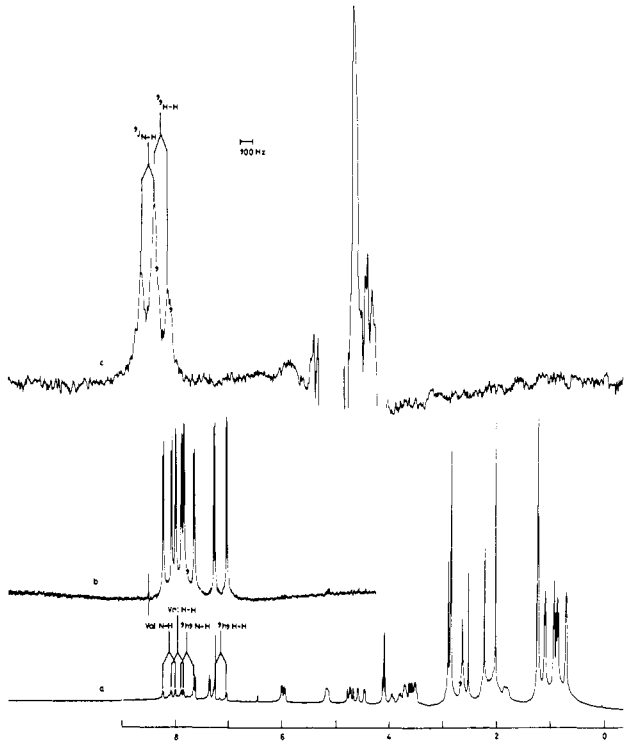

Figure 1. (a) ^1H NMR spectrum of 2 mg of uniformly enriched [^{15}N]actinomycin D in $CDCl_3$; 32 fids were accumulated. (b) ^1H NMR spectrum of actinomycin D in chloroform edited to select ^{15}N-H protons. (c) ^1H NMR spectrum of the actinomycin D complex with d(A-G-C-T) at 25 °C edited to select ^{15}N-H protons. A recycle time of 8 s was used, and 3600 fids were accumulated.

to a solution of ^{15}N-enriched actinomycin D is shown in Figure 1, a and b. ^{15}N decoupling has been used as an alternative approach to the same result in tRNAs.[5]

A solution of about 2 mg of d(A-G-C-T) with a quantity of ^{15}N-enriched actinomycin D equimolar with nucleotide duplex was made up in 10% D_2O/H_2O at pH 7.0 containing 100 mM NaCl and 50 mM phosphate buffer. Although the pulse sequence (1) should eliminate the strong H_2O signal after one cycle, a hard π-pulse followed by a wait period of 2.4 s was inserted before its implementation in order to avoid receiver overload. The ^1H spectrum of the complex at 25 °C is shown in Figure 1c, edited to show only protons directly bonded to ^{15}N. As no ^{15}N broadband decoupling was employed during acquisition, each N-H proton is manifested as a doublet. A maximum of six ^{15}N-H doublets are expected from actinomycin D; the two amino group N-Hs, however, certainly exchange too fast with H_2O to be observed. There are two pairs of peptide N-Hs that should be resolved under favorable exchange conditions, however, and ideally the two L-threonyl and two D-valyl N-Hs should be seen. Figure 1c shows only two doublets instead of the four expected, although the line widths are such that it is possible that the observed signal corresponds to overlap of the two pairs.

Crystal structure analyses of actinomycin D complexed with nucleosides[6] and oligonucleotides[7] suggest that the threonyl N-Hs are involved in hydrogen bond formation with the guanine bases of DNA, while the D-valyl N-Hs participate in hydrogen bonds linking the two pentapeptide rings of the drug. We conjecture that only one N-H pair is observed and that it corresponds to the intramolecularly hydrogen bonded N-Hs, i.e., the D-valyl N-Hs. Attempts to observe both pairs at reduced temperature are complicated if a hard pulse is used to initiate the inversion-recovery H_2O suppression procedure; the complex is outside the extreme narrowing regime, and a decrease in temperature increases T_1 in the complex while decreasing it for H_2O. Thus, whereas at 20 °C T_1s in the complex were much shorter than $T_{1(H_2O)}$, at temperatures low enough to slow the exchange, T_1s in the complex and of H_2O are comparable, and nulling of the complex signals occurs.

If the above conjecture is correct, some kinetic restrictions are placed upon the amide NH exchange processes. The D-valyl N-Hs are required to exchange with water at a rate considerably less than $^1J_{^{15}N-^1H}$ (i.e., 90 Hz), or this coupling would not be resolved (although this rate is not necessarily that of breaking of the intramolecular hydrogen bonds). On the other hand, the proposed intermolecular hydrogen bond to the nucleotide is broken at a rate faster than the chemical shift separation of H_2O and the threonyl N-Hs (in slow exchange), i.e., $> \sim 1000$ Hz at 400 MHz.

Experiments utilizing a soft pulse to selectively suppress the H_2O resonance at low temperature are in progress. We are also hoping to use selectively labeled drugs to assign the N-Hs unambiguously.

Acknowledgment. We thank S.E.R.C., the Royal Society, and M.R.C. (U.K.) for financial support. D.M.D. is on study leave from Griffith University and thanks the Royal Society for the award of a Nuffield Foundation Fellowship. The strain of *S. parvulus* was a generous gift from Joe Formica.

Registry No. Actinomycin D-^{15}N, 86688-52-0; d(A-G-C-T), 86688-53-1.

(5) Griffey, R. H.; Poulter, C. D.; Yamaizumi, Z.; Nishimura, S.; Hawkins, B. L. *J. Am. Chem. Soc.* **1983**, *105*, 143.
(6) Jain, S. C.; Sobel, H. M. *J. Mol. Biol.* **1972**, *68*, 1.
(7) Takusagawa, F.; Dabrow, M.; Neidle, S.; Berman, H. M. *Nature (London)* **1982**, *262*, 466.

Stereochemical Control in the Addition of Isothiocyanatoacetate Esters to Boron Trifluoride Activated 3-Thiazolines. A Novel Synthesis of *d*-Biotin

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Considerable effort has been directed recently toward the total synthesis of the biological cofactor *d*-biotin (**1**).[1] The three contiguous asymmetric centers of the molecule provide a key hurdle that a successful synthesis must surmount. We were intrigued with the possibility of establishing the requisite biotin stereochemistry by adding an ester enolate of specific geometry bearing a masked α-amino functionality to a suitably substituted imine containing the biotin valeric acid side chain (Scheme I). In so doing, we wished to determine whether the wealth of recent data surrounding the aldol condensation[2] could be extended to the chemistry of imines.[3]

Treatment of α-bromoheptanal[4] with sodium hydrogen sulfide followed by acetone and ammonia generated 3-thiazoline **2**[5,6]

(1) Baggiolini, E. G.; Lee, H. L.; Pizzolato, G.; Uskokovic, M. R. *J. Am. Chem. Soc.* **1982**, *104*, 6460-6462 and references therein.
(2) Evans, D. A.; Nelson, J. V.; Taber, T. R. In "Topics in Stereochemistry"; Allinger, N. L., Eliel, E. L., Wilen, S. H., Eds.; Wiley: New York, 1982; Vol. 13, Chapter 1.
(3) Ester enolate-imine condensations have been employed primarily in the preparation of β-lactams. The scope of the reaction is limited. See: (a) Gilman, H.; Speeter, M. *J. Am. Chem. Soc.* **1943**, *65*, 2255-2256. (b) Dardoize, F.; Moreau, J.-L.; Gaudemar, M. *Bull. Soc. Chim. Fr.* **1972**, 3841-3846. (c) Luche, J. L.; Kagan, H. B. *Ibid.* **1969**, 3500-3505. (d) Gluchowski, C.; Cooper, L.; Bergbreiter, D. E.; Newcomb, M. *J. Org. Chem.* **1980**, *45*, 3413-3416.
(4) Yanovskaya, L. A.; Teremtēv, A. P. *Zh. Obschch. Khim.* **1952**, *22*, 1598-1602; *Chem. Abstr.* **1953**, *47*, 9258.
(5) Thiel, M.; Asinger, F.; Schmiedel, K. *Liebigs Ann. Chem.* **1958**, *611*, 121-131. Asinger, F.; Offermanns, H. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 907-919.
(6) The NMR and IR spectra were entirely consistent with the assigned structure, and satisfactory C, H, and N analyses were obtained.

0002-7863/83/1505-5946$01.50/0

Scheme I

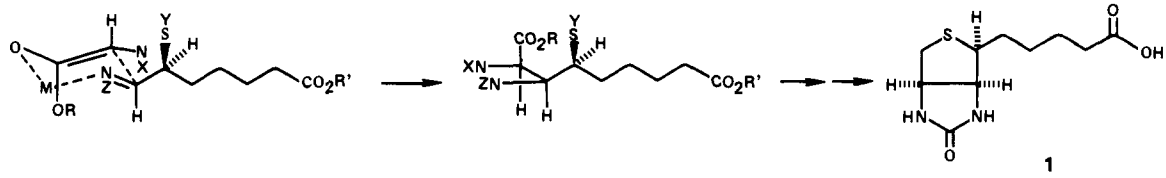
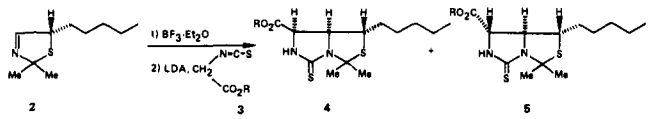
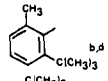
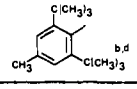


Table I

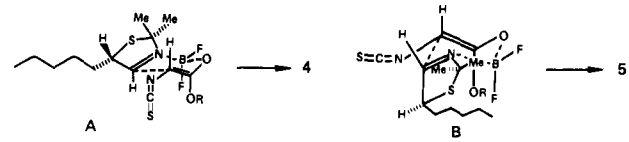


R	Ratio 4:5[a]	R	Ratio 4:5[a]
CH_3-[b]	1.4		1
CH_3OCH_2-[c]	2		3
CH_3CH_2-[b]	3		>5
$(CH_3)_2CH$-[b]	3		

[a] Determined by analysis of the *gem*-dimethyl region in the ^1H NMR of the crude reaction mixture. These values are consistent with the isolated product yields. [b] Prepared by treatment of the corresponding glycine ester derivatives with thiophosgene.[11] [c] Prepared by the treatment of *N*-[[(ethoxycarbonyl)thio]-thioxomethyl]glycine[12] with TEA (1 equiv) followed by chloromethyl methyl ether (1 equiv). [d] The glycine esters required for making these isothiocyanatoacetates were prepared by (1) the reaction of phthalylglycyl chloride[13] with the corresponding lithium phenoxides and (2) hydrazine treatment of the resultant phthalimides.

(Table I), which was selected as the imine component for our initial model studies. Isothiocyanatoacetate esters **3**[7,11,12] were selected for our ester enolate studies. Treatment of **2** with various metal enolates of **3** and a host of other nucleophiles resulted in no product formation. This result was not totally unexpected as the problem of poor imine reactivity is widespread and has limited the group's utility in organic synthesis. In many instances, activation of the imine moiety is achieved by the subsequent generation of acylimines or immonium salts.[8] Since the activating groups in these cases are not easily removed, they do not provide a general solution to this fundamental problem. We therefore focused our attention on alternate methods of enhancing the reactivity of **2** toward nucleophilic attack. As a result of these investigations, we now report that: (1) a broad range of nucleophiles[9] add to 3-thiazolines

Scheme II



under remarkably mild conditions if these imines are first *activated by the addition of an equivalent of boron trifluoride*[10], (2) the isothiocyanatoacetate ester moiety plays a significant role in determining the stereochemical outcome of the ester enolate–boron trifluoride thiazoline condensation, (3) this activated imine methodology can be employed for the synthesis of *d*-biotin (**1**).

Accordingly, if a solution of **2** (1 equiv) in THF containing BF_3·OEt_2 (1 equiv) is cooled to −78 °C and treated with the lithio enolate of isothiocyanatoacetate esters **3** (1 equiv of LDA, 1 equiv of **3**, THF, −78 °C), thiazolidines **4**,[6] which have the requisite biotin stereochemistry, and epimers **5**[6] can be isolated in ca. 75–85% yield after reaction workup and silica gel (Na_2HPO_4 buffered) chromatography (Table I).

The formation of thiazolidines **4** and **5** formally involves an ester enolate–imine addition (which establishes the product stereochemistry) followed by an intramolecular amine–isothiocyanate condensation. While the precise mechanism of the thiazoline addition is not known, the results are best accommodated by a pericyclic process[14] involving a vinyloxyborane–imine transition state[15] similar to that suggested for the kinetic aldol condensation (Scheme II). According to this analysis, factors that favor diastereomeric transition state A[16] (similar to the favoring of threo diastereoselection in the aldol condensation of *Z* enolates) should favor the formation of **4**. The fact that larger alkyl esters of **3** (R = Et, *i*-Pr) exert greater stereochemical influence than methyl ester **3** (R = Me) supports this hypothesis. In addition, the 2,6-di-*tert*-butyl-4-methylphenyl (BHT) ester group, which provides the best threo/erythro diastereoselectivity in the addition of ester enolates to aldehydes[17] also provides the best diastereoselectivity in this imine addition reaction. Esters capable of internal chelation[18] (R = CH_2OMe), however, exert no notable influence on product stereochemistry.

With the technology available for establishing in respectable yield the three biotin stereocenters, our attention was next focused on the molecule's synthesis (Scheme III). Bromination (bromine–dioxane, Et_2O, 0 °C) of ethyl 7-oxoheptanoate (**6**)[19] afforded

(7) Hoppe, D. *Angew. Chem., Int. Ed. Engl.* **1972**, *11*, 933–934. Hoppe, D.; Kloft, M. *Liebigs Ann. Chem.* **1976**, 1850–1861. Hoppe, D.; Follmann, R. *Chem. Ber.* **1976**, *109*, 3047–3061. Hoppe, I.; Hoppe, D.; Schöllkopf, U. *Tetrahedron Lett.* **1976**, 609–612.

(8) Meyers, A. I.; Collington, E. W. *J. Am. Chem. Soc.* **1970**, *92*, 6676–6678. Akiba, K.; Araki, K.; Nakatani, M.; Wada, M. *Tetrahedron Lett.* **1981**, 4961–4964.

(9) Grignard reagents, ketone and ester enolates, nitronate anions, and acid dianions all add to the BF_3-activated imines. The full scope of these and other additions are the subject of future manuscripts.

(10) The utility of boron trifluoride in promoting the condensation of acetone and benzalaniline was reported years ago. See: Snyder, H. R.; Kornberg, H. A.; Romig, J. R. *J. Am. Chem. Soc.* **1939**, *61*, 3556–3558. More recently, titanium tetrachloride has been used to effect the condensation of silated ester enolates and chiral Schiff bases. See: Ojima, I.; Inaba, S. *Tetrahedron Lett.* **1980**, 2077–2080. Ojima, I.; Inaba, S. *Ibid.* **1980**, 2081–2084.

(11) Floch, L.; Kovac, S. *Collect. Czech. Chem. Commun.* **1975**, *40*, 2845–2854.

(12) Higashimura, T.; Kato, H.; Suzuoki, K.; Okamura, S. *Makromol. Chem.* **1966**, *90*, 243–253.

(13) Sheehan, J. C.; Frank, V. S. *J. Am. Chem. Soc.* **1949**, *71*, 1856–1861.

(14) Zimmerman, H. E.; Traxler, M. D. *J. Am. Chem. Soc.* **1957**, *79*, 1920–1923. Evans, D. A.; Vogel, E.; Nelson, J. V. *Ibid.* **1979**, *101*, 6120–6123.

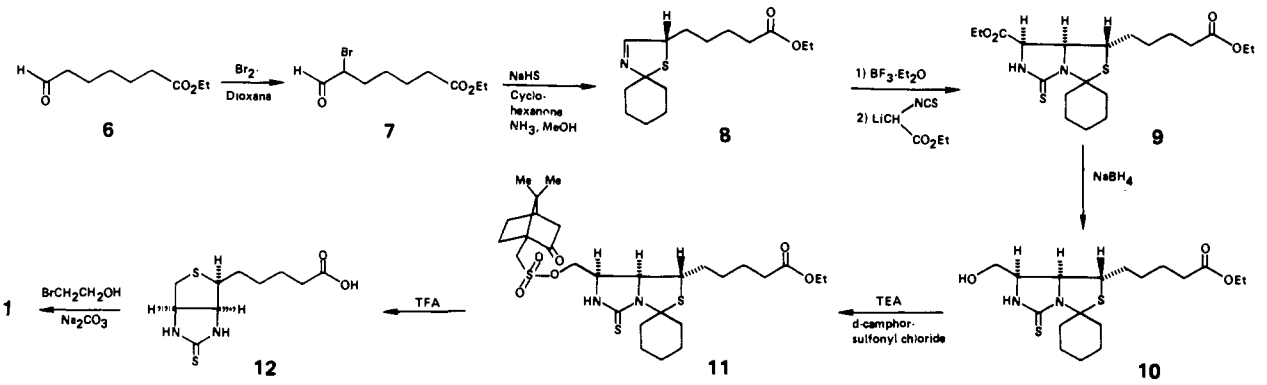
(15) Dialkylboron enolates play an important role in establishing the stereochemical course of many aldol condensations. While the existence of the difluoroboron enolate shown in Scheme II is unsubstantiated, it is not without precedent. β-Diketones, for example, react readily with BF_3 to generate HF and a stabilized vinyloxyborane species. See: (a) Morgan, G. T.; Tunstall, R. B. *J. Chem. Soc.* **1924**, *125*, 1963–1967. (b) Schiffman, B.; Staskun, B. *Tetrahedron, Suppl.* **1966**, *7*, 115–125.

(16) While we were unable to determine the geometry of the enolates of the isothiocyanatoacetate esters, we assume they possess the *Z* geometry on the basis of their structural similarity to known lithiopropionates (see: Heathcock, C. H.; Buse, C. T.; Kleschick, W. A.; Pirrung, M. C.; Sohn, J. E.; Lampe, J. *J. Org. Chem.* **1980**, *45*, 1066–1081).

(17) Heathcock, C. H.; Pirrung, M. C. *J. Org. Chem.* **1980**, *45*, 1727–1728.

(18) Meyers, A. I.; Reider, P. J. *J. Am. Chem. Soc.* **1979**, *101*, 2501–2502.

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.

Acknowledgment. We express our gratitude to colleagues at Pfizer for helpful suggestions. We also thank Professors E. J. Corey and D. S. Kemp for stimulating discussions.

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

(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.

(23) Singh, H.; Singh, P.; Malhotra, N. *Synth. Commun.* **1980**, *10*, 591–594. Barton, D. H. R.; Ley, S. V.; Meerholz, C. A. *Chem. Commun.* **1979**, 755–756. Mukherjee, R. *Indian J. Chem., Sect. B* **1977**, *15B*, 502–505. Additional references are cited in these papers.

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

(1) Wüthrich, K.; Wider, G.; Wagner, G.; Braun, W. *J. Mol. Biol.* **1982**, *155*, 311–319.

(2) Deisenhofer, J.; Steigemann, W. *Acta Crystallogr., Sect. B* **1975**, *31*, 238–250.

(3) Wagner, G.; Wüthrich, K. *J. Mol. Biol.* **1982**, *155*, 347–366.

(4) Brown, L. R.; DeMarco, A.; Richarz, R.; Wagner, G.; Wüthrich, K. *Eur. J. Biochem.* **1978**, *88*, 87–95. Brown, L. R.; DeMarco, A.; Wagner, G.; Wüthrich, K. *ibid.* **1976**, *62*, 103–197.

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

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