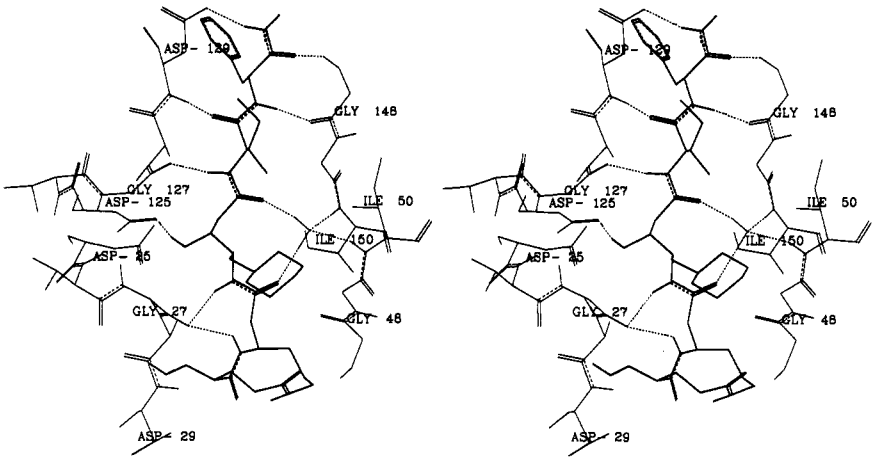

Figure 2. Stereoview of the hydrogen bonding pattern found for inhibitor **7** following molecular dynamics. This pattern is identical to that found for the HIV-1/acetylpepstatin complex.[20] For clarity, only polar hydrogens are shown.

the design are sufficient lipophilicity to allow for cell membrane penetration and resistance to proteolytic degradation.

We have designed a series of novel inhibitors that exhibit moderate to good potency against HIV-1 protease. We have incorporated a β-turn peptide mimetic unit which extends from the P_3-P_2 sites of the inhibitor. Despite the incorporation of less than optimal P_1-P_1' isosteres, which are known to play a significant role in binding affinity, and the known preference for branched residues in P_2, **7** displays 26 nM inhibition in vitro. This suggests that the turn mimetic framework may serve as an excellent foundation for the development of yet more potent HIV-1 PR inhibitors. There are several potential functions that the 11-membered ring may serve. The natural substrates of HIV-1 PR may have a propensity for a chain reversal adjacent to the cleavage site. Alternatively it may play a similar role to large heterocyclic species that can occupy the expanded S_3 site of HIV-1 PR.[29] Finally, it should be noted that recent work on the design of inhibitors for the closely related aspartic protease, renin, has also shown that replacement of the P_4-P_2 residues with various cyclic moieties provides good retention of activity, as compared to unconstrained, linear inhibitors.[38-41] Further investigations with extended substrates incorporating secondary structure mimetics will be required to evaluate their importance in HIV-1 PR specificity.

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Delineating the Pharmacophoric Elements of Huperzine A: Importance of the Unsaturated Three-Carbon Bridge to Its AChE Inhibitory Activity

While the acetylcholinesterase (AChE) inhibitor tetrahydroaminoacridine (THA) has been used in a number of clinical trials in the United States for the treatment of Alzheimer's disease (AD), results have been modest, and the studies have been hampered by its toxicity.[1] Indeed, in two primate model studies conducted by Iversen et al., THA was shown not to have a superior profile to physostigmine as a cognitive enhancer in primates.[2] Although physostigmine is also undergoing therapeutic trials, its usefulness appears to be limited by its short duration of action.[3] To the extent that AChE inhibitors can serve as useful adjuncts in the treatment of AD, two relatively new lycopodium alkaloids, huperzine A and B isolated from *Huperzia serrata* (Thunb.) Trev., a Chinese folk medicine, appear superior to THA and physostigmine.[4]

(1) Hallack, M.; Giacobini, E. Physostigmine, tacrine and metrifonate: the effect of multiple doses on acetylcholine metabolism in rat brain. *Neuropharmacology* **1989**, *28*, 199–206.
(2) Rupniak, N. M. J.; Field, M. J.; Samson, N. A.; Steventon, M. J.; Iversen, S. D. Direct comparison of cognitive facilitation by physostigmine and tetrahydroaminoacridine in two primate models. *Neurobiol. Aging* **1990**, *11*, 609–613.
(3) Becker, R. E.; Giacobini, E. Mechanisms of cholinesterase inhibition in senile dementia of the Alzheimer type: clinical, pharmacological, and therapeutic aspects. *Drug. Dev. Res.* **1988**, *12*, 163–195.

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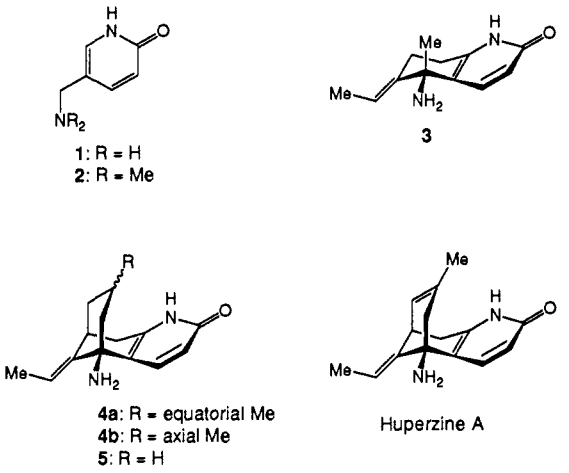
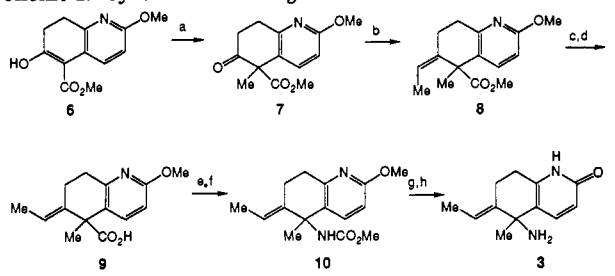


Figure 1. Structures of huperzine A and its analogues.

Scheme I. Synthesis of Analogue 3[a]

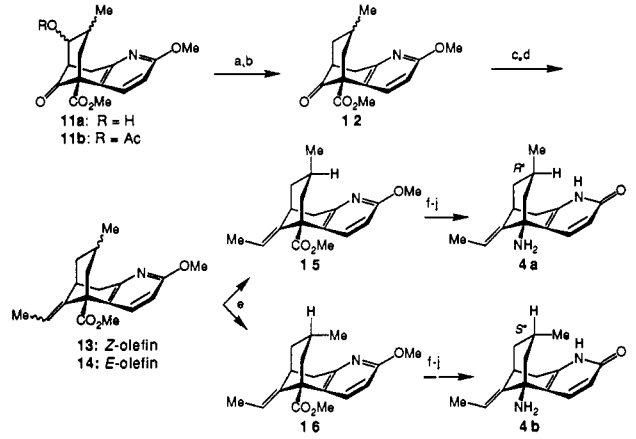


[a] Reagents and Conditions: (a) NaH, MeI, THF, room temperature, 19 h (68%); (b) $Ph_3P^+CH_2CH_3Br^-$, KO*t*-Bu, THF, −35 °C, 1 h, then room temperature, 16 h (*Z*:*E* = 10:1, 53%); (c) PhSH, AIBN, toluene, 90 °C, 21 h; (d) 20% NaOH, THF, MeOH, reflux, 20 h (71% overall); (e) $(PhO)_2P(O)N_3$, Et_3N, toluene, reflux, 1 h; (f) MeOH, reflux, 14 h (34%); (g) Me_3SiI, $CHCl_3$, reflux, 8 h; (h) MeOH, reflux, 18 h (58%).

To delineate the pharmacophoric elements of huperzine A with the possibility of arriving at more easily synthesized analogues still possessing AChE inhibitory activity, we have investigated recently the activity of the conformationally more flexible aminomethyl substituted pyridones **1** and **2** (see Figure 1).[5] Neither of these compounds was an effective cholinesterase inhibitor. The IC_{50}'s for **1** and **2** were determined to be >900 μM (the IC_{50} for natural huperzine A is 0.44×10^{-7} M). These findings would suggest that conformational constraints, hydrophobic binding forces, and steric and electrostatic fields provided by one or both of the other two rings present in the huperzine A structure must contribute to its AChE inhibitory activity.

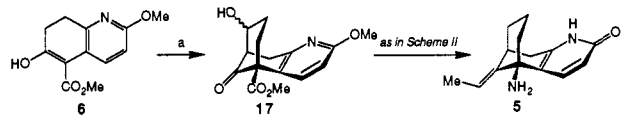
To discern the contributions that these two rings which are absent in **1** and **2** make to AChE inhibitory activity, we chose to synthesize four new analogues **3**, **4a**, **4b**, and **5**, for study. Compound **3** embodies similar conformational constraints as the quinolinone portion of huperzine A and would allow us to evaluate the necessity of the three-carbon bridge. Compounds **4a** and **4b** would permit an evaluation of the contribution of the endocyclic double bond to AChE inhibitory activity, while compound **5** would provide a measure of the C-15 methyl group contribution.

Scheme II. Synthesis of Analogues 4a and 4b[a]



[a] Reagents and Conditions: (a) $(imd)_2C{=}S$, THF, 70 °C, 24 h (88%); (b) *n*-Bu_3SnH, AIBN, PhMe, 125 °C, 2.5 h (92%); (c) $Ph_3P^+CH_2CH_3Br^-$, KO*t*-Bu, THF, 4 h, room temperature (74%); (d) PhSH, AIBN, PhMe, 100 °C, 24 h (93%); (e) separate by silica gel chromatography to get pure **15**, then resubmit mixture of **16** + **13**/**14** to conditions of d to get pure **16** after chromatography; (f) 20% NaOH, THF, MeOH, 90 °C, 24 h (70%); (g) $(PhO)_2P(O)N_3$, Et_3N, PhMe, 90 °C, 3 h; (h) MeOH, 70 °C, 11 h (67%); (i) TMSI, $CHCl_3$, 65 °C, 10 h; (j) MeOH, 65 °C, 2.5 h (68%).

Scheme III. Synthesis of Analogue 5[a]



[a] Reagents and Conditions: (a) acrolein, CH_2Cl_2, tetramethylguanidine, −78 °C, 30 min, then warm to room temperature over 1 h, and stir at room temperature for 2 h (84%).

Chemistry

To prepare analogue **3**, the known β-keto ester **6**[6] was first subjected to a C-methylation reaction using sodium hydride/methyl iodide in THF (Scheme I). Next, a *Z*-selective Wittig reaction was carried out to provide predominantly olefin **8** (*Z*:*E* = 10:1; in this series the *Z* isomer typically exhibits the higher field olefinic methyl group signal in the ^1H NMR spectrum). The olefin appendage was isomerized to the *E* isomer (*E*:*Z* = 9:1), and the ester was saponified to provide acid **9** of solely *E* stereochemistry after purification by silica gel chromatography. Curtius rearrangement and deprotection then completed the synthesis of **3**.

The synthesis of both **4a** and **4b** was carried out with the previously reported β-keto ester **11** used as the starting material. The hydroxyl group of **11** was removed by a Barton-type deoxygenation reaction employing 1,1′-(thiocarbonyl)diimidazole and tri-*n*-butyltin hydride.[7] Next a Wittig reaction was carried out on **12** employing ethylidenetriphenylphosphorane, and the 1:3 *E*/*Z* mixture initially formed was isomerized to an 8:1 *E*/*Z* mixture of **13**/**14** by the use of AIBN/PhSH. At this juncture it was possible to separate the *E* and *Z* isomers, and additionally to separate the C-15 *R** isomer **15** of *E*-olefin geometry from the C-15 *S** isomer **16** of *E* geometry (see Scheme II). These stereoisomers were converted individually to the corresponding dihydrohuperzine analogues **4a** and **4b** by

(4) Liu, J. S.; Zhu, Y. L.; Yu, C. M.; Zhou, Y. Z.; Han, Y. Y.; Wu, F. W.; Qi, B. F. The structures of huperzine A and B, two new alkaloids exhibiting marked anticholinesterase activity. *Can. J. Chem.* **1986**, *64*, 837–839.

(5) Kozikowski, A. P.; Xia, Y.; Reddy, E. R.; Tückmantel, W.; Hanin, I.; Tang, X. C. The synthesis of huperzine A, its analogues, and their anticholinesterase activity. *J. Org. Chem.* **1991**, *56*, 4636–4645.

(6) Xia, Y.; Kozikowski, A. P. A practical synthesis of the Chinese nootropic agent Huperzine A. *J. Am. Chem. Soc.* **1989**, *111*, 4116–4117.

(7) Barton, D. H. R.; Subramanian, R. Synthesis of deoxysugars and of deoxynucleosides from diol thiocarbonates. *J. Chem. Soc., Chem. Commun.* **1976**, 867.

Table I. AChE Inhibitory Activity of Huperzine A and Its Analogues

compound	AChE Inhibitory Activity (IC_{50})[a]
(−)-huperzine A	0.0445 ± 0.0029 μM (n = 5)
(±)-huperzine A	0.0715 ± 0.0024 μM (n = 5)
1	>900 μM (from ref 5)
2	>900 μM (from ref 5)
3	338 ± 5.2 μM (n = 3)
4a	1.63 ± 0.09 μM (n = 3)
4b	0.90 ± 0.078 μM (n = 3)
5	9.82 ± 0.4 μM (n = 3)

[a] Mean ± SEM.

base hydrolysis, Curtius rearrangement [$(PhO)_2P(O)N_3$],[8] and deprotection (TMSI). Since NMR proved inadequate for the purpose of assigning the stereochemistry of the C-15 center to these products, an X-ray analysis was carried out on one of the crystalline acetate derivatives of alcohol **11a**.[9] Subsequent chemical transformations correlated the structure of this crystalline derivative (**11b**, C-15 R^* stereochemistry) with the equatorial C-15 methyl analogue **4a**.

The chemistry required to produce analogue **5** was identical to that recorded in Scheme II with the exception that tricycle **17**, available in 84% yield from the reaction of the β-keto ester **6** with acrolein, was substituted for **11a** and step e was omitted (see Scheme III). Removal of the hydroxyl group from **17** by the Barton procedure proceeded in 49% overall yield. The Wittig reaction provided a 1:3 *E*:*Z* mixture of isomers in 84% yield which was isomerized and saponified to provide the pure *E* acid in 49% yield after silica gel chromatography. Curtius rearrangement and deprotection gave **5** in 47% overall yield for the two steps.

Biological Results

These newly synthesized compounds were tested for their abilities to inhibit the action of AChE isolated from rat brain cortex by using a modification of the protocol of Wilson et al.[10] with the exception that 10 μM ethopropazine was added to inhibit any action on butyrylcholinesterase (BuChE).[11] The IC_{50}'s of these compounds are presented in Table I together with the IC_{50}'s of (−)-huperzine A and (±)-huperzine A.

Discussion

As shown in Table I analogue **3** is at least 3800-fold less active than huperzine A. Although graphical overlay of

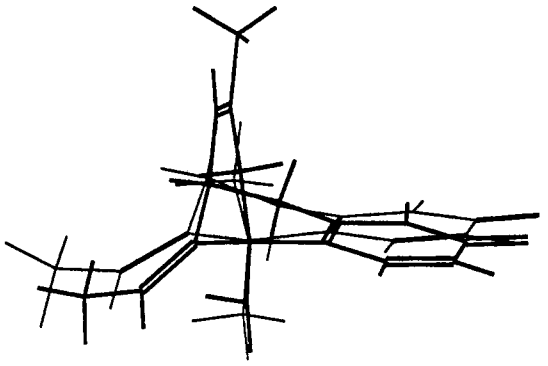


Figure 2. Superimposition of huperzine A (bold) and analogue **3** (plain).

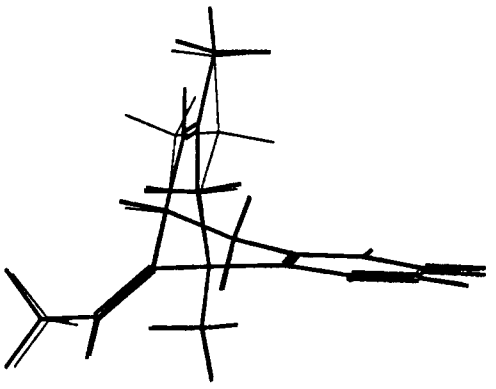


Figure 3. Superimposition of huperzine A (bold) and analogue **4a** (plain).

huperzine A with acetylcholine[5] would suggest that its three-carbon bridge may be superfluous to its AChE inhibitory activity, the poor activity of compound **3** clearly demonstrates the necessity of this bridge, since *conformationally* **3** closely mimics the quinolinone portion of huperzine A (see overlay Figure 2).[12]

The higher IC_{50} values of **4a** and **4b** compared to huperzine A which stem from the removal of the double bond of the three-carbon bridge could possibly be attributed to a combination of steric and electrostatic effects together with an entropic factor. Molecular modeling studies (SYBYL V5.41) on the evaluation of the steric field–fit energies of **4a** and **4b** reveal that the steric field of analogue **4a** closely resembles the steric field of huperzine A (see overlay Figure 3), while the steric field of **4b** deviates from that of huperzine A.[13] Since **4a** and **4b** possess similar AChE inhibitory activity, it is unlikely that steric factors originating

(8) Yamada, S.; Ninomiya, K.; Shioiri, T. Transfer of the azido function from diphenylphosphoryl azide to malonic acid half esters. *Tetrahedron Lett.* **1973**, 2343.

(9) Crystal data for 11b, acetate group axial, and methyl group equatorial: $C_{18}H_{21}NO_6$; M = 347.37; monoclinic space group $P2_1/c$; a = 14.115 (1), b = 11.419 (1), c = 10.999 (1) Å; β = 100.250 (9)°; V = 1744.5 Å^3; Z = 4; D_c = 1.322 g cm^{-3}; μ = 7.92 cm^{-1}; Cu Kα (λ = 1.54184 Å); 2θ = 30–52°. During the latter stages of refinement the non-hydrogen atoms were allowed to refine with anisotropic thermal parameters. However, due to the low data:parameter ratio only those atoms with high anisotropy were allowed to retain this anisotropic description into the final cycles. In the final cycle 182 parameters were refined using 1052 observations having $I \geq 3\sigma(I)$. The final agreement factors were R_1 = 0.063 and R_2 = 0.052. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre.

(10) Wilson, S. H.; Schrier, B. K.; Farber, E. J.; Thompson, R. W.; Rosenberg, R. W.; Blume, A. J.; Nirenberg, M. W. Markers for gene expression in cultered cells from the nervous system. *J. Biol. Chem.* **1972**, *147*, 3159–3169.

(11) McKinney, M.; Miller, J. H.; Yamada, F.; Tückmantel, W.; Kozikowski, A. P. Potencies and stereoselectivities of enantiomers of huperzine A for inhibition of rat cortical acetylcholinesterase. *Eur. J. Pharmacol.* **1991**, in press.

(12) Molecular modeling studies[13] were performed on huperzine A, **3**, and compound **3′**, an analogue of **3** lacking the methyl group at the NH_2-bearing carbon center. Since these studies revealed the conformation of **3** to be more huperzine-like than that of **3′**, it is for this reason that **3** was synthesized rather than **3′** in examining the ability of a bicyclic structure to exhibit anti-AChE activity. See supplementary material for complete details.

(13) SYBYL V5.41 (1991), Tripos Associates, Inc., 1699 S. Hanley Road, Suite 303, St. Louis, MO 63144. For quantitative details of the field fit analysis, see supplementary material.

(14) The X-ray structure of acetylcholinesterase from *Torpedo californica* has recently been published (Sussman, J. L.; Hårel, M.; Frolow, F.; Oefner, C.; Goldman, A.; Toker, L.; Silman, I. Atomic structure of acetylcholinesterase from *Torpedo californica*: a prototypic acetylcholine-binding protein. *Science* **1991**, *253*, 872–878), and we are carrying out docking studies between huperzine A and the enzyme in order to identify the amino acid residue(s) participating in the proposed electrostatic interaction of huperzine's three-carbon bridge.

from the three-carbon bridge are responsible for their reduced AChE inhibitory activity relative to that of huperzine A. On the other hand, comparison of the electrostatic fields of these compounds employing the semiempirical molecular orbital method, AM1, reveals differences in the electrostatic fields of huperzine A and analogues **4a** and **4b** in the region of the three-carbon bridge (isopotential maps and steric and electrostatic field-fit energies available in supplementary material). This result indicates that the reduced activities of **4a** and **4b** relative to that of huperzine A probably stem from the change in their electrostatic fields, and consequently reveals the function (i.e., to present the required electrostatic field to AChE) of the three-carbon bridge of huperzine A.

Again, as shown in Table I, the poor AChE activity of compound **5** can be attributed to the loss of the electrostatic contribution of the double bond together with the possible loss of hydrophobic binding due to the methyl group.

Taken together the foregoing physical properties and biological data of these newly synthesized compounds emphasize the importance of huperzine A's three-carbon bridge in presenting the required electrostatic field to the acetylcholinesterase enzyme. It is therefore unlikely that a potent AChE inhibitor can be found through extensive simplification of the huperzine A structure without considering the electrostatic field contributed by the unsaturated three-carbon bridge. Using some of the findings reported herein, progress on the design of more efficacious huperzine A analogues will be reported in due course.[14]

Acknowledgment. We are indebted to the National Institute on Aging (grant no. AG07591) for their support of our program. The authors also acknowledge helpful discussions with Dr. Werner Tückmantel.

Supplementary Material Available: Spectral data for compounds **3**, **4a**, **4b**, and **5**, full X-ray data report on compound **11b**, and complete details of the molecular modeling studies (34 pages); a listing of observed and calculated structure factors for all data (11 pages). Ordering information is given on any current masthead page.

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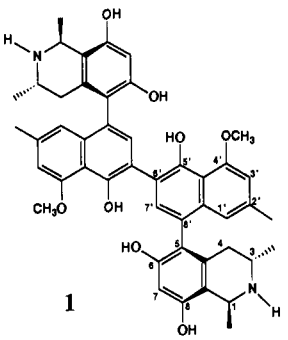
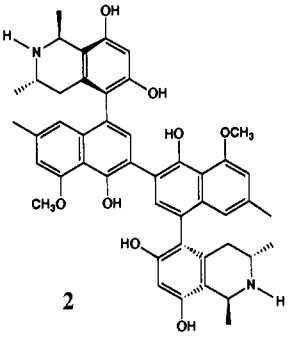
Novel Alkaloids from the Tropical Plant *Ancistrocladus abbreviatus* Inhibit Cell Killing by HIV-1 and HIV-2

The epidemic of acquired immune deficiency syndrome (AIDS) continues to present an urgent requirement for new drug development candidates with antiviral activity toward the human immunodeficiency virus (HIV). The U.S. National Cancer Institute has undertaken a major new initiative to discover novel anti-HIV agents from natural sources.[1]

In the present study, the NCI primary in vitro screen[1,2] initially disclosed anti-HIV-cytopathic activity in the organic extracts of the aerial parts of the tropical liana *Ancistrocladus abbreviatus* (Ancistrocladaceae), collected in Cameroon in March 1987. Bioassay-guided fractionation of those extracts provided the novel atropisomeric pair of anti-HIV-cytopathic alkaloids, michellamines A (**1**) and B (**2**).



1



2

The antiviral compounds were obtained in three steps. The crude extract was subjected to an acid-base partitioning scheme; the anti-HIV-cytopathic activity was concentrated in the basic fraction. This material was further separated by centrifugal partition chromatography ($CHCl_3$–CH_3OH–0.5% HBr/H_2O, 5:5:3, descending mode) and then by HPLC on an amino-bonded phase column [$CHCl_3$–0.075% $(NH_4)_2CO_3$/MeOH, 43:7] to give the two active compounds, **1** and **2**.

Plasma desorption mass spectrometry (^{252}Cf PDMS) demonstrated that the two compounds had identical molecular weights (m/z 756). The molecular formula was established as $C_{46}H_{48}N_2O_8$ by high-resolution, fast-atom-bombardment mass spectrometry. While the family Ancistrocladaceae is well known as a source of naphthalene–tetrahydroisoquinoline alkaloids,[3-5] the mass spectral data and the complex NMR spectra of our isolates suggested that they were heretofore unknown dimeric relatives of the Ancistrocladaceae alkaloids.[6]

The presence of only 23 resonances in the ^{13}C NMR spectrum of **1** indicated that the two naphthalene–isoquinoline components were equivalent. The structure and relative stereochemistry of the tetrahydroisoquinoline subunit could be readily discerned from ^1H–^1H coupling constant analyses and difference NOE experiments. The

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