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Molecular Recognition Studies on Supramolecular Systems. Part 38. Inclusion Complexation of Organic Dyes by Organoselenium Bridged Bis(β-cyclodextrin)s with a Short Linker

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Abstract

In order to further explore the inclusion complexation behavior with β-cyclodextrin dimers, the binding constants (K_S) of three organoselenium bridged bis(β-cyclodextrin)s (**2–4**) tethered with a short linker were determined with some representative dye molecules in aqueous phosphate buffer solution (pH 7.20) at 25 °C by fluorescence and UV-vis spectrometry. As compared with the parent β-cyclodextrin (**1**), the bridged bis(β-cyclodextrin)s (**2–4**) can not only significantly enhance the original binding affinity of the parent β-cyclodextrin by the cooperative binding of one guest molecule in the closely located two β-cyclodextrin cavities but also remarkably extend its molecular recognition abilities towards the different size/shape or substituent of model substrates. The higher binding ability and selectivity of dye molecules by bridged bis(β-cyclodextrin)s (**2–4**) are discussed from the viewpoint of the size/shape-fit concept and multiple recognition mechanism.

Introduction

Possessing two appropriately-located hydrophobic cavities in a single molecule, bridged bis(cyclodextrin)s linked with each other at the primary side by a simple tether feature a very high binding ability and molecular selectivity for specific bipedal guests through the cooperative multiple recognition. This fascinating property enables them to be employed successfully in several areas of science and technology as an excellent model system mimicking substrate-specific interaction of enzymes [1–6]. Hence, the study on the molecular recognition behavior of substrate (guest) by cyclodextrin dimers tethered by the spacer (or linker) of different sizes and shapes is one of the most current topics in supramolecular chemistry and biochemistry [7–8]. Recently, a variety of bis(β-cyclodextrin)s with considerable structural diversity have been prepared to elucidate their inclusion complexation behavior as well as the factors and mechanisms governing the multipoint recognition upon inclusion complexation with model substrates [9–16]. However, the work concerning the molecular recognition by cyclodextrin dimers has been concentrated mainly on the inclusion complexation by cyclodextrin dimers linked by alkanedioates [1, 17], disulfides [18, 19], dipyridines [20, 21], imidazole [22, 23], and oligo(ethylene diamino) [24], the synthetic and molecular recognition studies on organoselenium-bridged cyclodextrin dimers are still rare, except for recent studies by Liu *et al.* [16, 25, 26] and Shen *et al.* [27]. We have recently shown that organoselenium-bridged bis(β-cyclodextrin)s form more stable complexes with model substrates than native β-cyclodextrin through the cooperative binding of one guest molecule by two cyclodextrin moieties. These results promote our understanding of the multipoint recognition and the induced-fit interactions working between host and guest.

In the present investigation, we choose organoselenium bridged β-cyclodextrin dimers as specific host molecules. A simple reason is that selenium, possessing a larger radius and lower electronegativity than carbon, can provide a Se—Se bond that is longer and more flexible than a C—C bond. A more serious reason is that selenium compounds are known to function as mimics of glutathione peroxidase. It is considered that the organoselenium compounds are introduced to the primary or secondary rim of the cyclodextrin cavity, which can act as the catalytic functional group in artificial mimics to accelerate the reactions of substrates accommodated in the cyclodextrin cavity. Indeed, some organoselenium modified β-cyclodextrins and organoselenium bridged bis(β-cyclodextrin)s have been taken as an excellent model to mimic enzymes successfully [6, 27]. Therefore, the studies on organoselenium bridged β-cyclodextrin dimers can highlight the way for understanding the interaction between the cyclodextrin-based selenium-containing enzyme model and substrates. In this context, we wish to report our research results on the inclusion complexation behavior of some representative dye molecules by three organoselenium β-cyclodextrin dimers tethered by different spacers, shown in Chart 1. The complex stability constants (K_S) and Gibbs

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free energy changes ($-\Delta G^{\circ}$) for the 1 : 1 inclusion complexation of parent β-cyclodextrin and bis(β-cyclodextrin)s with guest molecules (Chart 2) have been determined at 25 °C by means of fluorescence and ultraviolet titrations in aqueous phosphate buffer solution (pH 7.20). These will provide our further understanding of the molecular recognition behavior in the light of the cooperative binding and the complementary geometrical relationship between the dimeric host and guest.

Experimental

Materials

β-Cyclodextrin (**1**) was purchased from Ensuiko Seito. 6,6′-*o*-Phenylene-diseleno-bridged bis(β-cyclodextrin) (**2**), 6,6′-trimethylenediseleno-bridged bis(β-cyclodextrin) (**3**), and 6,6′-[2,2'-diselenobis(benzoyloxyl)]-bridged bis(β-cyclodextrin) (**4**) were prepared according to the reported procedures [16, 25, 26], respectively. Acridine red (AR) was purchased from Chroma-Gesellshaft Schmid & Co. Neutral red (NR), methylene blue (MB) and methyl orange (MO) were purchased from Tianjin Chemical Reagent Plant. The mixture of solid methyl red, commercially available from Tianjin Chemical Reagent Plant, and sodium hydroxide (1:1 equiv.) was dissolved in the phosphate buffer solution, which was used as a guest molecule in spectral titration. Sodium dihydrogen phosphate and disodium hydrogen phosphate were dissolved in deionized, distilled water to make a 0.10 M buffer solution of pH 7.20, which was used as solvent throughout the spectral measurements.

Spectral measurements

Fluorescence spectra were measured in a conventional quartz cell (10 × 10 × 40 mm) on a JASCO FP-750 spectrofluorimeter, with the excitation and emission slits of 10 nm for all fluorescent dyes. The excitation wavelengths for AR, NR and MB were 490 nm, 510 nm and 495 nm, respectively. The sample solution containing fluorescent dye (1.6×10^{-6} mol dm^{-3} for AR, 7.2×10^{-6} mol dm^{-3} for NR, and 2×10^{-4} mol dm^{-3} for MB) and various concentrations of host (0–4.5×10^{-5} for AR, 0–2.6×10^{-4} for NR, 0–1.0×10^{-3} for MB) was kept at 25.0 ± 0.1 °C for spectral measurements by a circulating thermostated water-jacket.

Results and discussion

Spectral titration

Inclusion complexation of the guest molecule with cyclodextrin usually alters its original spectrum. Since AR, NR and MB are fluorescent in aqueous solution, and very sensitive to environmental changes, we quantitatively assessed the molecular recognition behavior of bis(β-cyclodextrin)s **2–4** with AR, NR and MB by the fluorescence spectral titration method. In the fluorescence spectral titration experiments, as shown in Figure 1, the relative fluorescence intensity of AR dramatically enhances upon gradual addition of dimeric host **2**. As a model spectral titration experiment of modified β-cyclodextrin, the addition of the host **2** to a diluted guest NR solution causes significant maximum hypsochromic shift of the fluorescence peak up to 36 nm, while a slight shift to the blue occurs for AR up to 5 nm. Analogous fluorescence behavior was also observed in the other case of inclusion complexation of hosts **1–4** with guest molecules AR and NR. Additionally, the guest MB shows no appreciable fluorescence peak in the wavelength range of 500–650 nm under these experimental conditions, but after adding host **2**, a new peak appears at ca. 549 nm and its relative intensity gradually increases with increasing host concentration. These phenomena jointly indicate that the guest molecule moves from the bulk water towards the interior of the hydrophobic β-cyclodextrin cavity, forming the inclusion complex with β-cyclodextrin dimers.

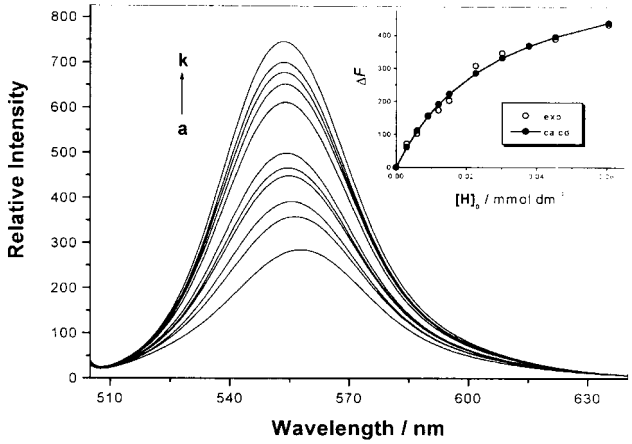

Figure 1. Fluorescence spectral changes of AR (1.5×10^{-6} mol dm^{-3}) and non-linear analysis (inset) upon addition of host **2** in aqueous phosphate buffer solution at pH 7.20. The concentration of **2** increases in the range 0.6–6.0×10^{-5} mol dm^{-3} from *a* to *k*. (Excitation wavelength was 490 nm.)

From the fluorescence intensity changes induced by adding the host molecule, we can determine the complex stability constants (K_S). With assumption of a 1 : 1 stoichiometry, where the two β-cyclodextrin moieties in **2–4** are treated as a unit, the inclusion complexation of guest (G) with host (H) is expressed by Equation (1).

$$H + G \overset{K_S}{\rightleftharpoons} G \cdot H. \tag{1}$$

The complex stability constants (K_S) [28] were calculated for each host–guest combination from the non-linear squares fit to Equation (2).

$$\Delta F = \{\alpha([H]_0 + [G]_0 + 1/K_S) \pm\sqrt{\alpha^2([H]_0 + [G]_0 + 1/K_S)^2 - 4\alpha^2[H]_0[G]_0}\}/2, \tag{2}$$

where $[G]_0$ and $[H]_0$ refer to the total concentrations of the guest and host and α the proportionality coefficient, which may be taken as a sensitivity factor for the fluorescence

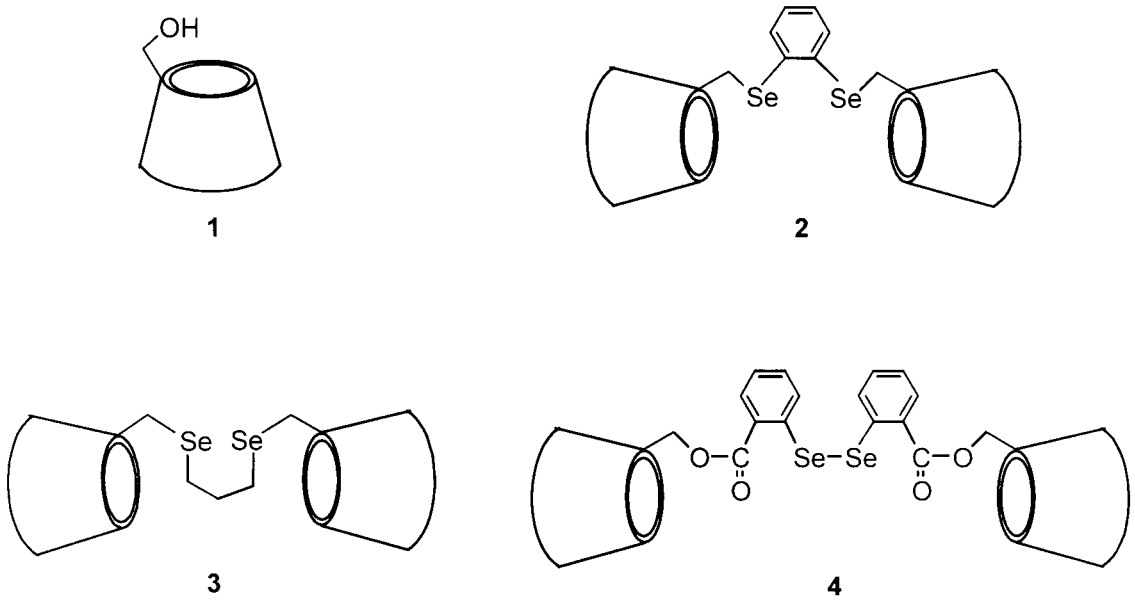

Chart 1.

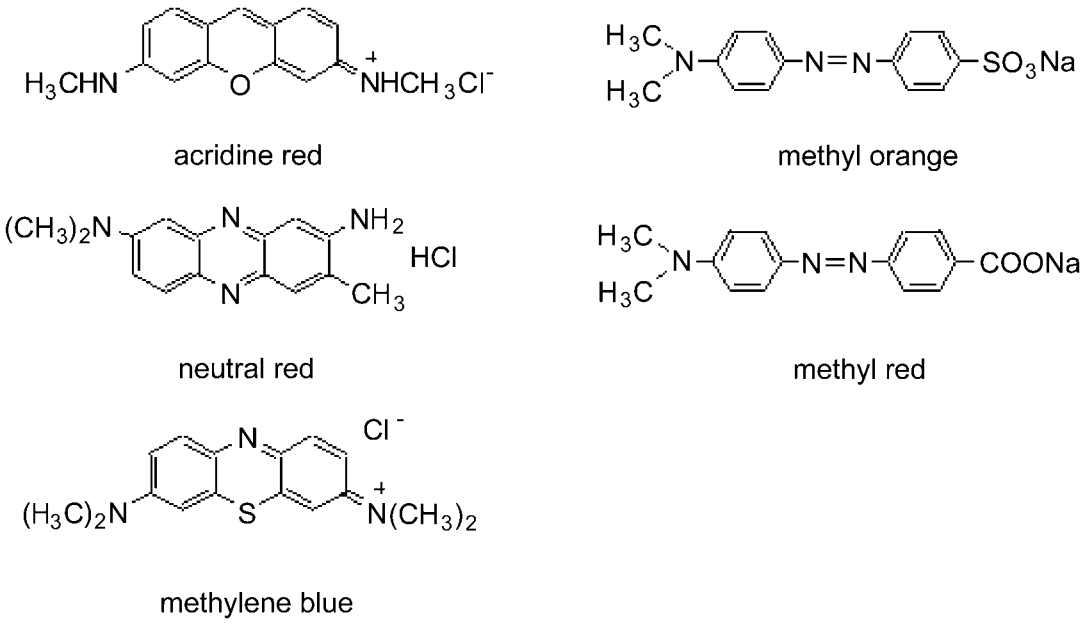

Chart 2.

change. For each host examined, the plot of ΔF as a function of $[G]_0$ gave an excellent fit, verifying the validity of the 1 : 1 complex stoichiometry assumed above. The experimental data do not show any significant deviations from the theoretical curve in each case. In the repeated measurements, the K_S values were reproducible within an error of $\pm 5\%$. The K_S values obtained are listed in Table 1, along with the free energy changes of complex formation ($-\Delta G^\circ$) and the ultimate fluorescence maximum (λ^F_{max}) obtained upon addition of a large excess of host. In order to visualize the inclusion complexation behavior of the host with dye molecules, the changing profiles of free energy changes ($-\Delta G^\circ$) upon complexation with host compounds **1–4** are shown in Figure 2.

Binding constants and molecular recognition

Native and simple modified cyclodextrins afford only limited binding constants upon inclusion complexation with model substrates probably due to the weak hydrophobic interactions between host and guest. Bridged cyclodextrin dimers, however, can greatly enhance the original molecular binding ability of native cyclodextrin through the cooperative binding of two adjacent cyclodextrin cavities with the single guest molecule. As can be seen from Table 1, the dimeric β-cyclodextrins **2–4** display significantly the higher binding constants (K_S) with dye molecules up to 1.3 $\sim$ 36 times as compared with native β-cyclodextrin, which in turn accounts for the inherent advantage of bis(β-cyclodextrin)s upon inclusion complexation with relatively large guest molecules. Moreover, further comparison of the structures of

Table 1. Complex stability constant (K_S), Gibbs free energy change ($-\Delta G^\circ$), and ultimate fluorescence maximum (λ^F_{max}) for 1 : 1 inclusion complexation of dye molecules with β-cyclodextrin **1** and bis(β-cyclodextrin)s **2–4** in aqueous buffer solution (pH 7.20) at 25.0 °C

Host	Guest	λ^F_{max}/nm[a]	K_S	log K_S	$-\Delta G^\circ$/kJ mol^{-1}	Method	Ref.
1	Acridine red (AR)	552	2630	3.42	19.52	FL	[b]
	Neutral red (NR)	576	480	2.68	15.30	FL	[b]
	Methylene blue (MB)	549	209	2.32	13.24	FL	[b]
	Methyl orange (MO)		3560	3.55	20.27	CD	[c]
	Methyl red (MR)		3450	3.54	20.19	UV	[b]
2	AR	554	36300	4.56	26.03	FL	[b]
	NR	562	17510	4.24	24.22	FL	[b]
	MB	549	6770	3.83	21.86	FL	[b]
	MO		63900	4.81	27.43	CD	[d]
	MR		9940	4.00	22.82	UV	[b]
3	AR	553	17810	4.25	24.26	FL	[b]
	NR	568	5090	3.71	21.26	FL	[b]
	MB	547	3990	3.60	20.55	FL	[b]
	MO		17400	4.24	24.20	CD	[c]
	MR		6120	3.79	21.64	UV	[b]
4	AR	558	3320	3.52	20.10	FL	[b]
	NR	572	2350	3.37	19.24	FL	[b]

[a]Ultimate fluorescence maximum obtained upon addition of large excess of host, while the λ^F_{max} of AR and NR are 559 and 598 nm, respectively. MB displays no original fluorescence peak within the measurement wavelength range.
[b] This work.
[c] Ref. [26].
[d] Ref. [25].

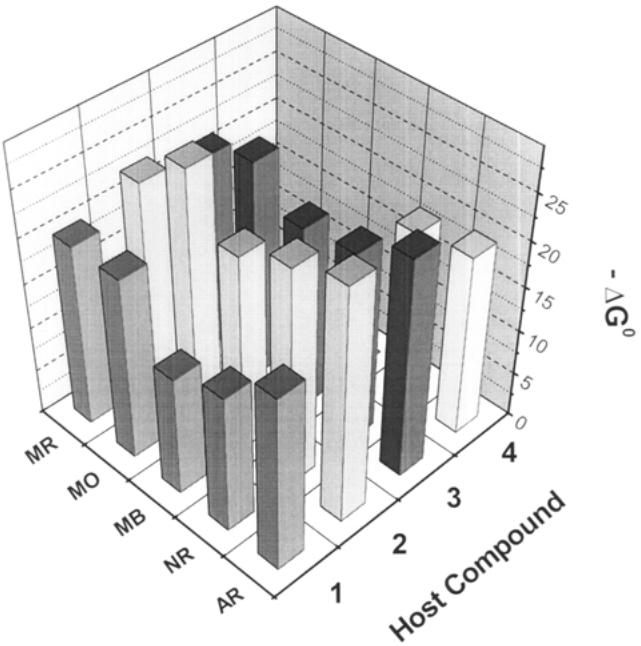

Figure 2. Gibbs free energy change ($-\Delta G^\circ$) of the inclusion complexation of hosts **1–4** with guest molecules.

the dye molecules examined shows that these guest molecules share some structural and functional similarities. For instance, either of AR, NR and MB possesses a heterocycle anthracene moiety, while both MR and MO bear an N=N double bond unit. Therefore, as illustrated in Table 1 and Figure 2, host compounds **1–4** display an interesting binding affinity sequence within two families of dyes, i.e., AR > NR > MB and MO > MR. Although both the parent β-cyclodextrin (**1**) and dimeric β-cyclodextrins (**2–4**) give lower K_S values for NR and MB rather than for AR, the effect of the cooperative binding by dimeric β-cyclodextrins is more remarkable for NR and MB, showing the enhanced higher binding constant upon inclusion complexation with **2–4**. This indicates that, although AR, NR and MB possess similar residues, they exhibit dramatically different complexation behavior with hosts. The guest AR with a small substituent can be well embedded in the cavity of β-cyclodextrin (**1**), and the second cavity added in the dimeric hosts **2–4** merely enhances the K_S value by 1.3–13.8 times. On the other hand, the guests NR and MB are only poorly accommodated in the cavity of **1**, attributed to the steric hindrance. Therefore, the contribution of the second cavity in **2–4** is much more pronounced to give an enhancement of K_S by a factor of 4.9 $\sim$ 36 for NR and 19 $\sim$ 32 for MB, respectively.

It is interestingly noted that the bridged bis(β-cyclodextrin)s not only greatly enhance the original binding ability of the parent β-cyclodextrin but also extend its molecular selectivity for guest molecules with different size/shape/substituent. As can be seen from Table 1, native β-cyclodextrin shows a similar binding ability for MO and MR. Examinations with CPK molecular models indicate that MO and MR can only partially penetrate into the cyclodextrin cavity to form an inclusion complex as a result of the steric hindrance. Thus, β-cyclodextrin gives a relatively low selectivity towards the MO/MR pair. In the case of the inclusion complexation of host compounds **2–4** with MO

or MR, since the sulfonic or carboxy group in the guest molecule can be partially included in the second cavity added in the dimeric hosts. Meanwhile, the electrostatic and/or hydrogen bond interactions between the sulfonic group and the cyclodextrin cavity are stronger than that of the carboxy group, therefore, host compounds **2** and **3** display the higher molecular selectivity for the MO/MR pair up to 2.8 and 6.4 times that of parent β-cyclodextrin. These results demonstrate that the bridged bis(β-cyclodextrin)s possess a relatively high binding ability as compared with native β-cyclodextrin, especially for large guest molecules.

It is worth noting that there exists a fairly general tendency of K_S values, which increase with the decrease of the tether length in hosts **2–4**, i.e., a bridged bis(β-cyclodextrin) linked by a rigid tether is larger than that with a flexible tether. As can be seen in Table 1, the binding constant (K_S) of host compounds **2–4** with the guest molecules examined varies in an order **2** > **3** > **4**. One possible explanation for the drastic variation of compounds **2–4** in their binding abilities towards relatively large guest molecules would be the inherent advantage in entropy gain upon inclusion complexation with bis(β-cyclodextrin) linked by a rigid tether, since the cooperative effect gradually declines upon extending the distance between two cyclodextrin moieties.

In conclusion, bridged bis(β-cyclodextrin)s **2–4** dramatically enhance the original binding ability of parent β-cyclodextrin through the cooperative binding of two closely located β-cyclodextrin cavities. The inclusion complexation behavior of host compounds **2–4** mainly depends on the conformation, length and flexibility of the organoselenium tether, which may control how the dual cyclodextrin cavities adjust their orientations and conformation to cooperatively bind guest molecules. Simultaneously, the size/shape matching concept between host and guest dominates also the stability of the inclusion complexes formed by bridged bis(β-cyclodextrin)s **2–4** with organic dye molecules. Therefore, the introduction of the different bridge organoselenium tether to the primary side of β-cyclodextrin can vary and control the orientation and binding ability/selectivity upon inclusion complexation of bridged bis(β-cyclodextrin)s with model substrates.

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References

1. R. Breslow and B. Zhang: *J. Am. Chem. Soc.* **114**, 5882 (1992).
2. B. Zhang and R. Breslow: *J. Am. Chem. Soc.* **119**, 1676 (1997).
3. F. Sallas, A. Marsura, V. Petot, I. Pintér, J. Kovács and L. Jicsinszky: *Helv. Chim. Acta.* **81**, 632 (1998).
4. R.R. French, J. Wirz and W.-D. Woggon: *Helv. Chim. Acta.* **81**, 1521 (1998).
5. I. Tabushi, Y. Kuroda and K. Shimokawa: *J. Am. Chem. Soc.* **101**, 1614 (1979).
6. (a) J.-Q. Liu, S.-J. Gao, G.-M. Luo, G.-L. Yan and J.-C. Shen: *Biochem. Biophys. Res. Commun.* **247**, 397 (1998); (b) X.-Y. Ma, Y.-H. Wu, L. Ding, D.-Q. Zhao, Z.-J. Ni and Y. Liu: *Chem. J. Chinese University* **20**, 1163 (1999).
7. J. Szejtli and T. Osa: in J.L. Atwood, J.E.D. Davies, D.D. MacNicol and F. Vögle (eds.), *Comprehensive Supramolecular Chemistry*, Vol. 3, Elsevier, Oxford (1996).
8. R. Breslow, S. Halfon and B. Zhang: *Tetrahedron* **51**, 377 (1995).
9. R. Breslow, Z. Yang and R. Ching: *J. Am. Chem. Soc.* **120**, 3536 (1998).
10. T. Jiang, D.K. Sukumaran, S.D. Soni and D.S. Lawrence: *J. Org. Chem.* **59**, 5149 (1994).
11. T. Jiang and D.S. Lawrence: *J. Am. Chem. Soc.* **117**, 1857 (1995).
12. R.C. Petter, C.T. Sikorski and D.H. Waldeck: *J. Am. Chem. Soc.* **113**, 2325 (1991).
13. M. Maletic, H. Wennemers, Q.D. McDonald, R. Breslow and W.C. Still: *Angew. Chem. Int. Ed. Engl.* **35**, 1490 (1996).
14. N. Brilirakis, B. Henry, P. Berthault, F. Venema and R.J.M. Nolte: *Tetrahedron* **54**, 3523 (1998).
15. Y. Liu, C.-C. You, R.-T. Chen, T. Wada and Y. Inoue: *J. Org. Chem.* **64**, 7781 (1999).
16. Y. Liu, B. Li, T. Wada and Y. Inoue: *Supramol. Chem.* **10**, 279 (1999).
17. R. Breslow, N. Greenspoon, T. Guo and R. Zarzycki: *J. Am. Chem. Soc.* **111**, 8296 (1989).
18. R. Breslow and S. Chuang: *J. Am. Chem. Soc.* **112**, 9659 (1990).
19. Y. Okabe, H. Yamamura, K. Obe, K. Ohta, M. Kawai and K. Fujita: *J. Chem. Soc. Chem. Commun.* 581 (1995).
20. R. Deschenaux, T. Ruch, P.-F. Deschenaux, A. Juris and A. Ziessel: *Helv. Chim. Acta.* **78**, 619 (1995).
21. H.F.M. Nelissen, A.F.J. Schut, F. Venema, M.C. Feiters and R.J.M. Nolte: *J. Chem. Soc. Chem. Commun.* 577 (2000).
22. R. Breslow and S. Halfon: *Proc. Natl, Acad. Sci. USA* **89**, 6916 (1992).
23. M.-M. Luo, R.-G. Xie, D.-Q. Yuan, W. Lu, P.-F. Xia and H.-M. Zhao: *Chin. J. Chem.* **17**, 384 (1999).
24. Y. Liu, C.-C. You and B. Li: *Chem. Eur. J.* **7**, 1281 (2001).
25. Y. Liu, C.-C. You, Y. Chen, T. Wada and Y. Inoue: *J. Org. Chem.* **64**, 7781 (1999).
26. Y. Liu, B. Li, C.-C. You, T. Wada and Y. Inoue: *J. Org. Chem.* **66**, 225 (2001).
27. (a) J.-Q. Liu, Y.-G. Ning, C.-B. Shi, G.-M. Luo, G.-C. Yan and J.-C. Shen: *Gaodeng Xuexiao Huaxue Xuebao (Chem. J. Chin. Univ.)* **19**, 1446 (1998). (b) J.-Q. Liu, G.-M. Luo, X.-J. Ren, Y. Mu, Y. Bai and J.-C. Shen: *Biochim. Biophys. Acta* **1481**, 222 (2000). (c) X.-J. Ren, J.-Q. Liu, G.-M. Luo, Y. Zhang, Y.-M. Luo, G.-L. Yan and J.-C. Shen: *Bioconjugate Chem.* **11**, 682 (2000).
28. H.-C. Becker and B. Norden: *J. Am. Chem. Soc.* **119**, 5798 (1997).