(1) The racemic modification resolved is an octahedral transition metal ion complex which can theoretically exist in as many as 12 diastereoisomeric pairs of enantiomers.[14] Equilibration of the ligands around the metal ions is very fast, however, compared to the rate of racemization of the ligands, as evidenced by the following facts. (a) Addition of 3 mol of L-ACL ($[\alpha]^{25}\mathrm{D}$ $-34°$) to a solution of 1 mol of **3** ($[\alpha]^{25}\mathrm{D}$ $-59°$) results in complete loss of optical activity in less than 10 s after mixing. (b) No mutarotation is observed in solutions containing 1 mol of nickel(II) and 3 mol of L-ACL, although the corresponding complex can exist in as many as four diastereoisomers.[14] It is obvious, therefore, that the rate determining step in the current process is not the interconversion of diastereoisomers involving the metal ion but the racemization of ACL.

(2) Although the exact structure of the crystals of formula **2** is not known,[15] it is certain that they are enantiomeric to the crystals of formula **3**. (In fact the current process can be carried out with equal success using **3** as seed crystals.) When spontaneous crystallization is allowed to take place from a solution of **1** containing 50% enantiomeric excess of L-ACL, the first crystals formed are **2**, although statistically the most abundant species is $(\text{L-ACL})_2(\text{D-ACL})\text{NiCl}_2$; correspondingly, **3** is obtained when D-ACL is in excess. Clearly, crystalline **2** and **3** (two enantiomers) are the stable solid phases in equilibrium with a solution of **1**. In view of the foregoing discussion, it is clear that the current process is a simultaneous resolution/racemization of enantiomers, although not the same pair of enantiomers are involved in each half of the process: with respect to the resolution the relevant enantiomers are crystalline **2** and **3**; with respect to racemization, these are L-ACL and D-ACL. The possible presence of 24 diastereoisomers in solution and four in each solid phase is kinetically irrelevant to the process.

Acknowledgment. We thank Mr. W. J. Lukasavage for technical assistance in the course of this work.

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(9) In a continuously operated asymmetric transformation the steady-state rate of resolution must equal the steady-state rate of racemization. If pseudo-first-order kinetics are obeyed, it can be shown that at steady state $R = 0.0069CD_{ee}/(t_{1/2})_r$, where R is the rate of crystallization of the L-enantiomer expressed in g l.$^{-1}$ h^{-1}, C is the total concentration of the two enantiomers in g l.$^{-1}$, D_{ee} is the percentage steady-state enantiomeric excess of the D enantiomer in solution and $(t_{1/2})_r$ is the half-life of racemization in h. For typical concentrations of 250 g l.$^{-1}$ and D_{ee} of 10%, $(t_{1/2})_r$ must be on the order of 1 h or less for the process to have a practical output.

(10) Kinetic studies and a discussion of the mechanism of racemization will be published in a forthcoming paper.

(11) The optical activity at the sodium D line measured in 1 N hydrochloric acid is due to the ACL and is not affected by the presence of Ni(II).

(12) The conversion computation was based on the maximum amount of **1** which can form in solution, taken as stoichiometric to the nickel(II) chloride charged.

(13) Ion exchange techniques were utilized in preparing solutions containing Ni^{2+} and ethoxide ion. Details will be presented elsewhere.

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(15) X-Ray work is currently under way to that purpose.

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A Stereoselective Synthesis of the 24(*R*),25-Dihydroxycholesterol Side Chain

Sir:

Introduction of the 24*R* - hydroxy group into a steroid side chain presents a significant challenge. The hydroxy group with this absolute configuration is characteristic of several natural products such as lyofoligenic acid,[1] lyofolic acid,[2] and the vitamin D_3 metabolites 24,25-dihydroxycholecalciferol[3,4] and 1,24,25-trihydroxycholecalciferol.[4,5] We now report a highly stereoselective method for producing this C-24 side chain functionality, which was developed for the vitamin D_3 metabolites but which should also be applicable to the synthesis of other natural products.

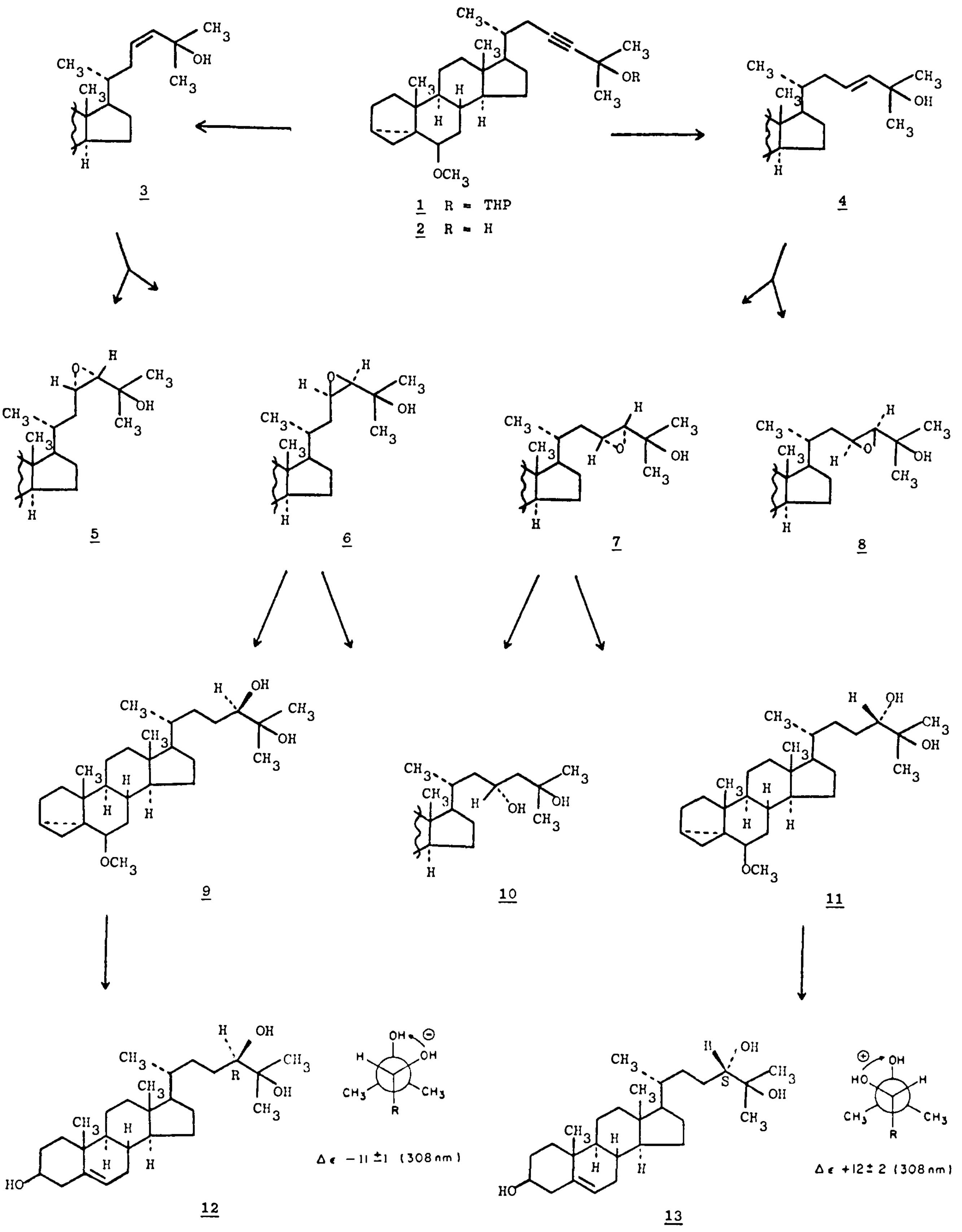
Initially, we considered it impractical to generate a specific chiral center on a long and flexible sidechain, but were encouraged by recent reports in the prostaglandin area.[6] Our previous results and those of Ikekawa[7] and Kodicek[8] have shown that no control of stereochemistry was possible in epoxidation and hydroxylation of the $\Delta^{24,25}$-double bond of desmosterol derivatives under a variety of conditions. Near 1:1 mixtures of products always resulted, indicating that this double bond was too far away from the C-17, C-20 chiral environment. Therefore, we decided to explore the chemistry of the (*Z*)- and (*E*)-$\Delta^{23,24}$-allylic alcohols expecting that the closer proximity of the double bond to the C-17 and C-20 chiral centers might have an influence on the stereoselectivity of the hydration reactions.

The two $\Delta^{23,24}$-allylic alcohols **3** and **4** were prepared from the acetylenic ether **1**, which was derived from stigmasterol in five steps (42% overall yield).[9] Compound **1** was cleaved in acidic methanol at 0° to give the acetylenic alcohol **2** ($[\alpha]\mathrm{D}$ $+50°$, 95% yield)[10] which was cleanly hydrogenated to the *Z*-allylic alcohol **3** ($[\alpha]\mathrm{D}$ $+37°$, 90% yield) over Lindlar catalyst in ethyl acetate (Scheme I). Alternatively, the acetylenic alcohol **2** was reduced with lithium aluminum hydride in tetrahydrofuran at reflux to give the *E*-allylic alcohol **4** (mp 126–127°, $[\alpha]\mathrm{D}$ $+46°$, 90% yield).

The *Z*-olefin **3**, when treated with several peracids, yielded a 1:1 mixture of epoxy alcohols **5** and **6**. However, when treated with anhydrous *tert*-butyl hydroperoxide in toluene and a catalytic amount of vanadyl acetoacetate[11] at −78°, followed by warming the mixture to −20° for 6 h, an 85:15 mixture of **6** and the undesired isomer **5** was obtained.[12] The 23*R*,24*R*-epoxy alcohol **6**, $[\alpha]\mathrm{D}$ $+57°$, was isolated by chromatography[13] and was reduced with lithium aluminum hydride (0°, tetrahydrofuran) to give the 24*R*,25-diol **9** contaminated only by 5% of the isomeric 23*R*,25-diol **10.** Pure diol **9**, mp 142–143°, $[\alpha]\mathrm{D}$ $+63°$, was obtained by direct crystallization and was exposed to acidic aqueous dioxane at 60° to yield the desired 24(*R*),25-dihydroxycholesterol (**12,** mp 200–202°, $[\alpha]\mathrm{D}$ $-11.3°$ (c 1.02, CH_3OH)).

Similarly, when the *E*-allylic alcohol **4** was epoxidized with *tert*-butyl hydroperoxide in toluene at −78° to −20° with vanadyl acetoacetate catalyst, an 85:15 mixture of epoxy alcohols **7** and **8** was obtained. The major epoxy alcohol **7**, mp

Scheme I



112–113°, $[\alpha]_D$ +53°, was purified by chromatography[13] and reduced with lithium aluminum hydride. However, in this case a 3:2 mixture of the 23*R*,25-diol **10** and the 24*S*,25-diol **11** was formed. The 24*S*,25-diol **11**, mp 167–168°, $[\alpha]$ +39°, was treated with acidic aqueous dioxane at 60° to give 24*S*,25-hydroxycholesterol (**13**, mp 196–198°, $[\alpha]_D$ −46° (*c* 1.00, CH_3OH)).

This significant difference in the mode of epoxide cleavage between epoxy alcohols **6** and **7** is surprising. The ratio of products was not changed by adding LiH, NaH, or KH prior to lithium aluminum hydride reduction. However, reduction of **6** and **7** with diisobutylaluminum hydride gave the 23*R*,25-diol **10** as the sole reduction product.

To determine the absolute configuration of the 24,25-diols, we initially employed the method of Nakanishi.[14] Using Pr(dpm)$_3$ under anhydrous conditions, we obtained the desired CD spectra which varied in intensity and duration. *However, by employing the stronger chelating reagent Eu(fod)$_3$, it was possible to obtain CD spectra exhibiting very large induced split Cotton effects which were essentially unchanged over a 10-day period in reagent grade chloroform or carbon tetrachloride solvents.*[15] On the basis of the empirical rule[14] α-diols **9** and **12** were shown to possess the 24*R*-absolute configuration and α-diols **11** and **13** the 24*S*-absolute configuration. These assignments were fully confirmed by a single-crystal x-ray structural determination of diol **9**.[16]

Thus, we have developed a short and efficient construction of the 24(*R*),25-dihydroxycholesterol side chain from readily available materials. The conversion of α-diols **9** and **12** into 24(*R*),25-dihydroxycholecalciferol and 1(*S*),24(*R*),25-trihydroxycholecalciferol will be discussed in a subsequent paper.

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(12) A mixture of 103 mg (0.25 mmol) of **3**, 3 ml of dry toluene, and 2 drops of a 2% VO(acac)$_2$ solution in toluene was cooled to −78° under nitrogen. A solution of 116 mg (1.20 mmol) of 94% *tert*-butyl hydroperoxide (Pennwalt Corp.) in 1 ml of toluene was briefly dried over anhydrous sodium sulfate and was then added to the reaction mixture. The pale pink mixture was briefly stirred at −78° and was warmed to −20° and stirred for 6 h. The product was isolated with methylene chloride and this solution was washed with 10% sodium bisulfite solution and water to remove any residual oxidant. A significant loss of stereoselectivity resulted when this process was carried out at room temperature.
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(16) The single-crystal x-ray determination was carried out by Dr. J. F. Blount and his staff at Hoffmann-La Roche Inc.

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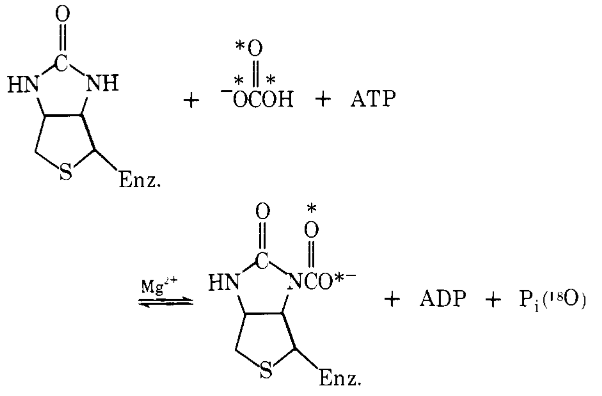
A Reaction Proceeding through Intramolecular Phosphorylation of a Urea. A Chemical Mechanism for Enzymic Carboxylation of Biotin Involving Cleavage of Adenosine 5′-Triphosphate[1]

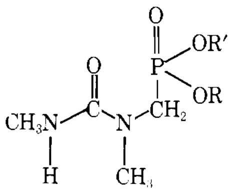
Sir:

The enzyme biotin carboxylase catalyzes the formation of *N*-carboxybiotin from biotin and bicarbonate with concomitant cleavage of ATP, leading to the ^{18}O labeling results shown in eq 1.[2,3] *N*-Carboxybiotin is the active form of coenzyme that is used in further biosynthetic reactions involving fixation of carbon dioxide. Bruice's studies on the carboxylation of ureas have demonstrated that *O*-carboxylated biotin would be the expected initial nonenzymic product.[4-6] Wood has suggested[3] that the observed enzymic product[7,8] and the unlikelihood of rearrangements imply that "simple model compounds are not always reliable indicators of reactivity in the environment of an enzyme." Thus, no entirely satisfactory mechanism for carboxylation of biotin has been proposed which would also account for the labeling and ATP-cleavage results in terms of known organic reactions. We have now observed that a urea moiety is nucleophilic toward a phosphate derivative in a manner consistent with what is reported for biotin and a mechanism can be formulated in common for both sets of reactions.

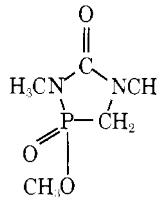


(1)

We prepared **1** as a model for the reactive portions of biotin and ATP bound in the same portion of an active site by modification of the procedure of Petersen and Reuther.[9] The formaldehyde-hydrogen chloride condensation product of *N*,*N*′-dimethylurea[10] was dissolved in trimethyl phosphite and heated to 70° for 1 h. After removal of phosphite, chromatography on silica gel with 5% methanol in chloroform gave **2** in 28% yield (NMR(CDCl$_3$): δ 3.86 (3 H, d, J = 11 Hz, P–OCH$_3$), 3.48 (2 H, d, J = 15 Hz, P–CH$_2$), 2.98 (3 H, d, J = 1 Hz, P–CH$_2$–N–CH$_3$), 2.90 (3 H, d, J = 8 Hz, P–NCH$_3$)). Treatment of **2** with 1 equiv of lithium hydroxide hydrate in methanol gave **1** in 83% yield. Anal. $C_5H_{12}N_2O_4PLi$(CHN): [NMR(D$_2$O) δ 2.75 (3 H, s, N–CH$_3$), 2.98 (3 H, s, N–CH$_3$), 3.57 (2 H, d, J = 10 Hz, P–CH$_2$), 3.62 (3 H, d, J = 10 Hz, P–OCH$_3$)]. Neut equiv: calcd 202, found 205 (pK_a ~ 1.3).



1, R = CH$_3$; R′ = Li
3, R = H; R′ = H
4, R = CH$_3$; R′ = CH$_3$
5, R = CH$_3$; R′ = H



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