Supramolecular Catalysis

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Artificial Molecular Clamp: A Novel Device for Synthetic Polymerases**

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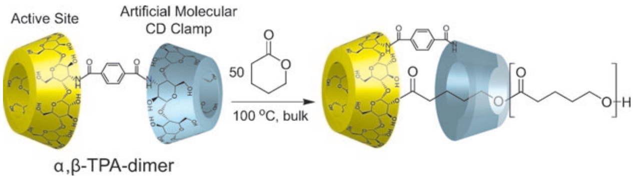
Renewable materials have attracted much attention from the viewpoints of environmental protection and efficient utilization of natural resources. Certain polyesters, polyamides, and polylactides, which are synthesized by either biological methods or chemical processes using a metal catalyst, have been extensively investigated as biodegradable and renewable polymers. However, biological methods are inefficient, and chemical processes involve harmful metals and organic solvents. Thus, more efficient and environmentally benign processes are necessary. In our studies, we hypothesized that innovative syntheses are best developed using chemical processes that take advantages of biological systems. Herein, we successfully obtained synthetic polymerases including an artificial molecular clamp to yield high-molecular-weight polymers without solvents or co-catalysts. This system is reminiscent of highly efficient DNA polymerases including a sliding clamp where the ring-shaped protein assembly of DNA polymerases plays an important role in the replication of polynucleotides.[1–7] Although the clamp does not have an active site, polymerization does not proceed well without the clamp. Similarly, cyclodextrins (CDs) are ring-shaped host molecules, which include various guests to form supramolecular complexes such as rotaxanes.[8–11]

An early example of supramolecular catalysis[12–14] is the hydrolysis of activated phenyl esters using CDs. These catalysts have also been utilized as enzyme models.[15–20] Moreover, modern supramolecular catalysts using host–guest interactions have achieved highly efficient and selective reactions, including hydrolysis reactions,[19,20] C−H bond activation,[23–25] epoxidation of olefins,[26–28] Diels–Alder reactions,[29–31] and 1,3-dipolar cycloadditions.[32,33] The bases of supramolecular catalysts are selective molecular recognition and substrate activation. One limitation of these catalysts is product inhibition because of their complex design, but introduction of an artificial molecular clamp into supramolecular catalysts can resolve the problems.

Herein, we show that cyclodextrins play an important role as an artificial molecular clamp in polymerization reactions. We selected β-CD as a supramolecular polymerization catalyst because it does not require a highly reactive catalytic center (metal complexes, cationic or anionic groups). CDs can include and activate lactones, yielding an oligomer tethered to a single CD at the end of the polymer chain.[34,35] However, the produced oligo(lactone)s bearing a β-CD unit did not initiate the polymerization reaction. We hypothesized that an artificial molecular CD clamp attached to the active site of the β-CD plays an important role in the polymerization by holding the polymer chain and consequently securing the active site.

First, we studied the polymerization activity of the α,β-TPA-dimer linked with terephthalamide between the α- and β-CDs for δ-valerolactone (δ-VL; Scheme 1). Polymerizations of δ-VL initiated by CD dimers were carried out by stirring and heating a bulk mixture of the CD dimers and δ-VL ([δ-VL]/[CD unit] = 50) at 100 °C.



Scheme 1. Polymerization of δ-VL initiated by the α,β-TPA-dimer linked with terephthalamide between the α-CD (gray-blue) and β-CD (yellow).

Table 1 summarizes the polymerization of δ-VL by CDs. Although intact α-CD did not initiate polymerization of δ-VL (Entry 1), intact β-CD and a mixture of α- and β-CD resulted in low polymerization activities for δ-VL under the same conditions (Entries 2 and 3). In contrast, the α,β-TPA-dimer displayed a significantly higher polymerization activity to give poly(δ-VL) with $M_n = 11\,000$ (Entry 5). Because of the absence of the active β-CD site, the α,α-TPA-dimer showed a much lower polymerization activity (Entry 4). Thus, the molecular α-CD clamp connected to the active β-CD site through a covalent bond is important for the polymerization.

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Table 1: Polymerizations of δ-VL initiated by CD derivatives in bulk.[a]

Entry	Initiator	Conversion [%]	Degree of polymerization	$M_n/10^3$[b]	M_w/M_n[c]
1	α-CD	0	–	–	–
2	β-CD	10	10	2.3	1.8
3	α-CD + β-CD (mixture)	11	11	2.9	1.1
4	α,α-TPA-dimer	5	3.0	2.5	1.1
5	α,β-TPA-dimer	74	87	11.0	1.6
6	α,β-TPA-dimer + Ad[d]	1	18	4.5	1.5

[a] [δ-VL]/[Initiator] = 50. CD derivative and δ-VL are heated at 100 °C for 120 h. [b] M_n is calculated based on the NMR ratio ([δ-VL units]/[CD terminal]). [c] M_w/M_n is determined by gel permeation chromatography (GPC) calibrated by polystyrene standards. [d] Adamantane (Ad) is added to the CD dimer prior to initiation of the polymerization. ([Ad]/[CD dimer] = 50).

Then we investigated the inhibition of polymerization of δ-VL using a competitive guest, adamantane (Ad), which is strongly bound in the cavity of β-CD.[36] Fifty equivalents of Ad guest molecules were mixed with the α,β-TPA-dimer and fifty equivalents of δ-VL were added to the reaction tube in solid state. The α,β-TPA-dimer/Ad mixture did not show a polymerization activity for δ-VL (see Entry 6 in Table 1). The initiation efficacy of the α,β-TPA-dimer/Ad mixture is lower than that of the pure α,β-TPA-dimer. Adamantane included in the β-CD unit of the α,β-TPA-dimer inhibited the polymerization of δ-VL in solid state. These observations confirm that the β-CD unit is the active site for the polymerization of δ-VL.

Next, CD dimers with different linker lengths were prepared to investigate the effect of linker length on the polymerization activity. The linkers of the α,β-*c*Sti-dimer (length of 8.5 Å) and the α,β-*t*Sti-dimer (length of 13.7 Å) were longer than that of the α,β-TPA-dimer (length of 7.0 Å), whereas the α,β-*c*Sti-dimer (length of 8.5 Å) had a shorter linker length than the α,β-*t*Sti-dimer. Additionally, we studied the polymerization activity of CD dimers with short linkers (less than 7 Å; Figure 1). The β,α-dimer lacked a linker, whereas the α,β-PA-dimer (length of 4.8 Å) and α,β-IPA-dimer (length of 6.7 Å) had shorter linkers than the α,β-TPA-dimer. Figure 2 shows the molecular weights of the polymers initiated by these CD dimers as a function of linker length. For linker lengths less than 8.5 Å long, the molecular weight of the resulting polymer decreased as the linker length of the CD dimer decreased. However, for linker lengths longer than 8.5 Å, the molecular weight of poly(δ-VL) decreased as the linker length increased. The molecular weight of the polymer was maximized when the α,β-dimers had linker lengths between 7.2 and 8.5 Å (Figure 2). These results indicate that the artificial molecular CD clamp spaces the active site of the β-CD unit and the spaced distance is adjusted by the linker length.

Afterwards we investigated the effects of the cavity size of the artificial molecular CD clamps on the polymerization activity. For combinations of α-CD/β-CD, β-CD/β-CD, and β-CD/γ-CD dimers with linker lengths between 7.2 and 8.5 Å we obtained polymers with maximum molecular weights (Figure 2). Compared to the α,β-*c*Sti-dimer, the β,γ-*c*Sti-dimer produced polymers with higher molecular weights ($M_n = 16400$). These results indicate that a linker length between 7.2–8.5 Å and introduction of γ-CD as molecular CD clamp afford high-molecular-weight poly(δ-VL) in good yields.

We then examined the differences in the polymerization activities of CD dimers with oligomer chains threaded through the molecular CD clamp and with dethreaded oligomer chains. The α,β-TPA-dimer with oligo(δ-VL) (1–2 units), which was prepared by reacting the α,β-TPA-dimer with two equivalents of δ-VL, was used to initiate the polymerization of δ-VL. The 2D ROESY NMR spectrum of the α,β-TPA-dimer with oligo(δ-VL) in D_2O showed that the proton peaks of the oligo(δ-VL) chain correlate to the inner protons (C(3)-H and C(5)-H) of the α-CD clamp, indicating that the oligomer chain is threaded through the molecular CD clamp (see Figure S26 in the Supporting Information). An aqueous solution of the α,β-TPA-dimer tethered to oligo(δ-VL) was freeze-dried to give a powder, which served as initiator. The α,β-TPA-dimer with oligo(δ-VL) treated in water initiated post-polymerization and gave a molecular weight of $M_n = 16500$ in 95 % yield (Figure 3 a). On the other hand, the 2D ROESY NMR spectrum of the α,β-TPA-dimer with oligo(δ-VL) in [D$_6$]DMSO did not show correlation peaks between the inner protons of the molecular α-CD clamp and the oligo(δ-VL) chain (see Figure S27 in the

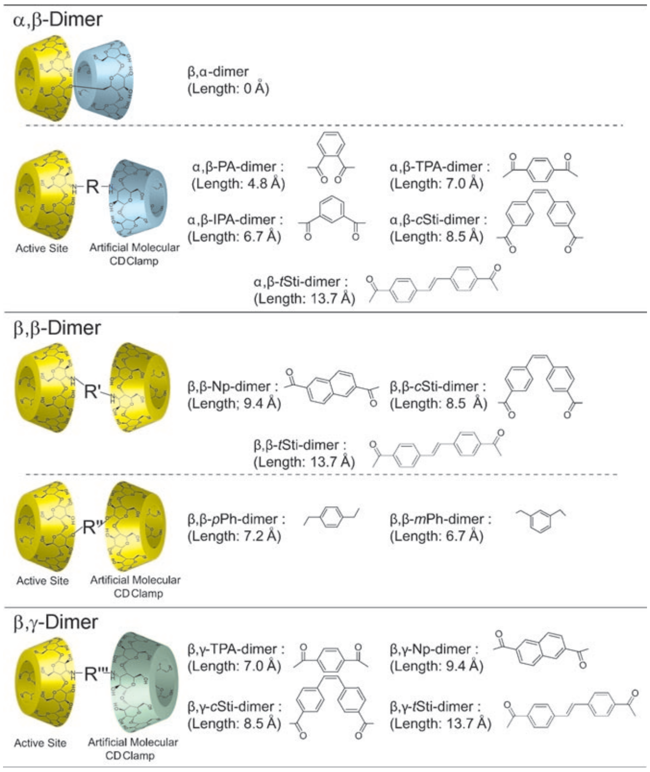

Figure 1. Chemical structures of CD dimers with linkers of different lengths. The linker length between the NH-amide groups is estimated by molecular modeling. Gray-blue: α-CD, yellow: β-CD, and gray-green: γ-CD.

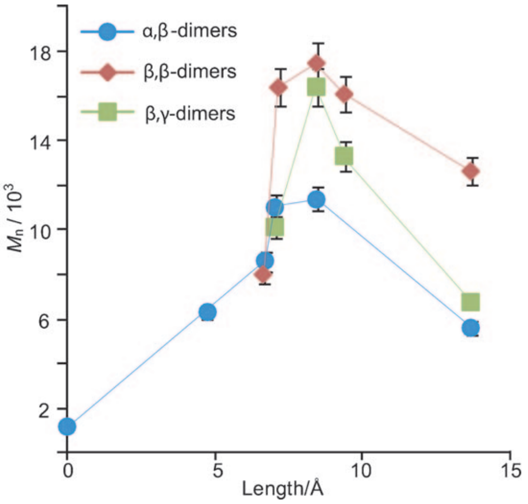

Figure 2. Influence of the linker length of the CD dimers on the molecular weight (M_n) of the resulting polymers.

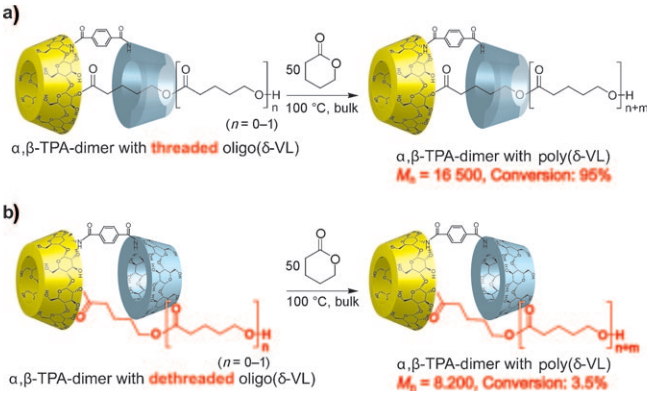

Figure 3. Effect of the oligomer chain threaded/not threaded through the molecular CD clamp. a) Polymerization of δ-VL initiated by the α,β-TPA-dimer with oligo(δ-VL) threaded through the molecular CD clamp. b) Polymerization of δ-VL initiated by the α,β-TPA-dimer with dethreaded oligo(δ-VL).

Supporting Information). These results indicate that the oligomer chain is not included in the cavity of the α-CD clamp in DMSO. The α,β-TPA-dimer with dethreaded oligo(δ-VL) was precipitated from a DMSO solution by pouring into an excess of acetone. The α,β-TPA-dimer treated in DMSO reinitiated polymerization of δ-VL in a yield of only 3.5 % (Figure 3b). These observations indicate that the CD dimer with dethreaded oligo(δ-VL) is inactive for the polymerization of δ-VL, and only the threaded dimer is active (Figure 3). The α,β-TPA-dimer with oligomer prepared from aqueous solutions showed a higher conversion relative to that prepared from organic solvents because the artificial molecular CD clamp effectively elongates the growing polymer chain and secures the active site of the β-CD unit. Thus, the molecular CD clamp plays an important role in controlling the polymerization activity.

A characteristic feature of the resulting polymers is that the CD dimer at the end of a polymer chain served as the active polymerization species. We investigated post-polymerization using purified CD dimers tethered to a polymer. The poly(δ-VL) ($M_n = 2300$) tethered to β-CD at the end of the polymer chain did not initiate post-polymerization of δ-VL (Entry 1 in Table 2) because of the absence of a threading CD on the propagating polymer chain. A prepolymer tethered to a α,β-TPA-dimer was prepared by reaction of the α,β-TPA-dimer and 50 equivalents of δ-VL in solid state (Entry 2 in Table 2). After purification of the prepolymer ($M_n = 11.0 \times 10^3$) tethered to the α,β-TPA-dimer, 100 equivalents of δ-VL were added to the prepolymer. Post-polymerizations gave poly(δ-VL) with $M_n = 15.2 \times 10^3$ under the same conditions (Entry 3 in Table 2).

A prepolymer tethered to a β,γ-*c*Sti-dimer was prepared by reaction of the β,γ-*c*Sti-dimer with 50 equivaltents of δ-VL (Entry 4 in Table 2). After purification of the prepolymer ($M_n = 16.4 \times 10^3$) tethered to the β,γ-*c*Sti-dimer, the prepolymer reinitiated polymerization of 500 equivalents of δ-VL to give poly(δ-VL) with $M_n = 45.2 \times 10^3$ (Entry 5 in Table 2). Upon further purification of the second polymer tethered to the β,γ-*c*Sti-dimer, the addition of 1000 equivalents of δ-VL led to a third polymer attached to the β,γ-*c*Sti-dimer, which reached $M_n = 85.8 \times 10^3$ (Entry 6 in Table 2). The products obtained from the CD dimers are polyesters with higher molecular weight. These results indicate that not only the β-CD unit acts as the active site but also the molecular CD clamp plays an important role in the propagation of polymerization.

In conclusion, we demonstrate that CD dimers behave like polymerases for cyclic esters without co-catalysts or solvents. Figure 4 shows the proposed polymerization mechanism of δ-VL initiated by the α,β-TPA-dimer. One CD moiety in the dimer acts as the active site for ring-opening and converts the monomer to produce a polymer chain. The other moiety serves as an artificial molecular clamp to effectively

Table 2: Post-polymerizations of δ-VL initiated by CD-dimer-tethered poly(δ-VL) in bulk.[a]

Entry	Initiator	[δ-VL]/[CD]	Conversion [%]	Degree of polymerization	$M_n/10^3$[b]	M_w/M_n[c]
1	β-CD with poly(δ-VL)	100	0	11	2.3	1.8
2	α,β-TPA-dimer[d]	50	74	87	11.0[d]	1.6
3	Product of entry 2	100	60	147	15.2	1.8
4	β,γ-*c*Sti-dimer	50	96	138	16.4	1.3
5	Product of entry 4	500	83	425	45.2	1.3
6	Product of entry 5	1000	43	831	85.8	1.3

[a] The CD derivative and δ-VL are heated at 100 °C for 120 h. The resulting polymer in entry 1 has the same properties as the prepolymer. [b] M_n is calculated based on the NMR ratio ([δ-VL units]/[CD terminal]). [c] M_w/M_n is determined by GPC calibrated by polystyrene standards. [d] The molecular weight of the prepolymer is given in Table 1, entry 5.

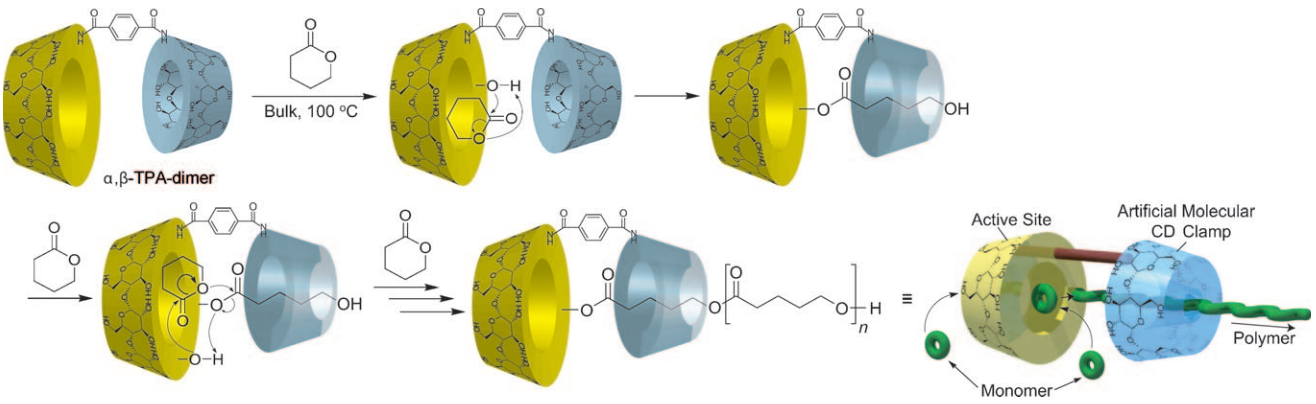

Figure 4. Proposed mechanism for the polymerization of δ-VL initiated by the α,β-TPA-dimer.

propagate the polymer chain. Although the molecular CD clamp does not show polymerization activity for δ-VL, it plays an essential role in the polymerization by holding the polymer chain and consequently securing the active site. Thus, another δ-VL unit is accessible to the β-CD at the end of polymer chain. The included δ-VL is inserted at the ester bond between the β-CD and the polymer chain. The polymerization activities of the CD dimers depend on the linker length. Linkers of proper lengths display high polymerization activities. If the linker is too short, then the CD dimer suppresses monomer recognition. In contrast, if the linker is too long, then the CD dimer cannot clamp the growing polymer chain. The proper length can be found by adjustment. This behavior is similar to a sliding DNA clamp–clamp loader complex, which plays an important role in propagating DNA in biological systems. These CD dimers efficiently initiate polymerization to produce polyesters. We are currently investigating the relationship between linker length and threading behavior. The artificial molecular clamp should cause a new paradigm in catalytic reactions of supramolecular catalysts as well as provide a template for syntheses of fine chemicals.

Experimental Section

Polymerization of δ-VL initiated by CD-dimers: Polymerization of δ-VL initiated by the α,β-TPA-dimer is given for example. The α,β-TPA-dimer (24 mg, 11 µmol) was dried in vacuum at 80 °C, and then δ-VL (50 µL, 55 mg, 550 µmol) was added. The bulk mixture was heated at 100 °C. After 120 h the heterogeneous mixture was dissolved in DMF (1 mL). This solution was added to 10 mL of THF to precipitate the α,β-TPA-dimer. Then the solution was evaporated and dried in vacuum to yield poly(δ-VL) (33 mg; 74 %). ^{1}H NMR ([D$_6$]DMSO, 30 °C, 500 MHz): $\delta = 8.16$–8.08 (br, 2H, –CON*H*–), 7.91–7.87 (br, 4H, 2,3–Ph), 5.89–5.45 (br, 23H, O_2H and O_3H), 4.87–4.61 (br, 13H, C_1H), 4.45–4.24 (br, 13H, O_6H), 3.95 (br, 145H, δ-polymer), 3.71–3.09 (br, overlaps with HOD, $C_{23456}H$), 2.30 (br, 165H, α-polymer), 1.51 ppm (br, 348H, β- and γ-polymer).

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[1] D. M. Carter, C. M. Radding, *J. Biol. Chem.* **1971**, *246*, 2502–2512.
[2] G. Nardone, J. George, J. G. Chirikjian, *J. Biol. Chem.* **1986**, *261*, 12128–12133.
[3] R. S. Brody, K. G. Doherty, P. D. Zimmerman, *J. Biol. Chem.* **1986**, *261*, 7136–7143.
[4] X. P. Kong, R. Onrust, M. O'Donnell, J. Kuriyan, *Cell* **1992**, *69*, 425–437.
[5] T. S. R. Krishna, X.-P. Kong, S. Gary, P. M. Burgers, J. Kuriyan, *Cell* **1994**, *79*, 1233–1243.
[6] R. Kovall, B. W. Matthews, *Science* **1997**, *277*, 1824–1827.
[7] M. A. Trakselis, S. C. Alley, E. Abel-Santos, S. J. Benkovic, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 8368–8375.
[8] J. P. Sauvage, C. Dietrich-Buchecker, *Molecular Catenanes, Rotaxanes and Knots: A Journey Through the World of Molecular Topology*, Wiley-VCH, Weinheim, **1999**.
[9] F. M. Raymo, J. F. Stoddart, *Chem. Rev.* **1999**, *99*, 1643–1664.
[10] A. Harada, M. Kamachi, *Macromolecules* **1990**, *23*, 2821–2823.
[11] A. Harada, M. Kamachi, *J. Chem. Soc. Chem. Commun.* **1990**, 1322–1323.
[12] J. M. Lehn in *Supramolecular Chemistry*, Wiley-VCH, Weinheim, **1995**, pp. 10–20.
[13] R. Breslow in *Artificial Enzymes*, Wiley-VCH, Weinheim, **2006**.
[14] P. W. N. M. van Leeuwen in *Supramolecular Catalysis*, Wiley-VCH, Weinheim, **2008**.
[15] M. F. Czarniecki, R. Breslow, *J. Am. Chem. Soc.* **1978**, *100*, 7771–7772.
[16] R. Breslow, *Acc. Chem. Res.* **1991**, *24*, 317–324.
[17] a) I. M. Mallick, V. T. D′Souza, M. Yamaguchi, J. Lee, P. Chalabi, R. C. Gadwood, M. L. Bender, *J. Am. Chem. Soc.* **1984**, *106*, 7252; b) V. T. D′Souza, K. Hanabusa, T. O'Leary, R. C. Gadwood, M. L. Bender, *Biochem. Biophys. Res. Commun.* **1985**, *129*, 727-732.
[18] V. T. D′Souza, M. L. Bender, *Acc. Chem. Res.* **1987**, *20*, 146–152.
[19] M. Komiyama, E. J. Breaux, M. L. Bender, *Bioorg. Chem.* **1977**, *6*, 127–136.
[20] R. Breslow, J. B. Doherty, G. Guillot, C. Lipsey, *J. Am. Chem. Soc.* **1978**, *100*, 3227–3229.
[21] S. Richeter, J. Rebek, Jr., *J. Am. Chem. Soc.* **2004**, *126*, 16280–16281.
[22] M. D. Pluth, R. G. Bergman, K. N. Raymond, *Science* **2007**, *316*, 85–88.

[23] D. H. Leung, D. Fiedler, R. G. Bergman, K. N. Raymond, *Angew. Chem.* **2004**, *116*, 981 – 984; *Angew. Chem. Int. Ed.* **2004**, *43*, 963 – 966.
[24] S. Das, C. D. Incarvito, R. H. Crabtree, G. W. Brudvig, *Science* **2006**, *312*, 1941 – 1943.
[25] D. H. Leung, R. G. Bergman, K. N. Raymond, *J. Am. Chem. Soc.* **2006**, *128*, 9781 – 9797 .
[26] P. Thordarson, E. J. A. Bijsterveld, A. E. Rowan, R. J. M. Nolte, *Nature* **2003**, *424*, 915 – 918.
[27] R. G. Coumans, J. A. A. Elemans, R. J. Nolte, A. E. Rowan, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 19647 – 19651.
[28] S. Jónsson, G. J. Odille Fabrice, P. O. Norrby, K. Wärnmark, *Chem. Commun.* **2005**, 549 – 551.
[29] C. J. Walter, H. L. Anderson, J. K. M. Sanders, *J. Chem. Soc. Chem. Commun.* **1993**, 458 – 460.
[30] M. Marty, Z. Clyde-Watson, L J. Twyman, M. Nakash, J. K. M. Sanders, *Chem. Commun.* **1998**, 2265 – 2266.
[31] M. Yoshizawa, M. Tamura, M. Fujita, *Science* **2006**, *312*, 251 – 254.
[32] W. L. Mock, T. A. Itta, J. P. Wepsiec, M. Adhya, *J. Org. Chem.* **1989**, *54*, 5302 – 5308.
[33] J. Chen, J. Rebek, Jr., *Org. Lett.* **2002**, *4*, 327 – 329.
[34] M. Osaki, Y. Takashima, H. Yamaguchi, A. Harada, *J. Am. Chem. Soc.* **2007**, *129*, 14452 – 14457.
[35] A. Harada, M. Osaki, Y. Takashima, H. Yamaguchi, *Acc. Chem. Res.* **2008**, *41*, 1143 – 1152.
[36] M. V. Rekharsky, Y. Inoue, *Chem. Rev.* **1998**, *98*, 1875 – 1917.