Cyclodextrins

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# Imidazolyl Cyclodextrins: Artificial Serine Proteases Enabling Regiospecific Reactions**

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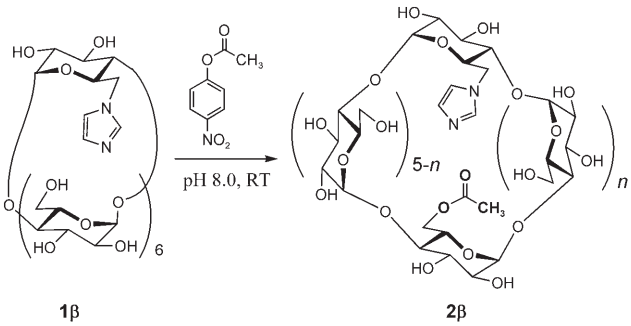
Inspired by the high selectivity and high efficiency of enzymes, scientists have made tremendous efforts in creating synthetic molecules that mimic the basic structural and catalytic features of enzymes.[1,2] Breslow and co-workers demonstrated that artificial enzymes can target a single functionality even in the presence of other more reactive functionalities without protective groups.[3] Wulff et al. prepared some imprinted polymer-based artificial enzymes that have strong catalytic activity that clearly surpasses the corresponding catalytic antibodies.[4] Many other artificial enzymes have also displayed interesting selectivity and remarkable rate acceleration.[1,2,5] However, little work has been done to demonstrate that *only the specifically located functionalities* in the presence of many others could actively take part in the catalysis, which is the general feature of natural enzymes. Chymotrypsin uses the Asp102-$COO^-$/His57-Im/Ser195-OH triad in catalyzing the hydrolysis of peptide substrates and the Ser195-OH group is acylated during the first step.[6] This enzymatic function has attracted a long-standing interest and has been extensively mimicked.[1,2] Cyclodextrins (CDs) remarkably accelerate (by a factor of up to six million) the hydrolysis of nitrophenyl esters by a mechanism closely related to that of proteases.[7] The acyl group is transferred to the secondary side of the CD, but it is not known whether the 2-OH or 3-OH group attacks the ester first.[8] Imidazolyl CDs demonstrate improved catalytic ability, whereas in this case, nothing is known about where the acyl group is transferred to.[9] On the other hand, it still represents a great challenge to functionalize CDs by introducing two or more different functional groups with high selectivity.[10] These drawbacks have hampered the rational design of more sophisticated artificial enzymes. Herein we demonstrate that among the many hydroxy groups of imidazolyl CDs, only the one at a specific position is engaged in the catalysis, which enables the establishment of a novel efficient strategy for the hetero-bifunctionalization and -trifunctionalization of CDs.

6-Imidazolyl-β-CD (**1β**) catalyzes the hydrolysis of *p*-nitrophenyl acetate. HPLC of the reaction mixture revealed the formation of a new CD species with a longer retention time than that of **1β**. Structural analysis indicated that the product is the isomerically pure monoacetate **2β** (Scheme 1),




1β 2β

***Scheme 1.*** Regioselective acylation of **1β**; $n = 0$–5.

thus implying that one of the 20 hydroxy groups can receive the acyl group of the substrate. Detailed NMR analysis (Figure 1S in the Supporting Information) revealed the acetylation of one primary hydroxy group (6-OH), but it did not allow the specification of that site.

Considering the significance of CD sulfonates in the functionalization of CDs, we examined the reaction of **1β** with mesitylenesulfonyl chloride to elucidate the cooperation between the imidazolyl and the hydroxy groups of CDs as well as to develop a new concept for the hetero-bifunctionalization or -multifunctionalization of CDs. The reaction was carried out at room temperature in a phosphate buffer at pH 8 containing 33% dimethylformamide (DMF). The sulfonate **3β**, among the estimated maximum 20 isomeric monosulfonates, was recognized and isolated in 13% yield together with unreacted **1β** (63%, Scheme 2). No obvious sulfonylation was observed with β-CD under these conditions. Structure determination of **3β** indicated the sulfonylaton of the $6^E$-OH group, implying that only the $6^E$-OH group is actively engaged in the reaction although the $6^D$-OH and $6^E$-OH groups are similarly located with respect to the imidazolyl moiety.[11] These results emphasize that precise geometrical matching is required for the cooperation between the hydroxy and imidazolyl groups.

Further information about the geometrical requirements for the cooperation between the hydroxy and imidazolyl groups was obtained from the reaction of the α and γ ana-

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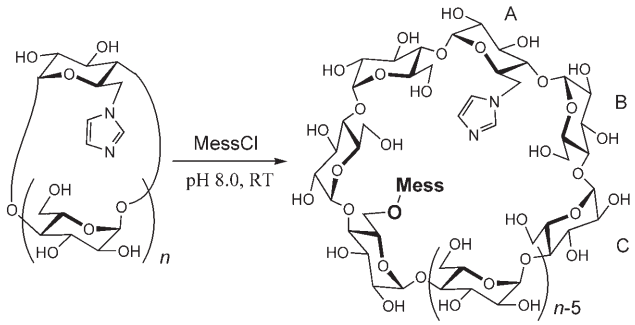



**1α**: *n* = 5 **3α**: *n* = 5
**1β**: *n* = 6 **3β**: *n* = 6
**1γ**: *n* = 7 **3γ**: *n* = 7

***Scheme 2.*** Regioselective sulfonylations of **1**; Mess = mesitylenesulfonyl.

logues **1α** and **1γ** under similar conditions (Scheme 2). Compound **1α** gave the mesitylenesulfonate **3α** as the only isolable product, which was converted to the $C_2$-symmetrical $6^A$,$6^D$-diimidazolyl-α-CD by treatment with imidazole (Figure 2S in the Supporting Information). The reaction of **1γ** afforded $6^A$-imidazolyl-$6^F$-mesitylenesulfonyl-γ-CD (**3γ**). The common regiochemistry of **3α**–**3γ** revealed that the imidazolyl group always cooperates with *the third* 6-OH group *on the anomeric side* of the imidazolylglucoside residue while the number of sugar units on the other side may vary. Obviously the imidazolyl group not only recognizes the hydroxy groups by distance (spatial specificity), but also differentiates hydroxy groups located in a clockwise–counterclockwise relationship (orientation specificity). Similar recognition ("vectorial recognition") was also performed by the carboxyl group of a cysteine unit flexibly linked to γ-CD.[12]

A plausible mechanism for the sulfonyl transfer may include the sulfonylation of the imidazolyl group, which delivers the sulfonyl group to the $6^E$-OH group of **1β** to effect a regioselective 1,22 remote functionalization, although two other hydroxy groups ($6^D$-OH and $2^E$-OH) are also situated apart from the imidazolyl moiety by the same number of single bonds as the $6^E$-OH group is.[13] The specificity of the reaction may stem from the intrinsic structure of the imidazolyl CDs. It is documented that an imidazolyl group attached to a methylene carbon atom of a CD induces strong shielding effects on the first subunit on the anomeric side while it does not significantly influence the other side.[14] These literature data together with the CPK model indicate that in the preferential conformation around the C5 and C6 atoms of the imidazolylglucoside residue, the imidazolyl group is *gauche* to O5 and *anti* to C4 (Figure 1). This structural information suggests that the sulfonyl group should be delivered along the anomeric side of the 6-imidazolylglucoside residue but never along the opposite side. The possible binding of the substrate in the CD cavity may also play an important role because the sulfonylation did not occur when the reaction medium was switched from water to DMF, which disfavors the hydrophobic binding.

On the basis of this enzyme-like catalysis, we developed an efficient methodology for the hetero-trifunctionalization of CDs. The $6^A$,$6^X$-diiodo-β-CDs (X = B, C, or D) were

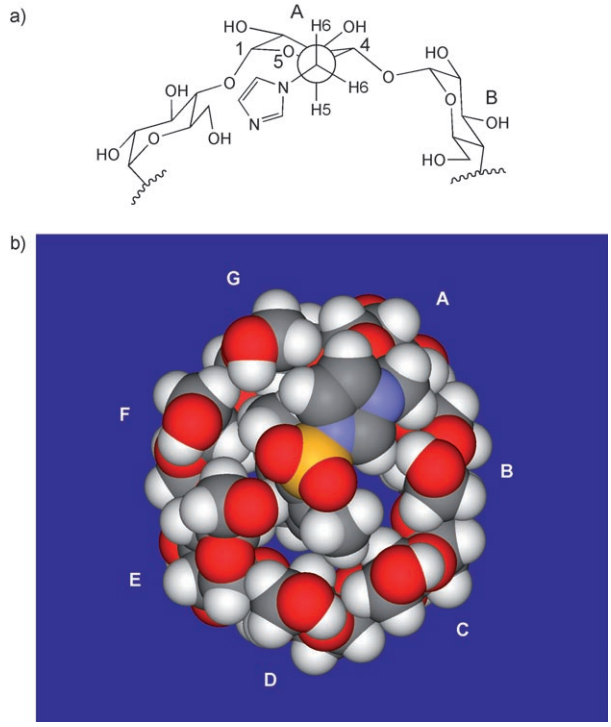


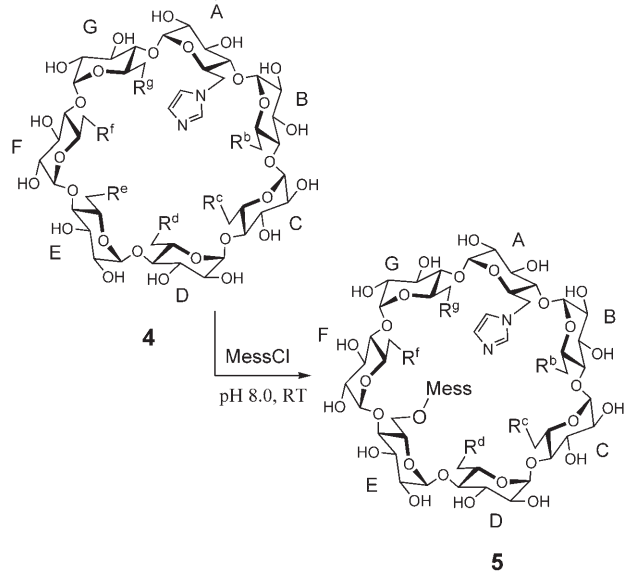

***Figure 1.*** a) The preferential conformation around the C5 and C6 atoms of the imidazolylglucoside residue suggested by the CPK model and NMR spectroscopy. The imidazolyl group is *gauche* to O5 and *anti* to C4. b) The structure of the possible reaction intermediate of **1β** generated by energy minimization using an MM2 force field. Only the $6^E$-OH group is located near the sulfonyl S atom on the rear side of the imidazolyl N atom; S yellow, N blue, O red, C gray, H white.

treated with imidazole to give the corresponding $6^A$-imidazolyl-$6^X$-iodo-β-CDs **4**.[15] Reactions of **4b**, **4c**, **4f**, and **4g** with mesitylenesulfonyl chloride were undertaken under similar conditions to that used for **1** (Table 1). In each case, one product was recognized and isolated in moderate to good yield and unreacted **4** could be recovered. Because it was difficult to separate **4d** from **4e**, they were used as a mixture in the sulfonylation. In this case, only **4d** reacted to generate one sulfonate. The sulfonyl group is attached to the $6^E$-OH group with no exception (Schemes 5S–8S and Figures 5S–7S in the Supporting Information). When the $6^E$-OH group is modified (**4e**), the sulfonylation does not occur.

Sequencing macrocyclic molecules such as cyclic peptides and cyclic carbohydrates requires chemical or enzymatic ring opening and subsequent spectroscopic analysis.[16] Structural characterization of the intact cyclic molecules is extremely difficult, even with the current state-of-the-art MS methods, because the multiple and indiscriminate ring-opening cleavages occur to generate superimposing random ions, thus making the interpretation difficult.[17] We have succeeded in straightforward sequencing of the hetero-trifunctional CDs by MALDI-PSD MS (PSD = post source decay). Compounds **5b** and **5g** are taken to illustrate the novel strategy. They were converted into the corresponding di(3,6-anhydro) species **6b** and **6g**, respectively, before PSD measurements in order to avoid the complexity caused by any side-chain cleavage. Both **6b** and **6g** fragmented and preferentially generated a series of

***Table 1:*** Selective sulfonylations of bifunctional CDs **4**.[a]




| Reactant | Position of I | Product | Yield [%] | Recovery of **4** [%] |
|---|---|---|---|---|
| **4b** | $R^b$ | **5b** | 57 | – |
| **4c** | $R^c$ | **5c** | 22 | 38 |
| **4d**+**4e**[b] | $R^d + R^e$ | **5d**[c] | 28 | 35 (mainly **4e**) |
| **4f** | $R^f$ | **5f** | 40 | 31 |
| **4g** | $R^g$ | **5g** | 42 | 46 |

[a] One of the $R^b$–$R^g$ groups is iodide, all others are OH. [b] A mixture was used. [c] Only one product was formed.

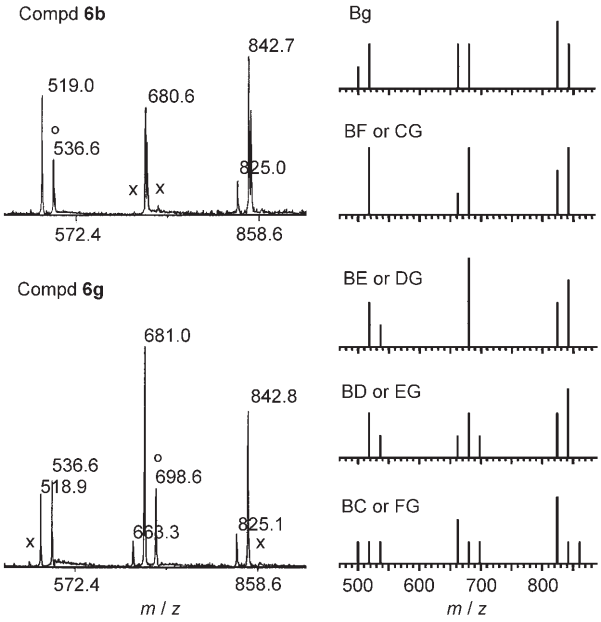
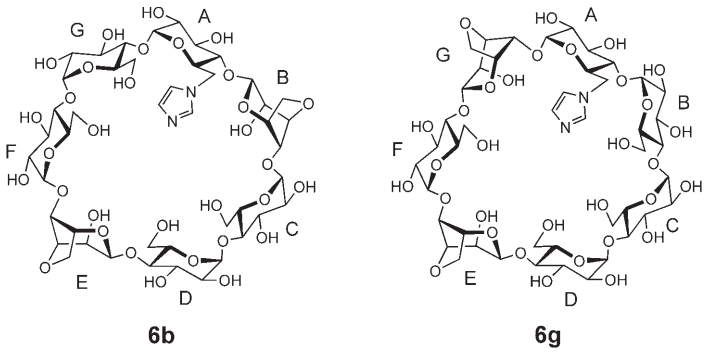



***Figure 2.*** MALDI-PSD mass spectra of **6b** and **6g** (left column), and the expected fragments relating the imidazolyl tri-, tetra-, and pentasaccharide segments of all the dianhydro-β-CDs that can be derived from **4b** and **4g** (right column). The expected relative abundance was deduced on an assumption of indiscriminate cleavage at any two glycosidic linkages. The capital letters denote the locations of the anhydro structure. The symbols ○ and × denote the presence and absence, respectively, of fragments that are important for differentiation of the regioisomers.




PSD ions containing the imidazolyl group with considerable abundance (Figure 2, left). For each compound, a library of the calculated fragmentation patterns, consisting of five di(3,6-anhydro)-β-CD candidates that can be derived from the precursor molecule **4b** or **4g**, was built by assuming that the cleavage occurs randomly at any two glycosidic linkages (Figure 2, right; the capital letters denote the locations of the anhydro moieties). Close resemblance was recognized between the PSD spectrum of **6b** and the expected spectrum of the BE dianhydro species, both characterized by the formation of the fragment $[MH-2(\text{anhydroGlc})-2\text{Glc}]^+$ ($m/z$ 537) and the lack of the fragments $[MH-2(\text{anhydroGlc})-\text{Glc}]^+$ ($m/z$ 699) and $[MH-3\,\text{Glc}]^+$ ($m/z$ 663). Compound **6g** demonstrated a fragmentation pattern that is in good agreement with that calculated for the EG dianhydro species, which is characterized by the formation of the fragment $[MH-2(\text{anhydroGlc})-\text{Glc}]^+$ at $m/z$ 699 and the absence of the fragments $[MH-2(\text{anhydroGlc})]^+$ ($m/z$ 861) and $[MH-4\,\text{Glc}]^+$ ($m/z$ 501). These observations indicated that both **6b** and **6g**, and therefore **5b** and **5g**, should have a modified E residue. The assignment was confirmed by the routine method employing enzymatic ring opening and subsequent MS analysis.

The regiospecific reaction, which mimics well the acyl transfer process of proteases, not only provides very important information for the design of more sophisticated artificial enzymes and receptors but also presents an excellent example of controlling efficiently the reaction selectivity by the combination of covalent and noncovalent weak interactions. The new concept described herein for the regiospecific hetero-bifunctionalization or -trifunctionalization of CDs may lead to a breakthrough in the selective modification of CDs. The first successful straightforward sequencing of the CD derivatives by mass spectrometry should be of significance in the structural characterization of cyclic carbohydrates, and it is expected to add considerable impact to the structural analyses of a wide range of biological and synthetic macrocyclic molecules.

## Experimental Section

General procedure for the sulfonylation of imidazolyl CDs: A solution of mesitylenesulfonyl chloride in DMF (0.6 M, 4 mL) was added to a pH 8.0 phosphate buffer solution (1 M, 8 mL) containing an imidazolyl CD (10 mM), and the resulting mixture was stirred at room temperature. When the pH dropped below 7, the reaction mixture was subjected to chromatography on a reversed-phase Lobar column (Rp-18, eluted with a gradient composed of water and ethanol) to afford the monosulfonate and the unreacted imidazolyl CD. All sulfonate products were characterized with MS and NMR spectroscopy and sequenced by MALDI-PSD mass spectrometry and/or by the combination of enzymatic ring-opening reactions and MS analysis.

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[11] The sugar units are numbered with the capital letters in the clockwise direction by starting from the imidazolylglucoside unit.

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