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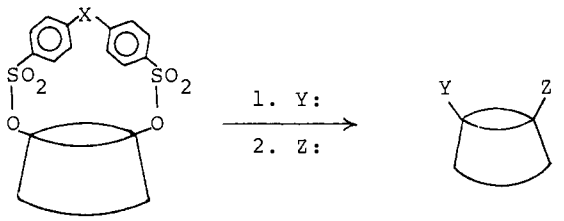
Communications to the Editor

The First Successful Carbonic Anhydrase Model Prepared through a New Route to Regiospecifically Bifunctionalized Cyclodextrin

Sir:

Cyclodextrins or their simple derivatives have now become very common as enzyme models in biomimetic chemistry.[1] For the construction of more refined and sophisticated enzyme models, however, the regiospecific multifunctionalization of cyclodextrins has become increasingly important since the convenient *capped cyclodextrin route*,[2,3] from **1** to **2**, has been developed,[3,4] as exemplified by a successful nuclease model[3] involving the use of bis(*N*-imidazolyl)cyclodextrin (**3**) or in the preparation of a promising multirecognition molecule,[5] duplex cyclodextrin (**4**). However, the attempted bifunctionalization of cyclodextrin directly from **1** through the replacement by weak nucleophile(s) often encounters difficulties because of the insufficient reactivity of **1** and, therefore, a new preparative route should be developed.



1a X = CH_2
b X = O

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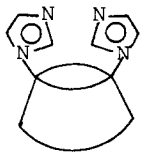

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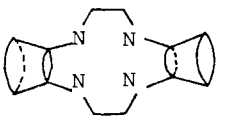

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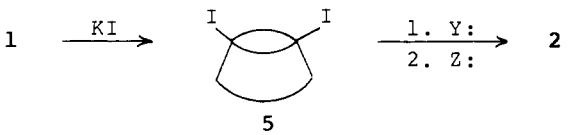
The authors report now a new and much more facile route to bifunctionalized cyclodextrins from **1a** through the corresponding diiodide (**5**) and its successful application to the preparation of the first *carbonic anhydrase model*, **6** and **3**, the former of which especially affords reasonable activity. Thus, **1a** (6.3 mmol) was treated with potassium iodide (180 mmol) in 300 mL of dry DMF at 80 °C for 2 h. After the usual workup, followed by the reprecipitation from water, the addition of tetrachloroethylene gave **5** in 95% yield. The structure of **5** was ascertained by its conversion into known disubstituted cyclodextrins including di(ω-aminoethylamino)-β-cyclodextrin[6] or di(*N*-imidazolyl)-β-cyclodextrin.[3] NMR and IR spectra of **5** were also satisfactory.



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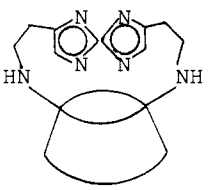

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The diiodide, **5**, treated with (an) appropriate nucleophile(s) gave a disubstituted cyclodextrin, **3** or **5**, very readily. Thus, **5** was treated with 10 molar excess of histamine in DMF at 45 °C for 4 h to give bis(*N*-histamino)-β-cyclodextrin (**6**) in 34% yield. After the reaction mixture cooled, the resultant precipitate was collected by filtration and dissolved in water. The addition of tetrachloroethylene gave a precipitate which was further purified by the preparative paper chromatography: IR 1550, 1480 cm^{-1} and other cyclodextrin absorptions; NMR δ 2.90 (8 H), centered at 3.75 (42 H), 5.05 (7 H), 6.82 (2 H), 7.57 (2 H). Bis(*N*-imidazolyl)-β-cyclodextrin (**3**) was similarly prepared by reaction of **5** with imidazole at 80 °C for 4 h in 46% yield.[7]

Carbonic anhydrase has Zn^{2+} surrounded by three imidazoles in its active site and CO_2 is bound to the active site in close proximity[8] to Zn^{2+} with assistance of hydrophobic interactions.[9] Water activated by Zn^{2+} coordination[10-12] attacks CO_2 bound where the base catalysis by an imidazole may be involved.[12] However, the detailed mechanism is still uncertain, and no appropriate model study has been carried out.[13,14] Our present compound, **6** or **3**, affords a hydrophobic pocket, Zn^{2+} bound to imidazoles located at the edge of the pocket, and also additional bases in the case of **6**, providing an appropriate model of carbonic anhydrase.

A thermostated solution of either enzyme model **3** or **6** was mixed with a thermostated solution of CO_2 by use of a stopped-flow spectrophotometer, and the formation of car-

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Table I. Rates of Hydration of CO_2 Catalyzed by Imidazole Derivatives[a] (pH 7.50 at 25 °C)

catalyst	10^{-3} M	buffer factor, 10^{-3} [b]	apparent k_{cat}, $M^{-1}\,s^{-1}$ [c]
imidazole	4.0	1.59	*d*
histamine	4.0	1.02	14.9
$(imidazole)_2Zn^{II}$ [e]	2.0	1.60	2.0
3-Zn^{II} [e]	2.0	0.60	16.2
$(histamine)_2Zn^{II}$ [e]	2.0	1.82	57.9
6-Zn^{II} [e]	2.0	1.95	166
human carbonic anhydrase B		k_{cat}/K_m, 1×10^7 [f]	
human carbonic anhydrase C		$k_{cat}K_m$, 8×10^7 [f]	

[a] The initial concentration of CO_2 was 1.25×1^{-2} M; see ref 15. [b] Determined by the direct titration and defined by equivalents of H^+ formed/absorbance increase of *p*-nitrophenol. [c] $[\Delta(HCO_3^-)_{formed}^{catalytic} - \Delta(HCO_3^-)_{formed}^{spontaneous}]/(CO_2)_0 \times (catalyst)_0 \times \Delta t$. This value is practically the same as k_{cat}/K_m. [d] Negligibly small. [e] Zinc chloride was used. [f] Reference 15.

bonate was followed with assistance of an indicator, *p*-nitrophenol.[15] The results obtained are summarized in Table I.

Although the catalytic activities of the present models are considerably lower than that of the native carbonic anhydrase (k_{cat}/K_m, 10^7 M^{-1} min^{-1}), it is significant and interesting to note that both the hydrophobic environment provided by cyclodextrin and the Zn^{2+} bound to the imidazoles[16] contribute to the carbonic anhydrase activity. Thus, as is seen from the Table I, each Zn^{2+} complex is more effective than the corresponding uncomplexed imidazole derivative and each cyclodextrin derivative is more effective than the corresponding catalyst without cyclodextrin. Another interesting finding is that the introduction of an additional base enhances the activity as is seen for the bis(histamino)cyclodextrin-Zn^{2+} (**6**-Zn^{2+}) compared with **3**-Zn^{2+}. Therefore, with regard to the present models, all three factors, Zn^{2+}-imidazole, hydrophobic environment, and base seem to help to generate the carbonic anhydrase activity.

References and Notes

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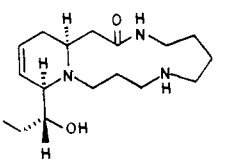
Stereochemistry of the Intramolecular Imino Diels–Alder Reaction

Sir:

The intramolecular version of the Diels–Alder reaction has recently been applied to synthesis of a wide variety of natural products and structurally interesting polycyclic molecules.[1,2] The stereochemistry of this process is now generally understood, and is predictable in most cases.[1-3] Recently, we demonstrated that the intramolecular imino Diels–Alder cyclization is a useful method for alkaloid synthesis.[4] Unlike the "all-carbon" case, the stereochemistry of the intramolecular imino cyclization has never been investigated.[5-7] We now report that this cyclization is, in fact, highly stereoselective but gives results *opposite* those usually found in closely analogous "all-carbon" systems.

As an initial target molecule for this study, we chose methyl dihydropalustramate (**2**), a degradation product of the *Equisetum* spermidine alkaloid palustrine (**1**).[8] This particular



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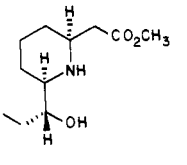

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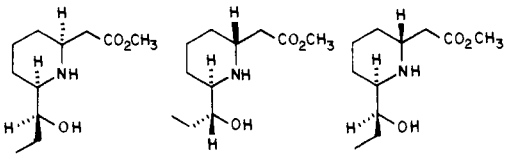
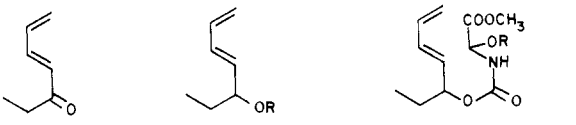
structure was chosen since all four possible diastereomers (**2–5**) have been prepared and fully characterized by Eugster and co-workers,[8] thus giving us the opportunity of making an unambiguous chemical correlation of relative stereochemistry.



3 **4** **5**

Treatment of butadiene with propionyl chloride in the presence of anhydrous stannic chloride (−42 °C, CH_2Cl_2), followed by treatment of the crude product with $CaCO_3$ (80 °C, 30 h), gave dienone **6** in 42% yield. Reduction of **6** with $LiAlH_4$ in ether gave diene alcohol **7** (92%; bp 26–30 °C (0.08) Torr; IR (film) 3360, 902 cm^{-1}) and this alcohol was converted into the carbamate **8** using sodium cyanate–trifluoroacetic acid in ether[9] (55%; mp 60–61.5 °C; IR (film) 1720, 1605, 908 cm^{-1}). Carbamate **8** condensed nicely with methyl glyoxylate[10] in refluxing acetone, giving the crystalline adduct **9** (83%; mp 65–67 °C; IR ($CHCl_3$) 3430, 3340, 1750, 1705 cm^{-1}).



6 **7** R=H; **8** R=$CONH_2$ **9** R=H; **10** R=$COCH_3$; **11** R=CONHPh

The hydroxyl group of **9** could be acetylated (acetic anhydride–pyridine–CH_2Cl_2, reflux) to afford the oily ester **10** (89%; IR (film) 3350, 1740 cm^{-1}; NMR ($CDCl_3$) δ 2.10 (3 H, s), 3.81 (3 H, s)). Similarly, bis carbamate **11** was prepared by treating alcohol **9** with phenyl isocyanate in refluxing methylene chloride and was used without purification.

Pyrolysis of acetate **10** (PhBr, 230–240 °C, 2.5 h, sealed tube) gave an inseparable 45:55 mixture of what proved to be

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