Pergamon

Bioorganic & Medicinal Chemistry 7 (1999) 709–714

BIOORGANIC & MEDICINAL CHEMISTRY

Reversal of Optical Induction in Transamination By Regioisomeric Bifunctionalized Cyclodextrins*

Elisabetta Fasella,† Steven D. Dong‡ and Ronald Breslow §

Department of Chemistry, Columbia University, New York, New York 10027, USA

Received 29 June 1998; accepted 6 August 1998

Abstract—Two isomeric compounds have been synthesized carrying a pyridoxamine on C-6 of β-cyclodextrin and an imidazole unit on C-6 of the neighboring glucose residue. Each one stereoselectively transaminates phenylpyruvic acid to produce phenylalanine, and with opposite stereochemical preferences. Structure determinations by X-ray crystallography and NMR spectroscopy indicate that the imidazole units serve to block proton addition from their side, rather than acting to protonate the transamination intermediates. Related cyclodextrin–pyridoxamine compounds had been reported carrying ethylenediamine units instead of imidazoles, and high enantioselectivities in transamination were claimed. Our work indicates that these claims are incorrect, and that only poor selectivities are seen that are often unrelated to the position of the ethylenediamine units. Neither of these transaminating systems yet approaches the enantioselectivity seen with a pyridoxamine carrying a chirally mounted internal base group.

Introduction

The ability of cyclodextrins to form inclusion complexes with a variety of hydrophobic guests in aqueous solution and the relative ease of functionalization of their primary and secondary hydroxyl groups makes them versatile enzymatic binding site mimics.[1] Although the chiral discriminating properties of cyclodextrins have long been recognized[2,3] and amply exploited in chromatographic separations of enantiomeric species,[4] there are only a few examples of optical induction by cyclodextrins in reactions at prochiral centers: in the cyanohydration of benzaldehyde,[5] in the reduction of ketones,[6] and in the transamination of α-keto acids.[7–12] In a few studies[13,14] detailed structural and modelling data have given insight into the mechanism of the molecular recognition process, but no unifying picture has emerged to account for the observed chiral discriminating properties of cyclodextrins.[15]

In the past three decades, a number of transaminase mimics combining the pyridoxamine cofactor **1** (Fig. 1) and cyclodextrin have been investigated in a continuing effort to reproduce in simple model systems the selectivity for substrate, reaction selectivity, and enantioselectivity of enzymatic transaminations. The first cyclodextrin–pyridoxamine conjugate examined, compound **2**,[7] displayed a 5 to 1 preference for formation of L-Phe over D-Phe in the transamination of phenylpyruvic acid (cf. Scheme 1).[8] In an effort to reproduce the catalytic role of the lysine residue at the active site of transaminase enzymes,[16] basic groups have been incorporated in enzyme mimics. Attachment of a basic side chain at the 5′-C of pyridoxamine in compound **3** resulted in an increased L-/D-Phe ratio of 6.8.[9] However, compound **4**, incorporating *N*,*N*-dimethylglycine in the linker between cyclodextrin and the cofactor, formed L-/D-Phe in only a modest 1.4/1 ratio.[17] These examples illustrate the difficulty in designing effective enantioselective transaminase models.

A remarkable increase in enantioselectivity (>95% ee) has been reported using the bifunctional cyclodextrin derivatives **5** and **6**, incorporating a pyridoxamine moiety and an ethylenediamine chain on adjacent glucose residues.[11,12] The structural information was insufficient to directly assign the regiochemistry of substitution (the clockwise versus anticlockwise arrangement of the ethylenediamine and of the pyridoxamine substituents on adjacent glucose residues). However, the substitution pattern was inferred from the reported stereochemistry of the product amino acids assuming participation of the ethylenediamine arm in the proton transfers needed for tautomerization of intermediate **A** to **B** in Scheme 1.[11]

Key words: Amino acids and derivatives; biomimetic reactions; cyclodextrins; stereospecificity.

* This paper is dedicated to the memory of Derek Barton, an extraordinary chemist and role model.

† Taken in part from the Ph.D. thesis of Elisabetta Fasella, Department of Chemistry, Columbia University, 1997. Current address: Department of Chemistry, University of Wisconsin, Madison Wisconsin.

‡ Taken in part from the Ph.D. thesis of Steven D. Dong, Department of Chemistry, Columbia University, 1998.

§Corresponding author. Tel.: +1 212 854 2170; fax: +1 212 854 2755.

0968-0896/99/$ - see front matter
PII: S0968-0896(98)00193-X

In this paper we report on the reversal in optical induction in transaminations by the unambiguously characterized regioisomeric bifunctionalized cyclodextrin derivatives **9** and **10**. The origin of the observed enantioinduction can be related to the structural data. Also, we have re-evaluated compounds **5** and **6**. Their enantioselectivities are modest at best, and often unrelated to the position of the ethylenediamine unit.

Results and Discussion

The key precursors to transaminase mimics **9** and **10** are the mono-imidazolyl-mono-iodo-β-cyclodextrin derivatives **7** and **8** differing in the clockwise or anti-clockwise arrangement of the C-6 substituents on adjacent glucose residues. Compounds **7** and **8** were synthesized by reaction of 6^A,6^B-diiodo-β-cyclodextrin with imidazole and separated by careful reverse phase chromatography. The first eluted isomer readily crystallized in water. X-ray diffraction data (Fig. 2) allowed its identification as compound **7**. NOESY experiments carried out on the second eluted isomer revealed cross peaks between the anomeric proton of the glucose ring carrying the iodo substituent and the C-6 methylene hydrogens adjacent to the imidazole substituent. Such an NOE is in agreement with structure **8**.

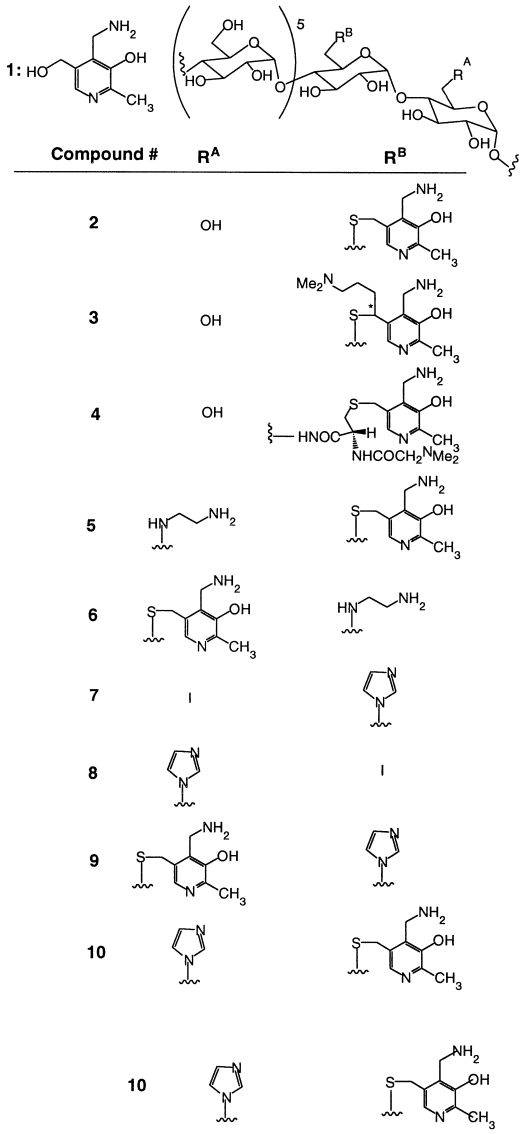
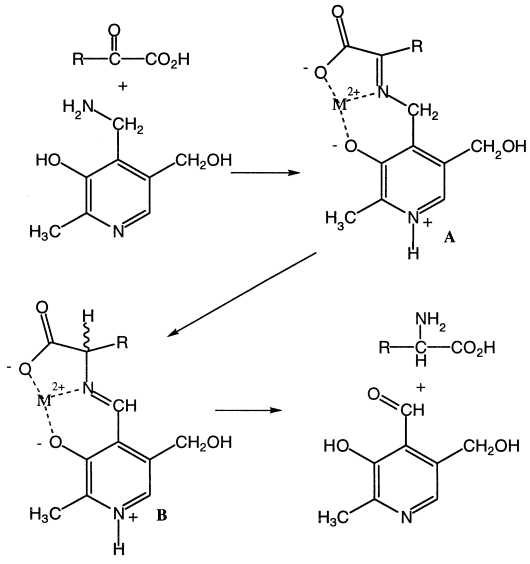


Figure 1. Pyridoxamine and pyridoxamine–cyclodextrin conjugates (and their precursors) evaluated as transaminase mimics. The lettering of glucose residues is clockwise and sequential A,B when the cyclodextrin is viewed from the primary face that carries the hydroxymethyl groups.

Reaction of the two separated regioisomers **7** and **8** with 5′-thiopyridoxamine dihydrobromide[9] in DMF in the presence of Cs_2CO_3 afforded transaminase mimics **9** and **10**, respectively. Compounds **9** and **10** have been evaluated in transamination reactions of phenylpyruvic acid and of indolepyruvic acid. After incubation, aliquots of the reaction mixture were treated with an *o*-phthalaldehyde/*N*-*boc*-cysteine derivatizing mixture and the diastereomeric isoindole derivatives analyzed by HPLC on a C-18 reverse-phase silica gel column.[18] Under the variety of conditions indicated in Table 1, compound **9** preferentially afforded L-Phe, and compound **10** preferentially yielded D-Phe. At most a 5.9/1 ratio of the enantiomeric amino acids was observed. Similar results were obtained in transamination of indolepyruvic acid to afford tryptophan.

Our reinvestigation of the reactions of compound **5** and **6** under the previously reported[11,12] conditions (2 M K_2HPO_4, adjusted to pH 8) showed that both isomers showed a preference for the formation of L- rather than D-Phe, and with at most a 4/1 ratio, as illustrated in Table 1. Furthermore, **5** and **6** *both* reversed their preference at pH 4 in water in favor of the D-enantiomer. These results indicate that the position of the ethylenediamine substituent plays only a minor role in determining the chirality of the product amino acid, outweighed by the buffer, protonation state, and intrinsic chirality of the cyclodextrin cavity.



Scheme 1. Transamination of a ketoacid by pyridoxamine. In the compounds described in this paper, the CH_2OH group is replaced by a CH_2-S-cyclodextrin group.

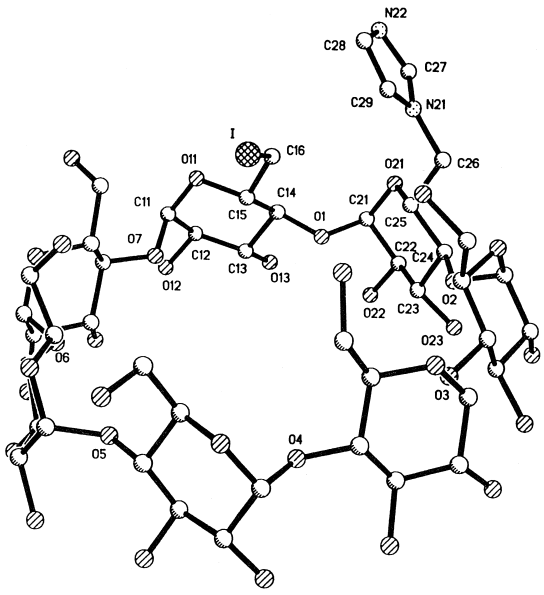


Figure 2. The crystal structure of compound **7**. The iodine is on the 6A position according to the convention and structure shown in Figure 1.

To ensure that no undesired racemization occurred in our assay, control reactions were analyzed in which the transamination mixtures were doped with known amounts of L-Phe. The ratio of L-/D-Phe observed after the standard incubation time reflected the addition of L-Phe in the transamination mixture with no evidence of racemization. The extraordinary results reported in refs 11 and 12, which we do not confirm, may have been caused by analytical artifacts.

Kinetic data on transamination by **1**, **2**, **4**, and **9** are reported in Table 2. They were obtained by monitoring the ketimine to aldimine conversion spectrophotometrically using the method developed by Matsushima and Martell[19] and modified by Zimmerman et al.[20] Compound **9**, incorporating an imidazole group, displays an eightfold rate acceleration compared with compound **2** in the transamination of phenylpyruvic acid. This modest rate acceleration is comparable to that observed for transaminase mimic **4** incorporating an *N*,*N*-dimethylglycine moiety.[17]

Table 1. L-/D-ratio of amino acid products in transamination by **5**, **6**, **9**, and **10** after 4 h incubation at 25 °C in water[a,b]

Transaminase, Amino Acid	K_2HPO_4 2 M pH8	pH9	pH8	pH7	pH6	pH5	pH4
9,Phe	3.9/1	2.1/1	2.9/1	3.5/1	4.3/1		5/1
10,Phe	1/1.8	1/5.9	1/1.8	1/3.6			1/5
9,Trp	1.8/1		2.9/1	3.4/1	3.6/1	4/1	
10,Trp	1/2			1/2		1/4	
5,Phe	3.7/1		2.1/1				1/4.8
6,Phe	1.4/1		1/2				1/1.2

[a]The absolute regiochemistry of **5** and **6** has not been determined.
[b]The pH of the 2 M K_2HPO_4 was adjusted to 8 with H_3PO_4. The other solutions were unbuffered except by the reagents. Their pHs were adjusted with NaOH or HCl. Measurements indicated that the pH did not change appreciably during the runs.

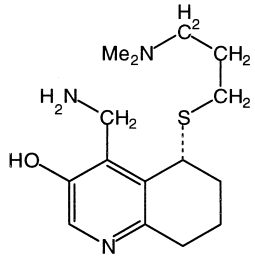
Table 2. Rates of ketimine to aldimine conversion as measured by monitoring the absorbance at 3845 nm. Transaminations were run in 50% MeOH/H_2O in the presence of 0.16 mM catalyst, 0.16 mM $Zn(OAc)_2$ and 1.6 mM phenylpyruvic acid at pH 7.3 and 30 °C

Transaminase	k_{obs} (s^{-1})	Relative rate
1	1.0×10^{-4}	1.7
2	6.0×10^{-5}	1.0
4[a]	4.4×10^{-4}	7.3
9	4.8×10^{-4}	8.0

[a]From ref 17.

Considering the modest enantioselectivity displayed by **9** and **10**, and the modest rate enhancement with respect to compound **2**, it does not appear that the imidazole is acting as an effective base catalyst for tautomerization in transamination. This is so even though most of the reactions were performed without external buffers, to maximize the chance that proton transfers would be performed by the imidazole rings. They may be catalyzing the formation or hydrolysis of the Schiff base intermediates, but cannot be catalyzing the proton transfers involved in the conversion of intermediate **A** to **B** (Scheme 1). Examination of CPK-molecular models indicates clearly that the observed preference of **9** for L-Phe formation and of **10** for D-Phe formation shows that the proton is being added to the new amino acid on the face of **B** *away* from the imidazole group. The observed stereochemical preference probably involves steric blocking of one face of the imine intermediate by the imidazole ring so that protonation by solvent occurs on the other face. Similarly, **4** displayed an optical induction opposite to that predicted if the side arm were mediating the proton transfer.[18]

High enantioselectivity in transamination, as much as 92% e.e., has been achieved previously by a rigid pyridoxamine **11** carrying a chirally mounted base group.[21] That base indeed transfers protons on a single face of intermediates like **A**, but the compound lacks a cyclodextrin binding group.



11

Conclusion

Our transamination studies with compounds **5**, **6**, **9**, and **10** show that the enantioselectivity is highly dependent on the pattern of substitution on the cyclodextrin rim, but the absolute optical induction is still modest and previous reports of high optical induction by bifunctionalized cyclodextrins cannot be confirmed. Considering what has been achieved with compound **11**, it should be

possible to create full mimics of transaminase enzymes with high enantioselectivity.

Experimental

All reactions were performed in oven-dried glassware under an Ar atmosphere.

6^A-Monoimidazolyl-6^B-monoiodo-β-cyclodextrin (7) and 6^B-Monoimidazolyl-6^A-monoiodo-β-cyclodextrin (8). 6^A,6^B-Diiodo-β-cyclodextrin (2.8 g, 2.1 mmol)[10] and imidazole (1.4 g, 20.8 mmol) were stirred in 30 mL anhydrous DMF under Ar at 85 °C for 7 h. The solvent was removed under reduced pressure and the residue suspended in 50 mL acetone. The precipitate was recovered by filtration, dissolved in a minimum amount of water and loaded onto a C-18 reverse-phase silica gel[22] column (5×13 cm). Elution was performed with the following MeOH/H_2O gradient: 400 mL each of 0, 10, 15 and 20% MeOH/H_2O followed by varying amounts of solvent (250–1200 mL) in 1% increments from 21 to 30% MeOH, and then in 2% increments from 32 to 40%, and finally in 5% increments from 40 to 60% MeOH. 6^A,6^B-Bisimidazolyl-β-cyclodextrin (R_f 0.1 on silica TLC plates, eluent: *i*-PrOH/EtOAc/H_2O/NH_4OH, 7/7/3.5/3) was eluted in the 21–24% MeOH fractions, followed by **7** (R_f 0.22) in the 27% MeOH fractions, and **8** in the 28–38% MeOH fractions (R_f 0.20). Finally unreacted starting material was eluted in the 45–60% MeOH fractions. Concentration and lyophilization of the appropriate fractions afforded **7** (0.81 g, 0.62 mmol, 30%) and **8** (0.50 g, 0.38 mmol, 18.5%).

Compound 7: ^{1}H NMR (400 MHz, D_2O) δ 2.50 (m, 1H, C-6′ iodo-glucose ring), 2.84 (d, J=10.98, 1H, C-6′ iodo–glucose ring), 3.08 (m, 1H, C-5′ iodo–glucose ring), 3.28 (t, J=9.43, 1H, C-4′ imidazolyl–glucose ring), 3.31 (t, J=9.89, 1H, C-4′ iodo–glucose ring), 3.48 (m, 12 H), 3.72 (m, 23H), 4.07 (dd, J=23.8, J=5.78, 1H, C-6′ imidazolyl–glucose ring), 4.48 (d, 14.28, 1H, C-6′ imidazolyl–glucose ring), 4.83 (d, J=3.66, 1H, C-1′, iodo–glucose ring), 4.91 (m, 4H, anomeric), 4.96 (d, J=3.66, 1H, anomeric), 5.02 (d, J=3.66, 1H, C-1′ imidazolyl–glucose ring), 6.91 (s, 1H, C-5 imidazole), 7.05 (s, 1H, C-4 imidazole), 7.57 (s, 1H, C-2 imidazole); ^{13}C NMR (75.4 MHz, DMSO) δ 11.02 (C-6′ iodo–glucose ring), 47.06 (C-6′ imidazolyl–glucose ring), 59.84, 59.98, 60.55, 67.98, 70.95, 71.78, 72.04, 72.38, 72.84, 72.97, 73.25, 81.33, 81.42, 81.67, 82.46, 83.73, 85.69, 101.70, 101.82, 102.00, 102.25, 120.29, 128.18, 137.89. MS(FAB): 1295 (M + 1). The crystal structure is shown in Figure 2.

Compound 8: ^{1}H NMR (400 MHz, D_2O) δ 2.89 (d, J=9.89, 1H), 3.15 (d, J=12.37, 1H, C-4′ imidazolyl–glucose ring), 3.38 (m, 15H), 3.57 (m, 1H, C-6′ iodo–glucose ring), 3.74 (m, 21H), 3.95 (t, J=9.23, 1H, C-5′ imidazolyl–glucose ring), 4.24 (dd, J=23.35, J=5.94, 1H, C-6′ imidazolyl–glucose ring), 4.42 (d, J=14.73, 1H, C-6′ imidazolyl–glucose ring), 4.85 (d, J=3.30, 1H, C-1′ imidazolyl–glucose ring), 4.90 (m, 5H, anomeric), 4.99 (d, J=3.37, 1H, C-1′ iodo–glucose ring), 6.87 (s, 1H, C-5 imidazole), 7.09 (s, 1H, C-4 imidazole), 7.59 (s, 1H, C-2 imidazole); ^{13}C NMR (75.4 MHz, DMSO) δ 9.16 (C-6′ iodo–glucose ring), 47.49 (C-6′ imidazolyl–glucose ring), 58.90, 59.81, 59.91, 59.99, 69.78, 70.02, 71.95, 72.08, 72.19, 72.38, 72.93, 73.06, 73.26, 80.48, 81.09, 81.44, 81.86, 83.84, 86.11, 100.77, 101.75, 102.01, 102.19, 102.38, 120.40, 127.85, 137.83. MS(FAB): 1295 (M + 1).

6^A-Monoimidazolyl-6^B-(5′-thiopyridoxaminyl)-β-cyclodextrin (9). Anhydrous DMF (10 mL) was added under Ar to a flask containing 6^A-monoimidazolyl-6^B-monoiodo-β-cyclodextrin **7** (400 mg, 0.31 mmol), 5-thiopyridoxamine dihydrobromide[9] (315 mg, 0.91 mmol) and Cs_2CO_3 (900 mg, 2.7 mmol). The solution was stirred at 40 °C for 3 h, and the solvent was removed under reduced pressure. The residue was dissolved in a minimum amount of water and the pH adjusted to 5 with 2 N HCl. The yellow mixture was loaded on a C-18 reverse-phase column (bed volume: 1.5×13 cm) and eluted with a 0 to 50% MeOH/H_2O gradient. The product was eluted in the 30% MeOH fractions. Concentration and lyophilization of the 30% MeOH fractions afforded 9 (R_f 0.14, 180 mg, 0.13 mmol, 42%). ^{1}H NMR (400 MHz, D_2O) δ 2.17 (s, 3H), 3.2–3.9 (m, 40H), 4.0–4.1 (m, 4H), 4.34 (d, J=14, 1H), 4.8–5.0 (m, 7H), 6.74 (s, 1H), 6.97 (s, 1H), 7.27 (s, 1H), 7.48 (s, 1H); ^{13}C NMR (75.4 MHz, DMSO-d_6) δ 18.5, 31.4, 32.1, 46.9, 59.9, 70–73, 81.3–83.6, 101.9–102.2, 120.2, 127.9, 128.8, 138.0, 138.8, 145.3, 152.7, 153.8. MS(FAB): 1351 (M + 1).

Compound **10** was prepared in an analogous manner by reaction of **8** with 5-thiopyridoxamine dihydrobromide. **10**: ^{1}H NMR (400 MHz, D_2O) δ 2.09 (s, 3H), 2.6–2.8 (m, 2H), 2.9–3.1 (m, 2H), 3.2–4.1 (m, 38H), 4.3–4.4 (m, 2H), 4.8–5.0 (m, 7H), 6.79 (s, 1H), 6.91 (s, 1H), 7.19 (s, 1H), 7.47 (s, 1H); ^{13}C NMR (75.4 MHz, DMSO-d_6) δ 18.5, 31.4, 33.2, 36.5, 47.1, 58.9, 69.8–73.1, 80.5–81.7, 83.9, 85.0, 100.8–102.2, 120.3, 127.4, 127.7, 128.8, 137.7, 138.6. MS(FAB): 1424 (M + 74), 1351 (M + 1).

Transamination reactions and HPLC analysis

In a typical experiment 50 μL of a 2 mM solution of catalyst in the appropriate medium (pH, solvent) was vortexed in a 100 μL vial with 50 μL of a 40 mM solution of phenylpyruvic acid in the appropriate medium (pH, solvent). The solution was then incubated at 25 °C. Except in the case of the reaction with phosphate buffer, no external buffer was added and the solutions were simply titrated to the correct pH with NaOH or HCl. The pH was observed not to change appreciably during the reaction.

At various time points 10 μL samples were removed, derivatized in the injector loop with 0.7 μL of a solution of 5.2 mg *o*-phthalaldehyde and 8.8 mg *N*-*boc*-cysteine in 100 μL MeOH and injected on a C-18 Rainin Microsorb™ column (4.6 mm×25 cm, particle size 5 μm). Elution was performed at a flow rate of 0.35 mL/min with an isocratic mixture of 65% MeOH and 35% citrate buffer (citrate buffer composition: 9 parts of a pH 4

solution 20 mM in sodium hydrogen citrate sesquihydrate and 6 mM in NaH_2PO_4 and 1 part MeOH). Detection was performed measuring the absorbance at 344 nm with a UV/vis diode array detector. The retention times for the L- and D-Phe isoindole derivatives were respectively 19.9 and 23.9 min.

Synthesis of compounds 5 and 6

In our reinvestigation, compounds **5** and **6** were prepared by a three step procedure involving: (1) reaction of 6^A,6^B-diiodo-β-cyclodextrin with 4′-*N*-*boc*-5′-thioacetyl-pyridoxamine, (2) reaction of the resulting mixture of monoiodo-monopyridoxaminyl derivatives with ethylenediamine and chromatographic separation of the two regioisomers, and (3) deprotection of the two isolated regioisomers. The two compounds had NMR and MS(FAB) in agreement with those reported for compounds **5** and **6**.[11,12]

4′-*N*-*Boc*-5′-Thioacetyl-pyridoxamine. A solution of PPh_3 (0.64 g, 2.4 mmol) in 60 mL anhydrous THF was cooled in an ice bath. Diisopropylazodicarboxylate (0.48 g, 2.4 mmol) was added under Ar. After 30 min a solution of thioacetic acid (170 μL, 2.2 mmol) and 4′-*N*-*boc*-pyridoxamine[24] (0.33 g, 1.2 mmol) in 25 mL THF was added via syringe. The solution was stirred at 0 °C for 1 h and then at room temperature for 1 h. The solvent was removed under reduced pressure, the residue was dissolved in Et_2O, and the Ph_3PO precipitate was removed by filtration. The solution was concentrated and the residue purified by silica gel chromatography ($EtOAc/CH_2Cl_2$, 3/7 eluent) to afford 4′-*N*-*boc*-5′-thioacetylpyridoxamine (188 mg, 0.58 mmol, 48%, R_f 0.42, eluent: $EtOAc/CH_2Cl_2$, 1/3). ^1H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 8H), 2.4 (s, 3H), 2.49 (s, 3H), 4.1–4.2 (m, 2H), 4.27 (d, $J = 6.7$ Hz, 2H), 5.5–5.6 (br t, 1H), 7.95 (s, 1H), 9.53 (s, 1H). MS (CI, NH_3): 32 7 (M + 1).

6^A-Monoiodo-6^B-[4′-*N*-*boc*-5′-thiopyridoxaminyl]-β-cyclodextrin and 6^B-monoiodo-6^A-[4′-*N*-*boc*-5′-thiopyridoxaminyl]-β-cyclodextrin. To a flask containing 6^A,6^B-diiodo-β-cyclodextrin (1.7 g, 1.25 mmol), 4′-*N*-*boc*-5′-thioacetyl-pyridoxamine (0.44 g, 1.25 mmol) and Cs_2CO_3 (1.5 g, 4.6 mmol) were added 150 mL anhydrous DMF under Ar. The mixture was stirred at room temperature for 10 h, and the solvent was removed under reduced pressure. The residue was dissolved in a minimum amount of water, acidified to pH 6 with 1 N HCl and loaded on 140 g of C-18 reverse-phase silica gel. The product (R_f 0.23 on reverse-phase silica plates, eluent $MeOH/H_2O$, 1/1) was eluted by a $MeOH/H_2O$ gradient. Concentration and lyophilization of the 28% MeOH fractions afforded the products as a mixture of regioisomers (0.51 g, 0.29 mmol, 27% yield). ^1H NMR (400 MHz, DMSO-d_6) δ 1.4 (s, 9H), 2.3 (s, 3H), 3.1–4.2 (m, 40H), 4.25–4.6 (m, 6H), 4.8–4.9 (m, 7H), 5.5–5.9 (br, 14H), 7.79–7.81 (br, 1H). MS(FAB): 1511 (M + 1).

***N*-*Boc* protected 5 and 6.** A mixture of 6^A-monoiodo-6^B-[4′-*N*-*boc*-5′-thiopyridoxaminyl]-β-cyclodextrin and 6^B-monoiodo-6^A-[4′-*N*-*boc*-5′-thiopyridoxaminyl]-β-cyclodextrin (110 mg, 0.078 mmol) was stirred in 1.5 mL degassed, anhydrous ethylenediamine at 60 °C for 90 min. Excess ethylenediamine was removed under reduced pressure and 15 mL EtOH added. The suspension was cooled overnight at 5 °C and the precipitate recovered by filtration. The precipitate was dissolved in a minimum amount of water and the pH adjusted to 6 with 1 N HCl. The reaction mixture was first purified by ion exchange chromatography on a Sephadex CM-25 column (1.8×19 cm). The following NH_4HCO_3 gradient was used: 150 mL H_2O (starting material was eluted in these fractions), 100 mL 0.005 M NH_4HCO_3, 100 mL 0.01 M NH_4HCO_3, 200 mL 0.015 M NH_4HCO_3, 100 mL 0.02 M NH_4HCO_3, 100 mL 0.022 M NH_4HCO_3, 100 mL 0.03 M NH_4HCO_3, 100 mL 0.04 M NH_4HCO_3, 100 mL 0.052 M NH_4HCO_3, 100 mL 0.084 M NH_4HCO_3. A mixture of the two regioisomers was eluted in the 0.02–0.022 M NH_4HCO_3 fractions (R_f 0.04 and 0.08 on silica TLC plates, eluent: *i*-PrOH/EtOAc/H_2O/NH_4OH, 5/5/3.5/3; R_f 0.73 for the starting material under these conditions). These fractions were concentrated and lyophilized. The residue was redissolved in a minimum amount of water and the pH adjusted to 5 with 1 N HCl. The mixture of bifunctionalized cyclodextrins was loaded on a reverse-phase silica gel column (1.5×6 cm). The two regioisomers were separated using the following gradient: 100 mL H_2O, 100 mL 4% MeOH, 100 mL 10% MeOH, 100 mL 20% MeOH, 100 mL 24% MeOH, 100 mL 28% MeOH, 300 mL 30% MeOH, 200 mL 35% MeOH, 200 mL 38% MeOH, 200 mL 40% MeOH. The first regioisomer was eluted in the 24–28% MeOH fractions, the second regioisomer was eluted with 30% MeOH. A few mixed fractions were collected in between. Concentration and lyophilization of the appropriate fractions afforded the separated *N*-*boc*-protected derivatives of **5** and **6** in 22% (24 mg, 0.017 mm) and 13% (15 mg, 0.01 mm) yield.

First eluted isomer (the absolute regiochemistry of substitution has not been determined): ^1H NMR (300 MHz, D_2O) δ 1.47 (s, 8H), 2.29 (s, 3H), 2.74–2.77 (m, 3H), 2.95–3.1 (m, 3H), 3.3–4.0 (m, 36H), 4.97–4.99 (m, 7H), 7.60 (s, 1H). FAB(MS): 1443 (M + 1).

Second eluted isomer: ^1H NMR (300 MHz, D_2O) δ 1.46 (s, 8H), 2.28 (s, 3H), 2.5–3.0 (m, 6H), 3.3–4.0 (m, 40H), 4–4.3 (m, 3H), 5–5.1 (m, 7H), 7.75 (s, 1H). FAB(MS): 1443 (M + 1), 1515 (M + 73).

6^A-Monoiodo-6^B-[5′-thiopyridoxaminyl]-β-cyclodextrin and 6^B-Monoiodo-6^A-[5′-thiopyridoxaminyl]-β-cyclodextrin, 5 and 6. The first eluted isomer mentioned above (24 mg, 0.017 mm) was stirred in 1 mL of trifluoroacetic acid at room temperature for 45 min. Ethyl ether (8 mL) was added and the suspension cooled on ice. The precipitate was recovered by filtration, dissolved in a minimum amount of water and loaded on a reverse phase silica gel column (1.5×8 cm). The product was eluted by a $MeOH/H_2O$ gradient. Concentration and lyophilization of the 20% MeOH fractions afforded **5** (6 mg, 0.0044 mm, 26% yield).

5: ^1H NMR (300 MHz, D_2O) δ 2.46 (s, 3H), 2.61–2.70 (m, 1H), 3.04 (d, $J = 12.3$ Hz, 1H), 3.2–4.1 (m, 45H), 4.3

(s, 2H), 5–5.1 (m, 8H), 7.72 (s, 1H). FAB(MS): 1343 (M + 1) and 1415 (M + 73).

6: (3 mg, 0.0022 mm, 22%) was obtained in a similar fashion from deprotection of the second isomer: ^{1}H NMR (300 MHz, D_2O) δ 2.46 (s, 3H), 2.63 (dd, J = 15.6 Hz, J = 6 Hz, 1H), 2.83 (d, J = 13.8 Hz, 1H), 3.2–4.1 (m, 55H), 4.3 (s, 2H), 4.9–5.1 (m, 8H), 7.70 (s, 1H). FAB(MS): 1343 (M + 1) and 1415 (M + 73).

Acknowledgements

We thank Dr J. Decatur, Columbia University, for assistance with the NMR experiments and Professor G. Parkin, Columbia University, for the crystal structure analysis. We acknowledge the support of the Kanagawa Academy of Science and Technology, the NIH, and the NSF, and a Merck graduate fellowship to S.D.D. We are grateful to U. Mayer, Columbia University, for helpful discussions and to Dr M. Yamada for kindly providing a copy of his Ph.D. thesis.

References

1. (a) Tabushi, I. *Acc. Chem. Res.* **1982**, *15*, 66; (b) Breslow, R. *Acc. Chem. Res.* **1995**, *28*, 146; (c) Komiyama, M. In *Comprehensive Supramolecular Chemistry*; Atwood, J. L.; Davies, J. E. D.; MacNicol, D. D.; Vogtle, F.; Suslick, K. S., Eds.; Pergamon: Oxford, 1996: Vol. 3, pp 401–422.
2. Cramer, F.; Dietsche, W. *Chem. Ber.* **1959**, *92*, 378.
3. Easton, C. J.; Lincoln, S. F. *Chem. Soc. Rev.* **1996**, *25*, 163.
4. Snopek, J.; Smolkova-Keulemansova, E.; Cserhati, T.; Gahm, K. H.; Stalcup, A. M. In *Comprehensive Supramolecular Chemistry*; Atwood, J. L.; Davies, J. E. D.; MacNicol, D. D.; Vogtle, F.; Suslick, K. S., Eds.; Pergamon: Oxford, 1996: Vol. 3, pp 515–572.
5. (a) Cramer, F.; Dietsche, W. *Chem. Ber.* **1959**, *92*, 1739; (b) Gountzos, H.; Jackson, W. R.; Harrington, K. J. *Aust. J. Chem.* **1986**, *39*, 1135.
6. (a) Fornasier, R.; Reniero, F.; Scrimin, P.; Tonellato, U. *J. Org. Chem.* **1985**, *50*, 3209; (b) Sakuraba, H.; Inomata, N.; Tanaka,Y. *J. Org. Chem.* **1989**, *54*, 3482.
7. Breslow, R.; Hammond, M.; Lauer, M. *J. Am. Chem. Soc.* **1980**, *102*, 421.
8. Breslow, R.; Czarnik, A. W.; Lauer, M.; Leppkes, R.; Winkler, J.; Zimmerman, S. *J. Am. Chem. Soc.* **1986**, *108*, 1969.
9. Breslow, R.; Chmielewski, J.; Foley, D.; Johnson, B.; Kumabe, N.; Varney, M.; Mehra, R. *Tetrahedron* **1988**, *44*, 5515.
10. Breslow, R.; Canary, J. W.; Varney, M.; Waddell, S. T.; Yang, D. *J. Am. Chem. Soc.* **1990**, *112*, 5212.
11. (a) Tabushi, I.; Kuroda, Y.; Yamada, M.; Higashimura, H.; Breslow, R. *J. Am. Chem. Soc.* **1985**, *107*, 5545; (b) Tabushi, I. *Pure Appl. Chem.* **1986**, *58*, 1529. R. Breslow was included as an author in reference 11(a) as a courtesy, since he had suggested the general approach used in this work.
12. Yamada, M. Ph.D. Thesis, Kyoto University (Japan), 1985.
13. (a) Trainor, G. L.; Breslow, R. *J. Am. Chem. Soc.* **1981**, *103*, 154; (b) Breslow, R.; Trainor, G.; Ueno, A. *ibid.* **1983**, *105*, 2739; (c) Daffe, V.; Fastrez, J. *J. Chem. Soc. Perkin Trans. II* **1983**, 789.
14. (a) Brown, S. E.; Coates, J. H.; Duckworth, P. A.; Lincoln, S. F.; Easton, C. J.; May, B. L. *J. Chem. Soc. Faraday Trans.* **1993**, *89*, 1035; (b) Lipkowitz, K. B.; Raghothama, S.; Yang, J. *J. Am. Chem. Soc.* **1992**, *114*, 1554.
15. (a) Kano, K. *J. Phys. Org. Chem.* **1997**, *10*, 286. (b) Topiol, S.; Sabio, M. *Enantiomer* **1996**, *1*, 251.
16. John, R. A. *Biochim. Biophys. Acta* **1995**, *1248*, 81.
17. Zimmerman, S. C. Ph.D. Thesis, Columbia University (USA), 1984.
18. Kuang, H.; Brown, M. L.; Davies, R. R.; Young, E. C.; Distefano, M. D. *J. Am. Chem. Soc.* **1996**, *118*, 10702.
19. Matsushima, Y.; Martell, A. E. *J. Am. Chem. Soc.* **1967**, *89*, 1331.
20. Zimmerman, S. C.; Czarnik, A. W.; Breslow, R. *J. Am. Chem. Soc.* **1983**, *105*, 1694.
21. Zimmerman, S. C.; Breslow, R. *J. Am. Chem. Soc.* **1984**, *106*, 1490.
22. Kühler, T. C.; Lindsten, G. R. *J. Org. Chem.* **1983**, *48*, 3589.
23. (a) Chmielewski, J. A. Ph.D. Thesis, Columbia University (USA), 1988; (b) Kumabe, N. Ph.D. Thesis, Columbia University (USA), 1990.