oxygenase was purified by using a modified method of Sabourin et al.[50] The incubation medium contained 50 mM potassium phosphate; pH 7.8, 0.5 mM $NADP^+$, 2.0 mM glucose 6-phosphate, 1 IU of glucose-6-phosphate dehydrogenase, 4 mM *n*-octylamine, and 0.5–0.9 mg of microsomes or 65–690 mg of purified flavin-containing monooxygenase as described previously.[51] Reactions were initiated by the addition of the 2-aryl-1,3-oxathiolane or the 2-aryl-1,3-oxathiolane *S*-oxide and the incubation was continued with constant shaking to maintain adequate oxygen concentrations. At timed intervals the reaction was quenched and analyzed for products by the procedures given below.

The reaction was quenched with 1 mL of cold dichloromethane and the aqueous reaction medium was separated by centrifugation. The dichloromethane fraction was filtered through a Sep Pak and then evaporated to dryness and redissolved in 0.5 mL of methanol. The metabolic products were quantitated by HPLC as described above. Quantitation of para-substituted benzaldehydes by HPLC utilized a mobile phase consisting of acetonitrile/water (53:46). HPLC can efficiently separate aryl-1,3-oxathiolanes, aryl-1,3-oxathiolane *S*-oxides, and benzaldehydes.[52] The material balance was 95% as judged by parallel experiments with authentic internal standards.

Heat inactivation of hog or rat liver microsomal protein was accomplished by purging the protein suspended in buffer, pH 7.8, with argon and placing the microsomal protein in a bath of 55 °C water for 60 s in the absence of NADPH. This procedure has been shown to completely destroy FMO-dependent N-oxygenation while preserving 85–100% of cytochrome P-450 mediated N-dealkylation.[29]

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(52) Cashman, J. R.; Proudfoot, J. *Anal. Biochem.* **1988**, *175*, 274–280.

A Stereoselective Synthesis of (−)-Perhydrohistrionicotoxin

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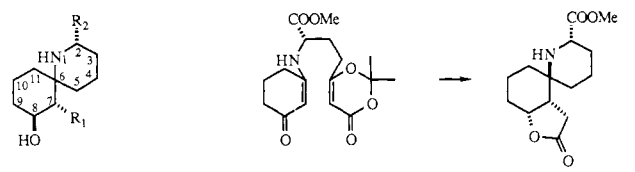
Abstract: The first synthesis of (−)-perhydrohistrionicotoxin, **2**, which proceeds without recourse to resolution of synthetic intermediates is reported. The absolute stereochemistry is derived from L-glutamic acid, and the key step, in which the critical relative stereochemical relationships are established, is the intramolecular photocycloaddition of a vinylogous amide with a dioxenone. The transformation of **5** into **2** proceeds in 16 steps in 9% overall yield.

In 1971, Witkop and co-workers reported the isolation of (−)-histrionicotoxin, **1** (Scheme I), from the skin extracts of the Neotropical poison frog *Dendrobates histrionicus*.[3] The attention given to the synthesis of the histrionicotoxin alkaloids[4] stems from their unique properties as neurotoxins in conjunction with their scarcity (ca. 200 μg per frog). It has been shown that both histrionicotoxin, **1**, and perhydrohistrionicotoxin, **2**, are potent blockers of acetylcholine-mediated ion conductance, thereby interrupting transsynaptic transmission of neuromuscular impulses. Both compounds are therefore of considerable importance in studying cholinergic receptor mechanisms in the neuromuscular system.[5,6]

Scheme I



1 R_1 = HC≡CH-C≡CH; R_2 = CH_2-HC≡CH-C≡CH

2 R_1 = C_4H_9; R_2 = C_5H_{11}

3

4

We have recently reported the transformation of **3**, derived from L-glutamic acid, into **4**, which possesses the azaspiroundecane skeleton of (−)-histrionicotoxin, **1**.[7] The key step in the conversion of **3** → **4**, in which the critical relative stereochemical relationships are established, is the intramolecular photocycloaddition of a

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(2) National Institutes of Health Predoctoral Trainee (Grant No. GM07151).

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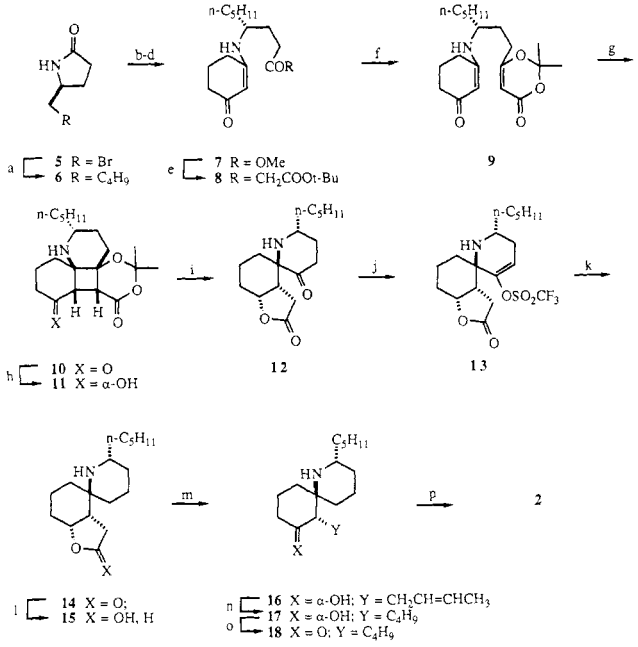
(5) Elliot, J.; Raftery, M. A. *Biochemistry* **1979**, *18*, 1968 and references cited therein; Aronstam, R. S.; Daly, J. W.; King, C. T., Jr.; Albuquerque, E. X; Feigl, D. M. *Biochem. Pharmacol.* **1985**, *34*, 3037. Daly, J. W.; Lovenberg, T. *Neurochem. Res.* **1986**, *11*, 1609.

(6) For excellent general reviews, see: Myers, C. W.; Daly, J. W. *Scientific American* **1983**, *248*, 120. Ibuka, T. *Chem. Pharm. Bull.* **1982**, *30*, 2840. Daly, J. W. In *Progress in the Chemistry of Natural Products*; Herz, W., Griesbach, H., Kirby, G. W., Eds.; Springer-Verlag: New York, 1980; Vol. 41, pp 204–340. Witkop, B.; Gossinger, E. In *The Alkalloids*; Academic Press: New York, 1983; Vol. 21, pp 139–247.

(7) Winkler, J. D.; Hershberger, P. M.; Springer, J. P. *Tetrahedron Lett.* **1986**, 5177.

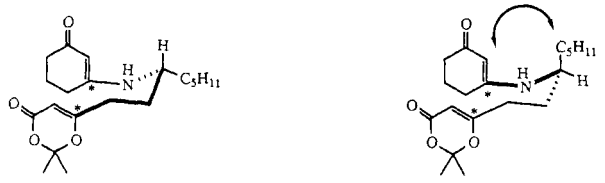
0002-7863/89/1511-4852$01.50/0

Scheme II[a]



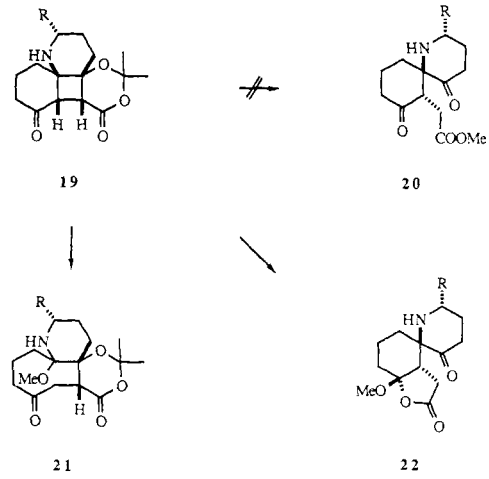
[a](a) *n*-Bu_2CuLi, Et_2O; (b) 6 N HCl; (c) 1,3-cyclohexanedione, C_6H_6; (d) dicyclohexylcarbodiimide, dimethylaminopyridine (catalyst), MeOH (67% overall yield from **5**; ref 26); (e) LDA, *tert*-butyl acetate, THF (60%); (f) acetone, trifluoroacetic anhydride, trifluoroacetic acid (84%); (g) *hν*; (h) $NaBH_4$, EtOH; (i) NaH, THF (60% overall yield from **9**; ref 26); (j) LDA, Tf_2NPh, THF (76%); (k) H_2, PtO_2, EtOH (96%); (l) (*i*-Bu)$_2$AlH, THF; (m) *n*-BuLi, $(Ph)_3PCH_3Br$, THF; (n) H_2, PtO_2, EtOH (72% overall yield from **14**; ref 26); (o) Dess–Martin periodinane, CH_2Cl_2 (87%); (p) LiAl(O*t*-Bu)$_3$H, THF (94%).

Scheme III



vinylogous amide[8] with a dioxenone.[9,10] We report herein the application of this strategy[11] to the first synthesis of (-)-perhydrohistrionicotoxin, **2**, which proceeds without recourse to resolution of synthetic intermediates.[12] The synthesis of the requisite photosubstrate **9** and its elaboration to (-)-perhydrohistrionicotoxin, **2**, is outlined in Scheme II.

Scheme IV



Reaction of **5**, which is readily available from L-glutamic acid,[13] with the cuprate derived from *n*-butyllithium and copper cyanide[14] [6 equiv, diethyl ether/tetrahydrofuran 6:1, °C (2 h) → −15 °C (18 h)] provided **6** in 79% yield after silica gel chromatography. The crude product was submitted to the following sequence of steps: (1) 6 N aqueous hydrochloric acid at reflux for 24 h to liberate the amino acid; (2) condensation with cyclohexane-1,3-dione (1 equiv, 12 h) in benzene to form the vinylogous amide; and (3) esterification (2.2 equiv of methanol, 1.1 equiv of dicyclohexylcarbodiimide, 0.1 equiv of (dimethylamino)pyridine, 25 °C)[15] in dichloromethane, which furnished the methyl ester **7** in 67% overall yield from **5**. Reaction of **7** with the lithium enolate of *tert*-butyl acetate[16] [2.5 equiv, tetrahydrofuran, −78 °C (1.2 h) → −40 °C (1.2 h) → 0 °C (1 h), 60% yield] provided the β-keto ester **8**, which was converted to the corresponding dioxenone, **9** (4 equiv of acetone, 4 equiv of trifluoroacetic anhydride, 25 equiv of trifluoroacetic acid, 25 °C, 24 h, 84% yield).[17]

Irradiation of **9** (450-W medium-pressure Hg lamp, Pyrex filter in 0.0076 M acetonitrile) provided **10** in 95% yield.[18] The stereoselective formation of **10** can be explained by examination of the diastereomeric conformations shown in Scheme III. Formation of the C-5/C-6 bond (histrionicotoxin numbering) between the two labeled carbon centers in A, with the pentyl chain pseudoequatorial on the six-membered ring being formed, leads to the relative stereochemistry shown in **10**. In this photochemical cycloaddition, the stereogenic center of the amino acid has led to the stereochemically controlled introduction of two of the three other stereogenic centers in (-)-perhydrohistrionicotoxin. This is the first successful case of efficient asymmetric induction in the formation of a [4.2.0]bicyclooctane via the intramolecular [2 + 2] photocycloaddition reaction, in which the stereogenic center is not directly attached to one of the alkenes which participate in the photocycloaddition.[19]

On the basis of our previous work with the intramolecular dioxenone photocycloaddition–fragmentation reactions,[10] we had originally anticipated that treatment of the photoadduct with acid would lead to the desired azaspiroundecane skeleton of histrionicotoxin. However, treatment of the photoadduct **19**[7] (Scheme

(8) (a) For other examples of the intramolecular photocycloaddition of vinylogous amides, see: Schell, F. M.; Cook, P. M. *J. Org. Chem.* **1984**, *49*, 4067. Tamura, Y.; Ishibashi, H.; Hirai, M.; Kita, Y.; Ikeda, M. *J. Org. Chem.* **1975**, *40*, 2702.

(9) The chromophore in this photocycloaddition is the vinylogous amide [λ_{max} = 292 nm, ϵ = 60000] and not the dioxenone [λ_{max} = 247 nm, ϵ = 8300], for which triplet sensitization is required.

(10) For examples of the use of the intramolecular dioxenone photocycloaddition reaction, see: Winkler, J. D.; Hey, J. P.; Williard, P. G. *J. Am. Chem. Soc.* **1986**, *108*, 6425. Winkler, J. D.; Hey, J. P.; Darling, S. D. *Tetrahedron Lett.* **1986**, 5959. Winkler, J. D.; Hey, J. P.; Hannon, F. J.; Williard, P. G. *Heterocycles* **1987**, *25*, 55. Henegar, K. E.; Winkler, J. D. *Tetrahedron Lett.* **1987**, 1051. Winkler, J. D.; Henegar, K. E. *J. Am. Chem. Soc.* **1987**, *109*, 2850. For the intermolecular photocycloaddition of dioxenones, see: Baldwin, S. W.; Wilkinson, J. M. *J. Am. Chem. Soc.* **1980**, *102*, 3634.

(11) Instead of elaborating **4** into **2**, which would have involved the conversion of the glutamate carboxyl to the requisite *n*-pentyl chain, we elected to complete the total synthesis of (-)-perhydrohistrionicotoxin via photosubstrate **9**, in which the *n*-pentyl chain of **2** is incorporated at the outset.

(12) For a synthesis of (-)-perhydrohistrionicotoxin which proceeds via resolution of the Kishi bicyclic lactam, see: Takashashi, K.; Witkop, B.; Brossi, A.; Maleque, M. A.; Albuquerque, E. X. *Helv. Chim. Acta* **1982**, *65*, 252.

(13) Silverman, R. B.; Levy, M. A. *J. Org. Chem.* **1980**, *45*, 815.

(14) Bertz, S. H.; Gibson, C. P.; Dabbagh, G. *Tetrahedron Lett.* **1987**, 4251.

(15) Hassner, A.; Alexanian, V. *Tetrahedron Lett.* **1978**, 4475.

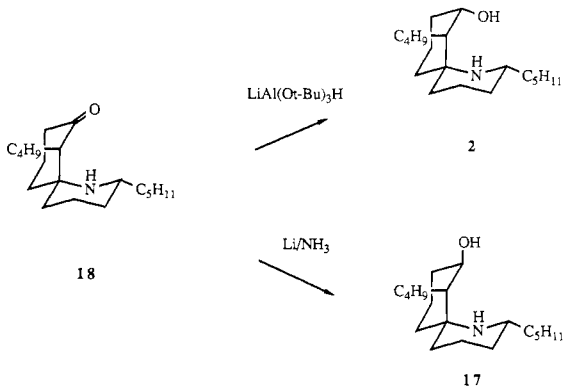
(16) Rathke, M. W.; Lindert, A. *J. Am. Chem. Soc.* **1971**, *93*, 2318.

(17) We have found these conditions for the preparation of the dioxenone photosubstrates to be superior in this and several other cases of the reaction conditions reported by Sato, M.; Ogasawara, H.; Oi, K.; Kato, T. *Chem. Pharm. Bull.* **1983**, *31*, 1896.

(18) Careful examination of the crude product revealed the presence of ca. 5% of an epimeric photoadduct, whose structure was assigned to be epimeric to **10** at C-7 (histrionicotoxin numbering, α to the ketone carbonyl group).

(19) For excellent recent reviews, see: Oppolzer, W. *Acc. Chem. Res.* **1982**, *15*, 132. Baldwin, S. W. In *Organic Photochemistry*; Padwa, A., Ed.; M. Dekker: New York, 1981; Vol. 5, p 123. Weedon, A. C. In *Synthetic Organic Photochemistry*; Horspool, W. M., Ed.; Plenum: New York, 1984.

Scheme V



IV, R = COOMe) from our earlier model study with catalytic *p*-toluenesulfonic acid in methanol led not the formation of the desired product **20** but instead to a mixture of retro-Mannich product **21** and the keto lactone **22**.

Liberation of the azaspiroundecane skeleton from **10** (Scheme II) proceeded in analogy to the previously described[7] two-step sequence: (1) reduction of the ketone (1.4 equiv of $NaBH_4$, 13:1 tetrahydrofuran/ethanol, −78 °C, 30 min, 65% yield), which furnished the α-alcohol **11**, as expected by addition of hydride from the convex face of the cis-fused bicyclo[4.2.0]octane moiety and (2) translactonization (3 equiv of NaH, tetrahydrofuran, 25 °C, 30 min) with concomitant ejection of acetone and carbon–carbon bond fragmentation to provide the keto lactone **12** in 60% overall yield from **9**. The relative stereochemical assignment for the keto lactone as shown in **12** was confirmed by the conversion of **12** into (−)-perhydrohistrionicotoxin, **2**, as outlined in Scheme II.

Keto lactone **12** was converted to the corresponding enol triflate **13** in 76% yield (1.5 equiv of lithium diisopropylamide, 0 °C, 1 h; 3 equiv of *N*-phenyltrifluoromethanesulfonimide, tetrahydrofuran/tetramethylethylenediamine (3:2), 25 °C, 1 h), which underwent smooth hydrogenolysis[20] (1 atm H_2, PtO_2, ethanol, 12 h) to give lactone **14** in 96% yield. Treatment of **14** with diisobutylaluminum hydride (4 equiv, tetrahydrofuran, −40 °C, 30 min) followed by extraction with ether and immediate reaction of the derived lactol **15** with 10 equiv of ethylidenetriphenylphosphorane in tetrahydrofuran at 25 °C for 3 h afforded **16**, as a 1:1 mixture of double bond isomers, which upon catalytic hydrogenation (1 atm H_2, PtO_2, 12 h, ethanol) provided **17** in 72% overall yield from **14**.[21] Attempted inversion of alcohol **17** under Mitsunobu conditions[22] was not successful. However oxidation of **17** with 1.5 equiv of the Dess–Martin periodinane[23] led to the formation of the ketone **18** in 87% yield. While reduction of ketone **18** with lithium in liquid ammonia[24] gave the equatorial alcohol **17**, as shown in Scheme V, treatment of **18** with lithium tri-*tert*-butoxyaluminohydride[25] (10 equiv, tetrahydrofuran, 0 °C, 24 h, quantitative yield) resulted in the formation of a 19:1 ratio of the axial alcohol **2** and the epimeric equatorial alcohol **17**, respectively. The spectral properties (^{13}C and ^1H NMR, high-resolution mass spectral data, IR) of the synthetic **2**, $[\alpha]^{22}_D$ −84.1° (*c* 0.024, dichloromethane), were identical in all respects with an authentic sample of (−)-**2**, $[\alpha]^{22}_D$ −83.1° (*c* 0.0067, dichloromethane), provided by Dr. John Daly of the National Institutes of Health.

The transformation of **5** into **2** in 16 steps in 9% overall yield[26] attests to the efficiency of this photochemical approach to the synthesis of (−)-perhydrohistrionicotoxin and demonstrates the exceedingly high levels of stereochemical control which are possible with the intramolecular [2 + 2] photocycloaddition reaction.[19] The application of the intramolecular vinylogous amide photocycloaddition to the synthesis of other nitrogen-containing ring systems is in progress in our laboratory and will be reported in due course.[27]

Experimental Section

General Procedures. ^1H NMR were recorded at 500 MHz in $CDCl_3$. ^{13}C NMR were recorded on a Varian XL-400 Spectrometer. The pH 7 buffer (3 M KH_2PO_4) which was used in many of the workups was prepared from 205 g of KH_2PO_4 and 35 g of NaOH in 1 L of water. nBuLi and MeLi were titrated against diphenylacetic acid.

5-Pentyl-2-pyrrolidone (6). To a slurry of CuCN (3.753 g, 41.9 mmol) in 98 mL of diethyl ether at −45 °C was added nBuLi (83.8 mmol, 2.5 M in hexanes). The resulting tan suspension was stirred at −40 °C for 90 min and then treated with bromide **5** (1.24 g, 6.97 mmol), which was added dropwise in 15 mL of tetrahydrofuran. The reaction mixture was stirred for 2 h at −40 °C and then stored in a freezer (ca. −15 °C) overnight (ca. 14 h). The reaction mixture was then partitioned between 800 mL of methylene chloride and 800 mL of 1:1 (v/v) concentrated NH_4OH/H_2O. The aqueous layer was extracted two more times with 300 mL of methylene chloride. Evaporation of the volatiles and flash chromatography (10% MeOH/EtOAc) provided a quantitative yield of **6** as a pale yellow oil, which used in the following reaction without further purification. ^1H NMR (500 MHz): 5.7 (s, 1 H), 3.64 (m, 1 H), 2.4–2.2 (m, 2 H), 1.8–1.7 (m, 1 H), 1.6–1.4 (m, 1 H), 1.4–1.3 (m, 8 H), 0.91 (t, 3 H, J = 6.7 Hz) ppm. FT-IR ($CHCl_3$): 3436, 2959, 2931, 2858, 1691 cm^{-1}. $[\alpha]^{22}_D$ −1.9° (*c* 0.042, CH_2Cl_2). Exact mass calculated for $C_9H_{17}NO$ 155.1310, found 155.1300.

Methyl Ester 7. A solution of **6** (2.47 g, 15.9 mmol) in 60 mL of 6 N aqueous HCl was heated at reflux for 24 h. The cooled solution was neutralized (to pH 7) with aqueous NaOH and concentrated under reduced pressure. The residue was triturated with methanol. Evaporation of the methanolic solution afforded the amino acid, which was heated at reflux under a Dean–Stark trap with 1,3-cyclohexanedione (1.78 g, 15.9 mmol) in 175 mL of benzene for 14 h. The crude product was dissolved in 200 mL of methylene chloride and treated with 17.5 mmol of dicyclohexylcarbodiimide, 17.5 mmol of MeOH, and 1.6 mmol of dimethylaminopyridine. After standing for 30 min at 25 °C, the reaction mixture was partitioned between 200 mL of water and 200 mL of methylene chloride. The crude product was chromatographed (silica, 5% MeOH/EtOAc) to furnish the desired ester **7**, as a yellow oil in 67% overall yield from bromide **5**. ^1H NMR (500 MHz): 5.11 (s, 1 H), 4.54 (m, 1 H), 3.67 (s, 3 H), 3.37 (m, 1 H), 2.4–2.2 (m, 6 H), 2.0–1.9 (m, 4 H), 1.8–1.7 (m, 2 H), 1.4–1.2 (m, 2 H), 0.89 (t, 3 H, J = 6.8 Hz) ppm. FT-IR ($CHCl_3$): 3412, 2955, 2933, 2873, 2860, 1731, 1581, 1521, 1257, 1191, 1142 cm^{-1}. $[\alpha]^{22}_D$ + 3.9° (*c* 0.030 CH_2Cl_2). Exact mass calculated for $C_{16}H_{27}NO_3$ (EI) 281.1991, found 281.1944.

Keto Ester 8. A solution of diisopropylamine (0.45 mL, 3.2 mmol) in 11 mL of THF was treated with nBuLi (3.0 mmol, 2.5 M in hexanes) at 0 °C. After stirring for 30–45 min at 0 °C, the solution was cooled to −78 °C, and a solution of *tert*-butyl acetate (0.40 mL, 3.0 mmol) in 11 mL of THF was added dropwise by syringe. After stirring of the mixture for 45 min at −78 °C, a solution of methyl ester **7** (0.337 g, 1.2 mmol) in 11 mL of THF was added dropwise. The resulting solution was stirred at −78 °C for 1.25 h and then warmed to −40 °C for 1.5 h. Finally, the reaction was stirred at 0 °C for 1 h. The reaction mixture was then treated with 65 mL of pH 7 buffer and extracted with 100 and 50 mL of methylene chloride. Chromatography (silica, 2% MeOH/EtOAc) provided 0.262 g (60%) of **8**. ^1H NMR: 5.12 (s, 1 H), 4.3 (m, 1 H), 3.36 (s, 1 H), 3.35 (s, 1 H), 3.4–3.3 (m, 1 H), 2.7–2.5 (m, 2 H), 2.3 (m, 4 H), 1.95 (m, 2 H), 1.9–1.7 (m, 2 H), 1.48 (s, 9 H), 1.4–1.2 (m, 8 H), 0.90 (t, 3 H, J = 7 Hz) ppm. FT-IR ($CHCl_3$): 3693, 3411, 2957, 2934, 1731, 1711, 1581, 1523, 1257, 1191, 1143 cm^{-1}. $[\alpha]^{22}_D$ +15.8° (*c* 0.0080 CH_2Cl_2). Exact mass calculated for $C_{21}H_{35}NO_4$ (EI) 365.2566, found 365.2599.

Photosubstrate 9. A solution of *tert*-butyl keto ester **8** (0.784 mg, 2.15 mmol), acetone (0.63 mL, 8.6 mmol), and trifluoroacetic anhydride (1.21 mL, 8.6 mmol) in trifluoroacetic acid (4.25 mL, 53.7 mmol) was stirred for 24 h at 25 °C. The reaction mixture was then neutralized with 300 mL of pH 7 buffer and extracted with 2 × 200 mL of methylene chloride. Evaporation of the volatiles under reduced pressure, followed by chromatography (silica, 10% MeOH/EtOAc) of the residue, provided 0.629

(20) Jiggajinni, V. B.; Wightman, R. H. *Tetrahedron Lett.* **1982**, 117.
(21) We were unable to perform the Dibal–Wittig sequence on enol triflate **13**, which would have made possible the reduction of the enol triflate and the butenyl side chain in the same step. It was therefore necessary to employ the sequence for **13** → **17**, with two separate hydrogenation steps, as described.
(22) Mitsonobu, O. *Synthesis* **1981**, 1. Varasi, M.; Walker, K. A. M.; Maddox, M. L. *J. Org. Chem.* **1987**, *52*, 4235.
(23) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155.
(24) Huffman, J. W.; Charles, J. T. *J. Am. Chem. Soc.* **1968**, *90*, 6486.
(25) Brown, H. C.; Dickason, W. C. *J. Am. Chem. Soc.* **1970**, *92*, 709.
(26) The overall yields for **5** → **7**, **9** → **12**, and **14** → **17** were higher than the products of the isolated yields reported in the Experimental Section if these sequences were performed without purification of the respective intermediates.
(27) Winkler, J. D.; Scott, R. D.; Muller, C. L. *J. Am. Chem. Soc.* **1988**, *110*, 4831. Winkler, J. D.; Scott, R. D.; Williard, P. G. Unpublished results.

g (84%) of the photosubstrate **9**, as a pale amber oil. ^{1}H NMR: 5.23 (s, 1 H), 5.14 (s, 1 H), 4.00 (m, 1 H), 3.40 (m, 1 H), 2.35 (m, 4 H), 2.27 (m, 2 H), 2.0 (m, 2 H), 1.9–1.8 (m, 2 H), 1.71 (s, 3 H), 1.70 (s, 3 H), 1.4–1.3 (m, 8 H), 0.91 (t, 3 H, J = 6.7 Hz) ppm. FT-IR ($CHCl_3$): 3694, 3415, 2955, 1722, 1585, 1515, 1255, 1191 cm^{-1}. $[\alpha]^{22}_D$ +44.0 (0.248, CH_2Cl_2).

Photoadduct 10. A solution of the photosubstrate **9** (0.192 g, 0.55 mmol) in 85 mL of MeCN was degassed by passing a stream of N_2 gas through the solution for 30 min at 0 °C. Irradiation for 30 min using a medium pressure Hg lamp in a Pyrex immersion well, followed by evaporation of the volatiles under reduced pressure, provided a 15:1 mixture of the desired photoadduct **10** and a stereoisomer (95% total yield). Chromatography (silica, 20% EtOAc/petroleum ether) effected conversion of **10** to its isomer.

Adduct 10. ^{1}H NMR: 3.19 (dd, 1 H, J = 1.7, 10.9 Hz), 3.05 (d, 1 H, J = 10.8 Hz), 2.60 (m, 1 H), 2.45–2.35 (m, 3 H), 2.30 (m, 1 H), 2.1–2.0 (m, 1 H), 2.0–1.8 (m, 4 H), 1.63 (s, 3 H), 1.60 (s, 3 H), 1.4–1.1 (m, 10 H), 0.90, (t, 3 H, J = 7 Hz) ppm. FT-IR ($CHCl_3$): 3693, 3685, 2957, 2932, 2858, 1737, 1703, 1271, 1265 cm^{-1}. ^{13}C NMR: 209, 170, 107, 73.9, 64.5, 51.7, 46.5, 43.4, 41.4, 38.0, 36.8, 32.9, 32.6, 29.6, 28.9, 28.7, 26.5, 23.6, 20.6, 15.1 ppm. Exact mass calculated for $C_{20}H_{31}NO_4$ (EI) 350.2331, found 350.2305.

C-7 Epimer of Photoadduct 10. MS (EI): 349. ^{1}H NMR: 3.48 (d, 1 H, J = 5.0 Hz), 3.45 (d, 1 H, J = 5.0 Hz), 3.12 (m, 1 H), 1.74 (s, 3 H), 1.67 (s, 3 H), 0.91 (m, 3 H) ppm. FT-IR ($CHCl_3$): 2955, 2932, 2860, 1740, 1701, 1265 cm^{-1}.

Hydroxydioxanone 11. Sodium borohydride (89 mg, 2.35 mmol) in 7.4 mL of EtOH was added to a solution of photoadduct **10** (0.687 g, 1.97 mmol) in 95 mL of THF at −78 °C. The mixture was partitioned between 400 mL of pH 7 buffer and 400 mL of methylene chloride, and the aqueous portion extracted with an additional 200 mL portion of methylene chloride. Concentration of the organic phase provided 713 mg of the crude alcohol, which was chromatographed (silica, 50% EtOAc/petroleum ether) to return 447 mg (65%) of pure **11**. ^{1}H NMR: 3.9–3.8 (m, 2 H); 3.16 (m, 1 H), 2.80 (d, 1 H, J = 10.4 Hz), 2.67 (m, 1 H), 2.39 (dt, 1 H, J^d = 14.7 Hz, J^t = 3.6 Hz), 2.0–1.8 (m, 4 H), 1.75–1.6 (m, 6 H), 1.63 (s, 3 H), 1.59 (s, 3 H), 1.5–1.3 (m, 8 H), 0.91 (t, 3 H) ppm. FT-IR ($CHCl_3$): 3420 (br), 2954, 2935, 1713, 1281, 1197 (vs), 1180 cm^{-1}. $[\alpha]^{22}_D$ +11.5 (c 0.0248, CH_2Cl_2). MS (EI): 351, 293, 265.

Keto Lactone 12. NaH (0.153 g, 60% suspension in oil, 3.8 mmol) was added as solid to a solution of the reduced photoadduct **11** (0.447 g, 1.27 mmol) in 70 mL of THF. The reaction mixture was placed under nitrogen and stirred at 25 °C for 30 min. The reaction mixture was partitioned between 120 mL of pH 7 buffer and 120 mL of chloroform. The organic phase was concentrated and chromatographed (silica, 20% EtOAc/petroleum ether) to furnish 0.354 g (95%) of the keto lactone **12**. ^{1}H NMR: 4.76 (dt, 1 H, J^d = 11 Hz, J^t = 6.6 Hz), 3.2 –3.1 (m, 1 H), 2.48 (m, 1 H), 2.33 (dd, 1 H, J = 17.2, 13.8 Hz), 2.3–2.1 (m, 4 H); 2.05 (dd, 1 H, J = 17.3, 8.3 Hz), 1.7–1.5 (m, 4 H), 1.5–1.3 (m, 8 H), 0.92 (t, 3 H, J = 6.8 Hz) ppm. FT-IR ($CHCl_3$): 2957, 2933, 2872, 2859, 1772, 1707, 1170 cm^{-1}. ^{13}C NMR: 211, 175, 77.4, 62.9, 49.2, 37.7, 37.5, 36.9, 36.3, 31.8, 29.2, 28.5, 28.1, 26.3, 22.6, 16.8, 14.1 ppm. $[\alpha]^{22}_D$ −86.6 (c 0.082, CH_2Cl_2). Exact mass calculated for $C_{17}H_{27}NO_3$ (CI) 294.2069, found 294.1972.

Enol Triflate 13. To a solution of diispropylamine (0.105 mL, 0.750 mmol) in 0.5 mL of THF at 0 °C was added nBuLi in hexanes (0.228 mL, 0.703 mmol), and the resulting solution was stirred at 0 °C for 30 min. To this solution was then added the keto lactone **12** (137.3 mg, 0.469 mmol) in 1 mL of 1:1 (v/v) tetramethylethylenediamine/THF. The reaction mixture was stirred for 1 h at 0 °C and was then rapidly transferred by cannula to a 25 °C slurry of Tf_2NPh (0.502 g, 1.40 mmol) in 1 mL of 1:1 (v/v) tetramethylethylenediamine/THF. After stirring for 1 h at 25 °C, the yellow reaction mixture was partitioned between 25 mL of pH 7 buffer and 25 mL of methylene chloride. Concentration of the organic layer followed by flash chromatography (silica, 5% EtOAc/petroleum ether to 10% and then 20%) provided 152.4 mg (76%) of **13**, the desired enol triflate. ^{1}H NMR: 5.85 (dd, 1 H, J = 2.6 Hz), 4.71 (dt, 1 H, J^d = 11.2 Hz, J^t = 6.3 Hz), 2.85 (m, 1 H), 2.55 (dd, 1 H, J = 14.0, 17.1 Hz), 2.34 (dd, 1 H, J = 8.1, 17.1 Hz), 2.31 (m, 1 H), 2.13 (m, 1 H), 1.95 (m, 1 H), 1.8–1.1 (m, 14 H), 0.90 (t, 3 H, J = 7 Hz) ppm. FT-IR ($CHCl_3$): 3450, 2934, 1772, 1414, 1197 (vs) cm^{-1}. $[\alpha]^{22}_D$ −163.2° (c 0.072, CH_2Cl_2). Exact mass calculated for $C_{18}H_{26}F_3NO_5S$ (CI) 426.15620, found 426.15573.

Deoxy Lactone 14. PtO_2 (11 mg, 0.048 mmol) was added to a solution of the enol triflate **13** (78 mg, 0.18 mmol) in 3.5 mL of ethanol, and the reaction mixture was stirred under 1 atm H_2 overnight. The reaction mixture was then partitioned between 25 mL of pH 7 buffer and 25 mL of methylene chloride. Concentration afforded 54 mg of crude product. Chromatography (silica, EtOAc) of the crude product returned the desired deoxy lactone **14** in 96% yield. ^{1}H NMR: 4.62 (dt, 1 H, J^d = 11.0 Hz, J^t = 6.5 Hz), 3.02 (m, 1 H), 2.82 (m, 1 H), 2.35–2.21 (m, 2 H), 2.11 (m, 1 H), 1.7–1.1 (m, 20 H), 0.89 (t, 3 H, J = 7 Hz) ppm. FT-IR ($CHCl_3$): 3363, 2932, 2861, 1767, 1423, 1238, 1212, 1147, 1142 cm^{-1}. $[\alpha]^{22}_D$ −77.1 (c 0.048, CH_2Cl_2). Exact mass calculated for $C_{17}H_{29}NO_2$ (EI) 279.2183, found 279.2202.

Lactols 15. DIBAL (1.5 M in toluene, 0.48 mL, 0.72 mmol) was added dropwise to a solution of deoxy lactone **14** (50.6 mg, 0.18 mmol) in 11 mL of THF at −40 °C. After stirring for 30 min at −40 °C, the reaction was quenched with 0.029 mL (4 equiv) of MeOH. Partitioning between 100 mL of ether and 100 mL of pH 7 buffer, followed by concentration of the ethereal layer, gave 39.6 mg (78%) of a nearly 1:1 mixture of lactols **15**. This material was best used immediately in the next reaction. ^{1}H NMR: 5.55 (t, 0.5 H, J = 5.8 Hz), 5.49 (d, 0.5 H, J = 5.2 Hz), 4.28 (d of t, 0.5 H, J^d = 10.8 Hz, J^t = 6.6 Hz), 4.05 (d of t, 0.5 H, J^d = 11.2 Hz, J^t = 6 Hz), 3.09 (m, 1 H); 2.86 (m, 1 H), 2.78 (m, 1 H), 2.50 (m, 1 H), 2.10 (m, 1 H), 1.9–1.1 (m, 19 H), 0.88 (m, 3 H) ppm.

Hydroxy Olefins 16. To a slurry of ethyltriphenylphosphonium bromide (735 mg, 1.98 mmol) in 7 mL of THF at 0 °C was added nBuLi (1.80 mmol, 2.5 M in hexanes). After stirring for 30 min at 0 °C, the orange ylide solution was transferred to a solution of lactols **15** (50.6 mg, 0.18 mmol) in 7 mL of THF. The reaction was stirred for 3.5 h, then partitioned between 75 mL of pH 7 buffer and 75 mL of methylene chloride. Concentration of the organic layer and chromatography (silica, neat EtOAc to 5% MeOH/EtOAc) provided 37.9 mg (72%) of hydroxyolefins **16** as a 1:1 mixture of double bond isomers. ^{1}H NMR: 5.57–5.40 (m, 2 H), 4.10 (m, 1 H), 2.80 (m, 1 H), 2.45–2.25 (m, 3 H), 1.94 (m, 1 H), 1.66 (m, 3 H), 1.8–1.2 (m, 21 H), 0.89 (t, 3 H, J = 7 Hz) ppm.

8-*epi*-Perhydrohistrionicotoxin (17). PtO_2 (2.3 mg, 0.01 mmol) was added to a solution of the hydroxy olefins **16** (11.7 mg, 0.040 mmol) in 1 mL of ethanol, and the resulting mixture was stirred under 1 atm of H_2 overnight at 25 °C. Filtration and concentration furnished 11.7 mg (100%) of 8-*epi*-perhydrohistrionicotoxin, **17**. ^{1}H NMR: 4.06 (dt, 1 H, J^d = 11.7 Hz, J^t = 4.3 Hz), 2.80 (m, 1 H), 2.19 (m, 1 H), 1.8–1.0 (m, 27 H), 0.90 (t, 3 H, J = 7 Hz), 0.88 (t, 3 H, J = 7 Hz) ppm. FT-IR ($CHCl_3$): 3417, 2957, 2935, 2871, 1255, 1197, 1178 cm^{-1}. $[\alpha]^{22}_D$ −20.4° (e 0.0169, CH_2Cl_2). Exact mass calculated for $C_{19}H_{35}NO$ (EI) 295.2875, found 295.2890.

8-Oxoperhydrohistrionicotoxin (18). Dess–Martin periodinane (52 mg, 0.12 mmol) was added as a solid to a solution of 8-*epi*-perhydrohistrionicotoxin, **17**, (24.7 mg, 0.084 mmol) in 1.2 mL of methylene chloride, and the resulting heterogeneous mixture was stirred for 90 min at 25 °C. The reaction was diluted with 8 mL of ether and 6 mL of saturated aqueous $NaHCO_3$ containing 5% sodium thiosulfate pentahydrate. The biphasic system was stirred vigorously for 10 min. Both layers became nearly clear, and the whole mixture was partitioned between 50 mL of pH 7 buffer and 50 mL of ether. Concentration of the organics followed by flash chromatography (silica, 50% EtOAc/petroleum ether) provided the desired ketone, **18**, in 87% yield as a pale yellow oil. ^{1}H NMR: 2.82 (dd, 1 H, J = 4.3, 11.6 Hz), 2.74 (m, 1 H), 2.37 (m, 1 H), 2.17 (m, 1 H), 2.05–1.9 (m, 2 H), 1.8 (m, 2 H), 1.67 (m, 1 H), 1.5–1.0 (m, 20 H), 0.89–0.85 (m, 6 H) ppm. FT-IR ($CHCl_3$): 2957, 2933, 2870, 1699, 1192 cm^{-1}. $[\alpha]^{22}_D$ −5.1 (c 0.023, CH_2Cl_2). Exact mass calculated for $C_{19}H_{33}NO$ (EI) 293.27186, found 293.27269.

(−)-Perhydrohistrionicotoxin (2). To a solution of oxoperhydrohistrionicotoxin, **18**, (22.6 mg, 0.077 mmol) in 0.85 mL of THF at 0 °C was added dropwise $LiAl(OtBu)_3H$ (0.77 mmol, 0.669 M in THF), and the resulting mixture was maintained at 0 °C for 24 h. The reaction was then partitioned between 20 mL of pH 7 buffer and 20 mL of methylene chloride. Concentration of the organics provided a quantitative yield of a 19:1 mixture of (−)-perhydrohistrionicotoxin, **2**, and the isomeric alcohol **17**. ^{1}H NMR: 3.88 (d, 1 H, J = 2.5 Hz), 2.90 (m, 1 H), 2.19 (br, d, 1 H, J = 5.3 Hz), 2.02 (q of t, 1 H, J^q = 13.7 Hz, J^t = 4.1 Hz), 1.80 (dt, 1 H, J^d = 14 Hz, J^t = 2.8 Hz), 1.73 (br dd, 1 H, J = 13, 2.9 Hz), 1.65–1.5 (m, 6 H), 1.47–1.3 (m, 13 H), 1.1–1.0 (m, 2 H), 0.91–0.86 (m, 6 H), 0.85–0.78 (md, 1 H, J^m = 12.4 Hz, J^d = 4.8 Hz), 0.71 (td, 1 H, J^t = 13.4 Hz, J^d = 4.2 Hz) ppm. FT-IR ($CHCl_3$): 3420, 2933, 2872, 2861, 1467, 1197, 1178 cm^{-1}. ^{13}C NMR: 69.8, 55.0, 49.9, 38.2, 38.0, 37.2, 37.2, 33.5, 32.1, 30.3, 27.8, 27.7, 25.6, 23.0, 22.5, 19.6, 15.2, 14.1, 14.0 ppm. Exact mass calculated for $C_{19}H_{35}NO$ (EI) 295.28751, found 295.28608. $[\alpha]^{22}_D$ −84.1 (c 0.024, CH_2Cl_2) for synthetic and $[\alpha]^{22}_D$ −83.1 (c 0.0067, CH_2Cl_2) for the authentic sample supplied by Dr. John Daly.

Attempted Acid Fragmentation of 19. The photoadduct **19** (69.4 mg, 0.206 mmol) was dissolved in 5.4 mL of 0.0075 M *p*-toluenesulfonic acid monohydrate in 9:1 MeOH/H_2O, and the resulting solution was warmed to reflux for 20 h. The reaction was partitioned between 25 mL of pH 7 buffer and 25 mL of methylene chloride, and the organic layer was concentrated to furnish 42.5 mg of crude product. The main products in this material were **21** and **22** in a 3:2 ratio. The yields of **21** and **22**

were 9.5% and 7.8%, respectively. Omission of the water made **22** the major product, but both products were always formed under a variety of acidic reaction conditions. Modest separation was realized by TLC with 50% ethyl acetate/petroleum ether as eluent.

Aminal 21. ^{1}H NMR: 3.94 (m, 1 H), 3.71 (s, 3 H), 3.35 (s, 3 H), 2.85 (d, 1 H, J = 9.7 Hz), 2.71 (d, 1 H, J = 14.7 Hz), 2.45-2.3 (m, 3 H), 2.1-1.9 (m, ca. 4 H), 1.8-1.5 (m, ca. 4 H), 1.66 (s, 3 H), 1.62 (s, 3 H) ppm. FT-IR: 2952, 1732 (br) cm^{-1}. MS (EI): 369, 311.

Acetal 22. ^{1}H NMR: 3.93 (dd, 1 H, J = 10.9, 3.2 Hz), 3.77 (s, 3 H); 3.45 (s, 3 H), 2.90 (m, 1 H), 2.65 (m, 1 H), 2.6-2.5 (m, 1 H), 2.5-2.2 (m, 5 H), 2.1-1.9 (m, 3 H), 1.6-1.4 (m, ca. 3 H) ppm. FT-IR: 1777, 1739 (contains a right shoulder) cm^{-1}. MS (EI): 311. ^{13}C NMR: 207.5, 173.0, 172.0, 110.1, 63.8, 52.9, 52.5, 51.8, 41.7, 37.2, 33.2, 29.6, 29.0, 28.9, 16.4 ppm.

Reduction of Ketone 18 with Lithium in Liquid Ammonia. To the blue solution prepared from 3 mg of lithium wire and 1 mL of liquid ammonia (distilled from Na^0) was added ketone **18** (3.4 mg, 0.012 mmol) in 0.5 mL of THF containing 2.5 μL of MeOH. After stirring for 1 h at -78 °C, the reaction mixture was partitioned between $CHCl_3$ and pH 7 buffer. Evaporation of the organic layer furnished 4 mg of crude product, whose ^{1}H NMR spectral data and TLC behavior (15% MeOH/EtOAc) were identical with those reported above for **17**.

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1,1′-Carbonylbis(3-methylimidazolium) Triflate: An Efficient Reagent for Aminoacylations

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Abstract: Amino acid carboxyl activation and subsequent coupling with nucleophiles frequently suffer from uncertain risks of racemization, complex reagent preparation, or troublesome side-product removal. All of these difficulties are eliminated with a new, simple reagent, 1,1′-carbonylbis(3-methylimidazolium) triflate (CBMIT), obtainable readily by bis-alkylation of carbonyldiimidazole with methyl triflate. Via a highly reactive acyl imidazolium intermediate, CBMIT couples amino acid components or amino acids and alcohols to give peptides and esters, easily isolated in high yield. The reaction medium remains free of any base, and no loss of optical activity is observed.

The subject of carboxyl activation of N-blocked amino acids, though explored in great detail, continues to attract new interest. The conventional coupling methods, utilizing reagents such as DCC, azides, carbonyldiimidazole, active esters, or anhydrides, though widely used, are not free of limitations.[1] Often, few or no options are available for demanding applications in both O-acylation (esters) and N-acylation (peptides). The esterification reaction, in particular, suffers from poor yields, long reaction times, and unacceptable amounts of racemization.

In studies on the synthesis of the 3′-terminal aminoacyl oligonucleotides of tRNA, we required a method for esterification that would satisfy the criteria of simple reagent preparation, mild coupling conditions, and retention of optical purity. Most conventional methods as well as those developed recently[2] did not meet these requirements. The reagent that has attracted most use in the synthesis of aminoacyl tRNA[3] is carbonyldiimidazole (CDI), due to the mild conditions under which it can be used. However, CDI has obvious drawbacks in that the intermediate acyl imidazolides are sluggish toward O-acylation,[4] and substantial racemization in the acyl component has also been reported.[5]

We have shown earlier, in past work from this laboratory, that alkylation of the imidazole nitrogen of (benzyloxycarbonyl)-imidazole with Meerwein's reagent greatly enhanced its reactivity as a CBZ-transfer reagent to poorly nucleophilic DNA bases.[6] Correspondingly, therefore, we projected that alkylation of the imidazole ring in acyl imidazoles should lead to highly reactive acyl-transfer species. The risks of racemization would be minimized if the coupling reaction could be conducted in the total absence of base. A direct route to acyl imidazolium salts such as **2** would be realized, if a bis-alkylated CDI could be obtained. One key feature in designing such a highly reactive version of CDI would have to be the choice of the counteranion as it must be nonbasic and nonnucleophilic. We herein describe the easily prepared reagent 1,1′-carbonylbis(3-methylimidazolium) triflate (CBMIT)[7] and its use in the preparation of complex, optically pure esters and peptides in high yields and under remarkably mild conditions.

Results and Discussion

The readily available, excellent alkylating reagent methyl triflate was chosen for alkylation of CDI, since the trifluoromethane-

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(7) The presumed preparation of a bis-alkylated CDI was postulated in ref 2c; however, no proof of its formation was given. Furthermore, a reactive halide counteranion was involved, and no applications in the coupling of chiral components were presented.

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