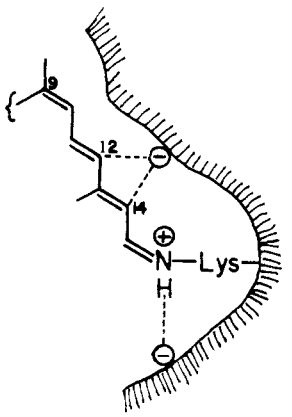

Figure 2. External point-charge model for the binding site of bovine rhodopsin. The dotted lines represent interactions over distances of ~3 Å. The negative charge near C-12/C-14 could be a member of a charge pair in a salt bridge or the negative end of a dipolar group.

then with 9-*cis*-retinal; comparisons of the absorptions at 410 vs. 485 nm (for 9-*cis*-rhodopsin, Table II) showed that formation of 9-*cis*-rhodopsin was ~3% for both **2b** and **2c**. Furthermore, submission of the hexane-washed pellet to the CH_2Cl_2-denaturation extraction procedure[9] regenerated the original retinal, thus proving that the chromophore had not changed upon binding. The chromophores **2a** and **2d** with 9-trans bonds failed to give pigments.

The 9-cis and 9,13-dicis adamantyl allenic retinals, despite their cage structures, thus bind to bovine opsin. This result proves the earlier deduction[1] that the β-ionone binding site is quite lenient. However, there is considerable difference in terms of stability between the cyclohexyl (Ch) and adamantyl (Ad) series, the latter being much less stable. Cyclohexyl-9-*cis*-rhodopsin underwent only 3, 10, and 20% decomposition when kept in 0.5% digitonin,[10] 1.5% Triton X-100, and 2% A-LO,[11] respectively, for 1 h at 25 °C in the dark. In contrast, adamantyl-9-*cis*-rhodopsin was completely destroyed after 2 h in digitonin, after 35 min in Triton, and immediately in A-LO. It is also not stable in 0.1 M hydroxylamine (Triton, pH 7.0). However, the stability of the pigment in Triton X-100 is not affected by change in pH from 5.2 to 8.3. In the form of pellets, adamantyl-9-*cis*-rhodopsin could be stored for at least 1 month without appreciable decomposition if kept at −20 °C, but 50% was destroyed after 3 h at 25 °C.

The data in Table II point to several interesting aspects. The maxima of trans aldehydes **1a** and **2a** are similar to that of *all-trans*-5,6-dihydroretinal (356) and are red shifted in comparison with *all-trans*-7,8-dihydroretinal (320) as expected. The 9-cis chromophores are at ~8 nm shorter wavelengths than the trans chromophores in retinals **9** and **10**, presumably owing to steric interaction between 8-H and 11-H. This difference in the cyclohexyl analogue is only 2 nm, but in the adamantyl analogue it is much larger, i.e., 20 nm. Examination of CPK molecular models allows one to suggest the following to account for these results. In **1b**, the 9-ene is locked into conjugation with the allenic 7-ene owing to steric hindrance between the 9-Me and the annular equatorial Me groups despite the steric interaction between 8-H/11-H. In contrast, in **2b**, which has no ring Me groups, the 8–9 single bond is free to rotate, and hence the 8-H/11-H interaction moves the 9-ene considerably out of conjugation with the allenic 7-ene. The λ_{max} (335 nm) thus falls between those of 5,6-dihydro- and 7,8-dihydroretinals, **10** (347 nm) and **11** (321 nm); a similar tendency is seen in the three protonated Schiff bases, i.e., 398 nm vs. 425 and 392 nm.

The instability of the adamantyl allenic pigment relative to the cyclohexyl allenic pigment is probably due to the spheric shape of the adamantyl moiety. It is to be noted that the 410-nm absorption maximum of the adamantyl pigment is only 12 nm red shifted from the SBH^+ value, and is at a shorter wavelength even than that of 7,8-dihydrorhodopsin. We have proposed[12,13] that external charges within the binding site are responsible for wavelength regulation in visual pigments. Namely, in the external point-charge model for bovine rhodopsins (Figure 2), the point-charge located at ~3 Å from C-12/C-14 is responsible for the red shifts of bovine visual pigments relative to their SBH^+. The short-wavelength absorption of the adamantyl allenic pigment implies that in the present case C-12/C-14 are farther away from this point charge.

Acknowledgments. We thank the National Institutes of Health, Grant EY 01253, and Hoffmann-La Roche for financial support.

References and Notes

(1) Nakanishi, K.; Yudd, A. P.; Crouch, R. K.; Olson, G. L.; Cheung, H.-C.; Govindjee, R.; Ebrey, T. G.; Patel, D. J. *J. Am. Chem. Soc.* **1976**, *98*, 236.
(2) Binding studies with model retinals have in general been confined to 9-cis isomers because opsin is known to bind readily to 9-*cis*-retinal and because of the relative ease of synthesis.
(3) Matsumoto, H.; Yoshizawa, T. *Nature (London)* **1975**, *258*, 523–526.
(4) A large excess of the reducing agent is required, unlike the original synthesis of cyclohexyl allenic retinal.[1] See Olson, G. L.; Cheung, H.-C.; Morgan, K. D.; Borer, R.; Saucy, G. *Helv. Chim. Acta* **1976**, *59*, 567–585.
(5) Still, W. C.; Khan, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
(6) Becker, R. S.; Inuzuka, K.; Balke, D. E. *J. Am. Chem. Soc.* **1971**, *93*, 38. Das, P. K.; Becker, R. S. *J. Phys. Chem.* **1978**, *82*, 2081.
(7) Gawinowicz, M. A.; Balogh-Nair, V.; Sabol, J. S.; Nakanishi, K. *J. Am. Chem. Soc.* **1977**, *99*, 7720.
(8) Arnaboldi, M.; Motto, M. G.; Tsujimoto, K.; Balogh-Nair, V.; Nakanishi, K. *J. Am. Chem. Soc.* **1979**, *101*, 7082–7084.
(9) Crouch, R.; Purvin, V.; Nakanishi, K.; Ebrey, T. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 1538. Pilkiewicz, F. G.; Pettei, M. J.; Yudd, A. P.; Nakanishi, K. *Exp. Eye Res.* **1979**, *24*, 421.
(10) Bridges, C. D. B. *Vision Res.* **1977**, *17*, 301.
(11) For a comparison of pigment properties in various detergents see the following: Waddell, W. H.; Yudd, A. P.; Nakanishi, K. *J. Am. Chem. Soc.* **1976**, *98*, 238–239.
(12) Honig, B.; Dinur, U.; Nakanishi, K.; Balogh-Nair, V.; Gawinowicz, M. A.; Arnaboldi, M.; Motto, M. G. *J. Am. Chem. Soc.* **1979**, *101*, 7084–7086.
(13) Sheves, M.; Nakanishi, K.; Honig, B. *J. Am. Chem. Soc.* **1979**, *101*, 7086–7088.

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Nonradical Intermediates in the Photolysis of Decacarbonyldimanganese

Sir:

Many metal–metal-bonded complexes are thought to react photochemically via homolytic cleavage of the metal–metal bond to form metal-centered radicals.[1] The following scheme is proposed[1a] for reaction of $Mn_2(CO)_{10}$ with carbon tetrachloride:[2]

$$Mn_2(CO)_{10} \overset{h\nu}{\rightleftharpoons} Mn_2(CO)_{10}^* \quad (1)$$

$$Mn_2(CO)_{10}^* \rightarrow 2Mn(CO)_5 \quad (2)$$

$$Mn(CO)_5 \xrightarrow[k_3]{CCl_4} ClMn(CO)_5 \quad (3)$$

$$2Mn(CO)_5 \xrightarrow[k_4]{} Mn_2(CO)_{10} \quad (4)$$

Kinetic studies fully consistent with such a scheme are conspicuously rare, almost all observations[1,3] being of limiting quantum yields that do not help to define the nature of the intermediates. Only when $[CCl_4]$ is low enough for reaction 4 to compete with 3 is kinetic evidence positively in favor of the

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Table I. Initial Quantum Yields for Loss of $Mn_2(CO)_{10}$ on Photolytic[a] Reaction with CCl_4 in Cyclohexane

I_a[b]	$[CCl_4]$[c]	ϕ_{obsd}[d]	Δ, %[e]	I_a[b]	$[CCl_4]$[c]	ϕ_{obsd}[d]	Δ, %[e]
3.35	1.5	0.329	8.9	8.23	5.0	0.379	3.3
4.62		0.313	9.4	13.1		0.336	−4.3
4.88		0.287	1.4	45.3		0.255	−12.7
9.55		0.251	0.8	45.8		0.283	−3.1
9.72		0.263	6.1	71.7		0.250	−7.1
12.9		0.237	0.9	55.7	1.5	0.145	−19.0
16.7		0.228	2.2	51.2	2.5	0.217	−0.9
27.7		0.202	−0.5	73.0		0.192	−6.3
29.8		0.190	−5.0	46.8	5.0	0.255	−12.1
40.2		0.181	−4.2	50.7	7.5	0.322	−1.5
52.8		0.170	−6.1	56.8	8.5	0.334	0.3
57.2		0.163	−8.4	56.8	10.0	0.359	3.5
77.8		0.171	0.6	52.7	20.0	0.386	−1.0
89.3		0.164	−1.2	54.5	40.0	0.436	7.9
94.2		0.187	13.3	53.7	80.0	0.410	0.2
1.77	5.0	0.408	2.5	55.0	160	0.412	0.5
4.97		0.370	−2.9				

[a] Irradiation with 436-nm light. [b] In units of 10^{-8} einstein L^{-1} s^{-1}. [c] In units of 10^{-4} mol L^{-1}. [d] In units of mol einstein^{-1}. [e] $100(\phi_{obsd} - \phi_{calcd})/\phi_{calcd}$.

scheme accessible.[3a] Provided that all of the solutions are homogeneous, the steady-state approximation leads to rate equation

$$\phi_{obsd} = \phi_{lr} - 4(k_4/k_3^2)\phi_{obsd}^2 I_a/[CCl_4]^2 \quad (5)$$

ϕ_{lr} is the limiting quantum yield for loss of $Mn_2(CO)_{10}$ when reaction 4 is negligible.

We have studied this reaction in cyclohexane over a range of values of I_a and $[CCl_4]$ (Table I) such that ϕ_{obsd} is generally less than the limiting value.[4] A plot of ϕ_{obsd} against $\phi_{obsd}^2 I_a/[CCl_4]^2$ (Figure 1) is not linear, however, there being a slower decrease of ϕ_{obsd} with increasing $I_a/[CCl_4]^2$ than expected. The data can be quantitatively analyzed if the excited species[5] $Mn_2(CO)_{10}$* can form, in addition to $Mn(CO)_5$, an isomer of $Mn_2(CO)_{10}$ that can also react with CCl_4 to form product:

$$Mn_2(CO)_{10}^* \rightarrow Mn_2(CO)_{10}^{\ddagger} \quad (6)$$

$$Mn_2(CO)_{10}^{\ddagger} \xrightarrow{CCl_4} 2ClMn(CO)_5 \quad (7)$$

Under the conditions used $Mn_2(CO)_{10}^{\ddagger}$ does not revert to $Mn_2(CO)_{10}$ and the quantum yield, ϕ_{lnr}, for reaction by this nonradical path is independent of $[CCl_4]$ and I_a. The rate equation for reactions 1–4 and 6–7 is

$$\phi_{obsd} = \phi_{lr} - 4(k_4/k_3^2)(\phi_{obsd} - \phi_{lnr})^2 I_a/[CCl_4]^2 + \phi_{lnr} \quad (8)$$

The line in Figure 1 was calculated with $\phi_{lr} = 0.30$ mol einstein^{-1}, $\phi_{lnr} = 0.11$ mol einstein^{-1}, and $k_3/k_4^{1/2} = 1.43$ $M^{-1/2}$ $s^{-1/2}$. The standard deviation, $\sigma(\phi_{obsd})$,[7] is 7.1% in comparison with 18.2% if all of the data are analyzed only in terms of the radical path. If the 19 data points with $\phi_{obsd}^2 I_a/[CCl_4]^2 < 0.3$ L einstein^{-1} s^{-1} are analyzed only in terms of the radical path, then a good linear plot of ϕ_{obsd} against $\phi_{obsd}^2 I_a/[CCl_4]^2$ is obtained with $\phi_{lr} = 0.41$ mol einstein^{-1}, $k_3/k_4^{1/2} = 2.13$ $M^{-1/2}$ $s^{-1/2}$, and $\sigma(\phi_{obsd}) = 7.3\%$. This corresponds to the initial slope of the curve in Figure 1. However, the 14 values of ϕ_{obsd} at $\phi_{obsd}^2 I_a/[CCl_4]^2 > 0.3$ L einstein^{-1} s^{-1} are all systematically higher (by 20–105%) than those predicted by these parameters. An additional, intensity-independent path such as the one shown in eq 6 and 7 must, therefore, exist, but its detection depends on obtaining data at sufficiently high values of $I_a/[CCl_4]^2$. This path accounts for ~25% of the limiting quantum yields, but at the highest values of $I_a/[CCl_4]^2$ it accounts for almost 70% of ϕ_{obsd}.

Meyer and co-workers[8] have shown that flash photolysis of

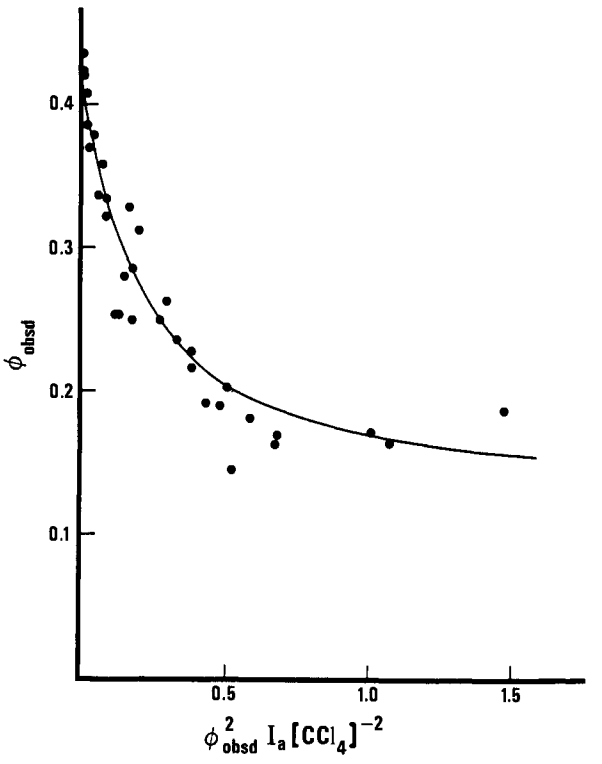
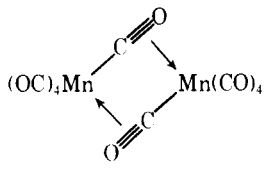


Figure 1. Dependence of ϕ_{obsd} (in mol einstein^{-1}) on $\phi_{obsd}^2 I_a/[CCl_4]^2$ (in L einstein^{-1} s^{-1}). The line is drawn with $\phi_{lr} = 0.30$ mol einstein^{-1}, $\phi_{lnr} = 0.11$ mol einstein, and $k_3/k_4^{1/2} = 1.43$ $M^{-1/2}$ $s^{-1/2}$.

$Mn_2(CO)_{10}$ in cyclohexane produces, in addition to $Mn(CO)_5$, a product that reverts to $Mn_2(CO)_{10}$ by a relatively slow first-order process. This species could be generated in detectable amounts by repeated flash photolysis and could well be the same as $Mn_2(CO)_{10}^{\ddagger}$. A possible formulation of $Mn_2(CO)_{10}^{\ddagger}$ is $(OC)_5Mn(\mu\text{-}CO)Mn(CO)_4$, a species that contains a bridging CO group but no Mn–Mn bond.[9] A close analogoue of this has been postulated[11] to be formed by photolysis of $Cp_2Fe_2(CO)_4$ (Cp = η^5-C_5H_5) in ethyl chloride or THF at −78 °C and it was suggested that this intermediate could account for the photolytic formation of $CpFe(CO)_2Cl$ in CCl_4. Photolytic reaction of $Cp_2Fe_2(CO)_4$ with CCl_4 in cyclohexane should then be intensity independent even at low enough values of $[CCl_4]$ for less-than-limiting quantum yields to be obtained. Such experiments remain to be done.[1f] Alter-

native formulations of $Mn_2(CO)_{10}^{\mp}$ can, of course, be postulated and I, for example, is by no means unlikely. Bridging



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carbonyls of the type shown have been characterized crystallographically[1,2] and the structure is analogous to the well-known $(OC)_4Mn(\mu\text{-}Cl)_2Mn(CO)_4$.

Intensity-dependence studies of such reactions are the exact counterparts of the studies of the dependence of initial rates on initial concentrations of complex that have played a definitive role in establishing the existence of reversible fragmentation pathways in thermal reactions of metal-metal-bonded carbonyls.[1,3] Their essential role in the study of photochemical reactions of metal carbonyl clusters is evident.

Acknowledgments. We thank Erindale College and the Natural Sciences and Engineering Research Council, Ottawa, for support of this research.

References and Notes

(1) (a) Wrighton, M. S.; Ginley, D. S. *J. Am. Chem. Soc.* **1975**, *97*, 2065. (b) Wrighton, M. S. *Top Curr. Chem.* **1976**, *65*, 37. (c) Huffadine, A. S.; Peake, B. M.; Robinson, B. H.; Simpson, J.; Dawson, P. A. *J. Organomet. Chem.* **1976**, *121*, 391. (d) Hudson, A.; Lappert, M. F.; Nicholson, B. K. *J. Chem. Soc., Dalton Trans.* **1977**, 551. (e) Abrahamson, H. B.; Wrighton, M. S. *J. Am. Chem. Soc.* **1977**, *99*, 5510. (f) Abrahamson, H. B.; Palazzotto, M. C.; Reichel, C. L.; Wrighton, M. S. *Ibid.* **1979**, *101*, 4123.

(2) The reversibility of equation 1 is indicated by the fact that limiting quantum yields for loss of the metal–metal-bonded carbonyls in such reactions are generally less than unity.[1,3] Participation of $Mn(CO)_4$ in the reaction scheme was not suggested[1a] and seems unlikely since none of the expected products, e.g., $Mn_2(CO)_8Cl_2$, was observed.

(3) (a) Laine, R. M.; Ford, P. C. *Inorg. Chem.* **1977**, *16*, 388. (b) Kidd, D. R.; Brown, T. L. *J. Am. Chem. Soc.* **1978**, *100*, 4095.

(4) Runs were carried out using standard techniques. Solutions in 3-cm^3 cuvettes were rapidly stirred; 20–50% of the incident light was absorbed. Great care was necessary to purify the solvent and to remove oxygen by several thorough freeze–pump–thaw cycles. Solvent was used only when the quantum yield for decomposition of $Mn_2(CO)_{10}$ in the solvent was <0.02 mol $einstein^{-1}$. The decrease in absorbance due to $Mn_2(CO)_{10}$ at 343 nm was monitored up to ~10% reaction. Yields of $Mn(CO)_5Cl$ were measured by infrared spectrophotometry. The quantum yields quoted in Table I are effectively initial quantum yields. Within the accuracy of the measurements, the quantum yields for formation of $Mn(CO)_5Cl$ were twice those for loss of $Mn_2(CO)_{10}$ over the whole range of conditions used. No evidence was obtained for direct formation of any other product.

(5) This is probably the lowest $d\pi\sigma^*$ state.[1a,6]

(6) Levenson, R. A.; Gray, H. B.; Ceasar, G. P. *J. Am. Chem. Soc.* **1970**, *92*, 3653.

(7) Values of $\sigma(\phi_{obsd})$ are standard deviations of individual measurements of ϕ_{obsd} estimated so that 95% confidence limits can be obtained by doubling them.

(8) Hughey, J. L., IV; Anderson, C. P.; Meyer, T. J. *J. Organomet. Chem.* **1977**, *125*, C49.

(9) Such intermediates have been invoked before[10] to explain features of thermal and photochemical reactions of metal–metal-bonded carbonyls.

(10) DeWit, D. G.; Fawcett, J. P.; Poë, A. J.; Twigg, M. V. *Coord. Chem. Rev.* **1972**, *8*, 81. Barrett, P. F.; Poë, A. J. *J. Chem. Soc. A* **1968**, 429. Barrett, P. F.; Sun, K. K. W. *Can. J. Chem.* **1970**, *48*, 3300. Barrett, P. F.; Clancy, R. R. *Ibid.* **1971**, *49*, 2627. Barrett, P. F.; Jacobs, W. J. *Ibid.* **1972**, *50*, 972. Barrett, P. F. *Ibid.* **1974**, *52*, 3773. Basato, M.; Poë, A. J. *J. Chem. Soc., Dalton Trans.* **1974**, 607.

(11) Tyler, D. R.; Schmidt, M. A.; Gray, H. B. *J. Am. Chem. Soc.* **1979**, *101*, 2753.

(12) Colton, R.; Commons, C. J. *Aust. J. Chem.* **1975**, 1673.

(13) Fawcett, J. P.; Poë, A. J.; Sharma, K. R. *J. Am. Chem. Soc.* **1976**, *98*, 1401. DeWit, D. G.; Fawcett, J. P.; Poë, A. J. *J. Chem. Soc., Dalton Trans.* **1976**, 528. Keeton, D. P.; Malik, S. K.; Poë, A. J. *Ibid.* **1977**, 1392. Chowdhury, D. M.; Poë, A. J.; Sharma, K. R. *Ibid.* **1977**, 2352. Fawcett, J. P.; Jackson, R. A.; Poë, A. J. *Ibid.* **1978**, 789. Fawcett, J. P.; Poë, A. J.; Sharma, K. R. *Ibid.*, **1979**, 1886.

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Stereochemical Analysis of the Homoserine Dehydrogenase Reaction and Preparation of Chiral 4-Deuteriohomoserines

Sir:

In the course of analyses of the stereochemical outcome of enzymic reactions at the γ carbon (C_4) of α-amino acids,[1] we have required a source of the physiological amino acid L-homoserine, stereospecifically deuterated at the prochiral C_4 alcoholic carbon. In this communication we report the preparation and the assignment of absolute stereochemistry to [4(*R*)- and [4(*S*)-^{2}H]-2(*S*)-homoserines from synthesis effected by homogeneous aspartokinase/homoserine dehydrogenase—a bifunctional enzyme from *E. coli*.[2] The useful activity for our purposes is the latter one, providing presumably chiral reduction of L-aspartate semialdehyde to L-homoserine at the expense of NADH oxidation. In turn this has required determination, reported here, of the previously unknown stereochemical outcome of this biosynthetic enzymic conversion of aldehyde to alcohol.

Two samples of [4-^{2}H]-L-homoserine were prepared enzymically. The first used [4(*S*)-^{2}H]-NADH[3] and L-aspartate semialdehyde with 5 units (5 μmol min^{-1} mg^{-1} of protein) of L-homoserine dehydrogenase[4] to yield 5.1 mg of pure [4-^{2}H]-L-homoserine (sample I) after LC purification (cation exchange, Whatman Partisil PXS 10/25 SCX). The second sample was obtained with unlabeled NADH and [4-^{2}H]-L-aspartate semialdehyde in a parallel incubation with analogous workup and a yield of 6.2 mg of [4-^{2}H]-L-homoserine (sample II). The [4-^{2}H]-L-aspartate semialdehyde was itself generated by ozonolysis of 4,5-dideuterio-L-allylglycine, in turn prepared by partial hydrogenation of L-propargylglycine with deuterium gas using Adams catalyst in ^2H_2O. The 4,5-dideuterio-L-allylglycine required rigorous purification from a small amount (5%) of unreacted acetylenic amino acid by LC, or else the ozonolysis products (presumably diketones) inhibit the homoserine dehydrogenase activity. The ozonolysis was carried out in 1 N $^2HCl/^2H_2O$ to yield, after enzymic aldehyde reduction, the anticipated [4-^{2}H]-L-homoserine (sample II).

That the two monodeuterio-L-homoserine samples did in fact have deuterium at the distinct (by virtue of isotopic substitution) diastereotopic methylene loci of carbon 4 was revealed by 270-MHz ^{1}H NMR as shown in Figure 1. The α and γ hydrogens have similar chemical shifts (α at 3.89 ppm and γ at 3.82 ppm). The spectrum of the γ hydrogens in nondeuterated L-homoserine appeared to be a triplet with secondary spliting into doublets by the α hydrogen. The coupling constants are 6.0 ($J_{\gamma\text{-}\beta}$) and 1.0 ($J_{\gamma\text{-}\alpha}$), respectively. The chemical shift of the γ hydrogens is 1031.4 Hz. The chemical shift of γ hydrogen of monodeuterio-L-homoserine (sample I) showed an upfield shift by 2.1 Hz, to 1029.3 Hz, from nondeuterated L-homoserine, and the other monodeuterio-L-homoserine (sample II) showed a further upfield shift to 1027.5 Hz. Line broadenings of the spectrum of γ hydrogens in sample I and sample II are due to deuterium coupling. Thus, *E. coli* homoserine dehydrogenase is stereospecific in reduction of the trigonal prochiral aldehyde group of L-aspartate semialdehyde. It remained then to determine absolute stereochemistry to assign upfield and downfield hydrogens in the NMR spectrum at γ (C_4) of the chiral [4-^{2}H]-2(*S*)-homoserine samples.

After some exploration we chose to degrade L-homoserine to 3-hydroxypropionate benzyl ester since it turned out to be an acceptable substrate for horse liver alcohol dehydrogenase, an enzyme known to remove only the *pro R* hydrogen from the oxidizable carbon of primary alcohols.[5] The degradation is shown in Scheme I. In the event we used a chiral [4-^{3}H]-L-homoserine sample **3** generated from homoserine dehydrogenase action on [4(*S*)-^{3}H]-NADH and L-aspartate semialdehyde, since we could then mix this species with [U-^{14}C]-

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