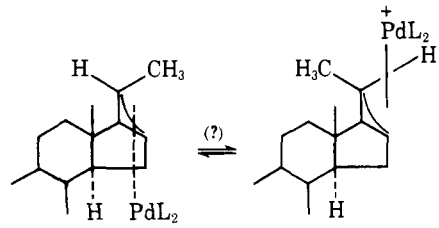
figuration (natural series) as assigned. Compound **6** reacts much more slowly, less cleanly, and in lower yield, under catalytic conditions to give **8**.

The stereochemistry of **3** obtained in the stoichiometric reaction is that expected from the anticipated preference for the bulky palladium to be on the less crowded α face of the steroid and the known preference for complexes to possess the syn rather than anti configuration.[4,5] We previously established that the nucleophile attacks the complex on the face opposite to palladium.[5b] On the other hand, the complementary stereochemistry obtained in the catalytic reaction is quite surprising. Firstly, the stereospecificity suggests that alkylation is faster than equilibration of the π-allyl complexes, and secondly, that the less stable diastereomeric complex **12a** or **12b**, which may be in dynamic equilibrium with each other, is probably involved. Considering the stereochemistry of **7**, this result indicates that the oxidative addition to the Pd complex is stereospecific and occurs with inversion of configuration.[13,14] Thus, the effect of the two inversions is an overall stereospecific SN2 displacement of the allylic acetate with a net retention of configuration, a result which should prove useful in organic synthesis.[15]



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(12) Control experiments establish that no alkylation occurs in the absence of the palladium complex as catalyst. Only an elimination product tentatively identified as the 16-vinyl compound is isolated.
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(14) Alternatively, σ complexes may be invoked. Nevertheless, since either allylic acetate gives the identical product and spectroscopic studies generally indicate that the π-allyl structure is more favorable than the σ isomer, rationalizations invoking the π-allyl species seem more appropriate.
(15) The overall retention of configuration in the catalytic allylic alkylation procedure has been demonstrated in simpler systems as well as other steroids and appears to be general.
(16) Camille and Henry Dreyfus Teacher-Scholar Grant Recipient, 1970-1975.

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The ^{15}N Nuclear Magnetic Resonance of Friend Leukemic Cell [Gly-^{15}N] Hemoglobin. The Resolution of Noncovalent Bonding Interactions

Sir:

A number of nitrogen NMR studies of amino acids[1] and short peptides[2] have indicated that nitrogen chemical shifts may be more easily correlated to macromolecular phenomena than those of ^{1}H and ^{13}C. However, the field of nitrogen-15 NMR of macromolecules has remained completely unexplored until now, due to the poor sensitivity routinely encountered in ^{15}N NMR, which is caused by the low natural abundance and relative insensitivity of nitrogen-15 nuclei. We here report the first nitrogen-15 NMR spectrum of a selectively ^{15}N enriched protein, which was achieved by using a Friend leukemic cell (FLC) culture to prepare hemoglobin 50% ^{15}N enriched in glycyl residues. The ^{15}N NMR spectra of [Gly-^{15}N]hemoglobin demonstrates that ^{15}N NMR can be used to distinguish between glycyl residues whose N-H groups are hydrogen bonded to water and those intramolecularly hydrogen bonded to peptide carbonyl groups. These results indicate that ^{15}N NMR might be a useful tool to study N-H noncovalent bonding interactions in oligopeptides, polyamino acids, and proteins.

[Gly-^{15}N]Hemoglobin (Hb-^{15}N) was obtained from the lysate of DMSO-treated Friend leukemic cells (FLC) (Clone 707)[3] grown on a medium containing 150 mg/l. of glycine-^{15}N (90% ^{15}N). The Hb-^{15}N (10% of the lysate proteins) was purified under CO on a CM-50 Sephadex column using a phosphate buffer pH gradient and shown by pH 6-8 polyacrylamide gel isoelectric focusing to have a pI = 7.52 and corresponded to the major compound of mouse DBA/2 hemoglobin, and the single band of mouse C57/BL hemoglobin. The freely reacting cysteine groups were carboxymethylated to prevent hemoglobin polymerization.[4]

The ^{15}N NMR spectrum of CO-Hb-^{15}N in H_2O (Figure 1A) displays a doublet, $^{1}J_{NH}$ = 94 Hz, centered at 81.4 ppm (downfield from 4 M $^{15}NH_4Cl$ in 2 M HCl) and a partially resolved shoulder at 90.0 ppm. Exchange of the peptide hydrogens for deuterium results in the collapse of the 81.4 ppm doublet into a singlet, and allows the second resonance at 90.0 ppm to be clearly resolved, as seen in the spectrum of CO-Hb-^{15}N in D_2O (Figure 1B). The proton-noise decoupled ^{15}N NMR spectrum of a FLC hydrolysate (Figure 2) displays a single intense resonance at 6.2 ppm corresponding to the chemical shift of glycine at pH 5.1 and indicates that no transfer of ^{15}N to other amino acids occurred and corraborates the mass spectrometric ^{15}N analy-

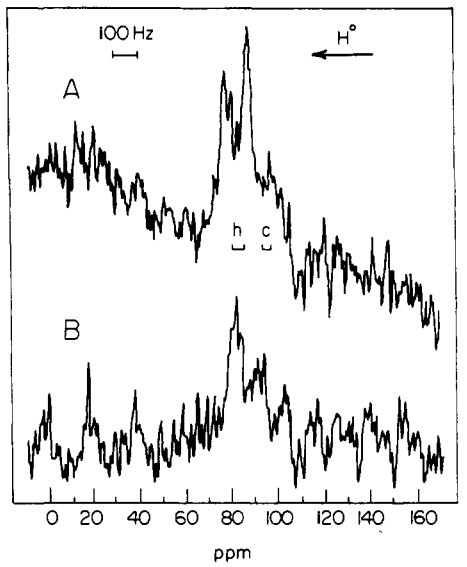

Figure 1. The FT ^{15}N NMR spectra of carbomonoxide FLC [Gly-^{15}N]hemoglobin (80 mg) in 1-cm^3 of H_2O (a) and D_2O (b), obtained from 64 000 accumulations of free induction decays following 90° pulses collected in 4K data points with a dwell time of 166 μs/point. The regions denoted by h and c correspond to the range of chemical shifts of *N*-methylacetamide in proton-donating (c) and proton-accepting (h) solvents.

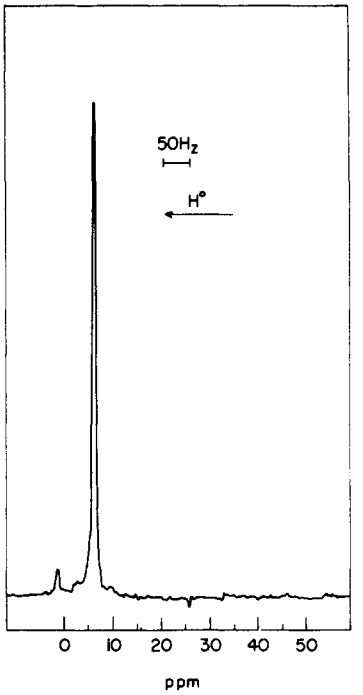

Figure 2. The FT ^{15}N NMR spectrum of the acid hydrolysate (200 mg/cm^3, pH 5.0) of the lysate of DMSO treated Friend leukemic cells grown in the presence of glycine-^{15}N. The spectrum was obtained from 2000 accumulations of the free induction decays following 90° pulses accumulated in 4K data points, over a range of 3000 Hz. A 10-sec delay between pulses was used.

sis of labeled [Gly-^{15}N]hemoglobin.[2] The ^{15}N NMR resonances of the heme nitrogens, that were also 50% ^{15}N enriched as determined by MS analysis, are estimated to resonate 60–70 ppm further downfield, but were not observed due to their long T_1 relaxation times.[5] We are forced to conclude that both ^{15}N resonances originate from glycyl residues alone.

The chemical shifts of a particular class of atoms in a protein, such as the peptide nitrogen, can be influenced by the neighboring residues, proximity to aromatic groups, and noncovalent bonding. Roberts and co-workers[6] have recently found that differences of up to 4.6 ppm in the ^{15}N chemical shift of the amide nitrogen in glycyl dipeptides can result from neighboring residue effects; however, the 8.6 ppm range of glycyl nitrogen in CO-Hb-^{15}N at present cannot be totally accounted for by this effect. The protein sequence data of C57/BL mouse hemoglobin,[7] which is analogous to the FLC hemoglobin sample studied here, and the noncovalent bonding map of myoglobin[8] can be used to determine the sites in hemoglobin from which the ^{15}N-glycyl resonances originate. These are 15 A13, 18 A16, 19 AB1, 22 B3, 25 B6, 51 D2, 57 E6, 70 E19, and 78 EF7. All of these positions are far from the heme group and their ^{15}N resonances should not be influenced by magnetic anisotropy effects, originating from the porphyrin π system. The location of the ^{15}N nuclei in the polypeptide backbone would shield them from magnetic anisotropy effects of individual aromatic side-chain groups. Although changes in the ψ–ϕ peptide dihedral angles result in changes in the charge density on nitrogen and the energy of the peptide electronic excited states,[9] there are no correlations between these and ^{15}N chemical shifts. Saito and co-workers[10] found that the nitrogen chemical shifts of *N*-methylacetamide occur in the range of 90–94 ppm in proton donating solvents, such as H_2O, and in the range of 80–85 ppm in proton acceptor solvents, such as acetone. As seen in Figure 1, the two ^{15}N resonances of CO-Hb-^{15}N fell precisely in these two ranges, designated by c and h in Figure 1. Inspection of the noncovalent bonding map of myoglobin reveals that the N–H groups of residues A2, B3, D2, and EF7 are solvated by water, while the N–H groups of residues A13, A16, AB1, B6, E6, and E19 are hydrogen bonded to C=O groups in either helices or bends. None of these residues is involved in additional noncovalent bonding interactions that occur at the $\alpha_1\beta_1$ and $\alpha_1\beta_2$ subunit contacts. We conclude that *the chemical shift differences of the glycyl residues in CO-Hb-^{15}N can be accounted by differences in the noncovalent bonding interactions of the N–H group.* These effects are observable only when selective isotopic labeling is used to thin out the peptide region of ^{15}N NMR spectra of macromolecules.

The fact that there is no clear segregation of ^1H resonances of the N–H group in formamide and *N*-methylacetamide according to the solvent donor-acceptor properties,[10] would seem to indicate that in polypeptides the amide ^1H resonance may be influenced by solvation effects, other than changes in hydrogen bonding. The similar ^{13}C chemical shifts of the enriched α-carbon in [Phe^8-^{13}C]S-ribonuclease-S′,[11] in which the N–H group is hydrogen bonded to C=O,[12] and in [Gly^6-^{13}C]S-ribonuclease-S′,[13] in which the N—H group is solvated by H_2O,[12] indicate that ^{13}C NMR cannot detect changes in noncovalent bonding at the peptide N—H.

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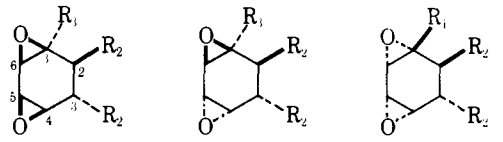
Synthesis of (±)-Crotepoxide, (±)-Epicrotepoxide, and (±)-Isocrotepoxide

Sir:

Crotepoxide (**1**), also known as futoxide, was isolated by Kupchan et al.[1] from *Croton macrostachys* and has been found to possess significant inhibitory activity against Lewis lung carcinoma and Walker intramuscular carcinoma. The structure of **1**,[2] confirmed by an x-ray crystallographic analysis,[3] reveals it to be a member of the small but pharmacologically interesting family of naturally occurring 1,3-diepoxides.[4] We wish to report the total synthesis of (±)-crotepoxide (**1**), its 4,5-epimer **2** (epicrotepoxide), and the 1,6;4,5-bis epi compound **3** (isocrotepoxide).[5]

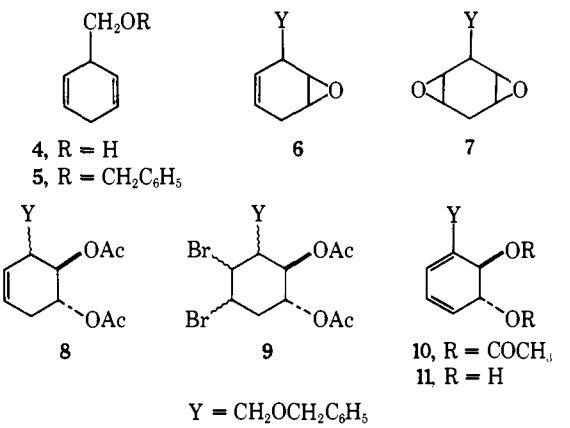


1 **2** **3**

R_1 = $CH_2OCOC_6H_5$, R_2 = $OCOCH_3$

1,4-Dihydrobenzyl benzyl ether (**5**), prepared from **4**[6] (NaH, benzyl bromide, glyme, 0 °C, 77%), underwent epoxidation upon treatment with *m*-chloroperbenzoic acid (MCPA) in CH_2Cl_2 (36 h, room temperature) to give **6** (79%) and only a trace of diepoxide **7**.[7] Exposure of **6** to acetic anhydride (HOAc, 36 h, reflux) produced trans diacetate **8** (79%) as a mixture of two diastereomers. This mixture was brominated (CH_2Cl_2) in the presence of pyridine yielding stereoisomeric dibromides **9** (93%) which, without separation, were dehydrohalogenated (LiCl, Li_2CO_3, HMPA, 105 °C, 16 h) to give a 90% yield of a single diene **10** (δ^{CDCl_3} 1.98 (3 H, s), 2.02 (3 H, s), 4.06 (2 H, s), 4.50 (2 H, s), 5.44 (1 H, t, J = 5 Hz), 5.74 (1 H, d, J = 5 Hz), 5.8–6.2 (3 H, broad m), 7.32 (5 H, s)). Reduction of **10** ($LiAlH_4$, ether, 0 °C) afforded diol **11** (84%). The efficient preparation of the relatively stable diene **10** (32% overall from benzoic acid) and corresponding diol **11** permitted a detailed study of their behavior under oxygenation ($^1\Delta_g$ O_2) and epoxidation conditions, and they therefore became the focal intermediates in the synthesis of crotepoxide and its stereoisomers.

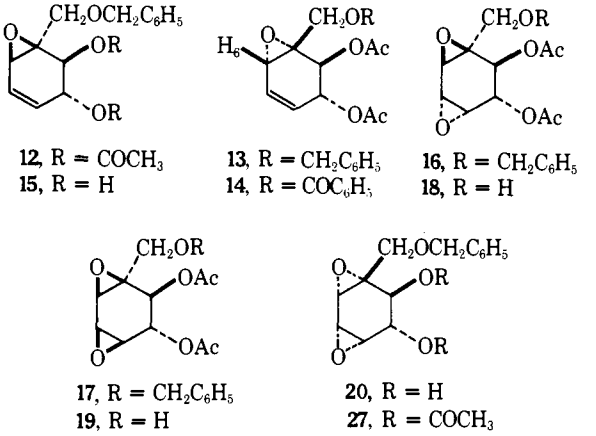
Epoxidation of **10** (MCPA, CH_2Cl_2) at 25 °C gave monoepoxides **12** and **13** exclusively in a 1:1 ratio. Configuration was assigned to these stereoisomers on the basis of a comparison of the chemical shift of H_6 (**12**, δ 3.60; **13**, δ 3.47) with the corresponding proton (δ 3.44) in senepoxide (**14**),[8] and also from the observation that epoxidation of **11** proceeded stereospecifically[9] to give **15** which, upon acetylation (Ac_2O, pyridine, 6 h, room temperature), yielded **12**. The difficulty associated with epoxidation of the 4,5 double bond of **10** was overcome by invoking the forcing conditions devised by Kishi.[10] Thus, treatment of **10** with MCPA in 1,2-dichloroethane in the presence of 2,6-di-*tert*-butyl-*p*-cresol (90 °C, 2 h) afforded in 55% yield a readily separable mixture of trans diepoxide **16** and cis diepoxide **17** in the ratio 8:1. Hydrogenolysis of **16** and **17** (10% Pd/C, EtOH) gave the corresponding primary alcohols **18** and **19** in quantitative yield, and subsequent benzoylation (C_6H_5COCl, $CHCl_3$) furnished (70% in each case) (±)-4,5-epicrotepoxide (**2**, mp 119–121 °C) and (±)-crotepoxide (**1**).[11] The stereochemistry of epicrotepoxide is revealed most convincingly by the chemical shift of H_2 (δ 5.74, d, J = 8 Hz; cf. δ 5.73 in **1**) and of H_4 (δ 3.39, d, J = 4 Hz; cf. δ 3.10 in **1**).



4, R = H; **5**, R = $CH_2C_6H_5$ **6** **7**

8 **9** **10**, R = $COCH_3$; **11**, R = H

Y = $CH_2OCH_2C_6H_5$



12, R = $COCH_3$; **15**, R = H **13**, R = $CH_2C_6H_5$; **14**, R = COC_6H_5 **16**, R = $CH_2C_6H_5$; **18**, R = H

17, R = $CH_2C_6H_5$; **19**, R = H **20**, R = H; **27**, R = $COCH_3$

Attempts to effect a direct bisepoxidation of **11** using the hydroxyl groups as controllers were unsuccessful with peracid oxidants. However, the reaction of **11** with *tert*-butyl hydroperoxide (2 equiv, benzene, reflux, 12 h) in the presence of $VO(acac)_2$ as catalyst[12] led stereospecifically to cis diepoxide **20** (15%). Acetylation followed by hydrogenolysis and benzoylation as for crotepoxide gave (±)-isocrotepoxide (**3**) as an oil (δ^{CDCl_3} 2.10 (3 H, s), 2.15 (3 H, s), 3.28 (1 H, m), 3.59 (1 H, m), 3.65 (1 H, m), 4.14 (1 H, d, J = 12 Hz), 4.72 (1 H, d, J = 12 Hz), 5.19 (1 H, t, J = 3 Hz), 5.43 (1 H, bs), 7.54–8.12 (5 H, m)). Formation of **20** exclusively can be rationalized assuming complexation of the vanadium oxidant with the more accessible C-3 hydroxyl of **11**. These epoxidations are known to be highly stereoselective in the case of allylic alcohols,[13] and based on the dimensions of a molecular model, should be likewise for homoallylic alcohols.[14]

Since endoperoxides derived from the reaction of singlet