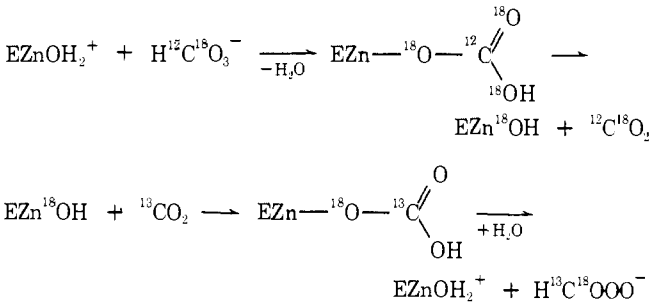
Scheme I



pattern very similar to Figure 2 is obtained using the buffers *N*-methylmorpholine at pH 7.5-7.8 and 2,4-lutidine at pH 6.9-7.3. Both θ_{cat} and ϕ_{cat} can be abolished by the carbonic anhydrase inhibitor ethoxzolamide at 10^{-7} *M*. Furthermore, no catalyzed type I or type II exchange can be observed using the apoenzyme of BCA at 1.6×10^{-9} *M*.

Figure 2 indicates that as the buffer increases θ_{cat} increases and ϕ_{cat} decreases proportionately. From the symmetry of these two curves and from the fact that they both measure a property of labeled oxygen, we conclude that the two exchange processes described by θ_{cat} and ϕ_{cat} are related. These characteristics are consistent with a scheme in which ^{18}O labels the active site, as postulated earlier.[8] Furthermore, this behavior combined with the results in Figure 1 suggests general features of the steps in the catalytic mechanism which involve proton transfer. Such a mechanism is presented in Scheme I, which shows ^{18}O bound to the zinc of the active site. Although there is no evidence from these experiments that bicarbonate forms an inner sphere complex with this metal, other experiments indicate that bicarbonate coordinates directly to zinc.[9,10] In the absence of added buffers there is a slow rate of protonation of $EZn^{18}OH$. Magnetic resonance relaxivity data[11,12] establish that the residence time of the proton on water or hydroxide bound to the metal in Co(II) or Mn(II) BCA is relatively long in a neutral or low pH region even in the presence of buffers. Consequently, this basic form of the labeled enzyme has a relatively long lifetime, increasing the likelihood that it reacts with CO_2 to form $HCOO^{18}O^-$. This step retains ^{18}O in the CO_2 system; that is, this is a step which does not exchange ^{18}O with water; it is a step which, if prevalent, would cause a low value of θ_{cat} and a high value of ϕ_{cat}. As buffer is added, the rate of proton transfer to the enzyme increases, and the rate of formation of $EZn^{18}OH_2^+$ increases. As shown in Scheme I, $^{18}OH_2$ is displaced from the active site by bicarbonate (also by hydroxide ion or certain other anions, or possibly by another water molecule), a step which results in the exchange of ^{18}O with water increasing θ_{cat} and, since ^{18}O is displaced from the active site, decreasing ϕ_{cat}. At higher buffer concentrations, the data show a change in rate-determining step; the maximum enzyme activity is reached and further buffer does not affect type I or II exchange. Consequently, a mechanism such as shown in Scheme I in which ^{18}O labels the active site and can exchange a proton with buffer is compatible with the data of Figure 2.

It is also pertinent to note in Figure 2 that the rate constant ϕ_{cat} is not abolished in solutions with larger buffer concentrations. Even with 50 m*M* imidazole at pH 7 the catalyzed type II exchange occurs. Apparently, under these conditions, the ^{18}O-labeled active site can react with CO_2 to give labeled bicarbonate at a rate which is still significant compared to the rate of equilibration of ^{18}O label with the solvent.

The importance of the data in Figure 2 then is to confirm the interpretation of θ_{cat} as indicative of a proton transfer step involving buffer and is to establish as a likely site of proton transfer the oxygen in the active site which is involved in catalytic hydration of CO_2. This need not be a direct proton transfer but may occur through intervening amino acid side chains and water bridges. Just as the proton transfer step can be rate determining in equilibrium oxygen exchange at low buffer concentration, we anticipate that this step will be rate determining in the *nonequilibrium* reaction at low buffer concentration. For example, in the catalytic dehydration, the catalysis will be limited by how rapidly the proton transfer can convert EZnOH into $EZnOH_2^+$. We consider these ^{18}O exchange experiments to be consistent with the hypothesis that the maximal activity of carbonic anhydrase-catalyzed hydration and dehydration of CO_2 is dependent on the presence of buffers capable of providing protons to or accepting protons from the carbonic anhydrase active site.

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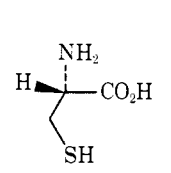
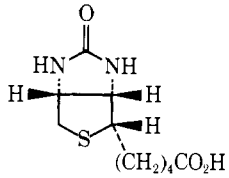
A Stereospecific Total Synthesis of *d*-Biotin from L-(+)-Cysteine

Sir:

We wish to record the total synthesis of d-biotin from its biogenetic precursor[1] L-(+)-cysteine[2] via a pathway which avoids a chemical resolution sequence characteristic of all previous syntheses.[3]



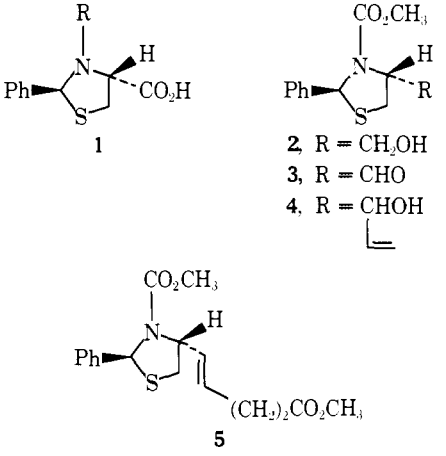
L-(+)-cysteine



d-biotin

To this end, L-(+)-cysteine was converted into (4*R*)-carboxy-(2*S*)-phenylthiazolidine (**1**, R = H),[4] mp 159-160°, $[\alpha]^{25}D$ −135.1 (*c* 1.02, DMSO), by condensation with benzaldehyde. The nitrogen atom was further protected by reaction with methyl chloroformate in aqueous base to yield the urethane **1** (R = CO_2CH_3), mp 129-130°, $[\alpha]^{25}D$

+122.8 (*c* 1.04, CH_3OH). Reduction with diborane in dry tetrahydrofuran at 25° afforded the alcohol **2**, mp 85-86°, $[\alpha]^{25}D$ +139.1 (*c* 1.08, acetone). Careful oxidation with chromium trioxide-pyridine produced the desired aldehyde **3** as a colorless oil, $[\alpha]^{25}D$ +151.7 (*c* 0.95, CH_3OH), in an overall yield of 70% based on L-(+)-cysteine. Reaction with excess vinyl magnesium chloride at −70° in methylene chloride produced the oily vinyl alcohol **4**, $[\alpha]^{25}D$ +101.6 (*c* 1.0, CH_3OH), which underwent a Claisen rearrangement at 85° in benzene with trimethyl orthoacetate in the presence of propionic acid as catalyst. The desired olefin **5**, $[\alpha]^{25}D$ +88.3 (*c* 1.0, $CHCl_3$), was isolated in 97% yield and found to be pure trans (NMR δ 5.62 (dd, 1, *J*(trans) = 16 and *J*(vic) = 6 Hz), 5.82 (dm, 1, *J*(trans) = 16 Hz)) without detectable contamination by the undesired cis isomer.

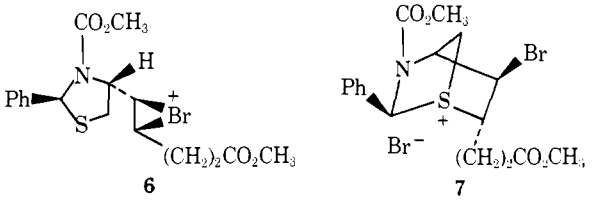


1

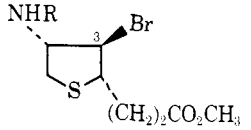
2, R = CH_2OH
3, R = CHO
4, R = CHOH

5

Bromination of the olefin **5** by pyridinium hydrobromide perbromide in methanol resulted in a dramatic and stereospecific rearrangement. Anchimeric participation by sulfur in order to stabilize the incipient bromonium ion **6** presumably yields the sulfonium cation **7**, which then expels benzaldehyde (isolated from the reaction mixture) to afford the tetrahydrothiophene **8**, mp 139-140°, $[\alpha]^{25}D$ −17.7 (*c* 1.04, CH_3OH), in 60% yield.[5] No other diastereomers were detected in the reaction mixture.[6] The urethane grouping was cleaved by hydrogen bromide in acetic acid (25°, 20 hr) to produce a quantitative yield of the amino bromide **9**, mp 160-161°, $[\alpha]^{25}D$ +18.2 (*c* 0.99, CH_3OH). Interestingly, the ester function was not affected by these conditions.



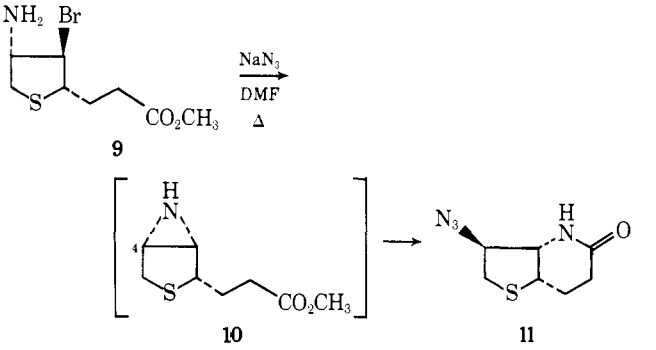
6 7



8, R = CO_2CH_3
9, R = H·HBr

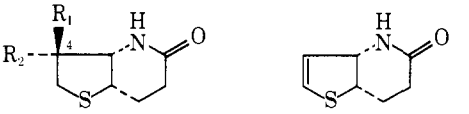
Attempts to directly displace the bromide of compound **9** at C_3 with excess sodium azide in DMF produced in 95% yield the undesired trans azide **11**, mp 174-175°, $[\alpha]^{25}D$ −100.0 (*c* 1.11, DMSO), arising from ring opening of the presumed aziridine intermediate **10** at C_4 followed by lactamization. The structure of the azide **11** was demonstrated unequivocably by an X-ray structure determination.

Systematic studies on this series of compounds, as well as



9 10 11

numerous results in model systems, forced the conclusion that the aziridine formation could not be avoided. We reasoned, therefore, that if the incoming nucleophile were to be subsequently a leaving group, a further SN2 displacement at C_4 would then afford the desired all cis stereochemistry. To this end the amino bromide **9** was heated at reflux in acetic acid for 3 hr to produce the trans bromolactam **12**, mp 208-209°, $[\alpha]^{25}D$ −90.7 (*c* 1.05, DMSO) in 92% yield. Treatment with lithium azide in dimethylformamide at 140° for 3.5 hr afforded the desired oily cis azido lactam **13**, $[\alpha]^{25}D$ −47.0 (*c* 0.99, CH_3OH), along with the dihydrothiophene **14**, a product of a competing E_2 elimination, in a ratio of 1:4 after chromatographic separation. Catalytic hydrogenation of **13** yielded the cis amino lactam **15**, mp 108-109°, $[\alpha]^{25}D$ −24.9 (*c* 0.49, CH_3OH), in quantitative yield.

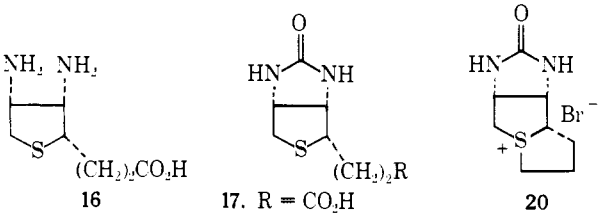


12, R_1 = Br; R_2 = H
13, R_1 = H; R_2 = N_3
15, R_1 = H; R_2 = NH_2

14

To complete the synthesis of *d*-biotin, **15** was hydrolyzed with aqueous barium hydroxide at reflux for 20 hr to the diamino acid **16**, which was immediately treated with gaseous phosgene in aqueous sodium bicarbonate at 25° to yield *d*-bisnorbiotin **17**, which was isolated as its methyl ester **18**, mp 163-164°, $[\alpha]^{25}D$ +55.68 (*c* 0.96, DMSO). An authentic sample of *d*-bisnorbiotin, itself a product of the microbiological degradation of biotin, was shown to be identical with our synthetic material.[7] Moreover, an independent synthesis[8] of optically pure *d*-bisnorbiotin methyl ester totally confirmed the structural assignments and verified that the optical purity of **18** was equal to that of the natural substance.

Reduction of **18** with lithium borohydride in refluxing tetrahydrofuran afforded *d*-bisnorbiotinol **19**, mp 189-190°, $[\alpha]^{25}D$ +60.29 (*c* 1.02, DMSO). Treatment with hydrogen bromide in acetic acid at reflux yielded the thiophanium bromide **20**, mp 221-222°, $[\alpha]^{25}D$ +14.30 (*c* 1.0,



16 17. R = CO_2H 20

18, R = CO_2CH_3
19. R = CH_2OH
21, R = $CH_2CH(CO_2Et)_2$
22. R = $CHCH_3(CO_2H)_2$

H_2O), in 80% overall yield from **18.** Addition of the two-carbon fragment was achieved by reaction of **20** with sodium diethyl malonate to yield the diester **21,** which was not isolated, but hydrolyzed by barium hydroxide to the corresponding diacid **22,** mp 190° dec. Decarboxylation occurred smoothly in hot water to give *d*-biotin, mp 232–233°, $[\alpha]^{25}D$ +91.2 (*c* 1.0, 0.1 *N* NaOH), identical in all respects with an authentic sample.

Acknowledgment. We would like to express our gratitude to the staff of the Physical Chemistry Department of Hoffmann-La Roche for their determination of spectral and analytical data.

References and Notes

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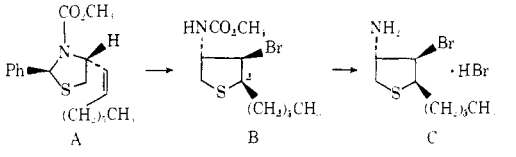
(4) All intermediates have the expected spectral properties and analytical data. These details will be reported in the full paper to follow.
(5) One of the referees suggested that this rearrangement is initiated by attack of bromine at sulfur. This plausible step would then be followed by a trans addition across the olefin functionality leading to the cation **7**.
(6) In a model study, the cis olefin A (NMR δ 5.58, 5.64 (m, 2, *J*(cis) = 6 Hz)) was brominated under similar conditions to give stereospecifically the epi isomer B, in which the alkyl substituent at C_2 is in the undesired β orientation. This was proven by an X-ray structure determination on the derived hydrobromide C.



This result formed the basis for the prediction that the oxidative cyclization on a trans olefin would afford an intermediate in which the chain at C_2 would be α as desired.
(7) We thank Professor D. B. McCormick, Cornell University, for supplying a sample of natural *d*-bisnorbiotin. Cf. D. B. McCormick, S. Iwahara, L. D. Wright, and H. C. Li, *J. Biol. Chem.,* **244,** 1393 (1969).
(8) This alternative route originates from an intermediate in the Sternbach biotin synthesis, U.S. Patent 2489235 (1949). Details will be given in the full paper.

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Nature of the Intermediates Involved in the [2 + 2] Cycloaddition of Tetracyanoethylene and Enol Ethers[1]

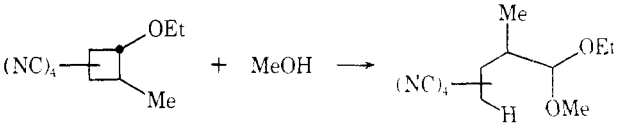
Sir:

In recent years overwhelming evidence has been published to support the proposition that the cycloaddition of tetracyanoethylene to enol ethers is a stepwise reaction. Thus, it has been found that the reaction is largely but not completely stereospecific in either direction,[2] that cis-trans isomerization of the enol ether accompanies the reaction,[3] and that an intermediate (with only one bond formed) can be intercepted by means of both alcohols[4] and 1,4-dipolarophiles.[5] It is furthermore clear that the intermediate has considerable zwitterionic character: the large negative activation volumes[6] and entropies,[7] the large solvent effect on the reaction rate,[8] and the effect of substituents in the enol ethers[9] all suggest this.

Table I. Pressure Effect on the Cycloreversion of the TCNE–Ethyl *E*-Propenyl Ether Adduct

p, kbar	$10^5 k_1$, sec^{-1}	No. of measurements
0.001	6.88	7
0.352	7.86	6
0.559	8.84	6
0.786	10.70	5
1.448	15.22	6
2.068	17.07	6

There is no known instance of the breakage of a carbon–carbon bond which is promoted by pressure.[10] It has been shown by Neuman[11] that the retardation which normally characterizes such reactions can be diminished if a polar component is present in the breaking bond. For this reason it seemed to us that the effect of pressure on the carbon–carbon bond cleavage in the reverse [2 + 2] cycloadditions just mentioned might well be the opposite of what is normally found and—on the assumption that the properties of the transition state foreshadow the incipient product—that the magnitude of the pressure effect would allow us to assess the zwitterionic character of the intermediate. Specifically, we hoped to learn whether the intermediate is truly a zwitterion as has been claimed on the basis of the transition state dipole moment (deduced from the solvent effect on the rate), or whether it is a diradical with dipolar character. If the former description is indeed correct, one may expect an activation volume in these reverse cycloadditions comparable to those observed in solvolysis, i.e., in the range of −20 cm^3/mol even in polar solvents;[12] if the latter is a better description, then a much more modest pressure enhancement or even mild retardation may be anticipated. The reaction studied is indicated below; the adduct was prepared as described by McKusick.[13] Methanol was used as the solvent



at 25°. The reaction was followed by NMR; the disappearance of the methinyl doublet at τ5.83. The data are recorded in Table I. The activation volume is −16.7 cm^3/mol; the precision is estimated to be ±1.2 cm^3/mol. This value may be compared with those obtained in solvolysis reactions in hydroxylic media; we recently noted that SN1 solvolysis in alcoholic media on the average has an activation volume of −21 ± 2 cm^3/mol.[12] Clearly this reaction is comparable in its electrostriction characteristics to limiting SN1 solvolysis, and the intermediate is as purely zwitterionic as the initial product of solvolysis.

The information gained in this experiment is in good agreement with the solvent effect from which Huisgen and Steiner[8] deduced a transition state dipole moment of 10–14 D; the calculated value for the intermediate is 17–21 D. Both approaches agree on a charge development of 60–70% in the activated complex.

Acknowledgment. This work is supported by the National Science Foundation. Professors H. Steinberg of the University of Amsterdam and H. Kelm of the University of Frankfurt have kindly informed us of similar studies in progress in their laboratories.

References and Notes

(1) Paper XXXV in the series "Kinetics of Reactions in Solutions under Pressure".