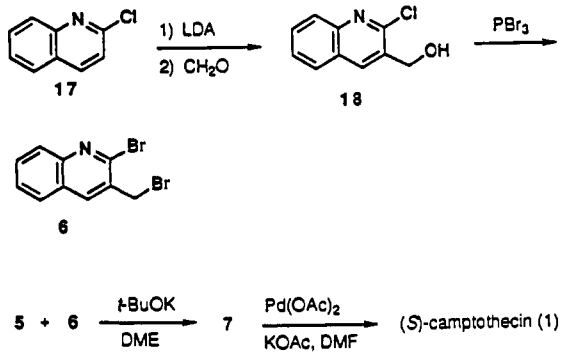
Scheme III



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-methoxypyridine (**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 lithiation[10] 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 (CH_3CN, 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; $[\alpha]^{23}_D$ +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; $[\alpha]^{23}_D$ +105.2° (*c* 1.0, MeOH)] in 95% yield.

The bromoquinoline **6** was prepared in two steps from 2-chloroquinoline (**17**) (Aldrich). Lithiation[13] at C-3 with LDA and trapping with formaldehyde gave the alcohol **18** (Scheme III). Bromination of **18** with PBr_3 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; $[\alpha]^{23}_D$ +48.7° (*c* 1.0, $CHCl_3$)]. The C ring was closed using a Heck reaction[15] ($Pd(OAc)_2$, $Bu_4N^+Br^-$, KOAc, DMF, 90 °C, 3 h), which provided a 59% yield of (*S*)-camptothecin (**1**) [mp 272–275 °C; $[\alpha]^{23}_D$ +42.3° (*c* 0.36, $CHCl_3$/MeOH, 4:1)] [lit.[7a] mp 275–278 °C dec; lit.[7c] $[\alpha]_D$ +42.0° (*c* 0.51, $CHCl_3$/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.

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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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Received October 1, 1992

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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‡ United States Air Force Academy.

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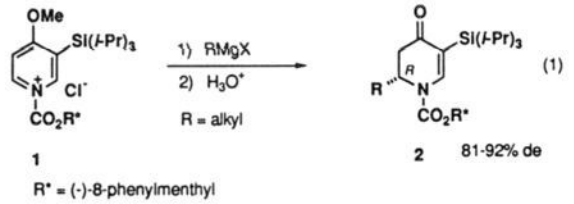


The absolute stereochemistry of the new stereogenic center at C-2 was shown to be the (*R*) configuration by NMR and single-crystal X-ray analysis.[1a] To expand the scope of this asymmetric synthesis, we investigated the reaction of (triphenylsilyl)magnesium bromide[3] (**3**) with chiral 1-acylpyridinium salts (Scheme I). The initial reaction was performed using 4-methoxy-3-(trimethylsilyl)pyridine[4] (**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 **7**[6] 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 Ph_3Si 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 $NaBH_4/CeCl_3$ gave a 98% yield of the cis alcohol **10**. When alcohol **10** in toluene was treated with $BF_3{\cdot}OEt_2$ (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.

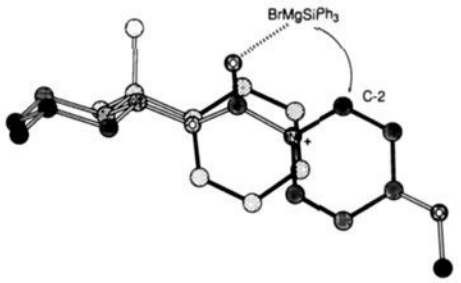
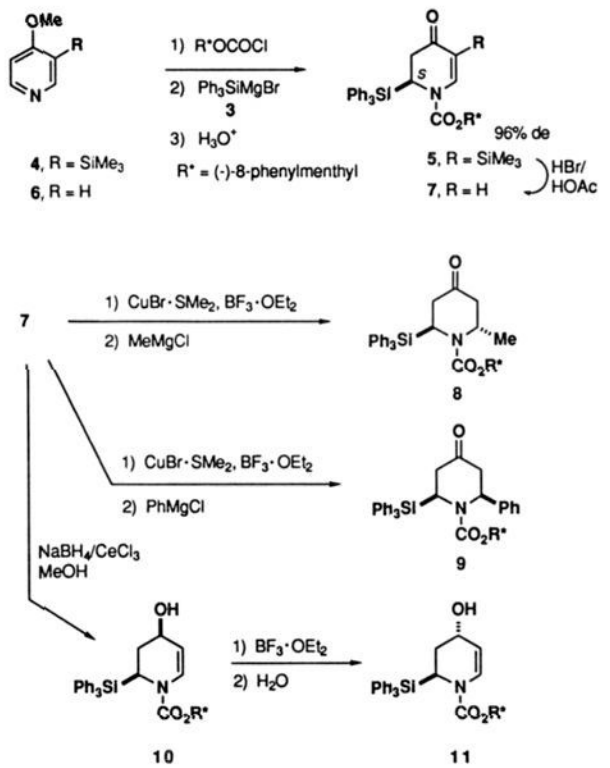
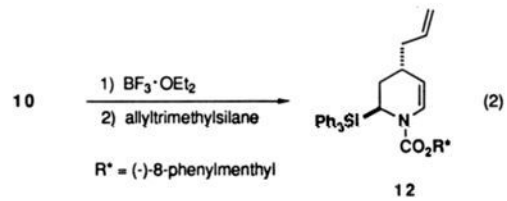


Figure 1. Working model derived from molecular mechanics (MMX).

Scheme I



Alcohols **10** and **11** are *N*-acyliminium ion precursors.[12] Treatment of **10** with $BF_3{\cdot}OEt_2$ and allyltrimethylsilane in CH_2Cl_2 (−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]

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(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 Ph_3Si anion to the β-face of the pyridinium ring at C-2 leads to the observed major diastereomer.

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

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Acknowledgment. We gratefully acknowledge support of this work by the National Institutes of Health (Grant GM 34442). M.K. also thanks the United States Air Force for support given under the Air Force Institute of Technology/Civilian Institute Program.

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 ±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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Received September 10, 1992

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 described[3] which overcome this problem by using one- and two-bond scalar couplings to correlate backbone (e.g., $^{13}C^{\alpha}$ and H^{α}) resonances of residue $i-1$ with backbone (e.g., ^{15}N and H^{N}) resonances of residue i. However, these experiments cannot be used reliably for the common situation in which there are chemical shift degeneracies of both the $^{13}C^{\alpha}$ and H^{α} resonances between amino acid residues. Recently, the CBCANH[4] and CBCA(CO)NH[5] experiments have been described, which overcome this problem using correlations between the backbone ^{15}N and H^{N} 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 CBCANH[4] experiment relies on relatively small $^{2}J(^{13}C^{\alpha}_{i-1}-^{15}N_i)$ 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)NH[5] 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 ^{15}N-edited HC(C)(CO)NH-TO-

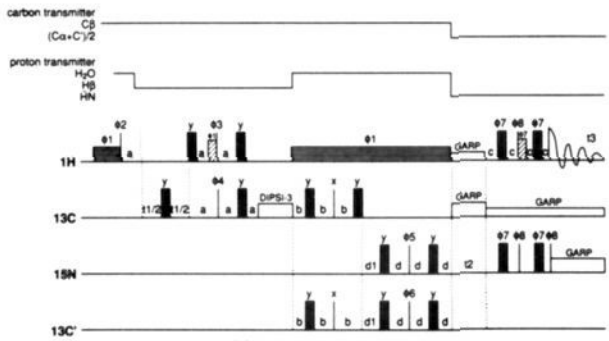

Figure 1. Pulse sequence of the 3D ^{15}N-edited HC(C)(CO)NH-TOCSY experiment. For pulses applied at transmitter frequencies corresponding to the center of the aliphatic (13C) or carbonyl (13C′) regions of the carbon-13 spectrum, the 90° and 180° pulse widths were adjusted to provide an excitation null at carbonyl and aliphatic resonance frequencies, respectively. Pulses were applied at the carbonyl resonance frequency by appropriate phase modulation through a waveform generator[6] while the synthesizer frequency was maintained at the $^{13}C^{\beta}$ 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 ^{1}H and ^{13}C spins were decoupled from ^{15}N, and during t_3 the aliphatic ^{13}C, carbonyl ^{13}C, and ^{15}N spins were decoupled from ^{1}H, using GARP.[8] GARP was also used to simultaneously decouple aliphatic and carbonyl ^{13}C spins from ^{15}N 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: $\phi1 = 16(+x), 16(-x)$; $\phi2 = +x$ with TPPI(^{1}H); $\phi3 = 4(+y), 4(-y)$; $\phi4 = +x, -x$; $\phi5 = +x, +x, -x, -x$; $\phi6 = 8(+x), 8(-x)$; $\phi7 = +y$ with TPPI(^{15}N); $\phi8 = +x$ with TPPI(^{15}N); and the receiver phase as $+x, -x, -x, +x, -x, +x, +x, -x, -x, +x, +x, +x, -x, -x, -x, +x$, with TPPI($^{15}N$). 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 H_2O resonance as shown in the pulse sequence.

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‡ UMDNJ—Robert Wood Johnson Medical School.

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