Cary Win UV software was also used with linear baseline corrections and second derivatives combined with 5 point Savizky–Golay smoothing of spectra being made using Origin 6.0 software.

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