120.2, 114.9, 60.7 60.2, 14.4, 14.3, 12.0; IR (Nujol) 3436, 3326, 1709, 1682, 1269, 1250, 1128, 1101, 1065, 1024, 783 cm^{-1}; EI-MS (70 eV) m/z (relative intensity) 530 (M^+, 6.2), 484 (10.1), 438 (12.3); UV (methanol) 340 (log ϵ 4.28), 275 nm (4.21); HRMS (EI) calcd for $C_{26}H_{30}N_2O_8S$ 530.1722, found 530.1694.

2,5-Bis(4-methyl-2-pyrryl)pyrrole (13). A deaerated solution of NaOH (3 N, 15 mL) in ethanol (25 mL) was charged with **11** (0.600 g, 1.2 mmol) and heated to a gentle reflux for 12 h. The reaction was cooled to room temperature, and ethanol was removed in vacuo. Water and CH_2Cl_2 were added. The aqueous layer was separated, cooled in an ice bath, and slowly acidified to pH 4 with HOAc. The resulting mixture was filtered to yield 540 mg of a black percipitate (crude tetraacid). The crude product was dried on a mechanical vacuum pump (0.05 mmHg) overnight. Sublimation of the dry solid (200 °C, 0.1 mmHg) provided 57 mg (22%) of **13** as a white powder: mp 229-231 °C; ^1H NMR (acetonitrile-d_3) δ 9.15 (br s, 1 H), 8.91 (br s, 2 H), 6.47 (s, 2 H), 6.14 (d, J = 2.6 Hz, 2 H), 6.10 (s, 2 H), 2.06 (s, 6 H); ^{13}C NMR (methanol-d_4) δ 127.3, 127.0, 119.7, 115.6, 104.9, 104.2, 11.7; EI-MS (25 eV) m/z (relative intensity) 225 (M^+, base), 210 (14.8), 111 (13.3), 97 (19.0); UV (methanol) 325 nm (log ϵ 4.46); HRMS (EI) calcd for $C_{14}H_{15}N_3$ 225.1266, found 225.1281.

2,5-Bis(4-methyl-2-pyrryl)thiophene (14). The same general procedure as for the formation of **13** was used. Saponification of **12** (0.350 g, 0.56 mmol) yielded 0.285 g of crude tetraacid as a dark green solid. Sublimation (200 °C, 0.1 mmHg) provided 83 mg (52%) of **14** as a pale yellow powder: mp 237-238 °C; ^1H NMR (THF-d_8) δ 9.96 (br s, 2 H), 6.85 (s, 2 H), 6.47 (s, 2 H), 6.10 (s, 2 H), 2.06 (s, 6 H); ^{13}C NMR (THF-d_8) δ 134.6, 127.5, 120.7, 120.1, 117.2, 108.1, 12.0; EI-MS (25 eV) m/z (relative intensity) 244 (M + 2, 5.7), 243 (M + 1, 17.3), 242 (M^+, base), 149 (10.6); UV (methanol) 362 nm (log ϵ 4.41); HRMS (FAB) calcd for $C_{14}H_{14}N_2S$ (M + H) 243.0956, found 243.0939.

Acknowledgment. This project was supported in part by a grant from the Center for Fundamental Materials Research at Michigan State University. B.A.M. thanks Michigan State University for a Lawrence Quill Fellowship and an H. T. Graham Fellowship. The NMR data were obtained on instrumentation purchased in part with funds from NIH Grant 1-S10-RR04750-01 and from NSF Grant CHE-88-00770.

Registry No. **1a**, 86688-93-9; **1b**, 123892-38-6; **2a**, 125902-01-4; **2b**, 125902-10-5; **3a**, 125902-02-5; **3b**, 125902-11-6; **4**, 3260-45-5; **5a**, 125902-03-6; **5b**, 125902-12-7; **6**, 125902-04-7; **7**, 125902-05-8; **8**, 2199-60-2; **10**, 125902-06-9; **11**, 125926-48-9; **12**, 125902-07-0; **13**, 125902-08-1; **14**, 125902-09-2; $H_2C{=}CHSO_2CH{=}CH_2$, 77-77-0; 2-formylpyrrole, 1003-29-8.

Supplementary Material Available: ^1H NMR and ^{13}C NMR spectra for all of the compounds reported in the Experimental Section (30 pages). Ordering information is given on any current masthead page.

An Unusual Fischer Indole Synthesis with 4-Keto Acids: An Indole Incorporating the Terminal Hydrazine Nitrogen

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Received August 28, 1989

During preparation of a pharmaceutically active, N-benzylated indole derivative from 4-keto acid and N_1-benzylated phenylhydrazine precursors, the N-unsubstituted indole analogue arose as a significant byproduct. The proportion of debenzylated indole was greater with α-alkylated rather than straight-chain keto acids and the byproduct was fully suppressed when a keto ester was substituted for the keto acid. The benzylic group was shown to have eliminated as the amine and ^{15}N label incorporation demonstrated terminal phenylhydrazine nitrogen incorporation in the indole byproduct only, an exception to the usual course of the Fischer indolization reaction. A ring-chain equilibration in the ketimino acid intermediate is proposed to account for the competing pathway.

Introduction

The mechanism of the Fischer indole synthesis has been the subject of investigations by numerous workers.[2] In particular, isotopic labeling studies clearly established the N_1 (aryl) nitrogen atom of phenylhydrazine precursors as that incorporated into the indole nucleus.[3] But when re-aromatization in the normal indolization route was purposely blocked, e.g., in 2,6-dialkylphenylhydrazines, non-indole or rearranged indole type products were isolated, usually in only poor to fair yield, with the terminal nitrogen incorporated.[4] We now report indolizations using simple phenylhydrazines and keto acids, in which significant indole byproducts arise bearing the terminal phenylhydrazine nitrogen while principal indole products concomitantly incorporate the usual N_1. Nitrogen-15 label studies fully corroborate the unusual mechanism.

Results

During a Fischer synthesis of a pharmacologically active indole compound, *N*-(*p*-chlorobenzyl)-3-methyl-5-fluoroindole-2-α,α-dimethylpropionic acid,[5] formation of con-

(1) Current address: The Pillsbury Company, Research and Development Laboratory, 311 Second Street SE, Minneapolis, MN, 55414.

(2) Robinson, B. *The Fischer Indole Synthesis* Wiley: New York, 1982. Houlihan, W. J., Ed. *Indoles*, Part I; Wiley-Interscience: New York 1972. Sundberg, R. J. *The Chemistry of Indoles*; Academic Press: New York, 1970.

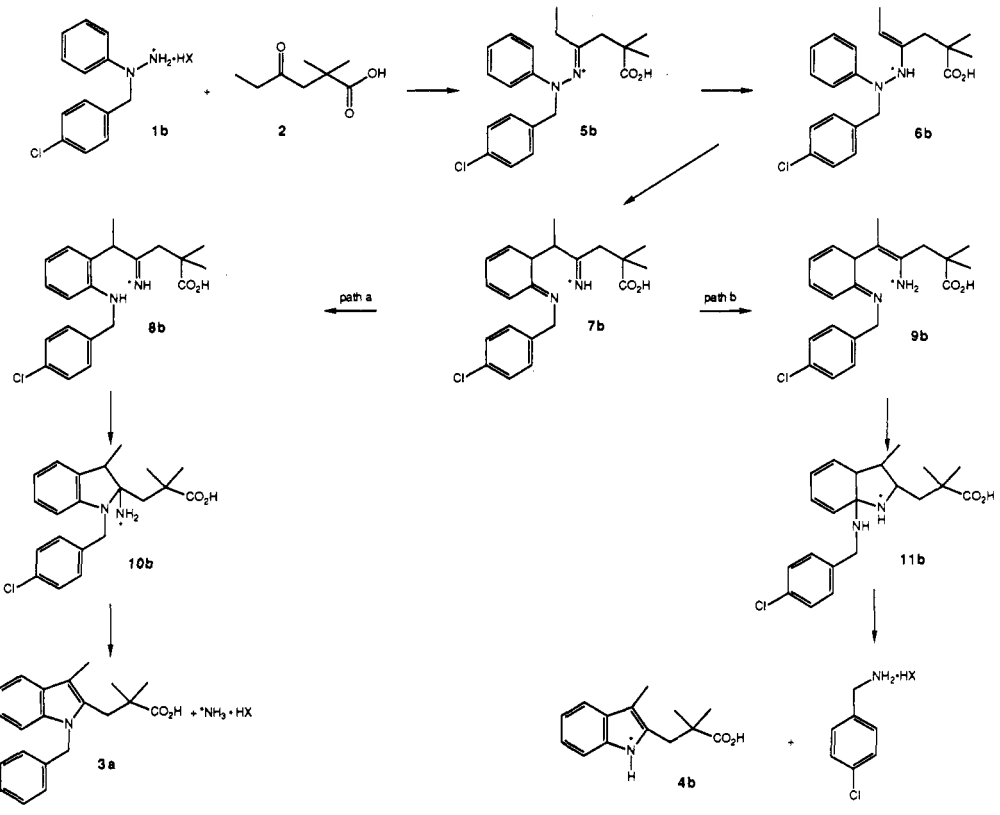
(3) (a) Allen, C. F. H.; Wilson, C. V. *J. Am. Chem. Soc.* **1943**, *65*, 611. (b) Clusius, K.; Weisser, H. R. *Helv. Chim. Acta* **1952**, *35*, 400.

(4) (a) Carlin, R. B.; Magistro, A. J.; Mains, G. J. *J. Am. Chem. Soc.* **1964**, *86*, 5300. (b) Bajwa, G. S.; Brown, R. K. *Can. J. Chem.* **1970**, *48*, 2293 and references cited therein.

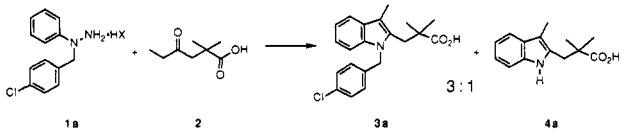
(5) (a) Guindon, Y.; Gillard, J. W.; Yoakim, C.; Jones, T. R.; Fortin, R. European Pat. Appl. EP 166591 A2. (b) Hall, R. A.; et al. *Eur. J. Pharm.* **1987**, *135*, 193.

0022-3263/90/1955-2908$02.50/0

Scheme I



siderable debenzylated indole byproduct was observed. Repetition of the reaction with a nonfluorinated substrate showed similar results and led us to examine reaction mixtures directly using ^{13}C NMR. Reaction product spectra indicated that a 3:1 mixture of **3a** and **4a** formed from N_1-(*p*-chlorobenzyl)phenylhydrazine and 2,2-dimethyl-4-oxohexanoic acid (**2**).[6] (eq 1).

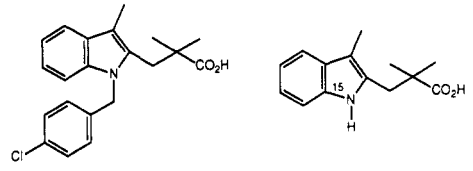


(1)

Surprisingly, a minor benzylic species present was identified as the protonated benzylamine. This was confirmed by addition of 4-chlorobenzylamine hydrochloride directly to the reaction mixture, incidentally identifying spectral positions of its carbons (cf. Table I). This suggested that N-unsubstituted indoles like **4** might be primary reaction products, coming via path b, Scheme I,[7] rather than secondary debenzylation byproducts.

β-^{15}N-labeled hydrazine **1b** was prepared to establish the identity of the nitrogen atom in the product. The fate of the label is shown in Scheme I. The experiment resulted in unlabeled ^{14}N-benzylindole **3a**, $M^+ = 355$, but labeled ^{15}N-unsubstituted indole **4b**, $M^+ = 232$. The recovered 4-chlorobenzylamine contained no label, $M^+ = 141$. Mass spectroscopy results were corroborated by NMR: indole **3a** and 4-chlorobenzylamine exhibited no ^{15}N splitting while **4b** showed ^{13}C–^{15}N coupling of 13.4 Hz to C_2 (δ_C = 133.4) and 15.2 Hz to C_9 (δ_C = 137.6). Proton spectra showed a one-bond ^{15}N–H (δ_H = 8.22) coupling of 95.3 Hz[8] and a three-bond coupling of 3.0 Hz to the 2-methylene

Table I. Carbon-13 Chemical Shifts of Unlabeled N-Benzylated and ^{15}N-Labeled Debenzylated Indoles 3a and 4b in a Reaction Mixture[a]



atom	3a	4b
C_2	133.2 or 133.5	132.4; J = 13.4 to ^{15}N
C_3	111.0	108.7
C_4	119.0 or 119.8	118.5 or 119.0
C_5	119.0 or 119.8	118.5 or 119.0
C_6	122.2	121.4
C_7	110.3	111.3
C_8	137.7 or 137.9	136.6; J = 15.2 to ^{15}N
C_9	129.8	not observed
NCH_2	46.9	43.2[b]
$C_{1'}$	137.7 or 137.9	135.4[b]
$C_{2',6'}$	128.0	131.5[b]
$C_{3',5'}$	129.2	129.6[b]
$C_{4'}$	133.2 or 133.5	132.6[b]
2-CH_2	34.5	36.6
CMe_2	44.9	44.3
CMe_2	25.8	26.2
CO_2H	180.4	181.2
3-CH_3	10.7	9.4

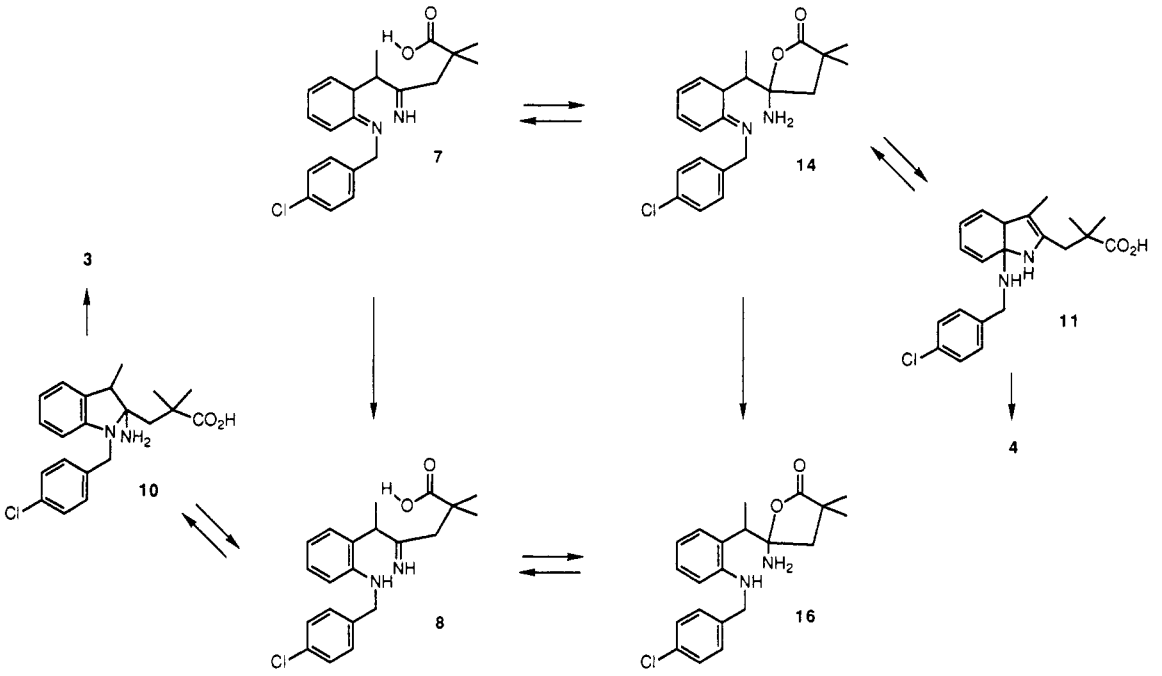
[a] Run in *t*-BuOH using added CD_3OD for field-frequency lock; *t*-BuOH CH_3 signal used as reference; δ_C = 31.60. [b] These shifts are for the free benzylamine salt in the reaction mixture.

(6) For the methyl ester of **2**, see: Miyashita, M.; Yanami, T.; Kumazawa, T.; Yoshikoshi, A. *J. Am. Chem. Soc.* **1984**, *106*, 2149. Scarpati, R.; Scherillo, G.; Imperato, F.; Nicolas, R. A. *Gazz. Chim. Ital.* **1967**, *97*, 654.

(7) An alternate possibility was that **4** was the result of nucleophilic attack on the benzyl group of protonated **3** under the reaction conditions. However, when HCl was employed as the acid, Cl^- was the available nucleophile and 4-chlorobenzyl chloride would have been formed.

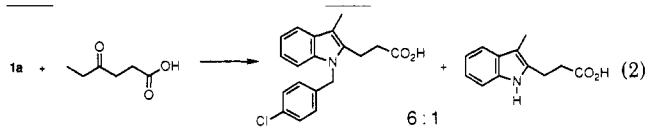
(8) Axenrod, T.; Wieder, M. J.; Berti, G.; Barili, P. L. *J. Am. Chem. Soc.* **1970**, *92*, 6066.

Scheme II



group (δ_H = 3.00). In addition, a proton NMR spectrum of the amine components isolated from the reaction showed splitting for the ammonium chloride signal of about 60 Hz[9] while the 4-chlorobenzylamine component showed none.

That the free acid was involved in the formation of the N-unsubstituted indole **4** was demonstrated by esterification of the keto acid prior to indole formation. Only the *N*-benzylindole ethyl ester was then obtained, with no evidence of the N-unsubstituted product. When a simpler keto acid lacking geminal dimethyl groups replaced **2**, the amount of the N-unsubstituted product diminished but it did not disappear. Thus, **1a** reacted with 4-oxohexanoic acid[10] to yield a 6:1 ratio of the N-benzylated indole **12** and the 1*H*-indole **13** (eq 2). Finally, when perdeuterated

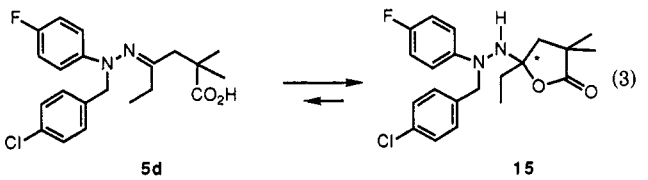


phenylhydrazine was allowed to react with the 2,2-dimethyl keto acid, no change in product ratio was observed.

Discussion

The two paths shown in Scheme I for the Fischer indole synthesis, (a) aromatization (**7** → **8** → **3**) and (b) side-chain imine to enamine tautomerization (**7** → **9** → **4**), are available in all cases. However, only in cases where aromatization is prevented by 2,6-disubstitution has the chemistry been shown to proceed as in path b.[4] In the present case, if the acid in **7** cyclizes onto the imine, normal indolization is prevented (Scheme II). In intermediate **14**, the terminal nitrogen is nucleophilic, allowing formation of **11**.

Five-membered lactones are energetically favored and those containing *gem*-dimethyl groups are known to cyclize rapidly.[11] The present system appears prone to cyclization; for example, the hydrazone **5d**[12] is in equilibrium with the cyclic hydrazino animal **15** (eq 3). At room temper-



ature, its NMR spectrum showed several broad signals, indicating a dynamic equilibrium. At −40 °C, the signals sharpened to show the cyclic structure **15**, δ_{C^*} = 100.5 ppm. No signal for the imine sp^2 carbon in **5d** was detected. The suggested intervention of **14** in the abnormal indolization observed would be analogous to existence of the hydrazone largely as **15**.

While aromatization, once it occurs, is essentially irreversible,[13] proton-transfer rates of carbon acids in protic media can be slow,[14] in this case allowing an alternate intramolecular cyclization (**7** → **14** → **11**) to take place as shown in Scheme II. This suggests that slowing the rate of aromatization (**7** → **8** and **14** → **16**) or increasing the propensity for the acid to cyclize at the imine (**7** → **14**) would increase the amount of the N-unsubstituted indole **4**. Aromatization might have been slowed by deuteration of the phenyl ring,[15] but a perdeuterated phenylhydrazine

(9) Binsch, G.; Lambert, J. B.; Roberts, B. W.; Roberts, J. D. *J. Am. Chem. Soc.* **1964**, *86*, 5564.

(10) Walton, E.; Jenkins, S. R.; Nutt, R. F. Holly, F. W. *J. Med. Chem.* **1968**, *11*, 1252.

(11) Eliel, E. L. *Stereochemistry of Carbon Compounds*; McGraw-Hill: New York, 1962; pp 197–202. Eliel, E. L.; Allinger, N. L.; Angyal, S. J.; Morrison, G. A. *Conformational Analysis*; Interscience: New York, 1965; p 191.

(12) The chemistry reported in this paper has been carried out also in the fluorine-substituted series: 1-(4-chlorobenzyl)-4-fluorophenylhydrazine hydrochloride reacting to give the *N*-benzyl and N-unsubstituted 5-fluoroindoles. All of the chemistry was similar; only small differences in the ratio of products were observed.

(13) Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*, 1st ed.; Harper & Row: New York, 1976; pp 450–2.

(14) Coetzee, J. F.; Ritchie, C. D. *Solute-Solvent Interactions*, Vol. 1; Dekker: New York, 1969; pp 248–52.

(15) A change in the overall rate of indole formation was not anticipated since C–D bond breaking would not be involved in the rate-determining step. However, a change in product distribution, due to slower aromatization, might have been expected.

gave no change in product distribution.

The alternative of decreasing the propensity of the acid to cyclize proved informative. Since the *gem*-dimethyl groups favor cyclization (Thorpe–Ingold effect[11]), removal of the methyl groups should slow the cyclization 7 → 14, allowing more of the reaction to proceed via aromatization to the expected *N*-benzylindole. Indeed, 4-oxohexanoic acid yielded a 6:1 ratio of *N*-benzylindole **12** and N-unsubstituted indole **13** (eq 2) rather than the 3:1 ratio of the analogous indoles containing *gem*-dimethyl groups. All the data presented are consistent with lactone animal **14** formation being responsible for the "abnormal" Fischer indolization.

Summary

Fischer indole synthesis with the benzylphenylhydrazine **1** and 2,2-dimethyl-4-oxohexanoic acid (**2**) led to a mixture of the expected *N*-benzylindole **3** and the unexpected N-unsubstituted indole **4**. The latter product **4** has been shown by ^{15}N labeling to incorporate the terminal hydrazine nitrogen atom and is the first example of such from a hydrazine bearing ortho protons.

Experimental Section

Solvents employed were dried over molecular sieves. Reactions were monitored on two HPLC systems: (1) gradient 50–100% B in 10 min, where A = 0.01 M KH_2PO_4, 0.001 M sodium heptanesulfonate, H_3PO_4 to pH 2.5; B = 20% A in CH_3CN; Altex ultrasphere octyl; 15 cm; 1.5 mL/min; 254 nm and (2) 70:30 CH_3CN/H_2O with 0.1% H_3PO_4, Zorbax C8, 2 mL/min, 254 nm. NMR experiments were performed on a Varian XL 100 or XL 300 or a Bruker WM 250 or AM 300 spectrometer as indicated. NMR samples of reaction mixtures were flushed with nitrogen when being transferred to the NMR tube and tightly capped. Mass spectra were obtained on a LKB Model 9000 spectrometer with direct inlet sampling and electron impact ionization at 70 eV. By temperature programming the probe, the indoles **3** and **4** could be selectively volatilized and examined individually.

1-(4-Chlorobenzyl)phenylhydrazine Hydrochloride (1a).[5,16] Phenylhydrazine hydrochloride (Aldrich, 20 g, 0.138 mol) was suspended in 300 mL of toluene in a nitrogen-flushed flask equipped with mechanical stirring. Triethylamine (Kodak, 27.9 g, 0.276 mol, 38.5 mL) was added, followed by 4-chlorobenzyl chloride (Aldrich, 22.3 g, 0.138 mol). The stirred reaction mixture was held at reflux for 6 h and aged overnight at room temperature. The reaction mixture was filtered to remove triethylamine hydrochloride and some unreacted phenylhydrazine hydrochloride. HCl (Matheson, gas) was bubbled into the filtrate to precipitate the product, which was contaminated by phenylhydrazine and the isomeric 1,2-disubstituted hydrazine hydrochlorides. The precipitate was filtered and dried at reduced pressure. The solid was slurried in 300 mL of cold water for 10 min to dissolve the phenylhydrazine hydrochloride, filtered, and dried at reduced pressure for 1 h. This material was recrystallized from 2-propanol (450 mL) to remove the unwanted 1,2-isomer and dried at reduced pressure to yield 20.4 g (55%) of white solid **1a**. NMR (300 MHz, d_6-DMSO): δ 10.5 (br s), 7.4–7.3 (6 H, m), 7.2 (2 H, m) 7.1 (1 H, m), and 4.7 (2 H, s). ^{13}C NMR (75 MHz, d_6-DMSO): δ 146.3, 134.0, 132.5, 130.7, 129.1, 128.4, 123.9, 118.8 and 57.9. MS: 234 (6, M^+, ^{37}Cl), 232 (16, M^+, ^{35}Cl), 127 (6, $C_7H_6{}^{37}Cl^+$), 125 (17, $C_7H_6{}^{35}Cl^+$), 107 (100, $-C_7H_6Cl$), and 77 (32). Anal. Calcd for $C_{13}H_{14}Cl_2N_2$: C, 58.01; H, 5.24; N, 10.41; Cl, 26.34. Found: C, 58.10; H, 5.46; N, 10.49; Cl, 26.27.

2,2-Dimethyl-4-oxohexanoic Acid (2). Lithium diisopropylamide was prepared from diisopropylamine (Aldrich, 15.7 g, 0.155 mol, 21.7 mL) and *n*-butyllithium (Aldrich, 1.6 M in hexanes, 0.147 mmol, 92.1 mL) in 74 mL of THF at −20 °C to −10 °C. The reaction mixture was cooled to −60 °C. Ethyl isobutyrate (Aldrich, sieve dried, 14.7 g, 0.127 mol) was added while the temperature was maintained below −50 °C, and the reaction mixture was then aged at −60 °C for 45 min. Epoxybutane (Aldrich, sieve dried, 10.62 g, 0.147 mol, 12.7 mL) was added in one portion and the reaction warmed to room temperature and aged overnight. The reaction was quenched by the addition of 240 mL of cold 1 N HCl, the layers were separated, and the aqueous phase was washed with 60 mL of ethyl acetate. The organic extract was washed with 40 mL of 1.5 N HCl and 40 mL of saturated aqueous NaCl. The organic layer was dried over Na_2SO_4 and filtered and the solvent removed at reduced pressure, keeping the temperature close to ambient. The residue was distilled at 115–120 °C (25 Torr) to yield 15.7 g (87%) of a colorless liquid, 2,2-dimethyl-4-hydroxyhexanoic acid γ-lactone. NMR (300 MHz, $CDCl_3$): δ 4.3 (1 H, m, O-C*H*), 2.1 (1 H, m, one of ring methylene protons), 1.8–1.5 (3 H, m, CH_2CH_3 + one of ring methylene protons), 1.26 (3 H, s, quat CH_3), 1.24 (3 H, s, quat CH_3), and 1.0 (3 H, t, J = 7 Hz, CH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 182.1 (*C*=O), 77.5 (*C*H-O), 43.1 (ring CH_2), 40.5 (quat C), 28.6 (CH_2CH_3), 25.1 (quat CH_3), 24.5 (quat CH_3), and 9.5 (CH_2CH_3). The lactone (7.11 g, 50 mmol) was suspended in 25 mL of water and treated with 5 N NaOH (11 mL). The mixture was aged at ambient temperature for 1 h to form a slightly turbid solution of the sodium salt. The reaction was cooled to 0 to 5 °C. Ruthenium dioxide dihydrate (30 mg) was added. Sodium hypochlorite solution (8.1%) was added dropwise, while the temperature was maintained below 10 °C.[17] After the theoretical amount of hypochlorite was added, the remaining hypochlorite was added in 1-mL aliquots until the color no longer turned black (RuO_2) but remained a greenish yellow. A total of 53 mL of NaOCl was required (40% over theory). The reaction was quenched with 2-propanol (3 mL) and filtered through Celite to remove the catalyst. The colorless filtrate was acidified with 6 M HCl (25 mL) and extracted with CH_2Cl_2 (2 × 100 mL). The organic layer was dried over $MgSO_4$ and filtered and the solvent removed at reduced pressure. The remaining solid was triturated with 50 mL of cold hexane and dried at reduced pressure to yield 6.3 g (80%) of white solid **2**, mp 86–87 °C. NMR (300 MHz, $CDCl_3$): δ 2.7 (2 H, br s), 2.4 (2 H, br q, J = 7 Hz), 1.2 (6 H, s), and 1.0 (3 H, t, J = 7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 209, 183, 52, 40, 36, 25, and 7.

Preparation of Indoles 3a and 4a by Reaction of 1a + 2. The hydrazine hydrochloride **1a** (538.4 mg, 2 mmol) was partitioned between 2 mL of toluene and 2.2 mL of 1 M NaOH. The toluene solution was carefully transferred to a clean flask, the solvent removed at reduced pressure, and the residue flushed with *t*-BuOH (2 × 2 mL). Six milliliters of *t*-BuOH was added, followed by trifluoroacetic acid (228.0 mg, 2 mmol, 0.16 mL) and 2,2-dimethyl-4-oxohexanoic acid (**2**) (316.4 mg, 2 mmol). The reaction was held overnight at 60–70 °C under a nitrogen atmosphere. The ^{13}C NMR spectrum was recorded in situ and is very similar to that reported in Table I for the ^{15}N-labeled case; vide infra. Upon completion, the reaction was partitioned between CH_2Cl_2 (10 mL) and 1 N HCl (5 mL). The solvent was removed from the organic layer at reduced pressure. MS **3**: 357 (12, M^+, ^{37}Cl), 355 (34, M^+, ^{35}Cl), 270 (33, $-C_4H_7O_2$, ^{37}Cl), 268 (100, $-C_4H_7O_2$, ^{35}Cl), 233 (29, $-C_4H_7O_2$, $-Cl$); 127 (14, $C_7H_6{}^{37}Cl^+$), and 125 (39, $C_7H_6{}^{35}Cl^+$). MS **4**: 231 (37, M^+), 145 (100, $-C_4H_7O_2$), 59 (70), and 42 (70). The aqueous layer was made basic with NaOH and extracted with toluene (2 × 5 mL). The organic extract was dried over Na_2SO_4 and filtered. HCl was bubbled into the toluene to precipitate 4-chlorobenzylamine hydrochloride, which was collected by centrifugation and dried at reduced pressure. MS: 143 (6, M^+, ^{37}Cl), 142 (20, −H, ^{37}Cl), 141 (18, M^+, ^{35}Cl), 140 (61, −H, ^{35}Cl), 127 (5, $-NH_2$, ^{37}Cl), 125 (14, $-NH_2$, ^{35}Cl), and 106 (100, −Cl).

Preparation of Hydrazone 5d. Hydrazine hydrochloride **1d** (269.1 mg, 1 mmol) was partitioned between 1 mL of toluene and 1.1 mL of 1 N NaOH. The toluene layer was carefully removed to a clean, nitrogen flushed flask. Molecular sieves (200 mg, 4A) were added and the solution was magnetically stirred for 0.5 h. Keto acid **2** (158.2 mg, 1 mmol) was added and the reaction mixture stirred at ambient temperature, under nitrogen, overnight. The reaction was filtered through Celite and washed with 2 mL

(16) For syntheses of similar hydrazines, see ref 10 and Perni, R. B.; Gribble, G. W. *Org. Prep. Proc. Int.* **1982**, *14*, 343.

(17) This oxidation may also be carried out with bromine. See: Wineberg, J. P.; Abrams, C.; Swern. D. *J. Heterocycl. Chem.* **1975**, *12*, 749.

Table II. Proton NMR of Neutral Products of Indolization Using N_2-Labeled 1-(4-Chlorobenzyl)phenylhydrazine (in $CDCl_3$)

group	3a	4b
$C(CH_3)_2$	1.28 (s)	1.30 (s)
3-CH_3	2.34 (s)	2.27 (s)
2-CH_2	3.06 (s)	3.00 (d, J = 3.0 to ^{15}N)
NCH_2	5.36 (s)	
aromatics ortho to N	6.72[a]	
NH		8.22 (d, J = 96.3 to ^{15}N)

[a] All other aromatic hydrogens of **3a** and **4b** were found between 7.05 and 7.60 ppm.

of toluene and the solvent was removed at reduced pressure.

[2-^{15}N]Phenylhydrazine Hydrochloride. The reaction mixture and all added solutions were maintained at 0 °C during this procedure. Aniline (0.67 g, 7.2 mmol, distilled) was added to vigorously stirred concentrated HCl (12.5 mL) and aged for 10 min. The diazonium salt was prepared by dropwise addition (ca. 15 min) of a solution of $Na^{15}NO_2$ (0.5 g, 7.1 mmol) in 3 mL of distilled water. The mixture was aged for an additional 15 min. A solution of $SnCl_2 \cdot 2H_2O$ (3.6 g, 16 mmol) in 3.6 mL of concentrated HCl was added dropwise. The colorless precipitate began to form immediately. The reaction mixture was aged for 30 min and filtered. The product was dried in vacuo overnight to yield 0.90 g (88%) of [2-^{15}N]phenylhydrazine hydrochloride.

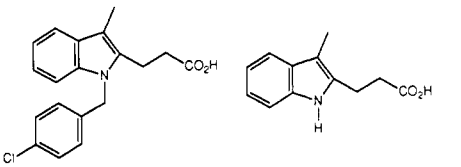
[2-^{15}N]-1-(4-Chlorobenzyl)phenylhydrazine Hydrochloride (1b). The labeled hydrazine was prepared in the same manner as the unlabeled material **1a** above using [2-^{15}N]phenylhydrazine (0.200 g, 1.4 mmol), 4-chlorobenzyl chloride (0.225 g, 1.4 mmol), and triethylamine (0.283 g, 2.8 mmol, 0.39 mL). NMR (250 MHz, d_6-DMSO): δ 7.3–7.6 (6 H, m, aromatics), 7.1–7.2 (3 H, m, aromatics) and 4.7 (2 H, d, J = 2.3 Hz to ^{15}N, CH_2). MS: 235 (6, M^+, ^{37}Cl, ^{15}N), 233 (15, M^+, ^{35}Cl, ^{15}N), 127 (6, $C_7H_6{}^{37}Cl^+$), 125 (17, $C_7H_6{}^{35}Cl^+$), 108 (100, $-C_7H_6Cl$, ^{15}N), and 77 (36).

Preparation of Indoles 3a and Labeled 4b by Reaction of 1b + 2. This reaction was carried out in the same manner as the unlabeled reaction above, using **1b** (72 mg, 0.27 mmol), trifluoroacetic acid (31 mg, 0.27 mmol, 21 μL), and **2** (43 mg, 0.27 mmol) in 0.8 mL of t-BuOH. The reaction was heated at 70 °C overnight. ^{13}C NMR data (62.5 MHz, t-BuOH) are reported in Table I. Solvent was removed from half the mixture at reduced pressure. $CDCl_3$ was added to the residue and the soluble portion (indoles **3a** and **4b**) removed for proton NMR (300 MHz), which is reported in Table II. The $CDCl_3$-insoluble portion, consisting of 4-chlorobenzylamine trifluoroacetate and [^{15}N]ammonium trifluoroacetate, was then dissolved in d_6-DMSO. NMR (300 MHz): δ ca. 8.3 (3 H, br s, $^+NH_3$), 7.5 (4 H, s, all aromatic H's), 4.06 (2 H, s, NCH_2), and ca. 7.2 (4 H, br d, J = ca. 60 Hz to ^{15}N, $^+NH_4$). The remaining half of the reaction mixture was partitioned between CH_2Cl_2 (2 mL) and 1 N HCl (1 mL). The solvent was removed from the organic layer at reduced pressure.

MS 3: 357 (11, M^+, ^{37}Cl), 355 (34, M^+, ^{35}Cl), 270 (34, $-C_4H_7O_2$, ^{37}Cl), 268 (100, $-C_4H_7O_2$, ^{35}Cl), 233 (32, $-C_4H_7O_2$, $-Cl$), 127 (19, $C_7H_6{}^{37}Cl^+$), and 125 (50, $C_7H_6{}^{35}Cl^+$). MS 4: 232 (25, M^+, ^{15}N), 145 (100, $-C_4H_7O_2$, ^{15}N) 59 (52), and 42 (59). The aqueous layer was made basic with NaOH and extracted with toluene (2 × 1 mL). The organic layer was dried over Na_2SO_4 and filtered. HCl was bubbled into the toluene to precipitate 4-chlorobenzylamine hydrochloride, which was collected by centrifugation and dried at reduced pressure. It was free of label; MS as previously described (vide supra).

Preparation of Ethyl Ester of Indole 3a by Reaction of 1a + Ethyl 2,2-Dimethyl-4-oxohexanoate. Ethyl 2,2-dimethyl-4-oxohexanoate was prepared by Fischer esterification. 2,2-Dimethyl-4-oxohexanoic acid (1.0 g, 5.4 mmol) was dissolved in 8 mL of absolute ethanol with 1 drop of concentrated sulfuric acid and refluxed, under nitrogen, overnight. The reaction was cooled, **1a** (1.2 g, 4.3 mmol) was added, and the reaction again left at reflux overnight. That none of the unsubstituted indole ester was present was verified by HPLC. An authentic sample was prepared by Fischer esterification of a mixture of indoles **3a** and **4a**. After cooling, the ethanol was removed at reduced pressure and the reaction mixture worked up by partitioning between CH_2Cl_2 and 1 N HCl. The organic layer was dried over Na_2SO_4 and filtered. The yellow oil was purified by Chromatotron,[18] eluting with 10% ethyl acetate in hexane. The solvent was removed at reduced pressure to yield 1.1 g (68%) of a colorless oil that crystallized on standing; mp 44–46 °C. NMR (300 MHz, $CDCl_3$): δ 7.56 (1 H, m), 7.18–7.09 (5 H, m), 6.70 (2 H, m), 5.32 (2 H, s), 4.08 (2 H, q, J = 7 Hz), 3.00 (2 H, s), 2.31 (3 H, s), 1.22 (6 H, s), and 1.20 (3 H, t, J = 7 Hz). Anal. Calcd for $C_{23}H_{26}ClNO_2$: C, 71.96; H, 6.83; N, 3.65; Cl, 9.23. Found: C, 71.63; H, 6.80; N, 3.84; Cl, 9.44.

Table III. Carbon-13 Chemical Shifts of Indoles 12 and 13 in a Reaction Mixture[a]



12 13

atom	12	13[b]
C_2	135.1	134.3
C_3	108.2	106.4
C_4	118.8 or 119.7	118.3 or not observed
C_5	118.8 or 119.7	118.3 or not observed
C_6	121.9	121.1
C_7	109.6	111.2
C_8	137.2 or 137.7	136.5
C_9	129.4	not observed
NCH_2	46.2	43.1[c]
$C_{1'}$	137.2 or 137.7	135.3[c]
$C_{2',6'}$	128.0	131.4[c]
$C_{3',5'}$	129.2	not observed
$C_{4'}$	133.3	132.5[c]
2-CH_2	20.6	22.1
CH_2CO_2H	34.9	not observed
CO_2H	175.1	176.1
3-CH_3	9.1	8.7

[a] Run in t-BuOH using added CD_3OD for field-frequency lock; t-BuOH CH_3 signal used as reference; δ_C = 31.60. [b] **13** is at so low a level that several of its signals cannot be recognized. [c] These shifts are for the free benzylamine salt in the reaction mixture.

Preparation of Indole 3a by Saponification of Its Ethyl Ester. Indole (**3a**) ethyl ester (2.2 g, 5.8 mmol) was dissolved in 50 mL of THF and treated with 3 N NaOH (4 mL) and the resulting solution aged at reflux overnight. After cooling, the solvent was removed at reduced pressure and the residue partitioned between 25% ethyl acetate in hexane (25 mL) and 1 N HCl (25 mL). The organic layer was washed with water, dried over $MgSO_4$, and filtered, and the solvent was removed at reduced pressure. The white solid obtained was triturated with hexane and dried to yield 1.98 g (96%) of **3a**. NMR (300 MHz, $CDCl_3$): δ 7.6 (1 H, m), 7.2–7.1 (5 H, m), 6.7 (2 H, m), 5.4 (2 H, s), 3.1 (2 H, s), 2.4 (3 H, s), and 1.3 (6 H, s). ^{13}C NMR (75 MHz, $CDCl_3$): δ 184.5, 136.9, 136.6, 132.9, 132.4, 129.0, 128.9 (2 C), 127.2 (2 C), 121.7, 119.2, 118.6, 111.1, 109.8, 46.5, 44.6, 33.5, 24.9 (2 C), and 10.4. Anal. Calcd for $C_{21}H_{22}ClNO_2$: C, 70.87; H, 6.23; N, 3.93; Cl, 9.96. Found: C, 70.66; H, 6.33; N, 4.16; Cl, 10.23.

1-(4-Chlorobenzyl)perdeuteriophenylhydrazine Hydrochloride (1c). The labeled hydrazine was prepared in the same manner as the unlabeled material **1a** above, using perdeuteriophenylhydrazine hydrochloride[19] (1.32 g, 8.8 mmol), 4-chlorobenzyl chloride (1.42 g, 8.8 mmol), and triethylamine (1.78 g, 17.6 mmol, 2.5 mL) in 19 mL of toluene to yield 1.0 g (41%) of **1c** as a white solid. NMR (250 MHz, d_6-DMSO): δ 7.4 (4 H, AA'BB'), 4.8 (2 H, s), and 3.5 (3 H, br s). Anal. Calcd for $C_{13}H_9Cl_2D_5N_2$: C, 56.93; H (H + $^1/_2$D), 5.11; N, 10.21; Cl, 25.86. Found: C, 56.80; H (H + $^1/_2$D), 5.36; N, 10.15; Cl, 25.67.

Preparation of Indoles 3c and 4c by Reaction of 1c + 2. This reaction was carried out in the same manner as the indole formation above, using **1c** (100 mg, 0.4 mmol), trifluoroacetic acid

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(45 mg, 0.4 mmol, 30 μL), and **2** (63 mg, 0.4 mmol) in 1.5 mL of *t*-BuOH. The reaction was heated at 70 °C for 8 h.

Preparation of Indoles 12 and 13 by Reaction of 1a + 4-Oxohexanoic Acid. This reaction was carried out in the same manner as the indole formation above, using **1a** (0.2 g, 0.75 mmol), trifluoroacetic acid (86 mg, 0.75 mmol, 57 μL), and 4-oxohexanoic acid (Aldrich, 0.1 g, 0.75 mmol) in 2 mL of *t*-BuOH. The reaction was heated at 80 °C for 23.75 h. ^{13}C NMR (25.2 MHz, *t*-BuOH) data are reported in Table III.

Acknowledgment. We acknowledge Ms. L. DiMichele for NMR spectra, Mr. J. Smith for the mass spectral data, and Dr. D. L. Hughes for helpful discussions. We also thank Ms. M. Spears for preparation of this manuscript.

Registry No. **1a**, 103252-87-5; **1b**, 125687-04-9; **1c**, 125687-05-0; **1d**, 103252-74-0; **2**, 15118-53-3; **3a**, 125687-06-1; **3a** ethyl ester, 125687-07-2; **3c**, 125687-08-3; **4a**, 125687-09-4; **4b**, 125687-10-7; **4c**, 125687-11-8; **5d**, 125687-12-9; **12**, 103252-86-4; **13**, 125687-13-0; **15**, 125687-14-1; $H_3CCH_2CO(CH_2)_2CO_2H$, 1117-74-4; phenylhydrazine hydrochloride, 59-88-1; 4-chlorobenzyl chloride, 104-83-6; ethyl isobutyrate, 97-62-1; epoxybutane, 106-88-7; 2,2-dimethyl-4-hydroxyhexanoic acid γ-lactone, 54491-23-5; 4-chlorobenzylamine hydrochloride, 42365-43-5; aniline, 62-53-3; [2-^{15}N]phenylhydrazine hydrochloride, 125687-15-2; 4-chlorobenzylamine trifluoroacetate, 125687-16-3; [^{15}N]ammonium trifluoroacetate, 125687-17-4; ethyl 2,2-dimethyl-4-oxohexanoate, 89509-76-2; perdeuteriophenylhydrazine hydrochloride, 125687-18-5.

Syntheses of 1- and 2-Naphthol Analogues of DL-Tyrosine. Potential Fluorescent Probes of Peptide Structure and Dynamics in Complex Environments

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Received November 21, 1989

The racemic 1- and 2-naphthol analogues of tyrosine, (±)-2-amino-3-(4-hydroxy-1-naphthyl)propanoic acid hydrochloride, **1**, and (±)-2-amino-3-(6-hydroxy-2-naphthyl)propanoic acid hydrobromide, **2**, have been synthesized in gram quantities from 4-hydroxy-1-naphthaldehyde and 6-methoxy-2-naphthaldehyde in overall yields of 29 and 41%, respectively. The naphthaldehydes were condensed with hippuric acid to form the (*Z*)-oxazolones stereoselectively and oxazolone ring opening to the (*Z*)-propenoic acid derivatives with ethoxide or hydroxide was stereospecific. Hydrogenation over 10% Pd/C and deprotection gave the products **1** and **2**. Single-crystal X-ray structures of ethyl (*Z*)-2-(*N*-benzoylamino)-3-(4-hydroxy-1-naphthyl)propenoate, **1c**, (*Z*)-2-(*N*-benzoylamino)-3-(4-hydroxy-1-naphthyl)propenoic acid, **1f**, and ethyl (±)-2-(*N*-benzoylamino)-3-(4-hydroxy-1-naphthyl)propenoate, **1d**, verified the *Z* double bond stereochemistry or were proof of structure. NOE ^1H NMR measurements were used to demonstrate the double bond stereochemistry for **1f** and the analogous (*Z*)-2-(*N*-benzoylamino)-3-(6-methoxy-2-naphthyl)propenoic acid, **2h**.

Two types of fluorescent probes have been used to report structure and dynamics of peptides and proteins: extrinsic probes, e.g. fluorescein[1] or dansyl,[2] and intrinsic probes, e.g. tryptophan or tyrosine.[3] Intrinsic fluorescent probes are inherently more reliable reporters of peptide or protein structure and dynamics because the native characteristics are not perturbed. However, intrinsic fluorescent probes suffer from hopelessly complex spectral overlap problems when several natural fluorophores occur in the native peptide or protein. Hybrids of extrinsic and intrinsic fluorescent probes are needed in order to determine the conformation(s) of flexible peptide hormone analogues during the lifetime of complexes with membrane-bound receptors. Otherwise, spectral overlap from similar fluorophores in the membrane-bound receptors will make the selective monitoring of the structure and dynamics of the bound peptide hormone very complex. This fundamental information may allow a completely rational rather than partly empirical approach to the design of peptide hormone analogues. The synthesis of hybrid fluorescent probes that structurally mimic the tyrosine residue of superpotent peptide hormone analogues of somatostatin are reported in this paper.[4]

Tyrosine analogues which have 1- and 2-naphthol fluorophores in place of the 4-hydroxyphenyl fluorophore of tyrosine absorb and emit at longer wavelengths than native amino acid fluorophores and may be substituted for the tyrosine residues of somatostatin analogues with minimal perturbation of peptide hormone structure and dynamics.

Results and Discussion

Synthesis of the 1-Naphthol Tyrosine Analogue. The synthesis of the 1-naphthol tyrosine analogue began with a Gatterman condensation of 1-naphthol, and subsequent imine hydrolysis afforded **1a**.[5] Condensation of **1a** with hippuric acid in a heated slurry of sodium acetate and acetic anhydride gave the acetylated oxazolone **1b** in a 53% yield (Scheme I). Decreased yields of **1b** were observed if the reagents were not anhydrous. Concomitant ring opening and deacetylation of **1b** with sodium ethoxide gave **1c** in 90% yield. We expected exclusive formation of the (*Z*)-oxazolone and stereospecific ring opening to the (*Z*)-dehydro amido carboxylic acid derivative under these conditions.[6] We verified the double bond stereochemistry at this point with a single crystal X-ray structure of **1c** and **1f** (Figures 1 and 2). **1f** was obtained by reaction of the oxazolone with refluxing 1% NaOH in 86% yield. See the discussion of NMR experiments later.

Hydrogenation of **1c** over Pd/C gave racemic **1d** in 75% yield. The single-crystal X-ray structure of **1d** was proof

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0022-3263/90/1955-2913$02.50/0