Pergamon

Tetrahedron Letters, Vol. 35, No. 25, pp. 4275-4278, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)00813-2

The Effect of Tether Length on the Affinity of Ligands for Bis(cyclodextrins)

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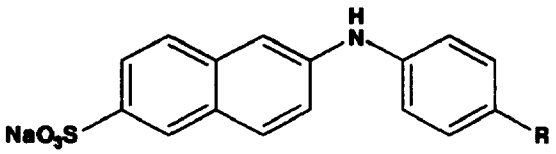
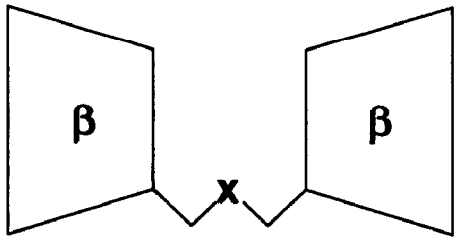
Summary: Bis(cyclodextrin)s linked by alkyl chains of varying lengths were prepared and their ability to bind to fluorophoric ligands such as 6-toluidino-2-naphthalenesulfonate (TNS) was evaluated.

β-Cyclodextrin (β-CD), a macrocyclic oligomer of seven [α-1,4]-linked glucoses, has been widely studied as a water-soluble receptor that binds small organic ligands by inclusion complexation.[2] High binding affinity to CDs generally requires good shape complementarity in the receptor/ligand complex. β-CD binds organic guests with equilibrium binding constants (K_a) in the range of 10^2 to 10^4 M^{-1} in water.[2] For example, K_a = 1800 M^{-1} for 6-toluidino-2-naphthalenesulfonate (TNS).[3-5] The cooperative action of two covalently linked cyclodextrins (bisCDs) has been shown by several groups[3,4,6-8] to provide more potent binding of ligands which exploit the extended recognition site, with K_a values of 10^5 to 10^9 M^{-1}. However, a study of the dependence of binding affinity of bisCDs on the length of a simple tether has not been reported. We have prepared bisCDs **1** through **8** and assayed their binding behavior by titration against fluorophoric ligands TNS and BNS[9] (6-(4-*tert*-butylanilino)-2-naphthalenesulfonate). In this series of bisCDs, the optimum tether for BNS as guest is -SCH_2CH_2S-, with $K_a = 8.2 \pm 1.2 \times 10^6$ M^{-1}.

BisCD **1**, previously reported by Fujita,[4] was prepared by treating β-CD-6-monotosylate with thiourea in DMF, desalting with Amberlite MB-3 (affording the 6-monomercapto-β-CD in 53% yield), and oxidative coupling with KOH/KI/I_2 followed by purification on a Sephadex G-25 column (eluted with water and monitored by the ORD of the collected fractions) to give the disulfide in 90% yield.[10] Alkylation of linear 1,ω-dimercaptoalkanes with β-CD-6-monotosylate in K_2CO_3/DMF followed by chromatography on BioGel P-2 gave bisCDs **2** (42%), **3** (29%), **4** (24%), **5** (38%), and **6** (47%).



TNS R=CH_3
BNS R=$C(CH_3)_3$



1 X=S-S
2 X=$S(CH_2)_2S$
3 X=$S(CH_2)_3S$
4 X=$S(CH_2)_4S$
5 X=$S(CH_2)_5S$
6 X=$S(CH_2)_6S$

Equilibrium binding isotherms were obtained by titration of the bisCD against a constant concentration of fluorophoric ligand in aqueous buffer (10 mM phosphate, pH 7.0 at 23 °C) and monitoring the enhanced fluorescence intensity (ΔF) at the emission λ_{max} exhibited upon binding. The concentrations of bisCD and fluorophore were chosen so as to maintain [complex]/[ligand] = 0.2 to 0.8, the range of the binding isotherm which is most informative.[12] The data were analyzed using HOSTEST-II[13] and K_a values are reported with ± figures that encompass their 90% confidence limits.

Table 1. Association Constants for BNS and TNS with β-CD and BisCDs.

receptor	linker	ligand	[ligand], μM	K_a (M^{-1})
β-CD	--	TNS	3.33	1200
1	S-S	TNS	0.33	$2.8 \pm 0.11 \times 10^4$
1	S-S	BNS	3.33	$7.9 \pm 2.1 \times 10^4$
2	S-$(CH_2)_2$-S	TNS	3.33	$7.4 \pm 0.79 \times 10^4$
2	S-$(CH_2)_2$-S	BNS	0.33	$8.2 \pm 1.2 \times 10^6$
3	S-$(CH_2)_3$-S	BNS	0.33	$2.5 \pm 0.6 \times 10^6$
4	S-$(CH_2)_4$-S	BNS	0.33	$6.8 \pm 1.1 \times 10^5$
5	S-$(CH_2)_5$-S	BNS	0.33	$4.9 \pm 1.0 \times 10^5$
6	S-$(CH_2)_6$-S	BNS	0.33	$1.5 \pm 0.4 \times 10^5$

Titrations were performed at 23 °C in 10 mM phosphate at pH 7.0, excitation λ_{max} = 325 nm, emission λ_{max} = 440 nm, and analyzed by HOSTEST-II.[13] K_a values are reported with intervals of 90% confidence.

As expected, BNS and TNS bound to the bisCDs in Table 1 more tightly than to β-CD. We measured a K_a for the formation of the binary complex of β-CD with TNS at 1200 M^{-1}, which is comparable to the previously reported value of 1800 M^{-1}.[4a]

Thus, **1** and **2** bind TNS more tightly by factors of 23 and 61, respectively. BNS exhibited a non-hyperbolic binding isotherm when titrated with β-CD under conditions identical to those used to titrate TNS; specifically, though ΔF of BNS vs. [β-CD] was qualitatively similar to that observed for TNS and β-CD, saturation was not achieved even when [β-CD]/[BNS] > 1000. Under our conditions formation of the ternary complex (β-CD_2•BNS) may be competitive with formation of the binary complex, thus leading to the observed nonideal binding isotherm. In contrast, all the bisCDs, when titrated against BNS, yielded hyperbolic binding isotherms reaching saturation at [bisCD]/[BNS] ≤ 100.

Of the bisCDs tested, the tightest binder of BNS was **2**, with $K_a = 8.2 \pm 1.2 \times 10^6$ M^{-1}. As the tether is lengthened beyond two methylenes, the affinity of the bisCDs for BNS diminishes; this is consistent with a model for complexation in which the two CD cavities must cooperate in binding the two ends of the ligand, in analogy to the 'chelation effect'.[14] That is, the longer the tether, the more entropy must be quenched to form the highly ordered bisCD/BNS inclusion complex. This rather facile account of the advantages of ditopic binding has been called into question by Zhang and Breslow,[7c] who determined that enthalpy—not entropy—was responsible for the tighter binding on the part of bisCD hosts with ditopic guests.

Finally, the binding affinities of bisCDs **2**-**6** for BNS fall off rather smoothly as the tether lengthens. The free energies of binding are plotted as a function of tether length in Figure 1; with the addition of each intervening methylene, the ΔG for binding drops by about 0.25 kcal•mol^{-1}. The significance of this ratio is unclear, but the prospect that similar relationships might be observed in other flexibly tethered two-site receptors is intriguing.

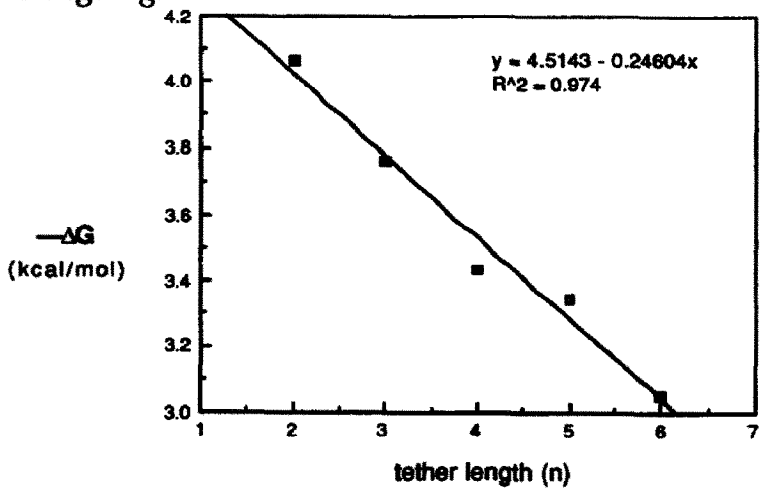


Figure 1. Plot of —ΔG of binding of BNS *vs.* tether length (n = number of methylenes) for bisCDs **2**-**6**.

Acknowledgment: This work was supported by the National Institutes of Health.

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(*Received in USA* 3 *March* 1994; *accepted* 20 *April* 1994)