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Synthesis of per(5-*N*-carboxamide-5-dehydroxylmethyl)-β-cyclodextrins and their selective recognition ability utilizing multiple hydrogen bonds

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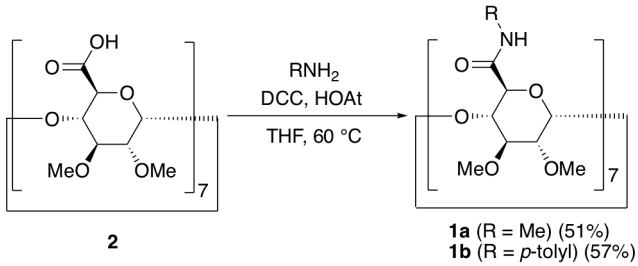
Per(5-*N*-carboxamide-5-dehydroxylmethyl)-β-cyclodextrin derivatives with seven equivalent amide groups directly attached to each pyranose ring were synthesized. The amide cyclodextrins show unique recognition properties toward hydrogen phosphonate anions. An X-ray crystallographic analysis revealed its recognition mode in which unsymmetrically arranged amide groups play distinctive roles both as a hydrogen bond donor and acceptor.

Natural receptor proteins achieve sophisticated molecular recognition by exerting multiple hydrogen bonds at their hydrophobic binding pockets, in which amide groups of the main chain often play an important role.[1] Learning from the excellent examples of the biomacromolecules, a number of artificial molecular receptors possessing multiple amide functionalities have been reported.[2] In particular, the accumulative use of amide groups as hydrogen bond donors is suitable for their employment as receptors for anions.[3] The introduction of multiple functional groups onto a scaffold is required to configure a recognition site. Due to synthetic reasons, many receptors are designed to possess equivalent interaction units, which usually leads to a high symmetry in the molecular structure. One strategy aiming at specific recognition is the introduction of different functional groups. In the case of anion binding, receptors equipped with both hydrogen bond donor and acceptor units are reported.[4] However, the differentiation of host structures by introducing different substituents often requires more synthetic steps. In this context, if a molecule with equivalent substituents exhibits a recognition mode in which each functional group plays distinctive roles,[5] it will be a simple approach for artificial receptors to achieve precise functions reminiscent of biological systems.



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1a (R = Me) (51%)
1b (R = *p*-tolyl) (57%)

Scheme 1 Synthesis of per(5-*N*-carboxamide-5-dehydroxylmethyl)-cyclodextrins **1a** and **1b**.

We now report the first synthesis of per(5-*N*-carboxamide-5-dehydroxylmethyl)-cyclodextrins with multiple amide groups directly attached to each pyranose ring, and their unique recognition ability utilizing multiple hydrogen bonds. β-Cyclodextrin, a cyclic heptamer of glucose, is one of the most widely used host compounds.[6] In many cases, the framework of the native and functionalized cyclodextrins is regarded as a circular conical cylinder. Toward more sophisticated applications, such as enzyme mimics, the introduction of one or more substituents at specific positions of the cyclodextrins has been investigated.[7] However, the separation of numerous isomers with different numbers and positions of the substituents is often formidable. It is known that native cyclodextrins maintain their circular scaffold through intramolecular hydrogen bonds between hydroxy groups, and conversion of the hydroxy groups increases the degree of freedom of each glucose unit thus making the entire macrocycle flexible.[8] By taking this mobility into account, we postulated that cyclodextrins possessing multiple equivalent amide groups can take a desymmetrized conformation as a snapshot, which can be utilized for unique molecular recognition.

The *N*-methylamide derivative **1a** and *N*-*p*-tolylamide derivative **1b** were synthesized by the condensation of the corresponding amines with per(5-carboxy-5-dehydroxymethyl)-β-cyclodextrin **2**[9] (Scheme 1). Among the several tested conditions, the employment of *N*,*N*-dicyclohexylcarbodiimide (DCC) together with 1-hydroxy-7-azabenzotriazole (HOAt) was

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†Electronic Supplementary Information (ESI) available: Detailed synthetic procedures, characterisation data, and ^{1}H NMR titration measurements. CCDC 1858034 (**2**), 1858035 (**1b**), and 1858036 ((PhPO$_3$H$^-$)⊂**1b**) contain the crystallographic data for this paper. See DOI: 10.1039/x0xx00000x

effective. A MALDI TOF mass spectrum of the reaction mixture to synthesize **1b** demonstrated that all the seven carboxylic groups of **2** were efficiently converted to *p*-tolylamide groups (Fig. S5).

We succeeded in the single-crystal X-ray diffraction analysis of the carboxylic acid **2** (Fig. 1). As suggested in a previous study based on vapor-pressure osmometry,[9] **2** forms a dimer upon intermolecular connection of the carboxy groups. Intriguingly, all the seven carboxy groups make tight hydrogen-bonded pairs with the counterpart of the other **2** (Fig. 1a,b), and the distances between the oxygens in the carboxy dimer are in the range of 2.60–2.66 Å (Fig. 1c). Focusing on the macrocyclic framework, the shape of **2** in the dimer is close to a true circle. The distances between the carboxy carbons of the adjacent units are in the range of 3.94–4.05 Å (Fig. 1d). Their small variation is noticeable when compared to the unfunctionalized β-cyclodextrin; the distances between the carbons of the corresponding hydroxymethyl groups in its crystal are in the range of 4.41–4.82 Å.[10]

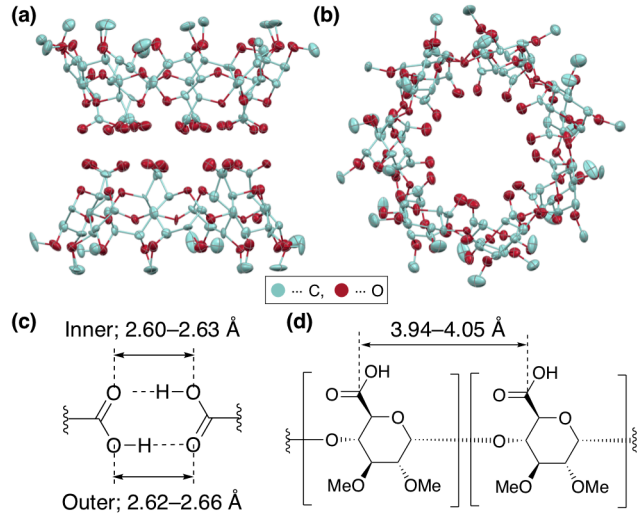

Fig. 1 Structure of $\mathbf{2}_2$ determined by X-ray crystallography (an ellipsoid model, 50% probability, hydrogens are omitted). Side view (a) and top view (b). (c,d) Representative distances.

The conversion of carboxy groups into amides drastically changes the macrocyclic structure. A single crystal of the *N*-*p*-tolylamide derivative **1b** suitable for an X-ray diffraction analysis was obtained by slow evaporation of a MeOH/H_2O solution. **1b** has a characteristic twist in the macrocyclic framework, which comes from the steric repulsion between the seven *p*-tolyl groups and from the hydrogen bonds of the amides (Fig. 2a,b). In particular, one of the seven repeating units is largely tilted with *p*-tolyl groups directed outside. Focusing on the participation of the amide groups in the structural conformation, three intramolecular and one intermolecular amide-amide hydrogen bonds are observed in the crystal (Fig. 2c). In the cavity of **1b**, five water molecules are found without disorder, and they form a hydrogen bond network with the amides and other functional groups. Elemental analysis of the isolated sample also matched with **1b**·5H_2O (see ESI).

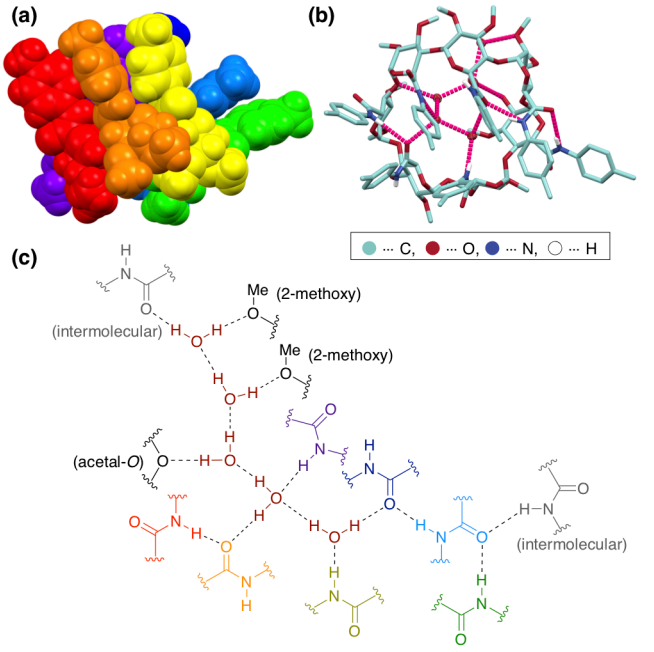

Fig. 2 Structure of **1b**·5H_2O determined by X-ray crystallography. (a) Colouring by repeating unit (a space-filling model). (b) Top view (a stick model). Hydrogens except for amides are omitted. H_2O depicted as ellipsoids (50% probability). Hydrogen bonds are shown in magenta. (c) A hydrogen bond network between amide groups and water molecules inside the cavity. Colours of amide groups correspond to (a).

Intriguingly, two conformational isomers of **1b** are observed by NMR (Fig. 3). For one isomer, the NMR signals corresponding to one repeating unit are observed, suggesting its time-averaged seven-fold symmetry. For the other isomer, seven sets of NMR signals are observed, suggesting that all seven repeating unit are in different environments on the NMR timescale. The ^{1}H and ^{13}C NMR signals for the two isomers have been successfully assigned by 2D NMR measurements (Fig. 3, Fig. S8–S14, Table S2 and S3). ^{1}H–^{1}H ROESY revealed that the isomer with a lower symmetry has a conformation where one of the *p*-tolyl substituents is self-included in the cyclodextrin cavity (**1b**$_{in}$).[11] The ^{1}H NMR signals of **1b**$_{in}$ in Fig. 3 are shown in a manner that assigns the *p*-tolyl group of the unit 1 (red) as the self-included one. Characteristic ROE correlations with i_1 (2-position of the *p*-tolylamide group) are observed for e_2, e_5, and e_6, and those with j_1 (3-position of the *p*-tolylamide group) are observed for c_3, e_3, and e_4 (Fig. 3b, Fig. S11). A ^{1}H DOSY measurement shows similar diffusion constants (D = 6.5–7.0 × 10^{-10} m^2s^{-1}) for the two isomers, thus supports that the isomer with a lower symmetry is a self-included monomer **1b**$_{in}$, not a doubly-threaded dimer[12] (Fig. S14). The ratio between **1b**$_{in}$ and the other non-included isomer (**1b**$_{out}$) is dependent on the solvent (Table S1, 298 K). Among the investigated solvents, the ratio of **1b**$_{in}$ is relatively high in CD_3CN (**1b**$_{in}$: **1b**$_{out}$ = 60 : 40) and acetone-d_6 (**1b**$_{in}$: **1b**$_{out}$ = 30 : 70). Meanwhile, the equilibrium shifted to **1b**$_{out}$ in solvents such as $CDCl_3$ (**1b**$_{in}$: **1b**$_{out}$ = 10 : 90) and DMSO-d_6 (**1b**$_{in}$: **1b**$_{out}$ = 10 : 90). At elevated temperatures (1,1,2,2-tetrachloroethane-d_2, 373–393 K), the ^{1}H NMR signals of **1b**$_{in}$ and **1b**$_{out}$ coalesced into one set of signals and are broadened, thus probably suggesting the interconversion of the cyclodextrin conformations (Fig. S6).

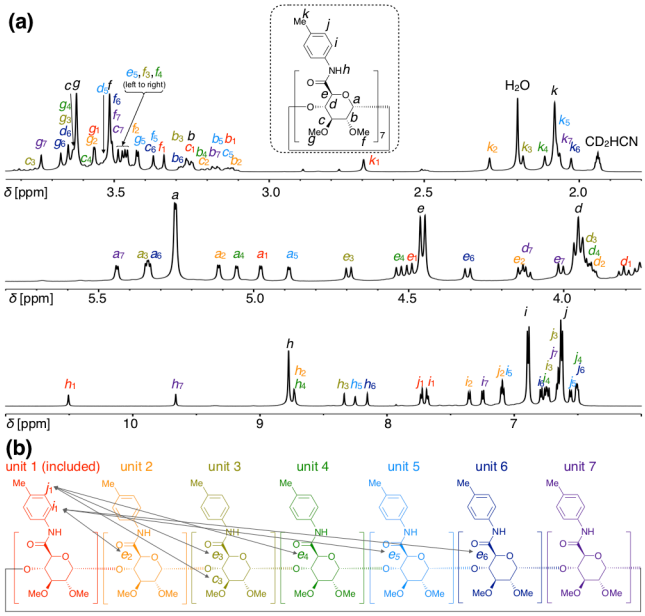

Fig. 3 (a) ^{1}H NMR spectrum of **1b** (600 MHz, CD_3CN). Coloured letters with subscripts that denote each unit indicate the signals of **1b**$_{in}$. Black letters indicate signals of **1b**$_{out}$. (b) Structure of **1b**$_{in}$. ^{1}H–^{1}H pairs that show characteristic interunit ROE peaks are denoted.

Furthermore, it was found that water in the solvents shifted the equilibrium toward **1b**$_{out}$ (Table S1, **1b**$_{in}$: **1b**$_{out}$ = 3 : 97 in CD_3CN/D_2O = 9/1 (v/v)). As shown in Fig. 2, **1b**·5H_2O in the crystal obtained from MeOH/H_2O solution takes a conformation in which all seven *p*-tolyl groups are positioned outside, i.e., **1b**$_{out}$. The water molecules are considered to stabilize the non-included conformer **1b**$_{out}$ via hydrogen bonds with the amides.

The amide cyclodextrins showed unique recognition properties of anions utilizing multiple hydrogen bonds inside the cavity. Table 1 summarizes the binding constants K_a [M^{-1}] of various anions with **1a**/**1b** in $CDCl_3$ or DMSO-d_6. A series of hydrogen phosphonates (R-PO_3H^-) and hydrogen phosphates (R-OPO_3H^-) are encapsulated in the amide cyclodextrins in a 1:1 ratio, which was determined from the integral values of the encapsulated guest. In chloroform, the hydrogen phenylphosphonate ($PhPO_3H^-$) and hydrogen methylphosphonate ($MePO_3H^-$) are bound the strongest with the *p*-tolylamide derivative **1b** (log K_a > 4). In contrast, the amide cyclodextrin derivatives interact only weakly (log K_a = 1–2) with the carboxylates (R-CO_2^-). As for the halides (Cl^-, Br^-, I^-) and diphenyl phosphate ($(PhO)_2PO_2^-$), the binding constants were too low to be determined (Fig. S32, S43, S44 and S55). Thus, a remarkable selectivity toward hydrogen phosphonates is achieved. As the anion binding was not observed for *per-O*-methyl-β-cyclodextrin in this condition (Fig. S56), the recognition ability of **1a**/**1b** can be attributed to the introduced amide groups. Comparing **1a** and **1b**, **1b** tends to more strongly bind anions. This is explained by the rigidity of **1b** derived from the steric restriction due to the multiple *p*-tolyl groups, which reduces the entropy loss upon guest binding.

The ^{1}H NMR spectrum of the host-guest complex (*n*-Bu_4N)[($PhPO_3H$)⊂**1b**] shows an amide proton signal at 10.03 ppm (downfield shift by 1.22 ppm compared to the guest-free **1b**), which supports the hydrogen bonds with $PhPO_3H^-$ (Fig. S16). Furthermore, the signal of an acidic proton of the encapsulated $PhPO_3\underline{H}^-$ is observed at 8.56 ppm. In fact, mixing a salt of the phenylphosphonate dianion ($PhPO_3^{2-}$) with **1b** also resulted in the inclusion as a hydrogen phenylphosphonate ($PhPO_3H^-$), which shows an interesting specificity toward the monoprotonated form (Fig. S41). The inclusion of $PhPO_3H^-$ was also confirmed by a ROESY measurement, in which the phenyl group showed ROE correlations with the introverted 3- and 5-protons of the pyranose rings and methoxy groups (*c*, *e*, and *g*, see Fig. S18).

Table 1. Binding constants log K_a [log(M^{-1})] of anionic guests with **1a**/**1b** (^{1}H NMR, 298 K).

Entry	Anions[a]	**1a** ($CDCl_3$)	**1b** ($CDCl_3$)	**1b** (DMSO-d_6)
1	$MeCO_2^-$	2.0	—[b]	3.3
2	$H_2PO_4^-$	2.9	3.0	2.3
3	$MePO_3H^-$	3.2	4.3	4.9
4	$PhCO_2^-$	—[b]	—[b]	2.0
5	$PhPO_3H^-$	3.1	4.5	4.5
6	$PhOPO_3H^-$	2.9	2.6	1.8
7	*p*-$TolSO_3^-$	—[b]	1.9	—[b]
8	$PhPO_3^{2-}$	1.9	2.8[c]	2.6
9	$PhCH_2PO_3H^-$	3.3	3.0	3.6

[a] *n*-Bu_4N^+ salts except for entry 7, in which a Et_4N^+ salt was used. [b] The binding constant is too low to be determined. [c] Bound as $PhPO_3H^-$.

The binding of $PhPO_3H^-$ is also strong in other solvents such as DMSO-d_6 (log K_a = 4.5), CD_3CN (log K_a > 5), and CD_2Cl_2 (log K_a = 5.0) (Fig. S19, S20 and S50). The $PhPO_3H^-$ was released from the cavity of **1b** in an aqueous mixed solvent CD_3CN/D_2O = 9/1 (v/v), probably due to the interaction of water with amide groups (Fig. S57). For a series of other anions, **1b** shows comparative or even higher binding constants in DMSO-d_6 in comparison to those in $CDCl_3$ (Table 1). The strong binding via hydrogen bonds in competing solvents such as DMSO-d_6 is of significant note. The thermodynamic parameters for the binding of $PhCH_2PO_3H^-$ (Table 1, entry 9) by **1b** in $CDCl_3$ and DMSO-d_6 were evaluated by variable-temperature ^{1}H NMR measurements (Fig. S58–S63): ΔH_{298} = –13 kJ·mol^{-1} and ΔS_{298} = 16 J·mol^{-1}·K^{-1} in $CDCl_3$; ΔH_{298} = –9.5 kJ·mol^{-1} and ΔS_{298} = 37 J·mol^{-1}·K^{-1} in DMSO-d_6. Thus, the entropy gain contributes to the strong binding in DMSO-d_6. The molecular origin of this entropy change is elusive, but the release of DMSO solvent molecules from amide moieties upon anion binding probably plays an important role.[13]

An X-ray crystallographic analysis of (*n*-Bu_4N)[($PhPO_3H$)⊂**1b**] revealed the detailed recognition mode (Fig. 4). $PhPO_3H^-$ is tightly bound in the cavity of **1b** by multiple hydrogen bonds with amides. Seven amide groups play distinct roles in the recognition. The four amide groups work as a hydrogen bond donor to the three oxygen atoms of $PhPO_3H^-$. Meanwhile, one amide group behaves as a hydrogen bond acceptor to the acidic proton of $PhPO_3H^-$. Thus, the unsymmetrical arrangement of both the hydrogen donor/acceptor sites in the binding pocket was confirmed in the recognition of hydrogen phosphonates (R-PO_3H^-). The other two amides do not directly interact with

$PhPO_3H^-$ but get involved in the structure formation by intramolecular hydrogen bonds with the adjacent amides. The pattern of the hydrogen bonds of **1b**·$5H_2O$ (Fig. 2c) and that of ($PhPO_3H^-$)⊂**1b** (Fig. 4d) share a common feature. The difference is the two *p*-tolylamide groups shown in orange and yellow in both figures; they work as one hydrogen bond acceptor to the H_2O inside the cavity, but as two hydrogen bond donors to the $PhPO_3H^-$. Thus, the intramolecular hydrogen bonds of the seven amide groups have a preferable pattern irrespective of the including guest to some extent, which can explain its unique recognition toward hydrogen phosphonates that possess both hydrogen donating and accepting moieties.

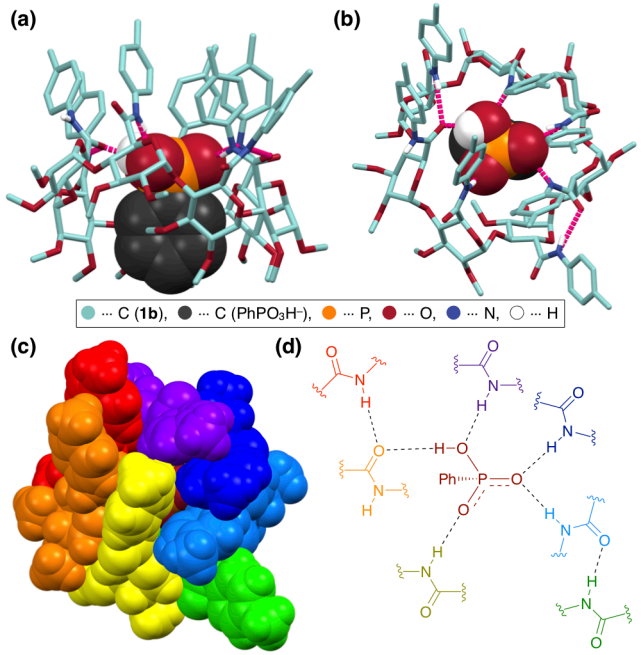

Fig. 4 Structure of ($PhPO_3H^-$)⊂**1b** determined by X-ray crystallography. (a,b) Side and top views (**1b**, a stick model; $PhPO_3H^-$, a space-filling model). Hydrogens except for hydrogen phosphono and amide group are omitted. Hydrogen bonds are depicted in magenta. (c) Colouring by repeating unit (a space-filling model). (d) Recognition mode of $PhPO_3H^-$ utilizing multiple hydrogen bonds.

In conclusion, novel cyclodextrin derivatives with seven amide groups were synthesized. The *p*-tolylamide derivative **1b** showed selective binding of hydrogen phosphonates, and the ambivalent use of the equivalent amide groups as a both hydrogen bond donor and acceptor is demonstrated from the single-crystal X-ray measurement. The reported amide cyclodextrin derivatives would be promising for applications, such as sensors and carriers of phosphorylated molecules.[2a,2d] Furthermore, desymmetrization of an apparently symmetric structure would bring a fresh view to the design strategy of artificial functional molecules.

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Conflicts of interest

There are no conflicts to declare.

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