

sequentially distant contacts are crucial in determining the folding of the TF1 dimer. Modeling studies based on the partially homologous *Bacillus stearothermophilus* HU protein⁷ suggest that these are intermonomeric contacts. These contacts are not seen in similar experiments for TF1-¹H.

The selective deuteration approach reported here should simplify the identification of spin systems in large proteins by using a complementary set of deuterated protein variants. The NOESY spectra of TF1-²H[FGIY] can be more accurately analyzed than those of the normally protonated protein. Spin-system identification in the TF1 23-kDa dimer (work in progress) relies more on NOE than *J*-derived connectivities, as in a recently published study of the *Escherichia coli* trp repressor, a 25-kDa dimer.⁸

In closing, we mention that the use of biosynthetically deuterated proteins for NMR studies has a long history.⁹ However, it is only recently through 2D correlated ¹H NMR spectroscopy that the benefits of deuteration for larger proteins may be fully appraised.^{10,11} The use of selectivity deuterated amino acids, instead of the fully protonated amino acids used here, as well as the use of homonuclear 3D NMR methods,¹² should increase the potential of our proposed strategy.

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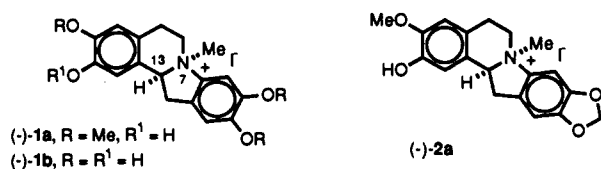
Total Synthesis of the Dibenzopyrrocoline Alkaloid (S)-(+)-Cryptaustoline. Revision of Absolute Configuration Due to an Unusual Inversion in Stereochemistry

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During studies designed to show further synthetic applications of chiral formamidines toward enantiomerically pure isoquinoline alkaloids,¹ we targeted (-)-cryptaustoline (**1a**) as a suitable goal. The latter, isolated in 1952² from *Cryptocarya bowiei* (Hook) Druce indigenous to Queensland, Australia, was one of only two dibenzopyrrocoline alkaloids obtained from this substance, the other being cryptowoline (**2a**). Stereochemical assignments and



(1) For recent representative illustrations of this methodology, see: (a) Meyers, A. I.; Guiles, J. *Heterocycles* **1989**, *28*, 295. (b) Meyers, A. I.; Bös, M.; Dickman, D. A. *Tetrahedron* **1987**, *43*, 5095. (c) Meyers, A. I.; Gottlieb, L. *J. Org. Chem.* **1990**, *55*, 5659.

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Scheme I

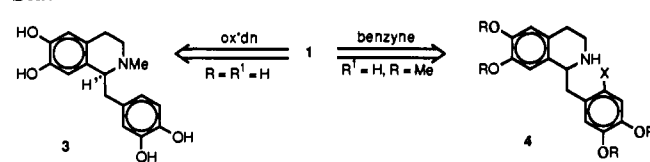


Table I. Cotton Effect of Intermediates^a

formamidine route		oxidative coupling route ^b	
compd	λ , nm (ΔE)	compd ^c	λ , nm (ΔE)
(+)- 6	286 (+1.58)	(+)- 3	298 (+1.67)
(+)- 7	286 (+0.97)	(-)- 1 (R = H)	298 (-0.94)
(+)- 1a	290 (+1.45)	(-)- 1 (R = Me)	293 (-1.18)

^a Taken in 95% ethanol. ^b References 3 and 7. ^c Refers to compounds in Scheme I.

total synthesis of **1** were reported by the Australian Group,^{2,3} two Japanese groups,^{4,5} and two American groups.^{6,7} Interestingly, the alkaloidal system was predicted by Robinson⁸ and Schopf⁹ in 1932 when they observed a facile ring closure of the racemic 1-benzylisoquinoline **3** upon treatment with oxidizing agents to give the benzopyrrocolines **1** or **2**. Many years later both Ewing et al.³ and Brossi⁷ showed that **3** can be transformed into the alkaloid by oxidative coupling using chloranil or horseradish peroxidase, respectively, and also reported that the absolute configuration of **1** was *S* at C-13. These assignments were based on completely sound conclusions since both laboratories began by using (+)-**3** with known absolute configuration and arrived at **1** with high optical activity and "complete retention of configuration" at C-13. Furthermore, a more recent study confirmed that the *N*-methyl group in **1** was *cis* to the C-13 proton using NOE techniques.^{5b} Another approach to the benzopyrrocoline system **1** is the use of a benzyne precursor,^{10,11} **4** (X = Cl, Br), which, when treated with a strong base, leads to the benzyne and undergoes an intramolecular addition by the amino group. Satisfied that the structure and stereochemistry of cryptaustoline **1a** was on firm ground, we embarked on a synthetic route to produce the "natural enantiomer *S*-(+)", in an asymmetric manner.¹

Transformation of **6**, prepared as described previously,¹ into the tetracyclic system **7** was readily accomplished by treating a solution in THF at -100 °C with 2.1 equiv of *n*-butyllithium. Following chromatography (silica gel, EtOAc-hexane-Et₃N, 3:6:1), (+)-**7** was obtained as a crystalline product [mp 131-132 °C (lit.¹⁰ mp 126-128 °C); [α]_D +48.5° (*c* 1.0, acetone)]. Methyl iodide was introduced to form the *N*-methyl quaternary salt [mp 224 °C (lit.¹⁰ mp 224-226 °C)], and then debenzoylation was performed (benzene, concentrated HCl, KI, 50 °C, 3 h) to furnish cryptaustoline **1a** [mp 256-258 °C dec (lit. racemate^{10,2} mp 258-260 °C dec)]. The specific rotation observed, however, was [α]_D +141° (*c* 0.2, EtOH), which was *opposite in sign* to the purported *S*-(-) natural product ([α]_D = -150°). On the basis of the magnitude of the rotation, the asymmetric synthesis was performed in 94% ee. Since the formamidine methodology has consistently afforded *S*-1-substituted tetrahydroisoquinolines,¹ the

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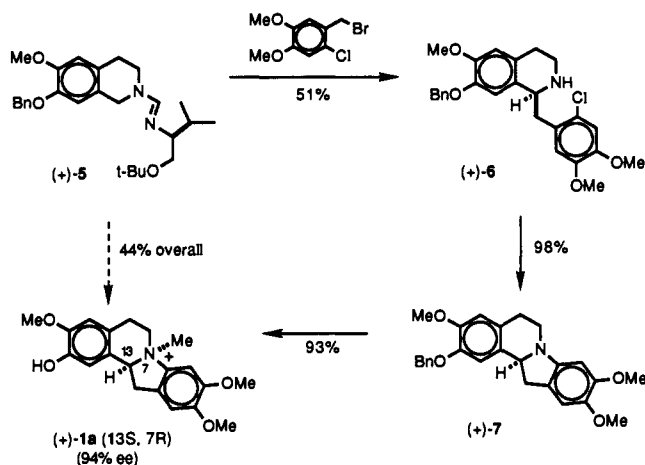
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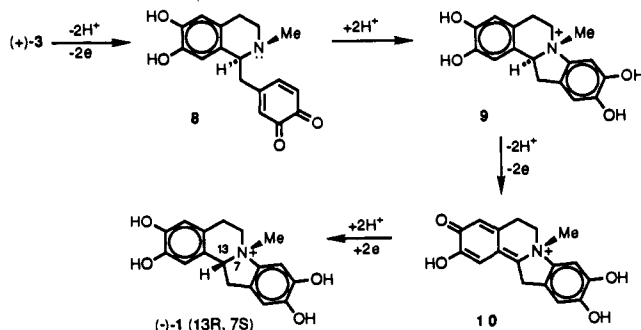
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(11) However, strong bases have been reported to racemize 1-substituted isoquinolines: Brossi, A.; Rahman, M.; Rice, K.; Gehrig, M.; Borer, R.; O'Brien, J.; Tietel, S. *Heterocycles* **1977**, *7*, 277. Tietel, S.; O'Brien, J. *J. Org. Chem.* **1976**, *41*, 1657.



question arose whether or not the natural alkaloid **1a** had been correctly assigned. As mentioned above, Brossi et al.⁷ had shown that (*S*)-(+)-laudanoline (**3**) had given optically active **1** (*R* = OH) when treated with horseradish peroxidase (Scheme I) and, after exhaustive methylation, provided the tetramethoxy derivative (Scheme I, **1**, *R* = Me). Furthermore, they methylated (-)-**1a**, the naturally isolated compound, to also provide the tetramethoxy derivative. Comparison of optical rotation of the material from both sources was in good agreement, which led to the reasonable conclusion that cryptaustoline possessed the *S* configuration at C-13. In attempts to clarify this obvious discrepancy, Cotton curves were examined for our intermediates (+)-**6**, (+)-**7**, and the final product, (+)-**1**. In all cases a positive Cotton curve was obtained (Table I). However, when we examined the Cotton curves for the route to cryptaustoline using horseradish peroxidase or the chloranil oxidation, we found that the coupled cyclized quaternary salts **1** (Scheme I) exhibited a negative Cotton effect. Furthermore, generation of the tetramethoxy derivative **1** (*R* = Me) also showed a negative Cotton effect (Table I). Thus, it was clear that *even though both routes to cryptaustoline derivatives began with a 1-benzylisoquinoline with the S configuration [(+)-3 and (+)-6], the oxidative route gave inverted stereochemistry at C-13 whereas the benzyne route retained stereochemistry.* Since the oxidative route may also be the most likely biosynthetic pathway,^{3,7-9} nature has played a devious game which caused two scientific groups to misassign the stereochemistry of these alkaloids, and only through a rational asymmetric synthesis was this uncovered.

We propose that the (1*S*)-(+)-1-benzylisoquinoline **3** undergoes oxidation to the quinone **8**, which adds in a Michael fashion to the enone in **8**, affording the trans-fused dibenzopyrrocoline **9**.



This is consistent with molecular mechanics calculations, which show that, of the four lowest energy conformations, only **8** has the correct orbital alignment to allow Michael addition. After ring closure had occurred, we examined the relative energies of trans-fused **9** and the cis-fused observed end product, **1**. According to annealed molecular dynamics calculations, the trans-fused system was 9.3 kcal (±2) less stable than the cis-fused system, the greatest contribution (~7 kcal) coming from angle strain in **9**. This energy difference could be responsible for a second

phenolic oxidation of **9** leading to the quinone, **10**. With the NMe quaternary center as the newly installed stereocenter, this anchors the absolute stereochemistry and then allows reprotonation of the reduced form, furnishing the more stable cis-ring-fused and final natural product, (-)-**1**.¹²⁻¹⁴ In summary, the natural alkaloid cryptaustoline is (1*R*,7*S*)-(-)-**1** due to the heretofore unprecedented inversion while the formamidine route led to the expected (1*S*,7*R*)-(+)-**1**.

Acknowledgment. We are grateful to Dr. Arnold Brossi for his cooperation and discussions during the course of this study. Thanks are due to Ms. Laurie Castonguay for her assistance in performing the MM2 calculations. Financial support for this work was provided by the National Science Foundation, to whom we express our gratitude.

(12) Another route of stereochemical inversion of (+)-**3** to (-)-**1** was suggested to us by Dr. Arnold Brossi. The negative gegenion (I⁻ or Br⁻) in **9** could attack the C-13 position to produce a nine-membered ring,¹³ which could reclose, giving the more stable optically active cis system. However, this experiment was attempted by placing (-)-**1** in aqueous solution, pH 4-5, similar to that employed⁷ when horseradish peroxidase was used. The molecule slowly racemized after 48 h at room temperature whereas racemization was virtually complete at reflux in 1 h. Thus, ring opening is a viable process, but only leads to racemization.

(13) Nine-membered rings have been known to be conformationally stable, which may allow for ring closure to an optically active product; cf.: (a) Magnus, P.; Ladlow, M.; Elliot, J.; Kim, C. S. *J. Chem. Soc., Chem. Commun.* 1989, 518. (b) Mislow, K.; Hyden, S.; Schaefer, H. *J. Am. Chem. Soc.* 1962, 84, 1449.

(14) Another possible inversion route could involve the ylide of **9**. However, treatment of **9** in D₂O-CDCl₃ or CF₃CO₂D for 1 h showed no evidence of D incorporation at C-13.

Stereoselectivity in Guest Release from Constrictive Binding in a Hemiacarceplex¹

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We report the syntheses of enantiomerically pure (*R*)₄-**1**-CHCl₃² (12%) and (*S*)₄-**1**-CHCl₃² (13% yield) from rigid bowl-shaped cavitand **2**³ and enantiomerically pure (*R*)-**3** and (*S*)-**3**, respectively.^{4,5} All protons in the 500-MHz NMR spectrum of (*R*)₄-**1**-CHCl₃ in CDCl₃ (except those of the eight phenyl groups) were assigned, where necessary, by ¹H-¹H COSY^{6a} or NOE difference experiments.^{6b} When **1**-CHCl₃ isomers in neat solvents were heated at the temperatures indicated, guest exchange occurred to give **1**-G (1:1 hemiacarceplexes, ¹H NMR integrations), with G = 1,4-(CH₃)₂C₆H₄ (100 °C, 18 h),⁷ CH₃CHICH₂CH₃ (70 °C, 4 h), CH₃CHOHCH₂CH₃ (in 5-(CH₃)₃C-1,3-(CH₃)₂C₆H₃, 90 °C, 24 h), and BrCH₂CH(CH₃)₂ (90 °C, 4 h). The hemiacarceplexes liberated their guests in CDCl₃ at 23 °C with *t*_{1/2} values (¹H NMR spectral changes) as follows: 1,4-

(1) We warmly thank the National Science Foundation for support through Grant NSF CHE 88 02800.

(2) Elemental analyses (C, H) were within 0.30% of theory, 500-MHz NMR spectra in CDCl₃ were structurally consistent, and FAB MS gave expected molecular ions.

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(5) The reaction was run in pure, dry (CH₃)₂NCOCH₃-Cs₂CO₃ (40 °C, argon, 5 days, double syringe pump). After isolation (evaporation at 50 °C, chromatography), the carceplex was dissolved in CHCl₃, where guest exchange occurred rapidly. The product was precipitated by addition of pentane and was dried at 25 °C at 10⁻² Torr, 3 h. For (*S*)₄-**1**-CHCl₃, [α]_D²³₄₄₅ -52.4° (c, 0.042, CHCl₃); for (*R*)₄-**1**-CHCl₃, [α]_D²³₄₄₅ +53.6° (c, 0.36, CHCl₃).

(6) (a) Derome, A. E. *Modern NMR Techniques for Chemical Research*; Pergamon Press: Oxford, 1987; Chapter 6; (b) Chapter 6.

(7) Heating **1**-CHCl₃ in CH₃CH₂C₆H₅ at 115-140 °C did not provide **1**-CH₃CH₂C₆H₅, whose CPK models cannot be assembled.