Tomas Bata University in Zlín
Faculty of Technology

Doctoral Thesis Summary

Study of adamantane-based supramolecular cross-linkage agents for cyclodextrin-modified biopolymers

Studium síťování cyklodextrinem modifikovaných biopolymerů supramolekulárními činidly na bázi derivátů adamantanu

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ABSTRACT

Polysaccharide-based biopolymers are produced by living organisms or chemically synthesised from basic biomolecules. Biopolymers have a great potential to be employed in development of therapeutic devices for biomedical applications. The benefits of using naturally occurring polymers instead of synthetic materials are biocompatibility, biodegradability, lower antigenicity, and renewability. To combine the above mentioned advantages and reversible formation of higher aggregates in host-guest manner, biopolymers have been widely utilised in supramolecular chemistry. Among various host-guest partners, interaction based on cyclodextrins has been extensively investigated for the construction of supramolecular aggregates due to their hydrophobic cavities, biocompatibility, and low toxicity.

The presented doctoral thesis is focused on the preparation of modified biopolymers (specifically, hyaluronane and chitosan) and study of their ability to form supramolecular networks with multitopic adamantane-based guest motifs. Due to low toxicity and sufficient biocompatibility, biopolymers like hyaluronic acid (HA) and/or chitosan (CS) are suitable for hydrogel preparation and make them excellent candidates for use in drug delivery, tissue engineering, etc. The new modified CD-HA polymer was prepared via a click reaction between propargyl-modified HA and monoazido-β-cyclodextrin, whereas modified CD-CS polymer was prepared via a Schiff base reaction between 6-*O*-(4-formylphenyl)-β-cyclodextrin and commercially available chitosan. Consecutively, we studied supramolecular behaviour of modified biopolymers with single-, two-, and three-site adamantane-based guest motifs to describe their ability to form supramolecular networks, which can be driven by competing signal molecules. The achieved results inferred that the modified biopolymers present promising components for construction of chemical stimuli-responsive architectures.

The second aim of this work was preparation of 1-adamantylalkylimidazolium and bis(benz)imidazolium salts with bicyclo[2.2.2]octane as a centerpiece. According to their binding ability towards macrocyclic molecules, they can be employed as potential multitopic guests for the construction of supramolecular systems. Last but not least, selected single-site model guests based on adamantane imidazolium salts were prepared and examined. We studied how the prolongation of carbon chain length between non-polar part of the molecule and cationic moiety would influence their binding behaviour with macrocyclic hosts. Stoichiometry and stability of resulting complexes have been determined by means of ^{1}H NMR spectroscopy and isothermal titration calorimetry.

ABSTRAKT

Biopolymery na bázi polysacharidů jsou produkovány živými organismy nebo jsou chemicky syntetizovány ze základních biomolekul. Biopolymery mají velký potenciál využití při vývoji terapeutických přístupů medicinálních aplikací. Ve srovnání se syntetickými materiály patří k výhodám přirozeně se vyskytujících polymerů biokompatibilita, biodegradabilita, nižší antigenicita a obnovitelnost zdrojů. Biopolymery jsou široce používány v supramolekulární chemii, poněvadž umožňují kombinovat výše uvedené výhody a reverzibilní vyznik vyšších agregátů na bázi "hostitel-host" interakcí. Mezi různými hostitel-host partnery zaujímají významné místo ve vélkem rozsahu zkoumané interakce založené na cyklodextrinech (CD) vzhledem k jejich hydrofobním kavitám, biokompatibiltě a nízké toxicitě.

Předkládaná dizertační práce je zaměřena na přípravu modifikovaných biopolymerů (konkrétně hyaluronanu a chitosanu) a na studium jejich schopností tvořit supramolekulární soustavy (systémy) na bázi multitopic adamantanu hosta. Nové modifikovaný CD-HA polymer byl připraven pomocí click reakce mezi propargyl-modifikovaného HA a monoazido-β-cyklodextrinu, zatímco modifikovaný CD-CS polymer byl připraven pomocí Schiffovy báze reakcí 6-*O*-(4-formylfenyl)-β-cyklodextrin a komerčně dostupného chitosanu. Následně jsme studovali supramolekulární chování modifikovaných biopolymerů na jednom, dvou nebo třech-místech adamantanu popisujících jejich schopnost tvořit supramolekulární sítě, které mohou být poháněny konkurenčními signálními molekulami. Dosažené výsledky naznačují, že modifikované biopolymery představují slibné komponenty pro konstrukci chemických architektur reagujících na chemické podněty.

Dalším cílem předkládané dizertační práce bylo připravit 1-adamantylalkylimidaziolové a bis(benz)imidazoliové soli s bicyklo[2.2.2]oktanovou středovou částí molekul. Vzhledem ke svým schopnostem vázat se na makrocyklické molekuly mohou pak tyto soli být použity jako potenciální vícevazebné hostující molekuly pro konstrukci supramolekulárních systémů. V neposlední řadě byly připraveny a prostudovány vybrané modelové jednovazebné ligandy na bázi adamantylimidaziolových solí. Studovali jsme, jak prodloužení délky uhlíkového řetězce mezi nepolární částí molekuly a kationtovou skupinou ovlivní jejich schopnost vázat se s makrocyklickými hostiteli. Stechiometrie a stabilita výsledných komplexů byly stanoveny pomocí ^{1}H NMR-spektroskopie a izotermické titrační kalorimetrie.

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LIST OF SYMBOLS AND ABBREVIATIONS

Ad	adamantane
CB[*n*]	cucurbit[*n*]uril
CDs	cyclodextrins
CS	chitosan
DA	degree of acetylation
DCM	dichloromethane
DD	degree of deacetylation
DME	dimetoxyethane
DMF	*N*,*N*–dimethylformamide
DMSO	dimethyl sulfoxide
DOSY	diffusion ordered spectroscopy
DS	degree of substitution
DBE	dibromoethane
EDTA	ethylenediaminetetraacetic acid
ESI-MS	electrospray ionization mass spectrometry
G	guest
GC-MS	gas chromatography mass spectroscopy
HA	hyaluronic acid/hyaluronane/sodium hyaluronate
HGs	hydrogels
ITC	isothermal titration calorimetry
PBS	phosphate buffered saline (1 mM KH_2PO_4/K_2HPO_4)
PBT	polybutylene terephthalate
PEG	polyethylene glycol
PVA	poly(vinyl) alcohol
SEC	size exclusion chromatography
TEMPO	(2,2,6,6-tetramethylpiperidin-1-yl)oxyl
Tg	glass transition temperature
THF	tetrahydrofuran
TLC	thin layer chromatography

1. INTRODUCTION

Supramolecular hydrogels are formed from polymers, which are modified by suitable supramolecular host and guest motifs. For instance, natural cyclodextrins and selective cucurbit[*n*]urils are the most extensively studied macrocyclic hosts, whose hydrophobic cavities successfully comprise specific guest molecules in aqueous solution to generate supramolecular polymers. Particulary, CDs and CB[*n*]s form ultrahigh-affinity host-guest pairs with cage of hydrocarbons (for instance, adamantane), where the first chapter is dedicated to brief description of adamantane.

Supramolecular hydrogels have significant role in the host-guest systems since the supramolecular cross-linking by diverse non-covalent interactions decreases the structural flexibility and modifies the macroscopic performance resulting in the construction of 3D cross-linked networks. Besides, the capability of hydrogels to keep significant volume of water, hydrogels also show reversible gel-sol transition behaviour. They can serve as intelligent carriers in drug delivery or promising matrices in tissue engineering. In addition, techniques for hydrogel preparation are given in the following chapters in more detail.

2. ADAMANTANE

Adamantane is an intriguing polycyclic hydrocarbon $C_{10}H_{16}$, which molecules have a three dimensional cage structure.[1] Adamantane is the smallest member of diamondoids family, i.e., hydrogen terminated hydrocarbons with a diamond-like structures.[2,3] Further, adamantane consists of three fused cyclohexane rings, which adopt a chair conformation. In addition, C–C–C angle values are close to the common tetrahedral angle (109.5°) and the total strain is estimated to be very low (6.950 kJ·mol^{-1}). The geometrical parameters of adamantane and its structure are shown in *Figure 1*.

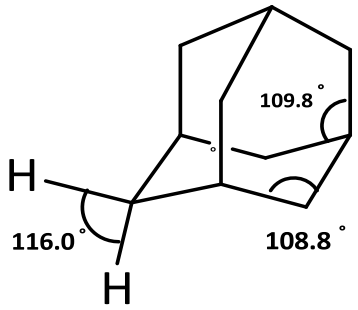
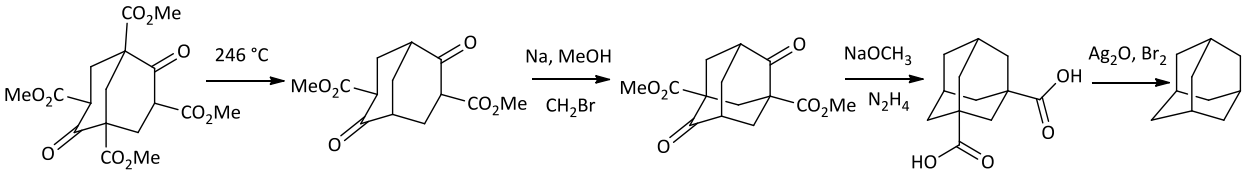


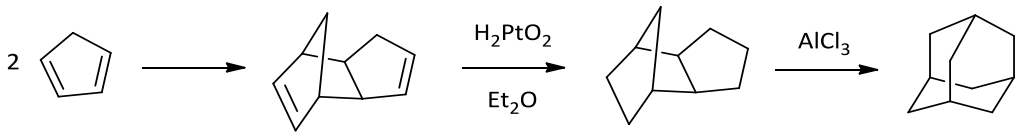
Fig. 1: The structure of adamantane.[4]

The adamantane was discovered and isolated from petroleum fractions of the Hodonin oilfields in Czechoslovakia in 1933.[1] The first chemical synthesis of adamantane was performed by Prelog and Seiwerth in 1941.[5] Meerwein's ester was used as a starting compound[5] and the synthetic approach is shown in *Scheme 1*.



Scheme 1: Synthesis of adamantane from Meerwein's ester.

Initially, the overall yield of adamantane was only 0.16 %, but yield was later increased up to 6.5 % by Heinsdecker and Hoffman reaction. However, this improved yield was still low and synthetic procedure was very complicated to provide enough of adamantane for an extensive research. Later, Schleyer found the convenient Lewis-acid catalysed rearrangement procedure leading to the adamantane cage.[6] In addition, Schleyer performed hydrogenation of dicyclopentadiene (*Scheme 2*), where hydrogenated product was converted to adamantane by thermal treatment with $AlCl_3$.[6] The overall yield was increased up to 40 %. Later, the Lewis acid and reaction conditions were optimised to achieve adamantane yield of 98 %.



Scheme 2: Lewis-acid catalysed rearrangement leading to adamantane.

Furthermore, the adamantyl moiety can serve as an ideal filling for cavities of various macrocyclic molecules such as cyclodextrins or cucurbit[*n*]urils.[7,8] Adamantane can be included in lipophilic part of the lipid bilayer that creates membranes.[9] This is a crucial step for the molecule transfer through the cell membranes.

2.1. Adamantyl-cyclodextrin complexes

Cyclodextrins are capable to generate inclusion complexes with diverse guest molecules.[10–13] The adamantyl moiety fits perfectly into β-cyclodextrin

cavity and complexes of adamantane derivatives with β-CD display high association constant of the order 10^3–10^5 M^{-1}.[14] Because of the highly strong interaction and facile inclusion complex construction between adamantane and cyclodextrins, various cyclodextrin-based self-assembled systems have important applications in supramolecular chemistry such as hydrogels,[15] cyclodextrin polymer-based particles,[16] surface-mediated gene delivery,[17] and/or supramolecular polymers.[18] In addition, particular attention is paid to the chemical synthesis of new carbohydrate-adamantane conjugates and supramolecular studies of their complexes with cyclodextrins.[19]

2.2. Adamantane containing polymers

The incorporation of adamantane into polymers provides the higher thermal stability and increasing of glass transition temperature (T_g) of polymers.[20,21] The adamantyl groups can be comprised in the various polymers (polysulphones, polyamides, polyesters) as a part of the main chain or as side groups. Incorporation of adamantane as a pendant group provides slightly modified properties such as the decreasing of crystallinity, the increasing of solubility, and higher glass transition temperature of the parent polymer. Alkylation of aromatic compounds with 1-bromoadamantane yields monomers (for instance, 4–adamantylphenyl methacrylate)[22] that incorporates adamantane as a pendant group on the polymer backbone as shown in *Figure 2*. If adamantane is incorporated on backbone of polymers than polymers will have better thermo-oxidative and hydrolytic stability. In addition, physical hydrogels are obtained by host-guest interaction of adamantyl-containing polymers with cyclodextrins.[23,24] Host-guest interaction of cyclodextrins with suitable guests is an ideal system for preparation of non-covalently cross-linked physical hydrogel.

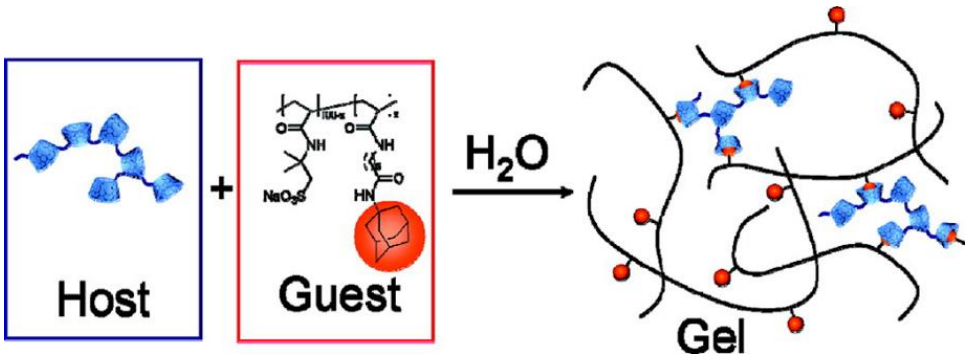


Fig. 2: Formation of physical hydrogel via a host-guest interaction of β-cyclodextrin polymers and copolymers bearing adamantyl groups.[15]

3. HYDROGELS

Hydrogels (HGs) are water-swollen polymeric networks, which are composed from hydrophilic polymers[25] (*Figure 3*). They have been defined during 1950's by Wichterle, who prepared poly(2-hydroxyethyl)methacrylate hydrogel for contact lens application. Based on their properties to swell in water under biological conditions, hydrogels have been applied in biomedicine research. Hydrogels present powerful scaffolding materials because of their swollen network structure (contain more than 90 % of water), which is capable to encapsulate cells and bioactive compounds.

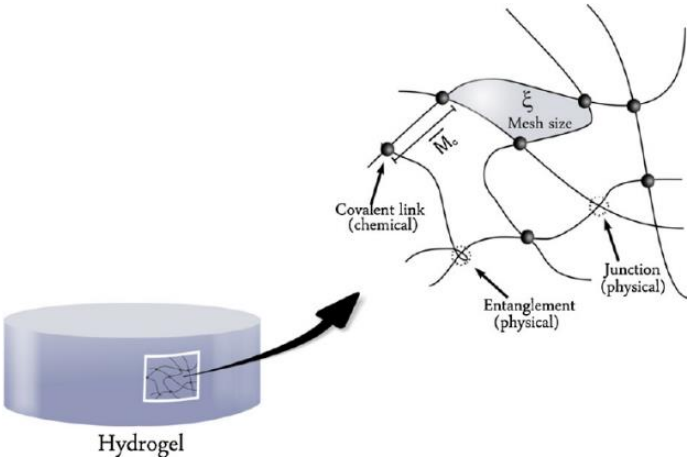


Fig. 3: Cross-linked polymer (hydrogel).[26]

Hydrogels can be cross-linked through covalent bonds or stuck together by physical intramolecular and/or intermolecular attractions.[25] Hydrogels are able to absorb high portion of water or other liquids. In swollen state, hydrogels appear soft or rubbery. There is a small difference between gels and hydrogels. Gels occur as semi-solid materials consisting of hydrophilic polymers, which contain small amount of solids. On the other hand, hydrogels are composed of cross-linked hydrophilic polymer chains. Hydrogels allow for swelling but retain their three-dimensional structure without dissolving. Natural, synthetic or hybrid polymers have been employed to form hydrogels via chemical or physical cross-linking.[25] The most familiar gels are chitosan-poly(ethylene glycol) hydrogel, chitosan-hyaluronic hydrogel, chitosan-glycerophosphate hydrogel, chitosan-alginate composite, chitosan-collagen composite, chitosan-hydroxyapatite composite, and chitosan-tricalcium phosphate composite.[27–33] These materials appear in various shapes or forms like films, fibers, sponges, beads, and solutions. They are mostly useful in bone tissue engineering scaffolds, drug delivery systems, wound healing materials, and metal and dye absorbents for polluted water.

3.1. Techniques for hydrogel preparation

Hydrogels can be classified into two groups based on their cross-linking nature like physically and chemically cross-linked hydrogels.[34,35] Increased interest in physically cross-linked hydrogels is due to the absence of cross-linkers for hydrogel preparation. Physically cross-linked hydrogels have transient junctions, while chemically cross-linked hydrogels have permanent junctions.[25] Methods for formation of physically cross-linking hydrogels are ionic interactions, hydrophobic interactions, stereocomplex formation, coiled-coil interactions, and antigen-antibody interactions[25,36] (*Figure 4*). *Table 1* shows some examples of physically cross-linked hydrogels.

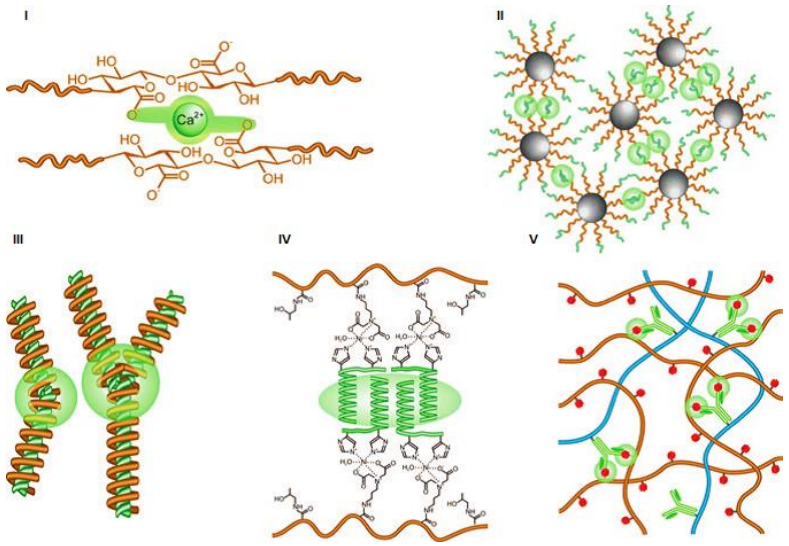


*Fig. 4: Physically cross-linked hydrogels via: **I** ionic interactions; **II** hydrophobic interactions; **III** self-assembling of stereocomplex formation; **IV** coiled-coil interactions; **V** antigen-antibody interactions.*[36]

Table 1: *Selected examples of physically cross-linked hydrogels.*

Polymer	Method type	Loaded drug	References
PEG and PBT	*Melt polycondensation*	*Lysozyme*	[37]
Pullulan	*HGs nanoparticles*	*Adriamycin*	[38]
Polyacrylamide	*Antigen-antibody interaction*	*IgG*	[39]

Physically cross-linked hydrogels have some limitations such as rapid degradation, long gelation time, network pore size, and chemical modification. In contrast, chemically cross-linked hydrogels have greater mechanical strength and extended degradation time. Increased interest in chemically cross-linked hydrogels is based on good mechanical strength of chemically cross-linked hydrogels. Methods for formation of chemically cross-linking hydrogels are free radical polymerisation of monomers and macromonomers, cross-linking with

pendant functional groups, and cross-linking by high energy radiation[25,36] (*Figure 5*). *Table 2* gives some examples of chemically cross-linked hydrogels.

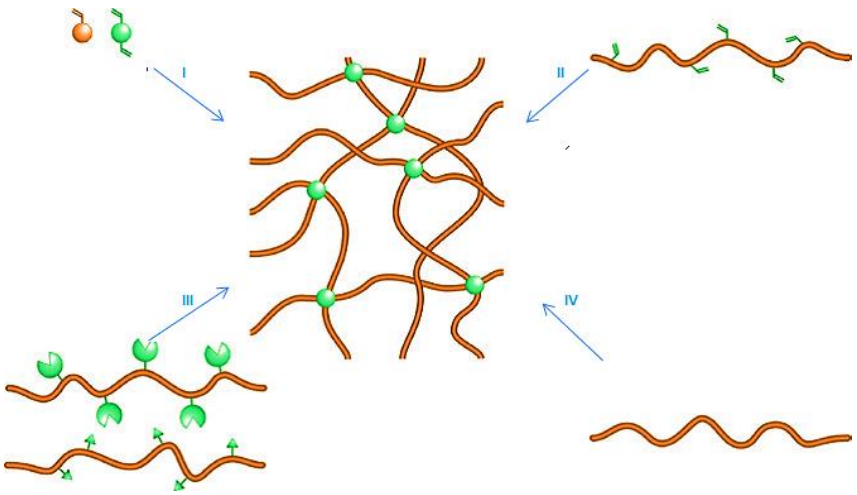


*Fig. 5: Chemically cross-linked hydrogels: **I** radical polymerisation of vinyl monomers; **II** radical polymerisation of macromonomers; **III** cross-linking with pendant functional groups; **IV** cross-linking by high energy radiation.*[36]

Table 2: *Selected examples of chemically cross-linked hydrogels.*

Polymer	Method type	Loaded drug	References
Chitosan-PVA	*Cross-linking with aldehyde*	*Nano-insulin*	[40]
Gelatin	*Cross-linking with aldehyde*	*TGF-B1*	[41]
Albumin	*Cross-linking with aldehyde*	*Adriamycin*	[42]
Chitosan	*Cross-linking with aldehyde*	*Mitoxantrone*	[43]
PVA	*Polycondensation reaction*	*Diltiazem HCl*	[44]
Gelatin	*Polycondensation reaction*	*Lysozyme*	[45]

4. MOTIVATION AND AIMS OF THE STUDY

Motivation of this research was to synthesise biopolymers modified with β-cyclodextrin units, which can be employed for preparation of chemical stimuli responsive supramolecular architectures, e.g., hydrogels. Particularly, supramolecular hydrogels are very attractive since they are consisting of host and guest components to enable modulation of gelation by competition with others supramolecular additives. According to low toxicity and adequate biocompatibility, biopolymers are appropriate for hydrogel preparation and make them excellent candidates for use in drug delivery, tissue engineering, etc.

Generally, aims of the research can be highlighted as follows:

1. Synthesis of model single-site adamantane binding motifs to enable tuning of binding strength of the guests towards suitable macrocyclic hosts based on cyclodextrins and cucurbit[*n*]urils.

2. Synthesis of bis(benz)imidazolium salts with bicyclo[2.2.2]octane as a centerpiece and adamantane scaffolds as terminal binding sites.

3. Preparation of host-modified hyaluronic acid and chitosan.

4. Supramolecular study of modified biopolymers.

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5. RESULTS AND DISCUSSION

5.1. Preparation of adamantane model guests

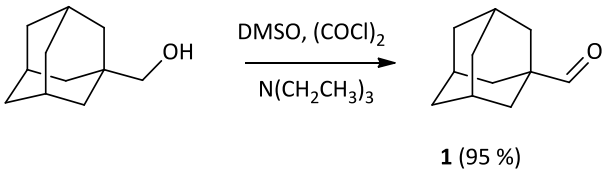
The 1-adamantylalkyl(benz)imidazolium salts were synthesised in order to estimate how their binding strength towards macrocyclic hosts such as cyclodextrins and cucurbit[*n*]urils is influenced by prolongation of the linker between non-polar part of the molecule and cationic moiety. The positive charge on the guest molecule, located in appropriate distance from the portal carbonyl oxygens, contributes to the complex stability via ion-dipol interactions with the portal of the CB[*n*] macrocycle. Therefore, we assumed that the length of the linker would have negative impact on the binding strength. On the other hand, prolongation of the linker could fix some synthetic issues related to the steric hindrance of the adamantane moiety during quaternisation steps leading to multitopic guests of our interest.

Cucurbit[*n*]urils are capable to form stable inclusion complexes with non-polar cationic guests having association constants up to 10^{17} M^{-1}.[46] Since, adamantane derivatives have a high affinity towards cucurbit[7]uril based on the extremely strong association of CB[7] interior cavity with adamantane cage, we prepared new series of 1-adamantylalkyl(benz)imidazolium salts with a longer spacer between adamantane and (benz)imidazolium part. The aim of this study was to estimate how the prolongation of carbon chain length would influence their binding behaviour with macrocyclic hosts.

5.2. Swern oxidation

Due to previous unsuccessful attempts to extend carbon chain length by employing CH_3—NO_2 we considered another way for extension of carbon chain such as Ad—C_2+C_1 based on Wittig approach. As Wittig reaction requests two components for the reaction, we used Ad—CHO (because of better obtainability) and the appropriate C2—phosphonium salt.

The 1-adamantylmethanol, which can be easily prepared from commercially available 1-adamantanecarboxylic acid (Sigma Aldrich) was used as a starting reagent for the synthesis of adamantane-based binding motifs. Although, there are many useful ways for the transformation of primary alcohols to aldehydes (chromium-based reagents, hypervalent iodine compounds, 4-acetamido-TEMPO/NaOCl, etc)[47] we employed Swern oxidation (*Scheme 3*) as the most useful and productive reaction.[48]

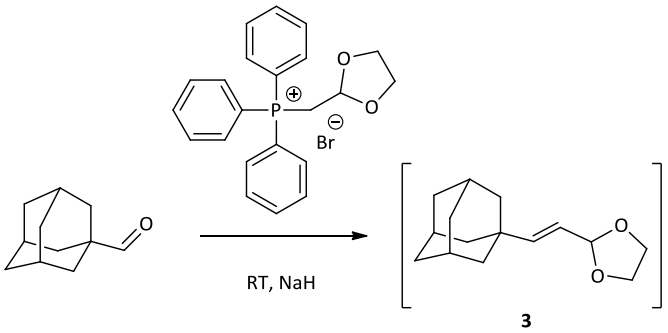


Scheme 3: *Swern oxidation of 1-adamantylmethanol.*

The Swern oxidation of 1-adamantylmethanol was carried out at −70 ℃, which led to the aldehyde in 95 % of yield. The purity of the aldehyde **1** was confirmed by ^{1}H NMR and the melting point was in the agreement with the literature data.[48]

5.3. Synthesis of a key intermediate for preparation of salts

Another approach based on Wittig reaction was to use commercially available, 1,3-dioxolan-2-ylmethyl)triphenylphosphonium bromide (Merck) and aldehyde **1** (*Scheme 4*). This reaction is well-recognised as an aldehyde C_2 homologation.[49] One interesting aspect of Wittig reactions is that the reaction mixture was intensely colored (grape-juice purple) after slowly addition of 1,3-dioxolan-2-ylmethyl)triphenylphosphonium bromide. Therefore, progress of Wittig reaction could be monitored by color changes.[50]



Scheme 4: *Synthesis of 2-(2-(1-adamantyl)ethenyl)-1,3-dioxolne (**3**).*

The reaction was carried out in a dry THF under inert atmosphere in the presence of NaH base, which was previously washed with several portions of freshly distilled pentane to remove a mineral oil. The side product triphenylphosphine oxide was removed by silica gel chromatography CH_3OH:$CHCl_3$ (2:98, v:v). Unfortunately, the compound **3** was too labile to allow for spectral characterisation and crude products were contaminated by compound **4**.

In order to remove heterocyclic acetal, deprotection of compound **3** was performed by acid catalysed hydrolysis in acetone (*Scheme 5*), which led to the compound **4** in 90 % yield. The compound **4** was isolated by extraction with toluene and used as a pure product in further reaction sequence. Because of the

semi-stabilised ylide, the final product can be obtained as a *cis/trans* mixture. As it can be seen from *Figure 6*, the stereochemistry of the C=C bond indicates preferentially presence of *E* isomer (97 %), where coupling constants of H(e) dd signal are J=15.5 Hz (H(e)—H(d)) and 7.5 Hz (H(e)—H(f)). Whereas, *Z* isomer is present in minority (3%), whose signals are close to noise at 5.70 ppm and 6.88 ppm.

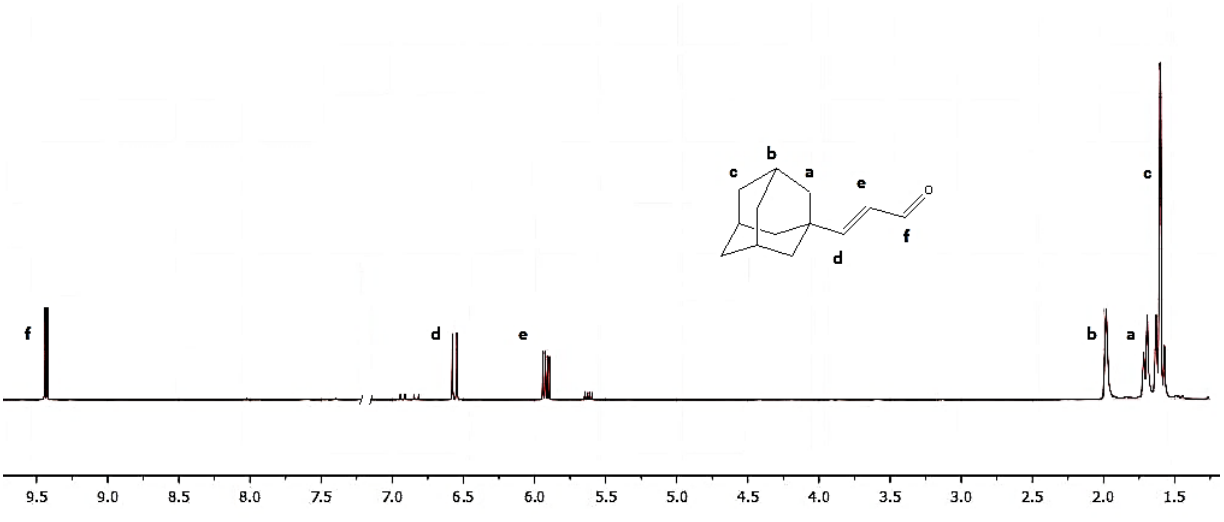
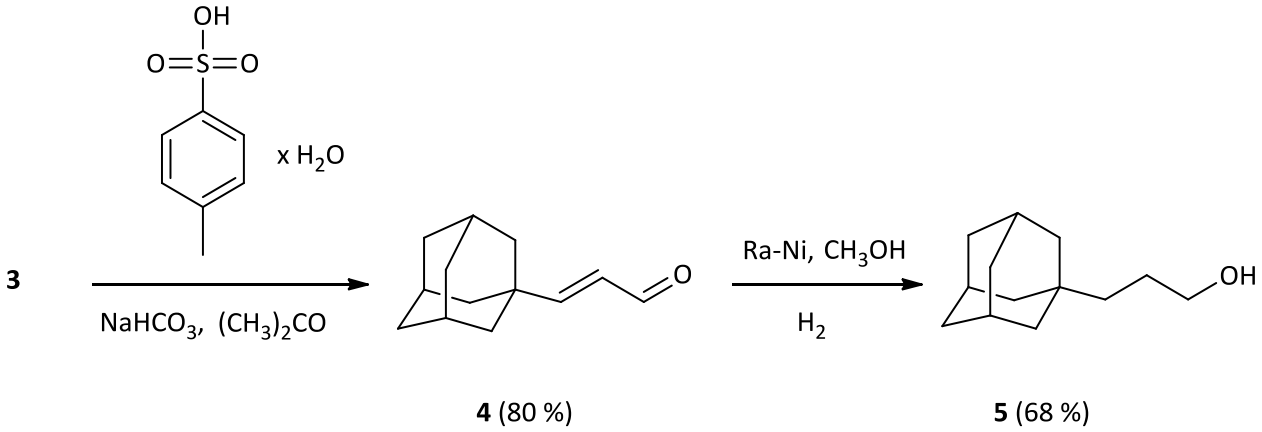


Fig. 6: *^{1}H NMR spectrum ($CDCl_3$) of 3-(1-adamantyl)prop-2-enal (**4**).*

The next step was preparation of alcohol **5**, where compound **4** was treated with hydrogen gas in the presence of catalyst Ra-Ni in methanol. As it can be seen in *Figure 7*, the C=C double bond was reduced and aldehyde was successfully converted to alcohol in one step, as the disappearing of the signals of H-atoms at aldehyde group and C=C double bond indicates.



Scheme 5: *Synthesis of 3-(1-adamantyl)propan-1-ol (**5**).*

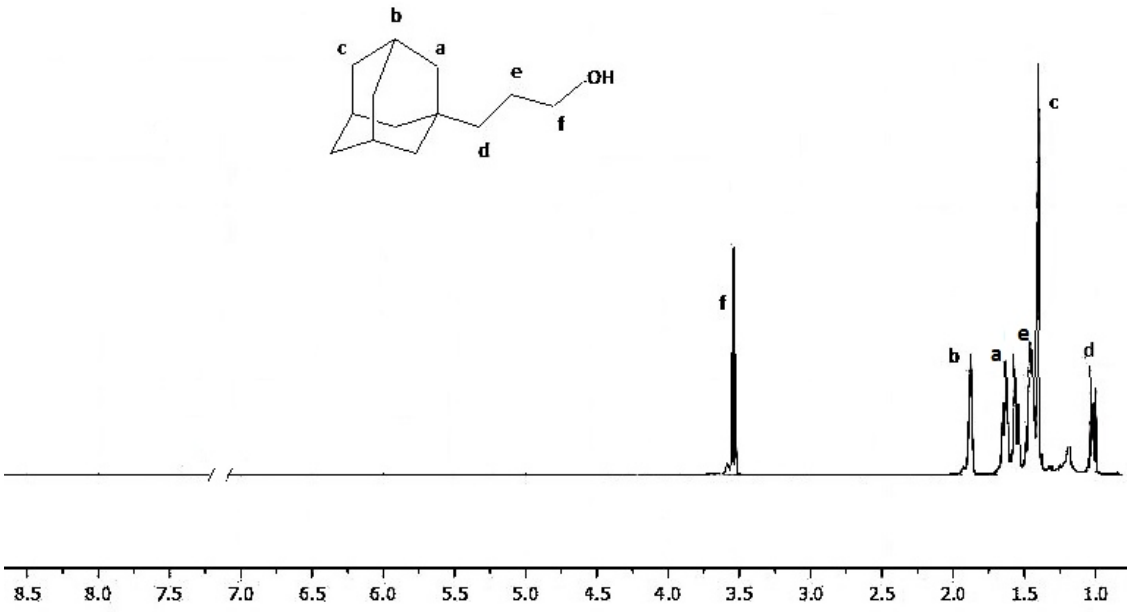
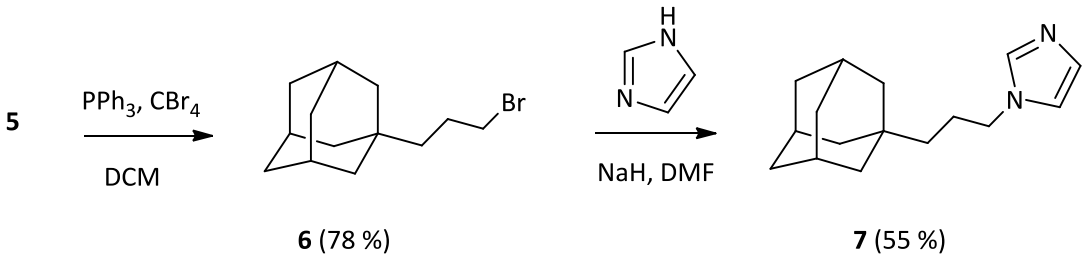


Fig. 7: *^{1}H NMR spectrum ($CDCl_3$) of 3-(1-adamantyl)propan-1-ol (**5**).*

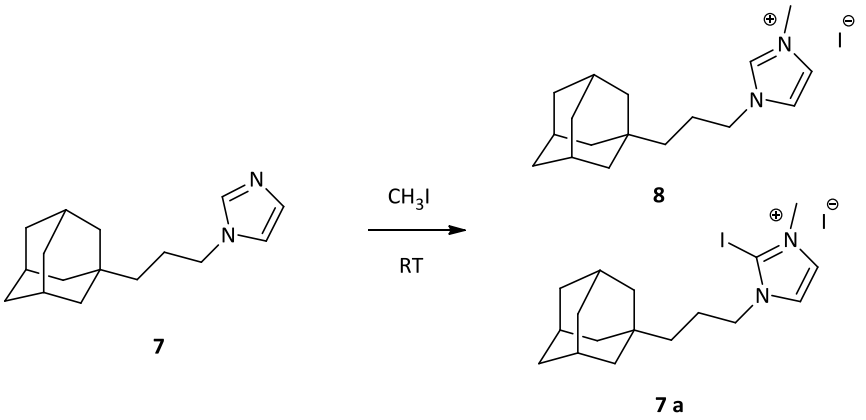
Furthermore, the obtained compound **5** was used for bromination reaction using CBr_4 and PPh_3 in a dry CH_2Cl_2 (Appel reaction, *Scheme 6*), which resulted compound **6** in 85 % of yield. The desired compound **6** was isolated from side product $Ph_3P=O$ by column chromatography with CH_3OH:$CHCl_3$ (1:5, v:v) as a mobile phase. The preparation of the intermediate **7** was performed in a dry DMF with alternately addition of NaH and imidazole. The progress of reaction was monitored by TLC. The obtained product **7** was isolated from side products by column chromatography on silica gel using a mixture CH_3OH:$CHCl_3$ (1:5, v:v) as a mobile phase. The identity of compound **7** was confirmed by NMR and ESI-MS and compound **7** was used in next reaction sequence for the preparation of the 1-(1-adamantylalkyl)-3-methyl(benz)imidazolium salts.



Scheme 6: *Synthesis of 1-(3-(1-adamantyl)propyl)imidazole (**7**).*

5.4. Synthesis of 1-adamantylalkylimidazolium salts

The initial plan was to prepare imidazolyl quarternary ammonium salt from the intermediate **7**. Quaternisation reaction was carried out in a neat CH_3I at room temperature under an inert atmosphere. The mechanism is based on nucleophilic substitution (S_N2), where CH_3I is a highly reactive reagent with a good leaving iodide group. Conversely, the high reactivity and related low selectivity of CH_3I can cause some unwanted reactions (*Scheme 7*). Unfortunately, we obtained the mixture of two products, where desired product was formed in a lower amount (ratio between **7a** and **8**, 55:45 % was calculated from ^{1}H NMR spectrum, *Figure 8*). Due to the very similar chemical nature, it was essentially impossible to isolate desired product from the mixture.



Scheme 7: *Methylation of the compound **7**.*

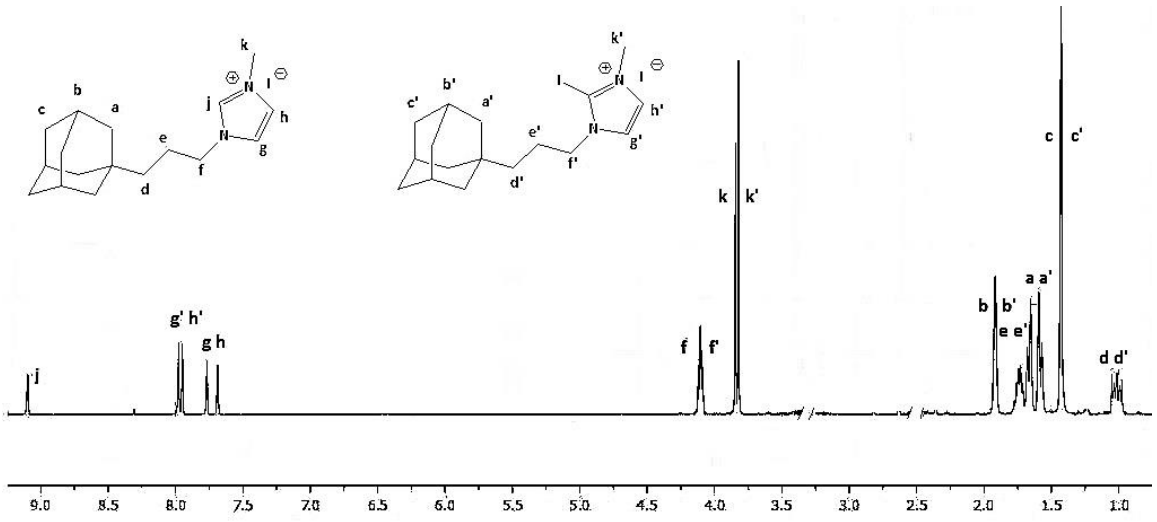
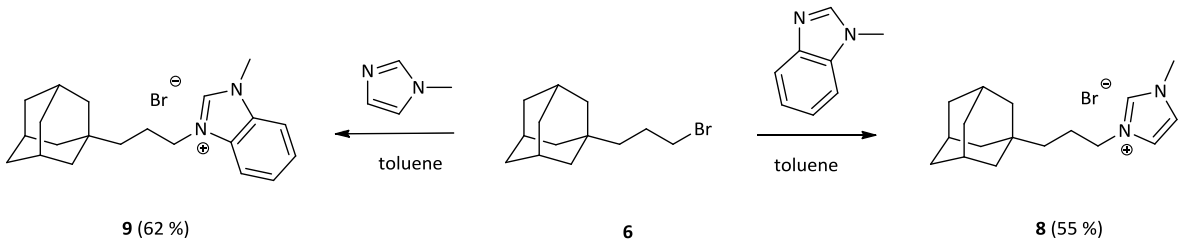


*Fig. 8: ^{1}H NMR spectrum (DMSO) of methylation of the compound **7**.*

Another approach was to perform quaternisation reaction directly from **6** to **8** using 1-methylimidazole in a dry DMF. Because of a high content of DMF in the crude product that was impossible to remove during purification, we replaced DMF solvent with toluene (*Scheme 8*). Quaternisation proceeded smoothly to result compound **8** in a yield of 55 %.



9 (62 %) **6** **8** (55 %)

Scheme 8: *Synthesis of 1-(1-adamantylalkyl)-3-methyl(benz)imidazolium salts.*

Furthermore, the compound **8** was identified by NMR, where the sharp singlet of the —CH_3 group appeared at 3.84 ppm, while two doublets and one singlet of H-atoms located at imidazole core were observed at 7.69, 7.77, 9.11 ppm, respectively. On the similar way, compound **6** was converted to compound **9** (62 %) using 1-methyl-1*H*-benzimidazole. Similarly to the imidazolium salt **8**, the structure of compound **9** was determined by NMR spectroscopy. The singlet of the —CH_3 group was observed at 4.07 ppm, multiplets of the benzimidazole core H-atoms appeared in the range of 7.70−8.07 ppm and singlet of C2—H atom of benzimidazolium ring was observed at 9.71 ppm.

5.5. Thermodynamic parameters of adamantylalkylimidazolium salts

The isothermal titration calorimetry (ITC) is a powerful method for a study of intermolecular interactions. It is based on the measurement of the heat generated or absorbed upon the interactions between molecular components of the system. As one of the most sensitive methods (characterisation of low–high affinity constants of $10–10^7$ M^{-1}), ITC can be employed for the determination of stoichiometry of the interaction (n), the affinity or association constant (K_a), and the enthalpy change (ΔH), which reflects the heat released or taken up during the interaction. Furthermore, the entropy (ΔS) and the Gibbs free energy of the process (ΔG) can be calculated from the ITC data.[51] In this part of the research, we basically focused on examining the binding behaviour of several structurally related guests (for structures, see *Figure 9*) towards macrocyclic hosts with suitable inner cavity dimensions such as CB[7], CB[8], and β-CD. We intended to estimate, how prolongation of alkyl chain between non-polar part of the molecule and cationic moiety of the guests influence the binding strength towards macrocyclic hosts. Generally, the ITC experiments were conducted in pure water at 30 °C (303 K) and the initial concentrations of the guest and host

have been adjusted to obtain titration curves that can be fitted properly. Consequently, ITC measurements confirmed the formation of 1:1 inclusion complex and that the binding strength positively correlates with the bulkiness of the cationic moiety.

Preliminary studies showed an extremely high binding strength of the CB[7]–(benz)imidazolium complexes. We observed higher variations in the binding strengths of imidazolium and benzimidazolium salts with CB[7]. For instance, the association constant for *Ia*@CB[7]Ad is 7732 times higher than for *Ib*@CB[7]Ad (Note, enteries 2 and 5 in *Table 3*). This can be attributed to high steric hindrance of benzimidazole cation in CB[7] cavity, based on incapability of the rigid CB[7] portal to accommodate the benzimidazolium cation efficiently. The prolongation of the alkyl chain between adamantane and cationic moiety should lead to reducing of the ion-dipole contribution. In contrast, we have noticed increasing of K_a values with the prolongation of the linker between non-polar part and cationic moiety. This phenomenon can be attributed to the supple linker that permits the cation to reach a position closer to the portal. Moreover, complexes formed between the same guests and CB[8] display similarly high stabilities. This phenomenon can be explained by better accommodation of guests in a wider cavity of CB[8]. In addition, the calculated K_a values between β-CD and adamantane-based guest motifs are increased with prolongation of the linker and this phenomenon could be recognised to the bulkier benzimidazolium core, which more negatively affects the binding strength sitting closer to the CD portal.

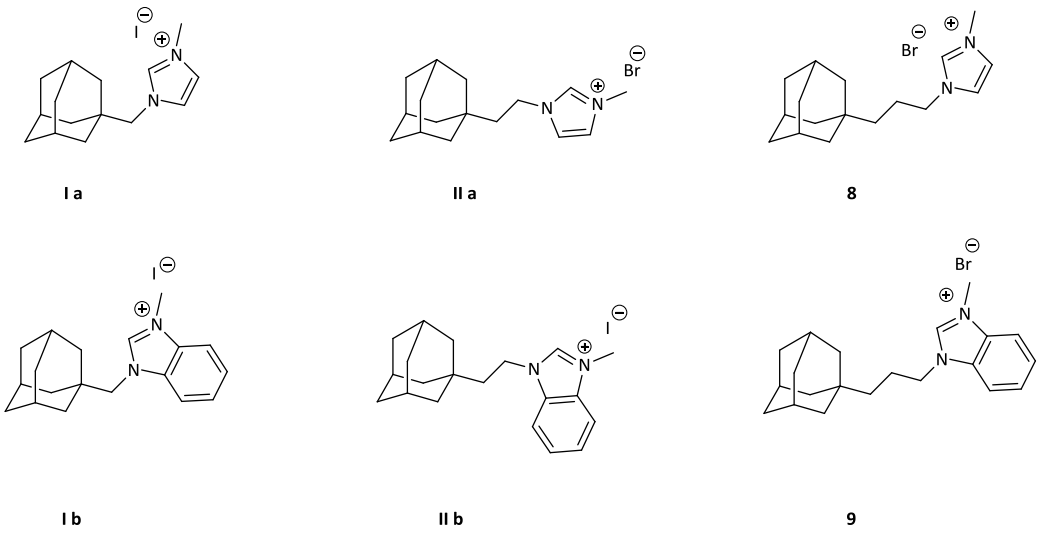


Fig.9: The chemical structure of 1-(1-adamantylalkyl)-3-methyl(benz)imidazolium salts.

Table 3: *Thermodynamic parameters of supramolecular complex formation.*

Entry	Guest	Host	Competitor	n	K_a [$dm^3\ mol^{-1}$]	ΔH[kJ mol^{-1}]	ΔS [$Jmol^{-1}\ K^{-1}$]
1	Ia	β-CD		0.99	6.07×10^4	−28.99	−4.08
2		CB[7]	HMDCl	1.01	8.97×10^{12}	−85.61	−51.99
3		CB[8]	MV	0.869	1.07×10^{11}	−39.13	−70.31
4	Ib	β -CD		0.99	5.43×10^4	−28.22	−2.51
5		CB[7]	α -Phe	0.92	1.16×10^9	−89.35	−64.28
6		CB[8]	MV	1.02	3.10×10^{10}	−49.01	39.29
7	IIa	β -CD		1.07	1.70×10^5	−28.66	5.60
8		CB[7]	HMDCl	1.00	1.20×10^{11}	−84.25	−47.69
9		CB[8]	MV	1.00	1.20×10^{11}	−84.25	−47.69
10	IIb	β -CD		1.02	1.66×10^5	−30.15	−0.52
11		CB[7]	α -Phe	1.04	3.39×10^{10}	−89.77	−50.63
12		CB[8]	MV	1.08	7.26×10^{10}	−50.85	−40.76
13	8	β -CD		0.96	4.87×10^5	−33.21	−0.58
14		CB[7]	HMDCl	1.00	8.11×10^{12}	−84.59	−47.82
15		CB[8]	MV	1.01	4.66×10^{10}	−35.56	86.50
16	9	β -CD		0.98	5.00×10^5	−32.25	−2.78
17		CB[7]	α-Phe	1.08	4.84×10^{10}	−85.75	−52.06
18		CB[8]	MV	1.05	5.67×10^{10}	−43.23	61.77

*HMDCl=hexamethylenediamine chloride; MV=methyl viologen dichloride hydrate; α-Phe=Phenylalanine. Experiments were conducted in water (CDs and CB[7]) or 50 mM NaCl solution (CB[8]) at 303.15 K. ITC titrations were done in three replicates. K_a values are calculated for a single binding site.

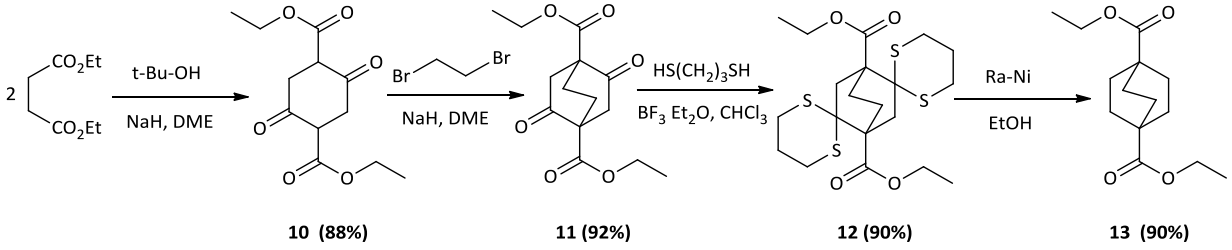
Contribution declaration note: All ITC titration experiments were conducted by Zdeňka Prucková, who is responsible for the calorimetric measurements in our research group. The compounds **Ia** and **Ib** have been already published with corresponding ITC data,[46] while the compounds **IIa** and **IIb** were prepared in our group within another project.

6. PREPARATION OF BICYCLO[2.2.2]OCTANE MODEL GUESTS

Bicyclo[2.2.2]octane-1,4-dicarboxylic acid has been of the interest of several studies and diverse applications.[52–62] The impact of the aliphatic backbone as well as spectroscopic and conformational properties have been extensively studied.[53,63] The rigid aliphatic backbone is also of interest because of its incorporation into metal organic frameworks[58] and polyesters.[54,55,59] New series of prepared compounds based on a bicyclo[2.2.2]octane core are designed to form complexes with cucurbit[*n*]urils (CB[*n*]s) or cyclodextrins (CDs) in aqueous solution. It has been known that guests combining a non-polar core with cationic substituents can be employed as ultrahigh affinity CB[7] guests.[64] The aim of our study was preparation of bisbenzimidazolium salts with bicyclo[2.2.2]octane as a centerpiece, which will be employed for study of their supramolecular behaviour with cucurbit[*n*]urils and cyclodextrins.

6.1. Synthesis of intermediates towards the linker

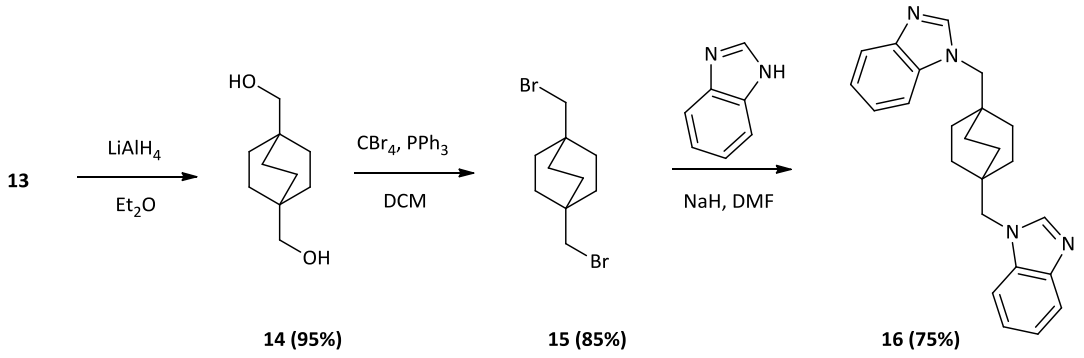
The synthesis of bicyclo[2.2.2] octane bisbenzimidazolium salts was started from the commercially available diethyl succinate, which was used for the preparation of compound **10** via Dieckmann condensation (*Scheme 9*).



Scheme 9: *Synthesis of bicyclo[2.2.2]octane cage.*

The next step in the sequence was preparation of compound **11** by alkylation/cyclisation.[65] The reaction was carried out in a dry DME with slow addition of 1,2-dibromoethane (DBE), which provided 92 % of yield. The principal drawbacks of this sequence included the long reaction time (3–5 days), which was necessary for the complete formation of compound **11**. In order to remove the oxo groups from the cage compound **12** was synthesised in a yield of 90 % using 1,3-propanedithiol and BF_3, which was used as an acid catalyst.[65] The excess of dithiol was removed by washing with 0.5 % aqueous NaOH. Afterwards, reduction of compound **12** was accomplished by desulfurisation in a yield of 90 % using Raney nickel (Ra-Ni). The long reaction time, which was necessary for the formation of compound **13** and a very large amount of Ra-Ni, were drawbacks of this step. However, this reaction provides required

bicyclo[2.2.2]octane skeleton with only bridgehead functionalisation (positions 1 and 4). Moreover, lithium aluminum hydride was used for the reduction[65] of compound **13** in a dry Et_2O providing compound **14** in a high yield of 95 %. Afterwards, we converted the diol **14** to **15** via Appel reaction[66] in the presence of CBr_4 and PPh_3 in a dry DCM, which resulted yield of 85 %. The obtained product was separated from the undesired side products, namely $Ph_3P{=}O$, by column chromatography in chloroform. The compound **15** was used for the preparation of the key intermediate **16**, which was employed for preparation of bicyclo[2.2.2]octane bisbenzimidazolium salts. Preparation of the key intermediate **16** was performed in a dry DMF with slowly portionwise addition of base NaH and benzimidazole (*Scheme 10*). The reaction was carried out at 80 °C and product was isolated by precipitation with cold H_2O. The obtained product was purified on column using CH_3OH:$CHCl_3$ (1:5, v:v) as a mobile phase. After ^1H NMR and ESI-MS confirmation the structure of the key intermediate **16,** it was used for the further quaternisation steps to produce compounds **17**, **18**, and **19** (*Scheme 11 and 12*).



Scheme 10: *Synthesis of intermediates and a key intermediate **16**.*

6.2. Synthesis of bicyclo[2.2.2] octane based bis(benz)imidazolium salts

Quaternisation reactions towards **17** and **18** were performed in a neat methyliodide at 30 °C and 1-iodobutane at 110 °C, respectively. The excess of CH_3I or BuI was removed by stream of argon. Traces of residual reagents were taken out under vacuum. The residues in the flask were precipitated with a dry distilled Et_2O and products were dried under vacuum until constant mass. The characterisation of compounds **17** and **18** included NMR, IR, and ESI-MS spectral patterns. The sharp singlet of the —CH_3 group, which appears at 4.08 ppm, was a useful diagnostic for compound **17**, as can be seen in *Figure 10*. In addition, multiplets of the benzimidazole core H-atoms appeared in the range 7.68−8.07 ppm and singlet related to the C(2)—H at 9.60 ppm**.**

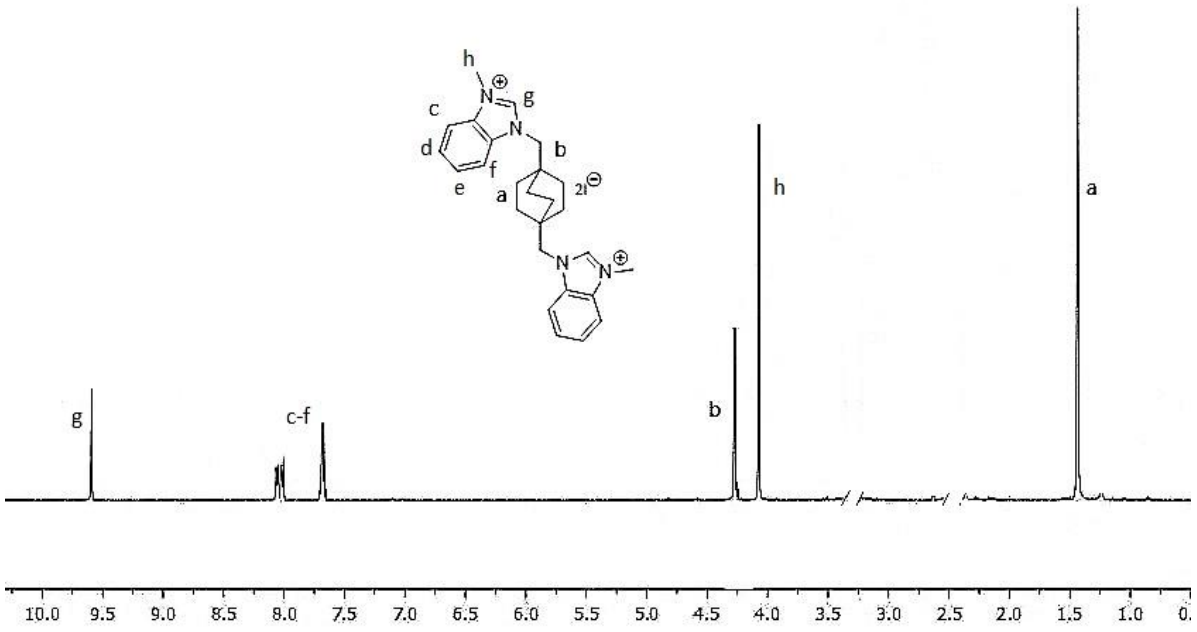


*Fig. 10: ^{1}H NMR spectrum 1,4-bis(3-methylimidazoliomethyl)bicycle[2.2.2]octane diiodide (**17**)*

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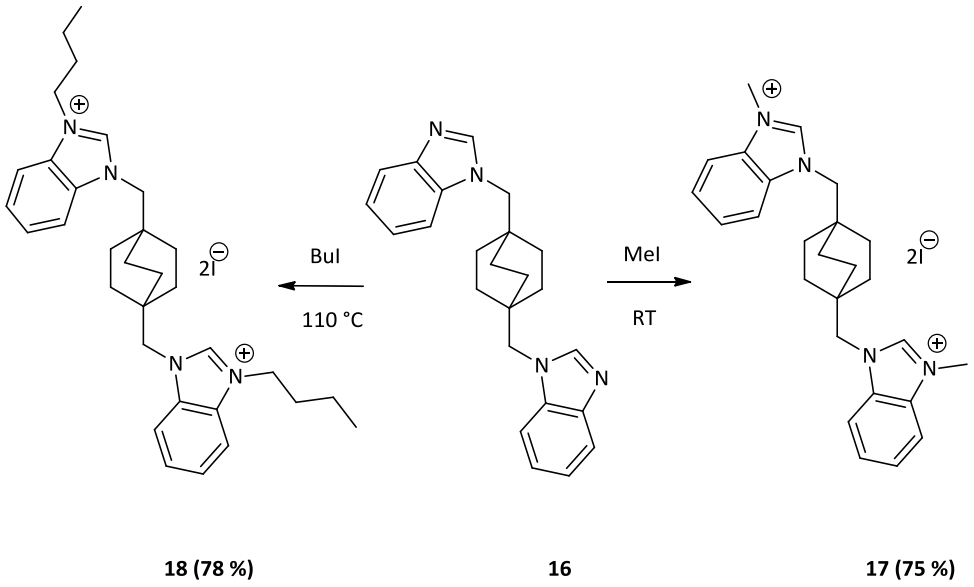


Scheme 11: *Synthesis of bisbenzimidazolium salts **17** and **18**.*

Signals such as multiplet of the benzimidazole core H-atoms observed in the range 7.67–8.07, singlet C2—H atom of benzimidazolium ring at 9.69 ppm and signals of alkyl chain at 4.50, 1.89, 1.33, and 0.91 ppm are characteristic for compound **18** (*Figure 11*).

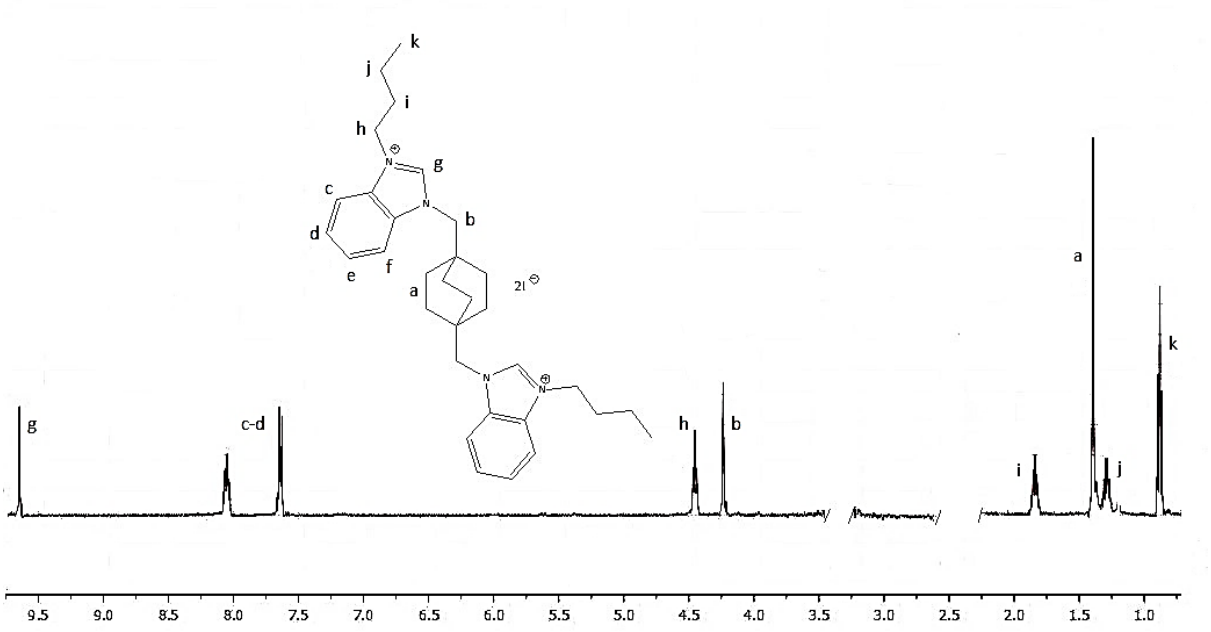
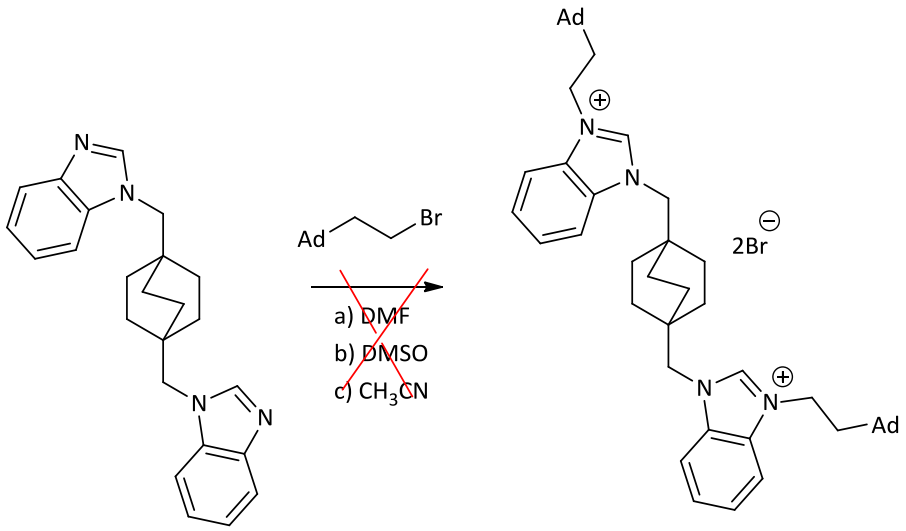


*Fig. 11: ^{1}H NMR spectrum 1,4-bis(3-butylbenzimidazoliomethyl)bicyclo[2.2.2]octane diiodide (**18**).*

Finally, we attempted to prepare the guest **19**. We performed the quaternisation reaction in a dry DMF at 110 °C with slowly addition of 2-(1-adamantyl)-1-bromoethane (2 eq). Since the chosen conditions did not shift reaction to the desired product, the amount of alkylating agent was gradually increased up to (8 eq) to support complete conversion of the intermediate **16**. The progress of reaction was monitored by TLC and precipitated with a dry distilled Et_2O in order to isolate the bisbenzimidazolium salt **19**. We managed to isolate crude product from the reaction mixture as a colourless powder, but recorded ^{1}H NMR spectrum did not correspond to the desired product **19**.

Moreover, quaternisation of compound **16** with 2-(1-adamantyl)-1-bromoethane was carried out in other polar aprotic solvents, such as DMSO and CH_3CN, but results of these reactions were essentially the same. Unfortunately, the isolated powder was probably inorganic solid, since the NMR spectrum contained a lot of unknown impurities with absence of adamantane signals. The possible reason of this failure can be attributed to the steric hindrance in the expected products or the lower reactivity of alkylating agent.



16 **19**

Scheme 12: *1,4-bis(3-(2-adamantyl)ethylbenzimidazoliomethyl)bicyclo[2.2.2]octane dibromide (**19**).*

7. BIOPOLYMERS MODIFIED BY β-CD UNITS

Biopolymers are defined as polymers that are produced under natural conditions, from natural sources or by chemical synthesis from biological materials. Biopharmaceutical properties, such as biocompatibility, biodegradability, and absence of immunotoxicity and cytotoxicity, make biopolymers ideal candidates for use in drug delivery as drug carriers,[67] to prolong the effects of a drug,[68] or for use as bioresorbable scaffolds in the tissue engineering.[69] Natural polymers can be chemically modified to introduce various functional groups in the polymer chain, to improve the properties of the polymer, and to enable new functions. Studies on the chemical modifications of biopolymers have been mainly concerned with cross-linking and grafting. In these cases, covalent bonds are used for the cross-linking of the polymer chains, and therefore, any reversible modification of the polymer properties is disabled. In contrast, when the supramolecular host-guest approach is employed, the degree of cross-linkage can be controlled due to the reversible nature of supramolecular interactions.[70]

Cyclodextrins (CDs), natural macrocyclic oligosaccharides with 6–8 D-glucose units linked by β-1,4 glycosidic bond, are feasible hosts for diverse guest molecules in aqueous medium driving by hydrophobic and van der Waals interactions into the non-polar cavity. According to biopharmaceutical properties of biopolymers and the molecular recognition ability of β-CD linked to polymer chain, cyclodextrins could generate interesting assemblies with a high biocompatibility and promising applications for the targeted and/or sustained delivery of drugs.[71] The grafting of CDs to HA has been accomplished by covalent approaches including conjugation of amine-modified CDs to carboxylic group of HA,[72,73] interaction of boronic acid grafted on HA with hydroxyl groups of CD[74] or by supramolecular assembly.[75,76] The conjugation of HA to macrocyclic molecules has been the most exploited method in supramolecular chemistry, for instance the anchoring of CD to HA via triazole linkage.[77] Thus, the cyclodextrin units were grafted onto carboxylate functional groups, which, however, are believed to play an important role in the recognition of HA by cells[78,79] In this part of the work present research the aim was to examine whether the natural hyaluronan, which was conveniently modified with β-cyclodextrin units via the primary hydroxyl groups of HA, can form supramolecular networks with adamantane based guest motifs in the reversible manner.

Moreover, chitosan is also well known as a promising biomaterial and has been extensively studied as a carrier matrix candidate for a drug delivery. The primary amino groups on chitosan provide sites for a variety of side groups attachment under mild reaction conditions, which makes chitosan an ideal

material for biofabrication.[80] Introduction of diverse functional groups into chitosan allows not only preparation of hydrogels based on chitosan via interaction of these groups, but also immobilisation of a drug on the polymeric matrix.[81] Therefore, we described the synthesis of modified biopolymers with β-CD units, which, are linked to the polymer chain via the primary hydroxy groups of HA or primary amino groups of CS. Subsequently, we examined the supramolecular behaviour of modified biopolymers with one-, two-, and three-site adamantane-based guest motifs to describe their ability to form supramolecular networks, which can be driven by competing signal molecules.

7.1. Chemistry

Initially, mono-6-(*p*-toluenesulphonyl)-β-cyclodextrin (**20**) was prepared from a natural β-CD, where selective monotosylation was performed in the C6–OH position (*Scheme 13*). The reaction was performed in alkaline aqueous solution according to a published procedure.[82] Covalent linkage between tosyl group and β-CD was indicated by DOSY spectrum. As it can be seen in *Figure 12*, signals in upper line are related to the 4-methylbenzene sulphonic acid, while signals in bottom line are related to the modified β-CD.

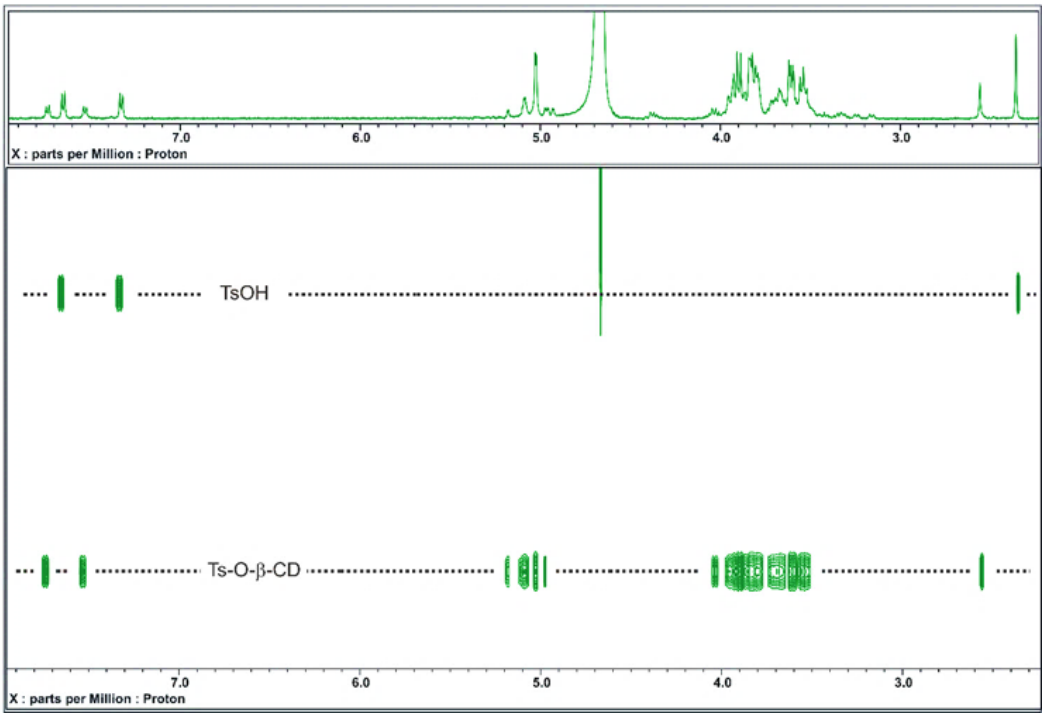
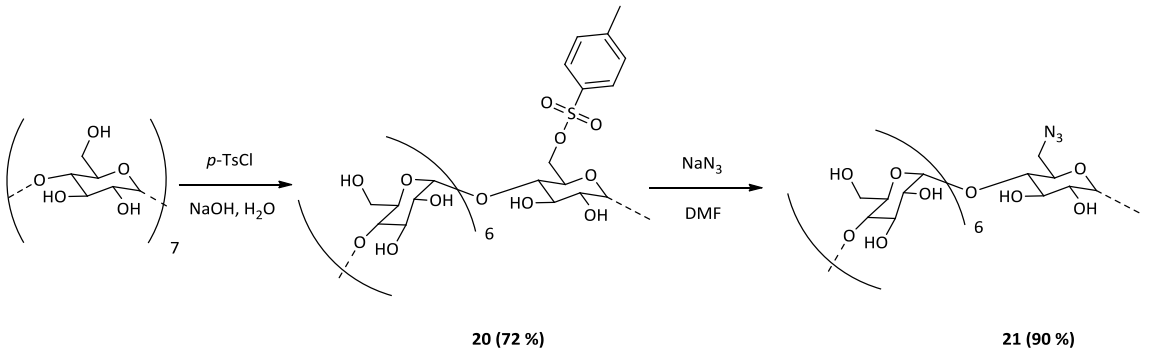


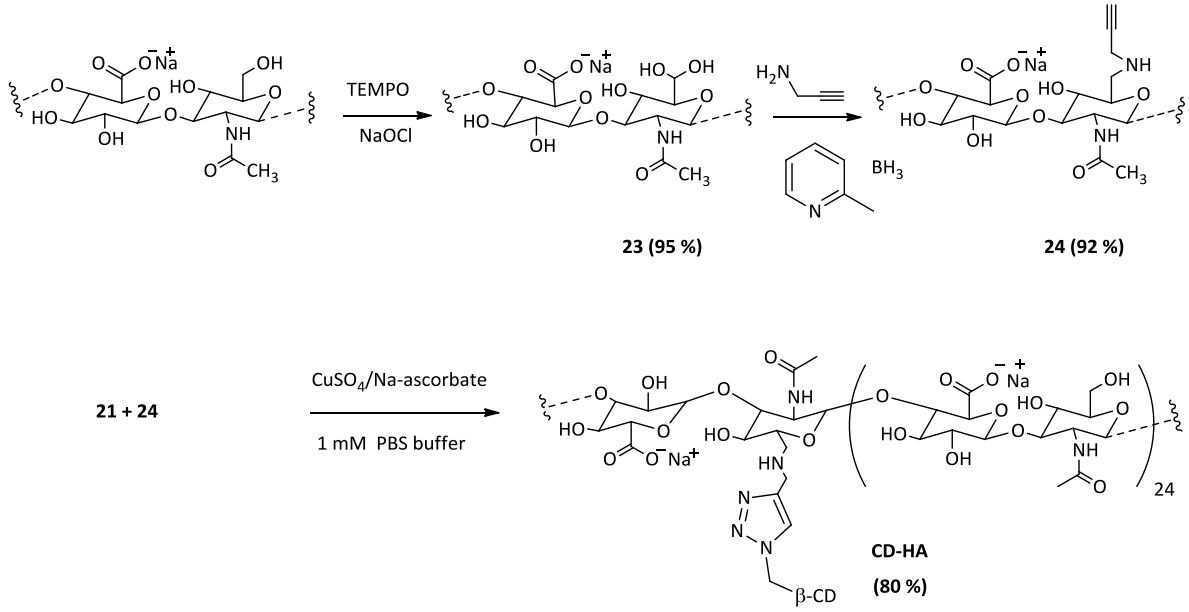
Fig. 12: *DOSY spectrum of the crude product of monotosylation of β-CD.*

Subsequently, mono-6-(*p*-toluenesulphonyl)-β-cyclodextrin was readily converted to monoazido-CD (**21**) using NaN_3 in a dry DMF, where the obtained crude product was recrystallised from water and precipitated by addition of acetone.[83]



Scheme 13: *Monosubstitution of CD intermediates at the primary hydroxy groups.*

The azido group located on the primary rim of the CD scaffold is a suitable anchoring point for the introduction of an alkyne into the structure. Thus, a new CD-HA polymer was synthesised that combine the chemical modification of the HA chain with terminal alkyne groups via the sequence of partial selective oxidation, reductive amination and final 1,3-dipolar cycloaddition between monoazido-β-CD and propargyl-modified HA (*Scheme 14*).



Scheme 14: *Synthesis of the CD-HA polymer via a click reaction.*

Initially, natural hyaluronan was oxidised with 4-acetamide-TEMPO[84] using sodium hypochlorite as a primary oxidant to produce aldehyde moieties (hydrated form is shown in *Scheme 14*). Oxidation occurred at the C6-OH position and the oxidised HA was grafted with primary amines carrying alkyne groups via imine intermediate. A new CD-HA polymer was prepared via the 1,3-dipolar cycloaddition between monoazido-CD and the propargyl derivative of HA. The coupling between monoazido-CD and the alkyne-terminated

derivative of HA was successfully performed in 1 mM PBS at ambient temperature (*Scheme 14*). The synthesis of aldehyde **22** (*Figure 13*) was based on two steps starting from the natural β-CD (*Scheme 15*) following a published procedure.[85] The reaction was performed using 4-hydroxybenzaldehyde with anhydrous K_2CO_3 in a dry DMF under an inert atmosphere. In order to remove side products such as *p*-TsOH, the compound **22** was isolated by crystallisation, which resulted in the formation of aldehyde **22** in a yield of 88 %.

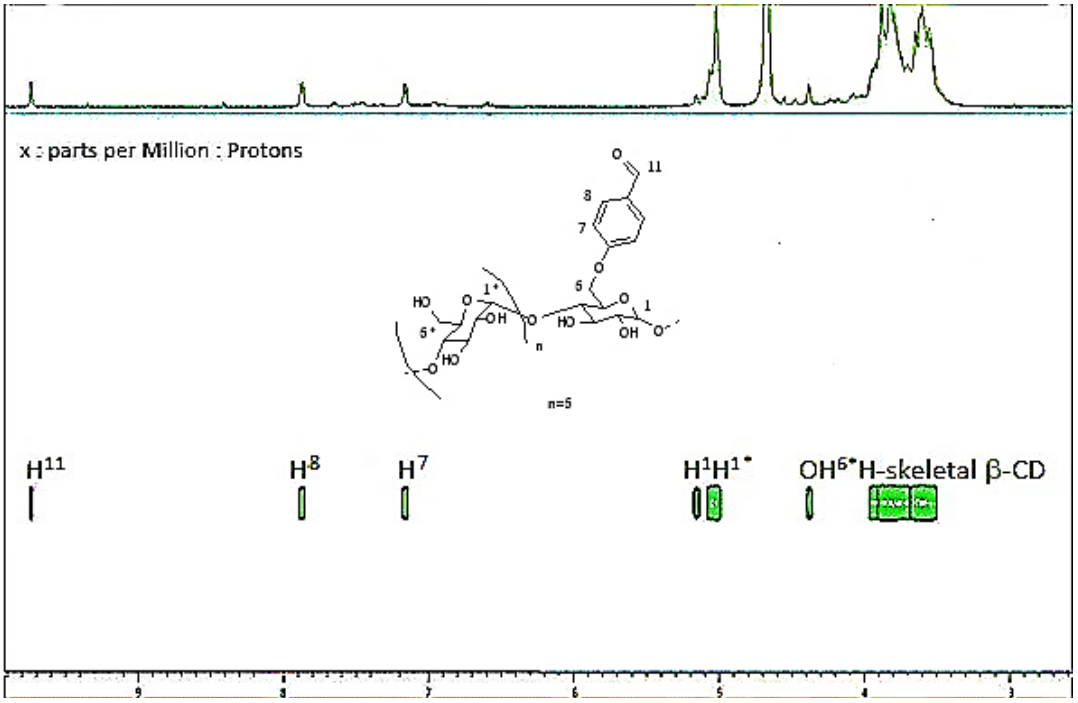
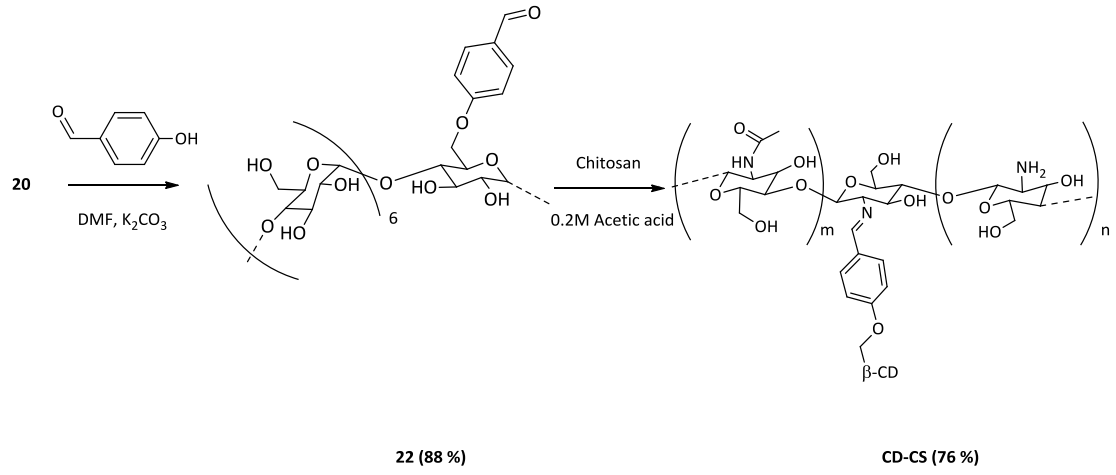


Fig. 13: DOSY spectrum of the pure product of 6-O-(4-formylphenyl)-β-CD.

Furthermore, the CD-CS polymer was synthesised via a Schiff base reaction (*Scheme 15*) between desired aldehyde **22** and commercially available chitosan (Sigma Aldrich) in the mixture of 0.2 M CH_3COOH and CH_3OH.[86]



Scheme 15: *Synthesis of the CD-CS polymer via a Schiff base reaction.*

7.2. Characterisation of biopolymers

7.2.1. Characterisation of modified CD-HA

The propargyl-modified HA (Prop-HA) was characterised by means of ^{1}H NMR. The ^{1}H NMR spectrum shows signals for the *N*-acetyl group at 2.02 ppm. As it is shown in *Figure 14*, the signals of H-atoms of the HA skeletal can be seen at 3.40–4.00 ppm. Other detected signals at 3.10 and 2.85 ppm can be assigned to the methylene protons at position C6.[84] These signals confirmed the successful formation of the covalent linkage between propargylamine and HA.

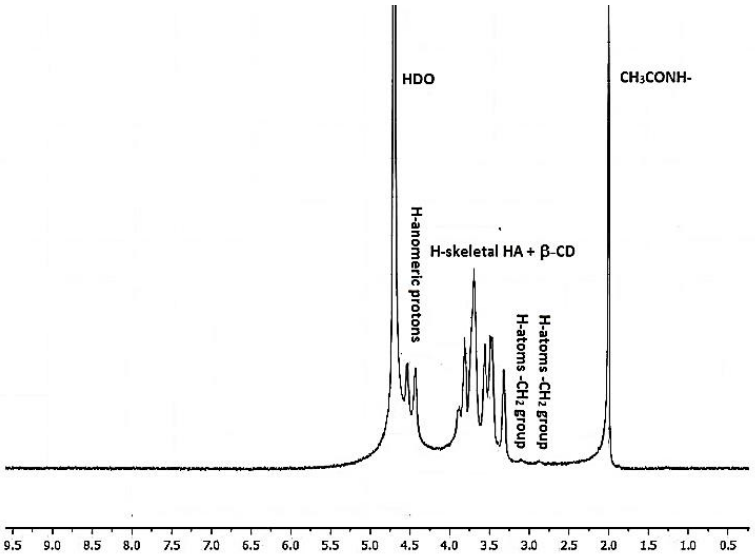


Fig. 14: *^{1}H NMR spectrum of propargylated hyaluronan (Prop-HA).*

The ^{1}H NMR spectrum of the new CD-HA polymer showed a peak at 5.06 ppm, which is attributed to the H1 (β-CD). The substitution degree of 4 % was calculated from the integration of the peak of the *N*-acetyl group at 2.00 ppm and the peak at 5.06 ppm, which is related to the anomeric proton (C1—H) of β-cyclodextrin (*Figure 15*).

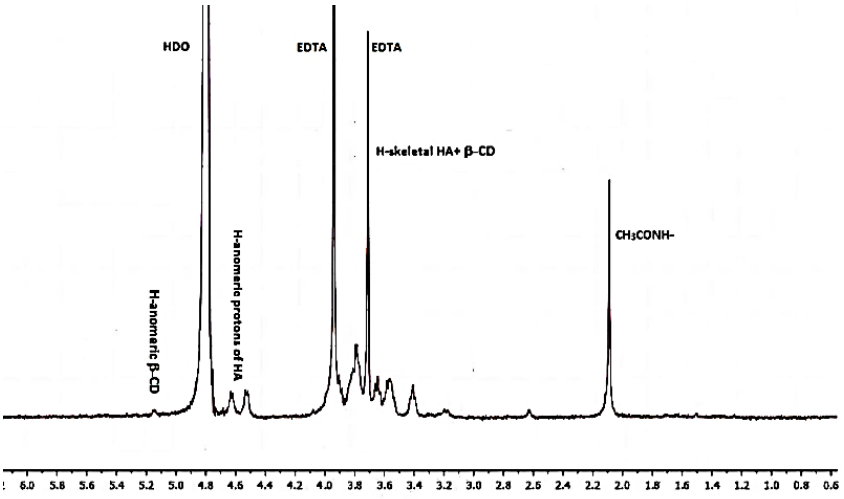


Fig.15: *^{1}H NMR spectrum of CD-HA.*

To verify whereas the β-CD unit is bound to the HA polymer chain, we performed DOSY experiment. It can be clearly seen in DOSY spectrum (*Figure 16*) that the signals of the anomeric H-atoms from the CD macrocycle, anomeric H-atoms from the HA chain, skeletal H-atoms, and —CH_3 groups from the acetamido substituent display the same diffusion coefficient. This observation implies that the CD units are covalently linked to the HA polymer.

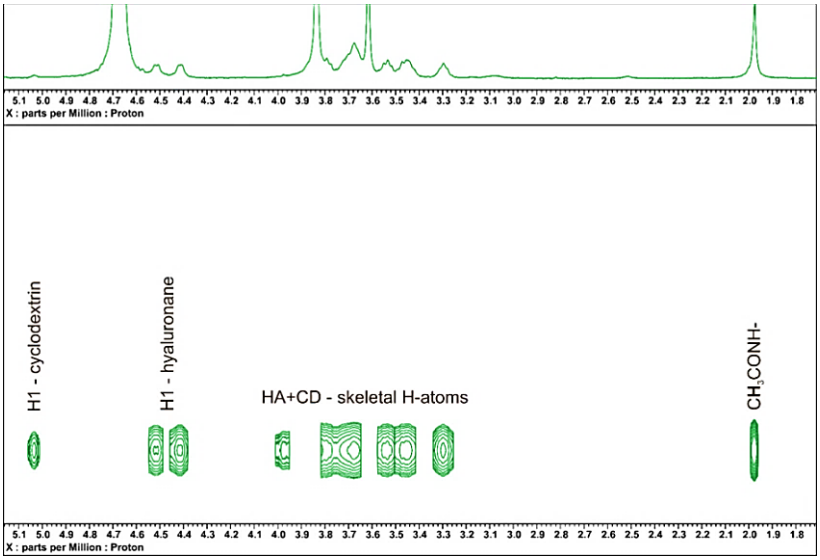


Fig. 16: *DOSY spectrum of CD-HA.*

Additionally, size exclusion chromatography (SEC) was used to determine whether the HA polymer chain was markedly cleaved during the chemical transformations. As can be seen in *Figure 17*, the peaks related to the original HA polymer, oxidised HA, propargylated HA, and CD-HA appeared at essentially the same retention time. Therefore, it can be inferred that no significant decomposition of the polymer backbone occurred.

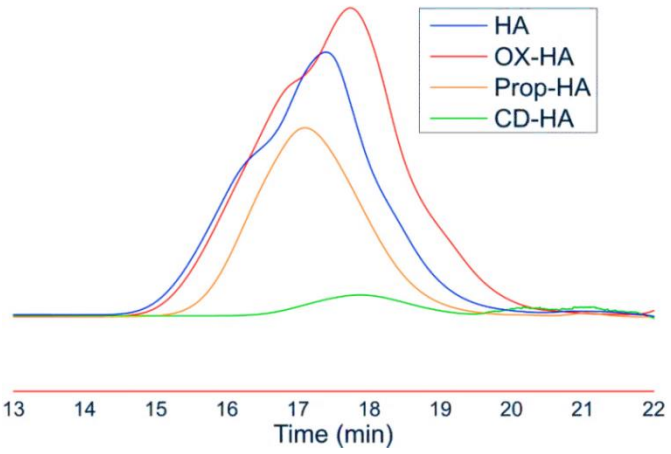


Fig. 17: *SEC results of original hyaluronan (HA), oxidised hyaluronan (OX-HA), propargylated hyaluronan (Prop-HA), and CD-HA.*

7.2.2. Characterisation of modified CD-CS

The degree of substitution (DS, %) of modified CD-CS polymer was determined from the ^{1}H NMR spectrum by comparing the integral intensities of the *N*-acetyl group at 2.03 ppm and the signals at 7.91 and 7.18 ppm, which are related to the H-atoms of phenyl group. Digital integration of the NMR signals arising from the *N*-acetyl group (CS) and H-atoms of phenyl group (6-*O*-(4-formylphenyl)-β-cyclodextrin) gave a substitution degree approximately 3 %. Whereas, the degree of deacetylation (66 %) of pure chitosan was calculated based on ^{1}H NMR spectrum using the following equation:

$$DD = 1 - \frac{\frac{1}{3} \cdot I_{CH_3}}{\frac{1}{6} \cdot I_{C_{2-6}}} \qquad (1)$$

(where ICH_3 is the integral intensity of —CH_3 group, whilst $Ic_{2–6}$ is the summation of integral intensities of C(2)H, C(3)H, C(4)H, C(5)H, and C(6)H_2).

The ^{1}H NMR spectrum in *Figure 18* depicts signals for *N*-acetyl group at 2.03 ppm and signals of skeletal H-atoms of CS, which are observed at 3.13 to 5.04 ppm. Other detected signals for phenyl groups are 7.18 and 7.91 ppm and the signal at 9.77 ppm can be attributed to the imine group. The presence of these signals in the spectrum suggests covalent linkage between chitosan and the β-CD units.

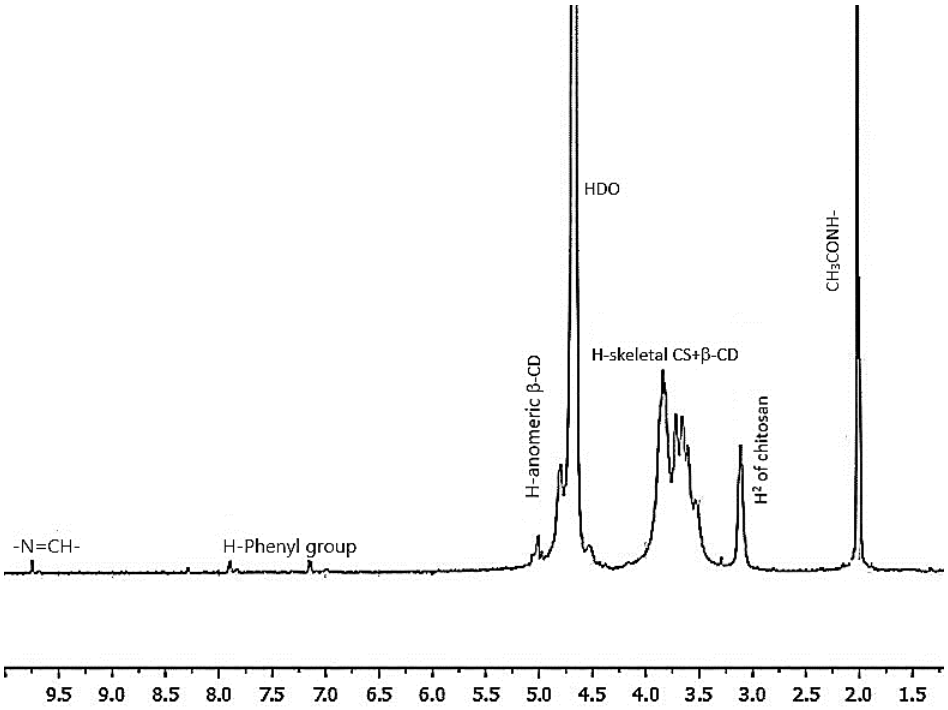


Fig. 18: *^{1}H NMR spectrum of chitosan modified by β-cyclodextrin units.*

The covalent linkage between the β-CD units and chitosan polymeric chain was strongly supported by DOSY spectrum. As can be seen in *Figure 19*, signals of phenyl group, CD-CS skeletal protons, and *N*-acetyl group display the same diffusion coefficient. This observation implies that the CD units are covalently linked to the CS polymer.

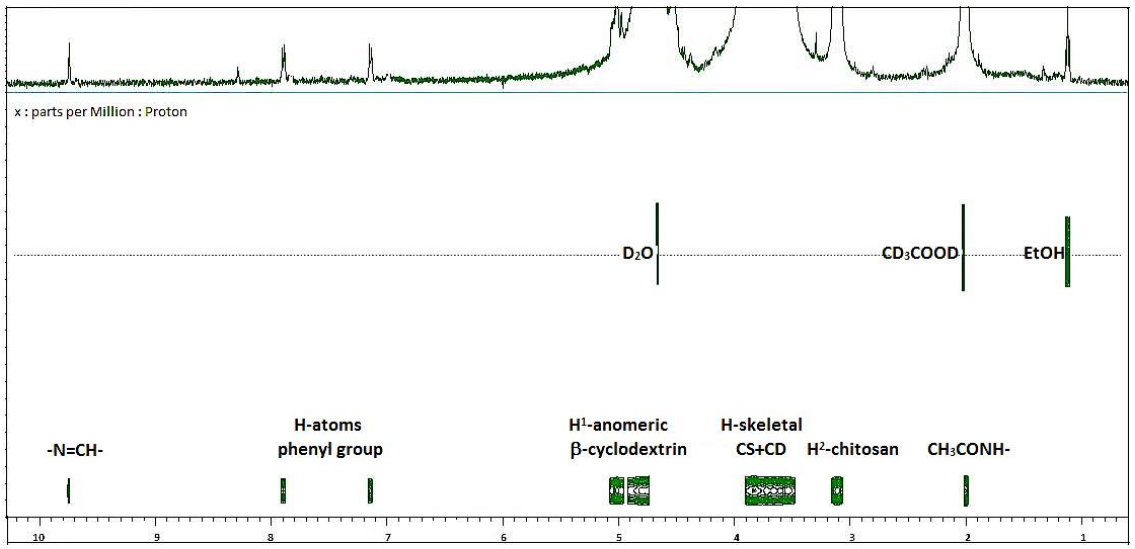


Fig. 19: *DOSY spectrum of chitosan modified by β-cyclodextrin units.*

Furthermore, size exclusion chromatography was used to determine whether the CS polymer chain was cleaved during the chemical modification. As it is shown in *Figure 20*, the peaks related to the original CS polymer and CD-CS appeared at essentially the same retention time. Thus, it can be inferred that no significant decomposition of the polymer backbone occurred.

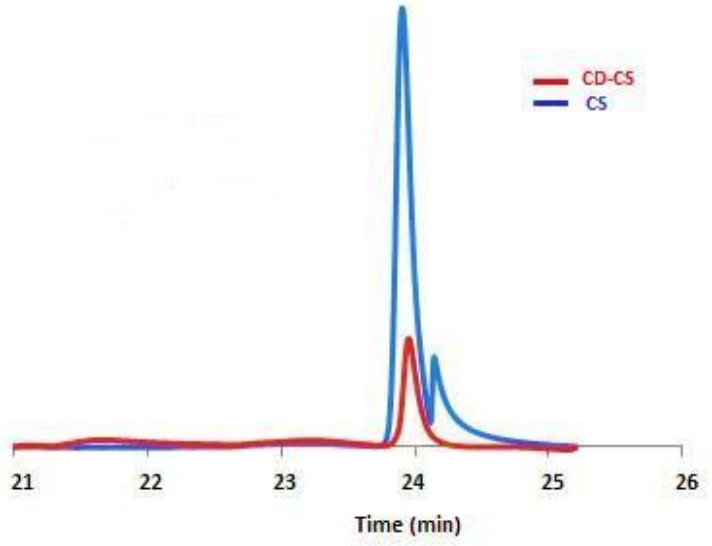


Fig. 20: *SEC results of a pure chitosan (CS) and modified chitosan (CD-CS) by β-CD units.*

7.3. Supramolecular studies on modified biopolymers

7.3.1. Supramolecular studies on CD-HA

Successively, we examined supramolecular behaviour of the new CD-HA polymer with single-site guests such as G1 and G2 (for structure, see *Figure 21*) in terms of titration calorimetry. The obtained ITC results inferred that the new CD-HA polymer presents a promising component for construction of chemical stimuli-responsive architectures (for instance, scaffolds). The ITC results are summarised in *Table 4*. Both used guests with a single-binding site (i.e., G1 and G2) form a 1:1 complex with a natural β-CD with a sufficiently high binding constant.[87] For instance, guest G1 forms a 1:1 complex with a natural β-CD with the exceptionally high binding constant, which exceed the order of 10^6 in its magnitude (these results are from our research group and they are not a part of this Thesis and results have not been published yet). The high binding strength allows for unambiguous analysis of titration data, namely stoichiometric parameter *n*, even at millimolar concentrations of supramolecular components. Therefore, we performed the ITC titration of the CD-HA polymer with G1 and G2 and calculated the actual concentration of available CD units ($C_{\beta\text{-}CD}=5.82\times10^{-5}$ mol dm^{-3}) using the above mentioned parameter *n* from ITC spectrum, while the degree of substitution was previously determined by integrating the ^{1}H NMR spectrum.

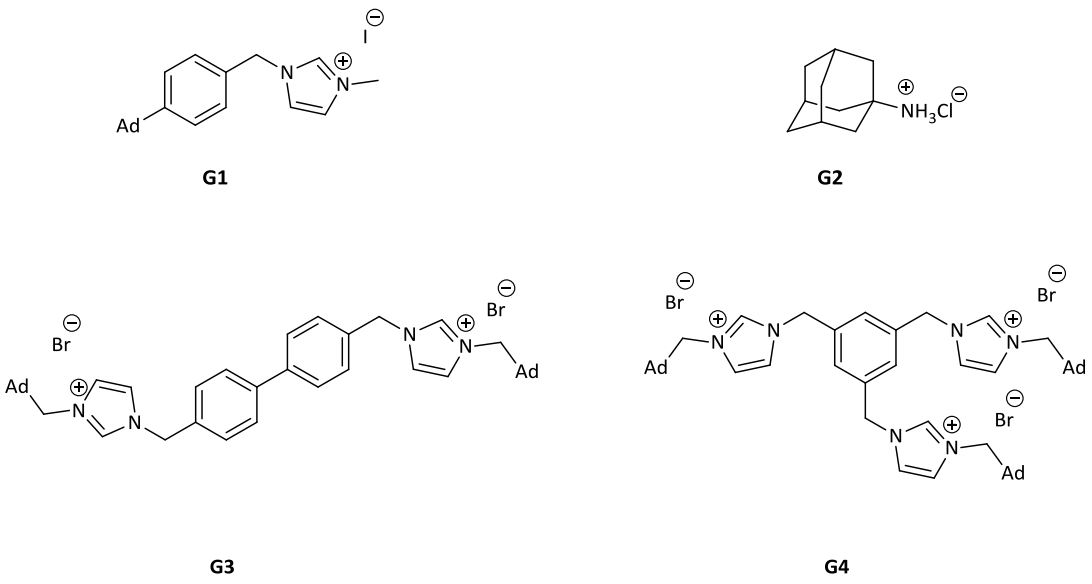


Fig. 21: *The adamantane-based supramolecular guest motifs.*

The calculated concentration value was used as the input concentration of CDs for the data processing of further titrations of CD-HA with G3 and G4. *Figure 22* clearly shows that relative to guest G1, an equivalency point was reached after the addition of one-half and one-third of the molar quantities of G3 and G4,

respectively. For instance, ditopic guest G3 employs both its binding sites to saturate the CD units of CD-HA to form a supramolecular network as it is depicted in *Figure 22.*

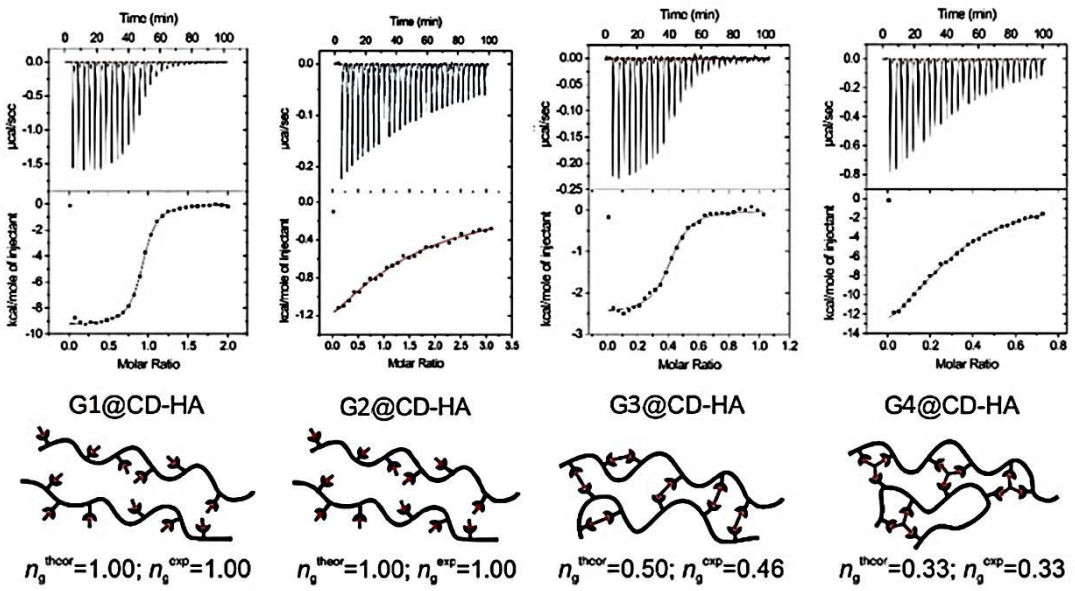


Fig. 22: ITC results of titrations of CD-HA with guests G1, G2, G3, and G4.

Table 4: *Thermodynamic parameters for guests G1–G4.*

Guest	Host	n	K_a[dm^{3}mol^{-1}]	ΔH[kJmol^{-1}]	ΔS[Jmol^{-1}K^{-1}]
G1					
	CDHA	0.99	2.08×10^{6}	−80.77	−7.33
G2	β-CD	0.96	7.05×10^{3}	−24.5	−7.0
	[c]CB[7]	ng	1.7×10^{14}	−80.6	0
	CDHA	1	1.12×10^{4}	−13.9	32.0
G3	[a]β-CD	0.49	9.20×10^{4}	−59.0	−99.0
	[a]CB[7]	0.56	1.35×10^{12}	ng	ng
	CDHA	0.43	1.14×10^{6}	−11.4	79.0
G4	[b]β-CD	0.37	1.02×10^{5}	−85.7	−187.0
	[b]CB[7]	0.39	4.84×10^{10}	−173.8	−369.0
	CDHA	0.31	1.00×10^{5}	−87.6	−193.0

[c b,a] K_a values are reported for guests G2,[88] G3[89] and G4;[90 d] K values for will be published elsewhere. ITC titrations were done in triplicate.

NMR titration of CD-HA with guests G1 and G2 (*Figures 23 and 24*) was carried out in D_2O solution at 30 °C. All signals of hydrogen atoms, which can be assigned to the adamantane cage, are significantly shifted downfield. This phenomenon is well-recognised and attributed to the deshielding effect of cyclodextrin interior cavity.[91]

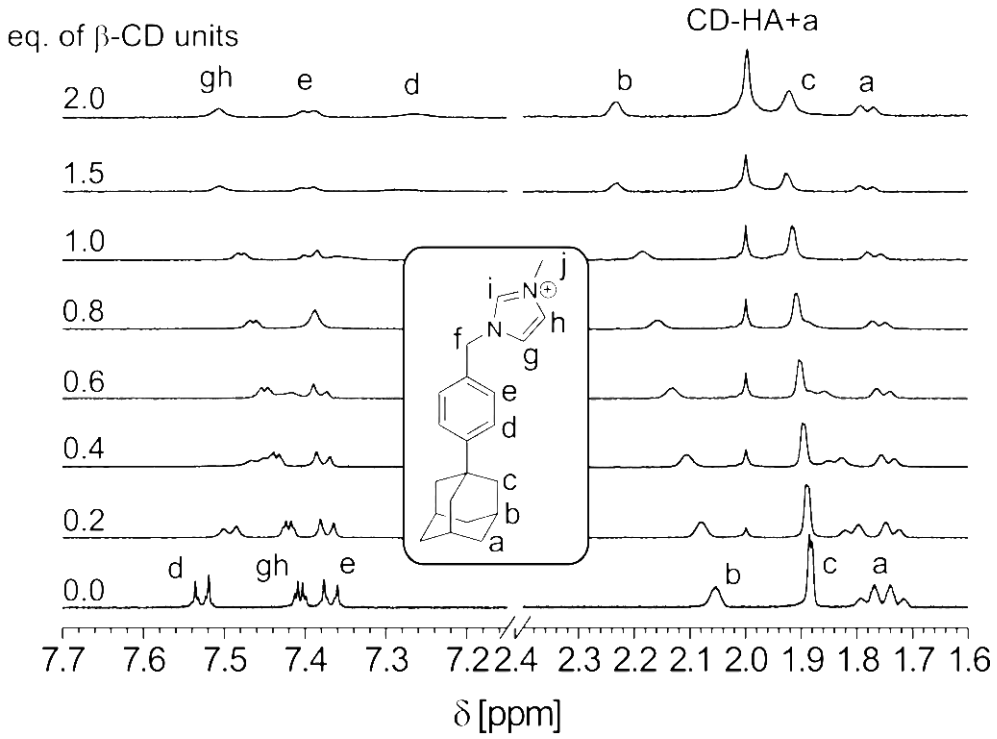


Fig. 23: *Stacking plot of [1]H NMR spectra recorded within titration of G1 with CD-HA.*

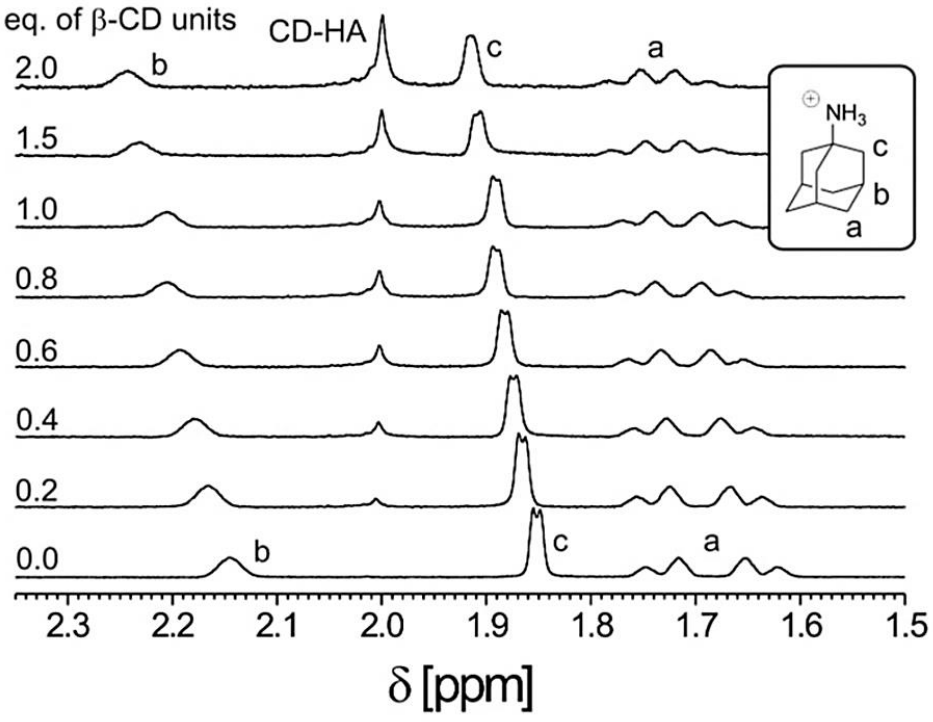


Fig. 24: *Stacking plot of [1]H NMR spectra recorded within titration of G2 with CD-HA.*

Similarly results were obtained within ^{1}H NMR titration of G3 and G4, respectively (*Figures 25 and 26*). Therefore, we can conclude that adamantane cage is included inside the CD cavity in all examined cases and the nature of the

interaction which was detected by ITC can be attributed to the formation of the supramolecular complex in a host-guest manner.

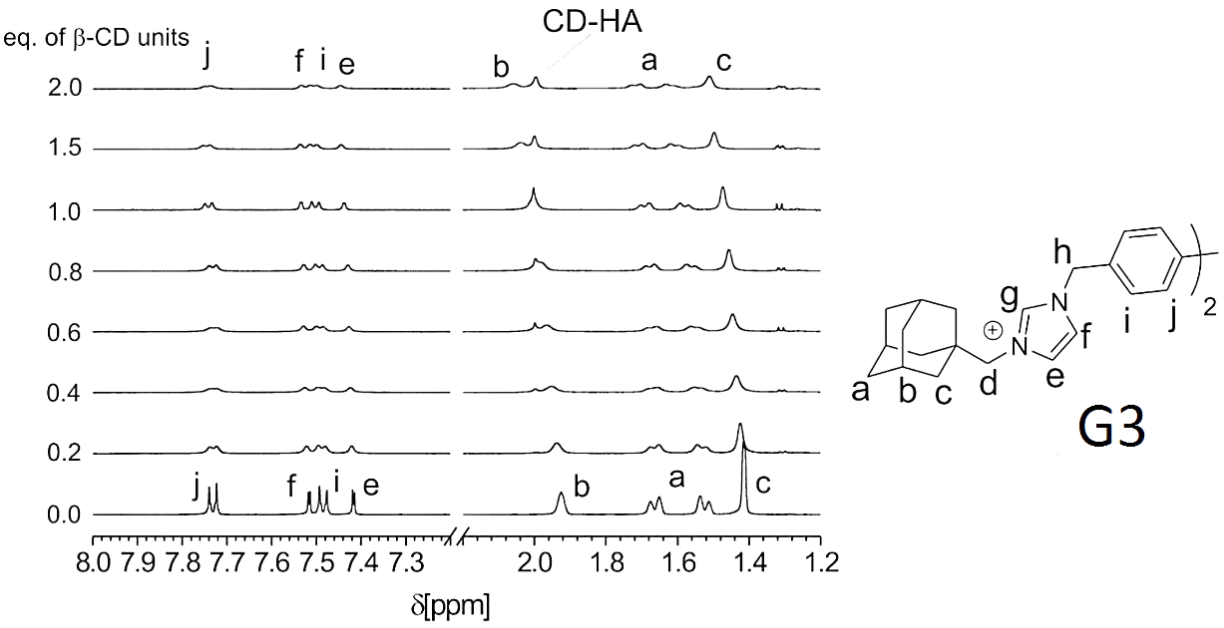


Fig. 25: *Stacking plot of the ^{1}H NMR spectra recorded w of G3 with CD-HA.*

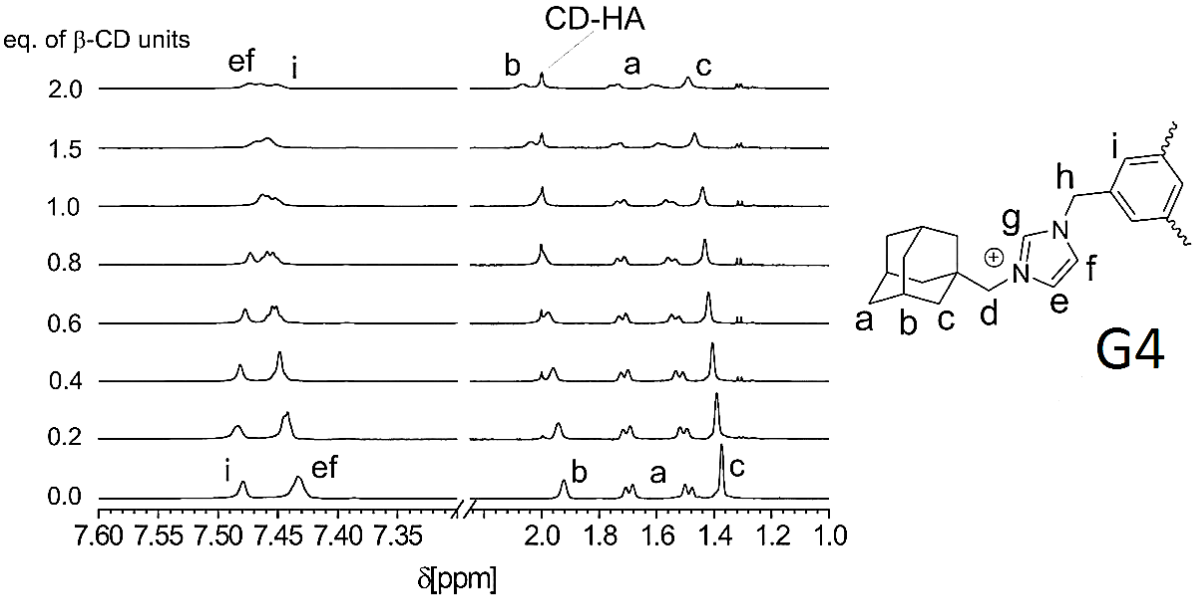


Fig. 26: Stacking plot of the ^{1}H NMR spectra recorded of G4 with CD-HA.

Concurrently, we performed a competitive experiment (*Figure 27*) to divulge whether the cross-linking agent G3 and/or G4 can be withdrawn from the CD-HA complex. We added an equimolar quantity of CD-HA to the solution of the guest G3 to form a supramolecular network, as it is described below (line

i and ii in *Figure 27*). Consequently, two molar equivalents of cucurbit[7]uril, with respect to G3 were added in two portions (line iii and iv in *Figure 27*). Considering that cucurbit[7]urils are well-known as strong binding agents for cationic derivatives of cage hydrocarbons,[92,93] we expected that complex of G3@CB[7] should be favored in the mixture.

The new set of G3 signals was observed, when we added one portion of CB[7]. We concluded that the complex G3@CB[7] was formed, because the new signals correlated to the adamantane cage were significantly shifted upfield (marked with ‡ in *Figure 27*). The remaining signals of the adamantane cage were markedly shifted downfield (asterisked in *Figure 27* line iii). This can be explained by a change of environment as the supramolecular network was partially cleaved. After the second addition of CB[7], the signals related to the adamantane cage complexed inside the β-CD units completely disappeared. It should be noted that the complex G3@CB[7] was sparingly soluble in the system and colourless precipitate was formed. Since we obtained similar results with the guest G4, we infer that our new hyaluronane derivate is capable to forming supramolecular networks which can be modulated by competitive approach.

Since the formation of the supramolecular network was assumed, we tried to support this hypothesis with rheological measurements. Because, we did not have sufficiently amount of polymer sample we decided to determine the D parameter of polymer during the titration using DOSY NMR approach. Thus, we performed conventional titration experiment and we determined the D coefficient after each addition of the guest G3 or G4. Additionally, we performed also blank titration, where only pure solvent was added to eliminate the effect of dilution. Unfortunately, we did not observe any significant changes in diffusion rates within titrations. This can be attributed to the low concentrations of components of our systems, relatively weak binding strength, low degree of substitution, and dynamic nature of the host-guest interactions.

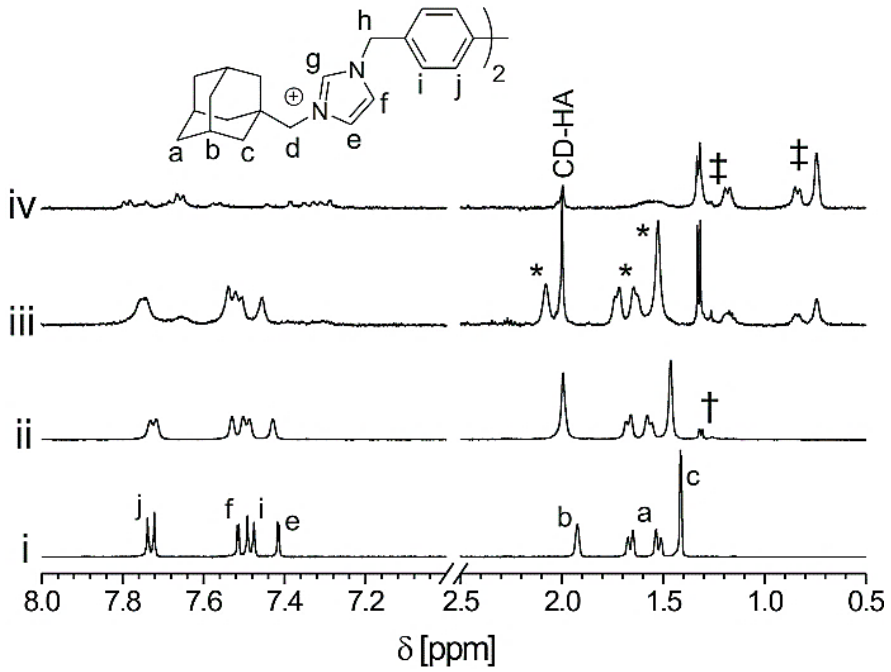


Fig. 27: *Stacking plot of portions of the ^{1}H NMR spectra recorded within the competitive experiment in D_2O at 30 °C. Free guest G3 (**i**); G3 and 1eq. of CD-HA (**ii**); G3, 1 eq. of CD-HA, and 1 eq. of CB[7] (**iii**); G3, 1 eq. of CD-HA, and 2 eq. of CB[7] (**iv**). The signal of the residual propan-2-ol is marked with †. The other symbols are explained in the text.*

7.3.2. Supramolecular studies of CD-CS

We further studied the interactions between modified chitosan CD-CS and multitopic adamantane-based guest motifs by isothermal titration calorimetry, while ^{1}H NMR titration was not performed due to the poor reproducibility of the CD-CS preparation and lack of the CD-CS polymer. Similar studies were done as in the case of CD-HA, we examined the supramolecular properties of modified CD-CS polymer with one-, two-, and three-site adamantane-based guest motifs, respectively. The isothermal titration experiment was carried out in 20 mM CH_3COONa solution (pH=2.61) at 30 °C. We started our examination with the single-site guest G1 and determined the actual concentration of CD units ($C_{\beta\text{-CD}}=5.176\times10^{-5}$ mol dm^{-3}) taking into consideration the parameter *n* from ITC and degree of substitution, which was assessed by integration of ^{1}H NMR spectrum (*Figure 18*). The calculated concentration was used as an input concentration of CDs for further titrations of CD-CS polymer with guests G3 and G4. As it is depicted in *Figure 28*, relatively to the guest G1, an equivalency point was reached by addition of one half and one third of molar quantity of G3 and G4, respectively. Thermodynamic parameters obtained from ITC experiments are summarised in *Table 5*.

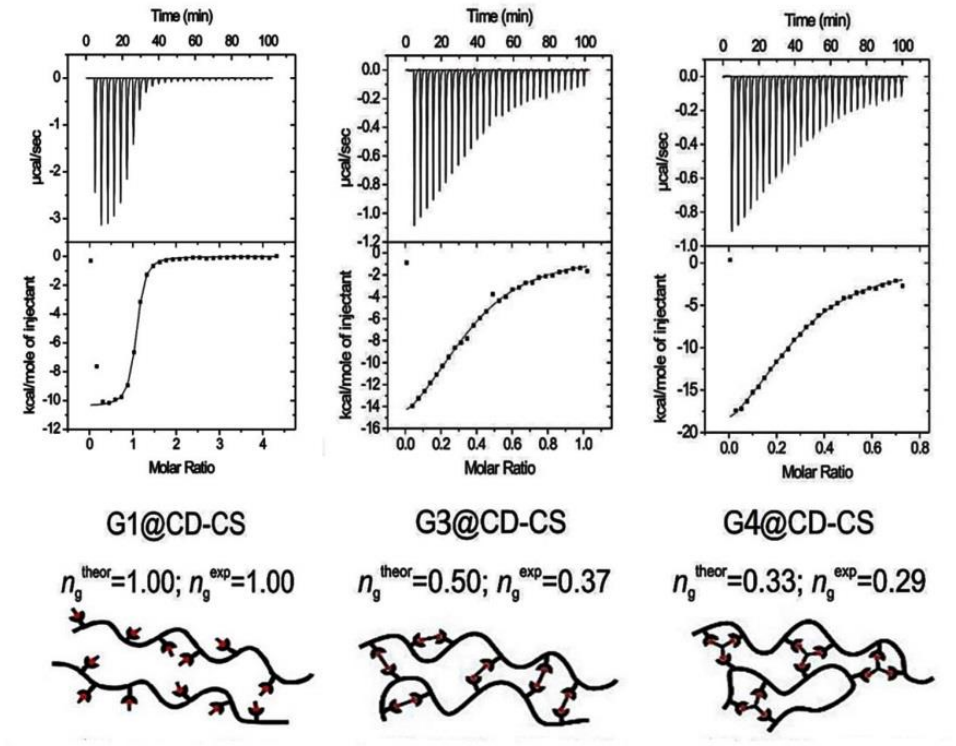


Fig. 28: ITC results of titrations of CD-CS with guests G1, G3, and G4.

Table 5: *Thermodynamic parameters for the guests G1, G3, and G4.*

Guest	Host	n	K_a[dm^3 mol^{-1}]	ΔH[kJ mol^{-1}]	ΔS[$Jmol^{-1}$ K^{-1}]
G1	CD-CS	1.00	2.03×10^6	−44.09	−24.58
G3	CD-CS	0.37	1.11×10^5	−69.5	−133.15
G4	CD-CS	0.29	1.75×10^5	−106.35	−250.8

*ITC titrations were performed in triplicate.

8. CONCLUSION

During the last decades, attention has been paid to develop and investigate various novel supramolecular systems. Developing of novel supramolecular assemblies presents very interesting trend for their biomedical applications in future. Supramolecular assemblies are ideal candidates for generating dynamic materials, because their building blocks are not covalently permanent and supramolecular forces that hold them together can be rearranged to change their properties.

Thesis is basically focused on supramolecular studies of intermolecular interactions between multitopic binding guest motifs and host molecules (specifically, cyclodextrins and/or cucurbit[*n*]urils). As it is known, guests that combine a non-polar core with cationic substituents can be employed as ultrahigh affinity guests towards macrocyclic hosts. For instance, cucurbit[*n*]urils can comprise cationic guest inside their hydrophobic cavity with very high binding constants in aqueous medium. Therefore, we synthesised series of guests comprising a non-polar adamantyl and/or bicyclo[2.2.2]octane core decorated with one and two cationic substituent, respectively. Subsequently, we described the complexation ability of prepared cationic derivatives towards macrocyclic hosts by isothermal titration calorimetry. According to the obtained results from ITC measurement, we determined that synthesised adamantylated (benz)imidazolium salts form 1:1 complexes with macrocyclic molecules such as β-CD, CB[7[, and CB[8]. In addition, we expected that prolongation of the linker between non-polar core (adamantane cage) and cationic moiety would reduce the contribution of the ion-dipole interaction and stability of the complex with CB[*n*]s. In contrast, obtained results inferred that some ligands with longer linker bind the CB[*n*] stronger than ligands with methylene linker. This phenomenon can be attributed to the better accommodation of the more flexible guest to optimise ion-dipole contributions.

The second part of Thesis is based on examination the supramolecular behaviour of modified biopolymers (precisely, hyaluronane and chitosan) with adamantane-based guests to describe their ability to form supramolecular networks. The new modified hyaluronan CD-HA polymer was prepared via a 1,3-dipolar cycloaddition between propargylated HA and monoazido-β-CD to reach a degree of substitution of 4 %, whose complexation ability was studied by isothermal titration calorimetry and ^{1}H NMR titrations. These performed experiments revealed that β-CD units on the CD-HA polymer form 1:1, 1:2, and 1:3 supramolecular aggregates in a host-guest manner with one-, two-, and three-binding sites adamantane-based guest motifs. Moreover, we demonstrated that the supramolecular network can be readily cleaved to restore the original

CD-HA by the addition of a suitable competitor, namely CB[7], for the multitopic guests. These results confirmed our assumption that the new modified CD-HA polymer is a promising component for the construction of chemical stimuli-responsive supramolecular architectures.

Simultaneously, modified chitosan CD-CS has been prepared via a Schiff base reaction between 6-*O*-(4-formylphenyl)-β-cyclodextrins and chitosan to reach a degree of substitution of 3 %. The complexation ability was analysed by isothermal calorimetry with adamantane-based guest motifs. This study revealed that β-CD units on the CD-CS polymer form 1:1, 1:2, and 1:3 supramolecular aggregates in a host-guest manner with adamantane-based guest motifs. Consequently, of a poor reproducibility of the CD-CS preparation ^{1}H NMR titration has not been performed unlike for the CD-HA polymer. Considering their biopharmaceutical properties, biopolymers are excellent candidates for drug delivery or tissue engineering providing insights and spurring new developments in supramolecular chemistry.

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Krystina Jelínkova, Jelica Kovačević, Eva Babjaková, Zdeňka Prucková, Michal Rouchal, Lenka Dastychová, Robert Vícha: Binding study on 1-adamantylalkylimidazolium salts towards cyclodextrins and cucurbit[*n*]urils, *New Journal of Chemistry*, 2020 (submitted).

CONFERENCE

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