using beef serum phosphotriesterase to hydrolyze any residual (−)-MBNP in an aliquot and following the hydrolysis at 400 nm. The MBNP (50–70 μg) was equilibrated in Tris–HCl buffer (3.10 ml) containing $CaCl_2$, EDTA, and CH_3CN (1.61% v/v), with partially purified phosphotriesterase. Residual (−)-MBNP was hydrolyzed in a pseudo-first-order reaction, concurrent with slow, apparently zero-order, hydrolysis of the (+)-MBNP by the enzyme. Adding a trace of racemic MBNP (1 μg) to such a reaction mixture already containing the optically active MBNP confirmed that the first-order reaction was hydrolysis of (−)-MBNP. In this case, a first-order increase in A_{400}, corresponding to hydrolysis of one-half of the added (±)-MBNP, occurred, with a rate constant the same as that for the reaction of the enzyme with a sample of optically active MBNP. These assays showed that one of the preparations of (+)-MBNP described above contained 0.50% (−)-MBNP and the other contained 0.04%.

At present we are studying the stereospecific inhibition of some serine hydrolases by MBNP and the stereochemistry of this and other asymmetric phosphotriesters.

Nicholas P. B. Dudman, Burt Zerner*
Department of Biochemistry, University of Queensland
St. Lucia, Queensland 4067, Australia
Received December 15, 1972

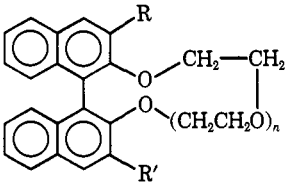
Complete Optical Resolution by Differential Complexation in Solution between a Chiral Cyclic Polyether and an α-Amino Acid[1]

Sir:

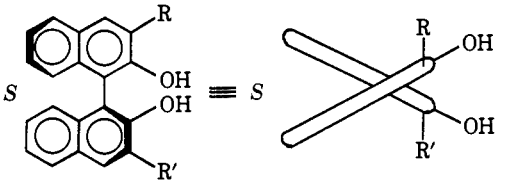
Complexation–decomplexation reactions between organic entities are necessary stages in enzyme-catalyzed reactions. At the active site, the host and guest parts of the complex are highly ordered. Studies of structured molecular complexes[2] between organic compounds in solution not involving enzymes have centered on cyclodextrins as host molecules[2a–f] and on catalysis or inhibition of reaction rates through complexation.[2a–h] Most optical resolutions involve differences in formation rates or in energies of crystal lattices.[2m–o,s,t,z] Others involve solid–liquid[2n–r,v] or gas–liquid chromatography[2n–r,v] or dialysis.[2w] Racemate distribution between water and optically active liquids[2u,x] gave a maximum optical purity of 2 ± 0.9%.[2u] One full resolution by countercurrent extraction has appeared.[2y]

We report the first optical resolution of a racemate by differential complexation in liquid–liquid chromatography to give optically pure enantiomers. The host molecule was designed for configurational differentiation in complexation in solution, and the relative configurations of the more stable diastereomeric complexes were predicted from examination of molecular models in advance of experiment.

The previous paper reported racemates **1–5** and the facts that acids **1**, **2**, and **4** complexed valine better than acid **3** or ester **5**.[3] Optically pure acids (*S*)-**1**,[4a,b] (*S*)-**3**,[4a,b] and (*R*)-**4**[4a,b] now have been prepared (comparable yields)[3] from optically pure (*S*)- or (*R*)-**6**.[5] Reduction of (*S*)-**6** and (*R*)-**6** gave (*S*)-**7**[4a,b] (79%) and (*R*)-**7** (77%),[4a,b] respectively. Diazomethane and (*R*)-**6** gave (76%) the dimethyl ester (*R*)-**8**[4a,b] mp 243–245°,



1, $n = 4$; R = R′ = $CH_2OCH_2CO_2H$
2, $n = 4$; R = H; R′ = $CH_2OCH_2CO_2H$
3, $n = 3$; R = R′ = $CH_2OCH_2CO_2H$
4, $n = 5$; R = R′ = $CH_2OCH_2CO_2H$
5, $n = 4$; R = R′ = $CH_2OCH_2CO_2CH_3$



	R	mp°C	$[\alpha]^{25}_{578}$	(*c* 1.1)
(*S*)-**6**	CO_2H	> 285	−198°	$(CH)_5N$
(*R*)-**6**	CO_2H	> 285	+195°	$(CH)_5N$
(*S*)-**7**	CH_2OH	190–193	−63.5°	$(CH_2)_4O$
(*R*)-**7**	CH_2OH	192–195	+64.1°	$(CH_2)_4O$

$[\alpha]^{25}D$ +172°,[4d] reported[6a] mp 239–240°, $[\alpha]^{25}D$ +159°.[4d] Reduction of (*R*)-**8** gave (*R*)-**7** (63%), mp 195–196°, $[\alpha]^{25}_{578}$ +63.8°.[4d] The absolute configura-

(1) This work was supported by the U. S. Public Health Service Research Grant No. GM 12690-8 from the Department of Health, Education and Welfare, and by a grant from the National Science Foundation, GP 33533X.

(2) (a) F. Cramer and W. Dietsche, *Chem. Ber.*, **92**, 378, 1739 (1959); (b) F. Cramer and W. Kampe, *J. Amer. Chem. Soc.* **87**, 1115 (1965); (c) F. Cramer, *Angew. Chem., Int. Ed. Engl.*, **5**, 601 (1966), and references cited therein; (d) R. L. van Etten, G. A. Clowes, J. F. Sebastian, and M. L. Bender, *J. Amer. Chem. Soc.*, **89**, 3253 (1967), and references cited therein; (e) R. Breslow and P. Campbell, *ibid.*, **91**, 3085 (1969); (f) K. Flohr, R. M. Paton, and E. T. Kaiser, *Chem. Commun.*, 1621 (1971), and references cited therein; (g) A. K. Colter, F. F. Guzik, and S. H. Hui, *J. Amer. Chem. Soc.*, **88**, 5754 (1966), and references cited therein; (h) R. Hershfield and M. L. Bender, *ibid.*, **94**, 1376 (1972); (i) M. S. Newman, W. B. Lutz, and D. Lednicer, *ibid.*, 77, 3420 (1955); (j) M. S. Newman and W. B. Lutz, *ibid.*, **78**, 2467 (1956), and references cited therein; (k) M. S. Newman, "Steric Effects in Organic Chemistry," Wiley, New York, N. Y., 1956, pp 472–478, and references cited therein; (l) A. C. D. Newman and H. M. Powell, *J. Chem. Soc.*, 3747 (1952); (m) M. Schlenk, Jr., *Experientia*, **8**, 337 (1952); (n) D. R. Buss and T. Vermeulen, *Ind. Eng. Chem.*, **60**, 12 (1968); (o) P. H. Boyle, *Quart. Rev., Chem. Soc.*, 323 (1971); (p) G. Losse and K. Kuntze, *Z. Chem.*, **10**, 22 (1970); (q) B. Feibush and E. Gil-Av, *Tetrahedron*, **26**, 1361 (1970); (r) W. Parr, J. Pleterski, C. Yang, and E. Bayer, *J. Chromatogr. Sci.*, **9**, 141 (1971); (s) M. Mikolajezyk, J. Drabowicz, and F. Cramer, *Chem. Commun.*, 317 (1971); (t) H. P. Benschop and G. R. Van den Berg, *ibid.*, 1431 (1970); (u) N. S. Bowman, G. T. McCloud, and G. K. Schweitzer, *J. Amer. Chem. Soc.*, **90**, 3848 (1968); (v) J. D. Morrison and H. S. Mosher, "Asymmetric Organic Reactions," Prentice-Hall, Englewood Cliffs, N. J., 1971, pp 16–17, and references cited therein; (w) V. O. G. Klingmüller and G. Gedenk, *Nature (London)*, **179**, 367 (1957); (x) K. Bauer, H. Falk, and u. K. Schlögl, *Monatsh. Chem.*, **99**, 2186 (1968); (y) S. J. Romano, K. H. Wells, H. L. Rothbart, and W. Rieman, III, *Talanta*, 16, 581 (1969); (z) S. H. Wilen, *Top. Stereochem.*, 6, 107 (1972).

(3) R. C. Helgeson, J. M. Timko, and D. J. Cram, *J. Amer. Chem. Soc.*, **95**, 3023 (1973).

(4) (a) Carbon and hydrogen analyses were within 0.30% of theory. Pmr spectra were consistent with assigned structures. (b) Mass spectra exhibited molecular ions. (c) Chloroform, *c* 1.0 ± 0.2. (d) Tetrahydrofuran, *c* 1.0 ± 0.2.

(5) (a) W. M. Stanley and R. Adams, *Recl. Trav. Chim. Pays-Bas*, **48**, 1035 (1929); (b) K. Weil and W. Kuhn, *Helv. Chim. Acta*, **27**, 1648 (1944); (c) H. J. Barber and K. Gaimster, *J. Appl. Chem.*, **2**, 565 (1952).

(6) (a) H. Akimoto, T. Shioiri, Y. Iitaka, and S. Yamada, *Tetrahedron Lett.*, 97 (1968); (b) H. Akimoto, T. Shioiri, Y. Iitaka, and S. Yamada, *ibid.*, 4617 (1971); (c) H. Akimoto and Y. Iitaka, *Acta Crystallogr., Sect. B*, **25**, 1491 (1969); (d) I. Hanazaki and H. Akimoto, *J. Amer. Chem. Soc.*, **94**, 4102 (1972); (e) H. Akimoto and S. Yamada, *Tetrahedron*, **27**, 5999 (1971).

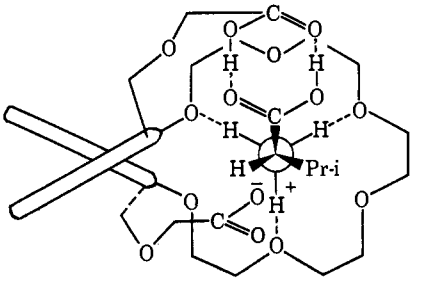
tion of (*R*)-**8** has been determined.[6] Thus the absolute configurations of all compounds related by reactions to (*R*)-**8** are established. The patterns of rotations obtained for several sets of reaction-related stereoisomers resolved by different methods[5a,6a,7] indicate maximum rotations are known. Optically pure (*S*)-**7** was converted[3] to (*S*)-**5**[4a,b] (glass), $[\alpha]^{25}_{546}$ −39.2°,[4c] which gave[3] (*S*)-**1**[4a,b] (glass), $[\alpha]^{25}_{546}$ +75.6°,[4c] $[\alpha]^{25}_{546}$ −24.9°.[4d] Optically pure (*S*)-**7** gave[3] (*S*)-**3**[4a,b] (glass), $[\alpha]^{25}_{546}$ −105°,[4c] $[\alpha]^{25}_{546}$ −107°.[4d] Optically pure (*R*)-**7** gave[3] (*R*)-**4**[4a,b] (glass), $[\alpha]^{25}_{546}$ −105°,[4c] $[\alpha]^{25}_{546}$ −15.0°.[4d] Only at ∼150° at <50 μ as films could these glasses be completely dried. Only (*S*)-**1** showed an inverted sign of rotation when chloroform was substituted for tetrahydrofuran as solvent.

In run 1, the (*R*) and (*S*) components of 76 mg of racemic **1** and 11.4 mg of optically pure (*R*)-valine[8] were partitioned at equilibrium between the two layers formed by shaking 0.2 ml of D_2O, 0.3 ml of $CDCl_3$ and 0.6 ml of CD_3CO_2D (at ∼30°). The pmr spectra (HA-100) of the aqueous (∼0.75 ml) and chloroform layers (∼0.25 ml) indicated the former contained ∼50% of the **1** and ∼95% of the valine used and the latter contained ∼50% of the **1** and ∼5% of the valine used. Appropriate pmr experiments[7a] indicated the **1** in the aqueous layer and the valine in the chloroform layer to be complexed, and for complexation–decomplexation to be fast on the pmr time scale. The aqueous layer was evaporated (vacuum), and the residue was shaken with chloroform and water. The organic layer was washed with water and evaporated to dryness (30°, 50 μ, 3 hr) to give **1**·0.5H_2O as a film, yield 33.5 mg (∼44%), $[\alpha]^{25}_{546}$ +6.7°.[4d] A 40-mg sample of optically pure (*S*)-**1** submitted to the above isolation conditions gave a 5% weight loss, $[\alpha]^{25}_{546}$ −24.6°.[4d] Thus the aqueous layer contained **1** of ∼27% optical purity, rich in the (*R*) isomer. The chiral recognition factor is defined as the ratio of the predominant to the subordinate enantiomer at equilibrium in the phase containing the complex. In run 1, the factor = [63% (*R*)-**1**]/[37% (*S*)-**1**] = 1.7. In run 2, 0.35 ml of CD_3CO_2D, 1 ml of benzene, and 0.25 ml of D_2O at 30° gave an aqueous layer (∼0.5 ml) that contained >95% of the valine and ∼50% of **1** rich in the (*R*) isomer by a factor of 1.25. In run 3, diester **5** was used. Although (*R*)-**5** dominated in the aqueous layer, the factor dropped to 1.06. Run 4 involved monoacid **2** of unknown absolute configuration and maximum rotation. The **2** isolated from the aqueous layer gave $[\alpha]^{25}_{546}$ +1.2°.[4c] The magnitudes of rotation of the molecular relatives of **2** suggest this rotation reflects a very small factor. Run 5 employed diacid **4**. The aqueous layer was enriched in (*R*)-**4** by a factor of 1.02. Run 6 with diacid **3** showed no enrichment in either enantiomer.

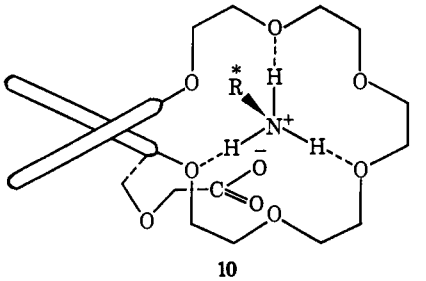
Run 7 determined the ability of optically pure acid (*S*)-**1** to discriminate between (*R*)- and (*S*)-valine when complexation occurred only in the chloroform layer. A mixture of 400 mg of (*S*)-**1**, 177 mg (2.5 equiv) of racemic valine, 1.0 ml of CD_3CO_2D, 1.5 ml of $CDCl_3$, and 0.5 ml of D_2O was shaken at 30° to produce two equilibrated layers. The chloroform layer (∼2.3 ml) contained >99% of the (*S*)-**1** and 40% (1.0 equiv) of the valine (pmr, HA-100). The chloroform layer was evaporated, and the residue was shaken with chloroform–water. This water layer was washed with chloroform and evaporated to give 37 mg of valine, $[\alpha]^{25}_{546}$ +4.0° (*c* 2.0, 5 *N* HCl). Thus (*S*)-**1** preferentially complexed (*S*)-valine in the chloroform layer by a factor of 1.3.

Racemic **1** was resolved to optical purity of both enantiomers by liquid–liquid chromatography. A solution of 6 g of (*S*)-valine ($[\alpha]^{25}_{546}$ +31.8°, *c* 2.00, 5 *N* HCl)[8] dissolved in a mixture of 40 ml of acetic acid–10 ml of water saturated with benzene (∼15 ml) was mixed with 60 g of Celite, which was dry packed into a column followed by 5 g of Celite to give a 60 by 3 cm stationary liquid phase. Racemic **1** (1.00 g) in 10 ml of 80% acetic acid–20% water (by volume) saturated with benzene was added and eluted with benzene saturated with aqueous acetic acid. Combined fractions 12–16 gave 336 mg of (*R*)-**1** (isolated as in run 1), $[\alpha]^{25}_{546}$ −69.2°,[4c] which when rechromatographed on a similar column containing optically pure (*R*)-valine gave 220 mg of optically pure (*R*)-**1**, all but the beginning fractions of which gave $[\alpha]^{25}_{546}$ −76.5°.[4c] Combined fractions 21–34 gave 363 mg of (*S*)-**1**, $[\alpha]^{25}_{546}$ +70.0°,[4c] which when rechromatographed on a similar column containing (*S*)-valine gave 271 mg of optically pure (*S*)-**1**, all but the beginning fractions giving $[\alpha]^{25}_{546}$ +76.5°.[4c]

Thus host molecules have been designed whose complete optical resolution by guest molecules involves only stability differences in solution of diastereomeric complexes. Run 7 indicates valine and probably other α-amino acids can be resolved similarly, thus reversing the roles of host and guest as resolving agents. Runs 1–5 indicate that only **1** possesses useful chiral recognition properties. Complexes of the (*R*,*R*) or (*S*,*S*) configurations were substantially more stable than those of the (*R*,*S*) or (*S*,*R*) configurations. An *a priori* examination of CPK molecular models of the ten nonenantiomeric complexes of the five cyclic ethers tested led to the prediction that only **1** would show chiral recognition, and that the (*R*,*R*) and (*S*,*S*) complexes would be more stable. Structure **9** was



9



10

(7) (a) E. P. Kyba, K. Koga, L. R. Sousa, M. R. Siegel, and D. J. Cram, *J. Amer. Chem. Soc.*, **95**, 2692 (1973); (b) E. P. Kyba, M. G. Siegel, L. R. Sousa, G. D. Y. Sogah, and D. J. Cram, *ibid.*, **95**, 2691 (1973).

(8) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Wiley, New York, N. Y., 1961, p 116, gives $[\alpha]^{25}_{546}$ +32.2° (*c* 2.5 *N* HCl) for (*S*)-valine.

envisioned for the (*S,S*) complex. Three hydrogen bonds and carboxylate–ammonium ion pairing hold the amino acid to the crown, and carboxyl–carboxyl hydrogen bonding presses the hydrogen of the chiral center of the amino acid to the chiral barrier of the crown. Monoacid **2** binds well to amino acids[3] (see **10**), but the absence of the second carboxyl of the host allows the chiral center of the guest too much conformational flexibility for the diastereomeric complexes to differ much in stability. In models, the hole of **3** is too small for an ammonium ion to penetrate the crown of oxygens and little complexation is observed.[3] Models of amino acid complexes of **4** suggest that the three alternate oxygens remote from the chiral barrier most stably bind the ammonium ion. Thus the two chiral elements are also remote. Complexes of diester **5** are less structured, since they lack two of the three binding features that characterize the complex of diacid **1**. Compound **1** is one of a family of host molecules that promise to be useful in resolution, determination of configuration, and optical purity.

(9) Public Health Service International Postdoctoral Research Fellowship, 1971–1972.

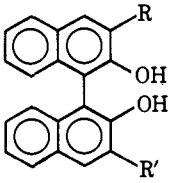
Roger C. Helgeson, Kenji Koga,[9] Joseph M. Timko
Donald J. Cram*
Contribution No. 3089, Department of Chemistry
University of California at Los Angeles
Los Angeles, California 90024
Received December 14, 1972

Structural Requirements for Cyclic Ethers to Complex and Lipophilize Metal Cations or α-Amino Acids[1]

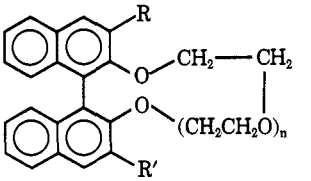
Sir:

Sixteen new multiheteromacrocycles are reported with cavities shaped to complex differentially metal ions and α-amino acids. Cycles **6–21**[2a,b] were prepared from ditosylates of polyethylene glycols and 3,3′-disubstituted-2,2′-dihydroxy-1,1′-binaphthyl compounds **1–5**,[2] which in turn were prepared from 2,2′-dihydroxy-1,1′-binaphthyl (no high dilution). Each unit of **17–21** was designed for a specific role in molecular complexation. The cycles' oxygens provide neutral ligands for metal or ammonium cation complexation.[3] The hole diameters with the oxygens turned inward vary with the naphthyl–naphthyl dihedral angles and the number of ethyleneoxy units. The binaphthyl unit is a chiral steric barrier whose two 3-positions direct attached side chains under or over the hole. The carboxyl groups of the side chains can center above or below the hole, and their anions can provide internal counterions for complexed cations. Amino groups or a second carboxyl attached to the side chain can serve as additional neutral ligands or as sites for hydrogen bonding or ion pairing the carboxyl of complexed amino acids. The binaphthyl and methylene units of the ring and side chain shape the area around the hole and provide a lipophilic skin for the hydrophilic metal or amino acid guest entities.

Complexes **24–32** were examined. Easily visible pmr spectral differences (deuterated solvents, HA-100) of the $ArCH_2$ and $ArOCH_2$ proton signals in complexed and noncomplexed polyethers provided one criterion of complexation in solution. Lipophilization of cat-

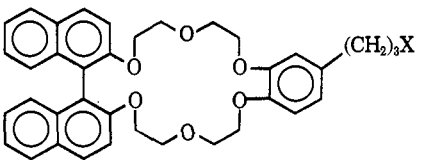


	R	R′	mp, °C	%
1, CH_2M		CH_2M	300 dec	75
2, H		CH_2M	226–228	15
3, CH_2OH		CH_2OH	222–224	65
4, CH_2M		CH_2OH	190–192	30
5, H		CH_2OH	206–207	80



n	R	R′	mp, °C	%
6, 4	CH_2M	CH_2M	Oil	65
7, 4	CH_2OH	CH_2OH	132–134	60
8, 4	H	CH_2OH	136–137	50
9, 4	CH_2M	CH_2OH	Oil	55
10, 3	CH_2OH	CH_2OH	Oil	10
11, 5	CH_2OH	CH_2OH	Oil	50
12, 4	CH_2E	CH_2E	Oil	60
13, 4	H	CH_2E	Oil	70
14, 4	CH_2M	CH_2E	Oil	35
15, 3	CH_2E	CH_2E	Oil	55
16, 5	CH_2E	CH_2E	Oil	50
17, 4	CH_2A	CH_2A	Oil	80
18, 4	H	CH_2A	Oil	70
19, 4	CH_2M	CH_2A	Oil	65
20, 3	CH_2A	CH_2A	Oil	85
21, 5	CH_2A	CH_2A	Oil	75
22, 4	H	H	130–130.5	60

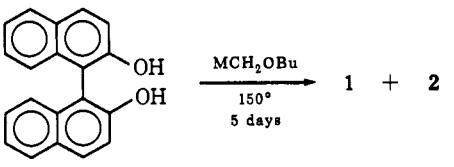
M = $N(CH_2CH_2)_2O$; E = $OCH_2CO_2CH_3$; A = OCH_2CO_2H; Ts = $SO_2C_6H_4CH_3$-*p*



23a, X = OH

23b, X = CO_2H, oil, 70%

Conversions



1 or **2** $\xrightarrow[2.\ LiAlH_4]{1.\ Ac_2O,\ 140°,\ 2\ weeks}$ **3**, **4**, or **5**

1, **3**, **4**, or **5** $\xrightarrow[2KO\text{-}t\text{-}Bu,\ THF]{Ts(OCH_2CH_2)_{n-1}OTs}$ **6**, **7**, **8**, **9**, **10**, or **11**

7, **8**, **9**, **10**, or **11** $\xrightarrow{BrCH_2CO_2CH_3}$ **12**, **13**, **14**, **15**, or **16**

12, **13**, **14**, **15**, or **16** $\xrightarrow[2.\ H^+]{1.\ OH^-}$ **17**, **18**, **19**, **20**, or **21**

(1) This work was supported by U. S. Public Health Service Research Grant No. GM 12640-08 from the Department of Health, Education and Welfare, and by a grant from the National Science Foundation.

(2) (a) Carbon and hydrogen analyses were within 0.30% of theory. Pmr spectra were consistent with assigned structures. (b) Mass spectra exhibited molecular ions. (c) Metal salt analyses were within 1.00% of theory.

(3) See C. J. Pedersen, *J. Amer. Chem. Soc.*, **89**, 2495, 7017 (1967), for first observations.