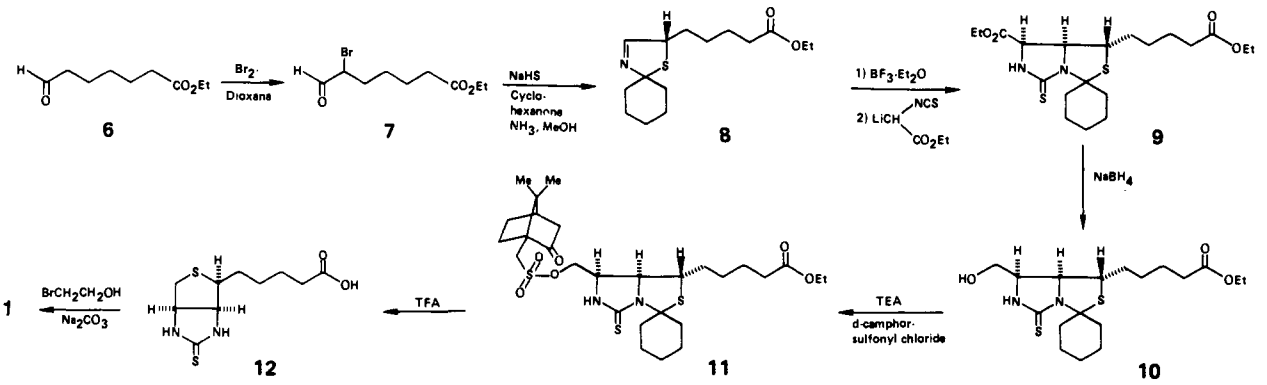
Scheme III. Biotin Synthesis



in 90% yield bromo aldehyde **7** (bp 95–100 °C (0.3 mm)), which was best not distilled but treated directly with sodium hydrogen sulfide, cyclohexanone, and ammonia to give imine **8**[5,6] in >90% yield. The BF_3-catalyzed imine addition reaction involving crude **8** and ethyl isothiocyanatoacetate **3** (R = Et) afforded diester **9**,[6] mp 98.5 °C, in ca. 50% yield as the major product following a silica gel chromatography. Treatment of diester **9** with sodium borohydride (MeOH/THF, 0 °C) resulted in a selective ester reduction[20] to give in >90% yield alcohol **10**,[6] mp 131.5–132 °C. Alcohol **10** was smoothly converted in >90% yield (TEA, *d*-camphorsulfonyl chloride, CH_2Cl_2, 0 °C) to a mixture of *d*-camphorsulfonates **11**,[6] which were separated by silica gel chromatography (85:15 CH_2Cl_2/EtOAc). The less polar isomer, mp 120 °C, $[\alpha]^{23}_D$ +15.6° (*c* 10, $CHCl_3$), was converted upon aqueous trifluoroacetic acid treatment (2.7:1 TFA/H_2O, 45 °C, 65 h; 3:1 H_2O/TFA, 100 °C, 1 h) to *d*-2-thiobiotin **12**,[6,21] mp 225–227 °C, $[\alpha]^{23}_D$ +99.2° (*c* 1, TFA), in 83% yield. The reaction conditions affected thiazolidine ring hydrolysis, thiophane ring formation, and ester hydrolysis.[22]

The remaining task required in the generation of *d*-biotin involved a thiourea/urea transformation. The standard literature techniques[23] were not effective in the conversion of **12** to *d*-biotin (**1**). We therefore devised a procedure that would exploit the nucleophilic character of the thiourea sulfur atom and would, in principle, deliver in an intramolecular fashion an oxygen atom to give ultimately *d*-biotin (**1**) (via a labile alkoxyimidazoline). Accordingly, treatment of *d*-2-thiobiotin (**12**) with 2.3 equiv of bromoethanol in *N*-methylpyrrolidinone (110 °C, 4 h) followed by Na_2CO_3 (110 °C, 18 h) afforded crude *d*-biotin, which was recrystallized from water to give *d*-biotin (**1**),[6] mp 229.5–230 °C, $[\alpha]^{25}_D$ 91.3° (*c* 1, 0.1 N NaOH), in 64% yield from **12**.

Thus, a total synthesis of *d*-biotin has been realized in ca. 9% overall yield from ethyl 7-oxoheptanoate (**6**). The novel boron trifluoride promoted thiazoline addition, in this synthesis, demonstrates a successful application of the aldol condensation literature to the chemistry of imines. We believe that the concept of imine activation via Lewis acid complexation will have widespread utility and, as a result, the "C=N" moiety will play a larger role in organic synthesis.

(19) Ethyl 7-oxoheptanoates can be conveniently prepared by: (a) ethoxycycloheptene ozonolysis. See: Bestmann, H. J.; Koschatzky, K. H.; Vostrowsky, O. *Chem. Ber.* **1979**, *112*, 1923–1925. (b) Cycloheptene ozonolysis. See: Schreiber, S. L.; Claus, R. E.; Reagan, J. *Tetrahedron Lett.* **1982**, 3867–3870. (c) Grignard coupling. See: Volkmann, R. A.; Davis, J. T.; Meltz, C. N. *J. Org. Chem.* **1983**, *48*, 1767–1769.

(20) Esters bearing α-substituted heteroatoms are often readily reduced by sodium borohydride. See: Schenker, E. "Newer Methods of Preparative Organic Chemistry"; Verlag Chemie: Weinheim, 1968; Vol. IV, 196–335.

(21) Jansen, A. B. A.; Stokes, P. J. *J. Chem. Soc.* **1962**, 4909–4914.

(22) We later discovered that while the camphorsulfonyl group provides a convenient means for a biotin resolution, it is not crucial to thiophane ring formation as acid treatment (3:1 TFA/H_2O, 100 °C, 4 h) of alcohol **10** in fact generated racemic 2-thiobiotin (**12**) *directly* in 83% yield.

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Supplementary Material Available: Additional experimental data for the compounds studied (9 pages). Ordering information is given on any current masthead page.

Hydrogen Bond Length and ^{1}H NMR Chemical Shifts in Proteins

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Recently we proposed novel NMR procedures for studies of the spatial structure of noncrystalline proteins.[1] In this scheme the initial structure determination relies primarily on measurements of intramolecular nuclear Overhauser effects between individually assigned hydrogen atoms. Subsequent refinements of the structure, however, depend further on correlations between additional NMR parameters and polypeptide conformation. The present communication reports on correlations observed between proton NMR chemical shifts and the length of intramolecular hydrogen bonds in a globular protein. Besides their potential usefulness for structure refinements these observations are of general interest because of the importance of hydrogen bonds in proteins.

For the basic pancreatic trypsin inhibitor (BPTI) a refined single-crystal X-ray structure[2] and almost complete, sequence-specific assignments of the ^{1}H NMR spectrum in solution[3] are available. There is much evidence that, with the exception of the chain termini and some long side chains,[4] the crystal structure is essentially preserved in aqueous solution.[5] BPTI is therefore a good "model protein" for studies of correlations between NMR parameters and spatial polypeptide structure. In the present investigation we adopt the hypothesis that the crystal structure[2] is strictly preserved under the conditions of the NMR experiments. Proton positions in the crystal structure were calculated from the heavy atom coordinates by attaching the hydrogens with the assumption of standard geometries for the individual amino acid residues.[6] From the BPTI structure thus obtained we computed

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(5) Wagner, G.; Wüthrich, K. *J. Mol. Biol.* **1982**, *160*, 343–361.

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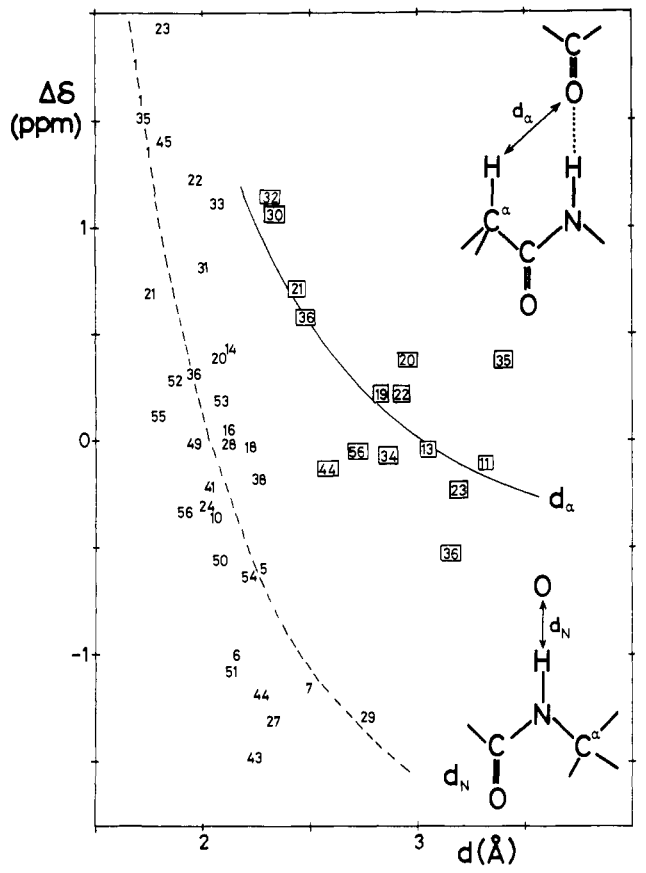

Figure 1. Plot of the conformation-dependent chemical shifts for the hydrogen-bonded amide protons in BPTI and for the C^α protons in the antiparallel β-sheets of this protein vs. the distance of these protons from the nearest oxygen atom. $\Delta\delta$ is the observed chemical shift at 50 °C and pH 3.5 minus the corresponding random-coil and ring-current shifts (see text). Positive and negative values of $\Delta\delta$ mean low-field and high-field shifts, respectively. The data points for the amide protons are identified by the sequence location of the amino acid residues to which they belong. All the amide protons have been included that exchange slowly with the solvent and/or are within a distance of 3.0 Å from an oxygen atom in the crystal structure.[3] The broken line represents the best fit to a $1/d_N^3$ dependence of $\Delta\delta$ (eq 2). The data points for C^α protons are indicated by squares that contain the sequence location of the residues. Only those C^α protons were considered that are within 3.5 Å of an oxygen atom. Most of these protons belong to residues in the antiparallel β-sheet. The solid line represents the best fit to a $1/d_\alpha^3$ dependence of $\Delta\delta$ (eq 3). d_N and d_α are defined by the structures on the right-hand side of the figure.

interatomic distances, such as d_N between amide protons and nearby oxygen atoms and d_α between C^α protons and nearby oxygens (Figure 1). Furthermore, from their positions relative to the aromatic rings we computed ring-current shifts for all the hydrogen atoms in BPTI, as was previously described in detail.[7]

The ^1H NMR chemical shifts for BPTI in aqueous solution at pH 4.6 and 68 °C have been published,[3] and a corresponding data set was obtained for pH 3.5 and 50 °C[14]. For the present investigation we evaluated the conformation-dependent effects on the chemical shifts at 50 °C that cannot be attributed to ring-current fields by subtracting for each hydrogen atom the random-coil chemical shift[8] and the ring-current shifts[7] from the experimental shift.[9] Positive and negative values for the resulting quantity $\Delta\delta$ mean downfield and upfield shifts, respectively. We then started a search for correlations between $\Delta\delta$ and structural parameters in BPTI. Two particularly interesting results of this search are presented in this paper. First, the chemical shifts, $\Delta\delta$, of amide protons were found to correlate with the hydrogen bond length, d_N (Figure 1). Only those amide protons were considered in the analysis that are in close proximity to a well-defined oxygen atom in the crystal structure. Short hydrogen bonds are correlated with low-field shifts, larger bond lengths with shifts to higher field. The chemical nature of the acceptor group seems not to play an important role, and the oxygen atom may belong to a carboxyl group, a carbonyl group, or an internal water molecule. All the data points for amide protons fall on the left side in Figure 1.

Second, we found for C^α protons that close proximity to oxygen atoms is correlated with large positive values of $\Delta\delta$ (low-field shifts). In BPTI short C^α-proton to oxygen distances, d_α, occur predominantly in antiparallel β-structures where the C^α protons are located near to backbone carbonyl oxygens of the opposite polypeptide strand (Figure 1). In Figure 1 $\Delta\delta$ is plotted vs. d_α for all those residues for which d_α is smaller than 3.5 Å. All these data points fall in the upper right area of the figure.

Likely physical origins for these correlations are electric field effects,[10] local magnetic anisotropies,[11] and/or a polarization of the electron cloud near the hydrogen atom by the proximity of an oxygen atom.[12] For all three effects a d^{-3} dependence of $\Delta\delta$ is expected.[10-12] From a linear regression of the experimental values for $\Delta\delta$ vs. d^{-3} with the equation

$$\Delta\delta = ad^{-3} + b \quad (1)$$

we obtained

$$\Delta\delta_N = 19.2d_N^{-3} - 2.3 \quad (2)$$

and

$$\Delta\delta_\alpha = 19.6d_\alpha^{-3} - 0.7 \quad (3)$$

with correlation coefficients of 0.75 and 0.76, respectively. In Figure 1 these relations are shown by the broken curve and the solid curve, respectively. The root-mean-square deviation of d_N from the calculated curve is 0.2 Å. This may be compared with the error limit of the atomic coordinates in the crystal structure, which, as a rule of thumb, is at most 0.3 × resolution, i.e., 0.3 × 1.5 Å for BPTI.[2] It is striking that very similar values for the coefficient a were obtained for $\Delta\delta_N$ and $\Delta\delta_\alpha$, which indicates that a common mechanism is responsible for both effects. In a theoretical study Ditchfield[12] has analyzed the influence of hydrogen bonding in a water dimer on the isotropic proton chemical shifts and obtained a d^{-3} dependence with a constant factor of 20.53 ppm·Å^3. This is in excellent agreement with the corresponding factor a in eq 2 and 3.

The constant factor b in eq 2 and 3 represents the influence of hydrogen bonding on the chemical shifts in the random coil state. By our definition $\Delta\delta = 0$ for random-coil polypeptide chains and with the use of the eq 2 and 3 we can estimate that the corresponding average proton to solvent-oxygen distances are d_N (random coil) = 2.0 Å and d_α (random coil) = 3.0 Å. Hence, while comparison of the shape of the two curves in Figure 1 provides evidence that the low-field shifted C^α protons of the antiparallel β-sheet in BPTI are also involved in hydrogen-bond-like interactions,[13] the vertical displacement by 1.7 ppm of the solid curve for $C^\alpha H$ relative to the broken curve for the amide protons is a clear indication that hydrogen bonding makes a much larger contribution to the amide proton random-coil chemical shifts than to those of the C^α protons.

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