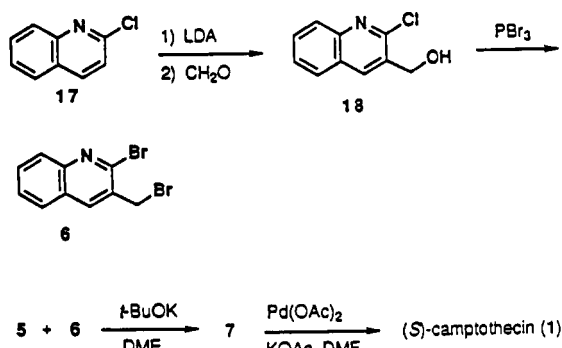


Scheme III



for large-scale preparation of active derivatives. Furthermore, only the (*S*) enantiomer of **1** exhibits biological activity, which limits the usefulness of racemic syntheses. To date three enantioselective syntheses of **1** have been reported,⁷ two of which involve a resolution.^{7a,b} Given the emerging medicinal value of the camptothecins, a practical asymmetric synthesis amenable to analog preparation is needed. We now report such a process that uses α -amino alkoxide directed lithiation and the Heck reaction in key steps.

The synthetic plan called for preparing enantiopure **D** and **E** rings **5** and bromoquinoline **6**, combining the two fragments through N-alkylation to provide intermediate **7**, and concluding with a C-ring cyclization to give **1** (Scheme I).

The hydroxylactone **5**⁸ was prepared enantioselectively in six steps starting from commercially available 2-chloro-6-methoxy-pyridine (**8**) as shown in Scheme II. Directed lithiation (*t*-BuLi, THF, -78 °C, 1 h) and trapping with formamide **9**⁹ gave an α -amino alkoxide in situ. Addition of *n*-butyllithium (-23 °C, 2 h) effected α -amino alkoxide directed lithiation¹⁰ to give dianion **10**, which on reaction with iodine provided a 78% yield of aldehyde **11**, mp 129–130 °C. Methyl ether **12**, mp 74–75 °C, was prepared in 92% yield from **11** in one step on treatment with MeOH, triethylsilane, and TFA.¹¹ Lithium halogen exchange (THF, -78 °C, 1 min) and addition of α -keto ester **13**, prepared from α -ketobutyric acid and (-)-8-phenylmenthol, afforded the addition product as a lithium alkoxide in situ.¹² Trapping with 4-phenylbenzoyl chloride (room temperature, 36 h) provided a solid product (87% de), which on recrystallization from petroleum ether gave diastereomerically pure **14** as a white crystalline solid, mp 100–103 °C, in 60% yield. Saponification of diester **14** with 2 N NaOH/EtOH provided a 76% yield of hydroxy acid **15** as a colorless oil. Prior to acidification, extraction of the saponification reaction mixture with ether gave a near-quantitative recovery of the chiral auxiliary, (-)-8-phenylmenthol. Without purification, **15** was treated with TMSCl/NaI (CH₃CN, Dabco, reflux, 5 h) followed by hydrolysis with 6 N HCl (100 °C, 4 h). Workup provided hydroxylactone **16** in 77% yield as an off-white solid [mp

219–220 °C; [α]_D²³ +58.5° (*c* 0.85, MeOH)]. Catalytic hydrogenation (Pd/C, NaOAc, MeOH) effected the removal of the C-6 chloro group to give the desired intermediate **5** [mp 233–234 °C; [α]_D²³ +105.2° (*c* 1.0, MeOH)] in 95% yield.

The bromoquinoline **6** was prepared in two steps from 2-chloroquinoline (**17**) (Aldrich). Lithiation¹³ at C-3 with LDA and trapping with formaldehyde gave the alcohol **18** (Scheme III). Bromination of **18** with PBr₃ provided the desired dibromo compound **6**¹⁴ in high yield. Intermediates **5** and **6** were joined through N-alkylation using *t*-BuOK/DME (reflux, 48 h) to give an 87% yield of the tetracyclic intermediate **7** [mp 115–116 °C; [α]_D²³ +48.7° (*c* 1.0, CHCl₃)]. The C ring was closed using a Heck reaction¹⁵ (Pd(OAc)₂, Bu₄N⁺Br⁻, KOAc, DMF, 90 °C, 3 h), which provided a 59% yield of (*S*)-camptothecin (**1**) [mp 272–275 °C; [α]_D²³ +42.3° (*c* 0.36, CHCl₃/MeOH, 4:1)] [lit.^{7a} mp 275–278 °C dec; lit.^{7c} [α]_D²³ +42.0° (*c* 0.51, CHCl₃/MeOH, 4:1)]. Our synthetic (*S*)-camptothecin was identical in every respect with authentic material.¹⁶

This synthesis was carried out in 10 steps from commercially available materials.¹⁷ The overall yield of **1** from pyridine **8** and bromoquinoline **6** was 12%. Work is in progress on the enantioselective synthesis of other members of the camptothecin family.

Acknowledgment. We gratefully acknowledge support of this work by Glaxo, Inc.

Supplementary Material Available: Listings giving full spectroscopic and analytical characterizations of **5**, **7**, **10**–**14**, and **16** (7 pages). Ordering information is given on any current masthead page.

(13) Marsais, F.; Godard, A.; Queguiner, G. *J. Heterocycl. Chem.* **1989**, 26, 1589.

(14) The dibromide **6** has previously been prepared in four steps from isatin. Lyle, R. E.; Portlock, D. E.; Kane, M. J.; Bristol, J. A. *J. Org. Chem.* **1972**, 37, 3967.

(15) Grigg, R.; Sridharan, V.; Stevenson, P.; Sukirthalingam, S.; Worakun, T. *Tetrahedron* **1990**, 46, 4003.

(16) A sample of natural camptothecin was kindly supplied by Glaxo, Inc., Research Triangle Park, NC.

(17) Comins, D. L.; Baevsky, M. F. U. S. Patent Appl. Nos. 07/632,970, 1990, and 07/900,650, 1992.

Stereoselective Addition of (Triphenylsilyl)magnesium Bromide to Chiral 1-Acyl-4-methoxypyridinium Salts. Synthesis and Reactions of Enantiopure 1-Acyl-2-(triphenylsilyl)-2,3-dihydro-4-pyridones

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Reaction of chiral 1-acylpyridinium salt **1**, prepared in situ from 4-methoxy-3-(triisopropylsilyl)pyridine^{1a} and the chloroformate of (-)-8-phenylmenthol,² with aliphatic Grignard reagents gives 2,3-dihydro-4-pyridones **2** in high yield and 81–92% de (eq 1).¹

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(1) (a) Comins, D. L.; Goehring, R. R.; Joseph, S. P.; O'Connor, S. *J. Org. Chem.* **1990**, 55, 2574. (b) Comins, D. L.; Dehghani, A. *Tetrahedron Lett.* **1991**, 32, 5697. (c) Comins, D. L.; Hong, H. *J. Am. Chem. Soc.* **1991**, 113, 6672. (d) Comins, D. L.; Hong, H.; Salvador, J. M. *J. Org. Chem.* **1991**, 56, 7197. (e) Comins, D. L.; LaMunyon, D. H. *J. Org. Chem.* **1992**, 57, 5807.

(2) Optically pure (-)-8-phenylmenthol was purchased from Aldrich Chemical Co. or prepared by a literature procedure: Ort, O. *Org. Synth.* **1987**, 65, 203.

(6) Recent synthetic efforts: (a) Curran, D. P.; Liu, H. *J. Am. Chem. Soc.* **1992**, 114, 5863. (b) Ejima, A.; Terasawa, H.; Sugimori, M.; Tagawa, H. *J. Chem. Soc., Perkin Trans. 1* **1990**, 27. (c) Earl, R. E.; Vollhardt, K. P. C. *J. Org. Chem.* **1984**, 49, 4786. (d) Ihara, M.; Noguchi, K.; Ohsawa, T.; Fukumoto, K.; Kametani, T. *J. Org. Chem.* **1983**, 48, 3150.

(7) (a) Corey, E. J.; Crouse, D. N.; Anderson, J. E. *J. Org. Chem.* **1975**, 40, 2140. (b) Wani, M. C.; Nicholas, A. W.; Wall, M. E. *J. Med. Chem.* **1987**, 30, 2317. (c) Ejima, A.; Terasawa, H.; Sugimori, M.; Tagawa, H. *Tetrahedron Lett.* **1989**, 30, 2639.

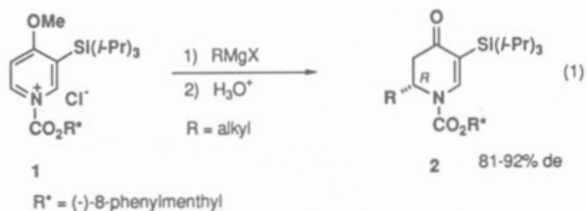
(8) Although not isolated, pyridone **5** was reported as a product, on the basis of UV absorption data, from the photolysis of 7-chloro-2-oxo-3-ethyl-3-hydroxy-3,6-dihydropyrido[5,4-*c*]pyran. Lyle, R. E.; Bristol, J. A.; Kane, M. J.; Portlock, D. E. *J. Org. Chem.* **1973**, 38, 3268.

(9) Einhorn, J.; Luche, J. L. *Tetrahedron Lett.* **1986**, 27, 1791.

(10) (a) Comins, D. L.; Killpack, M. O. *J. Org. Chem.* **1990**, 55, 69. (b) For a review of α -amino alkoxide directed lithiations, see: Comins, D. L. *Synlett* **1992**, 615.

(11) Doyle, M. P.; DeBruyn, D. J.; Kooistra, D. A. *J. Am. Chem. Soc.* **1972**, 94, 3658.

(12) For the diastereoselective addition of nucleophiles to α -keto-8-phenylmenthyl esters, see: (a) Whitesell, J. K.; Deyo, D.; Bhattacharya, A. *J. Chem. Soc., Chem. Commun.* **1982**, 989. (b) Whitesell, J. K.; Bhattacharya, A.; Henke, K. *J. Chem. Soc., Chem. Commun.* **1982**, 988.



The absolute stereochemistry of the new stereogenic center at C-2 was shown to be the (*R*) configuration by NMR and single-crystal X-ray analysis.^{1a} To expand the scope of this asymmetric synthesis, we investigated the reaction of (triphenylsilyl)magnesium bromide³ (3) with chiral 1-acylpyridinium salts (Scheme I). The initial reaction was performed using 4-methoxy-3-(trimethylsilyl)pyridine⁴ (4), (-)-8-phenylmenthyl chloroformate, and 3 in toluene/THF at -78°C to give the desired dihydropyridone 5 in 73% yield. The diastereoselectivity of this reaction was determined to be 96% by HPLC analysis. When the analogous reaction was carried out using 4-methoxypyridine (6), an 88% yield of 7 was obtained, again with a diastereoselectivity of 96%. Interestingly, unlike our earlier work,¹ a trialkylsilyl blocking group is not needed at C-3 of the pyridine ring to obtain a high degree of asymmetric induction.⁵ In addition, the absolute configuration of the newly formed stereogenic center at C-2 of dihydropyridones 5 and 7 is opposite that found in the major product of the reaction of 1 and alkyl Grignard reagents. This was determined by single-crystal X-ray analysis of 7⁶ and by conversion of 5 to 7 with HBr/HOAc.¹ The operating mechanism is obviously different than that of the asymmetric reaction of 1 and RMgX (eq 1). A working model that explains the observed stereochemistry through chelation control is shown in Figure 1.⁷

Due to the A^(1,3) strain present,⁸ the triphenylsilyl group of 7 occupies the axial position as shown by X-ray analysis and molecular modeling.⁹ Since the Ph₃Si group hovers directly above the enone system of 7, conjugate addition of an organometallic to 7 was anticipated to occur with a high degree of facial selectivity. The copper-mediated addition of methylmagnesium chloride to 7 gave a 78% yield of *trans*-piperidone 8. The reaction appears to be completely stereoselective as none of the *cis* diastereomer could be detected. To our amazement, the analogous reaction of 7 using phenylmagnesium chloride gave a 65% yield of the *cis* diastereomer 9.¹⁰ An explanation for this reversal of stereochemistry will have to await further study.

The keto function of 7 can be reduced with complete stereocontrol. Treatment of 7 with NaBH₄/CeCl₃ gave a 98% yield of the *cis* alcohol 10. When alcohol 10 in toluene was treated with BF₃·OEt₂ (2 equiv, -23°C , 5 min) followed by addition of water, a 99% yield of epimeric alcohol 11 was isolated.¹¹ The availability of both alcohol epimers holds potential for further stereocontrolled reactions directed by the C-4 hydroxyl group.

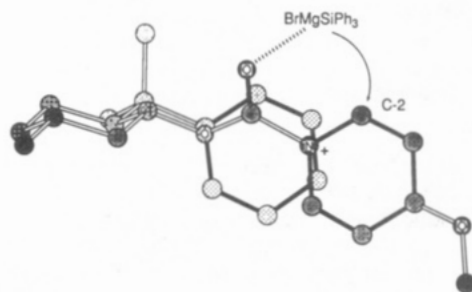
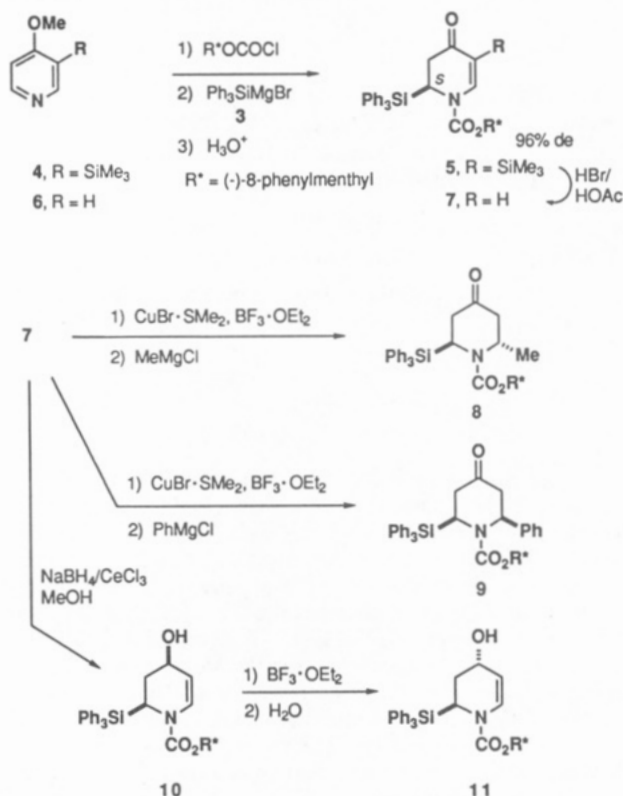
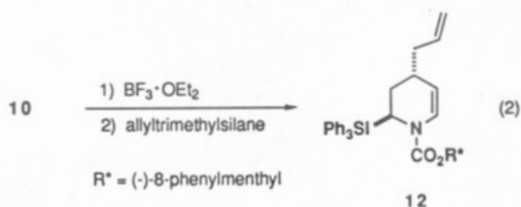


Figure 1. Working model derived from molecular mechanics (MMX).

Scheme I



Alcohols 10 and 11 are *N*-acyliminium ion precursors.¹² Treatment of 10 with BF₃·OEt₂ and allyltrimethylsilane in CH₂Cl₂ (-78 to 25°C) gave enecarbamate 12 in 83% yield (eq 2). By NMR analysis, the stereoselectivity and regioselectivity of this reaction are greater than 96%.



Once the C-2 triphenylsilyl group has been utilized to control stereoselective reactions at the C-6 or C-4 positions of 7, it can be removed from the piperidine ring by treatment with tetrabutylammonium fluoride.¹³ The high stereocontrol inherent in this methodology should be useful for the enantioselective synthesis of various alkaloid ring systems. Efforts in this direction are underway in our laboratories.¹⁴

(3) The Ph₃SiMgBr was formed by the addition of magnesium bromide to Ph₃SiLi. For the preparation of the lithium reagent, see: George, M. V.; Peterson, D. J.; Gilman, H. J. *Am. Chem. Soc.* **1960**, *82*, 403.

(4) Comins, D. L.; LaMunyon, D. H. *Tetrahedron Lett.* **1988**, *29*, 773.

(5) The reaction of 4-methoxypyridine, (-)-8-phenylmenthyl chloroformate, and phenylmagnesium chloride proceeds in only 30% de. See ref 1.

(6) Singh, P.; Comins, D. L.; Killpack, M. O. *Acta Crystallogr.* **1990**, *C46*, 1955.

(7) The dihedral angle between the carbonyl oxygen and the closest carbon (C-2) of the pyridinium ring was calculated (MMX) to be 47° . Chelation-controlled delivery of the Ph₃Si anion to the β -face of the pyridinium ring at C-2 leads to the observed major diastereomer.

(8) For a discussion of A^(1,3) strain in 1-acylpiperidines, see: Brown, J. D.; Foley, M. A.; Comins, D. L. *J. Am. Chem. Soc.* **1988**, *110*, 7445 and references therein.

(9) Molecular modeling was performed using PCMODEL (Serena Software, Bloomington, IN) and Chem3D (Cambridge Scientific Computing, Inc., Cambridge, MA). The MMX derived structure is very similar to that obtained by single-crystal X-ray analysis. See the supplementary material and ref 6.

(10) The structural assignment for 9 was made by single-crystal X-ray analysis. For data, see the supplementary material.

(11) The mechanism undoubtedly involves the formation of an *N*-acyliminium ion and attack by water at C-4 on the face anti to the Ph₃Si group.

(12) (a) Kozikowski, A. P.; Park, P.-u. *J. Org. Chem.* **1984**, *49*, 1674. (b) Comins, D. L.; Abdullah, A. H. *Tetrahedron Lett.* **1985**, *26*, 43. (c) Comins, D. L.; Foley, M. A. *Tetrahedron Lett.* **1988**, *29*, 6711.

(13) Comins, D. L.; Zeller, E. Unpublished results.

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Supplementary Material Available: Listings giving full spectroscopic and analytical characterization of 5 and 7-12 and tables of X-ray crystallographic data, including thermal and positional parameters, bond lengths, and bond angles for 8 and 9 (49 pages). Ordering information is given on any current masthead page.

(14) All new compounds were spectroscopically characterized and furnished satisfactory elemental analyses (C, H, N $\pm 0.4\%$) or high-resolution mass spectra. Details are provided in the supplementary material.

An Efficient Triple Resonance Experiment Using Carbon-13 Isotropic Mixing for Determining Sequence-Specific Resonance Assignments of Isotopically-Enriched Proteins

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Sequence-specific resonance assignments provide the basis for interpreting multidimensional NMR spectra and for determining 3D structures of proteins from these data.¹ A key step in this assignment procedure is the identification of amino acid spin systems which are sequential in the protein sequence. This is generally done using multidimensional nuclear Overhauser effect (NOE) spectroscopy (NOESY).^{1,2} However, as NOEs arise between proton pairs separated by less than about 5 Å, NOESY cross peaks are observed not only between resonances of sequential residues but also for intraresidue and longer-range interactions. In proteins with severe chemical shift degeneracies, this extra information complicates the identification of sequential connections between amino acid spin systems. Several experiments have been described³ which overcome this problem by using one- and two-bond scalar couplings to correlate backbone (e.g., ¹³C α and H α) resonances of residue $i-1$ with backbone (e.g., ¹⁵N and H α) resonances of residue i . However, these experiments cannot be

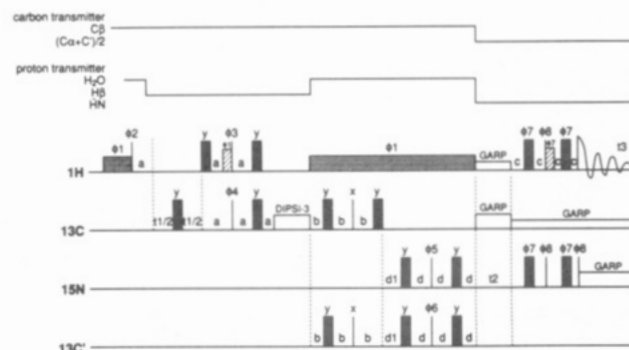


Figure 1. Pulse sequence of the 3D ¹⁵N-edited HC(C)(CO)NH-TOCSY experiment. For pulses applied at transmitter frequencies corresponding to the center of the aliphatic (¹³C) or carbonyl (¹³C') regions of the carbon-13 spectrum, the 90° and 180° pulse widths were adjusted to provide an excitation null at carbonyl and aliphatic resonance frequencies, respectively. Pulses were applied at the carbonyl resonance frequency by appropriate phase modulation through a waveform generator⁶ while the synthesizer frequency was maintained at the ¹³C β carbon resonance frequency. Coherence transfer delays were tuned to $a = 1.5$ ms, $b = 3.2$ ms, $c = 2.7$ ms, $d_1 = 10.3$ ms, and $d = 13.5$ ms, respectively. Carbon-13 isotropic mixing was done using DIPSI-3.⁷ During the evolution time t_2 the ¹H and ¹³C spins were decoupled from ¹⁵N, and during t_3 the aliphatic ¹³C, carbonyl ¹³C, and ¹⁵N spins were decoupled from ¹H, using GARP.⁸ GARP was also used to simultaneously decouple aliphatic and carbonyl ¹³C spins from ¹⁵N during the reverse-refocused INEPT transfer step that is between the t_2 and t_3 evolution times. These multipulse spin-lock and decoupling schemes were executed using waveform generators. Time proportional phase increments (TPPI) of 90° were used to obtain pure phase spectra. The pulse phases were cycled as follows: $\phi_1 = 16(+x)$, $16(-x)$; $\phi_2 = +x$ with TPPI(¹H); $\phi_3 = 4(+y)$, $4(-y)$; $\phi_4 = +x, -x$; $\phi_5 = +x, +x, -x, -x$; $\phi_6 = 8(+x)$, $8(-x)$; $\phi_7 = +y$ with TPPI(¹⁵N); $\phi_8 = +x$ with TPPI(¹⁵N); and the receiver phase as $+x, -x, -x, +x, -x, +x, +x, -x, -x, +x, +x, -x, +x, -x, +x$, with TPPI(¹⁵N). Solvent suppression was done using appropriately placed 0.5–1.0-ms trim pulses (diagonally-hatched bars) and weak selective irradiation (horizontally-hatched bars) of the H₂O resonance as shown in the pulse sequence.

used reliably for the common situation in which there are chemical shift degeneracies of both the ¹³C α and H α resonances between amino acid residues. Recently, the CBCANH⁴ and CBCA(CO)NH⁵ experiments have been described, which overcome this problem using correlations between the backbone ¹⁵N and H α resonances of residue i and the α and β resonances of residues $i-1$. While these experiments can provide data that is useful for determining sequential resonance assignments, the CBCANH⁴ experiment relies on relatively small $^2J(^{13}\text{C}_{\alpha-1}-^{15}\text{N}_i)$ coupling constants for coherence transfer across the peptide bond and provides sequential cross peaks for only 50–75% of the spin systems in small (<15 kDa) proteins. Although more efficient sequential coherence transfer is obtained in the CBCA(CO)NH⁵ experiment, this multiple relay experiment does not provide sequential connectivity information for peripheral side chain resonances beyond the C β position.

In order to provide a more complete set of connections between spin systems of sequential amino acids, we have developed a powerful experiment called HC(C)(CO)NH-TOCSY. This triple resonance experiment uses carbon-carbon isotropic mixing to move magnetization from the peripheral side chain proton and carbon nuclei to the backbone C α nucleus, through the carbonyl carbon, and to the backbone nitrogen and amide proton of the next residue in the sequence, resulting in selective detection of sequential magnetization transfer. A 3D ¹⁵N-edited HC(C)(CO)NH-TO-

[†] Rutgers University.

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(1) (a) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986. (b) Clore, G. M.; Gronenborn, A. M. *Science* 1991, 252, 1390–1399.

(2) (a) Dubs, A.; Wagner, G.; Wüthrich, K. *Biochim. Biophys. Acta* 1979, 577, 177–194. (b) Wagner, G.; Anil Kumar, B.; Wüthrich, K. *Eur. J. Biochem.* 1981, 114, 375–384. (c) Billeter, M.; Braun, W.; Wüthrich, K. *J. Mol. Biol.* 1982, 155, 321–346. (d) Wagner, G.; Wüthrich, K. *J. Mol. Biol.* 1982, 155, 347–366.

(3) (a) Montelione, G. T.; Wagner, G. *J. Am. Chem. Soc.* 1989, 111, 5474–5475. (b) Montelione, G. T.; Wagner, G. *J. Magn. Reson.* 1990, 87, 183–188. (c) Ikura, M.; Kay, L. E.; Bax, A. *Biochemistry* 1990, 29, 4659–4667. (d) Kay, L. E.; Ikura, M.; Tschudin, R.; Bax, A. *J. Magn. Reson.* 1990, 89, 496–514. (e) Clore, G. M.; Bax, A.; Driscoll, P. C.; Wingfield, P. T.; Gronenborn, A. M. *Biochemistry* 1990, 29, 8172–8184. (f) Powers, R.; Gronenborn, A. M.; Clore, G. M.; Bax, A. *J. Magn. Reson.* 1991, 94, 209–213. (g) Kay, L. E.; Ikura, M.; Zhu, G.; Bax, A. *J. Magn. Reson.* 1991, 91, 422–428. (h) Bax, A.; Ikura, M. *J. Biomol. NMR* 1991, 1, 99–109. (i) Kay, L. E.; Ikura, M.; Bax, A. *J. Magn. Reson.* 1991, 91, 84–92. (j) Wagner, G.; Schmieder, P.; Thanabal, V. *J. Magn. Reson.* 1991, 93, 436–440. (k) Clubb, R. T.; Thanabal, V.; Wagner, G. *J. Biomol. NMR* 1992, 2, 203–210. (l) Boucher, W.; Laue, E. D.; Campbell-Burk, S.; Domaille, P. J. *J. Am. Chem. Soc.* 1992, 114, 2262–2264. (m) Palmer, A. G.; Fairbrother, W. J.; Cavanagh, J.; Wright, P. E.; Rance, M. *J. Biomol. NMR* 1992, 2, 103–108. (n) Farmer, B. T.; Venters, R. A.; Spicer, L. D.; Wittekind, M. G.; Müller, L. *J. Biomol. NMR* 1992, 2, 195–202.

(4) Grzesiek, S.; Bax, A. *J. Magn. Reson.* 1992, 99, 201–207.

(5) Grzesiek, S.; Bax, A. *J. Am. Chem. Soc.* 1992, 114, 6291–6293.

(6) Patt, S. L. *J. Magn. Reson.* 1992, 96, 94–102.

(7) Shaka, A. J.; Lee, C. J.; Pines, A. *J. Magn. Reson.* 1988, 77, 274–293.

(8) Shaka, A. J.; Barker, P. B.; Freeman, R. *J. Magn. Reson.* 1985, 64, 547–552.

(9) Nilsson, B.; Moks, T.; Jansson, B.; Abrahmsén, L.; Elmlblad, A.; Holmgren, E.; Henrichson, C.; Jones, T. A.; Uhlén, M. *Protein Eng.* 1987, 1, 107–113.