Transaminations by Pyridoxamine Selectively Attached at C-3 in β-Cyclodextrin

Ronald Breslow* and Anthony W. Czarnik[1]

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

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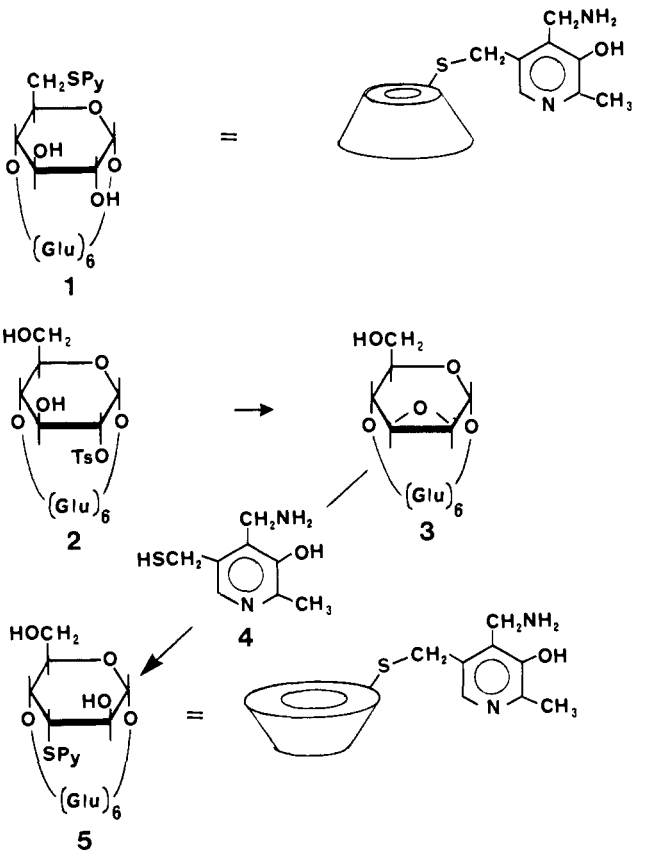
Pyridoxal and pyridoxamine are, as their phosphates, the coenzymes for numerous metabolic transformations involving amino acids and, in particular, for transaminations that interconvert amino acids and keto acids.[2] We have recently[3] described a "synthetic transaminase" **1** (Scheme I) in which pyridoxamine was linked to C-6 of β-cyclodextrin, putting it on the primary edge of the molecule. Compound **1** showed the ability to perform transaminations on keto acids, with preference for substrates such as phenylpyruvic acid or indolepyruvic acid, which can utilize hydrophobic binding of the aryl group into the cyclodextrin cavity. Interaction with the chiral cyclodextrin ring also led to induced optical activity in the product amino acids.

There is considerable interest in the contrasting properties of cyclodextrin attached respectively to the primary (C-6) and to the secondary (C-2, C-3) faces of the molecules.[4] The secondary side is more open and is the preferential locus for binding of large molecules; it is also the face on which the chirality of cyclodextrin is more apparent.[5] Since we have recently[6] been able to prepare authentic β-cyclodextrin-2-tosylate (**2**) we decided to use it to prepare an artificial transaminase with pyridoxamine attached to the cyclodextrin secondary side. The sequence involved conversion of **2** to the manno epoxide **3** and then opening with the pyridoxamine thiol **4** to afford **5**, in which the attachment is at C-3. Despite the double inversion in the glucose unit, which results in the pyridoxamine unit formally pointing into the ring cavity as an axial substituent, the new artificial transaminase **5** proved to have interesting catalytic and chiral induction properties.

An aqueous solution of **2** containing ammonium bicarbonate was heated at 60 °C for 3 h, affording the epoxide **3**, which was isolated by chromatography on Sephadex G-15 resin. The product was free of aromatic protons in the 300-MHz NMR and showed a one-proton doublet at 3.3 ppm (J = 3.7 Hz) for the C-2 proton on the glucose epoxide residue coupled to H-3 and an unsplit one-proton signal at 5.1 ppm for the C-1 proton of that residue, in addition to the normal cyclodextrin signals. In glucose-*manno*-epoxides, $J_{1,2}$ is normally[7] close to 0 Hz, while in *allo*-epoxides it is 2.5-4.5 Hz. Thus our epoxide spectrum confirms the assignment[6] that **2** is the C-2 tosylate.

The pyridoxamine derivative **5** was prepared directly from the above reaction mixture containing **3** by addition of pyridoxamine thiol dihydrobromide[3] **4** and further heating. After chromatography (Sephadex CM-25) the product **5** was isolated as a colorless solid.[8] The 300-MHz NMR spectrum in D_2O/dilute H_2SO_4 showed the expected signals from pyridoxamine and cyclodextrin protons and a one-proton signal at 3.05 ppm, which we assign[9] to the cyclodextrin C-H unit carrying the sulfur atom. This signal appeared as a doublet of doublets, J = 11.2 and 3.4 Hz. Decoupling occurred by irradiation at 3.95 (J = 3.4 Hz) and 3.8 ppm (J = 11.2 Hz), so the CH-S unit is not coupled to an anomeric proton. The anomeric signals for H-1 of β-cyclodextrin (and other glucosides) are normally[10] found at 4.9 ppm, while signals near 3.8 ppm are characteristic of H-2 through H-6. Thus the attachment of sulfur is not at C-2 and must be at C-3.

Scheme I



Diaxial opening is of course the rule[11] for sugar epoxides, so reaction of the *manno*-2,3-epoxide at C-3 was expected. However, such axial attack must occur from inside the cyclodextrin cavity; an exception to the rule might have been seen but was not. In the product **5**, though, the finding of a coupling constant as large as 11.2 Hz suggests that the C-3 proton is axial, not equatorial. Thus some conformational change has occurred at the substituted glucose residue, either a chair flip to the $^1C_4$ conformation or a flexure to nonchair geometry; the pyridoxamine unit is thus pseudoequatorial.

Transamination by **5** was examined by the method described previously[3] and compared with the data for **1**. Appropriate keto acids reacted with **5** to produce the corresponding amino acids, which were dansylated and analyzed by HPLC. Indolepyruvic acid was converted to tryptophan by **5** at a rate 25 times that for the conversion of pyruvic acid to alanine, while phenylpyruvic acid was converted to phenylalanine with an 18-fold acceleration. Thus again we observe selective reaction with aromatic keto acids which can utilize the cyclodextrin binding site, but this secondary-side derivative **5** is only about half[12] as effective as is the primary-side analogue **1**.

Remarkable effects are seen with respect to chiral induction in the product amino acids. On reexamination[13] we find that **1**

(1) NIH Postdoctoral Fellow, 1981-1983.
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(8) Anal. Calcd for $C_{50}H_{80}N_2O_{35}S\cdot11.5H_2O$ (found): C, 39.81 (40.06); H, 6.88 (6.70); N, 1.85 (1.70); S, 2.13 (1.93).
(9) This is the expected position, from standard NMR tables. A similar signal was produced on reaction of 4,6-*O*-benzylidene-1-*O*-methylglucose-2,3-*manno*-epoxide with pyridoxaminethiol.
(10) Since the anomeric proton is on the *outside* of the cavity, it would not shift because of anisotropic effects from a bound pyridine ring. Such binding effects are at most +0.3 ppm and only for *internal* protons.
(11) Williams, N. R. *Adv. Carbohyd. Chem.* **1970**, *25*, 109.
(12) We find a 50:1 preference for indolepyruvic acid by **1**, not the 200-fold preference of ref 3. The present analytical method seems more reliable.

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gives a 5:1 preference for the synthesis of L-phenylalanine over the D enantiomer, not the 3:1 reported earlier,[3] but **5** shows *no* detectable enantiomeric preference. However, in the conversion of indolepyruvic acid to tryptophan, **1** shows a 2:1 preference for the L enantiomer while **5** shows an 1.8:1 preference for the D product. This reversal of stereochemical selectivity can be rationalized if the cyclodextrin is thought of formally as a planar (i.e., clockwise or counterclockwise), not helical, chiral element.

Our results show that both the primary- and the secondary-side pyridoxamine cyclodextrins show cooperative catalysis of transaminations, but with differences in rate effects and chiral inductions. Furthermore, the axial opening of the epoxide **3** at C-3 means that other derivatives of cyclodextrin should be available with such axial groups pointing into the cavity or with an altered glucose conformation. Such derivatives could have very different properties from those of functionalized cyclodextrins available heretofore.[14]

Registry No. 2, 84216-71-7; **3**, 84648-78-2; **4**, 84648-79-3; **5**, 84648-80-6; indolepyruvic acid, 392-12-1; tryptophan, 73-22-3; pyruvic acid, 127-17-3; alanine, 56-41-7; phenylpyruvic acid, 156-06-9; phenylalanine, 63-91-2.

(13) By the method of Lam et al.: Lam, S.; Chow, F.; Karmen, A. *J. Chromatogr.* **1980**, *199*, 295.

(14) Support of this work by the NIH is gratefully acknowledged, as is the assistance of Dr. Christopher Turner with the NMR studies. The earliest studies on a β-cyclodextrin secondary tosylate and epoxide were performed here a few years ago by Dr. K. Nakasuji and Dr. M. F. Czarniecki, who protected the primary hydroxyls by *tert*-butyldimethylsilylation before tosylation. Their work will be described in the full publication.

Stereochemistry at the Chiral Ruthenium Atom in the Reaction of Diastereomeric $(\eta^5\text{-}C_5H_5)RuCl[(R)\text{-}Ph_2PCH(CH_3)CH_2PPh_2]$ with $SnCl_2$

Giambattista Consiglio*

Swiss Federal Institute of Technology
Department of Industrial and Engineering Chemistry
CH-8092 Zürich, Switzerland

Franco Morandini

CNR, Centro di Studio sulla Stabilità e
Reattività dei Composti di Coordinazione
Istituto di Chimica Analitica, 35100 Padova, Italy

Gianfranco Ciani and Angelo Sironi

Centro di Studio per la Sintesi e la
Struttura dei Composti dei Metalli di Transizione
nei Bassi Stati di Ossidazione, 20133 Milano, Italy

Matthias Kretschmer

Swiss Federal Institute of Technology
Department of Inorganic Chemistry
CH-8092 Zürich, Switzerland

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For a long time it has been known that the trichlorostannate ligand is able to modify or even to initiate catalytic activity in transition-metal complexes.[1,2] Recently there has been much interest devoted to the role of this ligand in organometallic reactions and in homogeneous catalysis.[3-10] Normally the $SnCl_3$ ligand is formed according to reaction 1, i.e., from transition-metal

$$M\text{-}Cl + SnCl_2 \rightleftharpoons M\text{-}SnCl_3 \qquad (1)$$

chloro complexes and $SnCl_2$[2] in a formal insertion reaction in an M-Cl bond and in a formal oxidation addition on Sn.

The stereochemical course of the reaction at the level of the transition metal has not yet been elucidated. It was recently proposed[11] (but not fully demonstrated) that reaction 1 can be highly stereospecific[12] when M-Cl is $(\eta^6\text{-}C_6H_6)RuCl(CH_3)[Ph_2PNHCH(CH_3)Ph]$ and when a molar ratio $SnCl_2$/M-Cl of 1.1 is used. However, it was not possible to determine whether the configuration at the ruthenium atom is retained or inverts during the reaction.[13] This also is of particular interest in view of the possible role of chiral complexes in which the metal is an asymmetry center in homogeneous asymmetric catalysis.[14-16] Indeed chiral diphosphine–platinum chloride–tin chloride catalytic systems have been used in asymmetric hydroformylation and hydrogenation.[17-19]

Recently we reported that in the displacement reaction of PPh_3 from pseudotetrahedral $(\eta^5\text{-}C_5H_5)RuCl(PPh_3)_2$ by (*R*)-1,2-propanediylbis(diphenylphosphine) ((*R*)-prophos)[20] two diastereomers form (**1** and **1′**) in nearly a 1:1 ratio, which differ in the configuration at the ruthenium atom.[21] In the meantime the two diastereomers have been separated;[22] the structure of diastereomer **1**, which has the higher frequency for the cyclopentadienyl protons in the ^1H NMR spectrum (δ 4.43 vs. 4.32 found for **1′**), has been determined by X-ray analysis[23] and has shown *S* configuration at the ruthenium atom, if we assume the priority order $\eta^5\text{-}C_5H_5 > Cl > PCH > PCH_2$[24] (Figure 1). We report here that the reaction of both diastereomers **1** and **1′** with $SnCl_2$ to give **2** and **2′** is highly stereospecific and takes place with retention of the configuration at the ruthenium atom (Scheme I).

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