Supramolecular Chemistry with Cyclodextrin Containing Polymers: Click Chemistry, Structures and Properties

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Die hier vorgelegte Dissertation habe ich eigenständig und ohne unerlaubte Hilfe angefertigt. Die Dissertation wurde in der vorgelegten oder in ähnlicher Form noch bei keiner anderen Institution eingereicht. Ich habe bisher keine erfolglosen Promotionsversuche unternommen.

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(Maricica Munteanu)

Es ist nicht wichtig woher du kommst, sondern wohin du gehst.
Autor unbekannt

TABLE OF CONTENTS

Acknowledgements

Abstract

Polymerizable cyclodextrin methacrylates were synthesized via the "click reaction" of mono-azido β-CD with propargyl derivatives. The microwave-assisted 1,3-dipolar cycloaddition assured the conversion into monomethacrylated cyclodextrin in excellent yields.Water-soluble oligomers were obtained via radical homopolymerization of methacrylated cyclodextrin.

Guest-copolymers were synthesized via radical copolymerisation of cyclodextrin methacrylate with N-isopropylacrylamide (NIPAAM) and methyl methacrylate (MMA), respectively. A CD-containing polymer was also obtained by the polymer analogous coupling of mono-azido β-CD onto poly(propargyl methacrylate). Supramolecular polymers were obtained by inclusion complexation with adamantane-derivatives and ionic liquids in aqueous solutions. The supramolecular complexes based on host-guest interactions were characterized by dynamic light scattering, microcalorimetric titrations, dynamic scattering calorimetry, UV-vis spectrofotometry or lower critical solution temperature measurements. The complexation of ionic liquids with the CD-containing copolymers generated a new type of polyelectrolyte, the so-called pseudopolyanions.

Supramoleculare structures were also generated by combining CD-dimers and adamantane guest-dimers synthesized via microwave-assisted "click" methodology. The water-soluble complexes were evidenced by dynamic light scattering measurements.

A combi-receptor with dual complexing properties was synthesized by coupling CD and cucurbituril (CB) via "click chemistry". The ability of the heterodimer to act as a supramolecular receptor in combination with a NIPAAM- and adamantane-containing polymer was investigated by dynamic light scattering measurements. The properties of the copolymer complexed with the CD-*click*-CB were influenced in the presence of the dicationic 4,4'-bipyridinium guest.

Zusammenfassung

Über die Click-Reaktion von Monoazid β-Cdyclodextrin mit Proporgyl-Derivaten leißen sich polymerisierbare Cyclodextrin-Methacrylate synthetisieren. Die Mikrowellen-unterstützte 1,3-dipolare Cycloaddition gewährleistet die Umsetzung in monomethacryliertes Cyclodextrin in ausgezeichneter Ausbeute.

Wasserlösliche Oligomere konnten mittels radikalischer Homopolymerisation erhalten werden.

Ebenso wurden Gast-Copolymere über die radikalische Copolymerisation con Cyclodextrin Methacrylat mit N-Isopropylacrylamid (NIPAAM) and Methylmethacrylat (MMA) synthetisiert.

Über eine polymeranaloge Kupplungsreaktion von β-CD-Azid an Poly(Propargylmethacrylat) konnte außerdem CD-enthalthendes Polymer erhalten werden.

Suprmamolekulare Polymere konnten durch Komplexierung von Adamantan-Derivaten und Ionischen Flüssigkeiten in wässriger Lösung erhalten werden.

Die auf Wirt-Gast-Welhselwirkung basierenden supramolekularen Komplexe werden durch Dynamische Lichtstreuung, Mikrokalorimetrische Titrationen, UV-vis Spektroskopie und LSCT-Messungen charakterisiert.

Die Komplexierung Ionischer Flüssigkeiten mit CD-enthaltenden Copolymeren stellt einen neuen Typ von Polyelektrolyten, sogenannten Polyanionen dar.

Supramolekulare Strukturen wurden auch durch kombination von CD-Dimeren und Adamantan-Gast-Dimeren, welche zuvor über Mikrowellen-unterstützte Click-Reaktion synthetisiert wurden durch LCST- und DLS-Messungen untersucht.

Desweiter wurde ein Kombi-Rezeptor mit dualen Komplexierungs-Eigenschaften durch Kupplung von CD und Cucurbituril über click-Chemie Synthetisiert.

Die Fähigkeit des Heterodiemers in Kombination mit NIPAAM- und Adamantan-enthaltenden Polymeren als supramolekularer Rezeptor zu fungieren, wurde durch DLS-Messungen untersucht.

Die Eigenschaften des mit CD-click-CB komplexierten Polymers werden in Anwesenheit des dikationischen 4,4′-Bipyridinium-Gast verändert.

1. Introduction

1.1. From molecules to supramolecular structures

The term "supramolecular chemistry" was introduced by Lehn to define "the chemistry of intermolecular bonds".[1-4] However, the "übermoleküle", i.e. supermolecules, designating molecular associations have been recognized and studied for a long time.[5]

Supramolecular chemistry, named also "the chemistry beyond molecules", refers to structures consisting of two or more molecules held together by intermolecular forces.[6] This is in contrast to molecular chemistry, which involves the formation of new covalent bonds between components to form a single molecule.

The smaller *guest* molecule is held within the internal cavity of a larger *host* molecule only by electrostatic forces, hydrogen bonding, π–π stacking, van der Waals interactions and hydrophobic effect.[7] The way from simple molecules to complex structures implies the primary stage of molecular "recognition", followed by self-assembling of components, with the spontaneous self-organization generating supramolecular architectures in the final stage.[8] The properties of supramolecular functioning structures depend on their nature, chemical constitution, and interaction between them.

Supramolecular oligomers formed by hydrogen bonds were reported for the first time by Lehn et al.[9-11] This direction is gradually becoming a new independent interdisciplinary science at the interface between biology and physical chemistry.[3, 10]

In recent years attention has been expanding from supramolecular chemistry to supramolecular polymer chemistry, with more sophisticated structures and functions.[12-14] In living systems, various supramolecular polymers play important roles in maintaining life. For example, the double helix DNA originates from self assembly and is directed by lateral hydrogen bonds between bases and stabilized by solvophobic interactions between the covalently linked bases perpendicular to the hydrogen bonds.[15] Microtubules, microfilaments, and flagella are helical supramolecular polymers formed by proteins. Hydrogen bonding is also involved in the selective signaling and interactions of carbohydrates, hormones, and other functional biomolecules.[16] Thus, the process of self-organization repeats algorithmically from the nano- to the macrolevel.

In recent years, much attention has been focused on supramolecular polymers formed by synthetic molecules, because of their unique structures and properties.

1.2. Cyclodextrins as hosts in supramolecular chemistry

The supramolecular structures involving macrocycles have been a fascinating research area because they not only serve as models for understanding natural supramolecular self-assembly and molecular recognition, but also provide precursors for designing novel nanomaterials for electronics, biomedical and pharmaceutical applications.[17]

The cage-like organic molecules, such as: cyclodextrins, cryptands, calixarenes, cyclophanes, spherands and crown ethers available with accessible internal cavities can serve as hosts.[18-20]

Among them, cyclodextrins (CDs) are the most important and promising macrocyclic hosts because they are water-soluble natural products, inexpensive, commercially available, nontoxic and readily functionalized.[21-23]

Cyclodextrins are versatile biocompatible macrocycles composed of D-glucopyranose residues bonded by α-(1→4) linkages.

IUPAC names of these macrocycles are cumbersome; 5,10,15,20,25,30,35-heptakis-(hydroxymethyl)-2,4,7,9,12,14,17,19,22,24,27,29,32,34-tetradecaoxaoctacyclo[31.2.2.2.^{3,6}.2^8, ^{11}.2^{13}, ^{16}.2^{18}, ^{21}.2^{23}, ^{26}.2^{28}, ^{31}]nonatetracontane-36,37,38,39,40, 41,42,43,44,45,46,47,48,49-tetradecol for β-CD makes the use of trivial names necessary.[24]

The most common cyclodextrins are referred as α-cyclodextrin, β-cyclodextrin, and γ-cyclodextrin, which consist of six, seven, and eight glucopyranose units, respectively. The nomenclature serves also to distinguish the different ring sizes. Other names for β-CD include: cyclomaltoheptaose, cyclomaltoglucan and cyclomaltoamylose.

Due to steric factors, cyclodextrins having fewer than six glucopyranose units cannot exist. However, cyclodextrins containing nine, ten, eleven, twelve, and thirteen glucopyranose units, which are designated δ-, ε-, ζ-, η-, and θ-cyclodextrin, respectively, have been reported.[25, 26]

Cyclodextrins are produced by the enzymatic degradation of starch, using cyclodextrin glycosyltransferase (CGTase). CDs are hydrolyzed by α-amylases, which can open the ring, forming a linear molecule with the corresponding number of glucose units.

β-CD has a lower water-solubility compared with α- and γ-CD. The solubility of CDs can be increased at elevated temperatures, as well as by using solvent/water mixture. Certain additives including bases, acids and salts have been also found to increase the aqueous solubility of β-CD.

Chemical and physical properties of the four most common cyclodextrins are given in Table 1.

5

Table 1. Some characteristics of α-, β-, and γ-Cyclodextrin

	α	β	γ
Number of glucopyranose units	6	7	8
Molecular weight	972	1135	1297
Outer diameter (nm)	1.53	1.66	1.72
Central cavity diameter (Å)	4.7-5.3	6.0-6.5	7.5-8.3
Water solubility at 24 °C (g/100 mL)	14.5	1.85	23.2
Melting temperature range (°C)	225-260	255-265	240-245
Water molecules in cavity	6	11	17

CDs are represented as a torus or truncated funnel (Figure 1). The primary hydroxyl groups are located on the narrow side of the torus while the secondary hydroxyl groups are located on the wider edge.[27]
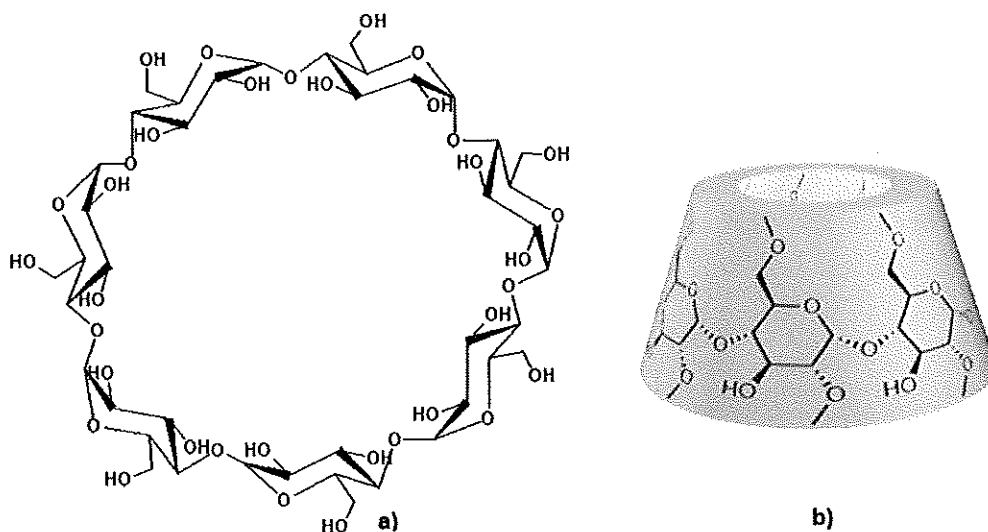
Figure 1. (a) The chemical structure and (b) the toroidal shape of the β-cyclodextrin

Since the hydroxyl groups exist on the wall, the cavity of CD is hydrophobic (Figure 2). In aqueous solutions, the hydrophobic ("semi-polar") cavity is filled with water, which can be easily replaced by other less polar guest molecules.[20]

The most important factor in the guest selectivity of CD is that the size of the cavity matches that of the guest, without forming covalent bonds.[28]
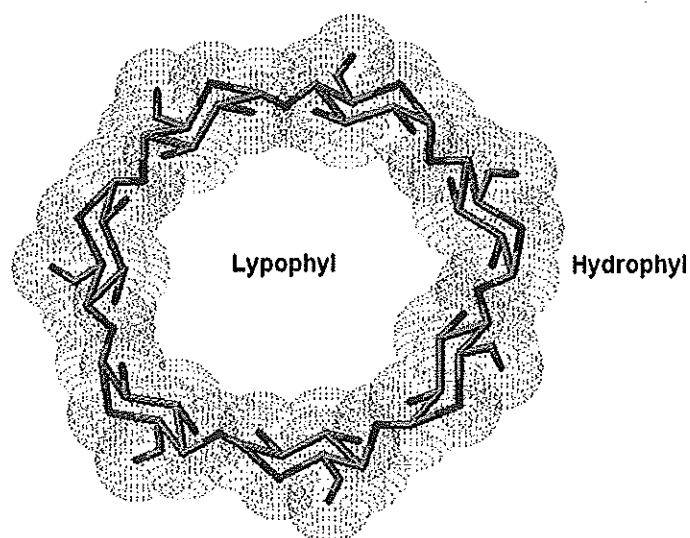
Figure 2. The hydrophobic cavity of cyclodextrin

The use of CDs as receptors in supramolecular chemistry has a wide a wide range of applications, such as:

- chromatographic separations;[29-31]
- drug delivery;[32-38]
- food industry;[39-41]
- solubilisation of environmental pollutants;[42,43]
- molecular reactors for the mediation of organic reactions;[44]
- fluorescent sensors.[45]

Numerous CD derivatives have been synthesized with the aim of improving their water-solubility and complexing properties and to make them suitable for various applications.[46, 47] These derivatives usually are produced by amination, esterification or etherification of primary and secondary hydroxyl groups of the CDs.[20, 48]

1.3. Cyclodextrin-containing supramolecular polymers

The supramolecular polymeric structures containing CDs have inspired interesting developments of novel supramolecular biomaterials.[17] The ability the host-polymer to encapsulate guest molecules, and particularly drugs, could afford the design of molecular

7

delivery systems with the potential of carrying drugs in a non-covalent way.[49-51] The incorporation of CDs within polymers is also important for the production of biodegradable materials, but can also assure flame retardant or antibacterial properties.[52, 53] CD applications should not only improve the properties of the products but, consistently with modern trends, they are also aiming at creating new needs by producing new types of materials with unheard of properties.[24]

Conversion of CDs to polymerizable materials is of great value for the construction of supramolecular structures. The design of cyclodextrin-containing polymers implies two procedures:

1) functionalization of OH groups;

2) inclusion complexation with polymeric chains or with the side chains of a polymeric backbone.

Since β-CD has 21 reactive sites on each of its molecule, understanding the chemical reactivity and reaction conditions are fundamental to designing cyclodextrin-based polymers. Two factors are important for modification reactions involving CD: the nucleophilicity of the hydroxyl groups and the ability of CDs to form complexes with the reagents used. Among three kinds of hydroxyl groups, primary side C6-OH groups are most basic and most nucleophilic, C2-OH groups are most acidic, and C3-OH groups are the most inaccessible. Therefore electrophilic reagents will initially attack C6-OH groups. The size of the CD cavity affects also the formation of the intermediate complex and the product structure.[54] The synthesis of monofunctional CDs monomers is difficult to achieve, because of the multiple hydroxyl groups. A selective acylation of CD at one of the secondary hydroxyl groups was conducted under mild conditions.[55] Highly substituted or derivatizated β-CD containing multiple methacrylate and/or acrylate moieties able to form cross-linked polymers are used for dental applications.[56. 57]

The water soluble materials synthesized by crosslinking CD with epichlorohydrin in alkaline media, referred as the first polymeric cyclodextrins[58] were used to prepare supramolecular structure based on host-guest interaction with suitable guests.[59-63]

Multiple cyclodextrin rings were found to thread over polymers and form a supramolecular polyrotaxane structure.[64-85] This field of research has inspired the development of interesting nanostructures.[86]

The supramolecular structures constituted by inclusion complexation between polyethylene glycols and cyclodextrin polymers are useful as multifunctional drug delivery matrices.[87]

1.4. Click reaction as a valuable tool in supramolecular chemistry

Recent progresses in the field of supramolecular chemistry are also the contribution of click chemistry, a versatile, powerful, and multiapplicable tool, intensively exploited in the past years. The Cu(I) catalysis of the well-known 1,3-dipolar cycloaddition reaction (CuAAC) between an azide and an alkyne[88] was reported independently by the groups of Meldal[89] and Sharpless[90] and become the most popular click reaction to date. Besides the 1,3-dipolar cycloaddition reaction (Figure 3), classical Diels-Alder-type reactions have been used extensively for the functionalization of polymeric materials and surfaces.[91, 92]

Although azides and alkynes display high mutual reactivity, individually these functional groups are two of the least reactive in organic synthesis. They have been termed bioorthogonal because of their stability and inertness towards the functional groups typically found in biological molecules.[91] However, the triazole products are more than just passive linkers; they readily associate with biological targets through hydrogen bonding and dipole interactions.
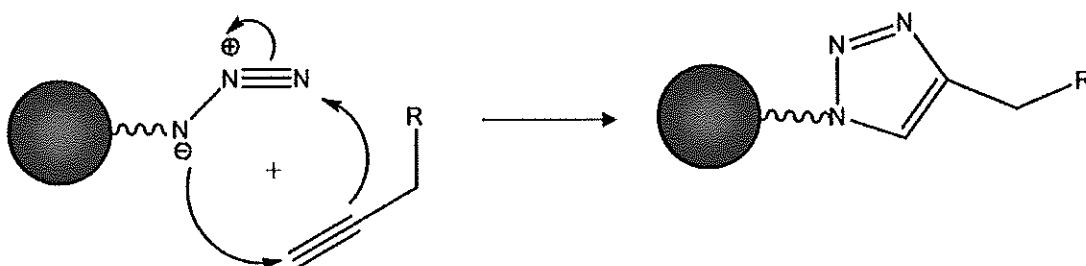
Figure 3. The basic process of 1,3-dipolar cycloaddition reaction

This type of coupling that affords the modular assembly of new molecular entities is also known in the cyclodextrin chemistry.[94-96]

CD monomers co-polymerized in the main polymer chain are typically synthesized via polycondensation of bifunctional monomers.[97] This polymerization technique generally yields structures with low molecular weight. High molecular weight β–CD copolymers were synthesized via "click reaction" of diazido-β-cyclodextrin with a series of dialkyne-oligoethyleneamine monomers.[98]

Click chemistry has been shown to be able to help bridge the gap between chemistry and biology, becoming a true interdisciplinary reaction.[99] Click chemistry as a concept of simplifying synthesis is very useful in polymer science to produce complex macromolecular structures, functional polymers and protein conjugates.[100]

1.5. Cyclodextrin heterodimers

Selective binding of guest molecules by synthetic receptors in an aqueous medium and the synthesis of selective functional systems is a major goal in molecular recognition field. In order to improve their binding affinity, CDs were conjugated with other kinds of hosts, e.g. with calixarene[101] or crown ether[102] (Figure 4).
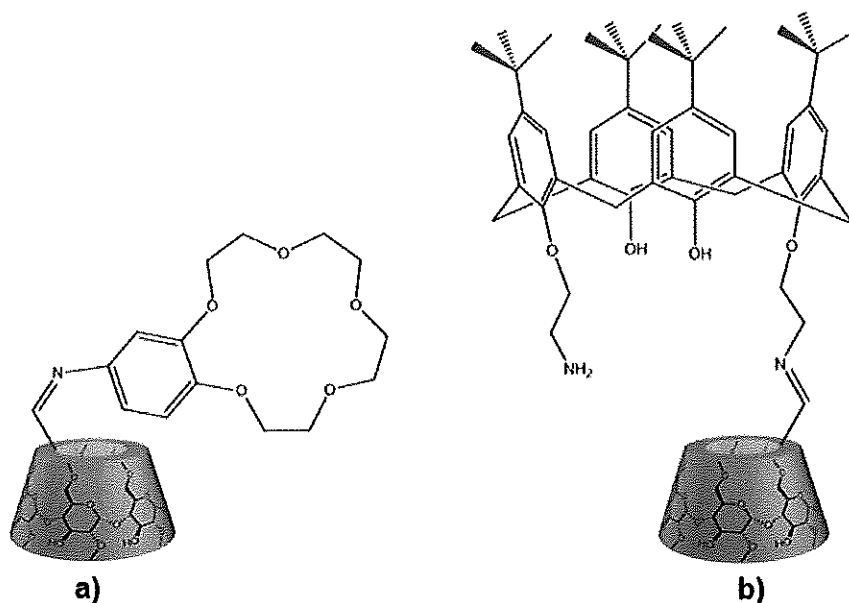
Figure 4. CD-crown ether (a) and CD- calix[4]arene conjugares

Due to the cooperative functioning of calix[4]arenes and β-CD, the heterodimers with two recognition sites: calix[4]arene and β-cyclodextrin found alreary applications for the separation of aromatic positional isomers and enantiomers of chiral compounds.[103]

CD–crown ether conjugates, which simultaneously exhibit the binding affinity these two kinds of are able to mimic the receptor sites of enzymes,[104] and also used for chromatographic separation.[105, 106]

2. Motivation and Aims of this Dissertation

The aim of this work is the synthesis of water-soluble supramolecular polymeric structures, based on inclusion complexation ability of the covalently attached cyclodextrins. Since the self-assembly of artificial functional molecular systems still remains a major challenge for supramolecular chemistry, the synthesis of new polymers with interesting structures and properties offers new opportunities in this field. Based on this motivating challenge, the general aims feature as central issue of the research:

1) Synthesis of polymerizable β-cyclodextrin and optimization of reaction parameters leading exclusively to monofunctional derivatives.

2) Synthesis of cyclodextrin containing copolymers by radical copolymerization of cyclodextrin monomers or polymer analogous reactions.

3) Synthesis of functional cyclodextrin dimers and heterodimers via microwave-assisted click reaction.

4) Preparation of new supramolecular polymers by complexation with hydrophobic guest molecules in aqueous media.

5) Investigation of the host–guest induced change of the solution and thermal properties of cyclodextrin containing copolymers by accommodating different hydrophobic guests.

The development of the proposed research directions, from the synthesis of cyclodextrin monomers and dimers to the preparation of supramolecular polymers are presented in the attached publication.

3. Conclusions

A monofunctional cyclodextrin monomer was synthesized via Cu-catalyzed click reaction of propargyl methacrylate with 6I-azido-6I-deoxycyclomaltoheptaose. Different reaction conditions were investigated, in order to achieve the complete conversion into monomethacrylated cyclodextrin. Investigating the kinetics of the cyclodextrin monomer synthesis via click chemistry showed that the microwave conditions assure a rapid and selectively reaction, with the high yield in the desired product, in comparison with the conventional heating. For the same reaction time and temperature, the yield of the microwave-assisted reaction was of 87%, while the classical reaction assured a conversion of only 67%.

Water soluble copolymers were prepared by the radical copolymerization of methacrylated cyclodextrin with NIPAAM. The formation of supramolecular structures by inclusion complexation with adamantane derivatives in aqueous media was proved by change of LCST and hydrodynamic diameter. The synthesized copolymers were also complexed with IL in aqueous solution, generating supramolecular polyelectrolyte-type structures.

Hydrophobic CD polymers synthesized by radical copolymerization with MMA were also complexed with IL. The variation of the glass transition temperature and hydrodynamic radius proved the inclusion complexation resulting in supramolecular structures. The solubility of the modified PMMA was switched from water insoluble to water soluble, due to the incorporation of the anions of ILs into the CD cavity, while the cations remained outside and were hydrated.

CD dimers and heterodimers were also synthesized. CD-CD and CD-Ad dimers were prepared via click reaction of 6I-azido-6I-deoxycyclomaltoheptaose with 1,7-octadiyne. DLS measurements indicated the self-assembly ability of the mix dimer, as well as the supramolecular interactions between the two types of structures.

Supramolecular structures were also prepared by complexing an adamantane containing polymer with a dual receptor CD-CB synthesized by click reaction.

CD-containing polymers are valuable building blocks for supramolecular chemistry, which can open new perspectives in the field of drug delivery systems.

4. References

1. J.-M. Lehn, *Struct. Bonding* (Berlin), **1973**, 16, 1.

2. J.-M. Lehn, *Science* **1985**, 227, 849-856.

3. J.-M. Lehn, *Pure Appl. Chem.*, **1978**, 50, 871-892.

4. J.-M. Lehn, *Act. Chem. Res.*, **1978**, 11, 49.

5. R. Pfeiffer, *Organische Molekülerverbindungen*, Stuttgart, **1927**.

6. B. Wagner, P. MacDonald, M. Wagner, *Journal of Chemical Education*, **2000**, 77, 178.

7. K. Nørgaard, T. Bjørnholm, *Chem. Commun.*, **2005**, 1812.

8. E. Rapis, *Technical Physics*, **2004**, *49*, 494.

9. J.-M. Lehn, *Adv. Mater.*, **1995**, 5, 254.

10. T. Gulik-Krzywicki, C. Fouquey, J.-M. Lehn, *Proc. Natl. Acad. Sci. USA*, **1993**, 90, 163.

11. J.-M . Lehn, *Makromol. Chem. Macromol. Symp*, **1993**, 69, 1.

12. L. Brunsveld, B. J. B. Folmer, E. W. Meijer, R. P. Sijbesma, *Chem. Rev.*, **2001**, 101, 4071.

13. J.-M. Lehn, *Polym. Int.*, **2002**, 51, 825.

14. W. H. Binder, R. Zirbs, *Adv. Polym. Sci.*, **2007**, 207, 1.

15. L. Brunsveld, J. A. J. M. Vekemans, J. H. K. K. Hirschberg, R. P. Sijbesma, E. W. Meijer, *Proc. Natl. Acad. Sci.*, **2002**, 99, 4977.

16. G. Brancato, F. Coutrot, D. Leigh, A. Murphy, J. Wong, Zerbetto F., *Proc. Natl. Acad. Sci.*, **2002**, 99, 4967.

17. J. Li, , X.H. Loh, *Advanced Drug Delivery Reviews*, **2008**, 60, 1000.

18. D. J. Cram, *Nature* **1992**, 356, 29.

19. M. M. Conn, J. Jr. Rebek, *Chem. Rev.* **1997**, 97, 1647.

20. M. Del Valle, *Process Biochemistry*, **2004**, 39, 1033.

21. S. Szejtli, *Cyclodextrin technology*; Kluwer Academic Publishers: Dordrecht, Netherlands, **1998**.

22. J. Szejtli, T. Osa, Eds. *Comprehensive cyclodextrin chemistry*; Pergmon: Oxford, U.K., **1996**.

23. S.A. Nepogodiev, J.F. Stoddart, *Chem. Rev.* **1998**, 98, 1959.

24. H. Dodziuk, Cyclodextrin and Their Complexes, *Wiley-VCH: Weinheim*, **2006**, 17.

25. D. French, *Adv. Carbohydr. Chem.* **1957**, 12, 189.

26. D. French, A. O. Pulley, J. A. Effenberger, M. A. Rougvie, M. Abdullah, *Arch. Biochem. Biophys.* **1965**, 111, 153.

27. J. Szejtli, *Chem. Rev.*, **1989**, 99, 1743.

28. K. Ariga, T. Kimtake, Supramolecular Chemistry – Fundaments and Applications, *Springer*, **2006**.

29. W. L. Hinze, , *Separ. Purif. Method.*, **1981**, 10, 159.

30. A.-H. Ahmed, S. El-Gizawy, N. Omar, *Analytical Letters*, **1991**, 24, 2207.

31. V. Schurig, H.-P. Nowotny, *Angew. Chem.*, **1990**, 102, 969; *Angew. Chem. Int. Ed. Engl.*, **1990**, 29, 939.

32. R. Challa, A. Ahuja, J. Ali, R. Khar, , *AAPS PharmSciTech.* **2005**, 6, 329.

33. U. Kaneto, H. Fumitoshi, A. Hidetoshi, *J. Incl. Phenom. Macrocycl. Chem.*, **2006**, 56, 3.

34. V. J. Stella, R. A. Rajewski, *Pharm. Res.*, **1997**, 14, 556.

35. T. Loftsson, T. Järvinen, *Adv. Drug Deliv. Rev.*, **1999**, 36, 59.

36. M. E. Brewster, T. Loftsson, *Adv. Drug Deliv. Rev.*, **2007**, 30, 645.

37. T. Loftsson, P. Jarho, M. Másson, T. Järvinen, *Expert Opin. Drug Drliv.*, **2005**, 2, 335.

38. S. Shimpi, B. Chauhan, P. Shimpi, *Acta Pharm.*, **2005**, 55, 139.

39. L. Szente, J. Szejtli, J., Trends in Food Science & Technology, **2004**, 15, 137.

40. G. Cravotto, A. Binello, E. Baranelli, P. Carraro, F. Trotta, Current Nutrition & Food Science, **2006**, 2, 343.

41. G. Astray, C. Gonzalez-Barreiro, J. C. Mejuto, , R. Rial-Otero, J. Simal-Gándara, *Food Hydrocolloids*, **2009**, 23, 1631.

42. K. C. Caroll, M. L. Brusseau, *J. Contam. Hydrol.*, **2009**, 106, 62.

43. S. Fourmentin, D. Landy, P. Blach, E. Piat, G. Surpateanu, *Global NEST Journal*, **2006**, 8, 324.

44. L. Lorna Barr, P. Dumanski, C. Easton, J. Harper, K. Lee, S. Lincoln, A. Meyer, J. Simpson, *J. Incl. Phenom. Macrocycl. Chem.*, **2004**, 50, 19.

45. T Ogoshi, A. Harada, *Sensors*, **2008**, 8, 4961.

46. J. Szejtli, *Starch/Starke*, **1984**, 36, 429.

47. J. Szejtli, *Pure Appl. Chem.*, **2004**, 76, 1825.

48. A. Villiers, *Compt. Rendu*, **1891**, 112, 536.

49. D. A. Fulton, J. F. Stoddart, *Bioconjug. Chem.* **2001**, 12, 655.

50. C. Ortiz Mellet, J. Defaye, J. M. García Fernández, *Chem. Eur. J.* **2002**, 8, 1982.

51. A. Vargas-Berenguel, F. Ortega-Caballero, J. M. Casas-Solvas, *Mini-Reviews in Organic Chemistry*, **2007**, 4, 1.

52. L. Huang, H. Taylor, M. Gerber, P. Orndorff, J. Horton, A. E. Tonelli, *J. Appl. Polym. Sci.,* **1990**, 74, 937.

53. L. Huang, M. Gerber, J. Lu, A. E. Tonelli, *Polym. Degrad. Stab.,* **2001**, 71, 279.

54. K. Takahashi, K. Hattori, F. Toda, *Tetrahedron Lett.,* **1984**, 25, 3331.

55. A. Harada, M. Furue, S. Nozakura, *Macromolecules,* **1976**, 9, 701.

56. R. L.Bowen, M. Farahani, S. H.Dickens, C. M. Guttman, *J. Dent. Res.,* **2000**, 79, 905.

57. R.L. Bowen, US Patent 5792821, **1998**.

58. V. J.Solms, R.H. Egli., *Helv. Chim. Acta,* **1965**, 48, 1225.

59. C. Amiel, B. Sébille, B *J. Inclusion Phenom. Mol. Recognit. Chem,.* **1996**, 25, 61.

60. C. Amiel, B. Sébille, B. *Adv. Colloid Interface Sci.,* **1999**, 79, 105.

61. A. Sandier, W. Brown, H. Mays, C. Amiel, *Langmuir,* **2000**, 16, 1634.

62. C. Amiel, L. Moine, A. Sandier, W. Brown, C. David, F. Hauss, E. Renard, M. Gosselet, B. Sébille, *ACS Symp. Ser.,* **2001**, 780, 58.

63. C. Koopmans, H. Ritter, *Macromolecules,* **2008**, 41, 7418.

64. A. Harada, M. Kamachi, *Macromolecules,* **1990**, 23, 2821.

65. A. Harada, J. Li, M. Kamachi, *Nature,* **1992**, 356, 325.

66. A. Harada, J. Li, M. Kamachi, *Nature,* **1993**, 364, 516.

67. A. Harada, H. Adachi, Y. Kawaguchi, M. Kamachi, *Macromolecules,* **1997**, 30, 5181.

68. I. Yamaguchi, K. Osakada, T. Yamamoto, *Macromolecules,* **1997**, 30, 4288.

69. I. Tomatsu, A. Hashidzume, A. Harada, *Macromol. Rapid Commun.,* **2005**, 26, 825.

70. I. Tomatsu, A. Hashidzume, A. Harada, *Macromolecules,* **2005**, 38, 5223.

71. A. Harada, F. Ito, I. Tomatsu, K. Shimoda, A. Hashidzume, Y. Takashima, H. Yamaguchi, S. Kamitori, S., *J. Photochem. Photobiol. A,* **2006**, 179, 13.

72. I. Tomatsu, A. Hashidzume, A. Harada, *Macromol. Rapid Commun.,* **2006**, 27, 238.

73. A. Hashidzume, A. Harada, *Polymer* **2006**, 47, 3448.

74. Y. Ohya, S. Takamido, K. Nagahama, T. Ouchi, R. Katoono, N. Yui, *Biomacromolecules,* **2009**, 10, 2261.

75. G. Wenz, B. Keller, *Angew. Chem.* **1992**, 104, 201; *Angew. Chem. Int. Ed. Engl.,* **1992**, 31, 197.

76. G. Wenz, B. H. Han, A. Muller, *Chem. Rev.,* **2006**, 106, 782.

77. M. Born, H. Ritter, *Makromol. Chem. Rapid Commun.,* **1991**, 12, 471.

78. M. Born, T. Koch, H. Ritter, *Acta Polym.,* **1994**, 45, 68.

79. H. Ritter, *Angew. Makromol. Chem.,* **1994,** 223, 165.

80. M. Born, H. Ritter, *Angew. Chem.,* **1995,** 107, 342; *Angew. Chem. Int. Ed. Engl.,* **1995,** 34, 309.

81. M. Born, T. Koch, H. Ritter, *Macromol. Chem. Phys.,* **1995,** 196, 1761.

82. M. Born, H. Ritter, *Adv. Mater.,* **1996,** 8, 149.

83. M. Born, H. Ritter, *Macromol. Rapid Commun.,* **1996,** 17, 197.

84. O. Noll, H. Ritter, *Macromol. Rapid Commun.,* **1997,** 18, 53.

85. O. Noll, H. Ritter, *Macromol. Chem. Phys.,* **1998,** 199, 791.

86. Y.-L. Wu, J. Li, *Angew. Chem. Int. Ed.* **2009,** 48, 3842.

87. S. Salmaso, A. Semenzato, S. Bersani, P. Matricardi, F. Rossi, P. Caliceti, *International Journal of Pharmaceutics,* **2007,** 345, 42.

88. R. Huisgen, *Angew. Chem., Int. Ed. Engl.* **1968,** 7, 321.

89. C.W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.,* **2002,** 67, 3057.

90. V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2002,** 41, 2596.

91. S. Itsuno, *Progr. Polym. Sci.,* **2005,** 540.

92. G. W. Goodall, W. Hayes, *Chem. Soc. Rev.,* **2006,** 35, 280.]

93. H. C. Kolb, K. B. Sharpless, *Drug Discovery Today,* **2003,** 8, 1128.

94. F. Perez-Balderas, M. Ortega-Munoz, J. Morales-Sanfrutos, F. Hernandez-Mateo, F. G. Calvo-Flores, J. A. Calvo-Asin, J. Isac-Garcia, F. Santoyo-Gonzalez, *Org. Lett.* **2003,** 5, 1951.

95. R. Hoogenboom, B. C. Moore, U. S. Schubert, *Chem. Commun.,* **2006,** 38, 4010.

96. L. Barr, S. Lincoln, C. Easton, *J. Supramol. Chem.,* **2005,** 17, 547.

97. S. J.Hwang, N. C.Bellocq, M. E.Davis, *Bioconjug. Chem.,* **2001,** 12, 280.

98. S. Srinivasachari, T. Reineke, T., *Biomaterials,* **2009,** 30, 928.

99. G. C. Tron, T. Pirali, R. Billington, P. R. Canonico, G. Sorba, A. Genazzani, *Medicinal Research Reviews,* **2008,** 28, 278.

100.   L. Nurmi, J. Lindqvist, R. Randev, J. Syrett, , D. Haddleton, *Chem. Commun.,* **2009,** 2727.

101.   Y. Liu, Y.W. Yang, L. Li, Y. Chen, *Org. Biomol. Chem.,* **2004,** 1542.

102.   Y. Liu, Y. Chen, L. Li, G. Huang, C. C.You, H. Y.Zhang, T. Wada, Y. Inoue, *J. Org. Chem.,* **2001,** 66, 7209.

103.   S. K. Thamarai Chelvi, E. L. Yong, Y. Gong, *J. Chromatogr. A.,* **2008,** 1203, 54.

104.   I. Willner and Z. Goren, *J. Chem. Soc., Chem. Commun.*, **1983**, 1469.

105.   W. X. Hunag, H. Xu, S. D. Fazio and R. V. Vivilecchia, *J. Chromatogr., B: Biomed. Appl.*, **1997**, 695, 157.

106.   Y. Gong, H. K. Lee, *Helv. Chim. Acta*, **2002**, 85, 3283.

5. Attachment

Cumulative Publications

1. Amajjahe S., Choi S.W., Munteanu M., Ritter H., "Pseudopolyanions Based on Poly(NIPAAM-co-β-Cyclodextrin Methacrylate) and Ionic Liquids", *Angew. Chem. Int. Ed.*, **2008**, 47, 3435; *Angew. Chem.*, **2008**, 120, 3484.

2. Munteanu M., Choi S.W., Ritter H., "Supramolecular structures based on dimeric combinations of cyclodextrin and adamantane via click chemistry", *J. Incl. Phenom. Macro. Chem.*, **2008**, 62, 197-202.

3. Munteanu M., Choi S.W., Ritter H., "Methacrylate Cyclodextrin via Microwave-assisted Click Reaction", *Macromolecules*, **2008**, 41 (24), 9619-9623.

4. Choi S.W., Munteanu M., Ritter H., "Monoacrylated cyclodextrin via "click" reaction and copolymerization with *N*-isopropylacrylamide: guest controlled solution properties", *J. Polym. Res.*, **2009**, 16, 389–394.

5. Amajjahe S., Munteanu M., Ritter H., "Switching the Solubility of PMMA Bearing Attached Cyclodextrin-Moieties by Supramolecular Interactions with Ionic Liquids", *Macromol. Rapid Commun.*, **2009**, 30, 904–908.

6. Munteanu M., Choi S.W., Ritter H., "Cyclodextrin-*click*-cucurbit[6]uril: Combi-Receptor for Supramolecular Polymer Systems in Water", *Macromolecules*, **2009**, 42, pp 3887–3891.

7. Maciollek A., Munteanu M., Ritter H., "New Generation of Polymeric Drugs: Copolymer from NIPAAM and Cyclodextrin methacrylate containing Supramolecular-attached Antitumor Derivative", *Macromol. Chem. Phys.*, in press.
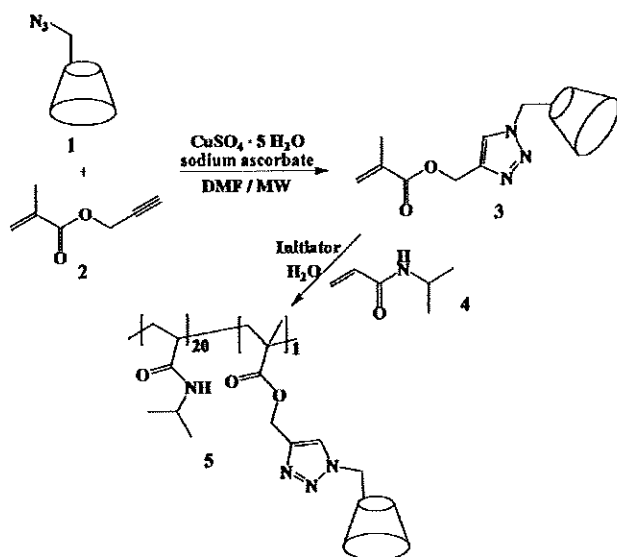
Cyclodextrin Complexes

Pseudopolyanions Based on Poly(NIPAAM-*co*-β-Cyclodextrin Methacrylate) and Ionic Liquids

Sadik Amajjahe, Soowhan Choi, Maricica Munteanu, and Helmut Ritter*

Cyclodextrins (CD) have gained much attention because of their ability to form inclusion complexes in aqueous solutions.[1] Herein we describe the synthesis of cyclodextrin-containing polymers by 1,3-dipolar cycloaddition. This type of click reaction is not only devoid of side reactions, but also provides access to complex structures in high yields. The process is experimentally simple and has enormous scope.[2]

We investigated the influence of different ionic liquids as guests on the lower critical solution temperature (LCST) and hydrodynamic volume of copolymers consisting of *N*-isopropylacrylamide (NIPAAM) and methacrylated CD. The triazole-CD-containing methacrylate 3 was obtained by a click-type reaction of propargyl methacrylate 2 with CD-azide 1 under microwave-assisted reaction conditions.[3] The monomer 3 and NIPAAM 4 were radically copolymerized in a molar ratio of 1:20 using a water-soluble azo initiator in an aqueous medium (Scheme 1). The number-average molar mass (M_n) of the obtained copolymer 5 was about

16000 g mol^{-1} according to GPC measurements, using polystyrene as standard. The copolymer 5 was further characterized by ^{1}H NMR spectroscopy, turbidity measurements, and dynamic light scattering (DLS).

The temperature-dependent solubility of aqueous solutions of copolymer 5 was investigated by monitoring changes of transparency as a function of temperature. Copolymer 5 had a slightly increased LCST at 36°C, which is about 3°C higher than that of homopoly(NIPAAM).[4] This increased value is caused by the influence of hydrophilic CD components in the copolymer, which leads as expected to an increased water solubility in comparison to the corresponding homopolymer of NIPAAM.[5]

The attached CD rings suggest that the copolymer 5 can form supramolecular polyelectrolyte-type structures through host–guest interactions with suitable ionic guests. To test this assumption, turbidity measurements were carried out. We used three ionic liquids[6] (ILs) 6–8 to investigate their influence on the LCST values of copolymer 5. The ionic liquids were synthesized by alkylation of *N*-vinylimidazole, followed by exchange of the anions (Scheme 2). In recent

Scheme 1. Synthesis of the CD-NIPAAM copolymer. MW = microwave.
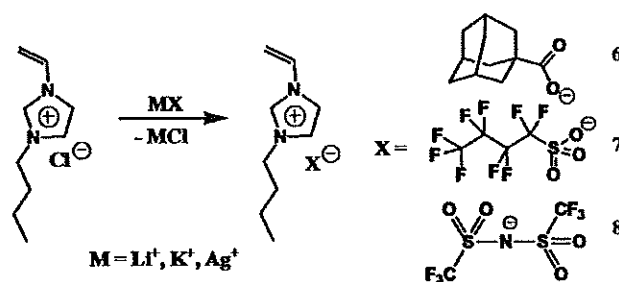
Scheme 2. Synthesis of the three vinylimidazolium ionic liquids (IL) with the anions 6, 7, and 8.

model studies, we evaluated the complex stabilities and stoichiometries of these ionic liquids 6, 7, and 8 with CD (Table 1).[7] Until now, mainly adamantyl guests have been reported to form exceptionally stable complexes with CD.[8]

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

Table 1: Stability constants, ΔH and $T\Delta S$ values of 6, 7, and 8 with CD at 25°C.

IL anion	K_s[a] [kJ mol^{-1}]	$-\Delta H$ [kJ mol^{-1}]	$T\Delta S$ [kJ mol^{-1}]
6	5300	9.75	11.52
7	21000	23.51	1.18
8	8100	32.25	−9.92

[a] Determined by microcalorimetric titrations.

However, in our studies, we could show conclusively that the fluorinated anions **7** and **8** have a high tendency to form inclusion complexes with CD (Table 1).

As shown in Table 2, the cloud points (LCST) of the aqueous NIPAAM-CD copolymer **5** change significantly with ILs **6**, **7**, and **8** as guest molecules. The LCST values of
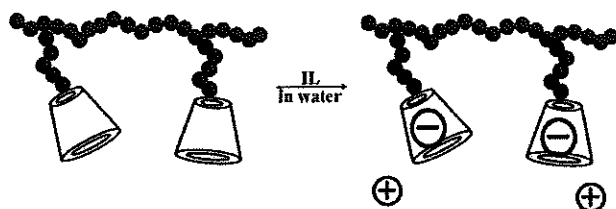
Table 2: LCST of copolymer **5** and its complexes with **6**, **7**, and **8** in water.

Polymer	5	5+6	5+7	5+8
LCST [°C][a]	36	43	53	34

[a] Determined by turbidity measurements.

complexed copolymers **5** increase after addition of ionic guest molecules **6** and **7**. This increased hydrophilicity of pseudo-polyanions results from the free carboxylate (IL **6**) and sulfonate (IL **7**) groups, which are preferentially located in the aqueous phase and therefore responsible for these increased LCST values of 43 °C and 53 °C, respectively.

Complexation of the bis(trifluoromethylsulfonyl)imide anion **8** leads to a compound in which the hydrophobic trifluoromethylsulfonyl group is preferentially located in the centre of the CD-cavity, leading to a decreased LCST value. The cloud points of the copolymer **5** decrease with increasing hydrophobicity of the ionic liquids, as expected (Table 2).[4] This observed influence of ILs on the LCST values is a result of the complexation of negatively charged guests in CD cavity. In other words, starting from a neutral polymer, we obtained a pseudopolyanion (Scheme 3).
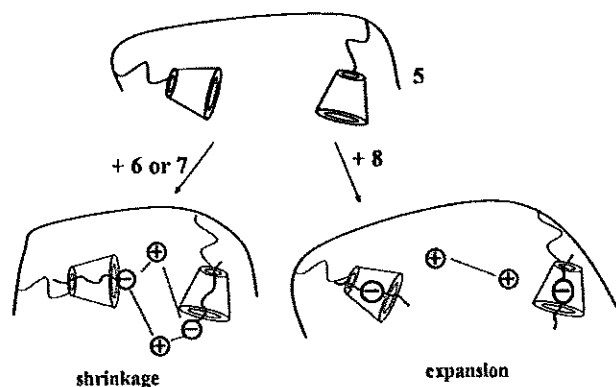
Scheme 3. Generation of pseudopolyanions.

Table 3: Determination of hydrodynamic diameter of the complexes of **5** with **6**, **7** and **8** at 25 °C in water.

System	Diameter [nm]
5	15.1
5+6	11.5
5+7	9.8
5+8	16.5

Scheme 4. Intramolecular interactions of guest molecules (IL **6** and IL **7**) with covalently bonded CD lead to shrinkage with IL anions **6** and **7** (left), or to expansion of the polymer chains with IL **8** (right) For details, see text.

The hydrodynamic diameters of copolymer **5** without guests, and copolymer **5** with charged guest molecules included, were measured by DLS (Table 3). Surprisingly, the results indicate that polymer **5** has a larger mean coil size than that of its complexes with IL **6** and **7**. In both cases (**5** + **6** and **5** + **7**), the negative charges of the anions are in contact with the water phase, owing to the hydrophobic cavity of CD. A possible reason for this situation is the increasing ionic strength, which leads to some intramolecular interactions between the CD-complexed anions and the cations in solution, resulting in a largely reduced intra- and intermolecular repulsion of the chains. These attractive forces obviously lead to a decrease of the hydrodynamic diameter of the complexes **5** + **6** and **5** + **7** (Scheme 4). We can thus conclude that electrostatics override entropy effects.[9]

In contrast, the hydrodynamic diameter of the complex **5** + **8** slightly increases in comparison to the pure copolymer **5**

(Scheme 4), owing to incorporation of the anion group of guest monomer **8** into the CD ring. Furthermore, as shown in Table 1, the entropy of the complex-formation process of IL **8** with CD is very negative, which indicates that a noncoordinating character of the covered anion dominates. Thus, the cation–cation repulsion plays a major role and determines the extended polymer coil structure.

The light-scattering results are consistent with the outcomes of the turbidity measurements. The complexes **5** + **6** and **5** + **7** are more hydrophilic than the pure copolymer **5**, resulting in an increase of the LCST. A decrease in hydrodynamic diameter is due to counterion effects. In case of IL **8**, a lower cloud point was obtained, because the ions are covered by the CD ring and therefore spatially separated from the cation. A higher hydrodynamic diameter is a result of electrostatic repulsion between the cations. These results are due to the delocalized negative charge of the anion of **8**.

Thus, the inclusion of ionic liquids into the cavity of a neutral copolymer consisting of NIPAAM and methacrylated CD generate a new type of polyelectrolyte, so-called pseudopolyanions. This result may open a new field of research in the area of polyelectrolytes. One important application would be in the field of hydrogels. The shrinkage and expansion behavior of cyclodextrin-containing networks could be controlled by supramolecular interaction of the CD components with ionic liquids. Moreover, the solubility behavior of CD-containing polymers could be controlled by complex formation.

Experimental Section

5: Monomer **3** (0.174 g, 1.4 mmol, 0.118 mg) was added to a solution of NIPAAM **4** (0.100 g, 0.07 mmol) in 0.7 mL H_2O. The solution was flushed with argon for 15 min, and the initiator VA-044 (2,2′-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride) (0.03 mmol) was added under argon atmosphere. After stirring at 50°C overnight, the copolymers were separated from the hot reaction mixture by filtration and dried under vacuum.

Characterization and further synthetic procedures are given in the Supporting Information.

[1] a) A. Harada, M. Furue, S. Nozakura, *Macromolecules* **1976**, *9*, 701–704; b) G. Wenz, B.-H. Han, A. Müller, *Chem. Rev.* **2006**, *106*, 782–817.

[2] a) V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 2708–2711; *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599; b) H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem.* **2001**, *113*, 2056–2075; *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021; c) P. Wu, A. K. Feldman, A. K. Nugent, C. J. Hawker, A. Scheel, B. Voit, J. Pyun, J. M. J. Frechet, K. B. Sharpless, V. V. Fokin, *Angew. Chem.* **2004**, *116*, 4018–4022; *Angew. Chem. Int. Ed.* **2004**, *43*, 3928–3932.

[3] S. W. Choi, M. Munteanu, H. Ritter, unpublished results.

[4] a) M. Heskins, J. E. Guillet, *J. Macromol. Sci. Chem.* **1968**, *2*, 1441–1455; b) O. B. Ptitsyn, A. K. Kron, Y. Y. Eizner, *J. Polym. Sci. Part C* **1968**, *16*, 3509–3517; c) H. G. Schild, *Prog. Polym. Sci.* **1992**, *17*, 163–249.

[5] H. Ritter, O. Sadowski, E. Tepper, *Angew. Chem.* **2003**, *115*, 3279–3281; *Angew. Chem. Int. Ed.* **2003**, *42*, 3171–3173; Corrigendum: H. Ritter, O. Sadowski, E. Tepper, *Angew. Chem.* **2005**, *117*, 6253; *Angew. Chem. Int. Ed.* **2005**, *44*, 6099.

[6] P. Bonhôte, A.-P. Dias, N. Papageorgiou, K. Kalyanasundaram, M. Grätzel, *Inorg. Chem.* **1996**, *35*, 1168–1178.

[7] S. Amajjahe, H. Ritter, *Macromolecules*, **2008**, 47, 716–718.

[8] M. V. Rekharsky, Y. Inoue, *Chem. Rev.* **1998**, *98*, 1875–1917.

[9] C. Gao, S. Leporatti, S. Moya, E. Donath, H. Möhwald, *Chem. Eur. J.* **2003**, *9*, 915–920.

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Supporting Information

Pseudo Polyanions based on Poly [NIPAAM-co-β-Cyclodextrin methacrylate] and Ionic Liquids

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Materials

Cyclodextrin (β-CD) was obtained from Wacker-Chemie GmgH, Burghausen, Germany and used after drying overnight in vacuum oil-pump, on P_2O_5. Propargyl methacrylate was purchased from Alfa Aesar GmbH & CoKG, Germany. *N*-isopropylacrylamide (NIPAAM) and sodium azide were obtained from Aldrich Chemicals, Germany and used as received. Copper (II)-sulfate pentahydrate was obtained form Carl Roth GmbH & Co, and sodium L(+)-ascorbate from AppliChem, Germany. α,α-Azo-isobutyronitril and *N,N*-dimethylformamide (DMF) were purchased from Fluka, Germany. 1-Vinylimidazol (Fluka), butyl bromide (Fluka), lithium bis(trifluoromethylsulfonyl)imide (Fluka), potassium nonafluor-1-butansulfonate (Fluka), potassium adamantylcarboxylate (from adamantane-1-carboxylic acid (Fluka)) were used as received. The structures of the synthesized monomers and polymers were proven by ^{1}H-, ^{13}C- and ^{19}F-NMR spectroscopy using a Bruker Avance DRX 500 spectrometer at 500.13 MHz for proton, 125.77 MHz for carbon and 470.55 MHz for fluorine, using $CDCl_3$ and DMSO-d6 as solvents.

Measurements

IR spectra were recorded with a Nicolet 5 SXB FT-IR Spectrometer, equipped with an ATR unit. NMR spectra were recorded with a Bruker AC 500 at 20 °C. Chemical shifts were referenced to the solvent value δ 2,51 for DMSO-d$_6$. MALDI-TOF-MS was performed on a Bruker Ultraflex TOF time-of-flight mass spectrometer using a 337 nm nitrogen laser. Gel permeation chromatography (GPC) analyses were performed on a GPC-System from PSS with PSS-WIN-GPC software 4.01, 6.1 with DMF as eluent. The system was calibrated with polystyrene standards with a molecular weight range from 374 up to 1 000 000 Da. The low rate was 1 ml·min^{-1}. One hundred μL of a 0.1% (w/w) polymer solution was given to a HEMA column combination that consisted of a pre-column of 40 Å and main columns of 40, 100 and 3000 Å porosities. Cloud points were determined by transmission changes (500 nm) of solutions heated at 1.0 °C·min^{-1} in a magnetically stirred cell. The values of the cloud points were taken as the temperature where the transmission decreases by 50%.

Concentration of the solutions: 4 g of Copolymer/L; 0,38 g/L of **6**; 0,52 g/L of **7**; 0,5 g/L of **8**. By considering the concentrations the Copolymer forms a 1:1 complex with the ILs.

Dynamic Light Scattering (DLS) experiments were carried out with a Malvern HPPS-ET in the temperature range 17-75 °C. The particle size distribution was derived from a deconvolution of the measured intensity autocorrelation function of the sample by the general purpose mode algorithm included in the DTS software. Each experiment was performed at least five times to obtain statistical information.

Concentration of the solutions: 4 g of Copolymer/L; 0,38 g/L of **6**; 0,52 g/L of **7**; 0,5 g/L of **8**. By considering the concentrations the Copolymer forms a 1:1 complex with the ILs.

Microwave-assisted synthesis was performed utilizing a CEM-Discover monomodal system, with stirring and cooling on and IR sensor for the temperature control.

Microcalorimetric titrations

Microcalorimetric titrations were accomplished with an isothermal calorimeter of type VP-ITC of the company Microcal. Millipore water was used for the solutions of host and guest. The concentration of the host solution was 1 mmol $* L^{-1}$, the concentration of the guest solution 8 mmol $* L^{-1}$ in each case. In the experimental process the guest solution in the syringe was placed over 25 injections with a respective volume of 10 µl into the measuring cell, which was filled with the host solution. The temperature was 298 K with stirrer rotational speed of 300 min^{-1}. Each injection was made over a period of 20 s, whereas the time between two injections was 4 min. The dilution heat was determined in a separate measurement by injection of the guest in millipore water and subtracting it from the determined heat flow. The evaluation of the received data was carried out with a Microcal modified version of the software Origin.
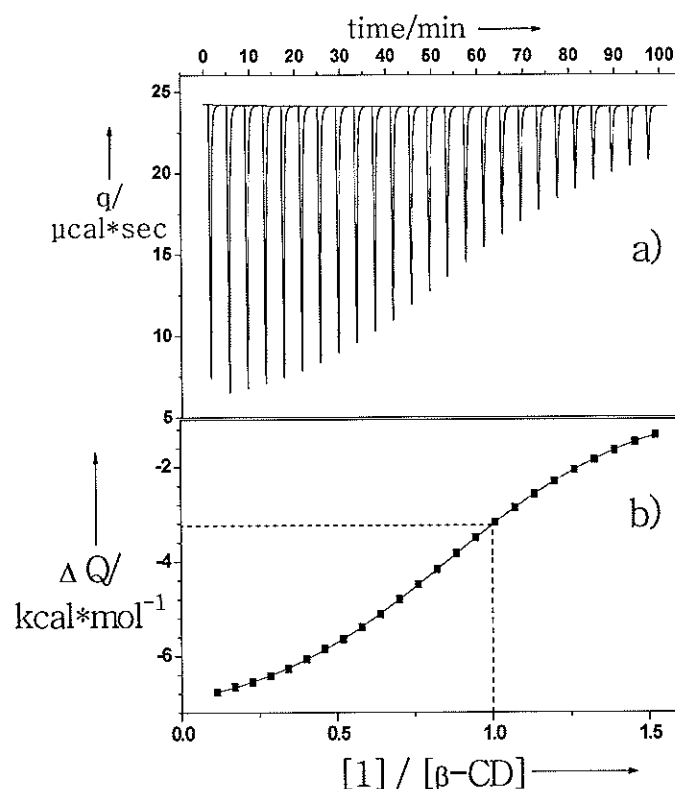
Figure 1. a) Heat flow q as a function of time detected by a microcalorimeter upon the addition of a 8 mM solution of monomeric ionic liquid to a 1mM solution of β-CD b) Inclusion enthalpies in dependence of the molar ratio calculated by the integration of the heat flows q for the consecutive peaks

Synthesis of Mono-(6-O-(p-tolylsulfonyl))-β-Cyclodextrin. Mono-(6-O-(p-tolylsulfonyl))-β-cyclodextrinwas synthesized according to the knownprocedure.[1] β-CD (50 g, 44,0 mmol) was solved in 500 mL solution sodium hydroxide 0.4 N and cooled under 5 °C on ice-bath. To the vigorously stirred solution was added (35 g, 184 mmol) p-toluensulfonyl chloride in small portions, over a period of 5 min. The mixture was stirred at 5 °C for 30 min, than was filtrated. The filtrate was neutralized with concentrated hydrochloric acid and stirred for 1 h. After filtering the reaction mixture and washing 3 times with water, the white precipitate obtained was dried overnight at 60 °C. The fine product occurred (15.32 g, 27% yield) was dried in oil-pump over P_2O_5.

FT-IR (film): 3316 (OH), 2924 (CH), 2160 (CH), 2030, 1358 (S=O), 1152 (S-O), 1077 (OH), 1023 (CH), 945, 841, 756 (OH), 684 (CH).

^{1}H NMR (DMSO-d_6): δ (ppm) 2.45 (s, 3H, H-11), 3.18-3.78 (br overlapped, H-2,3,4,5,6, 42H), 4.79 (2H, H-1), 4,86 (t, 5H, H-1), 5.74 (br, 14H, OH-2,3), 7,45 (d, ^3J_H = 8,2 Hz, 2H, H-9), 7.77 (d, ^3J_H = 8,2 Hz, 2H, H-8).

MALDI-TOF: m/z 1311.3 [M+Na$^+$].

Synthesis of Mono-(6-azido-6-desoxy)-β-Cyclodextrin (1). 10 g (7.76 mmol) Mono-(6-O-(p-tolylsulfonyl))-β-CD were solved in 100 mL water and heated to 80 °C. To this solution was added 2.52 g (38.8 mmol) sodium azide. After stirring 6 h at 80 °C, the clear solution was poured in 600 mL acetone. The white precipitate was isolated by filtering and dried over P_2O_5, to give 7.49 g (85% yield) solid product.

FT-IR (film): 3316 (OH), 2924 (CH), 2160 (C-C), 2102 (N=N), 2033, 1644 (C=C), 1364 (OH), 1152 (C-N), 1077 (OH), 1025 (CH), 945, 853, 756 (NH), 704 (CH).

^{1}H NMR (DMSO-d_6): δ (ppm) 3.32 (14H, H-2,4), 3.60-3.84 (br, 28H, H-3,5,6), 4.44-4.60 (br, 6H, OH-6), 4.85 (d, 6H, H-1), 4.92 (H, H-1), 5.73 (14H, OH-2,3).

MALDI-TOF: m/z 1182.4 [M+Na$^+$].

Synthesis of Triazol-CD-Monomer (3). The reaction of mono-(6-azido-6-desoxy)-β-CD 1 (100 mg, 0.086 mmol) with 2-propynyl 2-methacrylate 2 (32.02 mg, 0.256 mmol) was carried out in water, DMF or a mixed solvent of water and *tert*-butyl alcohol under reflux, in the presence of Cu(I) generated in situ by the reduction of copper sulphate (0.01-0.5 equiv.) with sodium ascorbate (0.05-1 equiv.). The product was separated by simple filtration, after precipitation with acetone. We approached also microwave-assisted cycloaddition, by adding 2-propynyl 2-methacrylate 2 (32.02 mg, 0.256 mmol) to a solution of mono-(6-azido-6-desoxy)-β-CD 1 (100 mg, 0.086 mmol) in 2 mL DMF in a pressure-resistant test tube. To the clear solution were added sodium ascorbate (5.07 mg, 0.025 mmol) and copper (II) sulfate pentahydrate (3.29 mg, 0.013 mmol). The tube was sealed and placed in the CEM monomode microwave and irradiated at 150 °C and 130 W for 30 min. The products were collected by filtration, after precipitating with acetone (50 mL). In order to obtain optimal reaction conditions we used different amounts of catalyst, varying the reaction time. We find that the presence of water may increase the yield of the reaction, due its favourable effect on the catalytic system.

FT-IR (film): 3351 (OH), 2927 (CH), 2160 (C-C), 2026, 1714 (C=O), 1657 (C=C), 1331 (OH), 1153 (C-N), 1078 (OH), 1026 (CH), 946 (C=C), 755 (NH), 706 (CH).

^{1}H NMR (DMSO-d_6): δ (ppm) 1.86 (3H, CH$_3$), 3.34 (br, 14H, H-2,4), 3.65 (br, 28H, H-3,5,6), 4.52 (br, 6H, OH-6), 4.84 (d, 7H, H-1), 5.03 (2H, -C$\underline{H}_2$-), 5.19 (H, -C$\underline{H}$=), 5.73 (br, 14H, OH-2,3), 6.07 (H, -C$\underline{H}$=), 8.13 (1H, CH).

MALDI-TOF: *m/z* 1306.6 [M+Na$^+$].

Characterisation of copolymer (5).

FT-IR (film):3286 (OH), 2970 (CH), 2930 (CH), 2160, 2025, 1649 (C=C), 1546 (C-C), 1439 (C-C), 1386 (CH), 1254 (C=O), 1154 (CN), 1095 (OH).

^{1}H NMR (DMSO-d_6): δ (ppm) 1.05 (br, H, CH$_3$), 1.44 (br, H, CH$_3$), 1.78-2.20 (br, H, CH$_3$), 3.50-3.75 (br, 14H, H-2,4,) 3.84 (H, H-3,5,6), 4.46 (br, H, OH-6), 4.84 (H, H-1), 5.69 (H, -CH$_2$-), 5.73 (H, OH-2,3), 7.21 (H, NH), 8.13 (1H, CH). Ratio of NIPAAM:CD is 22:1.

GPC (PS standard): M_n = 16.000 gmol^{-1}

Synthesis of 1-butyl-3-vinylimidazolium bis(trifluoromethylsulfonyl)imide (6)

Monomeric 1-butyl-3-vinylimidazolium bis(trifluoromethylsulfonyl)imide ([bvim][Tf$_2$N]) was synthesized by a modified procedure of Grätzel and co. [2] by using vinylimidazole and 1-butylbromide.

^{1}H-NMR (CDCl$_3$): δ (ppm) 0.96 (t, 3H, -CH_3), 1.38 (m, 2H, -CH_2CH$_3$), 1.88 (m, 2H,-NCH$_2$CH_2), 4.20 (t, 2H, -NCH_2-), 5.43 (dd, 1H, trans-CHCH_2), 5.80 (dd, 1H, cis-CHCH_2), 7.12 (dd, 1H,-CHCH$_2$),7.48 (s, 1H, N(1)CHCH-),7.66 (s, 1H, -N(1)CHCH-), 9.01 (s, 1H, -NCHN-).

^{19}F-NMR (CDCl$_3$): δ (ppm) -79.63 (6F, -CF_3).

Synthesis of 1-butyl-3-vinylimidazolium adamantylcarboxylate (7)

2.7 g (9.6 mmol) of freshly prepared silver adamantylcarboxylate (filtered and washed with water from a solution of potassium adamantlycarboxylate and silver nitrate) was added to an aqueous solution of 2.2 g (9.3 mmol) 1-vinyl-3-butyl-imidazolium bromide and stirred for approximately 2 h. The solid precipitate was filtered off, and the water removed by freeze drying. The resultant sample was dissolved in dichloromethane to precipitate a small quantity of silver adamantylcarboxylate, which was filtered off, and the filtrate evaporated to dryness. 1-butyl-3-vinylimidazolium adamantylcarboxylate was then dried in vacuum to yield 2.9 g (94 %) of white product.

^{1}H-NMR (DMSO-d$_6$): δ (ppm) 0.94 (t, 3H, -CH_3), 1.36 (m, 2H, -CH_2CH$_3$), 1.68 (s(br), 6H, Ad-H), 1.86 (m, 2H,-NCH$_2$CH_2), 1.90 (s(br), 6H, Ad-H), 1.95 (s(br), 3H, Ad-H), 4.35 (t, 2H, -NCH_2-), 5.27 (dd, 1H, trans-CHCH_2), 5.78 (dd, 1H, cis-CHCH_2), 7.30 (s, 1H, N(1)CHCH-), 7.59 (dd, 1H, -CHCH$_2$), 7.64 (s, 1H, -N(1)CHCH-), 11.79 (s, 1H, -NCHN-).

^{13}C-NMR (DMSO-d$_6$): δ (ppm) 13.8 (-CH$_3$), 19.9 (H$_3$C-CH$_2$), 29.3 (Ad-C), 32.5 (-NCH$_2$$CH_2$), 37.6 (Ad-C), 40.9 (-N$CH_2$-), 42.2 (Ad-C), 50.3 (Ad-C), 108.5 (H$_2$$C$=CH), 118.3 (-N(1)$C$HCH), 122.1 (-N(1)CH$C$H), 129.6 (H$_2$C=$C$H), 185.2 (-N$C$HN-).

Synthesis of 1-butyl-3-vinylimidazolium nonafluorobutansulfonate (8)

The solutions of 10 g (29.6 mmol) of potassium nonafluor-1-butansulfonate (not completely soluble) in 20 mL water and 6.7 g (29 mmol) 1-vinyl-3-butyl-imidazolium bromide in 20 mL water are mixed together in a 100 mL one-necked flask. Immediately a precipitate forms and the suspension is stirred for further 20 minutes. The white solid is filtrated and washed with water before drying in high vacuum. (yield: 70 %)

^{1}H-NMR (DMSO-d$_6$): δ (ppm) 0.91 (t, 3H, -CH_3), 1.29 (m, 2H, -CH_2CH$_3$), 1.80 (m, 2H,-NCH$_2$CH_2), 4.20 (t, 2H, -NCH_2-), 5.41 (dd, 1H, trans-CHCH_2), 5.94 (dd, 1H, cis-CHCH_2), 7.27 (dd, 1H,-CHCH$_2$), 7.92 (s, 1H, N(1)CHCH-), 8.19 (s, 1H, -N(1)CHCH-), 9.46 (s, 1H, -NCHN-).

^{19}F-NMR (DMSO-d$_6$): δ (ppm) -81.0 (3F,-CF_3), -115.4 (2F, F$_3$C-CF_2), -121.9 (2F, CF$_3$F$_2$C-CF_2), -126.2 (2F, F_2C-SO$_3$)

References

[1] Ohga, K., Takashima, Y.; Takahashi, H.; Kawaguchi, Y.; Yamaguchi; H.; Harada, A. *Macromolecules* **2005**, *38*, 5897-5904.

ORIGINAL ARTICLE

Supramolecular structures based on dimeric combinations of cyclodextrin and adamantane via click chemistry

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Abstract We describe the synthesis of 1-mono-β-cyclodextrin-4-[4-(1-mono-β-cyclodextrin-1H-1,2,3-triazol-4-l)butyl]-1H-1,2,3-triazole (CD–CD) **3**, 1-mono-β-cyclodextrin-4-[4-(1-adamantane-1H-1,2,3-triazol-4-yl)butyl]-1H-1,2,3-triazole (CD–Ad) **4**, and 1-adamantane-4-[4-(1-adamantane-1H-1,2,3-triazol-4-yl)butyl]-1H-1,2,3-triazole (Ad–Ad) **5** via microwave-assisted cycloaddition of 6I-azido-6I-deoxycyclomaltoheptaose and 1-azidoadamantane with 1,7-octadiyne. Dynamic light scattering (DLS) was used to investigate the host-guest interactions and the self-assembly properties of the complexes formed by the compound **3** with the dimers **4** and **5**.

Keywords Cyclodextrin and adamantane dimers ·
Microwave-assisted cycloaddition ·
Supramolecular structures

Introduction

Supramolecular chemistry based on fundamental principles like non covalent interactions, molecular recognition and self-assembly processes received a great consideration in the last years mainly due its role to connect the world of single molecules with that of nano-biotechnology [1]. The recent progresses in this field are also the contribution of 'click' methodology, a powerful strategy that affords the modular assembly of new molecular entities [2–7]. The strong interrelation between the two research areas

imposed the new concept of 'supramolecular click chemistry' [8], as a valuable tool in bioconjugation [7, 9], drug discovery [10–13] and material science [14–16]. The reference host compounds and the most important molecular receptors studied in supramolecular chemistry are cyclodextrins (CD), being appealing and versatile structures for the design of inclusion complexes with hydrophobic guests [17–21]. The strong binding of hydrophobic ditopic substrates into dimeric cyclodextrins, with potential application as antibody or enzyme mimics was demonstrated by Breslow [22–30]. Regarding the guest-modified CD, the couple cyclodextrin/adamantane has been intensive investigated, due its complementarity and reversible interaction [17], these features being of great interest for the design of self-associating biopolymers [31–33]. The use of microwave (MW)-assisted 'click' methodology to prepare CD-dimers and guest-dimers has not been reported. In the present research we thus describe the synthesis of three types of dimers via Cu(I)-mediated cycloaddition of 6I-azido-6I-deoxycyclomaltoheptaose and 1-azidoadamantane with 1,7-octadiyne (Scheme 1), respectively the study of their water-soluble inclusion complexes.

Experimental

IR spectra were recorded with a Nicolet 5 SXB FT-IR Spectrometer, equipped with an ATR unit. NMR spectra were recorded with a Bruker AC 500 at 20 °C. Chemical shifts were referenced to the solvent value δ 2.51 for DMSO-d_6, δ 4.79 for D_2O and δ 7.26 for $CDCl_3$. MALDI-TOF-MS was performed on a Bruker Ultraflex TOF time-of-flight mass spectrometer using a 337 nm nitrogen laser. GC-MS measurements were realised with a Thermo Finnigan Trace DSQ system. Dynamic Light Scattering (DLS) experiments

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Scheme 1 Dimer synthesis via
click methodology
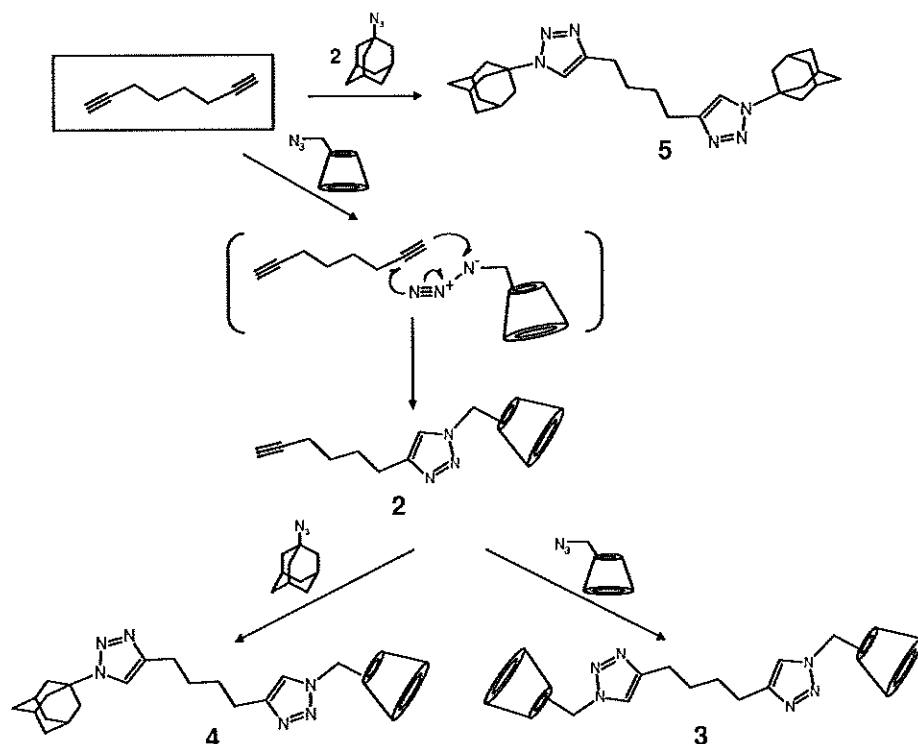
were carried out with a Malvern HPPS-ET at a temperature value of 25 °C. The particle size distribution was derived from a deconvolution of the measured intensity autocorrelation function of the sample by the *general purpose mode* algorithm included in the *DTS* software. Each experiment was performed five times to obtain statistical information. MW-assisted synthesis was performed using a CEM Discover Synthesis Unit (monomode system). The temperature was measured by infrared detection with continuous feedback temperature control, and maintained at a constant value by power modulation. Reactions were performed in closed vessels under controlled pressure. Commercially available reagents and solvents were used without further purification. Cyclodextrin (β-CD) was obtained from Wacker-Chemie GmbH, Burghausen, Germany and used after drying overnight in vacuum oil-pump, on P_4O_{10}. 6I-Azido-6I-deoxycyclomaltoheptaose (**1**) was prepared according to a method described in literature [34].

6I-Azido-6I-deoxycyclomaltoheptaose 1

6I-Azido-6I-deoxycyclomaltoheptaose was prepared from mono-(6-O-(p-tolylsulfonyl))-β-Cyclodextrin and sodium azide [34]. The product was dried in oil-pump and maintained in dessicator over P_4O_{10}. IR: ν 1,025, 1,077, 1,152, 1,364, 2,043, 3,316 cm^{-1}; ^{1}H-NMR (500 MHz; DMSO-d$_6$) δ: 3.32 (14H, CH), 3.60–3.84 (br, 28H), 4.44–4.60 (br, 6H), 4.85 (d, 6H), 4.92 (H, CH), 5.73 (14H); *m/z* 1,182.4 [M + Na$^+$].

Mono-(1H-1,2,3-triazol-4-yl)-4-hex-5-ynyl-β-cyclodextrin 2

The compound **2** was prepared according to the following method: 1,7-octadiyne (4.55 mg, 0.043 mmol) was added to a solution of 6I-azido-6I-deoxycyclomaltoheptaose (100 mg, 0.086 mmol) in 2 mL DMF in a pressure-resistant test tube. To the clear solution were added sodium ascorbate (0.85 mg, 0.0043 mmol) and copper (II) sulfate pentahydrate (0.5 mg, 0.0021 mmol). The tube was sealed and placed in the CEM monomode MW and irradiated at 150 °C and 130 W for 30 min. The product was collected by filtration, after precipitating with acetone (50 mL). In order to remove the unreacted 6I-azido-6I-deoxycyclomaltoheptaose, the white solid was re-dissolved in methanol (50 mL), than the compound **2** was isolated by evaporating the methanolic solution. IR: ν 1,020, 1,081, 1,153, 1,388, 1,655, 2,930, 3,309 cm^{-1}; ^{1}H-NMR (500 MHz; DMSO-d$_6$) δ: 1.24 (1H), 1.51–1.69 (8H), 3.32 (14H), 3.57–3.65 (br, 28H), 4.48 (6H), 4.84 (7H), 5.79 (14H), 8.47 (1H); *m/z* 1,288.5 [M + Na$^+$].

1-Mono-β-cyclodextrin-4-[4-(1-mono-β-cyclodextrin-1H-1,2,3-triazol-4-yl)butyl]-1H-1,2,3-triazole 3

The compound **3** was obtained from compound **2** (54 mg, 0.043 mmol) and compound **1** (100 mg, 0.086 mmol), which were solved in 2 mL DMF in a pressure-resistant test

tube. To the reaction mixture was added the catalytic system, consisting of sodium ascorbate (0.85 mg, 0.0043 mmol) and copper (II) sulfate pentahydrate (0.5 mg, 0.0021 mmol). The tube was irradiated at 150 °C and 130 W for 30 min. in the CEM monomode MW. The white solid collected by filtration, after precipitating with acetone (50 mL) was suspended in methanol (50 mL) and the dimer 3 was isolated by filtration. We tried also to obtain the compound 3 from the direct reaction of 1,7-octadiyn (4.55 mg, 0.043 mmol) with 6I-azido-6I-deoxycyclomaltoheptaose (100 mg, 0.086 mmol), solved in 2 mL DMF, in the presence of sodium ascorbate (1.7 mg, 0.0086 mmol) and copper (II) sulfate pentahydrate (1 mg, 0.0043 mmol). After irradiating the reaction mixture at 150 °C and 130 W for 60 min, we obtained a mixture of compounds 2 and 3, with a ratio of 2:1. IR: v 1,025, 1,086, 1,148, 1,337, 1,655, 2,925, 3,319 cm^{-1}; ^{1}H-NMR (500 MHz; D_2O) δ: 1.49–1.94 (16), 3.59–3.66 (28 H), 3.85–3.96 (br, 6H), 4.44–4.60 (br, 12H), 4.87 (d, 12H), 4.92 (2H), 5.07 (28H), 8.38 (2H); m/z 2,447.7 [M + Na^+].

1-Mono-β-cyclodextrin–4-[4-(1-adamantane-1H-1,2,3-triazol-4-yl)butyl]-1H-1,2,3-triazole 4

The compound 4 was prepared using the monomer 2 (176.6 mg, 0.14 mmol) and azido-adamantane (100 mg, 0.56 mmol) as starting materials, solved in 2 mL DMF, in a pressure-resistant test tube, in the presence of sodium ascorbate (5.5 mg, 0.028 mmol) and copper (II) sulfate pentahydrate (3.4 mg, 0.014 mmol). The reaction mixture was irradiated 120 °C and 130 W for 30 min, than poured in 50 mL acetone, and the solid product isolated by filtration and dried at room temperature. IR: v 1,020, 1,076, 1,143, 1,352, 1,655, 1,685, 2,852, 2,930, 3,309 cm^{-1}; ^{1}H-NMR (500 MHz; DMSO-d_6) δ: 1.51 (6H), 1.65 (6H), 2.17 (4H), 3.32 (14H), 3.57–3.65 (br, 28H), 4.48 (6H), 4.84 (6H), 4.88 (2H), 4.92 (H), 5.70 (14H), 7.79, 8.44; m/z 1,465.8 [M + Na^+].

1-Adamantane-4-[4-(1-adamantane-1H-1,2,3-triazol-4-yl)butyl]-1H-1,2,3-triazole 5

The compound 5 was obtained from 1,7-octadiyne (14.87 mg, 0.14 mmol) and azido-adamantane (100 mg, 0.56 mmol) as starting materials, which were solved in 2 mL DMF, in a pressure-resistant test tube. After adding sodium ascorbate (5.5 mg, 0.028 mmol) and copper (II) sulfate pentahydrate (3.4 mg, 0.014 mmol), the reaction mixture was heated at 120 °C and 130 W for 20 min. The solid product precipitated during the reaction was isolated by filtration and washed with acetone, in order to remove the unreacted azido-adamnantane, than was and dried at room temperature. IR v: 1,015, 1,255, 1,358, 1,434, 1,542, 1,675,

2,858, 2,904, 3,099 cm^{-1}; 1H-NMR (500 MHz; $CDCl_3$) δ: 1.20–1.41 (br, 12H, CH_2), 1.71 (4H), 2.24 (4H) 2.74 (br, 12H, CH_2), 7.36 (6H, CH), 7.70 (2H, CH); m/e 461.

Results and discussions

6I-Azido-6I-deoxycyclomaltoheptaose was synthesized according to a method described in literature [34]. In order to obtain CD–CD and CD–adamantane (CD–Ad) dimers, we prepared first the monomer 2 from 6I-azido-6I-deoxy-cyclomaltoheptaose and 1,7-octadiyne, applying Cu(I)-catalysed cycloaddition under microwave conditions (Scheme 1). Using this monomer for the synthesis of CD–CD dimer (3) in a two steps-procedure instead of the direct reaction of 6I-azido-6I-deoxycyclomaltoheptaose with 1,7-octadiyne provided the complete conversion into dimer, while the direct reaction led to a mixture of monomer (2) and dimer (3), with a ratio of 2:1. CD-monomer 2 was further used for the synthesis of the mix dimer CD–Ad (4). The guest adamantane-adamantane (Ad–Ad) dimer 5 was also obtained by MW-assisted 'click' reaction of azido-adamantane with 1,7-octadiyne, the reaction being easier to perform than the procedure applying to 6I-azido-6I-de-oxycyclomaltoheptaose. The compounds isolated after cycloaddition were characterised by ^{1}H-NMR, FT-IR, MALDI-TOF and GC-MS spectroscopy. The ^{1}H-NMR spectra confirmed all proposed structures, the chemical shifts of the triazolic proton being observed at 8.47 ppm (2), 8.38 ppm (3), 7.79 and 8.44 ppm (4), respective 7.70 ppm (5) (Fig. 1). The successful completion of each reaction was also proven by the absence of the specific N_3 vibrations at 2,043 cm^{-1} for 6I-azido-6I-deoxycyclomal-toheptaose and 2,090 cm^{-1} for 1-azidoadamantane and also of the band detected at 2,100 cm^{-1} for 1,7-octadiyne in the FT-IR spectra. Instead, we could observe new peaks at 1,655 cm^{-1}, specific for carbon-carbon double bond which, associated with the vibration of carbon-nitrogen bond at 1,020 cm^{-1}, proving the existence of triazole. For the mixed dimer 4 we identified, beside the specific peaks for triazole, both the vibrations for hydroxyl groups of CD at 3,344 cm^{-1} and for methylene groups of adamantane at 2,853 cm^{-1}. The molecular weight of the compounds 2, 3 and 4 was measured by MALDI-TOF mass spectroscopy, the values [M + Na^+] being 1,288.4, 2,447.7, respectively 1,465.8. Regarding the dimer 5, we obtained a molecular weight of 461 g/mol.

We investigated the tendency of synthesized dimers to form supramolecular complexes in water, using dynamic light scattering. An interesting behavior was observed for distinct water-soluble CD–CD (3) and CD–Ad (4) dimers. Therefore, the mean hydrodynamic radius size of 40 nm and 49 obtained for a complexation time of 1 h and for a

Fig. 1 ^{1}H-NMR (500 MHz, D_2O) spectra showing signals for the protons of the triazole on CD–CD dimer (a) as well as on Ad–Ad (b) complexed with CD–CD (▼: signals of solvent (DMF))
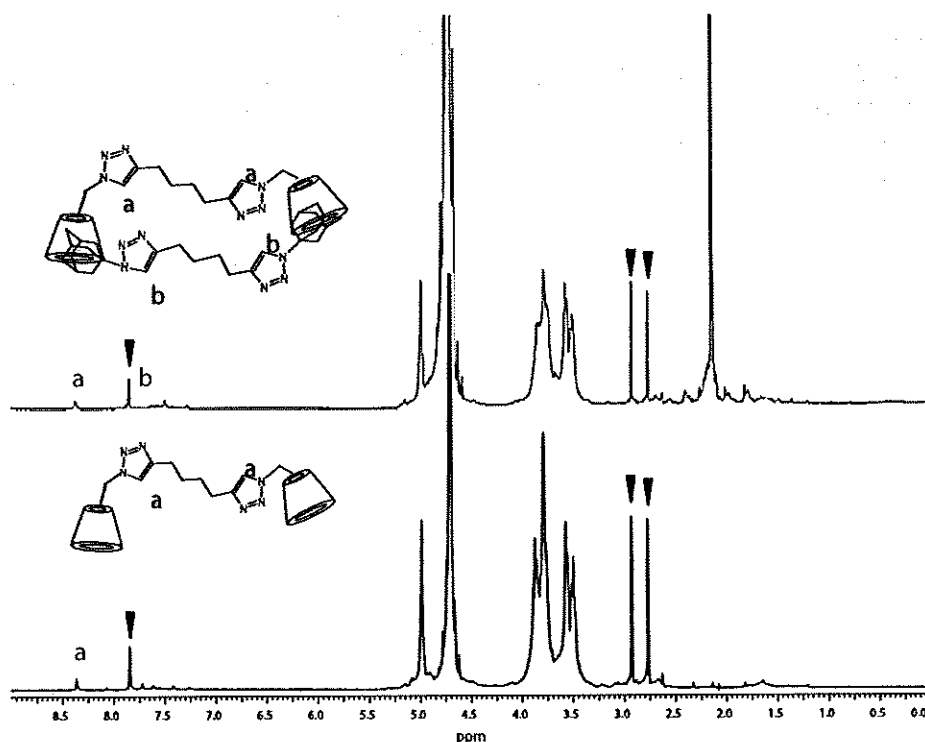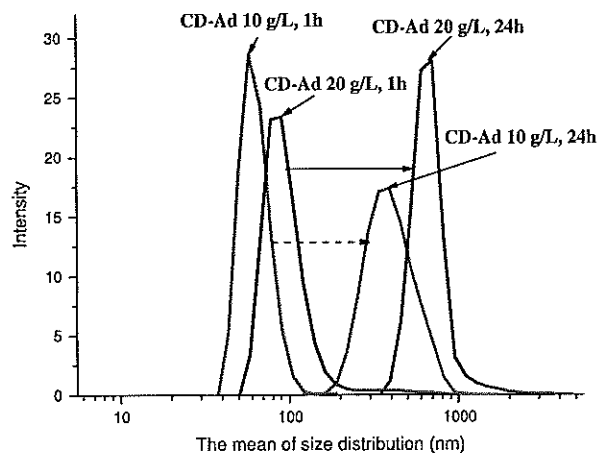

Table 1 Size distribution of synthesized dimers

Compound (No.)	Concentration (g/l)	Time of complexation (h)	Mean of size distribution by number (nm)
CD–CD (3)	10	1	40
		24	240
CD–CD (3)	20	1	49
		24	310
CD–Ad (4)	10	1	65
		24	415
CD–Ad (4)	20	1	92
		24	695

Fig. 2 Hydrodynamic volumes of CD–Ad dimer depending on concentration and complexation time

concentration of 10 g/L, respective 20 g/L, which increased up to 240 nm and 310 nm after 24 h, suggests that this dimer is self-associated through hydrogen bonds in water (Table 1).

The results confirm these reported by Bonini et al., according with β-CD monomers do aggregate in water at room temperature, with a mean value of hydrodynamic radius size of 90 nm [35, 36]. The same effect was observed for the mix dimer CD–Ad (4), the increased concentration and complexation time being favorable for the self-assembly process in water (Fig. 2). A greater hydrodynamic radius of 695 nm was obtained for higher concentration (20 g/L) after 24 h, in contrast to 310 nm obtained for the CD–CD (3) dimer in the same conditions.
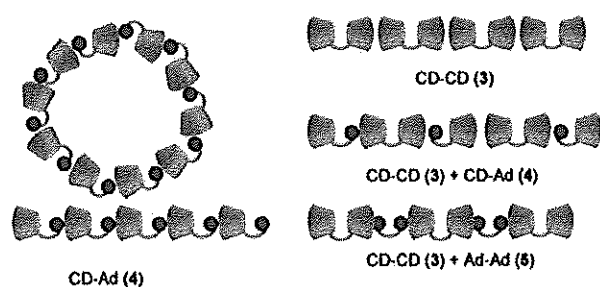
These results suggest the preference for self-association to hydrogen bonds detriment. This preferential behavior of CD–Ad dimer (4) is proved also by the decreased value of hydrodynamic radius in the presence of CD–CD (3) dimer for a low time of complexation, while increasing the time to 24 h lead to higher radius size (700 nm for a concentration of 10 g/L and 1,820 nm for 20 g/L). In this case we can postulate that the two dimers CD–CD (3) and CD–Ad (4) are associated, beside specific host-guest interactions, through hydrogen bonds between the cyclodextrin units.

Table 2 Size distribution of supramolecular complexes based on synthesized dimers

Compound	Concentration (g/l)	Time of complexation (h)	Mean of size distribution by number (nm)
CD–CD + CD–Ad	10	1	53
		24	700
CD–CD + CD–Ad	20	1	62
		24	1,820
CD–CD + Ad–Ad	10	1	55
		24	1,740
CD–CD + Ad–Ad	20	1	64
		24	3,610

Fig. 3 Proposed supramolecular structures for synthesized dimers and their complexes

We investigated also the specific host-guest interactions of CD–CD dimer (3) with Ad–Ad (5). An interesting behaviour of these complexes was observed by comparing them with the self-assembly CD–CD (3) dimer in water. Lower concentration and complexation time strongly suggested the specific host-guest interaction, while increasing the concentration and the time of guest complexation had as consequence association and aggregation phenomena, expressed by a mean of hydrodynamic volume of 1,740 nm for a concentration of 10 g/L and 3,610 nm for 20 g/L (Table 2).

These values clearly indicate the presence of host-guest processes, which are favoured not only by the complementarity cyclodextrin-adamantane, but also by the flexibility of the butylene (tetramethylene) which connects each two units. Based on hydrodynamic radius size values obtained by DLS measurements we proposed the supramolecular structures of synthesized dimers and their complexes (Fig. 3).

Conclusions

Cyclodextrin- and guest adamantane-dimers were successfully prepared via click methodology, under MW

conditions, using Cu(I) as catalyst. DLS measurements performed for CD–CD (3) and CD–Ad (4) dimers and also for the supramolecular complexes of CD–CD (3) with CD–Ad (4) and Ad–Ad (5) confirmed the ability of cyclodextrin to self-assembly in water, as well as its capacity to recognize and include adamantane moieties placed on the same dimer (as in the case of CD–Ad (4) compound) or on a different one (the interaction of CD–CD (3) with Ad–Ad (5)). By varying the solution concentration and the complexation time, we showed that the dimer CD–CD (3) has the tendency to self-associate through hydrogen bonds, while the inclusion phenomena are specific for CD–Ad (4) compound. Regarding the complexes of CD–CD (3) with the other dimers, we postulate that both types of interactions are possible in the presence of CD–Ad (4) and Ad–Ad (5) compounds, the host-guest complexation being characteristic for the supramolecular structures based on these dimeric combinations.

References

1. Albrecht, M.: Supramolecular chemistry—general principles and selected examples from anion recognition and metallosupramolecular chemistry. Naturwissenschaften 94, 951–966 (2007)
2. Rostovtsev, V.V., Green L.G., Fokin, V.V., Sharpless, K.B.: A stepwise Huisgen cycloaddition process: copper(1)-catalyzed regioselective "ligation" of azides and terminal alkynes. Angew. Chem. 114, 2708–2711 (2002); Angew. Chem. Int. Ed 41, 2596–2599 (2002)
3. Mobian, P., Collin, J.-P., Sauvage, J.-P.: Efficient synthesis of a labile copper(I)-rotaxane complex using click chemistry. Tetrahedron Lett. 47, 4907–4909 (2006)
4. Chittaboina, S., Xie, F., Wang, Q.: One-pot synthesis of triazole-linked glycoconjugates. Tetrahedron Lett. 46, 2331–2336 (2005)
5. Perez-Balderas, F., Ortega-Munoz, M., Morales-Sanfrutos, J., Hernandez-Mateo, F., Calvo-Flores, F.G., Calvo-Asin, J.A., Isac-Garcia, J., Santoyo-Gonzalez, F.: Multivalent neoglycoconjugates by regiospecific cycloaddition of alkynes and azides using organic-soluble copper catalysts. Org. Lett. 5, 1951–1954 (2003)
6. Ortega-Muñoz, M., Morales-Sanfrutos, J., Perez-Balderas, F., Hernandez-Mateo, F., Giron-Gonzalez, M.D., Sevillano-Tripero, N., Salto-Gonzalez, R., Santoyo-Gonzalez, F.: Click multivalent neoglycoconjugates as synthetic activators in cell adhesion and stimulation of monocyte/machrophage cell lines. Org. Biomol.Chem. 5, 2291–2301 (2007)
7. Chan, T.R., Hilgraf, R., Sharpless, K.B., Fokin, V.V.: Polytriazoles as copper(i)-stabilizing ligands in catalysis. Org. Lett. 6, 2853 (2004)
8. Hoogenboom, R., Moore, B.C., Schubert U.S.: Synthesis of star-shaped poly(epsilon-caprolactone) via 'click' chemistry and 'supramolecular click' chemistry. Chem. Commun. 4010–4012 (2006)
9. Breinbauer, R., Köhn, M.: Azide-alkyne coupling: a powerful reaction for bioconjugate chemistry. ChemBioChem 4, 1147–1149 (2003)
10. Moses, J.E., Moorhouse, A.D.: The growing applications of click chemistry. Chem. Soc. Rev. 36, 1249–1262 (2007)

11. Gupta, S.S., Raja, K.S., Eiton, K.S., Kaltgrad, E., Strable, E., Finn, M.G.: Virus-glycopolymer conjugates by copper (I) catalysis of atom transfer radical polymerization and azide-alkyne cycloaddition. Chem. Commun. 4315–4317 (2005)

12. Wang, Q., Chan, T.R., Hilgraf, R., Fokin, V., Sharpless, K.B., Finn, M.G.: Bioconjugation by copper(I)-catalyzed azide-alkyne [3 + 2] cycloaddition. J. Am. Chem. Soc. 125, 3192–3193 (2003)

13. Kolb, H.C., Sharpless, B.: The growing impact of click chemistry on drug discovery. Drug Discovery Today 8, 24 (2003)

14. Diaz, D.D., Punna, S., Holzer, P., McPherson, A.K., Sharpless, K.B., Kokin, V.V., Finn, M.G.: Click chemistry in materials synthesis. 1. Adhesive polymers from copper-catalyzed azide-alkyne cycloaddition. J. Polym. Sci.: Part A: Polym. Chem. 42, 4392–4403 (2004)

15. Binder, W.H., Sachsenhofer, R.: Click chemistry in polymer and materials science. Macromol. Rapid Commun. 28, 15–54 (2007)

16. Lutz, J.-F., Borner, H.G., Weichnehan, K.: Combining ATRP and "click" chemistry: a promising platform toward functional biocompatible polymers and polymer bioconjugates. Macromolecules 39, 6376–6383 (2007)

17. Villalonga, R., Cao, R., Fragoso, A.: Supramolecular chemistry of cyclodextrins in enzyme technology. Chem. Rev. 107, 308–311 (2007)

18. Easton, C.: Cyclodextrin-based catalysts and molecular reactors. J. Pure Appl. Chem. 77, 1865–1871 (2005)

19. Storsberg, J., Ritter, H., Pielartzik, H., Groenendaal, L.: Cyclodextrins in polymer synthesis: supramolecular cyclodextrin complexes of pyrrole and 3, 4-ethylenedioxythiophene and their oxidative polymerization. Adv. Mater. 12, 567–569 (2000)

20. Choi, S., Ritter, H.: Novel polymerization of myrcene in aqueous media via cyclodextrin-complexes. e-Polymers no. 045 (2007)

21. Harada, A.: Supramolecular polymers based on cyclodextrins. J. Polym. Sci.: Part A: Polym. Chem. 44, 5113–5119 (2006)

22. Breslow, R., Greenspoon, N., Guo, T., Zarzycki, R.: Very strong binding of appropriate substrates by cyclodextrin dimers. J. Am. Chem. Soc. 111, 8296–8297 (1989)

23. Zhang, B., Breslow, R.: Enthalpic domination of the chelate effect in cyclodextrin dimers. J. Am. Chem. Soc. 115, 9353–9354 (1993)

24. Breslow, R., Halfon, S., Zhang, B.: Molecular recognition by cyclodextrin dimers. Tetrahedron 51, 377–388 (1995)

25. Breslow, R., Zhang, B.: Cholesterol recognition and binding by cyclodextrin dimers. J. Am. Chem. Soc. 118, 8495–8496 (1996)

26. Leung, D.K., Yang, Z., Breslow, R.: Selective disruption of protein aggregation by cyclodextrin dimers. Proc. Natl. Acad. Sci. USA 97, 5050–5053 (2000)

27. Breslow, R., Belvedere, S., Gershell, L., Leung, D.: The chelate effect in binding, catalysis, and chemotherapy. Pure Appl. Chem. 72, 333–342 (2000)

28. Baugh, S.P.D., Yang, Z., Leung, D.K., Wilson, D.M., Breslow, R.: Cyclodextrin dimers as cleavable carriers of photodynamic sensitizers. J. Am. Chem. Soc. 123, 12488–12494 (2001)

29. Moore, S.: Askew J. A., Gibson, G.L., Aborgrein, A., El-Agnaf, O., Allsop, D., Leung, D.K, Breslow, R.: Novel cyclodextrin dimers and trimers as inhibitors of amyloid peptide aggregation. Neurobiol. Aging 23, S105–S397 (2002)

30. Wilson, D., Perlson, L., Breslow, R.: Helical templating of oligopeptides by cyclodextrin dimers. Bioorg. Med. Chem. 11, 2649–2653 (2003)

31. Bistri, O., Mazeau, K., Auzely-Velty, R., Sollogoub, M.: A hydrophilic cyclodextrin duplex forming supramolecular assemblies by physical cross-linking of a biopolymer. Chem. Eur. J. 13, 8847–8857 (2007)

32. Kretschmann, O., Steffens, C., Ritter, H.: Cyclodextrin complexes of polymers bearing adamantyl groups: host–guest interactions and the effect of spacers on water solubility. Angew. Chem. Int. Ed. 46, 2708 (2007)

33. Kretschmann, O., Choi, S.W., Miyauchi, M., Tomatsu, I., Harada, A., Ritter, H.: Switchable hydrogels obtained by supramolecular cross-linking of adamantyl-containing LCST copolymers with cyclodextrin dimers. Angew. Chem. Int. Ed. 45, 4361–4361 (2006)

34. Ohga, K., Takashima, Y., Takahashi, H., Kawaguchi, Y., Yamaguchi, H., Harada, A.: Preparation of supramolecular polymers from a cyclodextrin dimer and ditopic guest molecules: control of structure by linker flexibility. Macromolecules 38, 5897 (2005)

35. Bonini, M., Rossi, S., Karlson, G., Almgren, M., Lo Nostro, P., Baglioni, P.: Self-assembly of β-cyclodextrin in water. Part 1: Cryo-TEM and dynamic and static light scattering. Langmuir 22, 1478–1484 (2006)

36. Rossi, S., Bonini, M., Bonini, P., Baglioni, P.: Self-assembly of β-cyclodextrin in water. 2. Electron spin resonance. Langmuir 23, 10959–10967 (2007)

Cyclodextrin Methacrylate via Microwave-Assisted Click Reaction

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ABSTRACT: Click reaction of propargyl methacrylate (**1**) with 6I-azido-6I-deoxycyclomaltoheptaose (**2**) was carried out to synthesize mono-(1*H*-1,2,3-triazol-4-yl)(methyl)2-methylacryl-*β*-cyclodextrin (**3**). The process was investigated by varying the reaction time, temperature profiles, and copper catalyst. Microwave irradiation was compared with conventional heating. The microwave-assisted Cu(I)-catalyzed cycloaddition affords the complete conversion of (**2**) into 1,4-disubstituted triazole in a significant decreased reaction time. Under microwave conditions, the cycloaddition of (**2**) onto poly(propargyl methacrylate) (**5**) was conducted in excellent yields. The regioselectivity of click reactions in dependence of reaction conditions was evaluated by use of NMR spectroscopy. The reactions performed under microwave conditions led exclusively to 1,4-disubstituted triazole, while the conventional heating led to a regioisomeric mixture.

Introduction

In recent years, cyclodextrins have been extensively used in polymer chemistry. For example, they are used to complex water-insoluble monomers and polymers.[1–8] In this field, cyclodextrin monomethacrylates have not yet been studied extensively. Thus, we were encouraged to synthesize such polymerizable cyclodextrin monomers via "click chemistry". This type of coupling introduced by Sharpless and summarized as "all searches must be restricted to molecules that are easy to make"[9] has proven to be a versatile, powerful, and multiapplicable tool, intensively exploited in the past years.[10] Cu(I)-catalyzed Huisgen-type 1,3-dipolar cycloaddition of azides and alkynes extended its area of applications in carbohydrate research, organic and supramolecular chemistry, bioconjugation,[11–14] or drug discovery.[15] According to Huisgen[16] and confirmed by Meldal,[17] thermal 1,3-dipolar cycloaddition of alkynes and azides is not a regiospecific reaction. A structural control can be achieved under mild conditions by using active Cu(I) species.[18,19] The microwave-assisted copper-catalyzed 1,3-dipolar cycloaddition received recently much attention due its ability to afford a complete and regioselective conversion with a significant decrease in reaction time. Although click reactions are known in the cyclodextrin chemistry,[20–24] their use to obtain cyclodextrin monomethacrylate has not been reported. Attempts to synthesize monofunctional cyclodextrin esters led to mixtures, and the desired compound was obtained in low yield afther chromatographic purification.[25] By applying click reaction, cyclodextrin monomethacrylate can be obtained in high yields; the purification by chromatographic methods is not required, and the installation and removal of protecting groups are avoided. The triazol linker is relatively stable toward cleavage, oxidation, or reduction. Since 1,3-cycloaddition of 6-monoazido-6-monodeoxy-*β*-cyclodextrin with propargyl derivatives under the synergistic effects of copper catalysis and microwave irradiation was less investigated, we decided to explore different reaction parameters in order to provide an easy and efficient way to obtain polymerizable cyclodextrin monomethacrylates. Furthermore, we describe the click reaction of 6I-azido-6I-deoxycyclomaltoheptaose (**2**) onto poly(propargyl methacrylate) (**5**) to obtain a polymer with potential applications in supramolecular chemistry.

Experimental Section

Materials. Cyclodextrin (*β*-CD) was obtained from Wacker-Chemie GmbH, Burghausen, Germany, and used after drying overnight in vacuum oil pump on P_4O_{10}. 2-Propynyl 2-methacrylate (98%) was purchased from Alfa Aesar GmbH & CoKG, Germany. Sodium azide (99%) was obtained from Aldrich Chemicals, Germany, and used as received. Copper(II) sulfate pentahydrate (99%) was obtained form Carl Roth GmbH & Co. and sodium L(+)-ascorbate (99%) from AppliChem, Germany. α,α'-Azoisobutyronitrile (AIBN) (96%) and *N,N*-dimethylformamide (DMF) were purchased from Fluka, Germany. Dimethyl-d_6 sulfoxide (99.9 atom % D) was obtained from Deutero GmbH, Germany. 6I-Azido-6I-deoxycyclomaltoheptaose (**2**) was prepared according to a method described in the literature.[6]

Measurements. IR spectra were recorded with a Nicolet 5 SXB FTIR (Fourier transform infrared) spectrometer equipped with an ATR unit. The measurements were performed in the range of 4000–300 cm^{-1} at room temperature. ^{1}H spectra were recorded with a Bruker AC 500 at 20 °C. Chemical shifts were referenced to the solvent value δ 2.51 for dimethyl-d_6 sulfoxide. Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF-MS) was performed on a Bruker Ultraflex TOF mass spectrometer. Ions formed with a pulsed nitrogen laser (25 Hz, 337 nm) were accelerated to 25 kV, the molecular masses being recorded in linear mode. 2,5-Dihydroxybenzoic acid (DBH) in acetonitrile/water (25 mg mL^{-1}) was used as a matrix. The samples (1 mg mL^{-1} in water) were mixed with the matrix solution at volumetric ratios of 1:2. Gel permeation chromatography (GPC) analyses were performed on a GPC system from PSS with PSS-WIN-GPC software 4.01, 6.1 with *N,N*-dimethylformamide as eluent. The flow rate was 1 mL min^{-1}, and the column temperature was maintained at 60 °C. 100 μL of a 0.1% (w/w) polymer solution was given to a hydroxyethyl methacrylate (HEMA) column combination that consisted of a precolumn of 40 Å and main columns of 40, 100, and 3000 Å porosities. The number-average molecular weight (M_n) and the polydispersity (PD) were calculated by a calibration curve generated by polystyrene standards with a molecular weight range from 374 to 1 000 000 Da. Dynamic light scattering (DLS) experiments were carried out with a Malvern HPPS-ET in the temperature range 17–75 °C. The particle size distribution was derived from a deconvolution of the measured intensity autocorrelation function of the sample by the general purpose mode algorithm included in the DTS software. Each experiment was

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performed at least five times to obtain statistical information. Microwave-assisted synthesis was performed using a CEM Discover Synthesis Unit (monomode system). The temperature was measured by infrared detection with continuous feedback temperature control and maintained at a constant value by power modulation. Reactions were performed in closed vessels under controlled pressure as well as in standard open vessels under reflux conditions.

Synthesis of the Triazol-CD-Monomer (3). The reaction of 6I-azido-6I-deoxycyclomaltoheptaose (2) (116 mg, 0.1 mmol) with 2-propynyl 2-methacrylate 2 (24.80 mg, 0.2 mmol) was carried out in DMF, in the presence of Cu(I) generated in situ by the reduction of copper sulfate (1.2 mg, 0.005 mmol) with sodium ascorbate (1.98 mg, 0.01 mmol). The product was separated by simple filtration after precipitation with acetone (50 mL). We carried out microwave-assisted cycloaddition, by adding 2-propynyl 2-methacrylate (1) (24.80 mg, 0.2 mmol) to a solution of 6I-azido-6I-deoxycyclomaltoheptaose (2) (116 mg, 0.1 mmol) in 2 mL of DMF in a pressure-resistant test tube. Sodium ascorbate (4 mg, 0.02 mmol) and copper(II) sulfate pentahydrate (2.50 mg, 0.01 mmol) were added to the clear solution. The tube was placed in the CEM monomode microwave and irradiated at 140 °C and 100 W for 30 min. After precipitating the reaction mixture with acetone, 108 mg of product was isolated (84% yield). In order to ensure the equivalence of the reaction parameters, we conducted the reaction under reflux conditions, in oil bath as well as under microwave irradiation. The conventional reaction was performed by preheating the solvent, the reaction mixture being maintained 30 min at reflux temperature, after adding the reagents and catalytic system. The standard open vessel was placed in the CEM monomode microwave, and the reaction was conducted under reflux for 30 min, with a preheating time of 2 min. The product was collected by filtration, after precipitating with 50 mL of acetone (57% yield). By increasing the reaction time under conventional heating, the ratio monomer 3:monomer 4 was 3:1 after 24 h.

FT-IR (film, cm^{-1}): 3351 (OH), 2927 (CH$_2$), 1714 (C=O), 1657 (C=C), 1153 (C−O−C), 1078 (OH), 1026 (C−O). ^{1}H (DMSO-d_6): δ (ppm) 1.86 (3H, CH$_3$), 3.34 (br, 14H, H-2,4), 3.65 (br, 28H, H-3,5,6), 4.52 (br, 6H, OH-6), 4.84 (d, 6H, H-1), 5.03 (2H, −CH$_2$−), 5.19 (H, −CH=), 5.73 (br, 14H, OH-2,3), 6.07 (H, −CH=), 8.13 (1H, CH). MALDI-TOF: m/z 1306.5 [M + Na$^+$].

Homopolymerization of the Triazol-CD-Monomer 3. Mono-(1H-1,2,3-triazol-4-yl)(methyl)2-methylacryl-β-cyclodextrin (3) (128 mg, 0.1 mmol) was solved in 1 mL of DMF and flushed with argon for 5 min. AIBN (α,α'-azoisobutyronitrile) (1.6 mg, 0.005 mmol) was added under an argon atmosphere. The mixture was heated to 65 °C and stirred overnight. The solvent was removed under reduced pressure to afford 60 mg of product (46% conversion).

FT-IR (film, cm^{-1}): 3303 (OH), 2929 (CH), 1720 (C=O), 1653 (C=C), 1558 (N−H), 1153 (C−O), 1023 (C−O). ^{1}H (DMSO-d_6): δ (ppm) 1.89 (3H, CH$_3$), 3.32−3.56 (br, 14H, H-2,4, 28H, H-3,5,6), 4.51 (br, 6H, OH-6), 4.84 (d, 6H, H-1), 5.03 (2H, −CH$_2$−), 5.76 (br, 14H, OH-2,3), 8.13 (1H, CH). $M_n = 1.30 \times 10^4$ g mol^{-1}, PD = 1.4.

Poly(propargyl methacrylate) (5). Propargyl methacrylate (1) (1.241 g, 0.1 mol) was solved in 4 mL of dioxane and flushed with argon for 5 min, and AIBN (α,α'-azoisobutyronitrile) (16.4 mg, 0.01 mmol) was added under an argon atmosphere. The reaction was stopped after stirring 2 h at 65 °C (25% conversion). The reaction mixture was precipitated into methanol and filtered, and the solid was dried under vacuum.

FT-IR (film, cm^{-1}): 3284 (≡C−H), 3002 (C−H), 2942 (CH$_3$), 2135 (C≡C), 1723 (C=O), 1454 (CH$_3$), 1391 (CH$_3$), 1261 (C−O), 1128 (C−O−C). ^{1}H (DMSO-d_6): δ (ppm) 1.9 (3H, CH$_3$), 3.5 (C≡CH), 4.78 (2H, −CH$_2$−), 5.76 (H, −CH=), 6.07 (H, −CH=). $M_n = 1.04 \times 10^5$ g mol^{-1}, PD = 1.6.

Coupling Reaction of Poly(propargyl methacrylate) (5) with 6I-Azido-6I-deoxycyclomaltoheptaose (2). A solution of 128 mg of poly(propargyl methacrylate) (5) in 10 mL of DMF was prepared, and 6I-azido-6I-deoxycyclomaltoheptaose (2) (1.16 g, 1 mmol) was added under vigorous stirring. Sodium ascorbate (19.8 mg, 0.1 mmol) and copper(II) sulfate pentahydrate (12.45

mg, 0.05 mmol) were added to the clear solution. The reaction was conducted under conventional reflux conditions as well as under microwave irradiation. The reaction in oil bath was performed by preheating the polymeric solution, the reaction mixture being maintained 30 min at reflux temperature, after adding 6I-azido-6I-deoxycyclomaltoheptaose (2) and catalytic system. The standard open vessel was placed in the CEM monomode microwave, and the reaction was conducted under reflux for 30 min, with a preheating time of 2 min. The product was collected by precipitating the reaction mixture with 100 mL of acetone followed by filtration. The dry polymeric material was dissolved in water, dialyzed 3 days against distilled water using MWCO 3500 membrane, and freeze-dried. The polymer was obtained in a low yield (16%) under conventional heating, while the microwave conditions afforded a higher yield (27%).

FT-IR (film, cm^{-1}): 3317 (OH), 2923 (CH), 1723 (C=O), 1653 (C=C), 1558 (N−H), 1348 (C−N), 1153 (C−O), 1021 (C−O). ^{1}H (DMSO-d_6): δ (ppm) 1.89 (3H, CH$_3$), 3.32−3.56 (br, 14H, H-2,4, 28H, H-3,5,6), 4.51 (br, 6H, OH-6), 4.84 (d, 6H, H-1), 5.03 (2H, −CH$_2$−), 5.76 (br, 14H, OH-2,3), 8.13 (1H, CH).

Results and Discussion

We carried out the "click reaction" of propargyl methacrylate (1) with 6I-azido-6I-deoxycyclomaltoheptaose (2) to synthesize the new monomer mono-(1H-1,2,3-triazol-4-yl)(methyl)2-methylacryl-β-cyclodextrin (3) useful for the design of polymeric supramolecular architectures. Because of our interest in optimizing the process, we focused on the synergistic effects of microwave irradiation and Cu(I) catalysis. Although the conditions of the microwave-assisted reaction cannot be easily reproduced by the conventional heating, we duplicated some controllable parameters, such as temperature, time, catalyst, and solvent in order to ensure the comparability of the procedures. The active copper(I) catalytic species were generated in situ by reduction of CuSO$_4$ with sodium ascorbate. We used N,N-dimethylformamide as solvent due its high dielectric constant, high boiling point, and ability to solve both starting materials. We performed also the process in water, but no reaction occurred under microwave irradiation. Regarding our interest to investigate the microwave effect on the Cu(I)-mediated cycloaddition and the possible interaction between the dielectric irradiation and the metal catalysis, we approached three different procedures. Cu(I)-free cycloaddition conducted in N,N-dimethylformamide took place rapidly under MW irradiation. Applying the conventional heating, we obtained slowly a relatively low conversion, which remained constant with increasing reaction time (Figure 1).

Cu(I)-mediated click reaction conducted under microwave irradiation favored the selective 1,4-conversion in 3 (Figure 2). The reaction was completed after 30 min. In contrast, the oil bath procedure under similar conditions afforded a mixture of 3 and 4 with a relatively high conversion in 3 after increased reaction time (Figure 3).

The regioisomeric proportion was determined by ^{1}H NMR spectroscopy. The chemical shifts of the triazole ring protons were used to distinguish isomers 3 and 4. The H4 proton of the 1,5-disubstituted triazole is located at 8.23 ppm. The resonance of the H5 proton of the 1,4-disubstituted triazol isomer is at 8.13 ppm.

Without any heating, the cycloaddition proceeded regioselectively; however, extremely increased reaction time is required (74% conversion after 1 week).

As mentioned above, the Huisgen cycloaddition is not a selective process, while Cu(I)-mediated reactions afford exclusively 1,4-disubstituted regioisomers.[16,17] As the mild catalyzed reaction resulted in extremely long reaction time, we
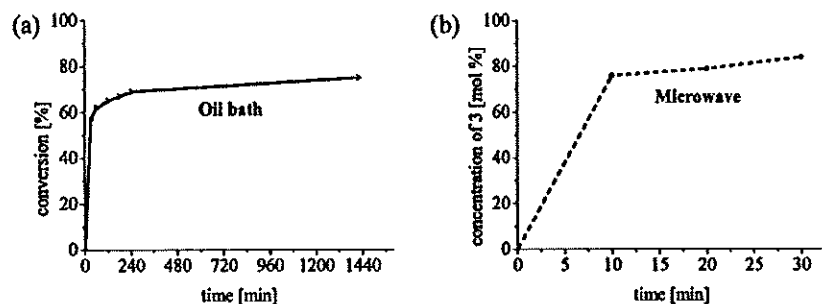
Figure 1. Kinetics of the click reaction under conventional heating (a) and microwave conditions (b). The temperature in microwave and oil bath was 140 °C. Respective reactions were performed in N,N-dimethylformamide, using $CuSO_4 \cdot 5H_2O$ and Na ascorbate as catalytic system. Conversion was determined by ^1H analysis of the isolated product.
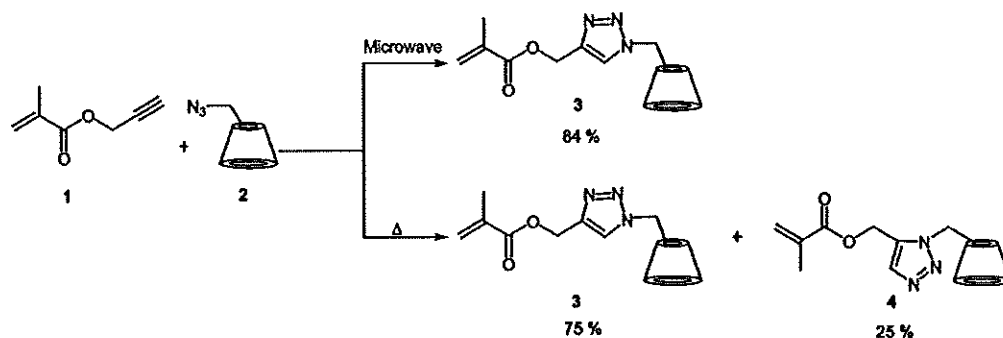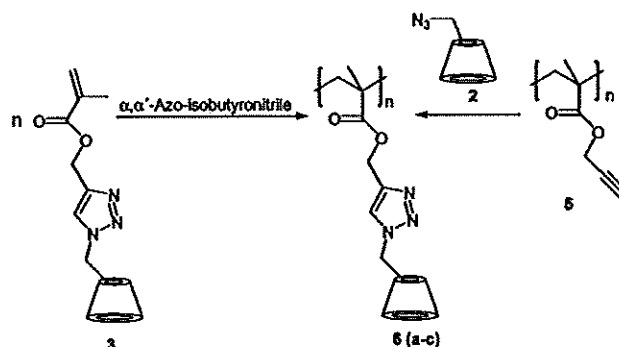

Figure 2. Influence of microwave and conventional conditions conditions on the regioselectivity of the reaction. The reactions were performed at 140 °C in N,N-dimethylformamide, using $CuSO_4 \cdot 5H_2O$ and Na ascorbate as catalytic system. The reaction time was 30 min under microwave heating and 24 h in an oil bath.
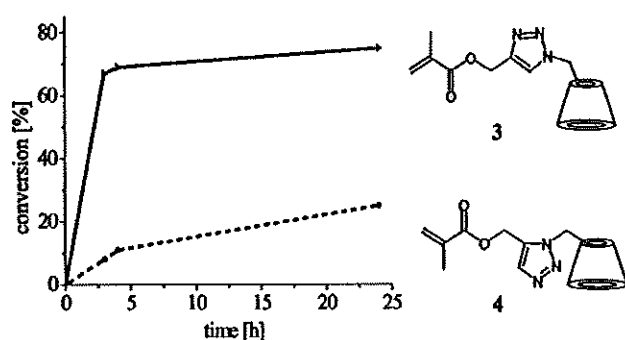
Figure 3. Regiosomeric proportion afforded by Cu-mediated cycloaddition under conventional heating. Reactions were performed in N,N-dimethylformamide at 140 °C.

Figure 4. Homopolymerization of monoacrylate cyclodextrin monomer (3) and click type coupling reaction of poly(propargyl methacrylate) (5) with 6I-azido-6I-deoxycyclomaltoheptaose (2). 6a: the polymer obtained after the coupling reaction in oil bath, in N,N-dimethylformamide, at 140 °C, after 30 min; 6b: the polymer obtained after the coupling reaction under microwave heating, using similar conditions; 6c: the polymer obtained after the free-radical polymerization of cyclodextrin methacrylate.

pursued the workup under thermal conditions. In order to explore and compare the effects induced by the microwave irradiation and the conventional heating under copper catalysis, we applied a fixed temperature for the same reaction time in both cases. We found that under conventional heating, even mediated by Cu(I), the reaction affords a mixture of 1,4- and 1,5-disubstituted regioisomers, while the tandem Cu(I) microwave assures structural control and a significant higher conversion for the same time. Furthermore, we performed the Cu(I)-mediated click reaction under reflux in MW and also in oil bath. The same reaction time was achieved by preheating the solvent under conventional reflux conditions. A higher conversion of 87% was obtained under microawe irradiation in comparison with 62% obtained under conventional heating. By comparing the results obtained under different reaction conditions (microwave, oil bath, and room temperature, with or without catalyst), we can conclude that the synergy Cu(I) microwave assured the highest conversion in compound 3 with a significant decrease in reaction time (Figure 2).

Attempts to polymerize the monomethacrylate cyclodextrin (3) resulted only in water-soluble oligomers (6) ($M_n = 1.30 \times 10^4$ g mol^{-1}, PD = 1.4, MALDI-TOF: m/z 9150.1 [M + Na$^+$]). The reason for the low molecular weight may be due to the bulky cyclodextrin ring. Thus, a polymer analogous coupling of 6I-azido-6I-deoxycyclomaltoheptaose (2) onto poly(propargyl methacrylate) (5) was carried out (Figure 4). Since atom transfer radical polymerization (ATRP) of propargyl methacrylate was reported to result in high polydispersities (PD > 3) and cross-linked networks,[26] we used classical free-radical-initiated polymerization in dioxane. The obtained polymer ($M_n = 1.04 \times 10^5$ g mol^{-1}, PD = 1.6) was completely soluble in N,N-dimethylformamide.

The coupling reaction of 2 onto 5 was monitored by IR and NMR spectroscopy. The successful reaction was confirmed by
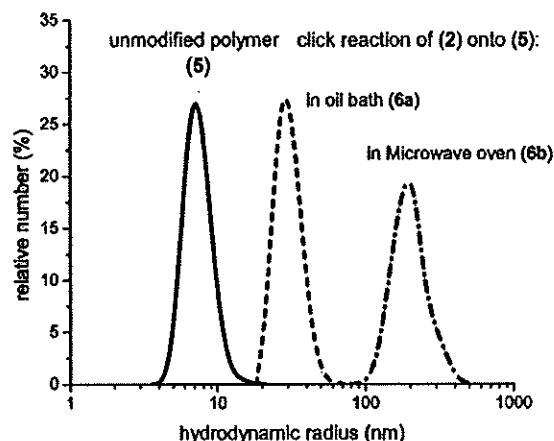
Figure 5. Hydrodynamic volumes of polymer 5 in N,N-dimethylformamide solution before and after coupling with 2 under conventional reflux conditions (6a) and MW irradiation (6b) (polymer concentration = 10 g/L, 25 °C).
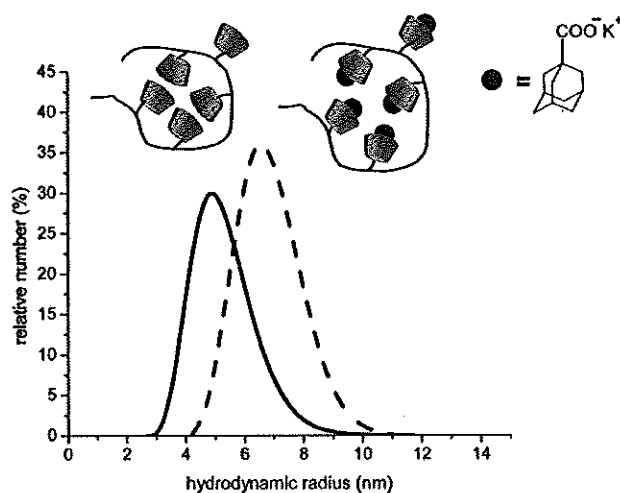
Figure 6. Hydrodynamic volume of poly(methacrylate cyclodextrin) (6c) in N,N-dimethylformamide solution before (—) and after (- - -) complexation with guest molecules (polymer concentration = 10 g/L, 25 °C).

Scheme 1. Synthesis of Triazol-CD-Monomer (3) via "Click Chemistry"
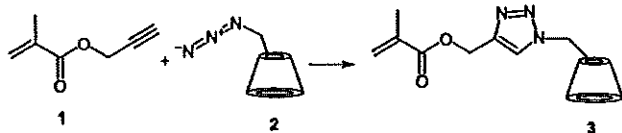
disappearance of the specific IR bands for azide and C≡CH bonds at 2100 and 2135 cm^{-1} and the presence of the peaks for the hydroxyl groups of cyclodextrin at 3317 cm^{-1} as well as of the new peaks at 1558 and 1387 cm^{-1}, indicating the appearance of N—H and C—N bonds. The presence of the triazole ring was also confirmed by NMR spectroscopy at 8.13 ppm.

The size of polymers **6a** and **6b** was investigated in N,N-dimethylformamide solution by DLS measurements. As expected, the hydrodynamic radius increased significantly after the cycloaddition of **2** onto poly(propargyl methacrylate) (**5**). Surprisingly, however, the yield of the product resulted after microwave coupling reaction was significantly higher compared to conventional coupling under oil bath conditions (Figure 5).

DLS measurements were also carried out for poly-(monomethacrylate cyclodextrin) (**6c**) in DMF solution. The low hydrodynamic radius (5 nm) suggests intrachain interactions due to cyclodextrin units. By repeating the measurement using adamantyl carboxylate as guest molecules, we noticed a slightly increased hydrodynamic radius (6 nm), which can be the result of electrostatic repulsion between the carboxylate groups and because of reducing the H-bonds interaction between the dimerized cyclodextrin rings (Figure 6). Similar results were obtained by exposure to LiCl (10%) as a consequence of breaking the intrachain H-bond interactions.

Conclusions

On the basis of the advantages conferred by the MW-assisted Cu-catalyzed "click reaction", we developed an elegant and efficient approach for the synthesis of polymerizable cyclodextrin monomethacrylate, under complete structural control and with an important decrease in the reaction time. IR and NMR measurements correlated with DLS investigations confirmed the polymerization of cyclodextrin monomethacrylates as well as the coupling of 6I-azido-6I-deoxycyclomaltoheptaose (**2**) with the acetylene units placed onto poly(propargyl methacrylate) (**5**). The resulting polymer with a significant content of covalently attached cyclodextrins has potential applications in supramolecular chemistry, drug delivery, and analytics.

References and Notes

(1) Kretschmann, O.; Steffens, C.; Ritter, H. *Angew. Chem., Int. Ed.* **2007**, *46*, 2708–2711.
(2) Koopmans, C.; Ritter, H. *J. Am. Chem. Soc.* **2007**, *129*, 3502–3504.
(3) Kretschmann, O.; Choi, S. W.; Miyauchi, M.; Tomatsu, I.; Harada, A.; Ritter, H. *Angew. Chem., Int. Ed.* **2006**, *45*, 4361–4365.
(4) Köllisch, H.; Barner-Kowollik, Ch.; Ritter, H. *Macromol. Rapid Commun.* **2006**, *27*, 848–853.
(5) Schmitz, S.; Ritter, H. *Angew. Chem., Int. Ed.* **2005**, *44*, 5658–5661.
(6) Ohga, K.; Takashima, Y.; Takahashi, H.; Kawaguchi, Y.; Yamaguchi, H.; Harada, A. *Macromolecules* **2005**, *38*, 5897–5904.
(7) Harada, A.; Hashidzume, A.; Takashima, Y. *Adv. Polym. Sci.* **2006**, *201*, 1–43.
(8) Wenz, G. *Angew. Chem., Int. Ed.* **1994**, *33*, 803–822.
(9) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, 2004–2021.
(10) (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem.* **2002**, *114*, 2708–2711. (b) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599.
(11) Chittaboina, S.; Xie, F.; Wang, Q. *Tetrahedron Lett.* **2005**, *46*, 2331–2336.
(12) Breinbauer, R.; Köhn, M. *ChemBioChem* **2003**, *4*, 1147–1149.
(13) Gupta, S. S.; Raja, K. S.; Eiton, K. S.; Kaltgrad, E.; Strable, E.; Finn, M. G. *Chem. Commun.* **2005**, 4315–4317.
(14) Wang, Q.; Chan, T. R.; Hilgraf, R.; Fokin, V. V.; Sharpless, K. B.; Finn, M. G. *J. Am. Chem. Soc.* **2003**, *125*, 3192–3193.
(15) Kolb, H. C.; Sharpless, B. *Drug Discovery Today* **2003**, *8*, 1128–1137.
(16) Huisgen, R. *Angew. Chem., Int. Ed.* **1963**, *2*, 565–598.
(17) Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057–3064.
(18) Kaval, N.; Ermolat'ev, D.; Appukkuttan, P.; Dehaen, W.; Kappe, O.; Van der Eycken, E. *J. Comb. Chem.* **2005**, *7*, 490–502.
(19) Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, B. K.; Fokin, V. V. *J. Am. Chem. Soc.* **2005**, *127*, 210–216.
(20) Perez-Balderas, F.; Ortega-Munoz, M.; Morales-Sanfrutos, J.; Hernandez-Mateo, F.; Calvo-Flores, F. G.; Calvo-Asin, J. A.; Isac-Garcia, J.; Santoyo-Gonzalez, F. *Org. Lett.* **2003**, *5*, 1951–1954.
(21) Ortega-Muñoz, M.; Morales-Sanfrutos, J.; Perez-Balderas, F.; Hernandez-Mateo, F.; Giron-Gonzalez, M. D.; Sevillano-Tripero, N.; Salto-Gonzalez, R.; Santoyo-Gonzalez, F. *Org. Biomol. Chem.* **2007**, *5*, 2291–2301.

(22) Chan, T. R.; Hilgraf, R.; Sharpless, K. B.; Fokin, V. V. *Org. Lett.* **2004**, *6*, 2853–2855.

(23) Hoogenboom, R.; Moore, B. C.; Schubert, U. S. *Chem. Commun.* **2006**, *38*, 4010–4012.

(24) Barr, L.; Lincoln, S.; Easton, C. *J. Supramol. Chem.* **2005**, *17*, 547–555.

(25) Harada, A.; Furue, M.; Nozakura, S. *Macromolecules* **1976**, *9*, 701–704.

(26) Sumerlin, B. S.; Tsarevsky, N. T.; Louche, G.; Lee, R. Y.; Matyjaszewski, K. *Macromolecules* **2005**, *38*, 7540–7545.

Monoacrylated cyclodextrin via "click" reaction and copolymerization with N-isopropylacrylamide: guest controlled solution properties

SooWhan Choi · Maricica Munteanu · Helmut Ritter

Abstract Mono-(1 *H*-1, 2, 3-triazol-4-yl) (methyl) 2-methylacryl-β-cyclodextrin (CD) **3** is synthesized by microwave assisted 'click' reaction of propargyl (meth) acrylate and mono-(6-azido-6-deoxy)-β-CD using Cu (I) catalysis. Free radical copolymerizations of monomer **3** with *N*-isopropylacrylamide **5** (NIPAAM) of different portions are carried out. The properties of polymers are characterized by use of gel permeation chromatography (GPC), dynamic light scattering (DLS) and turbidity measurements. The Lower Critical Solution Temperatures (LCSTs) of the copolymers with different portions of NIPAAM and influence of guest molecules addition by adamantly carboxylate and adamantly methyl methacrylate are evaluated.

Introduction

The construction of molecular architectures by self-organization is an attractive subject not only for chemists but also for material scientists [1]. Cyclodextrin (CD) is one of the most popular host molecules to create molecular assemblies [2]. CD can form an inclusion complex with molecules having an apolar segment [3, 4]. Low-molecular-weight substrates as well as smart polymeric chains can be complexed with one or more CD molecules [5–18].

'Click' reactions are per definition modular, tolerant towards a wide range of solvents and functional groups, simple to perform, and very high yielding. Click reactions [19–27] have already been used successfully to prepare enzyme inhibitors in situ [19], to functionalize surfaces [20–22], and to synthesize dendritic polymers [23, 24]. For example, Zhao et al. reported the preparation of water-soluble calixarenes using Huisgen 1, 3-dipolar cycloaddition of an azide and an alkyne to form a triazole [26], one of the most efficient click reactions to date [27].

Recently, the click-type synthesis of star-shaped heptakis-poly (ε-caprolactone)-β-cyclodextrin was reported [28]. Easton et al. observed that the propylamido-CD derivative serves as a molecular reactor for the regioselective click-type synthesis of 1, 5-disubstituted-1, 2, 3-triazols [29]. However, although β-CD symmetrical macrocycles with enhanced water solubility were prepared using 1, 3-dipolar cycloaddition [30, 31], up to now, the synthesis of a 'mono substituted CD derivative through microwave assisted click-chemistry is not reported. In the present paper, we thus describe the effective synthesis of a (meth) acryl group functionalized β-CD derivative via click reaction under microwave assisted conditions and its copolymerization with *N*-isopropylacrylamide (NIPAAM). In addition, we investigated the effect of inclusion of suitable guest molecules into the cavity of polymer attached CD ring on the thermo-sensitivity and coil dimensions of corresponding aqueous polymer solutions.

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Experimental section

Materials

Cyclodextrin (β-CD) was obtained from Wacker-Chemie GmbH, Burghausen, Germany and used after drying overnight in vacuum oil-pump, on P_2O_5. 2-propynyl 2-methacrylate was purchased from Alfa Aesar GmbH & CoKG, Germany. 2-propynyl acrylate, N-isopropylacrylamide (NIPAAM) and sodium azide were obtained from Aldrich Chemicals, Germany and used as received. Copper (II)-sulfate pentahydrate was obtained form Carl Roth GmbH & Co, and sodium L (+)-ascorbate from Appli-Chem, Germany. α, α-Azo-isobutyronitril and N, N-dimethylformamide (DMF) were purchased from Fluka, Germany. Deuterated DMSO was obtained from Deutero GmbH, Germany.

Synthesis of monomers

Synthesis of mono-(6-O-(p-tolylsulfonyl))-β-Cyclodextrin: Mono-(6-O-(p-tolylsulfonyl))-β-cyclodextrin was synthesized according to the known procedure [10]. The fine product occurred (15.32 g, 27% yield) was dried in oil-pump over P_2O_5.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 2.45 (s, 3 H, H-11), 3.18–3.78 (br overlapped, H-2, 3, 4, 5, 6, 42H), 4.79 (2 H, H-1), 4,86 (t, 5 H, H-1), 5.74 (br, 14 H, OH-2, 3), 7,45 (d, ^3J_H=8,2 Hz, 2 H, H-9), 7.77 (d, ^3J_H=8,2 Hz, 2 H, H-8), FT-IR (film): 3,316 (OH), 2924 (CH), 2,160 (CH), 1,358 (S = O), 1,152 (S-O), 1,077 (OH), 1,023 (CH), 945, 841, 756 (OH), 684 (CH), MALDI-TOF: m/z 1311.3 [M+Na$^+$].

Synthesis of mono-(6-azido-6-desoxy)-β-Cyclodextrin 1: Mono-(6-azido-6-desoxy)-β-cyclodextrin was synthesized according to the known procedure [10]. The white precipitate was isolated by filtering and dried over P_2O_5, to give 7.49 g (85% yield) solid product.

^{1}H NMR (DMSO-d_6): δ (ppm) 3.32 (14 H, H-2, 4), 3.60–3.84 (br, 28 H, H-3, 5, 6), 4.44–4.60 (br, 6 H, OH-6), 4.85 (d, 6 H, H-1), 4.92 (H, H-1), 5.73 (14 H, OH-2, 3), FT-IR (film): 3,316 (OH), 2,924 (CH), 2,102 (N = N), 1,644 (C = C), 1,364 (OH), 1,152 (C-N), 1,077 (OH), 1,025 (CH), 945, 853, 756 (NH), 704 (CH), MALDI-TOF: m/z 1,182.4 [M+Na$^+$].

General procedure for the synthesis of the triazol-CD-monomer 3 (or 4): The reaction of mono-(6-azido-6-desoxy)-β-CD 1 (100 mg, 0.086 mmol) with 2-propynyl 2-methacrylate 2 (32.02 mg, 0.256 mmol) or 2-propynyl acrylate 2 (28.30 mg, 0.256 mmol) was carried out in water, DMF or a mixed solvent of water and tert-butyl alcohol under reflux, in the presence of Cu(I) generated in situ by

the reduction of copper sulphate (0.01–0.5 equiv.) with sodium ascorbate (0.05–1 equiv.). The product was separated by simple filtration, after precipitation with acetone. We approached also microwave-assisted cycloaddition, by adding 2-propynyl 2-methacrylate 2 (32.02 mg, 0.256 mmol) or 2-propynyl acrylate 2 (28.30 mg, 0.256 mmol) to a solution of mono-(6-azido-6-desoxy)-β-CD 1 (100 mg, 0.086 mmol) in 2 mL DMF in a pressure-resistant test tube. To the clear solution were added sodium ascorbate (5.07 mg, 0.025 mmol) and copper (II) sulfate pentahydrate (3.29 mg, 0.013 mmol). The tube was sealed and placed in the CEM monomode microwave and irradiated at 150 °C and 130 W for 30 min. The products were collected by filtration, after precipitating with acetone (50 mL). In order to obtain optimal reaction conditions we used different amounts of catalyst, varying the reaction time. We find that the presence of water may increase the yield of the reaction, due its favourable effect on the catalytic system.

Monomer 3: ^{1}H NMR (DMSO-d_6): δ (ppm) 1.86 (3 H, CH$_3$), 3.34 (br, 14 H, H-2, 4), 3.65 (br, 28 H, H-3, 5, 6), 4.52 (br, 6 H, OH-6), 4.84 (d, 6 H, H-1), 5.03 (2 H, -CH$_2$-), 5.19 (H, -CH=), 5.73 (br, 14 H, OH-2, 3), 6.07 (H, -CH=), 8.13 (1 H, CH), FT-IR (film): 3,351 (OH), 2,927 (CH), 1,714 (C = O), 1,657 (C = C), 1,331 (OH), 1,153 (C-N), 1,078 (OH), 1,026 (CH), 946 (C = C), 755 (NH), 706 (CH), MALDI-TOF: m/z 1,306.6 [M+Na$^+$].

Monomer 4: ^{1}H NMR (DMSO-d_6): δ (ppm) 3.35 (br, 14 H, H-2, 4), 3.64 (s, br, 28 H, H-3, 5, 6), 4.50 (br, 6 H, OH-6), 4.84 (d, 6 H, H-1), 5.20 (2 H, -CH$_2$-), 5.80 (br, 14 H, OH-2, 3), 6.18 (H, -CH=), 6.37 (H, -CH=), 8.10 (1 H, CH), FT-IR (film): 3,349 (OH), 2,926 (CH), 1,713 (C = O), 1,652 (C = C), 1,387 (OH), 1,253, 1,152 (C-N), 1,078 (OH), 1,024 (CH), 944 (C = C), 754 (NH), 622 (CH), MALDI-TOF: m/z 1,294.5 [M+Na$^+$].

Synthesis of polymers

General procedure for copolymer synthesis (copolymer 6–10): The copolymerization of the monomer 3 with NIPAAM 5 was carried out using redox ($K_2S_2O_8$/ $Na_2S_2O_5$), AIBN (α, α-azo-iosobutyronitrile) and VA-044 (2, 2-azobis [2-(2-imidazolin-2-yl) propane] dihydrochloride) initiator, respectively, as shown in Table 1. The copolymers were obtained according the following method: Monomer 5 (0.174 g, 1.4 mmol, 0.118 g, 1.05 mmol or 0.079 g, 0.7 mmol, respectively) was added to a solution of corresponding comonomer (0.100 g, 0.07 mmol) in 0.7 mL solvent. The solution was flushed with argon for 15 min and the initiator (0.07 mmol,

Table 1 Click reaction under different conditions using Cu (I) catalysis (M: CD-monomer)

M	Condition	Solvent	Time [h]	temp. [°C]	yield [%]
3	Microwave*	DMF	0.5	140	83
4	Microwave*	DMF	0.3	140	60
3	Microwave*	H₂O	0.3	140	–***
3	oil bath	DMF	24	160**	70
3	oil bath	H₂O	72	100**	20

*microwave power was 130 W, **fixed temperature in oil bath, ***no conversion
M: CD-Monomer

respective 0.05 mmol or 0.03 mmol) was added under argon atmosphere. After stirring at initiated temperature overnight, the corresponding copolymers were separated by precipitating with methanol (7), by filtering the hot reaction mixture (7, 8) or by drying it under reduced pressure in freezing machine (10).

^{1}H NMR (DMSO-d$_6$): δ (ppm) 1.05 (br, H, CH$_3$), 1.44 (br, H, CH$_3$), 1.78–2.20 (br, H, CH$_3$), 3.50–3.75 (br, 14 H, H-2, 4,) 3.84 (H, H-3, 5, 6), 4.46 (br, H, OH-6), 4.84 (H, H-1), 5.69 (H, –CH$_2$-), 5.73 (H, OH-2, 3), 7.21 (H, NH), 8.13 (1 H, CH), FT-IR (film):3,286 (OH), 2,970 (CH), 2,930 (CH), 2,160, 2,025, 1,649 (C = C), 1,546 (C-C), 1,439 (C-C), 1,386 (CH), 1,254 (C = O), 1,154 (CN), 1,095 (OH).

General procedure for guest monomer synthesis: Adamantyl methylacrylamide was synthesized according to the known procedure [18].

Characterization

IR spectra were recorded with a Nicolet 5 SXB FT-IR Spectrometer, equipped with an ATR unit. NMR spectra were recorded with a Bruker AC 500 at 20 °C. Chemical shifts were referenced to the solvent value δ 2.51 for DMSO-d$_6$. MALDI-TOF-MS was performed on a Bruker Ultraflex TOF time-of-flight mass spectrometer using a 337 nm nitrogen laser. Gel permeation chromatography (GPC) analyses were performed on a GPC-System from PSS with PSS-WIN-GPC software 4.01, 6.1 with DMF as eluent. The system was calibrated with polystyrene standards with a molecular weight range from 374 up to 1,000,000 Da. The flow rate was 1 mlmin^{-1}. 100 µL of a 0.1% (w/w) polymer solution was given to a HEMA column combination that consisted of a pre-column of 40 Å and main columns of 40, 100 and 3,000 Å porosities. Cloud points were determined by transmission changes (500 nm) of solutions heated at 1.0 °Cmin^{-1} in a magnetically stirred cell. The values of the cloud points

were taken as the temperature where the transmission decreases by 50%. Dynamic Light Scattering (DLS) experiments were carried out with a Malvern HPPS-ET in the temperature range 17–75 °C. The particle size distribution was derived from a deconvolution of the measured intensity autocorrelation function of the sample by the general purpose mode algorithm included in the DTS software. Each experiment was performed at least five times to obtain statistical information. Microwave-assisted synthesis was performed utilizing a CEM-Discover monomodal system, with stirring and cooling on and IR sensor for the temperature control.

Results and discussion

Mono-6-azido-cyclodextrin (CD) 1 was prepared by known procedures [10].The alkyne compound of 2-propynyl (meth)acrylate 2 was 'clicked' via Cu-mediated cycloaddition with 1 (Scheme 1). The triazole containing CD monomer 3 was prepared under MW-irradiation and conventional heating, by duplicating some controllable parameters, such as: time, temperature, catalyst and solvent. The active copper (I) catalytic species were generated *in situ* by reduction of CuSO$_4$ with sodium ascorbate. DMF was used as solvent due its high boiling point, its high dielectric constant and ability to solve the both starting materials. The "click" reaction performed by duplicating the reaction conditions resulted in higher conversion under MW-irradiation (83% in comparison with 60% obtained under conventional heating, after 30 min at 140 °C and in the presence of copper catalyst). No MW effect could be observed in aqueous medium, a low conversion being achieved after extremely increased reaction time under conventional conditions (20% after 72 h). Interestingly, the 'click' reaction of azido-CD 1 and monomer 2 dominantly shows a much faster reaction pathway under microwave irradiation in comparison with conventional heating (Table 1). Based on these results, we investigated recently in detail the MW-assisted Cu (I)-catalyzed cycloaddition of azido-CD 1 with propargyl (methyl) methacrylate 2, as well as the stereochemistry of the reaction [32].

The products obtained via cycloaddition were characterized by ^{1}H-NMR, FT-IR spectroscopy and MALDI-TOF mass spectrometry. The molecular weight of 3 and 4 was observed by MALDI-TOF mass spectrometry, respectively (1,306.6 [M+Na$^+$] and 1294.5 [M+Na$^+$]) (Fig. 1).

Triazole containing CD-monomer 3 and NIPAAM 5 were copolymerized radically using different initiators under various reaction conditions, respectively (Scheme 2, Table 2). The copolymers were characterized by ^{1}H-NMR, tubidity measurement and dynamic light scattering (DLS). Table 2 shows the reaction conditions and characteristic
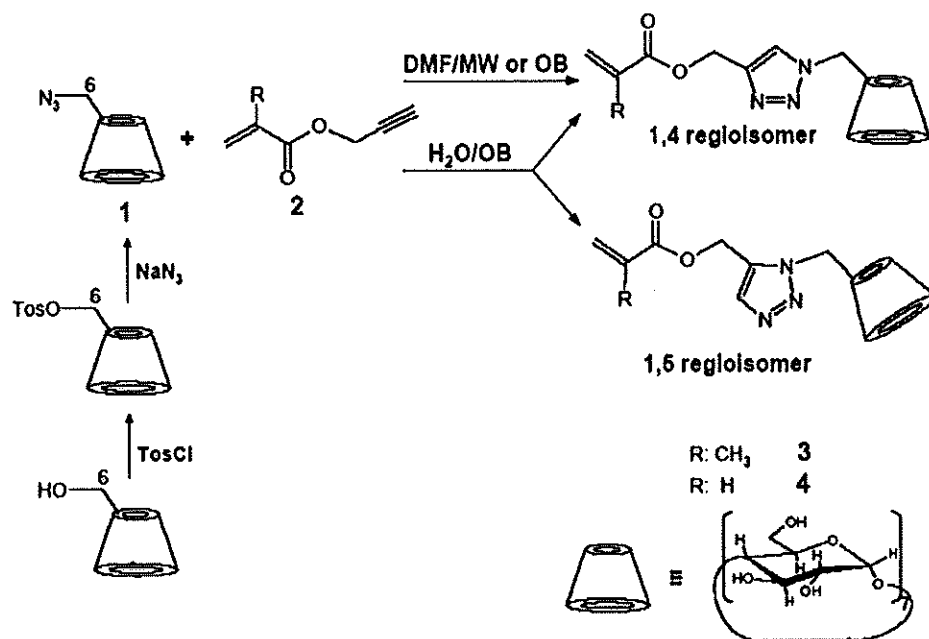
data of the copolymers used in this study. As determined by
GPC measurements, the average molar masses (M_n) of the
obtained copolymers are in the range between 13.000 and
17.000 $gmol^{-1}$.

The thermosensitive properties of aqueous solutions of
copolymers were investigated by monitoring the changes of
transparency as a function of temperature. As expected, *N*-
isopropylacrylamide containing copolymers (**6, 7, 8**, CD-
monomer/NIPAAM = 1/20) show identical LCST transitions
at 36 °C (Table 2) which are significantly higher than that of
poly(NIPAAM) homopolymer at 32 °C [33–36]. This
increased values are caused simply by the influence of
hydrophilic CD-components in the copolymers, which leads

to a better water-solubility of copolymers **6, 7** and **8** in
comparison to a homopolymer of NIPAAM [5–18].

Turbidity measurements were also performed to investi-
gate the influence of guest molecules on the LCST of the
cyclodextrin bearing copolymer **7** as example. The cloud
points of the copolymers studied decreased with increasing
hydrophobicity which corresponds to the general assump-
tions of Taylor and Cerankowski [37]. This characteristic
increase or decrease of the LCST in comparison to the
LCST value of pure copolymer is clearly a result of the
complexation of the hydrophobic or hydrophilic guests in
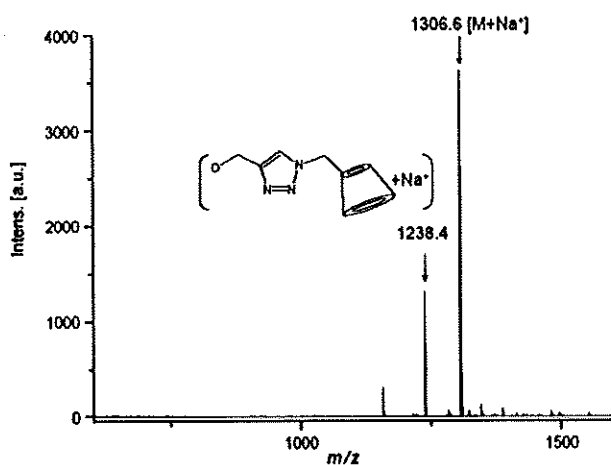
Fig. 1 MALDI-TOF MS spectrum of the monomer **3**
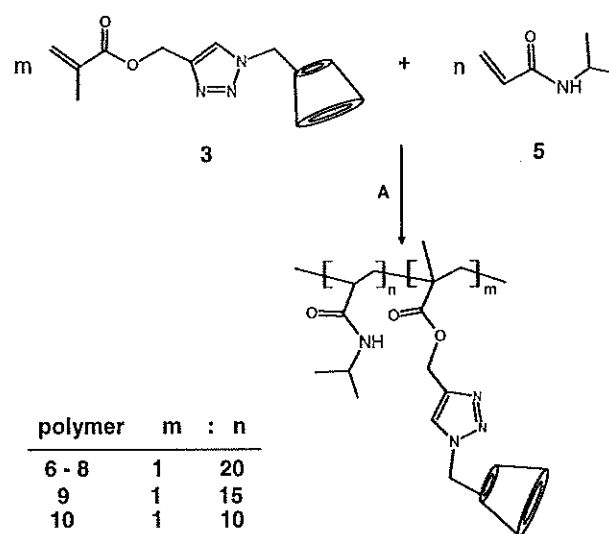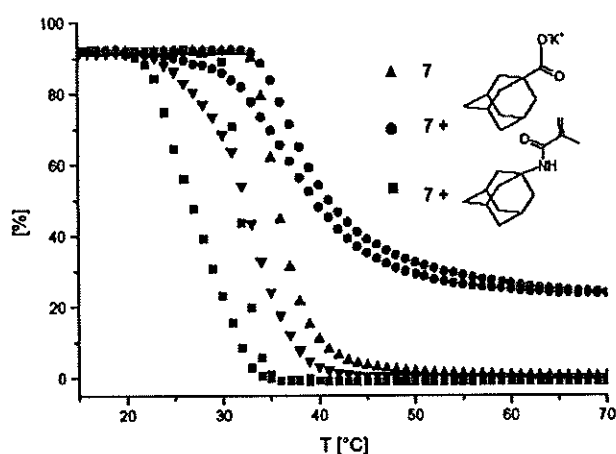
Scheme 2 Preparation of NIPAAM-CD copolymers

Table 2 Copolymerization of triazole CD-monomer 3 and N-isopropylacrylamide (NIPAAM) 5 using different initiators in different solvent systems

Obtained polymer	Initiator	solvent/temp. [°C]	M_n [gmol^{-1}]	LCST [°C]
6	Redox*	H$_2$O/r.t.	13000	36
7	AIBN	DMF/65	14000	36
8	VA-044**	H$_2$O/50	16000	36
9	VA-044**	H$_2$O/50	15000	45
10	VA-044**	H$_2$O/50	17000	—***

*K$_2$S$_2$O$_8$/Na$_2$S$_2$O$_5$, **2, 2-azobis [2-(2-imidazolin-2-yl) propane]-dihydrochloride, *** no LCST found

CD cavity. Investigations to prove these phenomena were carried out with a relatively hydophilic guest molecules, adamantly carboxylate (AdC) and the more hydrophobic polymerizable guest adamantly methylacrylamide (AdM).
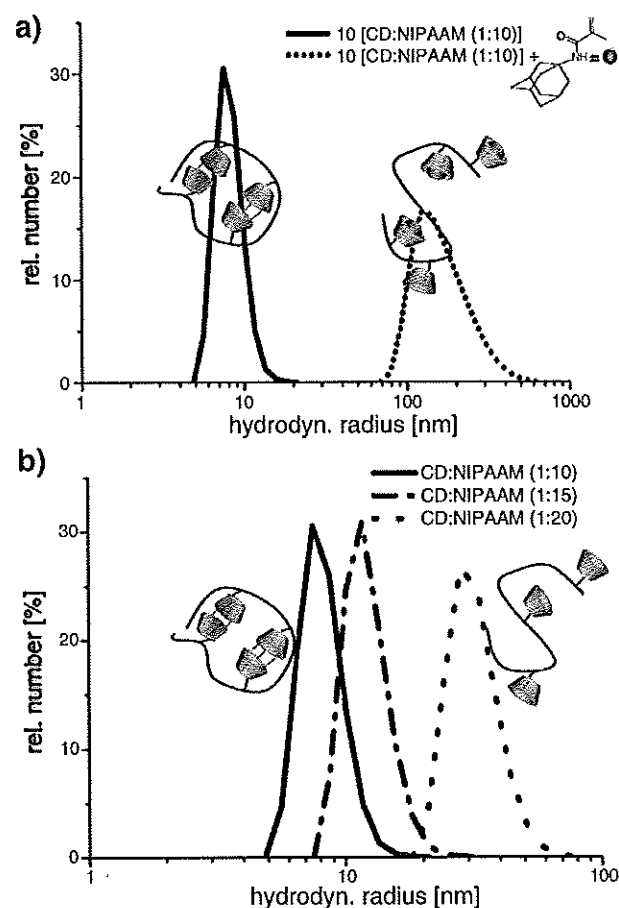
Figure 2 shows the changes of turbidity with temperature of an aqueous NIPAAM copolymer 7 with AdC and AdM as guest molecules. The LCST of complexed copolymer after addition of AdC became higher than that of copolymer 7. Through the inclusion of the hydrophobic part of the guest into the CD cavity, the polymer becomes more hydrophilic because the free carboxylic group of AdC is located preferently in the water phase and therefore responsible for a slightly increased solubility of that complexed system in water. This leads to higher LCST values of 53 °C for (7 + AdC) complex. Interesingly, the LCST of (7 + AdM) complex is definitely lower than that of uncomplexed polymer 7. In this case, the complexation of adamantly component of AdM in CD cavity leads to a supramolecular structure bearing a guest in which the more

hydrophobic methyl methacryl group is located in the water phase.

The effects of turbidity depending on the amounts of CD-monomer in the copolymer were also studied. Table 2 represents the LCST values for the copolymers (copolymer 8–10) with different portions of NIPAAM monomer unit. It is interesting to point out that, the turbidity points of copolymers were influenced by the amount of CD-monomers since highest portions of hydrophilic CD-monomers (1 to 10 ratio of CD: NIPAAM) within the copolymer chain led to no LCST effect. Additionally, the hydrodynamic volume of both copolymers and copolymers with guest molecules were measured by use of dynamic light scattering (DLS) to prove the existence of molecular dispersed complexes. Results clearly indicate that polymer 10 with high CD-monomer content shows smaller coil size than polymer 8 with lower CD-amount. It can be concluded that the copolymer 10 formed some intra interactions because of strong hydrogen bond interactions between the CD moieties on polymer

Fig. 2 Turbidity measurements of aqueous solutions of copolymer 7 (CD:NIPAAM = 1:20, ▲) and complex with guest molecule adamantly carboxylate (AdC, ●) and adamantly methylacrylamide (AdM, ■) (C$_P$=4 g/L, 20 °C)

Fig. 3 a Hydrodynamic volumes of polymer 10 before (-) and after (–) complexation with guest molecule AdM (C$_p$=10 g/L, in H$_2$O, 25 °C). b Hydrodynamic volumes of polymers 8, 9 and 10 (C$_P$=10 g/L, in H$_2$O, 25 °C

chains. In contrast, due to incorporation of the guest monomer **AdM** the hydrodynamic volume of the complex with copolymer **10** increases in comparison to copolymer (Fig. 3a). This effect is not valid in the case of copolymer **8** because of the content of CD-monomer is not high and there is no strong interaction between polymer attached CD rings (Fig. 3b).

Conclusion

Supramolecular CD containing copolymers were constructed by copolymerization between NIPAAM and CD monomer obtained after click reaction. Investigations for the interactions between the copolymers and different kinds of guest molecules have been studied by LCST- and hydrodynamic volume-measurements. Adamantyl carboxylate as guest molecule was placed in CD cavity of copolymer. Through the threading on CD the hydrophobic part was shielded, here on admantyl moiety, the LCST shows increasing comparing with this of pure copolymer. The resulting supramolecular structures containing high CD monomer units showed the increased LCST due to higher water solubility. In addition, the hydrodynamic volume was decreased because of an intra interaction between CD monomer units. After adding of the guest monomer it is obvious that the hydrodynamic volume was increased strongly because of incorporation between guest molecule and CD.

## References

1. Lehn JM (1995) Supramolecular Chemistr. Wiley-VCH, New York 1995
2. Raymo FM, Stoddart JF (1999) Chem Rev 99:1643 doi:10.1021/cr970081q
3. Szejtli J (1998) Chem Rev 98:1743 doi:10.1021/cr970022c
4. Wenz G, Han BH, Müller A (2006) Chem Rev 106:782 doi:10.1021/cr970027+
5. Harada A, Kamachi M (1990) Macromolecules 23:2821 doi:10.1021/ma00212a039
6. Harada A, Li J, Kamachi M (1992) Nature 356:325 doi:10.1038/356325a0
7. Wenz G, Keller B (1992) Angew Chem Int Ed Engl 31:197 doi:10.1002/anie.199201971
8. Harada A, Okada M, Kamachi M (1995) Macromolecules 28:8406 doi:10.1021/ma00128a060
9. Harada A, Kawaguchi Y, Nishiyama T, Kamachi M (1997) Macromol Rapid Commun 18:535 doi:10.1002/marc.1997.030180701
10. Ohga K, Takashima Y, Takahashi H, Kawaguchi Y, Yamaguchi H, Harada A (2005) Macromolecules 38:5897 doi:10.1021/ma0508606
11. Huang L, Allen E, Tonelli AE (1999) Polymer (Guildf) 40:3211 doi:10.1016/S0032-3861(98)00529-1
12. Rusa CC, Tonelli AE (2000) Macromolecules 33:1813 doi:10.1021/ma9918831
13. Rusa CC, Luca C, Tonelli AE (2001) Macromolecules 34:1318 doi:10.1021/ma001868c
14. Lu J, Mirau PA, Tonelli AE (2001) Macromolecules 34:3276 doi:10.1021/ma001820z
15. Choi SW, Ritter H (2004) J. Macromol. Sci Part A 42:321 doi:10.1081/MA-200050463
16. Ritter H, Sadowski O, Tepper E (2003) Angew. Chem. Int. Ed. 42:3171; Corrigendum: Ritter H, Sadowski O, Tepper E (2005). Angew Chem Int Ed 44:6099 doi:10.1002/anie.200590128
17. Schmitz S, Ritter H (2005) Angew Chem Int Ed 44:5658 doi:10.1002/anie.200501374
18. Kretschmann O, Choi SW, Miyauchi M, Tomatsu I, Harada A, Ritter H (2006) Angew Chem Int Ed 45:4361 doi:10.1002/anie.200504539
19. Lewis WG, Green LG, Grynszpan F, Radic Z, Carlier PR, Taylor P et al (2002) Angew Chem Int Ed 41:1053
20. Fazio F, Bryan MC, Blixt O, Paulson JC, Wong CH (2002) Am. Chem. Soc. 124:14397 doi:10.1021/ja020887u
21. Bryan MC, Fazio F, Lee HK, Huang CY, Chang A, Best MD et al (2004) J Am Chem Soc 126:8640 doi:10.1021/ja048433f
22. Collman JP, Devaraj NK, Chidsey CED (2004) Langmuir 20:1051 doi:10.1021/la0362977
23. Wu P, Feldman AK, Nugent AK, Hawker CJ, Scheel A, Voit B et al (2004) Angew Chem Int Ed 43:3928 doi:10.1002/anie.200454078
24. Helms B, Mynar JL, Hawker CJ, Fréchet JMJ (2004) J Am Chem Soc 126:15020 doi:10.1021/ja044744e
25. Huisgen R, Knorr R, Mobius L, Szeimies G (1965) Chem Ber 98:4014 doi:10.1002/cber.19650981228
26. Ryu EH, Zhao Y (2005) Org Lett 7:1035 doi:10.1021/ol047468h
27. Rostovtsev VV, Green LG, Fokin VV, Sharpless KB (2002) Angew Chem Int Ed 41:2596 doi:10.1002/1521-3773(20020715)41:14<2596::AID-ANIE2596>3.0.CO;2-4
28. Hoogenboom R, Moore BC, Schubert US (2006) Chem Comm 4010
29. Barr L, Lincoln S, Easton C (2005) J Supramol Chem 17:547–555
30. Kraus T, Budesinsky M, Zavada J (1998) Collect Czech Chem Commun 63:534 doi:10.1135/cccc19980534
31. Roehri-Steockel C, Dangles O, Brouillard R (1997) Tetrahedron Lett 38:1551 doi:10.1016/S0040-4039(97)00145-7
32. Munteanu M, Choi SW, Ritter H Macromolecules (submitted)
33. Heskins M, Guillet JE (1968) J Macromol Sci Chem A2:1441 doi:10.1080/10601326808051910
34. Schild HG (1992) Prog Polym Sci 17:163 doi:10.1016/0079-6700(92)90023-R
35. Ohashi H, Hiraoka Y, Yamaguchi T (2006) Macromolecules 39:2614 doi:10.1021/ma052509q
36. Wang HD, Chu LY, Yu XQ, Xie R, Yang M, Xu D et al (2007) Ind Eng Chem Res 46:1511 doi:10.1021/ie061265a
37. Taylor LD, Cerankowski LD (1975) J. Polym Sci., Polym Chem Ed 13:2551

Switching the Solubility of PMMA Bearing Attached Cyclodextrin-Moieties by Supramolecular Interactions with Ionic Liquids

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Methacrylate-modified β-cyclodextrin (β-CD) and methyl methacrylate (MMA) have been radically copolymerized to obtain hydrophobic CD copolymers. The water-insoluble copolymers are able to form highly stable inclusion complexes with anions of ionic liquids. Surprisingly, the inclusion of the anions in the CD cavity results in a significant change of thermal and solution properties. Furthermore, it can be shown that the structure of the ionic liquid anions influences the coil structure of the copolymers. The obtained results could be proven by means of microcalorimetry, differential scanning calorimetry, and dynamic light scattering.

Hydrophobic
CD-Copolymer

Hydrophobic IL
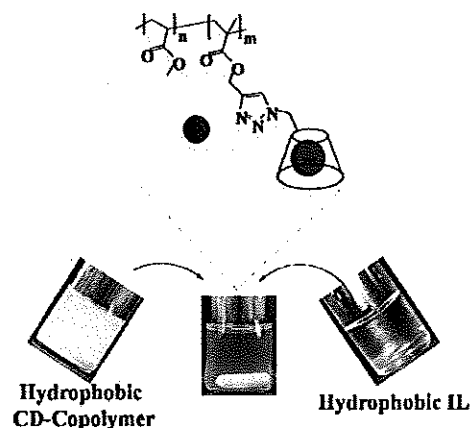
Introduction

Cyclodextrins (CDs) have drawn much attention because of their ability to form inclusion complexes with a variety of organic molecules in aqueous solutions.[1] α-Cyclodextrin is composed of six glucose residues, while β-cyclodextrin has seven and γ-cyclodextrin has eight. This means that each type of cyclodextrin provides a cavity with slightly different geometric properties and a preference for particular-sized molecules. This leads to widespread applications in pharmaceutical chemistry, food technology, analytical chemistry, chemical synthesis, and catalysis.[2]

One of their interesting properties is the inclusion of ionic liquids (ILs) into the CD cavity.[3–7] In recent years, ILs have became highly important because of their distinguished properties such as no vapour pressure, excellent thermal stability, high electrical conductivity, wide electrochemical window, and potential as a solvent for various applications which include the improved solubility of CDs in ILs.[8] The interaction between ILs and CDs plays a crucial role in analytical chemistry and material synthesis.[9]

Moreover it is described in the literature that the solution properties of copolymers can be influenced by temperature, pH, and light. For instance, copolymers that consist of N,N-dimethylacrylamide and 4-phenylazophenyl acrylate, also known as photoresponsive polymers, can

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Switching the Solubility of PMMA Bearing Attached Cyclodextrin-Moieties by...

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be influenced by means of light as a result of reversible isomerization of the azobenzene moieties.[10,11]

In this paper we accomplish the synthesis of PMMA that contains cyclodextrin moieties. The CD monomer was synthesized by a 1,3-dipolaric cycloaddition, which is also known as a 'click-reaction'. This type of click-reaction provides access to complex structures in high yields. The process is experimentally simple and has an enormous scope.[12]

The goal of the present work is the investigation of the host–guest induced change of the solution and thermal properties of hydrophobic copolymers that consist of methyl methacrylate (MMA) and methacrylated CD by accommodating anions of different hydrophobic ionic liquids.

Experimental Part

Materials and Methods

Radomly methylated β-cyclodextrin (β-CD) was obtained from Wacker-Chemie GmbH, Burghausen, Germany and used after drying overnight in a vacuum oil pump over P_4O_{10}. MMA was obtained from Aldrich Chemicals, Germany and used after distillation under reduced pressure. Asoisobutyronitrile (AIBN), N,N-dimethylformamide (DMF-d_7), and Taka-Amylase A (Taka-Diastase from Aspergillus oryzae) were purchased from Fluka, and acetic anhydride was obtained from Acros, Germany. Deuterated dimethyl sulfoxide (DMSO-d_6) was purchased from Deutero GmbH, Germany. The triazol-CD monomer was prepared according to a method described previously.[13]

IR spectra were recorded with a Nicolet 5 SXB FT-IR Spectrometer, equipped with an ATR unit. NMR spectra were recorded with a Bruker AC 500 at 20 °C. Chemical shifts were referenced to the solvent value δ 2.51 for DMSO-d_6.

Gel permeation chromatography (GPC) was performed on a GPC-System from PSS with PSS-WIN-GPC software 4.01, 6.1 with DMF-d_7 as eluent. The system was calibrated with polystyrene standards with a molecular weight range from 374 to 1 000 000 Da. The flow rate was 1 mL·min^{-1}. One hundred microlitres of a 0.1% (w/w) polymer solution was given to a HEMA column combination that consisted of a pre-column of 40 Å and main columns of 40, 100, and 3 000 Å porosities.

Dynamic light scattering (DLS) experiments were carried out with a Malvern HPPS-ET at 25 °C. The particle size distribution was derived from a deconvolution of the measured intensity autocorrelation function of the sample by the general purpose mode algorithm included in the DTS software. DLS measurements were performed in water; the concentration of the copolymers was 10 mg·mL^{-1}. The concentrations of ILs 5 and 6 were: 2.1 and 2.7 mg·mL^{-1} for 3a, 1.9 and 2.5 mg·mL^{-1} for 3b, and 1.4 and 1.8 mg·mL^{-1} for 3c. Each experiment was performed at least five times to obtain statistical information.

Differential scanning calorimetry (DSC) measurements were performed on a Mettler DSC-30 instrument in a temperature range of −25 to 200 °C at a heating rate of 15 °C·min^{-1}. For calibration, standard tin, indium, and zinc samples were used. The T_g values

are reported as the average of five measurements using the midpoint method.

Microcalorimetric titrations were accomplished with an isothermal calorimeter of type VP-ITC from Microcal. Millipore water was used for the solutions of the host and guest. The concentration of the host solution was 1 mmol·L^{-1}, the concentration of the guest solution was 8 mmol·L^{-1} in each case. In the experimental process the guest solution in the syringe was placed over 25 injections with a respective volume of 10 μL into the measuring cell, which was filled with the host solution. The temperature was 298 K with a stirrer rotational speed of 300 min^{-1}. Each injection was made over a period of 20 s, whereas the time between two injections was 4 min. The dilution heat was determined in a separate measurement by injection of the guest in millipore water and subtracting it from the determined heat flow. The evaluation of the received data was carried out with a Microcal modified version of the software Origin.

General Procedure for Copolymer Synthesis

The copolymerization of the CD monomer with MMA was carried out using AIBN as initiator. The copolymers were obtained according to the following method: to a solution of MMA (1.00 g, 0.78 mmol) in 4 mL of DMF-d_7, compound 2 (0.234 g (2.34 mmol), 390 mg (3.9 mmol), 780 mg (7.8 mmol)) was added. The solutions were flushed with argon for 15 min and the initiator (0.062 mmol, 0.093 mmol, 0.17 mmol) was added under argon atmosphere. After stirring the clear solution overnight at 65 °C, the corresponding copolymers were collected by precipitating the product in 100 mL of diethyl ether, followed by filtration. The dry polymeric material was dissolved in water, dialyzed for 3 d against distilled water using MWCO 8000 membrane, and freeze-dried.

FT-IR (film): 3 450 (OH), 2 993 (CH), 2 949 (CH), 1 723 (C=O), 1 657 (C=C), 1 484, 1 436, 1 386, 1 243, 1 140, 1 092 cm^{-1} (C–O).

^{1}H NMR (DMSO-d_6): $\delta = 0.73$–0.91 (br d, 3H, CH$_3$), 1.79 (br, 3H, CH$_3$), 3.37 (br, 1H, H-2,4), 3.56–3.65 (br d, 3H, H-3,5,6, 3H, –O–CH$_3$), 4.47 (br, 6H, OH-6), 4.84 (d, 1H, H-1), 5.08 (2H, –CH$_2$–), 5.74 (br, 12H, OH-2,3), 8.05 (1H, CH).

$\overline{M}_n$ for 3a, 3b, and 3c is 24, 66, and 32 kDa, respectively (polystyrene as standard).

Results and Discussion

The CD-containing methacrylate 2 was obtained by a click-type reaction of propargyl methacrylate with CD-azide under microwave assisted reaction conditions.[13] The triazole-containing CD monomer 2 and MMA (4) were radically copolymerized in DMF-d_7 at different molar ratios using AIBN as the initiator to result in copolymers 3a to 3c (Scheme 1). The CD-methacrylate (2)/MMA molar ratios in the obtained copolymers are 1: 3, 1: 5, and 1: 10, respectively. The average molar mass ($\overline{M}_n$) of the hydrophobic CD-copolymers were around 24 to 66 kDa according to GPC measurements (polystyrene as standard). The
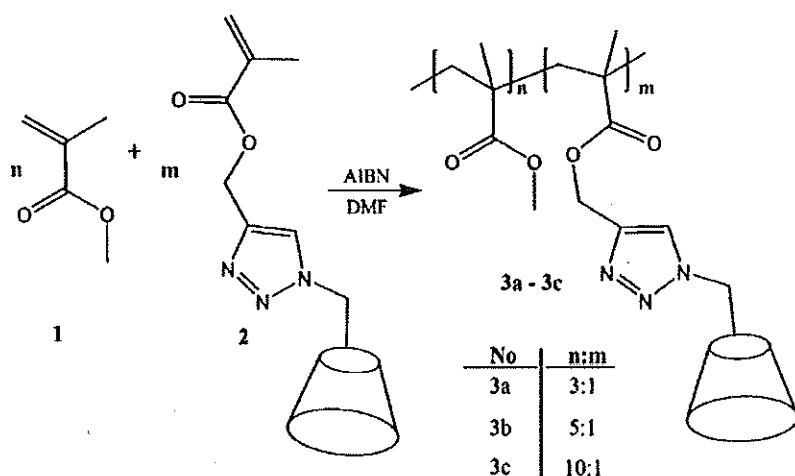
■ *Scheme 1.* Illustration of the synthesis of poly(MMA-*co*-CD-methacrylate) **3a–3c**.

No	n:m
3a	3:1
3b	5:1
3c	10:1

copolymers were further characterized by ^{1}H NMR spectroscopy, DSC, DLS, and host–guest formation experiments.

In our previous publications we have already mentioned the host–guest formation of anions of hydrophobic ionic liquids with CD and CD-copolymers.[3–5]

The hydrophobic ILs used in this study are illustrated in Scheme 2 and were synthesized according to the literature.[8f]

The ILs exhibit a high driving force to form host–guest **complexes with CD as confirmed by measuring the** complex stability constant K with different CDs (Table 1). It is obvious that the supramolecular complexes with ILs are quite stable because of the higher value of K.

In addition, it is interesting to note that the structure of **the CD has a distinct influence on the host properties.**[14] While, in general, native CD exhibits relatively low K values, some CD derivatives show much higher K values concerning the inclusion of hydrophobic ILs. For instance, for the CD monomer **2**, K was 10 300 M^{-1} with IL **5** and 3 500 M^{-1} with IL **6**, respectively, which indicates the

ability to form highly stable host–guest complexes. Thus, copolymerization of CD-monomer **2** with MMA at different ratios results in copolymers with host–guest formation properties. The copolymers are hydrophobic and not water soluble.

Glass transition temperatures of the PMMA copolymers **3a** to **3c** were determined by means of DSC (Table 2). With an increase of the CD-monomer content in the copolymer, the glass temperature also increased. This is a result of the increase of chain stiffness by intermolecular hydrogen bonds[15] between the free hydroxy groups of CD and carbonyl group of MMA.

Surprisingly, the addition of IL **5** led to a further increase of the glass temperature of copolymers **3a** to **3c**. This clearly indicates a complex formation and consequently an electrostatic effect on the chain mobility of the copolymer system.

However, the inclusion of IL **5 did not only influence the thermal properties of the dry polymer, it led to a significant** change of the solution properties as well (Figure 1). As mentioned above, **3a–3c** are not soluble in water because of the dominating hydrophobic MMA units. Surprisingly, the addition of hydrophobic ILs **5** and **6** to the turbid starting copolymer dispersion resulted in clear transparent solutions while the addition of **4** did not have any effect on the solubility.

This incident is clearly a result of the inclusion of **5** and **6** into the copolymer. The IL **4** does not form any inclusion **complexes at all. This could be confirmed by means of** microcalorimetry experiments and also from the literature.[16] This observation is consistent with the fact that the relatively hydrophobic anions are included in the cavity of the attached CD moiety and the free cations thus contribute preferentially to the hydrophilicity of the
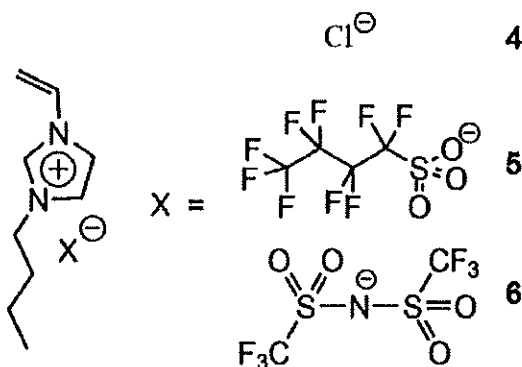
■ *Scheme 2.* Polymerizable ILs used in this study.

Table 1. Complex stability constant K of different CDs with ILs **5** and **6**.

Host	K with guest	
	M^{-1}	
	5	6
β-CD[a]	9 500	3 230
CD monomer (2)	10 300	3 500
RAMEB-CD[b]	21 000	8 100

[a]Ref.[2]; [b]ref.[4].

Switching the Solubility of PMMA Bearing Attached Cyclodextrin-Moieties by...

Macromolecular
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Table 2. Glass transition temperature of 3a to 3c before and after complexation with 5.

Copolymer	Glass temperature[a]	
	°C	
	Without 5	Inclusion complex with 5
3a	122.4	126.1
3b	120.4	125.8
3c	119.7	124.0

[a]Determined by means of DSC (10 K·min^{-1}).

Table 3. Hydrodynamic diameter of 3a–3c with 5 and 6 as guest molecules.

Copolymer	Hydrodynamic diameter[a]	
	nm	
	Copolymer with 5	Copolymer with 6
3a	30	90
3b	94	145
3c	131	236

[a]Determined by means of DLS.

copolymer (Scheme 3). However, the chloride ion of IL 4 is no guest for the CD ring.

Further treatment of the obtained clear solutions of the CD-copolymer/IL complexes with an α-amylase from *Aspergillus oryzae,* a CD-degrading enzyme, led to the precipitation of the PMMA residue (Figure 1, right picture). This process underlines the interaction of the CD moiety in the copolymer with the ILs.

Interestingly, the addition of 5 led to a higher transparency of the water-insoluble copolymers than
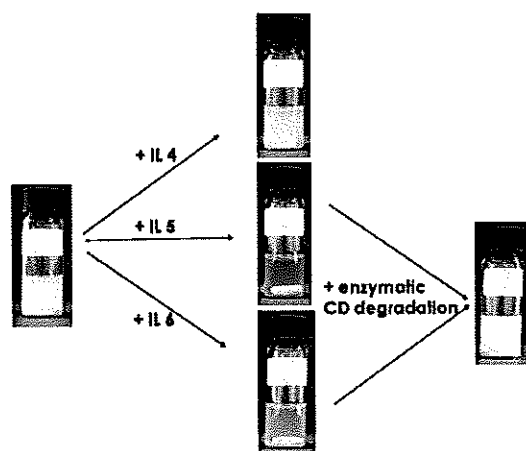
Figure 1. Switching the solubility of hydrophobic poly(MMA-co-CD-methacrylate) (20 mg·mL^{-1}) with hydrophobic ILs.

water insoluble CD-Copolymer   water soluble IL containing CD-Copolymer

- ● MMA
- ○ CD-monomer
- · water
- ⊖⊕ IL
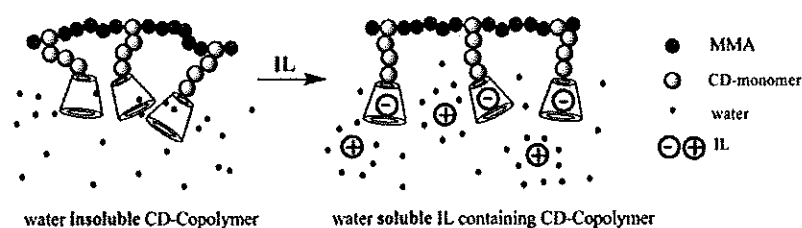
Scheme 3. Proposed mechanism of the influence of hydrophobic ILs 5 and 6 on the solution properties of 3a–3c.

the addition of 6. This observation is attributed to the different structure and localization of the anions of the ILs in the CD cavity.

Furthermore, the hydrodynamic diameters of the transparent CD-copolymer/IL complexes in water (3a–3c with 5 and 6) were measured by use of DLS to prove the hydrodynamic volume of molecular-dispersed polymeric complexes (Table 3).

The results shown in Table 3 surprisingly indicate that the copolymers with IL 6 exhibit a much larger mean coil size than that of the complexes with IL 5. In the case 5 the negative charge of the anions is in contact with the water phase because of the hydrophobic cavity of the CD.

A possible reason for this circumstance is the increasing ionic strength, which leads to some intramolecular interactions between the CD-complexed anions and the cations in the solution. This results in a largely reduced intramolecular repulsion of the chains. These attractive forces obviously lead to a decrease of the hydrodynamic diameter of 3a to 3c with IL 5. We can thus conclude that electrostatics override entropy effects.[17]

In contrast, as a result of incorporation of the anion group of guest monomer 6 into the CD cavity, the hydrodynamic diameter of the complex with hosts 3a to 3c showed a significant increase. Furthermore, it is known that the entropy of the complex formation process of IL 6 with CD is exceptionally negative.[5] This indicates that a non-coordinating character of the covered anion dominates because of this disorder effect. Thus, the cation–cation repulsion plays a major role and determines the extended polymer coil structure.

These results correspond with our previous outcomes for other CD-copolymers and explain the distinguished influence of 5 and 6 on the copolymers and the resulting change of the water solubility.[4] Moreover the given reasons explain the above described variation in

the transparency after addition of **5** and **6**. This is a further example of the influence of anions on the properties of ILs, which is extensively discussed in the literature.[18-20]

Conclusion

From the above reported results we could clearly show that the solubility of hydrophobic MMA-CD copolymers can be switched from water insoluble to water soluble. This surprising host–guest induced effect is a result of the incorporation of the anions of ILs into the CD cavity while the cations remain outside and are hydrated. Moreover, the inclusion led to a variation of the glass transition temperature. Finally, we provided an explanation for the different hydrodynamic diameters of the MMA-CD copolymer/IL complexes. It results from the distinguished anion properties of the ILs.

[1] [1a] G. Wenz, B.-H. Han, A. Müller, *Chem. Rev.* **2006**, *106*, 782; [1b] H. Ritter, M. Tabatabai, *Prog. Polym. Sci.* **2002**, *27*, 1713.

[2] [2a] J. Szejtli, *Chem. Rev.* **1998**, 1743; [2b] K. Uekama, F. Hirayama, T. Irie, *Chem. Rev.* **1998**, 2045.

[3] S. Amajjahe, H. Ritter, *Macromolecules* **2008**, *41*, 3250.

[4] S. Amajjahe, S. W. Choi, M. Munteanu, H. Ritter, *Angew. Chem. Int. Ed.* **2008**, *47*, 3435; *Angew. Chem.* **2008**, *120*, 3484.

[5] S. Amajjahe, H. Ritter, *Macromolecules* **2008**, *41*, 716.

[6] N. Noujeim, L. Leclercq, A. S. Schmitzer, *J. Org. Chem.* **2008**, *73*, 3784.

[7] Y. Gao, X. Zhao, L. Zheng, N. Li, S. Zhang, *J. Phys. Chem. B* **2006**, *110*, 8576.

[8] [8a] J. D. Holbrey, K. R. Seddon, *Clean Products and Processes* **1999**, *1*, 223; [8b] T. Welton, *Chem. Rev.* **1999**, *99*, 2071; [8c] J. S. Wilkes, M. J. Zaworotkot, *J. Chem. Soc., Chem. Commun.* **1992**, 965; [8d] K. R. Seddon, *J. Chem. Technol. Biotechnol.* **1997**, *68*, 351; [8e] P. Wasserscheid, W. Keim, *Angew. Chem.* **2000**, *112*, 3926; [8f] P. Bonhôte, A.-P. Dias, N. Papageorgiou, K. Kalyanasundaram, M. Grätzel, *Inorg. Chem.* **1996**, *35*, 1168; [8g] Q. B. Liu, M. H. A. Janssen, F. van Rantwijk, R. A. Sheldon, *Green Chem.* **2005**, *7*, 39.

[9] [9a] S. D. Qi, S. Y. Cui, X. G. Chen, Z. Hu, *J. Chromatogr. A* **2004**, *1059*, 191; [9b] B. Jing, X. Chen, J. C. Hao, H. Y. Qiu, Y. C. Chai, G. D. Zhang, *Colloids Surf. A* **2007**, *292*, 51; [9c] K. Tian, Y. S. Wang, Y. L. Chen, Y. G. Chen, Z. D. Hu, *Talanta* **2007**, *72*, 587.

[10] D. Kuckling, H.-J. P. Adler, K.-F. Arndt, L. Ling, W. D. Habicher, *Macromol. Chem. Phys.* **2000**, *201*, 273.

[11] R. Kroger, H. Menzel, M. L. Hallensleben, *Macromol. Chem. Phys.* **1994**, *195*, 229.

[12] [12a] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 2708; [12b] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem.* **2001**, *113*, 2056; [12c] P. Wu, A. K. Feldman, A. K. Nugent, C. J. Hawker, A. Scheel, B. Voit, J. Pyun, J. M. J. Frechet, K. B. Sharpless, V. V. Fokin, *Angew. Chem. Int. Ed.* **2004**, *43*, 3928.

[13] S. W. Choi, M. Munteanu, H. Ritter, *J. Polym. Res.* **2008**, DOI: 10.1007/s10965-008-9240-0

[14] T. Höfler, G. Wenz, *J. Incl. Phenom. Macrocycl. Chem.* **1996**, *25*, 81.

[15] S. W. Kuo, H. C. Kao, F. C. Chang, *Polymer* **2003**, *44*, 6873.

[16] Y. He, X. Shen, *J. Photochem. Photobiol. A: Chem.* **2008**, *197*, 253.

[17] C. Gao, S. Leporatti, S. Moya, E. Donath, H. Möhwald, *Chem. Eur. J.* **2003**, *9*, 915.

[18] R. Lungwitz, S. Spange, *New J. Chem.* **2008**, *32*, 392.

[19] S. Amajjahe, H. Ritter, *Macromol. Rapid Commun.* **2008**, *30*, 94.

[20] A. G. Avent, P. A. Chalconer, M. P. Day, K. R. Seddon, T. Welton, *J. Chem. Soc., Dalton Trans.* **1994**, 3405.

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ARTICLE

Cyclodextrin-*click*-cucurbit[6]uril: Combi-Receptor for Supramolecular Polymer Systems in Water

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ABSTRACT: We describe the synthesis of a combi-receptor via the click reaction of 6I-azido-6I-deoxycyclomaltoheptaose onto (propargyl-O)₆cucurbit[6]uril under microwave-assisted conditions. The process was investigated by ^{1}H NMR, FT-IR spectroscopy, and MALDI-TOF mass spectrometry. The ability of the synthesized compound to act as a molecular receptor and supramolecular building block in combination with NIPAM- and adamantane-containing methacrylates was investigated by turbidity measurement and dynamic light scattering (DLS).

Introduction

The molecular recognition based on macrocyclic receptors such as cyclodextrins (CDs) and cucurbiturils (CBs) is of great interest in supramolecular chemistry.[1] Similar to CDs, CBs can hold small organic molecules through hydrophobic interaction. Unlike CDs, however, the carbonyl groups at the portals allow CB[6] to form stable complexes with various ions and molecules through charge–dipole as well as hydrogen-bonding interactions.[2,3] Because of their host–guest chemistry, CDs and CBs proved to be very attractive not only as molecular receptors but also as building blocks for the construction of supramolecular architectures.[4–9] Combinations of CDs and CBs are also used for the design of supramolecular structures.[10–12] Recent progresses in the field of supramolecular chemistry are also the contribution of click chemistry, a versatile and powerful tool that affords the modular assembly of new molecular entities.[13,14] Both CDs and CBs have already found applications in click chemistry. Click reactions are known in CD chemistry,[15–19] whereas the use of CB[6] is based on its remarkable ability to catalyze 1,3-dipolar cycloadditions in a regioselective manner within the cavity.[20,21] However, the coupling of CBs and CDs via "click reaction" has not been reported. Therefore, we describe herewith the first synthesis of a dual CD-*click*-CB receptor by the successful cycloaddition of 6I-azido-6I-deoxycyclomaltoheptaose (3) onto (propargyl-O)₆CB[6] (2) under microwave-assisted conditions (Scheme 1). Furthermore, the ability of the CD-*click*-CB receptor to recognize and include guest molecules was investigated by turbidity measurement and dynamic light scattering (DLS).

Experimental Section

Materials. Cyclodextrin (β-CD) was obtained from Wacker-Chemie GmbH (Burghausen, Germany) and used after drying overnight in vacuum oil pump on P₄O₁₀. *N*-Isopropylacrylamide (NIPAAM) 97% and sodium azide (99.5%) were obtained from Aldrich Chemicals (Germany) and were used as received. Copper(II)-sulfate pentahydrate (99%) was obtained

form Carl Roth GmbH & Co., and sodium ʟ(+)-ascorbate (99%) was obtained from AppliChem (Germany). α,α′-Azoisobutyronitrile (96%) and *N,N*-dimethylformamide (DMF) were purchased from Fluka (Germany). Dimethylsulfoxide-*d₆* 99.9 atom % D was obtained from Deutero GmbH and DMSO p.a. from Carl Roth GmbH & Co. (Germany). Commercially available reagents and solvents were used without further purification. 6I-Azido-6I-deoxycyclomaltoheptaose and 6-acrylamido-*N*-adamantyl-hexane amide were prepared according to methods described in literature[22] or applied previously by us.[23]

Measurements. IR spectra were recorded with a Nicolet 5 SXB FTIR (Fourier transform infrared) spectrometer equipped with an ATR unit. The measurements were performed in the range of 4000–300 cm⁻¹ at room temperature. ^{1}H NMR spectra were recorded with a Bruker AC 500 at 20 °C. Chemical shifts were referenced to the solvent value *δ* 2.51 for DMSO-*d₆*. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS) was performed on a Bruker Ultraflex TOF mass spectrometer. Ions formed with a pulsed nitrogen laser (25 Hz, 337 nm) were accelerated to 25 kV, the molecular masses being recorded in linear mode. 2,5-Dihydroxybenzoic acid (DBH) in acetonitrile/water (25 mg·mL⁻¹) was used as a matrix. The samples (1 mg·mL⁻¹ in water) were mixed with the matrix solution at volumetric ratios of 1:2. Gel permeation chromatography (GPC) analyses were performed on a GPC system from PSS with PSS-WIN-GPC software 4.01, 6.1 with *N*, *N*-dimethylformamide as eluent. The flow rate was 1 mL·min⁻¹, and the column temperature was maintained at 60 °C. A 0.1% (w/w) polymer solution (100 μL) was given to a hydroxyethyl methacrylate (HEMA) column combination that consisted of a precolumn of 40 Å and main columns of 40, 100, and 3000 Å porosities. The number-average molecular weight (*M*ₙ) and the polidispersity (PD) were calculated by a calibration curve generated by polystyrene standards with a molecular weight range from 374 to 1 000 000 Da. DLS experiments were carried out with a Malvern HPPS-ET apparatus at a temperature value of 20 °C. The particle size distribution was derived from a deconvolution of the measured intensity autocorrelation function of the sample by the general purpose mode algorithm included in the DTS software. Each experiment was performed five times to obtain statistical information. Cloud points were determined by transmission changes (at 500 nm) of the

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Scheme 1. Synthesis of CD-CB Receptor via *click* Chemistry
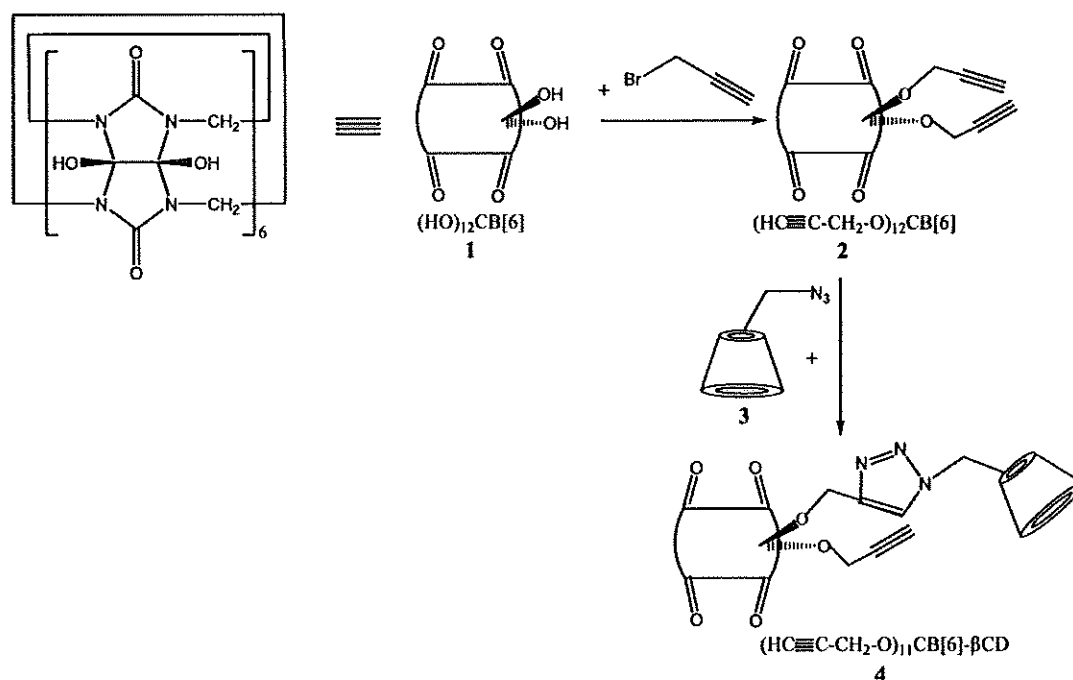
solutions heated at 0.1 K·min⁻¹ in a magnetically stirred cell; values of the cloud points were defined as the temperature at which the transmission decreases by 50%. Microwave-assisted synthesis was performed using a CEM Discover synthesis unit (monomode system). The temperature was measured by infrared detection with continuous feedback temperature control and maintained at a constant value by power modulation. Reactions were performed in closed vessels under controlled pressure.

Synthesis of Perhydroxycucurbit[6]uril (CB[6]OH) (1). PerhydroxyCB[6] was prepared according to a method described by Kim.[24] FT-IR (film): 1745 (C=O), 1437 (C–H), 1372 (N–H), 1264 (O–H), 1102 (C–O), 985 (C–H), 906 (–CH=CH₂). ^{1}H NMR (DMSO-d_6, δ): 4.43 (d, 12H, CH_2), 5.33 (d, 12H, CH_2), 7.96 (s, 12H, OH). MALDI-TOF: m/z 1227.238 [M + K⁺].

Synthesis of (Propargyl-O)₆CB[6] (2). Sodium hydride (0.304 g, 7.6 mmol) was solved in 20 mL of dry DMSO and stirred for 15 min. PerhydroxyCB[6] (0.76 g, 0,63 mmol) was added to the solution cooled to 0 °C, and the reaction mixture was stirrred at room temperature for 8 h. After the solution was cooled to 0 °C, propargylbromide (1.50 g, 12.6 mmol) was added, and the mixture was stirred overnight at room temperature. The reaction was stopped by the addition of 125 mL of cold water. The precipitate was collected by filtration and washed with water and ether on the filter (47% yields). FT-IR (film): 3280 (HC≡), 2924 (CH), 2112 (HC≡), 1728 (C=O), 1454 (C–H), 1409 (CH₃), 1319 (N–H), 1029 (CH), 906 (–CH=CH₂). ^{1}H NMR (DMSO-d_6, δ): 1.23 (s, 12 H, HC≡), 2.04 (s, 24 H, –CH_2–), 4.43 (d, 12H, CH_2), 5.34 (d, 12H, CH_2). MALDI-TOF: m/z 1667.3 [M + Na⁺].

Synthesis of CD-*click*-CB (4). The microwave-assisted click reaction of (propargyl-O)₆CB[6] (82 mg, 0,05 mmol) with 6I-azido-6I-deoxycyclomaltoheptaose (116 mg, 0.1 mmol) was carried out in DMSO in the presence of Cu(I) generated in situ by the reduction of copper sulfate (0.002 mmol) with sodium ascorbate (0.005 mmol). The tube was sealed and placed in the CEM monomode microwave and irradiated at 140 °C and 100 W for 40 min. The product was separated by simple filtration after precipitation with 50 mL of acetone (86 mg, 71%). FT-IR

(film): 3382 (OH), 2920 (CH₂), 2112 (HC≡), 1739 (C=O), 1644 (–CH=CH₂), 1473 (C–H), 1365 (N–H), 1318 (O–H), 1150 (C–O–C, OH), 1080 (C–O–C), 995. ^{1}H NMR (DMSO-d_6, δ): 1.23 (s, 11 H, HC≡), 2.04 (s, 24 H, –CH_2–), 3.35 (br, 14H, H-2,4), 3.64 (s, br, 28H, H-3,5,6), 4.43 (d, 12H, CH_2), 4.54 (br, 6H, OH-6), 4.83 (d, 6H, H-1), 4.92 (H, H-1), 5.43 (d, 12H, CH_2), 5.73 (br, 14H, OH-2,3), 8.14 (1H, CH). MALDI-TOF: m/z 2843 [M + K⁺].

Copolymer (5) Synthesis. N-Isopropylacrylamide (1.13 g, 10 mmol) and 6-acrylamido-N-adamantyl-hexane amide (159 mg, 0.5 mmol) were dissolved in DMF (4 mL). The solution was flushed with argon for 30 min, and AIBN (8.5 mg, 0.05 mmol) was added under an argon atmosphere. The mixture was heated to 65 °C and stirred overnight. The solvent was removed under reduced pressure. The dry polymeric material was dissolved in water, dialyzed, and freeze dried (M_n = 3.6 × 10⁴ g·mol⁻¹, PD = 1.6.)

Results and Discussion

The synthesized compounds were characterized by ^{1}H NMR, FT-IR spectroscopy, and MALDI-TOF mass spectrometry. The successful microwave-assisted cycloaddition of (propargyl-O)₆-CB[6] onto 6I-azido-6I-deoxycyclomaltoheptaose was IR spectroscopically proven by the disappearance of the azide band at 2100 cm⁻¹. We identified the characteristic peaks of CB[6] at 1739 and 1473 cm⁻¹ corresponding to C=O and C–N stretching vibration, respectively, the peaks corresponding to OH stretching at 3370 cm⁻¹ and C–O–C stretching and OH bending at 1150 cm⁻¹ for CD. Furthermore, the formation of triazole ring was confirmed by the appearance of the new peak at 1644 cm⁻¹ as well as by ^{1}H NMR data, the triazolic proton being distinguished at 8.14 ppm (Figure 1).

The MALDI-TOFMS measurement confirmed the molecular masses of all synthesized compounds. DLS measurements accomplished in THF indicated a hydrodynamic diameter of CB-*click*-CD compound of nearly 2.5 nm. In comparison, the outer diameter of β-CD is about 1.5 nm, and that of CB[6] is only 0.6 nm. An increased hydrodynamic diameter of 112 nm obtained
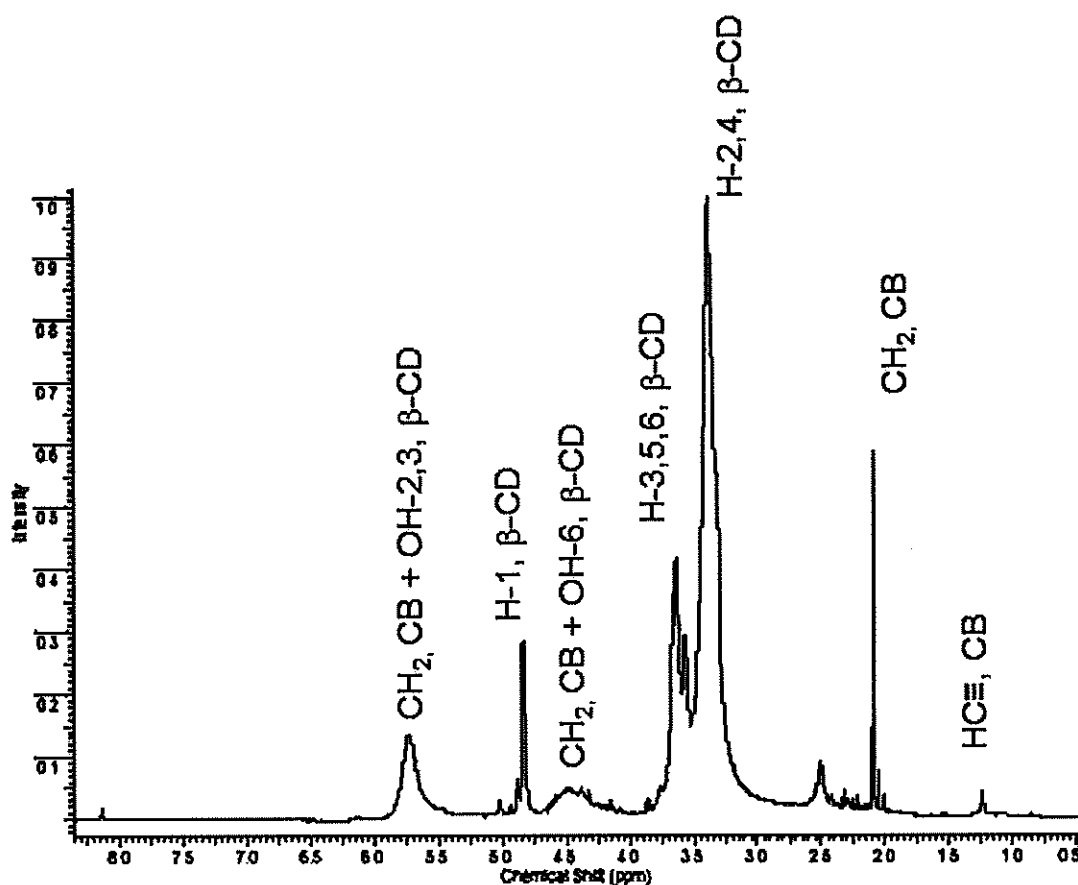
Figure 1. ^{1}H NMR spectrum of the CB–CD heterodimer.

by performing the DLS measurements in aqueous solution suggested the formation of heterodimer aggregates.

Additionally, the ability of the synthesized CD-*click*-CB compound **4** to act as a receptor and supramolecular building block was investigated. We performed DLS and cloud point measurements for an adamantane-containing copolymer **5**, which was then complexed with an equivalent ratio of **4**. The increasing of the hydrodynamic diameter of copolymer **5** from 15 to 31 nm in the presence of **4** suggests the inclusion of adamantane moiety into CD. By the addition of N,N'-dimethyl-4,4'-bipyridinium (methyl viologen, MV^{2+}), the hydrodynamic volume decreased to 20 nm as a result of the shrinkage effect of the complexed copolymer because of the interaction of the dicationic 4,4'-bipyridinium nucleus with CB (Figure 2).

The turbidity point of the copolymer **5** at 23 °C is significantly lower than that of the unmodified poly(NIPAAM) itself (34 °C) because of the influence of hydrophobic adamantyl units. To investigate the effect of CD-*click*-CB compound **4** on the cloud point of the adamantane-containing copolymer **5**, we performed turbidity measurements in the presence of an equivalent amount of CD-*click*-CB (Table 1, Figure 3). The upward shift of cloud point temperature from 23 to 29 °C confirms the inclusion of the hydrophobic adamantyl units by CD-*click*-CB. Relative to the cloud points of poly(NIPAAM) itself, this value also suggests the presence of the hydrophobic CB units.

To investigate the influence of the CB moiety, we further measured the cloud point of the copolymer complexed with CD-*click*-CB in the presence of MV^{2+}. As the DLS measurements already confirmed, the CB units can interact with the guest MV^{2+}. The complex formed by CD-*click*-CB with MV^{2+} has

increased stability and precipitates above the turbidity point of copolymer **5**. In terms of the phase transition temperature, this can be assumed to be the decrease in turbidity point to 25 °C (Table 1).

Because the adamantane moiety belongs to the best guest of β-CD known so far,[25] with a high complex stability constant around 5000 M^{-1},[26] an equimolecular mixture of the copolymer **5** with CD-*click*-CB **4** led to a stable supramolecular complex. Regarding the possible influence of MV^{2+}, the literature data suggest no inclusion of the dicationic 4,4'-bipyridinium nucleus in the CD host,[27,28] whereas a weak interaction with the CB[6] is confirmed by a complex constant of 21 M^{-1}.[29] As control experiments, we performed turbidity and DLS measurements for the copolymer **5** in the presence of MV^{2+} and β-CD/MV^{2+}, using the same concentrations of **5** and MV^{2+} as those for the first experiment. In both cases, the cloud point and hydrodynamic radius were not modified by MV^{2+}, but the combination β-CD/MV^{2+} increased the turbidity point up to 35 °C. In our former work, we showed that the addition of β-CD to this copolymer also led to a cloud point of 35 °C, which correlates to the LCST of pure poly(NIPAAM).[30] Also, the DLS measurement proved the formation of aggregates with a hydrodynamic diameter around 300 nm. We can conclude that because only β-CD interacts with the polymer chain in the control experiment, MV^{2+} can influence the turbidity point and hydrodynamic radius of the polymer **5** by interacting with CB[6]. Ion–dipole interactions are possible between the dicationic 4,4'-bipyridinium guest and the carbonyl groups at the portals of CB[6]. The successive supramolecular interactions have a shrinkage effect on the polymer network, confirmed by the DLS and turbidity measurements.
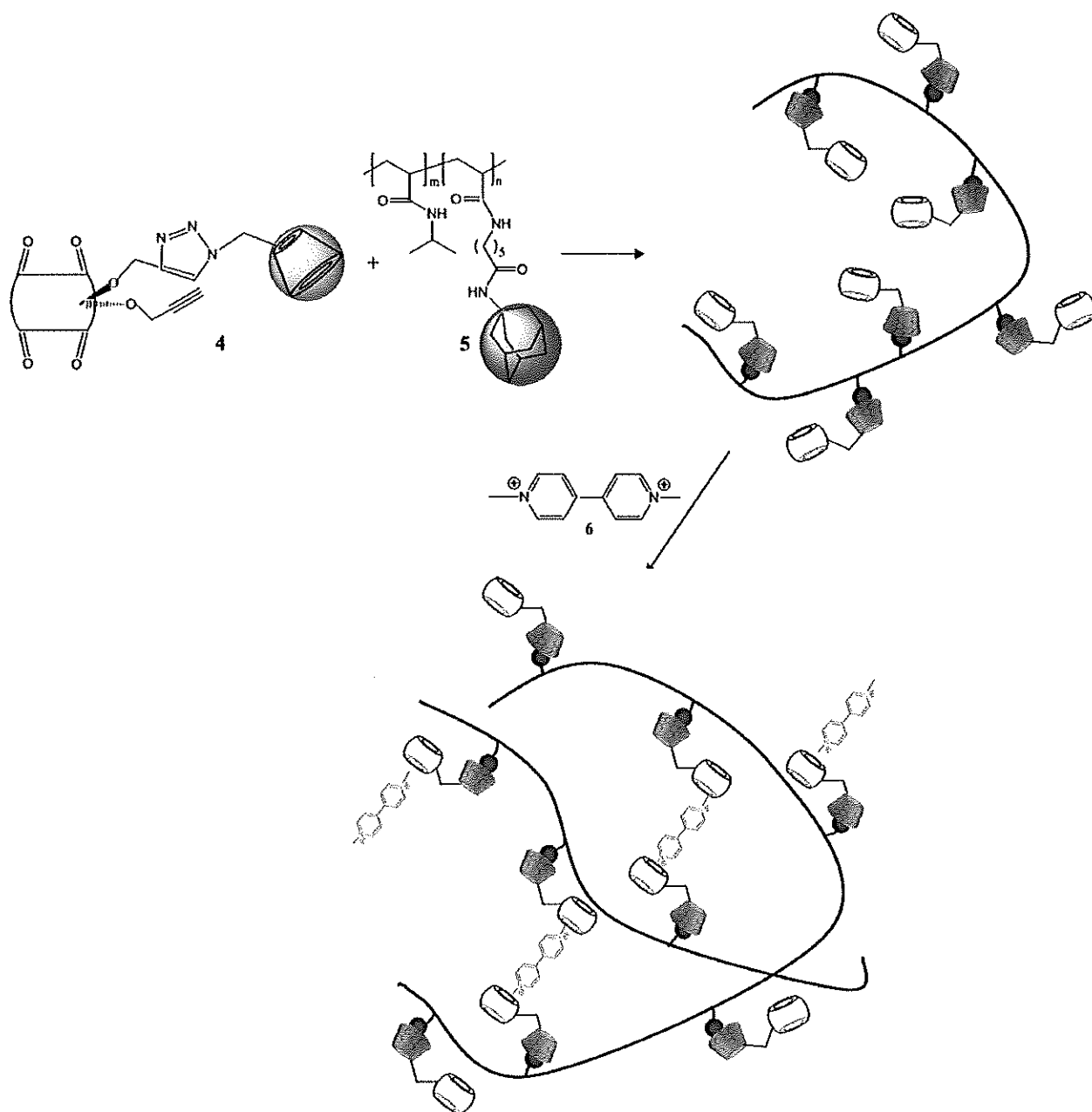
Figure 2. Supramolecular interaction of CD-*click*-CB with the adamantane-containing polymer network.

Table 1. Experimental Cloud Point Temperature Dependence on the Balance of Hydrophobic/Hydrophilic Interactions of CB—CD with the Copolymer and the Influence of CB—CD on the Hydrodynamic Volumes of Copolymer[a]

compound	turbidity point (°C)	hydrodynamic diameter [nm]
copolymer 5	23	15
copolymer 5 + CD-*click*-CB 4	29	31
copolymer 5 + CD-*click*-CB 4 + MV²⁺ 6	25	20

[a] DLS measurements were performed at 20 °C, below the turbidity point of the copolymer, using the following concentrations of copolymer and guests: $C_P = 20$ g L⁻¹, $C_{CD-CB} = 26$ g L⁻¹, $C_{MV}^{2+} = 2$ g L⁻¹. Turbidity measurements were performed in aqueous solutions using the following concentrations of copolymer and guests: $C_P = 10$ g L⁻¹, $C_{CD-CB} = 13$ g L⁻¹, $C_{MV}^{2+} = 1$ g L⁻¹.

Conclusions

A cyclodextrin-cucurbit[6]uril (CD-*click*-CB) combi-receptor was synthesized via copper-catalyzed Huisgen 1,3-dipolar cycloaddition. DLS and turbidity measurements were performed after complexing an adamantane-containing copolymer with the synthesized heterodimer and the dicationic 4, 4'-bipyridinium guest MV²⁺. As a control experiment, the same polymer was investigated in the presence of MV²⁺ and β-CD/MV²⁺. Because the dicationic 4,4'-bipyridinium guest can influence the polymer chain only in the presence of CD-*click*-CB compound, this could be valuable not only by being a molecular receptor, because of its dual complexing ability, but also by controlling the shrinkage of polymer networks by supramolecular interactions.
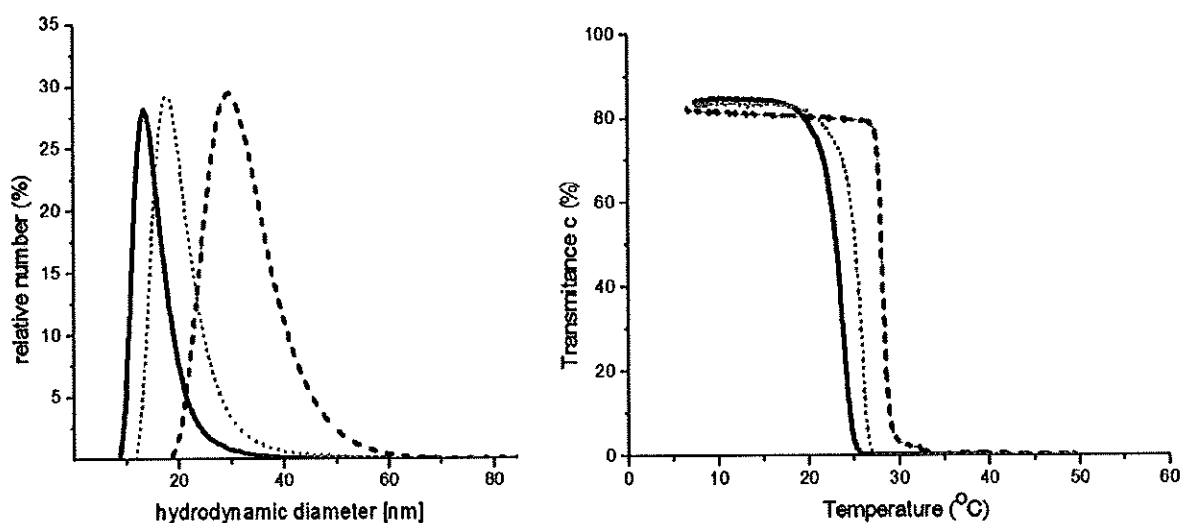
Figure 3. (a) Effect of CB–CD compound on hydrodynamic volume and (b) the change of phase transition temperature of the copolymer (—, copolymer 5; —, copolymer 5 + CD-*click*-CB 4; ···, copolymer 5 + CD-*click*-CB 4 + MV^{2+} 6).

References and Notes

(1) Liu, Y.; Li, C. J.; Gou, D. S.; Pan, Z. H.; Li, Z. *Supramol. Chem.* **2007**, *19*, 517.

(2) Marquez, C.; Hudgins, R. R.; Nau, W. M. *J. Am. Chem. Soc.* **2004**, *126*, 5806–5816.

(3) Mock, W. L. *Top. Curr. Chem.* **1995**, *175*, 1–24.

(4) Kim, K. *Chem. Soc. Rev.* **2002**, *31*, 96–107.

(5) Mock, W. L.; Irra, A.; Websiec, J. P.; Adhya, M. *J. Org. Chem.* **1989**, *54*, 5302–5308.

(6) Tuncel, D.; Steinke, J. H. G. *Chem. Commun.* **2002**, 496–497.

(7) (a) Jeon, W. S.; Bharadwaj, P. K.; Choi, S. W.; Lee, J. W.; Kim, K. *Angew. Chem.* **2002**, *114*, 4654–4656. (b) *Angew. Chem., Int. Ed.* **2002**, *41*, 4474–4476.

(8) Lee, J. W.; Kim, K.; Choi, S. W.; Ko, Y. H.; Sakamoto, S.; Yamaguchi, K.; Kim, K. *Chem. Commun.* **2002**, 2692–2693.

(9) Choi, S. W.; Lee, J. W.; Ko, Y. H.; Kim, K. *Macromolecules* **2002**, *35*, 3526–3531.

(10) Liu, Y.; Li, X. Y.; Heng-Yi Zhang, H. Y; Li, C. J.; Ding, F. *J. Org. Chem.* **2007**, *72*, 3640–3645.

(11) Rekharsky, M. V.; Yamamura, H.; Kawai, M.; Osaka, I.; Arakawa, R.; Sato, A.; Ko, Y. H.; Selvapalam, N.; Kim, K.; Inoue, Y. *Org. Lett.* **2006**, *8*, 815–818.

(12) Ooya, T.; Inoue, D.; Choi, S. H.; Kobayashi, Y.; Loethen, S.; Thompson, D. H.; Ko, Y. H.; Kim, K.; Yui, N. *Org. Lett.* **2006**, *8*, 3159–3162.

(13) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, 2004–2021.

(14) (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem.* **2002**, *114*, 2708–2711. (b) *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599.

(15) Perez-Balderas, F.; Ortega-Munoz, M.; Morales-Sanfrutos, J.; Hernandez-Mateo, F.; Calvo-Flores, F. G.; Calvo-Asin, J. A.; Isac-Garcia, J.; Santoyo-Gonzalez, F. *Org. Lett.* **2003**, *5*, 1951–1954.

(16) Ortega-Muñoz, M.; Morales-Sanfrutos, J.; Perez-Balderas, F.; Hernandez-Mateo, F.; Giron-Gonzalez, M. D.; Sevillano-Tripero, N.; Salto-Gonzalez, R.; Santoyo-Gonzalez, F. *Org. Biomol.Chem.* **2007**, *5*, 2291–2301.

(17) Chan, T. R.; Hilgraf, R.; Sharpless, K. B.; Fokin, V. V. *Org. Lett.* **2004**, *6*, 2853–2855.

(18) Hoogenboom, R.; Moore, B. C.; Schubert, U. S. *Chem. Commun.* **2006**, *38*, 4010–4012.

(19) Munteanu, M.; Choi, S. W.; Ritter, H. *Macromolecules* **2008**, *41*, 9619–9623.

(20) (a) Tuncel, D.; Steinke, J. H. G. *Chem. Commun.* **2002**, 496–497. (b) Tuncel, D.; Steinke, J. H. G. *Macromolecules* **2004**, *37*, 288–302.

(21) Krasia, T. C.; Steinke, J. H. C. *Chem. Commun.* **2002**, 22–23.

(22) Ohga, K.; Takashima, Y.; Takahashi, H.; Kawaguchi, Y.; Yamaguchi, H.; Harada, A. *Macromolecules* **2005**, *38*, 5897–5904.

(23) Kretschmann, O.; Steffens, C.; Ritter, H. *Angew. Chem., Int. Ed.* **2007**, *46*, 2708–2711.

(24) Jon, S. J.; Selvapalam, N.; Oh, D. H.; Kang, J. K.; Kim, S. Y.; Jeon, Y. J.; Lee, J. W.; Kim, K. *J. Am. Chem. Soc.* **2003**, *125*, 10186–10187.

(25) Rekharsky, M. V.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875–1917.

(26) Amajjahe, S.; Ritter, H. *Macromolecules* **2008**, *41*, 716–718.

(27) Okuno, Y.; Chiba, Y.; Yonemitsu, O. *J. Chem. Soc., Chem. Commun.* **1984**, 1638.

(28) (a) Mirzoian, A.; Kaifer, A. E. *Chem.—Eur. J.* **1997**, *3*, 1052–1058. (b) Mirzoian, A.; Kaifer, A. E. *Chem. Commun.* **1999**, 1603–1604.

(29) Ong, W.; Gomez-Kaifer, M.; Kaifer, A. E. *Org. Lett.* **2002**, *4*, 1791–1794.

(30) Kretschmann, O.; Choi, S. W.; Miyauchi, M.; Tomatsu, I.; Harada, A.; Ritter, H. *Angew. Chem., Int. Ed.* **2006**, *45*, 4361–4365.

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Full Paper

New Generation of Polymeric Drugs: Copolymer from NIPAAM and Cyclodextrin methacrylate containing Supramolecular-attached Antitumor Derivative

Dedicated to Professor Helmut Ringsdorf in occasion of his 80th anniversary

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Abstract. We describe the synthesis of an adamanatne-bearing analogue of 5-fluorocytosine. The process was investigated by ^{1}H-NMR, FT-IR spectroscopy, MS-EI mass spectrometry, and elemental analysis. The non covalent inclusion complexation of the synthesized compound with a copolymer containing NIPAAM and cyclodextrin methacrylate was investigated by turbidity measurement, dynamic light scattering and UV-vis spectrofotometry.

Introduction

Polymeric drugs were originally prepared in a broad manner by Ringsdorf et al. in the early seventies of the last century. The drugs were covalently attached to the hydrophilic polymer backbone preferentially via a spacer group.[1]

Since the biological and tumor-inhibitory properties of 5-fluorinated pyrimidines were reported,[2] 5-fluorouracil (5-FU) is still one the frequently used chemotherapeutic agents. 5-FU is the active metabolite of 5-fluorocytosine (5-FC), the prodrug being converted by the bacterial and fungal cytosine deaminase (CD). Although the human cells lack this enzyme, the role of the intestinal flora in the fluorocytosine conversion process was postulated.[3] In order to avoid the toxicity and side effects induced by 5-FU, new derivatives of 5-fluorocytosine were designed and synthesized.[4] Also, lipophilic derivatives of 5-FU bearing cholesterol moieties or carbon chains were proved to have increased efficacy as a result of entrapment into carriers. In contrast to 5-fluorouracil, 5-fluorocytosine is not toxic to human cells.[5] However, the prodrug can be used in association with the bacterial cytosine deaminase (CD) gene, as a "suicide system" in gene therapy.[6] The non-toxic 5-fluorocytosine derivates could also be used in intestinal cancer therapy. In order to develop new potential anticancer agents, we synthesized a 5-fluorocytosine analogue bearing an adamantyl moiety (**Scheme 1**). Numerous scientific articles describe the advantages of drugs complexed with cyclodextrins, such as: increased solubility, enhanced bioavailability and improved stability.[7] Since adamantyl groups are known to be included and held strongly in β-CD, the hydrophobic compound was further complexed with a cyclodextrin-containing polymer. Beside the stable complexes formed with the CD cavity, adamantane moieties could also facilitate the partition into the lipid cell membrane, as a result of increased lipophilicity. Thus, we wish to describe in this paper the synthesis of an adamantane-bearing analogue of 5-FC and its non covalent inclusion complexation with CD covalently attached to a polymer.

Experimental Part

Materials. Cyclodextrin was obtained from Wacker-Chemie GmbH (Burghausen, Germany) and used after drying overnight in vacuum oil pump on P_4O_{10}. Flucytosine 98% was purchased from ABCR. Adamantylisocyanate 97% and 1,4-diazabicyclo[2.2.2]octane (DABCO) were obtained from Sigma Aldrich. Dimethylsulfoxide-d_6 99.9 atom % D was obtained from Deutero GmbH and N,N-dimethylformamide (DMF) extra dry from Fisher Scientific. Commercially available reagents and solvents were used without further purification. Triazol-CD-monomer was prepared according to methods applied previously by us.[8]

Measurements. IR spectra (4000-300 cm^{-1} at room temperature) were recorded with a Nicolet 5 SXB FTIR (Fourier transform infrared) spectrometer equipped with an ATR unit. ^1H NMR spectra were recorded with a Bruker AC 500 at 20 °C. Chemical shifts were referenced to the solvent value δ 2.51 for DMSO-d_6. Gel permeation chromatography (GPC) analyses were carried out on a GPC system from PSS with PSS-WIN-GPC software 4.01, 6.1 with N,N-dimethylformamide as eluent. The flow rate was 1 mL·min^{-1}, and the column temperature was maintained at 60 °C. A 0.1% (w/w) polymer solution (100 µL) was given to a hydroxyethyl methacrylate (HEMA) column combination that consisted of a precolumn of 40 Å and main columns of 40, 100, and 3000 Å porosities. The number-average molecular weight (Mn) and the polidispersity (PD) were calculated by a calibration curve generated by polystyrene standards with a molecular weight range from 374 to 1 000 000 Da. UV-VIS spectra were recorded on a Nicolet UV540 spectrometer. DLS experiments were carried out with a Malvern HPPS-ET apparatus at a temperature value of 20 °C. The particle size distribution was derived from a deconvolution of the measured intensity autocorrelation function of the sample by the general purpose mode algorithm included in the DTS software. Each experiment was performed five times to obtain statistical information. Cloud points were

determined by transmission changes (at 500 nm) of the solutions heated at 0.1 K·min⁻¹ in a magnetically stirred cell; values of the cloud points were defined as the temperature at which the transmission decreases by 50%.

Synthesis of N-1-adamantyl-4-amino-5-fluoro-2-oxopyrimidine-1(*2H*)-carboxamide (3)

5-Fluorocytosine (2) (129.09 mg, 1 mmol) and DABCO (11.22 mg, 0.1 mmol) were solved in 10 mL of dry DMF and stirred and heated up to 100 °C. Adamantylisocyanat (1) (177.24 mg, 1 mmol) dissolved in 10 mL of DMF was added and the reaction mixture was stirrred for 1h. After cooling down the solution to room temperature, the precipitate was collected by filtration and washed 3 times with hot ethanol on the filter. The isolated product was dried in high vacuum (84.6 % yield).

IR (film): 3369 (NH), 3313 (NH), 2910 (CH_2), 2848 (CH_2), 1659 (C=O), 1560 (NH), 1460 (CH_3), 1336 (CN), m/z = 306. ^{1}H NMR (DMSO-d_6): δ = 1.66 (m, 6 H), 1.99 (m, 6 H), 2.06 (m, 3H), 7.95 (d, 1H), 9.53 (s, 1H), 11.3 (hydrogen-bonded structure).

Elemental analysis calculated for $C_{15}H_{19}FN_4O_2$: C 59.21; H, 6.26; N 18.62. Found: C 58.95; H 6.410; N 18.23.

Results and Discussion

The synthesis and of N-1-adamantyl-4-amino-5-fluoro-2-oxopyrimidine-1(*2H*)-carboxamide (3) from adamantylisocyanat (1) and 5-fluorocytosine (2) is described in **Scheme 1.**

Scheme 1

The potential antitumor drugs were characterized by ^{1}H-NMR, FT-IR spectroscopy and MS-EI mass spectrometry and elemental analysis. The successful reaction was IR spectroscopically proven by the disappearance of the isocyanat band at 2250 cm⁻¹. We identified the characteristic peaks of adamantane at 2848 cm⁻¹, 2910 cm⁻¹, 1460 cm⁻¹ and 1336

cm^{-1} corresponding to CH_2 and CH stretching vibration, respectively the peak corresponding to NH stretching vibrations at 3369 cm^{-1} and 3313 cm^{-1}. The two amid bands were also identified at 1659 cm^{-1} and 1560 cm^{-1}. Furthermore, the formation of the 5-FC bearing adamantane moiety was confirmed by ^{1}H NMR data, as well as by MS EI and elemental analysis.

In respect of the aim of the present work, we combined the adamantly modified antitumor agent (3) with the water soluble copolymer (4) bearing covalently-attached cyclodextrin. In contrast to the former work of Ringsdorf et al.[1] we obtained non colavently attached polymeric drugs. In order to increase the solubility of 5-fluorocytosine analogue and make it suitable for biological applications, we designed a supramolecular structure based on inclusion compelxation of adamantane with a polymer containing covalently attached cyclodextrins (**Figure 1**).

Figure 1

We performed DLS, UV/vis and cloud-point measurements for the cyclodextrin-containing copolymer, which was then complexed with an equivalent ratio of fluorocytosine-derivative. The inclusion complexation of the adamantane moiety into CD was confirmed by UV-vis spectrofotometry (**Figure 2**). The absorbtion curve of the complexed polymer resemble that for drug analogue included into m-β-CD and corresponds to cytosine moiety.

Figure 2

The type of inclusion complexes and the complex formation constant were also investigated based on the phase solubility technique. As Higuchi–Connors[9] phase diagram indicates, an initial 1:1 complex was formed by including the adamantyl moieties into CDs cavity, while at higher concentration of CD a 1:2 complex is formed (**Figure 3**). The relatively high value of the formation constant calculated for the 1:1 complex, at around 1.9×10^4 M^{-1} indicates the

presence of stable supramolecular structures based on typical adamantane-CD interaction. The low 1:2 complex constant around 1.2×10^2 M^{-1} corresponds to fluorocytosine inclusion into CD.

Figure 3

The complexation of the drug analogue with the CD-containing polymer was also indicated by increasing the hydrodynamic diameter of the copolymer from 7 nm to 14 nm **(Figure 4)**.

Figure 4

The thermosensitive properties of aqueous solutions of cyclodextrin-bearing copolymer **(4)** and of the supramolecular polymer network containing the drug analogue **(5)** were investigated by monitoring the changes of turbidity as a function of temperature. The cyclodextrin-containing copolymer shows a turbidity point at 30 °C, which correlates to the LCST of poly(NIPAAM). By complexing the copolymer with the synthesized compound we obtained a cloud point of 36 °C **(Figure 5)**.

Figure 5

The increased hydrodynamic radius and LCST value after inclusion of **(3)** could be explained as the result of hydrogen bonding involving the amino groups of the guest molecule, with a favourable effect on the mobility and solubility of the supramolecular complex **(5)**.

To achieve drug activity it is important to decomplex the included guest. Accordingly, we were interested to check the interaction of an enzyme with the polymeric drug system. Some α-amylases, such as human salivary α-amylase and human pancreatic α-amylase are able to hydrolyze cyclodextrins to glucose and maltooligosaccharides, by combining the ring-opening with the repetitive attack on the product.[10] Since several bacterial and fungal α-amylases

have been shown to be able to hydrolyze cyclodextrins,[11] the supramolecular polymer system (5) was treated with α-amylase from *Aspergillus oryzae* in water. As demonstrated in **Figure 6**, the clear polymeric solution of (5) became turbid after 36h due to the enzymatic degradation (**Figure 6**).

Figure 6

The drug releasing from the polymer network in the presence of the enzyme was also spectrophotometrically investigated. After incubating the complexed polymer with α-amylase, we performed UV-vis measurement of the supernatant isolated by centrifugation (**Figure 7**).

Figure 7

The decrease of absorbance at 292 nm corresponds to reduced drug concentration, as a result of CD enzymatic hydrolysis.

Conclusion

Supramolecular complexes were constructed by inclusion process between CD-containing copolymer and a 5-FC analogue. Interactions between the copolymer and the synthesized compound have been studied by DLS, LCST and UV/vis measurements. The guest molecules can be released by enzymatic hydrolysis.

Keywords: drug delivery systems, supramolecular structures

References

[1] [1a] H. Gros L., Ringsdorf H., Schupp H.; *Angew Chem. Int. Ed. Engl.* **1981**, 305; [1b] H. Ringsdorf; *J. Polym. Sci.* **1975**, 135.

[2] [2a] R. Duchinsky, E.Pleven, C. Heidelberg; *J. Am. Chem. Soc.* **1957**, 79, 4559; [2b] C.Heidelberg, N. K. Chaudhuri, P. B. Danneberg, D. Mooren, L. Griesbach, R. Duchinsky, R. J. Schnitzer, C. Heidelberg; *Nature* **1957**, 197, 663.

[3] M. C.Malet-Martino, R.Martino, M. de Forni, A.Andremont, O.Hartmann, J. P. Armand; *Infection* **1991**, 19, 178.

[4] [4a] K. H. Kim, J. Y. Kim, K. H. Lee, M. J. Noh, Y. C. Kim, H. J. Park; *Bioorganic & Medicinal Chemistry Letters* **2002**, 12, 483; [4b] L. N. Kaliberov, D. L. Della Manna, V. Krendelchtchikova, M. E. Black, D.J. Buchsbaum, S.A. Kaliberov *Mol Cancer Ther.* **2008**, 7, 2845-54; [4c] L. Negroni, M. Samson, J. M. Guigonis, B. Rossi, V. Pierrefite-Carle, C. Baudoin, *Mol Cancer Ther.* **2007**, 6, 2747-2456; [4d] X. L. Gao , Q. L. Li , Z. L .Peng , H. Wang, *Zhonghua Fu Chan Ke Za Zhi* **2005**, 40, 761-764; [4e] H. Kato , K. Koshida, K. Yokoyama, A. Mizokami, M. Namiki, *Int. J. Urol.*, **2002**, 9, 567-576; [4f] L. Yao , Y. Li , Y. Wu , A. Liu ,H. Yan, *Biochemistry* **2005**, 44, 5940-5947.

[5] [5a] M. Hashida, K. Sato, Y. Takakura, H. Sezaki; *Chem. Pharm. Bull.* **1988**, 36, 3186; [5b] H. Sasaki, Y. Matsukawa, M. Hashida, H. Sezaki, *Int. J. Pharm.* **1987**, 36, 147; [5c] A. Hoshi, M. Iigo, A. Nakamura, M. Inomata, K. Kuretani; *Chem. Pharm. Bull.* **1978**, 26, 161.

[6] L. Negroni, M. Samson, J. M. Guigonis, B. Rossi, V. Pierrefite-Carle, C. Baudoin, *Mol. Cancer Ther.* **2007**, 6.

[7] [7a] M. Rekharsky, Y. Inoue; *Chem. Rev.* **1998**, 98, 1875-1917; [7b] T. Loftsson, M. Brewester; *J Pharm Sci.* **1996**, 85, 1017; [7c] K. Connors; *Chem. Rev.* **1997**, 97, 1325.

[8] M. Munteanu, S.W. Choi, H. Ritter; *Macromolecules* **2008**, 41, 9619.

[9] T. Higuchi, K. A. Connors; *Adv. Anal. Chem. Instrum.* **1965**, 117.

[10] [10a] D. French; *Adv. Carbohydr. Chem.* **1957**, 189-260; [10b] J. J. Marshall, I. Miwa; *Biochim. Biophys Acta.* **1981**, 142; [10c] H. Kondo, H. Nakatani, K. Hiromi; *Carbohydr. Res.* **1990**, 207.

[11] I. Jodal, L. Kandra, J. Harangi, P. Nanasi, Debrecen, J. Szejtli; *Starch/Stärke*, **1984**, 140.

Figure captions

Scheme 1. Synthesis of the adamantly-modified 5-fluorocytosine **3**

Figure 1. Supramolecular polymer containing 5-FC derivative

Figure 2. UV/vis spectra of drug analogue complexed with methylated-CD and polymer **5** compared with the uncomplexed copolymer **4**

Figure 3. Higuchi–Connors phase diagram of the polymer-drug complex

Figure 4. Hydrodynamic radius of the polymer before (—) and after (--) supramolecular drug-complaxation

Figure 5. Turbidity measurement of aqueous solutions of the CD-containing polymer **4** and measurement of the supramolecular complexed polymer **5**

Figure 6. Drug precipitation by treating the complexed polymer **5** with α-amylase

Figure 7. UV-vis spectrophotometrically investigation of drug releasing from **5**

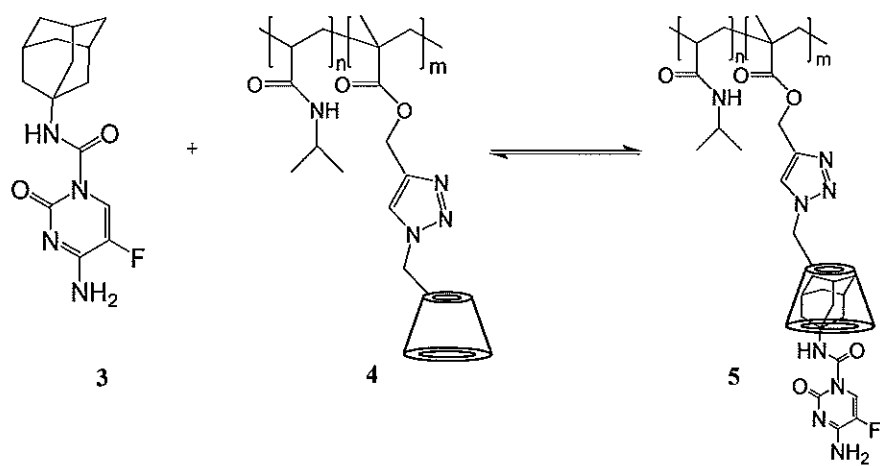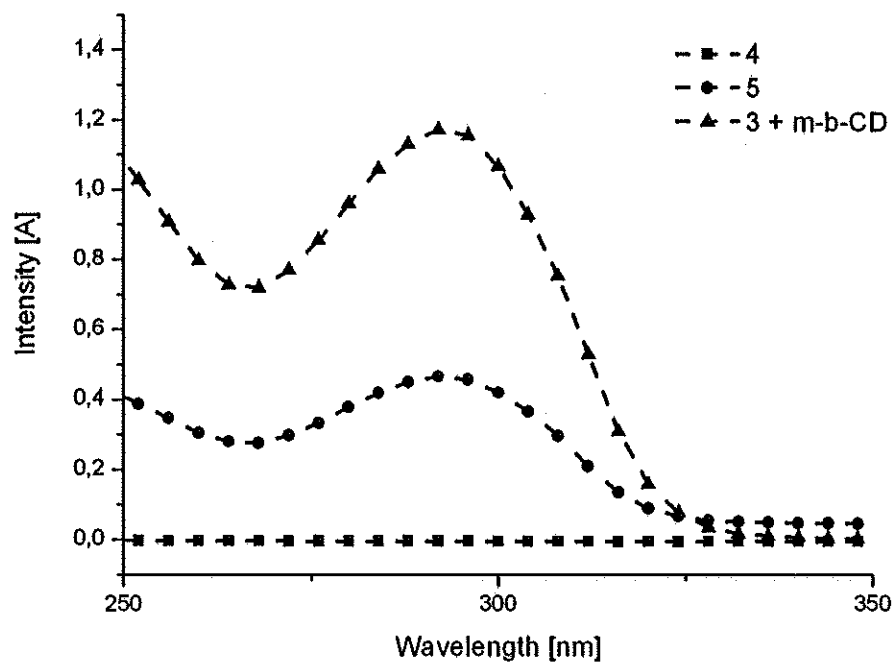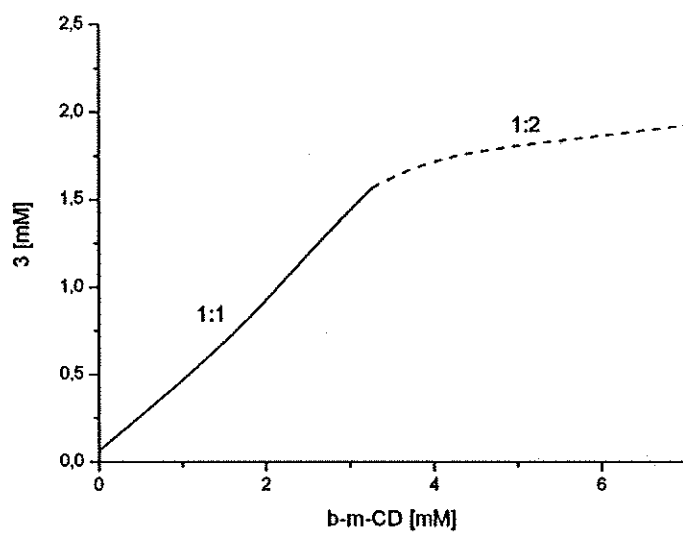Arkadius Maciollek, Maricica Munteanu, Helmut Ritter*

Scheme 1.
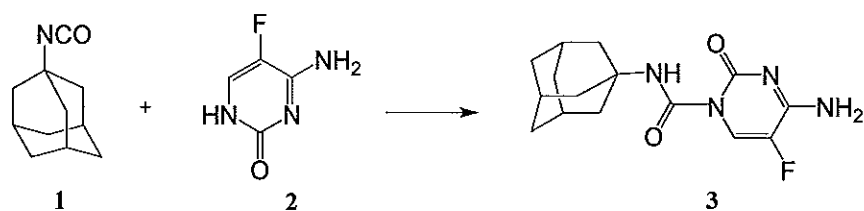
Figure 1.

Figure 2

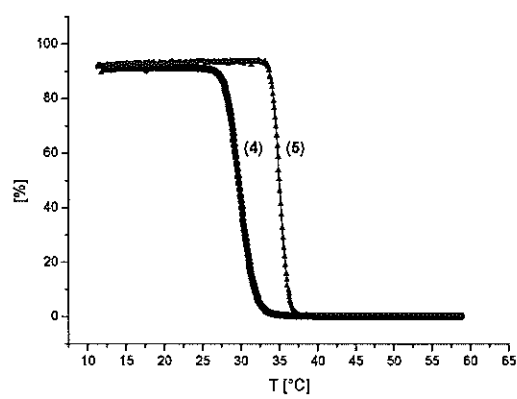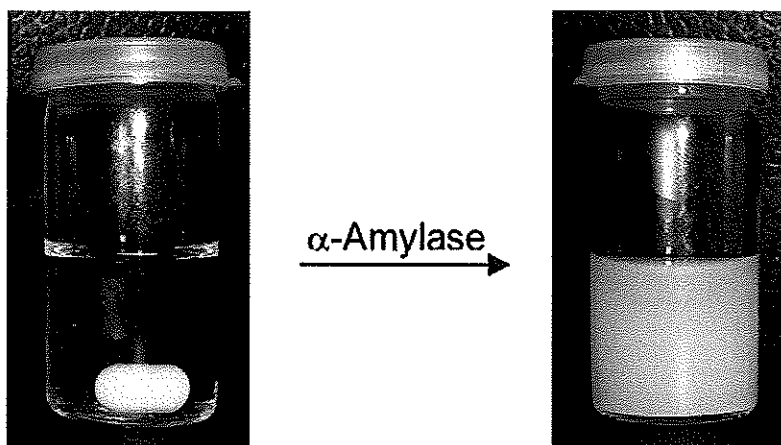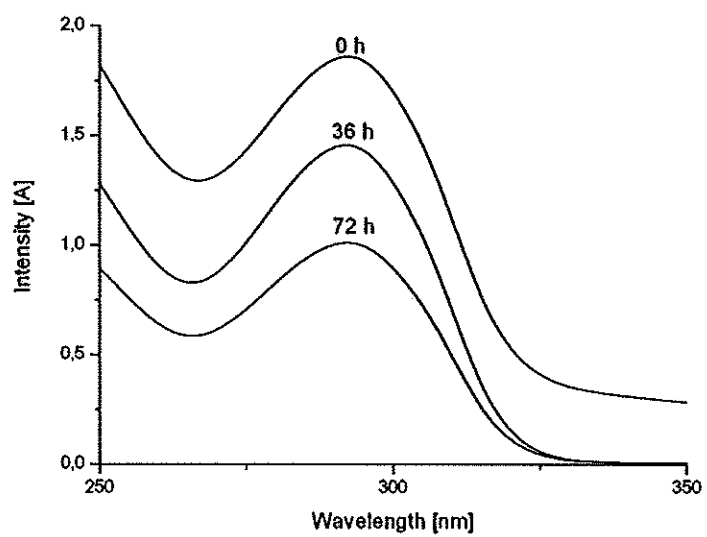Figure 3.

Figure 4
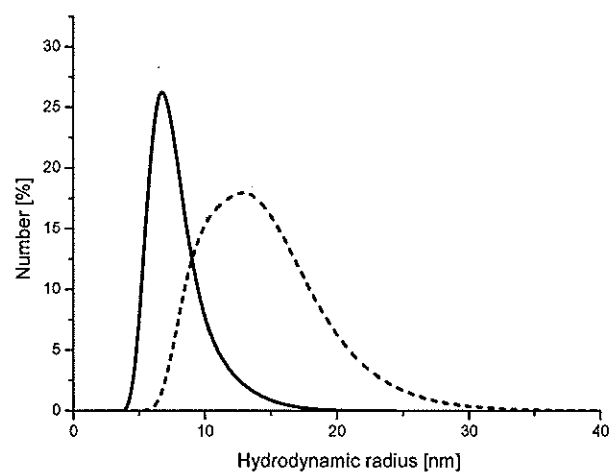
Figure 5.

Figure 6.

Figure 7

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Supramolecular complexes were constructed by non covalent inclusion complexation between a cyclodextrin-containing copolymer and an adamanatne-bearing analogue of 5-fluorocytosine. The adamantly-modified antitumor agent can be released from the polymer by enzymatic hydrolysis of cyclodextrin.
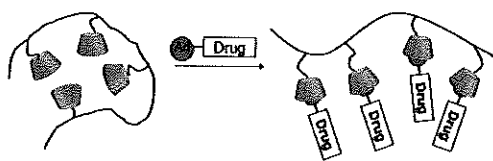
Arkadius Maciollek, Maricica Munteanu, Helmut Ritter*

New Generation of Polymeric Drugs: Copolymer from NIPAAM and Cyclodextrin methacrylate containing Supramolecular-attached Antitumor Derivative

Supporting Information for New Generation of Polymeric Drugs: Copolymer from NIPAAM and Cyclodextrin methacrylate containing Supramolecular-attached Antitumor Derivative

Arkadius Maciollek, Maricica Munteanu, Helmut Ritter*

Table 1. Concentration of 5-fluorocytosine derivative **3** and polymer **4** used for DLS/LCST measurements

Concentration [Mol·L^{-1}]		Molar Ratio
3	**4**	
2,17·10-3	2,17·10-3	1 : 1

Table 2. Concentration of 5-fluorocytosine derivative **3** and methylated-b-cyclodextrin (m-β-CD) used for UV-vis measurements

Concentration [Mol·L^{-1}]		Molar Ratio
3	**m-β-CD**	
3,26·10^{-3}	0·10^{-3}	1 : 0
3,26·10^{-3}	0,65·10^{-3}	1 : 0,2
3,26·10^{-3}	1,3·10^{-3}	1 : 0,4
3,26·10^{-3}	1,95·10^{-3}	1 : 0,6
3,26·10^{-3}	2,61·10^{-3}	1 : 0,8
3,26·10^{-3}	3,26·10-3	1 : 1
3,26·10^{-3}	4,89·10-3	1 : 1,5
3,26·10^{-3}	6,52·10^{-3}	1 : 2

Table 2. Concentration of 5-fluorocytosine derivative **3** and CD-containing polymer **4** used for UV-vis measurements

Concentration [Mol·L^{-1}]		Molar Ratio
3	**4**	
2,17·10^{-3}	0·10^{-3}	1 : 0
2,17·10^{-3}	0,43·10^{-3}	1 : 0,2
2,17·10^{-3}	0,86·10^{-3}	1 : 0,4
2,17·10^{-3}	1,30·10^{-3}	1 : 0,6
2,17·10^{-3}	1,73·10^{-3}	1 : 0,8
2,17·10^{-3}	2,17·10^{-3}	1 : 1
2,17·10^{-3}	2,60·10^{-3}	1 : 1,2
2,17·10^{-3}	3,04·10^{-3}	1 : 1,4
2,17·10^{-3}	3,47·10^{-3}	1 : 1,6
2,17·10^{-3}	3,90·10^{-3}	1 : 1,8
2,17·10^{-3}	4,34·10^{-3}	1 : 2