Steric Course of N^5,N^{10}-Methylenetetrahydrofolate Formation from Glycine by the Glycine Cleavage System in *E. coli*†

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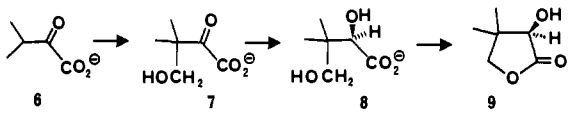
Abstract: The steric course of the conversion of glycine to 5,10-methylenetetrahydrofolate (5,10-CH_2-H_4folate) by the glycine cleavage system in *E. coli* has been elucidated. 2-H_{re} of glycine is the precursor of 11-H_{re} of 5,10-CH_2-H_4folate, and 2-H_{si} of glycine is the precursor of 11-H_{si} of 5,10-CH_2-H_4folate. The overall transformation is at least 78% stereospecific (possibly higher).

The catabolism of glycine via the glycine cleavage system (Scheme I) constitutes one of the principal biochemical sources of single-carbon units in a wide variety of organisms.[1] In this process, glycine is reversibly cleaved to form CO_2, NH_3, NADH + H^+, and N^5,N^{10}-methylenetetrahydrofolate (5,10-CH_2-H_4folate). Both C-2 hydrogens of glycine are retained in the overall transfer of the methylene group from glycine to tetrahydrofolate (H_4folate) to form 5,10-CH_2-H_4folate.[2] The methylene group transfer takes place in two steps involving three proteins. In step one, catalyzed by *P-protein*, a pyridoxal phosphate dependent decarboxylation of glycine (**1**) occurs, with transfer of an aminomethyl group to one of the sulfurs of lipoic acid, itself in turn bound to a relatively small *H-protein*[3] (**2**), to form **3**. A similar process can be catalyzed by serine hydroxymethyltransferase.[4] In step two, *T-protein*, an H_4folate-dependent enzyme, catalyzes the transfer of the methylene group with loss of ammonia to form 5,10-CH_2-H_4folate (**4**), releasing *H-protein* which now bears a reduced lipoic acid unit (**5**). In the final step of this cycle, the NAD^+-dependent *L-protein* catalyzes the reoxidation of the reduced lipoic acid unit.[5,6] We now present evidence which demonstrates the overall steric course of the methylene group transfer from glycine to form 5,10-CH_2-H_4folate by this enzyme system in *E. coli*.

Results

In the accompanying paper,[7] we report the determination of the steric course of the methylene group transfer catalyzed by the 5,10-CH_2-H_4folate-dependent enzyme ketopantoate hydroxymethyltransferase (KHMT) in the formation of ketopantoate (**7**)



6 **7** **8** **9**

from α-ketoisovaleric acid (α-KIVA) (**6**) in *E. coli*. After the in vivo reduction of ketopantoate by ketopantoate reductase, (2*R*)-pantoate (**8**) is formed which is subsequently isolated as (2*R*)-pantolactone (**9**). Thus, in principle, the steric course of formation of 5,10-CH_2-H_4folate from glycine could be elucidated by determining the C-4 labeling pattern of pantolactone resulting from the incubation of stereospecifically labeled glycine.

In preliminary experiments, [2-^{14}C]glycine (admixed with unlabeled DL-valine) was incubated with *E. coli* ATCC 23783. After addition of calcium pantothenate as carrier, followed by acidic hydrolysis, (2*R*)-pantolactone (**9**) was isolated in 1.5% radiochemical yield, the dilution of isotope being ca. 1:22. Thus, as in the accompanying work,[7] it appeared that we could easily use deuterium labeling in conjunction with deuterium NMR for our stereochemical studies.

Parallel incorporations of glycine-2,2-d_2 plus [2-^{14}C]glycine, admixed with either DL-valine or α-KIVA as coprecursor, revealed a substantial advantage (3.2% radiochemical yield, 1:15 dilution with α-KIVA) in using α-KIVA instead of valine, thereby bypassing the preliminary transamination step. Therefore, all subsequent incubations, both in this and in the accompanying work, were carried out with α-KIVA as coprecursor.

Table I. Incorporation of [2-^{3}H,2-^{14}C]Glycines into Pantolactone in *E. coli*

glycine labeling stereochemistry	^{3}H/^{14}C ratio: incubated glycine	^{3}H/^{14}C ratio: isolated pantolactone	% ^{3}H retention
2*RS*	10.7	5.02	47
2*R*	11.8	9.2	78
2*S*	11.7	2.2	19

The ^{2}H NMR in benzene solution of the pantolactone isolated after the above incubation (Figure 1A) showed two peaks, δ 3.23 and 3.33, corresponding in chemical shift to the C-4 protons of pantolactone. Thus, deuterium from C-2 of glycine was specifically incorporated only into C-4 of pantolactone. However, the observed 2:1 peak ratio contrasted with our observation of two equal peaks in the product obtained from DL-serine-3,3-d_2[7] and indicated a partial loss of some of the C-2 deuterium of glycine at some stage in the transformation to pantolactone.[8,9] The mass spectrum of this product indicated the presence of both pantolactone-d_2 (4%) and pantolactone-d_1 (13%). Since the glycine cleavage system has been reported to proceed without loss of glycine C-2 hydrogens,[4] it was apparent that some extraneous process was responsible for this partial washout of the deuterium. Since our experiments with serine[7] suggested that negligible losses of deuterium occurred in the formation from serine of 5,10-CH_2-H_4folate or its conversion to ketopantoate, the observed deuterium loss must have resulted from the interaction of glycine with other enzymes active in *E. coli*. Several enzymes are known to stereospecifically remove glycine C-2 hydrogens. Besmer and Arigoni[10] have reported that

† This paper is dedicated to Professor P. de Mayo on the occasion of his 60th birthday.

(1) (a) Hampson, R. K.; Barron, L. L.; Olson, M. S. *J. Biol. Chem.* **1983**, *258*, 2993–2999. (b) Fujiwara, K.; Motokawa, Y. *Ibid.* **1983**, *258*, 8156–8162. (c) Fujiwara, K.; Okamura, K.; Motokawa, Y. *Arch. Biochem. Biophys.* **1979**, *197*, 454–462.

(2) Motokawa, Y.; Kikuchi, G. *Arch. Biochem. Biophys.* **1974**, *164*, 624–633, 634–640.

(3) H-Protein can be replaced by lipoic acid alone, or for the reverse reaction, by dihydrolipoic acid. Kochi, H.; Kikuchi, G. *Arch. Biochem. Biophys.* **1976**, *173*, 71–81.

(4) Zieske, L. R.; Davis, L. *J. Biol. Chem.* **1983**, *258*, 10355–10359.

(5) Proteins P, H, L, and T are also referred to as P_1, P_2, P_3, and P_4.[6].

(6) Klein, S. M.; Sagers, R. D. *J. Biol. Chem.* **1967**, *242*, 297–300, 301–305; **1966**, *241*, 197–205, 206–209.

(7) Aberhart, D. J.; Russell, D. J. *J. Am. Chem. Soc.*, preceding paper in this issue.

(8) In a total of seven conversions of glycine-d_2 to pantolactone in this work, including the large scale (10 × 1 L) run used to prepared deuterated pantolactone for degradation, the ratio of ^{2}H peaks was always the same (±10%). Although we did not explore the effects of changing incubation conditions on the peak ratio, in one additional run with a modified precursor ratio (glycine/α-KIVA = 3/1 instead of 1/1, weight basis), the signal intensity ratio was unchanged.

(9) The presence of two deuterium signals in these spectra is not the result of partial racemization of pantolactone at C-2. The methyl signals of (2*R*)- and (2*S*)-pantolactone are well separated in the presence of Eu(hfbc)$_3$, and the biosynthetic product did not show the presence of any (2*S*)-pantolactone.

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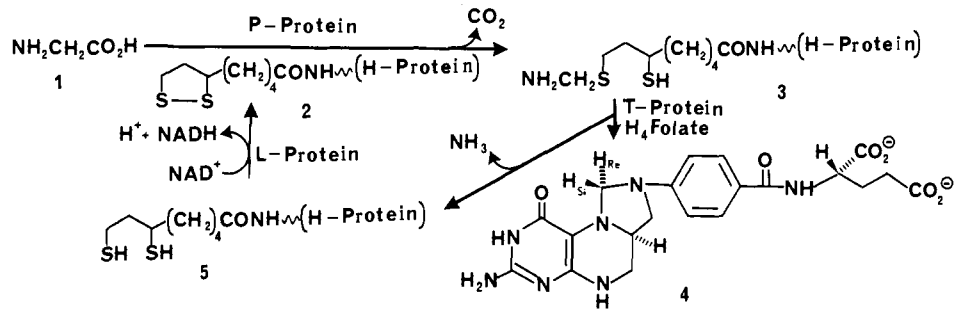
Scheme I. Glycine Cleavage System



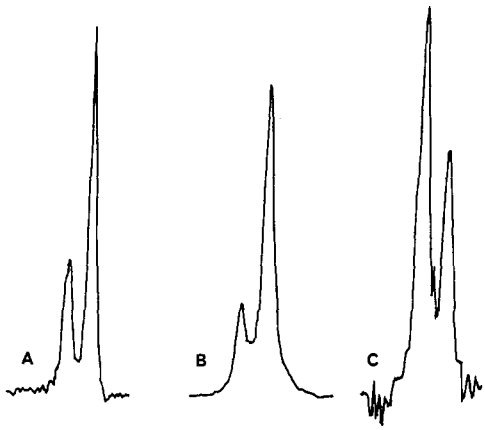
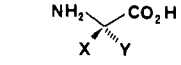


Figure 1. ^{1}H-Decoupled ^{2}H NMR spectra: (A) pantolactone (ca. 1:2 mixture of **17** (one part) and **18** (two parts), plus a small amount of pantolactone-4,4-d_2); (B) pantolactone (ca. 22:78 mixture of **17** to **18**, respectively) biosynthesized from (2*R*)-glycine-d_1 (**10c**); (C) pantolactone (ca. 62:38 mixture of **17** to **18**, respectively) biosynthesized from (2*S*)-glycine-d_1 (**10d**). Conditions for part A: 76.77 MHz, SW 2000 Hz, LB zero, PW 55 μs, repetition rate 2.048 s, Hz/pt 0.488, RD zero. Conditions for parts B and C: 38.40 MHz, SW 1000 Hz, LB zero, PW 25.0 μs, repetition rate 2.048 s, Hz/pt 0.488, RD 1.0 s.

L-alanine aminotransferase catalyzes the stereospecific exchange of the 2-H_{re} atom[11] of glycine with solvent hydrogens. On the other hand, Jordan and Akhtar[12] reported that serine hydroxymethyltransferase (SHMT) labilizes the 2-H_{si} atom of glycine.

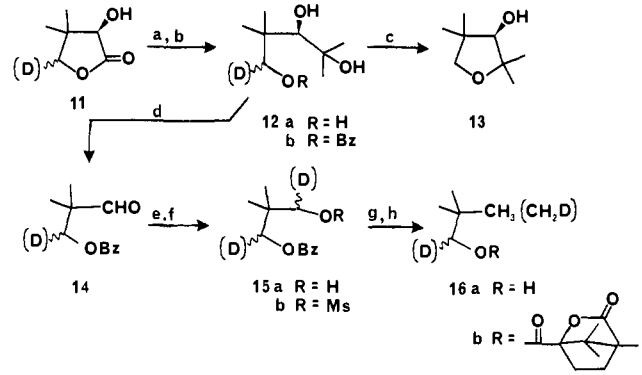
To evaluate which hydrogen(s) of glycine was (were) undergoing exchange in our incubations, (2*R*)- and (2*S*)-[^{3}H$_1$]glycines **10a** and **10b** were prepared by incubation of (2*RS*)-[^{3}H]glycine



10 a X = T, Y = H
b X = H, Y = T
c X = D, Y = H
d X = H, Y = D

with alanine aminotransferase in H_2O or by incubation of unlabeled glycine with alanine aminotransferase in ^3H_2O, respectively.[10,13] These products, and also (2*RS*)-[^{3}H]glycine, admixed with [2-^{14}C]glycine, were incubated with *E. coli*. After the usual workup,[7] the resultant pantolactone was isolated and recrystallized to constant activity. The results (Table I) indicated the predominant, but not exclusive, loss of the 2-H_{si} tritium of glycine.

Scheme II. Degradation of Biosynthetic Pantolactone-4-d_1 (**11**) to Neopentanol (-)-Camphanate Ester (16b)[a]



[a] Reagents: a, CH_3MgBr; b, BzCl/pyridine; c, heat; d, $NaIO_4$; e, $NaBH_4$; f, MsCl/pyridine; g, $LiAlH_4$; h, (-)-camphanyl chloride/pyridine.

Although the losses of deuterium from 2-H_{re} or 2-H_{si} might be somewhat different from those of tritium as a consequence of different isotope effects, it was probable that we had, fortuitously, biosynthesized predominately (2*R*)-glycine-d_1 in vivo from glycine-d_2. Thus, the stereochemistry of the overall process could be elucidated from the deuterium NMR spectrum of the derived pantolactone, provided that the C-4(H) signal assignment of pantolactone in C_6H_6 solution could be secured.

This signal assignment was secured by degradation (Scheme II) of asymmetrically deuterated pantolactone (**11**), biosynthesized as above from glycine-d_2, into neopentanol-1-d_1 (**16a**),[14] whose configuration at C-1 could be unambiguously assigned. In order to obtain enough deuterated pantolactone for degradative purposes, the biosynthesis was repeated on a large scale (10 × 1 L cultures), from which 650 mg of deuterated pantolactone was isolated after workup. The ^{2}H NMR of this product showed the usual 2:1 deuterium peak distribution, as in Figure 1A. Of this material, 600 mg was mixed with additional unlabeled D-pantolactone (400 mg) as carrier, and the mixture was then treated with methylmagnesium bromide[15] (3.3 equiv) to give (2*R*)-1,1,3,3-tetramethyl-1,2,4-butanetriol (**12a**). On attempted Kugelrohr distillation of an unlabeled sample of **12a**, it underwent cyclization to (3*R*)-2,2,4,4-tetramethyltetrahydrofuran-3-ol[16] (**13**). Benzoylation of **12a** cleanly afforded the primary monobenzoate (**12b**). This product showed, in the ^{1}H NMR spectrum, a well-spaced AB pattern, δ_A 3.93, δ_B 4.45 for the C-4 protons. The ^{2}H NMR also showed two broad signals at these positions in a ca. 2:1 ratio (downfield signal more intense), thus showing retention of the deuterium labeling asymmetry to this point. The benzoate (**12b**) was treated with excess $NaIO_4$, and ther resultant 2,2-dimethylhydracraldehyde benzoate[17] (**14**) was reduced with $NaBH_4$

(10) Besmer, P.; Arigoni, D. *Chimia* **1968**, *22*, 494. See also: Wellner, D. *Biochemistry* **1970**, *9*, 2307–2310 and Palekar, A. G.; Tate, S. S.; Meister, A. *Ibid.* **1970**, *9*, 2310–2315.

(11) Nomenclature: Prelog, V.; Helmchen, G. *Helv. Chim. Acta* **1972**, *55*, 2581–2598. Rêtey, J.; Robinson, J. A. "Stereospecificity in Organic Chemistry and Enzymology"; Verlag Chemie: Weinheim, 1982; pp 1–16.

(12) Jordan, P. M.; Akhtar, M. *Biochem. J.* **1970**, *116*, 277–286.

(13) Armarego et al. have issued a cautionary note that the chiral purity of enzymatically prepared chiral glycines should be confirmed in each case: Armarego, W. F.; Milloy, B. A.; Pendergast, W. *J. Chem. Soc., Perkin Trans. 1* **1976**, 2229–2237. Although we did not confirm the chiral purity of these tritiated glycines, our subsequent preparation of chiral deuterated glycines confirmed that the method used leads to stereospecifically labeled glycines.

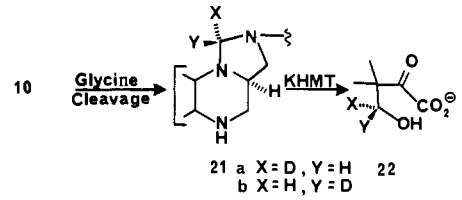
(14) The structures in Scheme II are shown as d_1 compounds, but in fact some d_2 materials are also present as shown by the mass spectrum.

(15) Stiller, E. T.; Keresztesy, J. C.; Finkelstein, J. *J. Am. Chem. Soc.* **1940**, *62*, 1779–1785.

(16) McConnell, W. V.; Moore, W. H. *J. Org. Chem.* **1963**, *28*, 822–827.

(17) Merger, F.; Fuchs, W.; Dockner, T. German Patent 2000511; *Chem. Abstr.* **1971**, *75*, 88113*w*.

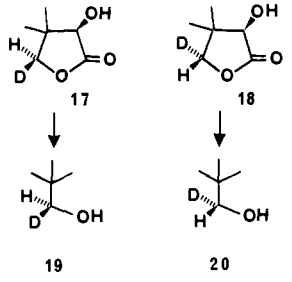
Scheme III. Steric Course of Ketopantoate Hydroxymethyltransferase (KHMT) As Determined in Ref 7[a]



[a] Although the results of the accompanying paper do not establish that the KHMT reaction is completely stereospecific, the predominant steric course of the reaction is as shown.

to the alcohol[18] (**15a**). The reduction was accompanied by complete ester interchange between the two oxygen-bearing carbons, as established by the ^2H NMR spectrum which showed two peaks of equal intensity at δ 3.35 and 4.17. The alcohol (**15a**) was converted to the mesylate (**15b**), which was reduced with $LiAlH_4$ to a mixture of neopentanol (**16a**) and benzyl alcohol. The mixture was partially separated by Kugelrohr distillation. Then the crude neopentanol (**16a**) was converted to the (−)-camphanate ester (**16b**) accompanied by benzyl (−)-camphanate, the latter of which was easily removed by preparative TLC. The ^1H NMR spectrum of **16b** was then obtained in the presence of $Eu(hfbc)_3$ (which gave better resolution of the C-1 proton signals than did $Eu(fod)_3$), which shifted the C-1 proton signals to an AB pattern, δ_A 5.68 and δ_B 5.99. The ^2H NMR showed broadened peaks at the same positions in a ratio of ca. 2:1, the *upfield* peak being the more intense. The spectrum also showed a sharper peak at δ 1.29 (*tert*-butyl; relative area 3). The results established that the C-4 deuterium labeling asymmetry originally present in the biosynthetic product was preserved throughout the degradative sequence. It was possible a priori that intramolecular participation processes, especially in the mesylate displacement step, could have resulted in complete racemization at the labeled center, but this clearly did not occur. On the other hand, it is inconceivable that such processes could invert the original configuration.

It has been reported by Gerlach and Zagalak[19] that 1-H_{si} of (−)-camphanate esters of primary alcohols is more strongly shifted by $Eu(dpm)_3$ than is 1-H_{re} (and we have found that this also applies with $Eu(hfbc)_3$[20]). Furthermore the (−)-camphanate ester of authentic (1*S*)-neopentanol-1-d_1, prepared by Midland's method,[21] exhibited this same shifting behavior. We therefore conclude that the (mainly) monodeuterated neopentanol (**16a**) obtained in this degradative sequence consisted of a mixture of ca. 1 part of **19** to 2 parts of **20**.[22] It follows that the biosynthetic



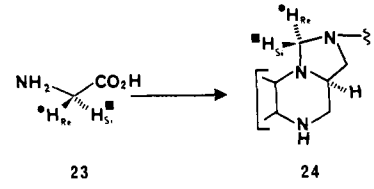
pantolactone was labeled as in **17** (1 part) and **18** (2 parts) (plus a small amount of pantolactone-4,4-d_2).

Since the steric course of the KHMT-catalyzed reaction is now known[7] (*subject, as previously discussed,[7] to the assumption that serine hydroxymethyltransferase in liver and in E. coli have the same overall steric courses*); Scheme III, **21** → **22**, we may tentatively conclude that deuterated glycine, formed in vivo from glycine-d_2 and consisting predominantly of (2*R*)-glycine-d_1 (**10c**) plus a lesser amount of (2*S*)-glycine-d_1 (**10d**), was metabolized by the glycine cleavage system to form a mixture of labeled 5,10-$C^2H_1{}^1H_1$-H_4folates **21a** (two parts) plus **21b** (one part).

To put this assignment of steric course on a more secure basis, stereospecifically deuterated glycines (2*R*)-glycine-d_1 (**10c**) and (2*S*)-glycine-d_1 (**10d**) were prepared by the method of Besmer and Arigoni,[10] previously used to prepare **10a** and **10b**. A portion of each product was converted as described[13] to the methyl ester *N*-(−)-camphanamide derivative. Each derivative, in the presence of $Eu(dpm)_3$, showed a single ^1H and ^2H resonance for the C-2 proton (deuteron). Thus, the exchange reactions, when allowed to proceed for a sufficient length of time,[23] went to completion and were completely stereospecific.

The products were then incubated with *E. coli*, and the resultant pantolactones were examined by ^2H NMR (Figure 1B,C). Qualitatively the results were in accord with expectations based on the tentatively deduced steric course above. However, both products, particularly that derived from (2*S*)-glycine-d_1 (**10d**) (Figure 1C), showed some nonstereospecificity in the overall process. Probably this nonstereospecificity is the result of the action of the SHMT present in the *E. coli* causing partial racemization of the 5,10-$C^2H_1{}^1H_1$-H_4folate which is, in turn, being produced stereospecifically, or nearly so, from glycine. The result obtained in the incubation of (**10c**) (Figure 1B) shows that the overall process must be at least 78% stereospecific.

Thus, in summary, our results show that the methylene group transfer in the overall transformation of glycine to 5,10-CH_2-H_4folate by the glycine cleavage system proceeds at least partially (≥78%) stereospecifically. 2-H_{re} of glycine becomes 11-H_{re} of 5,10-CH_2H_4folate and 2-H_{si} of glycine becomes 11-H_{si} of 5,10-CH_2-H_4folate, **23** → **24**. Further studies with isolated enzymes



with be necessary to ascertain whether, in fact, the process is completely stereospecific. A further interesting challenge, under study in our laboratory, will be to attempt to elucidate the steric courses of the two individual steps (Scheme I) in this process.

Experimental Section

General. See the accompanying paper[7] for instrumentation used and for procedures used for carrying out incubations of labeled substrates with *E coli* and for isolating pantolactone. Some ^2H NMR spectra were run at 38.40 MHz on a Bruker WM-250 instrument. Glycine-d_5 (used to prepared glycine-d_2 by exchange with H_2O) was obtained from MSD Isotopes. [2-^{14}C]Glycine was obtained from ICN. [2-^{3}H]Glycine was obtained from New England Nuclear. [^{3}H]H_2O (5 mCi/mL) was obtained from Amersham. Alanine aminotransferase (glutamic–pyruvic transaminase) was obtained from Sigma.

Degradation of Pantolactone (11) Derived Biosynthetically from Glycine-*2,2*-d_2. Pantolactone was biosynthesized from glycine-d_2 as previously described.[7] The mass spectrum of the biosynthetic product had m/z 71 (M − CO_2 − CH_3) (relative intensity 100), 72 (24.8 ± 1.0), 73 (9.2 ± 0.3); unlabeled pantolactone had m/Z 71 (100), 72 (9.2 ± 1.2), 73 (4.3 ± 0.6).

Biosynthetic pantolactone (**11**) (600 mg) was mixed with unlabeled D-pantolactone (400 mg) (total 7.68 mmol) and treated in ether (50 mL)

(18) Duke, R. B.; Perry, M. A. French Patent 1 414 216; *Chem. Abstr.* **1966**, *64*, 11090*d*.
(19) Gerlach, H.; Zagalak, B. *J. Chem. Soc., Chem. Commun.* **1973**, 274–276.
(20) Aberhart, D. J.; Gould, S. J.; Lin, H.-J.; Thiruvengadam, T. K.; Weiller, B. H. *J. Am. Chem. Soc.* **1983**, *105*, 5161–5170.
(21) Midland, M. M.; Greer, S.; Tramontano, A.; Zderic, S. A. *J. Am. Chem. Soc.* **1979**, *101*, 2352–2355.
(22) The derived pantolactone C-4(H) signal assignment is consistent with a closer approach of the benzene to the face of the ring remote from the hydroxyl group, thus effecting a stronger upfield shift of 4-H_{re} (of (2*R*)-pantolactone) through the ring current effect.
(23) After equilibration of glycine-d_2 in H_2O (25 mL) and pyridoxal phosphate (0.4 mg) with alanine aminotransferase (400 units) at 37 °C for 3 days, followed by derivatization, the presence of a small amount (10–15%) of unexchanged 2-H_{re} could be easily detected by ^2H NMR, although this impurity was not apparent from the ^1H NMR. However, after prolonged equilibration (10 days), no trace of the 2-H_{re} deuteron was detectable.

under N_2 at 0 °C with CH_3MgBr (2.9 M, 9 mL, 25.4 mmol) added over 15 min. The resultant gummy mass was broken up with a spatula, stirred at 25 °C for 30 min, and then refluxed for 1 h. After the material was cooled to 0 °C, H_2O (100 mL) was added, slowly at first. The mixture was then acidified (HCl), saturated with NaCl, and extracted continuously overnight with ether. Evaporation of the ether under reduced pressure gave **(2*R*)-1,1,3,3-tetramethyl-1,2,4-butanetriol**[15] (**12a**), as a viscous oil, 1.37 g, which was not further purified: NMR ($CDCl_3$) δ 0.97 (3 H, s), 1.03 (3 H, s), 1.33 (6 H, sl br s), 3.1–3.9 (6 H, m).

On attempted Kugelrohr distillation of an unlabeled sample of **12a** at ca. 100–110 °C (1 mmHg), it was converted into **(2*R*)-tetrahydro-2,2,4,4-tetramethyl-3-furanol**[16] (**13**): NMR ($CDCl_3$) δ 1.03 (3 H, s), 1.1 (3 H, s), 1.20 (3 H, s), 1.30 (3 H, s), 2.64 (1 H, s, D_2O exchangeable), 3.45 and 3.58 (2 H, AB, J_{AB} = 9 Hz), 3.55 (1 H, s); $[\alpha]^{25}_D$ +4° (*c* 2.2, $CHCl_3$).

The triol (**12a**) (1.35 g, 8.33 mmol) was dissolved in pyridine (20 mL) and treated at 25 °C with benzoyl chloride (1.14 g, 1 equiv). The mixture was then stirred at 60 °C for 3 h. By use of a standard workup, **(2R)-1,1,3,3-tetramethyl-1,2,4-butanetriol 1-benzoate** (**12b**), 1.94 g, was isolated as a viscous oil: ^1H NMR ($CDCl_3$), δ 1.13 (3 H, s), 1.20 (3 H, s), 1.37 (6 H, s), 2.97 (1 H, br s, $W_{1/2}$ = 5 Hz, –OH), 3.34 (1 H, s), 3.93 and 4.45 (2 H, AB, J_{AB} = 11 Hz), 7.2–8.1 (5 H, m). An unlabeled sample of **12b** was purified by preparative TLC (solvent, 30% EtOAc/hexane) and then Kugelrohr distilled at 200 °C (1 mmHg).

Anal. Calcd for $C_{15}H_{22}O_4$: C, 67.64; H, 8.33. Found: C, 67.82; H, 8.46.

The above product (**12b**) (1.8 g, 6.77 mmol) in MeOH (40 mL) was treated at 25 °C with $NaIO_4$ (1.85 g, 1.2 equiv) in H_2O (10 mL) for 65 h. The mixture was filtered, and the MeOH was evaporated under reduced pressure. Water (50 mL) was added, and the mixture was extracted with ether (3 × 50 mL). The extract was washed with saturated NaCl, dried (Na_2SO_4), and evaporated under reduced pressure to yield **2,2-dimethylhydracraldehyde benzoate**[17] (**14**), 1.38 g, as an oil: NMR ($CDCl_3$) δ 1.21 (6 H, s), 4.32 (2 H, s), 7.2–7.9 (5 H, m), 9.52 (1 H, s).

Aldehyde (**14**) (1.33 g, 6.46 mmol) in absolute EtOH (50 mL) at 25 °C was treated with $NaBH_4$ (0.65 g) for 1 h. $(NH_4)_2SO_4$ (1 g) and H_2O (15 mL) were added, the mixture was filtered, and the EtOH was evaporated under reduced pressure. Water (50 mL) was added and the mixture was extracted with ether (3 × 50 mL). The extract was washed with H_2O and saturated NaCl, dried (Na_2SO_4), and evaporated to give **2,2-dimethyl-1,3-propanediol 1-benzoate**[18] (**15a**) as an oil, 1.13 g: ^1H NMR ($CDCl_3$) δ 1.01 (6 H, s), 2.33 (1 H, br s, $W_{1/2}$ = 12 Hz, –OH), 3.35 (2 H, br s, $W_{1/2}$ = 8 Hz); ^2H NMR ($CHCl_3$) δ 3.35, 4.17 (equal intensities)

The product (**15a**) (1.10 g, 5.29 mmol) in CH_2Cl_2 (40 mL) at 25 °C was treated with CH_3SO_2Cl (667 mg, 5.82 mmol), and the solution was then cooled to –78 °C. Triethylamine (589 mg, 5.82 mmol) was added dropwise, and the mixture was allowed to warm spontaneously to room temperature and then stirred for 2 h. The mixture was then poured into cold H_2O (25 mL), and the CH_2Cl_2 phase was separated, dried (Na_2SO_4), and evaporated to yield the mesylate (**15b**) as an oil, 1.5 g: NMR ($CDCl_3$) δ 1.11 (6 H, s), 2.93 (3 H, s), 4.02 (2 H, s), 4.10 (2 H, s), 7.2–8.1 (5 H, m); several unidentified minor impurities were visible as well as some unreacted starting material (25%).

The crude product (1.4 g) in ether (50 mL) was treated with $LiAlH_4$ (600 mg) at reflux under N_2 for 48 h. The mixture was cooled and then treated dropwise with saturated Na_2SO_4, and the resultant white precipitate was filtered. The filtrate was evaporated under reduced pressure at ca. 25 °C to an oil, 790 mg. GLC analysis (3% SE-30, initially at 40 °C programmed to 90 °C at 5 °C/min) showed a mixture of benzyl alcohol (ca. 70%) and neopentanol (**16a**) (ca. 30%). The latter component was concentrated by bulb to bulb distillation at ca. 130 °C, giving 410 mg still containing substantial quantities (ca. 40%) of benzyl alcohol. The mixture was dissolved in pyridine (7 mL) and treated with (–)-camphanyl chloride[19] (1.0 g) at 70 °C for 90 min. After the mixture cooled to 25 °C, H_2O (50 mL) was added, and the mixture was extracted with ether (3 × 50 mL). The ether extract was washed with diluted HCl, diluted K_2CO_3, and saturated NaCl, dried (Na_2SO_4), and evaporated under reduced pressure to give a crystalline product consisting of a nearly equal mixture of the (–)-camphanate esters of benzyl alcohol and neopentanol. Preparative TLC (silica gel $HF_{254+366}$ (Merck); solvent, 10% EtOAc/hexane) was then used to isolate the neopentanol (–)-camphanate (**16b**), 103 mg, needles from MeOH, mp 66–67 °C: ^1H NMR ($CDCl_3$) δ 1.0 (12 H, s), 1.08 (3 H, s), 1.11 (3 H, s), 1.5–2.8 (4 H, m), 3.88 (2 H, s). An unlabeled sample of **16b** was directly prepared from neopentanol.

Anal. Calcd for $C_{15}H_{24}O_4$: C, 67.14; H, 9.01. Found: C, 67.27; H, 9.13.

Also the (–)-camphanate ester of (1*S*)-neopentanol-1-d_1[21] (**19**) was similarly prepared. Its ^1H NMR was as above except that the signal at δ 3.88 was reduced to 1 H.

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