Cbz-Val-O-*t*-Bu (19). Flash chromatography (eluant, 2% Et_2O/PhH) gave **19** (134 mg, 42%) as an oil: $[\alpha]_D$ = −7.7° (*c* = 2.2, MeOH) [lit.[11] $[\alpha]_D$ −4.7° (*c* = 2, MeOH)]; ^1H NMR ($CDCl_3$) δ 7.40–7.26 (m, 5 H), 5.28 (d, 0.9 H, *J* = 8.8 Hz), 5.11–5.06 (s, 2 H), 5.06–4.98 (s, 0.1 H), 4.19 (dd, 0.9 H, *J* = 4.2, 9.0 Hz), 4.08–4.00 (s, 0.1 H), 2.20–2.05 (m, 1 H), 1.46 (s, 9 H), 0.96, 0.88 (d, 6 H, *J* = 6.8 Hz).

(2*S*)-1-(Benzyloxycarbonyl)-2-[[(*tert*-butyldimethylsilyl)oxy]methyl]pyrrolidine (21). Flash chromatography (eluant, 0–4% Et_2O/PhH) gave **21** (160 mg, 46%) as an oil: $[\alpha]_D$ = −44° (*c* = 1.6 , $CHCl_3$); IR (neat) 2960, 1705, 1415, 1095, 835, 775 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.40–7.25 (m, 5 H), 5.02–5.00 (m, 2 H), 3.95–3.34 (m, 5 H), 2.05–1.70 (m, 4 H), 0.88, 0.85 (2 s, 9 H), 0.04, 0.02, −0.04 (3 s, 6 H); ^{13}C NMR ($CDCl_3$) 154.8, 137.1, 136.8, 128.4, 128.1, 127.9, 127.7, 66.7, 66.4, 63.8, 63.11, 63.06, 59.0, 58.9, 58.4, 58.3, 47.2, 46.9, 28.2, 27.5, 25.8, 23.8, 22.8, 18.2, −5.47, −5.52 ppm; HRMS (EI) calcd for $C_{19}H_{31}NO_3Si$ (M − Me^+) 334.1839, found (M − Me^+) 334.1833. Anal. Calcd for $C_{19}H_{31}NO_3Si$: C, 65.29; H, 8.94; N, 4.01. Found: C, 65.59; H, 9.11; N, 3.95.

General Synthesis of the Mosher Amides for Entries 11, 13, and 15. 10% Pd/C (10 wt % of the Cbz derivative) in MeOH was prehydrogenated under 1 atm of H_2 for 1 h before adding a MeOH solution of the Cbz derivative and camphorsulphonic acid (1 equiv). After the reduction was complete, the reaction mixture was filtered through Celite and rotary evaporated to a solid. To this solid was added dicyclohexylcarbodiimide (2 equiv), 4-(dimethylamino)pyridine (1.8 equiv), and (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (2 equiv) in CH_2Cl_2 (0.25 M in amino ester). When the reaction was complete, the solids were removed by filtration, and the filtrate was washed with 1 M $NaHSO_4$, H_2O, 10% aqueous $NaHCO_3$, and brine. The organic layer was dried with $MgSO_4$ and rotary evaporated to leave a syrup. Additional dicyclohexylurea was removed by dissolving the syrup in Et_2O and filtering. Evaporation gave the crude Mosher amide as an oil, and this was assayed for diastereioisomeric purity by ^1H NMR spectroscopy. For all three Mosher amides, no signals for the *R,R* diastereoisomer were observed. However, signals for the *R,R* diastereoisomer were clearly visible in the spectrum of a mixture of the *R,S* diastereoisomer containing 6% of a 1:1 mixture of *R,R* and *R,S* diastereoisomers. Thus, less than 3% of the *R,R* diastereoisomer could be detected.

***N*-[(*R*)-2-Methoxy-2-(trifluoromethyl)phenylacetyl]-(*R,S*)-Phe-O-Me.** Prepared from DL-phenylalanine methyl ester hydrochloride (0.30 mmol) according to the procedure above. Flash chromatography (eluant, 7–13% EtOAc/hexanes) of the residue gave the amide (105 mg, 88%) as a thick syrup that solidified on standing: $[\alpha]_D$ = −18° (*c* = 2.6, $CHCl_3$); IR (KBr) 3320, 2970, 1750, 1670, 1525, 1360, 1330, 1170, 740, 695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.55–6.85 (m, 11 H), 4.98, 4.91 (2 m, 1 H), 3.76, 3.73 (2 s, 3 H), 3.434, 3.430, 3.217, 3.214 (4 s, 3 H), 3.27–2.98 (m, 1 H).

***N*-[(*R*)-2-Methoxy-2-(trifluoromethyl)phenylacetyl]-(*S*)-Phe-O-Me.** Flash chromatography of the crude amide (eluant, 7–13% EtOAc/hexanes) gave the pure *R,S* amide as a solid: mp 99–101 °C; $[\alpha]_D$ = +6° (*c* = 1.1, $CHCl_3$); IR (neat) 3340, 2980, 1755, 1675, 1545, 1265, 1235, 1185, 1175, 740, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.60–7.10 (m, 11 H), 4.96–4.87 (m, 1 H), 3.73 (s, 3 H), 3.213, 3.210 (2 s, 3 H), 3.24 (dd, 1 H, *J* = 5.6, 14.0 Hz), 3.12 (dd, 1 H, *J* = 6.8, 14.0 Hz); HRMS (EI) calcd for $C_{20}H_{20}F_3NO_4$ ($M^{\cdot+}$), 395.1344, found ($M^{\cdot+}$) 395.1345.

***N*-[(*R*)-2-Methoxy-2-(trifluoromethyl)phenylacetyl]-(*R,S*)-Pro-O-Me.** Prepared from DL-proline methyl ester hydrochloride (0.30 mmol) according to the procedure above. Flash chromatography (eluant, 15% EtOAc/hexanes) of the reaction mixture gave the amide (80 mg, 77%) as a thick syrup: $[\alpha]_D$ = +144° (*c* = 0.7, $CHCl_3$); IR (neat) 2980, 1760, 1670, 1425, 1185, 720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.68–7.32 (m, 5 H), 4.65–4.55 (m, 1 H), 3.827, 3.822, 3.655, 3.650 (4 s, 3 H), 3.78, 3.77 (2 s, 3 H), 3.49–3.39 (m, 1 H), 2.84–2.71 (m, 1 H), 2.18–2.06 (m, 1 H), 1.90–1.72 (m, 2 H), 1.58–1.47 (m, 1 H).

***N*-[(*R*)-2-Methoxy-2-(trifluoromethyl)phenylacetyl]-(*S*)-Pro-O-Me.** Flash chromatography of the crude amide (eluant, 20–25% EtOAc/hexanes) gave the pure *R,S* amide as a thick syrup: $[\alpha]_D$ = +73° (*c* = 1.1, $CHCl_3$); IR (neat) 2980, 1755, 1665, 1420, 1270, 1180, 715 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.68–7.35 (m, 5 H), 4.58, 4.56 (2 d, *J* = 4.4 Hz), 3.79 (s, 3 H), 3.658, 3.654 (2 s, 3 H), 3.49–3.39 (m, 1 H), 2.79–2.71 (m, 1 H), 2.19–2.07 (m, 1 H), 1.90–1.71 (m, 3 H); HRMS (EI) calcd for $C_{16}H_{18}F_3NO_4$ ($M^{\cdot+}$) 345.1188, found ($M^{\cdot+}$) 345.1201.

***N*-[(*R*)-2-Methoxy-2-(trifluoromethyl)phenylacetyl]-(*R,S*)-Leu-O-Me.** Prepared from DL-leucine methyl ester hydrochloride (0.30 mmol) according to the procedure above. Flash chromatography (eluant, 15–20% EtOAc/hexanes) gave the amide (73 mg, 68%) as a thick syrup that solidified on standing: $[\alpha]_D$ = +1° (*c* = 1.8, $CHCl_3$); IR (KBr) 3310, 2960, 1755, 1740, 1670, 1540, 1170, 720, 700 cm^{-1}; ^1H NMR ($CDCl_3$) 7.60–7.23 (m, 5.5 H), 6.93 (d, 0.5 H, *J* = 4.4 Hz), 4.75–4.63 (m, 1 H), 3.76, 3.73 (2 s, 3 H), 3.562, 3.558, 3.371, 3.368 (4 s, 3 H), 1.80–1.40 (m, 3 H), 0.97, 0.96 (2 d, 3 H, *J* = 6.0 Hz), 0.89, 0.86 (2 d, 3 H, *J* = 6.4 Hz).

***N*-[(*R*)-2-Methoxy-2-(trifluoromethyl)phenylacetyl]-(*S*)-Leu-O-Me.** Flash chromatography of the crude amide (eluant, 15–20% EtOAc/hexanes) gave the pure *R,S* amide as a solid: mp 47–49 °C; $[\alpha]_D$ = −25° (*c* = 1.0, $CHCl_3$); IR (neat) 3320, 2970, 1765, 1740, 1670, 1525, 1180, 1165, 725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.60–7.24 (m, 6H), 4.72–4.67 (m, 1 H), 3.74 (s, 3 H), 3.373, 3.370 (2 s, 3 H), 1.80–1.60 (m, 2 H), 0.97, 0.96 (2 d, 6 H, *J* = 6.4 Hz); HRMS (EI) calcd for $C_{17}H_{22}F_3NO_4$ (M + H^+) 362.1579, found (M + H^+) 362.1579.

Acknowledgment. We thank the National Institutes of Health (AI-23034) for the support of this work and for the 400-MHz NMR spectrometer (RR-01672) and high-resolution mass spectrometer (RR-03245) used in these studies.

(11) Dhaon, M. K.; Olsen, R. K.; Ramasamy, K. *J. Org. Chem.* **1982**, *47*, 1962.

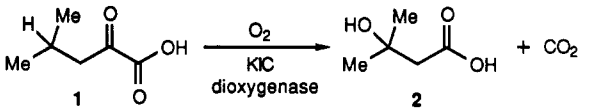
α-Ketoisocaproate Dioxygenase: The Stereochemical Course of the Hydroxylation Reaction

Hyunsoo Han and Robert A. Pascal, Jr.*

Department of Chemistry, Princeton University, Princeton, New Jersey 08544

Received February 26, 1990

α-Ketoisocaproate (KIC) dioxygenase catalyzes the oxidation of α-ketoisocaproate (1) to β-hydroxyisovalerate (2) in mammals.[1] This cytosolic enzyme is not generally



considered to be part of the "normal" pathway for the degradation of branched-chain α-keto acids, but it has been suggested to function as a "safety valve" to prevent the accumulation of the toxic keto acid **1**, which is produced by the transamination of leucine.[1] The enzyme has been highly purified from rat liver,[1] and ^{18}O-labeling experiments have shown that it is indeed a dioxygenase.[2] Both the C-3 hydroxyl group and one of the carboxyl oxygens of product **2** are derived at least in part from molecular oxygen; labeling of the carboxyl is nearly complete, but more than half of the product molecules contain no label in the hydroxyl group.[2]

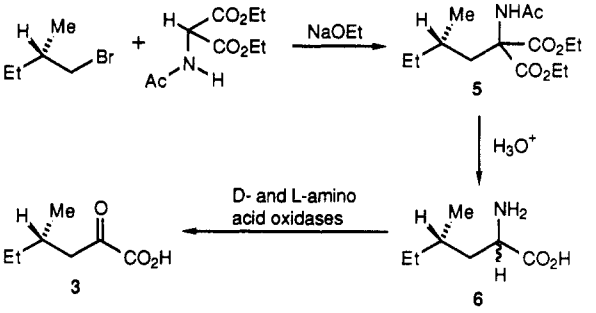
The vast majority of biological hydroxylations of aliphatic carbons proceed with retention of configuration,[3] although some stereochemical infidelity has been observed

(1) (a) Sabourin, P. J.; Bieber, L. L. *J. Biol. Chem.* **1982**, *257*, 7460–7467 and references cited therein. (b) Sabourin, P. J.; Bieber, L. L. *Methods Enzymol.* **1988**, *166*, 288–297.
(2) Sabourin, P. J.; Bieber, L. L. *J. Biol. Chem.* **1982**, *257*, 7468–7471.
(3) Hanson, K. R.; Rose, I. A. *Acc. Chem. Res.* **1975**, *8*, 1–10.

0022-3263/90/1955-5173$02.50/0

in reactions catalyzed by the cytochromes P-450.[4] Among the α-keto acid dependent dioxygenases,[5] prolyl hydroxylase and γ-butyrobetaine hydroxylase have been shown to catalyze hydroxylations with overall retention of configuration at carbon.[6,7] These enzymes are typical three-substrate α-keto acid dioxygenases: both utilize α-ketoglutarate and oxygen as cosubstrates for the hydroxylation of methylene groups. More unusual are mammalian KIC dioxygenase and the better known 4-(hydroxyphenyl)pyruvate dioxygenase (which has been isolated from numerous sources[8]), the only α-keto acid dependent dioxygenases for which a single substrate molecule contains both the α-keto acid and the carbon to be hydroxylated. The latter enzyme catalyzes the hydroxylation of an aromatic ring, so there is no overt stereochemical issue in the oxygen transfer, but KIC dioxygenase catalyzes the hydroxylation of an aliphatic methine group, and we report herein the overall stereochemical course of this oxygenation.

To determine the stereochemical course of the KIC dioxygenase catalyzed aliphatic hydroxylation, both a chiral substrate and a chiral product of known configuration are required. Unfortunately, both the normal substrate (1) of KIC dioxygenase and the hydroxylated product (2) are achiral. The desymmetrization of compounds 1 and 2 could be accomplished by isotopic substitution of one of the methyl groups in each, but the preparation of enantiomerically pure samples of the isotopically labeled species would be a formidable task. However, in a preliminary study, 4-methyl-2-oxohexanoic acid, a chiral homologue of 1, was observed to be a good substrate for KIC dioxygenase, and the preparation of an enantiomerically pure sample of this alternate substrate, (*S*)-4-methyl-2-oxohexanoic acid (3), proved to be a relatively simple matter.



In a conventional α-amino acid synthesis, diethyl acetamidomalonate was alkylated with commercially available (*S*)-(+)-1-bromo-2-methylbutane, and the product diester 5 was in turn hydrolyzed to form (2*RS*,4*S*)-2-amino-4-methylhexanoic acid (6). Aerobic incubation of 6 with D- and L-amino acid oxidases gave the desired α-keto acid 3 in good yield. Obviously, the configuration at carbon 4 of 3 is unchanged from that in the starting material.

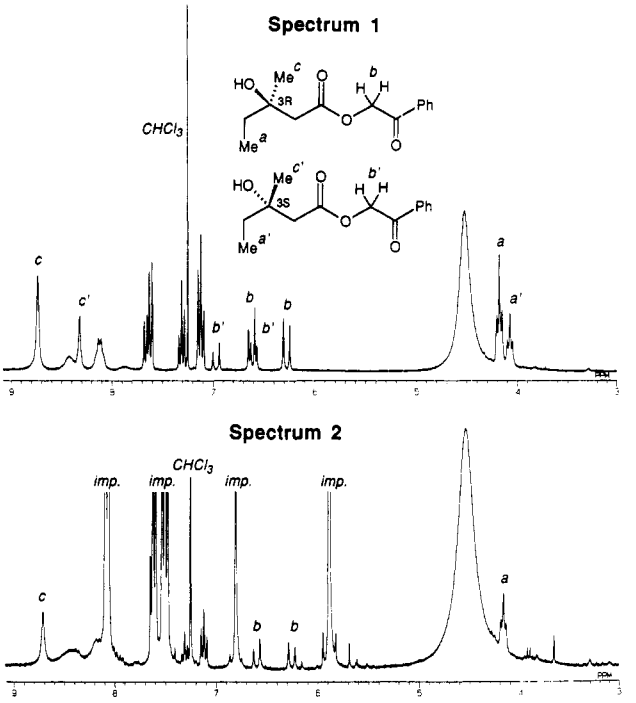
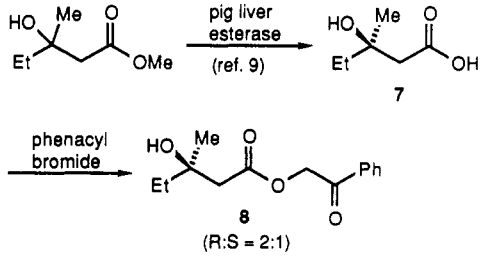


Figure 1. Partial ^{1}H NMR spectra of the phenacyl esters of samples of 3-hydroxy-3-methylpentanoate obtained by porcine liver esterase catalyzed hydrolysis of racemic methyl 3-hydroxy-3-methylpentanoate (spectrum 1) and by KIC dioxygenase catalyzed oxygenation of (*S*)-2-oxo-4-methylhexanoic acid (spectrum 2). The spectra were recorded at 300 MHz in deuteriochloroform containing the chiral shift reagent (−)-Eu(hfc)$_3$.

The preparation of an enantiomerically enriched sample of the expected product of the enzymatic oxygenation of 3, 3-hydroxy-3-methylpentanoic acid, was facilitated by the prior observation of Wilson et al.,[9] who reported that porcine liver esterase preferentially (but not exclusively) hydrolyzes the *R* isomer of methyl 3-hydroxy-3-methylpentanoate. Treatment of the racemic hydroxy ester with the commercial esterase under the reported conditions gave acid 7, whose absolute configuration had been determined by Wilson et al.[9] This material was converted to its phenacyl ester 8, and the optical purity of 8 was analyzed



by ^{1}H NMR spectroscopy in the presence of the chiral shift reagent tris[3-[(heptafluoropropyl)hydroxymethylene]-(−)-camphorato]europium(III) [(−)-Eu(hfc)$_3$]. A portion of this ^{1}H NMR spectrum is illustrated as spectrum 1 of Figure 1, and a 2:1 ratio of *R* and *S* enantiomers is clearly evident.

The enantiomerically pure α-keto acid 3 is oxidized by KIC dioxygenase at approximately 50% of the rate of the normal substrate 1. The reaction product 4 was isolated

(4) (a) Groves, J. T.; McClusky, G. A.; White, R. E.; Coon, M. J. *Biochem. Biophys. Res. Commun.* **1978**, *81*, 154–160. (b) Gelb, M.; Heimbrook, D. C.; Malkonen, P.; Sligar, S. G. *Biochemistry*, **1982**, *21*, 370–377.

(5) Abbott, M. T.; Udenfriend, S. α-Ketoglutarate-Coupled Dioxygenases. In *Molecular Mechanisms of Oxygen Activation*; Hayaishi, O., Ed.; Academic Press: New York, 1974; pp 167–214.

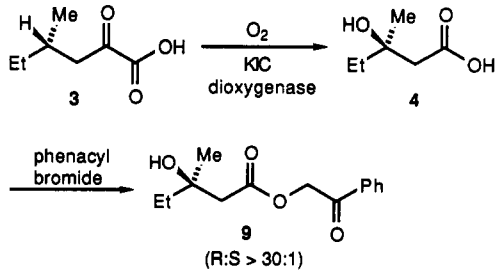
(6) Fujita, Y.; Gootlieb, A.; Peterkofsky, B.; Udenfriend, S.; Witkop, B. *J. Am. Chem. Soc.* **1964**, *86*, 4709–4716.

(7) Englard, S.; Blanchard, J. S.; Midelfort, C. F. *Biochemistry* **1985**, *24*, 1110–1116.

(8) (a) Goodwin, S.; Witkop, B. *J. Am. Chem. Soc.* **1957**, *79*, 179–185. (b) Wada, G. H.; Fellman, J. H.; Fujita, T. S.; Roth, E. S. *J. Biol. Chem.* **1975**, *250*, 6720–6726. (c) Nakai, C.; Nozaki, M.; Hayaishi, O. *Biochem. Biophys. Res. Commun.* **1975**, *67*, 590–595. (d) Lindblad, B.; Lindstedt, G.; Lindstedt, S.; Rundgren, M. *J. Biol. Chem.* **1977**, *252*, 5073–5084. (e) Lindstedt, S.; Odelhog, B.; Rundgren, M. *Biochemistry* **1977**, *16*, 3369–3377. (f) Leinberger, R.; Hull, W. E.; Simon, H.; Retey, J. *Eur. J. Biochem.* **1981**, *117*, 311–318. (g) Roche, P. A.; Moorehead, T. J.; Hamilton, G. A. *Arch. Biochem. Biophys.* **1982**, *216*, 62–73.

(9) Wilson, W. K.; Baca, S. B.; Barber, Y. J.; Scallen, T. J.; Morrow, C. J. *J. Org. Chem.* **1983**, *48*, 3960–3966.

and converted to its phenacyl ester 9. The ^1H NMR



spectrum of this material in the presence of (–)-Eu(hfc)$_3$ is spectrum 2 of Figure 1. (The several large impurity peaks in the spectrum are due to phenacyl maleate from the enzymatic reaction buffer, but they do not interfere with the analysis.) Only the *R* enantiomer is observed, and the *S* isomer cannot be present in more than 1 part in 30, if at all.

The conversion of the 4*S* isomer **3** to the 3*R* isomer **4** corresponds to *overall retention of configuration* at carbon for the KIC dioxygenase catalyzed hydroxylation reaction. Because the complementary result [oxidation of (*R*)-2-oxo-4-methylhexanoic acid to (*S*)-3-hydroxy-3-methylpentanoic acid] has not been demonstrated, we cannot discriminate between stereospecific and stereoselective hydroxylation processes, but the latter possibility must be considered unlikely.

Experimental Section

Enzymes and Assays. Porcine liver esterase, D-amino acid oxidase, L-amino acid oxidase, and catalase were purchased from Sigma Chemical Co. α-Ketoisocaproate (KIC) dioxygenase was purified from rabbit liver by using the same procedure reported by Sabourin and Bieber for the purification of the same enzyme from rat liver.[1] The purified protein was judged to be >90% pure and to have a monomer molecular weight of 45 000 by sodium dodecyl sulfate polyacrylamide gel electrophoresis. K_M for 1 was 0.22 mM; V_{max} was estimated to be 0.8 nmol of 1 oxidized to 2/min per mg of enzyme at 25 °C.

In the present work, KIC dioxygenase was assayed by gas chromatographic determination of the hydroxylated product β-hydroxyisovalerate (**2**). The standard assay mixture contained 0.2 M Tris, 0.2 M maleate, 1 mM $FeSO_4$, 0.5 mM ascorbate, 1 mM dithiothreitol, and 0.5 mg of KIC dioxygenase in a total volume of 0.4 mL at pH 6.0, and it was preincubated for 45 min at 25 °C. The reaction was initiated by addition of the substrate **1**, and the solution was shaken in air at 25 °C. The reaction was terminated by addition of 10 μL of a solution of 2,2′-bipyridyl (0.5 M in ethanol), the mixture was applied to a small column of Dowex 50X8 (H^+), and it was eluted with distilled water (1.6 mL). The eluent was neutralized with 10% NaOH (33 μL), a solution of phenacyl bromide (15 mg) in acetone (2 mL) was added, and the reaction mixture was refluxed for 2 h. The acetone was removed by rotary evaporation, and the remaining aqueous phase was extracted with ether. The ether extract was dried over sodium sulfate, and the residue was dissolved in 200 μL of ethyl acetate. This mixture of phenacyl esters was analyzed by gas chromatography on a capillary column [25 m × 0.53 mm, BP1 stationary phase (Scientific Glass Engineering, Inc.)]. An essentially identical method was used to assay the enzymatic oxidation of alternate substrate **3** to product **4**.

Ethyl (*S*)-2-Acetamido-2-(ethoxycarbonyl)-4-methylhexanoate (5). Diethyl acetamidomalonate (2.50 g, 11.5 mmol) was added to a solution of sodium (0.27 g, 11.7 mmol) in ethanol (100 mL). After 5 min (*S*)-(+)-1-bromo-2-methylbutane (2.0 g, 13.2 mmol; Aldrich Chemical Co.) was added, and the solution was refluxed for 24 h. After cooling, the reaction mixture was acidified with 1 N HCl, poured into water, and extracted with chloroform. The organic extract was dried and concentrated, and the resulting brown oil was purified by silica gel column chromatography (7:3 chloroform–ether). Concentration of the appropriate fractions gave compound **5** as a pale yellow solid: 2.57 g, 9.0 mmol, 78% yield; mp 48–50 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.81 (d, *J* = 6 Hz, 3 H, 4-methyl), 0.81 (t, *J* = 7 Hz, 3 H, terminal methyl), 1.24 [overlapping t (*J* = 7 Hz) and m; 9 H total; ethoxy methyls, 4-H, and 5-H_2], 2.02 (s, 3 H, acetyl methyl), 2.16 (dd, *J* = 15, 7 Hz, 1 H, 3-H), 2.44 (dd, *J* = 15, 4 Hz, 1 H, 3-H), 4.23 (m, 4 H, ethoxy methylenes), 6.83 (br s, 1 H, NH); MS, *m/z* 287 (M^+, 0.3%), 242 (6, M – OEt), 214 (89, M – CO_2Et), 172 (100, M – OEt – CO_2Et); exact mass 287.1723, calcd for $C_{14}H_{25}NO_5$ 287.1733.

(2*RS*,4*S*)-2-Amino-4-methylhexanoic Acid Hydrochloride (6). Diester **5** (1.00 g, 3.48 mmol) was refluxed in 6 N HCl overnight. Amino acid hydrochloride **6** crystallized upon concentration of the reaction mixture: 0.48 g, 2.64 mmol, 76% yield; mp 225–230 °C (sublimation); ^1H NMR (270 MHz, D_2O, DSS reference) δ 0.87 (t, *J* = 7 Hz, 3 H, terminal methyl), 0.94 (d, *J* = 6 Hz, 3 H, 4-methyl), 1.2–2.0 (overlapping m's, 5 H), 4.04 (m, 1 H, α-H); ^{13}C NMR (75.5 MHz, D_2O, dioxane reference; pairs of resonances resulting from the presence of the two diastereomers are bracketed) δ [10.8, 11.1], [18.4, 18.9], [28.9, 29.6], [30.9, 31.0], [37.6, 37.8], 52.3, 173.8; MS, *m/z* 146 (M – Cl [the protonated amino acid], 4%), 100 (M – HCl – CO_2H, 100), 74 (83, M – HCl – C_5H_{11}); exact mass (M – Cl ion) 146.1185, calcd for $C_7H_{16}NO_2$ 146.1181.

(*S*)-2-Oxo-4-methylhexanoic Acid (3). Compound **6** (0.48 g, 2.64 mmol) was dissolved in 50 mL of Tris buffer (0.2 M, pH 8.0) which also contained D-amino acid oxidase (5 mg), L-amino acid oxidase (3 mg), and catalase (2 mg), and the solution was stirred overnight at 25 °C. The reaction mixture was acidified and then extracted three times with ether. The combined organic extracts were dried and concentrated to give compound **3** as a colorless oil: 0.26 g, 1.81 mmol, 68% yield; ^1H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, *J* = 7 Hz, 3 H, terminal methyl), 0.90 (d, *J* = 7 Hz, 3 H, 4-methyl), 1.30 (m, 2 H, 5-H_2), 1.97 (m, 1 H, 4-H), 2.69 (dd, *J* = 17, 8 Hz, 1 H, 3-H), 2.87 (dd, *J* = 17, 6 Hz, 3-H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 11.2, 19.2, 29.4, 30.7, 44.5, 161.2, 195.6; MS, *m/z* 144 (M^+, 1.4%), 118 (17), 99 (79), 83 (100); exact mass 144.0778, calcd for $C_7H_{12}O_3$ 144.0786.

Phenacyl Ester of (*R*)-3-Hydroxy-3-methylpentanoic Acid Produced by Hydrolysis of Racemic Methyl 3-Hydroxy-3-methylpentanoate by Porcine Liver Esterase. Wilson et al. have previously established that porcine liver esterase preferentially hydrolyzes the *R* isomer of methyl 3-hydroxy-3-methylpentanoate.[9] In the present work, a solution of 3-hydroxy-3-methylpentanoate (0.37 g, 2.53 mmol) and porcine liver esterase (2.9 mg) in 1 M potassium phosphate buffer (5.8 mL, pH 8.9) was stirred at room temperature for 9 h. The reaction mixture was adjusted to pH 9 with dilute NaOH, and the reaction mixture was extracted with ether (to remove unhydrolyzed ester). The reaction mixture was then adjusted to pH 2 with 1 N HCl, and it was extracted with ether (to obtain the free acids). This extract was dried and concentrated. The residue was treated with a solution of phenacyl bromide (0.2 g) and triethylamine (0.2 mL) in acetone (5 mL), and this solution was refluxed for 2 h. The solvent was evaporated, and the residue was purified by preparative thin-layer chromatography (silica gel GF, Analtech Uniplate Taper Plates, chloroform solvent) to give the phenacyl ester of 3-hydroxy-3-methylpentanoic acid, which proved to be an approximately 2:1 mixture of the *R* and *S* isomers as judged by ^1H NMR in the presence of the chiral shift reagent (–)-Eu(hfc)$_3$ (Aldrich) (see Figure 1): ^1H NMR (270 MHz, $CDCl_3$) (no shift reagent) δ 0.96 (t, *J* = 7 Hz, 3 H, terminal methyl), 1.29 (s, 3 H, 3-methyl), 1.63 (q, *J* = 7, 2 H, 4-H_2), 2.58 (d, *J* = 14 Hz, 1 H, 2-H), 2.67 (d, *J* = 14 Hz, 1 H, 2-H), 3.61 (s, 1 H, OH), 5.38 (d, *J* = 17 Hz, 1 H, phenacyl methylene), 5.42 (d, *J* = 17 Hz, 1 H, phenacyl methylene), 7.46 (t, *J* = 7 Hz, 2 H), 7.60 (t, *J* = 7 Hz, 1 H), 7.89 (d, *J* = 7 Hz, 2 H); MS, *m/z* 235 (M – CH_3, 17%), 233 (M – OH, 9), 221 (M – CH_2CH_3, 46), 178 (38), 137 (93), 128 (76), 105 (100); exact mass (for M – CH_3 ion) 235.0978, calcd for $C_{13}H_{15}O_4$ 235.0970.

Phenacyl Ester of (*R*)-3-Hydroxy-3-methylpentanoic Acid Produced by Oxidation of Compound 3 by KIC Dioxygenase. A reaction mixture containing 0.2 M Tris, 0.2 M maleate, 1 mM $FeSO_4$, 0.5 mM ascorbate, 1 mM dithiothreitol, and 15.5 mg of KIC dioxygenase in a total volume of 1.8 mL at pH 6.0 was preincubated for 1 h at 25 °C. The reaction was initiated by addition of compound **3** (to a concentration of 0.5 mM, 0.13 mg

total), and the solution was stirred for 12 h at 25 °C. The reaction mixture was applied to a small column of Dowex 50X8 (H^+), and it was eluted with distilled water (2.5 mL). The eluent was neutralized with 10% NaOH (67 μL), a solution of phenacyl bromide (43.4 mg) in acetone (2.5 mL) was added, and the reaction mixture was refluxed for 2 h. The acetone was removed by rotary evaporation, and the remaining aqueous phase was extracted with ether. The ether extract was dried and concentrated, and the residue was purified by preparative thin-layer chromatography (silica gel GF, Analtech Uniplate Taper Plates, chloroform solvent). A band with R_f 4.5–5.5 was scraped off the plate and eluted with chloroform to yield a mixture of the phenacyl esters of 3-hydroxy-3-methylpentanoic acid and maleic acid. The former compound proved to be exclusively the *R* isomer as judged by ^1H NMR in the presence of the chiral shift reagent (-)-Eu(hfc)$_3$ (see Figure 1).

Acknowledgment. This work was supported by National Institutes of Health Grants GM31801 and AI24146 and by an Alfred P. Sloan Research Fellowship (to R.A.P.).

Supplementary Material Available: NMR spectra of compounds **3**, **5**, **6**, and **8** (5 pages). Ordering information is given on any current masthead page.

Calix[4]arenes Bridged at the Lower Rim

Jan-Dirk van Loon,† Dagmar Kraft,‡ Marc J. K. Ankoné,† Willem Verboom,† Sybolt Harkema,§ Walter Vogt,‡ Volker Böhmer,*,‡ and David N. Reinhoudt*,†

Laboratories of Organic Chemistry and Chemical Physics, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands, and Institute of Organic Chemistry, Johannes Gutenberg University, D-6500 Mainz, West Germany

Received March 1, 1990

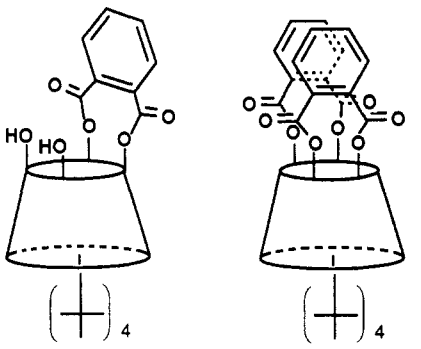
Calix[4]arenes[1] are building blocks constructed from phenols and formaldehyde like cyclodextrins are built from glucose units. An important method to discriminate between the sugar units of cyclodextrins is to use capping reagents of different size.[2] Depending on the shape of the bridge, selective A,C or A,D capping can be accomplished. In calixarene chemistry only a limited number of bridged calix[4]arenes are known. Two examples exist of calix[4]arenes with a bridge at the upper rim.[3,4] At the lower rim *p-tert*-butylcalix[4]arene has been bridged with a poly(oxyethylene) chain (calix crowns),[5,6] with a terphenyl unit (calix spherands),[6] and with a ferrocene unit.[7]

Our objective was to cap *p-tert*-butylcalix[4]arene (**1**) at the lower rim with bridges of different shape and rigidity to be able to discriminate between the different phenolic hydroxyl groups and to fix the calix[4]arene in a rigid cone conformation. Such a discrimination is a prerequisite for the potential use of calix[4]arenes as building blocks for larger synthetic (receptor) systems.

Results and Discussion

CPK models suggested that benzophenone-3,3′-disulfonyl dichloride (**2a**), which is used in cyclodextrin chemistry for selective A,C capping, should also be a suitable bridging reagent for calix[4]arenes. Indeed, we found that slow addition of a solution of **2a** in THF to a solution of the dipotassium salt of *p-tert*-butylcalix[4]arene (**1**) in THF resulted in the formation of the desired intrabridged compound **3a** in 28% isolated yield (Scheme I). However, in addition to 20% of starting material, a second product was isolated in 9% yield, which was identified as the doubly interbridged bis(calix[4]arene) **4a** by positive ion FAB mass spectrometry, consisting of two calix[4]arene units connected by two benzophenone-3,3′-disulfonyl bridges. The ^1H NMR spectra of both compounds are very similar, showing two singlets for the *tert*-butyl groups, an AB system for the methylene bridge protons, and two singlets for the aromatic protons of the calix[4]arene, which was typical for a diametrically disubstituted calix[4]arene in a rigid cone conformation.

Chart I



3f **5**

In order to study the effect of the nature of the bridge on the formation of mono- and bis(calix[4]arenes) we used the two capping units **2b** and **2c**, which have a structure similar to the benzophenone-3,3′-disulfonyl dichloride bridge (**2a**). (For structures and yields see Table I.) With use of diphenyl sulfone 3,3′-disulfonyl dichloride (**2b**) as a capping reagent, the overall yield dropped but the relative amount of bis(calix[4]arene) **4b** increased significantly. In the case of benzophenone-3,3′-dicarboxylic acid dichloride (**2c**) only the singly intrabridged calix[4]arene **3c** was isolated. A rationalization of the above results may be given in terms of the shape of the bridging unit. Probably the ring-closure reaction in the case of capping reagent **2b** is less efficient due to a nonoptimal fit, and therefore relatively more bis(calix[4]arene) is formed.

The 3,3′-substituted biphenyls **2d** and **2e** have a good fit, but they are more rigid than compounds **2a**–**2c**; therefore, only singly intrabridged calix[4]arenes **3d** and **3e** were isolated. In the case of 3,3′-bis(bromomethyl)-biphenyl (**2e**) the reaction conditions were changed to K_2CO_3/CH_3CN because with KOtBu/THF no bridged products could be isolated.

† Laboratory of Organic Chemistry, University of Twente.
‡ Johannes Gutenberg University.
§ Laboratory of Chemical Physics, University of Twente.

(1) For a review see: Gutsche, C. D. *Calixarenes*; Stoddart, F. J., Ed.; Monographs in Supramolecular Chemistry; The Royal Society of Chemistry: Cambridge, 1989; Vol. 1.
(2) Tabushi, I.; Shimokawa, K.; Fujita, K. *Tetrahedron Lett.* **1977**, 1527. Tabushi, I.; Kuroda, Y.; Yokota, K.; Chi Yuan, L. *J. Am. Chem. Soc.* **1981**, *103*, 711. Tabushi, I.; Chi Yuan, L.; Shimokawa, K.; Yokota, K.; Mizutani, T.; Kuroda, Y. *Tetrahedron Lett.* **1981**, *22*, 2273. Tabushi, I.; Nabeshima, T.; Kitaguchi, H.; Yamamura, K. *J. Am. Chem. Soc.* **1982**, *104*, 2017. Tabushi, I.; Yamamura, K.; Nabeshima, T. *J. Am. Chem. Soc.* **1984**, *106*, 5267. Tabushi, I.; Nabeshima, T.; Fujita, K.; Matsunaga, A.; Imoto, T. *J. Org. Chem.* **1985**, *50*, 2638.
(3) Goldmann, H.; Vogt, W.; Paulus, E.; Böhmer, V. *J. Am. Chem. Soc.* **1988**, *110*, 6811.
(4) To be published.
(5) Alfieri, G.; Dradi, E.; Pochini, A.; Ungaro, R.; Andreetti, G. D. *J. Chem. Soc., Chem. Commun.* **1983**, 1075.
(6) Dijkstra, P. J.; Brunink, J. A. J.; Bugge, K.-E.; Reinhoudt, D. N.; Harkema, S.; Ungaro, R.; Ugozzoli, F.; Ghidini, E. *J. Am. Chem. Soc.* **1989**, *111*, 7567.
(7) Beer, P. D.; Keefe, A. D. *J. Inclusion Phenom.* **1987**, *5*, 499.

0022-3263/90/1955-5176$02.50/0