to afford 50.5 mg (91%) of **17** and 5.0 mg (9%) of **18**. Spectral data of **17** were as follows: NMR ($CDCl_3$, δ) 0.81 (d, 3 H, J = 7 Hz), 0.98–1.42 (m, 6 H), 1.52–2.00 (s + m, 7 H), 2.08 (dd, 1 H, J = 6, 13 Hz), 2.24 (s, 3 H), 2.81 (m, 1 H), 3.06–3.20 (s + m, 4 H), 3.28–3.44 (s + m, 4 H), 3.49 (s, 3 H), 3.71 (s, 3 H), 4.75 (s, 1 H), 5.21 (q, 1 H), 5.69 (d, 2 H, J = 6 Hz), 7.30–7.50 (m, 5 H); FAB mass spectrum, m/e 533 ($M^+ + 1$). Anal. Calcd for $C_{30}H_{44}O_8$: C, 67.64; H, 8.32. Found: C, 67.70; H, 8.44.

(7*R*,12*R*,13*R*)-(*E*,*E*)-Methyl 12-[(*S*)-α-Methoxyphenylacetoxy]-14-methoxy-13-(methoxycarbonyl)-3,5,7-trimethyl-2,4-tetradecadienoate (18). The compound was prepared from **16** and (*S*)-(+)-α-methoxyphenylacetic acid according to the method described for **17**. Spectral data were as follows: NMR ($CDCl_3$, δ) 0.75 (d, 3 H, J = 7 Hz), 0.76–0.94 (m, 3 H), 0.96–1.12 (m, 3 H), 1.38–1.54 (m, 3 H), 1.67–1.79 (s + m, 4 H), 2.00 (dd, 1 H, J = 6, 12 Hz), 2.23 (s, 3 H), 2.90 (m, 1 H), 3.30 (s, 3 H), 3.38–3.50 (s + m, 4 H), 3.56–3.74 (2 s + m, 7 H), 4.74 (s, 1 H), 5.21 (q, 1 H), 5.68 (s, 2 H), 7.30–7.50 (m, 5 H); FAB mass spectrum, m/e 533 ($M^+ + 1$). Anal. Calcd for $C_{30}H_{44}O_8$: C, 67.64; H, 8.32. Found: C, 67.66; H, 8.31.

Synthesis of a Tritium-Labeled Photoaffinity Analogue of the Tussock Moth Pheromone: Tritium NMR of Vinyl Tritons of (*E*)- and (*Z*)-Alkene Isomers

Bachir Latli and Glenn D. Prestwich*

Department of Chemistry, State University of New York, Stony Brook, New York 11794-3400

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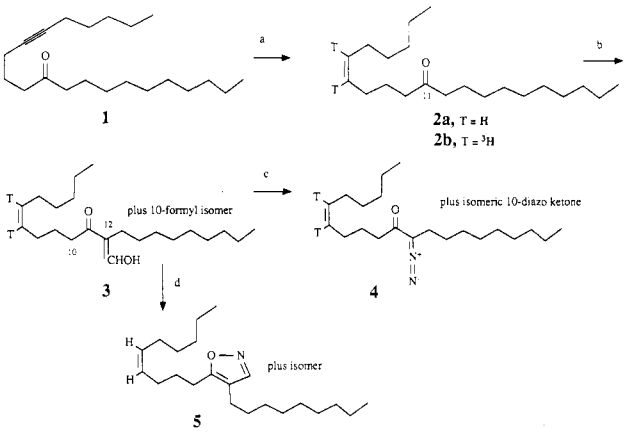
Introduction

The molecular mechanisms of pheromone perception[1] and degradation in insect olfactory sensilla can be examined in detail by using high specific activity tritium-labeled pheromones and pheromone analogues.[2] We recently identified pheromone binding proteins and putative pheromone receptors in the wild silkmoth, *Antheraea polyphemus*, using tritium-labeled (*E*,*Z*)-6,11-hexadecadienyl diazoacetate, a photoaffinity analogue of the natural hexadecadienyl acetate pheromone.[3] Many lepidopteran forest pests belong to the family Lymantriidae, which includes the gypsy moth and the tussock moths. We were intrigued by the unique ketonic pheromone of the Douglas fir tussock moth, *Orgyia pseudotsugata*, as a model system to examine the use of diazo ketone photoaffinity labels in this economically important group of insects.

The major pheromone component for *O. pseudotsugata* was identified in 1975 as (*Z*)-6-heneicosen-11-one,[4] and its occurrence in other tussock moths and the activity of several isomers and analogues have been briefly investigated.[5] Two syntheses[6] of this alkenone have been described in which the Eschenmoser fragmentation of an epoxy ketone is used to produce an acetylenic intermediate; the pheromone is obtained by semihydrogenation of an alkynone. These two schemes were most suitable for the introduction of tritium at high specific activity, i.e., by semitritiation with carrier-free tritium gas.

Scheme I. Synthesis of Labeled and Unlabeled Pheromone Photoaffinity Labels[a]



[a] Reagents: (a) 1 atm of $^nH_2$ (n = 1 or 3), 5% $Pd/BaSO_4$, THF, quinoline, 1 h, 20 °C; (b) NaH, $HCO_2C_2H_5$, ether, EtOH, 10 h, 30 °C; (c) TsN_3, CH_2Cl_2, Et_3N, 3 h, 0–20 °C; 1 N KOH, 0.5 h, 20 °C; (d) NH_2OH·HCl, EtOH, K_2CO_3, 80 °C.

The unlabeled mixture of diazo ketones **4a** was obtained as shown in Scheme I. Thus, alkynone **1** was obtained by using either the Kocienski[6a] or Mori[6b] procedures and was semihydrogenated in THF using 5% $Pd/BaSO_4$ poisoned with quinoline to give TLC- and GC-homogeneous enone **2a**. Formylation with sodium hydride and ethyl formate[7] provided a mixture of the 10- and 12-formylated ketones **3a** in a ratio of approximately 2:1 based on the ratios of enolic carbon resonances. In deuteriochloroform, approximately 10% of the β-dicarbonyl form was detectable by proton NMR; however, the formyl compounds decomposed during extended data acquisition. Thus, the crude formyl ketones **3a** were subjected to the diazo transfer reaction with tosyl azide[8] to provide the mixed 10- and 12-diazo ketones **4a** in 54% overall yield. The diazo ketone mixture induces upwind flight behavior at 20 ng and shows approximately 40% of the electroantennogram response[5] relative to the actual pheromone (at a 2000-ng dose) in male tussock moths (G. E. Daterman, personal communication).

The mixture of formyl compounds was converted in 81% yield to a complex mixture of the isoxazoles **5** by treatment with hydroxylamine hydrochloride to obtain thermally stable derivatives of the 10 and 12 isomers. Capillary GC analysis showed eight peaks, consistent with the presence of both (*E*)- and (*Z*)-alkenes (see below), the 10- and 12-formylated species, and two modes of addition of the hydroxylamine. Nonetheless, the high resolution mass spectroscopy confirmed the elemental composition of this mixture of isoxazole isomers.

The tritium-labeled pheromone **2b** and its derivatives were produced similarly. Catalytic tritiation of 10 mg of alkynone **1** using carrier-free tritium gas furnished 1.73 Ci of [^{3}H]-**2b**, specific activity 50–58 Ci/mmol. A 156-mCi fraction of purified [^{3}H]alkenone **2b** was formylated and

(1) (a) Vogt, R. G. In *Pheromone Biochemistry*; Prestwich, G. D., Blomquist, G. J., Eds.; Academic: New York, 1987; pp 385–431. (b) Bestmann, H.-J. In *Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Springer-Verlag: Berlin, 1986; p 45. (c) Kaissling, K.-E. *Ann. Rev. Neurosci.* **1986**, *9*, 121.

(2) (a) Prestwich, G. D. *Science (Washington, D.C.)* **1987**, *235*, 999. (b) Prestwich, G. D. In *Pheromone Biochemistry*; Prestwich, G. D., Blomquist, G. J., Eds.; Academic: New York, 1987; p 473, 527.

(3) (a) Prestwich, G. D.; Golec, F. A.; Andersen, N. H. *J. Labelled Cmpnd. Radiopharm.* **1984**, *21*, 593. (b) Vogt, R. G.; Prestwich, G. D.; Riddiford, L. M. *J. Biol. Chem.* **1988**, *263*, 3952.

(4) Smith, R. G.; Daterman, G. E.; Daves, G. D., Jr. *Science (Washington, D.C.)* **1975**, *188*, 63.

(5) (a) Daterman, G. E.; Peterson, L. J.; Robbins, R. G.; Sower, L. L.; Daves, G. D., Jr.; Smith, R. G. *Environ. Entomol.* **1976**, *5*, 1187. (b) Grant, G. G. *Environ. Entomol.* **1977**, *6*, 739.

(6) (a) Kocienski, P. J.; Cernigliaro, G. J. *J. Org. Chem.* **1976**, *41*, 2927. (b) Mori, K.; Uchida, M.; Matsui, M. *Tetrahedron* **1977**, *33*, 385.

(7) Ainsworth, C. *Org. Synth.* **1963**, *4*, 536.

(8) (a) Regitz, M.; Ruter, J.; Liedhegener, A. *Org. Synth.* **1971**, *51*, 86. (b) Hendrickson, J. B.; Wolf, W. A. *J. Org. Chem.* **1968**, *33*, 3610. (c) Regitz, M.; Hocker, J.; Liedhegener, A. *Org. Synth.* **1968**, *48*, 36.

0022-3263/88/1953-4603$01.50/0

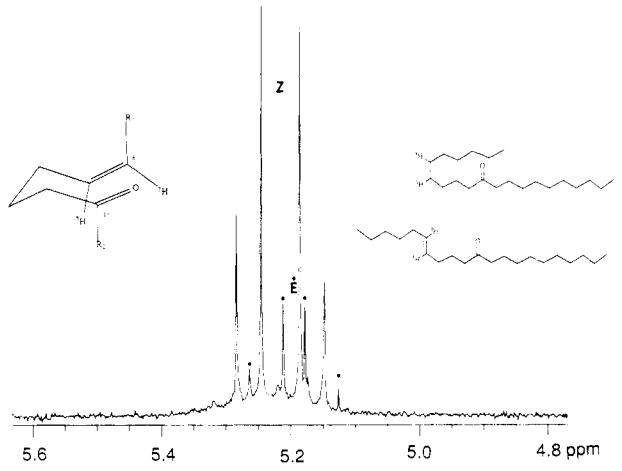


Figure 1. Tritium NMR spectrum of [6,7-$^3H_2$]-(*Z*)-6-heneicosen-11-one. Proton-decoupled 320-MHz ^3H NMR spectrum showing expanded vinyl region. Inset: proposed conformation of ketone illustrating interaction of carbonyl with alkene tritons.

converted to the diazo ketone mixture [^{3}H]-**4b** in 24% radiochemical yield after miniflash chromatography. The labeled pheromone and photoaffinity label were employed in biochemical experiments which will be described elsewhere.

The tritium-labeled pheromone **2b** (approximately 250 mCi in deuteriobenzene) was examined by proton NMR and by tritium NMR.[9] Vinyl protons were completely absent from the ^1H NMR spectrum of **2b**. The tritium NMR spectrum of **2b** allowed exclusive observation of the triton resonances and thus provided a unique and clear assessment of the extent of overreduction, the amount of *Z* and *E* isomers, and the presence or absence of partially tritiated materials. About 5% of the total tritium signal appears in a small peak at δ 1.15, corresponding to some production of overreduced material. The proton-decoupled 320-MHz ^3H NMR spectrum (Figure 1) shows one predominant AB quartet for the chemically nonequivalent C-6 and C-7 vinyl tritons of the (*Z*)-alkene. This nonequivalence must result from the 11-oxo substituent, perhaps via interactions as shown in the inset (Figure 1). Most interestingly, the expanded vinyl region clearly shows *two* AB quartets. The major pattern (δ_A 5.261, δ_B 5.171, J_{AB} = 12.44 Hz) corresponds to the expected *Z* isomer, while the minor pattern (δ_A 5.230, δ_B 5.160, J_{AB} = 16.59 Hz) is due to the *E* isomer. Note that the *Z* isomer has a larger chemical shift difference but a smaller coupling constant than the *E* isomer. It is a unique feature of tritium NMR that these vinyl AB patterns are so readily discerned in a long-chain alkene, since no homonuclear virtual coupling is present. Clear AB patterns for the vinyl *proton* region in the unlabeled alkenone **2a** could not be observed, even when extensive homonuclear decoupling of the allylic proton region is used.

Experimental Section

General. All solvents were distilled before use. Flash chromatographic purifications were carried out on Woelm silica, 32–63 μm. TLC was performed with MN Polygram Sil G/UV 254 (4 × 8 cm) TLC plates, and yields are reported for compounds which appear >95% homogeneous by TLC and ^1H NMR. Important ^1H NMR and ^{13}C NMR resonances are reported, and integrated intensities are consistent with the assigments indicated. Multiplicity of carbon signals was verified by the attached proton test (APT). Tritium NMR spectra were obtained at 320 MHz by Dr. P. G. Williams (National Tritium Labeling Facility) on a Bruker WM-300 or by Mr. S. McG. Graham and Dr. T. M. Barbara (Stony Brook) on a custom-built probe for the NT-300 (Doty Scientific). Mass spectra (HRMS) were obtained at 70-eV ionization potential. Autoradiography was performed on Kodak XAR-5 film. Radio TLC scanning (RTLCS) was performed on a Bioscan System 500 imaging scanner. Radioactive samples were counted in an LKB 1218 RackBeta liquid scintillation counter by using a PPO–PO-POP–toluene scintillation cocktail.

6-Heneicosyn-11-one (1). The required ynone was prepared by using both the Kocienski (method A) and Mori (method B) methods; the latter was more convenient for analogue preparation. We summarize our final routes to the ynone here.

Method A.[6a] The known[6] ynone **1** (19.5 mg, 67%) was obtained from 31 mg of 2-*n*-pentyl-3-*n*-decyl-2,3-epoxycyclohexan-1-one[6a] following the reported procedures: ^1H NMR ($CDCl_3$) δ 2.64 (t, J = 7.3 Hz, 2 H), 2.50 (t, J = 7.4 Hz, 2 H), 2.1–2.24 (m, 4 H), 1.85 (m, 2 H), 1.2–1.7 (m, 22 H), 0.9 (2 overlapping t, 6 H); IR 1720 cm^{-1} (C=O).

Method B.[6b] To a solution of the ethylene ketal of 1-hexadecyn-6-one (0.95 g, 3.4 mmol) in dry THF (20 mL) at −78 °C was added 5 mL of a 2.5 M solution of *n*-BuLi in hexanes. After being stirred for 1 h at −78 °C, 5 mL of dry HMPA was added and the red solution was stirred for 0.5 h. Next, excess 1-bromopentane (3 g) was added to the reaction mixture, and the solution was stirred at 0 to 20 °C for 2 h. This differs slightly from Mori's procedure in which the bromopentane is added in HMPA solution, which gave low yields in our hands. The reaction was worked up as usual to give 0.958 g (81%) of the ethylene ketal of ynone **1**. A portion of the ketal (530 mg) was hydrolyzed in 5 mL of THF and 1 mL of 3 N $HClO_4$ for 24 h at 20 °C. The usual workup afforded homogeneous ynone **1** in 89% yield after chromatography, and the physical and spectral properties were identical with literature values and with material prepared above.

(*Z*)-6-Heneicosen-11-one (2a). A mixture of ynone **1** (410 mg, 1.3 mmol), 70 mg of 5% Pd/$BaSO_4$, and 0.4 mL of a solution of quinoline in hexane (100 mg/mL) in dry THF (15 mL) was stirred under 1 atm of H_2 for 1–3 h. The reaction was followed by TLC to minimize overreduction. Although the reaction proceeds more rapidly in MeOH, THF was employed to minimize H–T exchange which decreases the specific activity of the product in the semitritiation described below.[2b] The reaction was filtered through a short pad of Celite, washed with ether, and concentrated; the residue was dissolved in ether and washed (aqueous HCl (2%), aqueous $NaHCO_3$, brine), dried ($MgSO_4$), filtered, concentrated in vacuo, and purified by flash chromatography (1 to 10% EtOAc/hex) to give 390 mg (95%) of homogeneous enone **2a**, which gave spectral data identical with those reported:[6] ^1H NMR ($CDCl_3$) δ 5.2–5.5 (m, 2 H), 2.4 (t, J = 7.4 Hz, 4 H), 2.0 (m, 4 H), 1.6 (m, 4 H), 1.25, (br s, 20 H), 0.87 (br t, 6 H, J = 6 Hz); ^{13}C NMR ($CDCl_3$) δ 211.39, 128.65, 129.16, 42.06, 42.79, 31.89, 31.58, 29.57, 29.49, 29.42, 29.29, 27.20, 26.56, 25.25, 23.89, 23.75, 23.58, 22.65, 22.53, 20.60, 14.07; IR 1720 cm^{-1} (C=O).

[6,7-$^3H_2$]-(*Z*)-6-Heneicosen-11-one (2b). Semitritiation was carried out at the National Tritium Labeling Facility (NTLF; Berkeley, CA) with carrier-free tritium gas[2b] by scaling down the conditions for the unlabeled reaction. Thus, 10 mg of ynone **1**, 6 mg of 5% Pd/$BaSO_4$, 20 μL of 10% quinoline–hexane, and 2.5 mL of dry THF were freeze–thaw–degassed three times, stirred under 1 atm of carrier-free $^3H_2$ gas for 1 h, concentrated, filtered, lyophilized, and purified by flash chromatography (1 to 10% EtOAc/hex) in a disposable pipette to give ca. 9 mg (1.73 Ci) of the labeled enone **2b** (specific activity 50–58 Ci/mmol). This material was subdivided into several vials of 86 or 172 mCi each, stored in 0.5 mL–2.0 mL of heptane/toluene (1:1) at −20 °C. Proton-decoupled ^3H NMR (C_6D_6): δ 1.15 (br s), major AB quartet, 5.261 and 5.171 (d, d, J_{AB} = 12.44 Hz), minor AB quartet, 5.230 and 5.160 (d, d, J_{AB} = 16.59 Hz). The integrated intensities correspond to an *E*:*Z* ratio of 1:3.45.

Mixture of (*Z*)-10-Formyl-6-heneicosen-11-one and 12-Formyl Isomer (3a). A suspension of 13 mg of NaH (60% oil dispersion) in 1 mL of dry ether containing ca. 50 μL of absolute ethanol was stirred at 0 °C during dropwise addition of a solution of **2a** (100 mg, 0.32 mmol) and 40 mg of ethyl formate in 1 mL of ether. The mixture was warmed to 30 °C and stirred for 8 h.

(9) Evans, E. A.; Warrell, D. C.; Elvidge, J. A.; Jones, J. R. *Handbook of Tritium NMR Spectroscopy and Applications*; Wiley: New York, 1985.

A drop of ethanol was added, and the mixture was stirred for 1 h and then poured into water. The ether layer was washed (H_2O) and the combined aqueous layers were acidified with 0.5 mL of 6 N HCl and extracted with ether. The ether solution was washed (aqueous $NaHCO_3$, brine), dried ($MgSO_4$), and concentrated in vacuo. The crude product was flash chromatographed (1 to 10% EtOAc/hex) to give 95.5 mg of an unstable colorless oil (87%): TLC R_f 0.55 (10% EtOAc/hex); ^1H NMR ($CDCl_3$) δ 15.00–15.12 (m, 0.9 H, enolic H), 9.58 (d, 0.05 H, J = 1.7 Hz), 9.56 (d, 0.05 H, J = 4.0 Hz), 7.88–7.98 (m, 1 H), 5.20–5.52 (m, 2 H), 2.40 (br t, 2 H), 1.8–2.32 (m, 4 H), 1.50–1.70 (m, 4 H), 1.25 (br s, 20 H), 0.87 (br t, 6 H, J = 6 Hz); ^{13}C NMR ($CDCl_3$) δ 197.62, (177.75, 177.00, 176.71, 176.55), (132.25, 131.70, 131.58, 131.18), (128.99, 128.48, 128.22, 127.52), (112.65, 112.59, 111.83), 31.87, 31.51, 31.39, 31.26, 29.55, 29.41, 29.29, 29.15, 27.35, 25.01, 22.64, 22.59, 14.08; IR 1580–1640 cm^{-1} (broad peak). Analytical data were obtained on the more stable isoxazole derivatives (see below).

Mixture of [6,7-$^3H_2$]-(*Z*)-10-Formyl-6-heneicosen-11-one and 12-Formyl Isomer (3b). The labeled ketone (143 mCi, ca. 0.76 mg) was formylated with 50 μL of ethyl formate and 5 mg of NaH in 1.00 mL of dry ether and 1 μL of absolute ethanol. The mixture was allowed to stir at 30 °C overnight. The reaction was worked up by using the same procedure described for the unlabeled reaction. Flash chromatography of the product (1 to 10% EtOAc/hex) gave 103 mCi (ca. 0.6 mg) (72% radiochemical yield) of the homogeneous formylated ketone isomers **3b**.

Mixture of (*Z*)-10-Diazo-6-heneicosen-11-one and 12-Diazo Isomer (4a). To a stirred and ice-cold solution of **3a** (85.5 mg, 0.25 mmol) and 50 mg of Et_3N (0.5 mmol) in dry CH_2Cl_2 (4 mL) was added a solution of *p*-toluenesulfonyl azide (50 mg, 0.25 mmol) in 1 mL of CH_2Cl_2 dropwise. Stirring was continued for 3 h as the ice melted, then 1 mL of 1 N KOH was added and the mixture was stirred for 0.5 h at room temperature. The methylene chloride layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with dilute aqueous KOH and water, dried over $MgSO_4$, and concentrated in vacuo. The crude product was chromatographed four times with 0.5% EtOAc/hex in a long thin column of silica gel packed in *n*-hexane to eliminate the unreacted tosyl azide and to give 55 mg of a yellow oil (65%): TLC R_f 0.48 (10% EtOAc/hex); ^1H NMR ($CDCl_3$) δ 5.2–5.6 (m, 2 H), 2.2–2.5 (m, 4 H), 1.8–2.1 (m, 2 H), 1.5–1.8 (m, 4 H), 1.25 (br s, 20 H), 0.85 (br t, 6 H, J = 6 Hz); IR 2050 (strong), 1730, 1640 cm^{-1}; UV (ethanol) λ_{max} 287 nm ($n \rightarrow \pi^*$, ϵ 6120); 248 nm ($\pi \rightarrow \pi^*$, ϵ 12 740); LR-MS, 70 eV, m/z (rel abund) 306 (0.7, $M^+ - N_2$), 279 (1.2, $M^+ - N_2 - C_2H_3$), 249 (2.8, $M^+ - N_2 - C_4H_7$), 235 (4.8), 221 (10.5), 165 (13.7, $M^+ - N_2 - C_{10}H_{21}$), 149 (57.3, $M^+ - N_2 - C_{10}H_{21}O$), 109 (39.8), 97 (54.2), 95 (69.6), 81 (65.2), 69 (77), 55 (100, $C_3H_3O^+$); HR-MS, calcd for $C_{21}H_{38}O$ (loss of N_2) 306.2922, found 306.2922.

Mixture of [6,7-$^3H_2$]-(*Z*)-10-Diazo-6-heneicosen-11-one and 12-Diazo Isomer (4b). The reaction was conducted as described above in a conical microflex vial by using the crude formylated ketone (103 mCi, ca. 0.6 mg), excess tosyl azide (ca. 10 mg), and 20 μL of Et_3N in 1 mL of dry CH_2Cl_2. After aqueous workup, the crude product was chromatographed four times as above to eliminate the excess tosyl azide and Et_3N to give ca. 35 mCi (ca. 0.2 mg) of the labeled diazo ketone **4b** (34% radiochemical yield).

Mixture of Isoxazoles. The crude formyl ketone mixture (10 mg, 0.03 mmol) was diluted with 1.5 mL of absolute ethanol, 70 mg of dry K_2CO_3, and 70 mg of hydroxylamine hydrochloride at 0 °C and then stirred for 1 h at 20 °C and 16 h at reflux.[10] The reaction was quenched with 1 mL of 2 N HCl, the isoxazoles **5** were extracted with 1:1 hexane/ether, and the crude product was purified by alumina chromatography (1% EtOAc–hexane) to give 8 mg (81%) of TLC-homogeneous isoxazoles: TLC R_f 0.5 (10% EtOAc/hex). While GC on a DB-5 Megabore column (15 m × 1 mm) showed a single peak, analysis on a 30 m × 0.25 mm DB-5 column (T_i = 150 °C, T_f = 250 °C, T_p = 10 °C/min) showed eight peaks with relative abundances of 38.2, 28.4, 15.3, 11.9, 2.3, 1.7, 1.5, 0.6. These were not assigned to individual regio- or stereoisomers. ^1H NMR ($CDCl_3$): δ 8.09 (s, 0.3 H), 8.06 (s, 0.7 H), 5.2–5.6 (m, 2 H), 2.70 (t, 2 H, J = 7.4 Hz), 2.15–2.45 (m, 2 H), 1.95–2.20 (m, 4 H), 1.2–1.86 (m, 20 H), 0.89 (br t, 6 H, J = 6 Hz). FT-IR (neat): 1736, 1628, 1467, 1378 cm^{-1}. HR-MS of mixed TLC-homogeneous isoxazole isomers: calcd for $C_{22}H_{39}ON$ 333.3033, found 333.3030.

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(10) Prestwich, G. D.; Collins, M. S. *J. Org. Chem.* **1981**, *46*, 2383.

Facile Synthesis of Protected β,β-Dialkylcysteine Derivatives Suitable for Peptide Synthesis

Nelson C. F. Yim, Heidemarie Bryan, William F. Huffman, and Michael L. Moore*

Department of Peptide Chemistry, Smith Kline & French Laboratories, King of Prussia, Pennsylvania 19406

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The use of β,β-dialkylcysteine derivatives has begun to find widespread use in conformation–activity studies of peptides due to the conformational constraint imposed by the *gem*-dialkyl substituent on the adjacent disulfide bond.[1] This type of substitution has produced antagonists of oxytocin[2,3] and vasopressin[4,5] and has produced enkephalin agonist analogues with δ-receptor subtype selectivity.[6]

While β,β-dimethylcysteine (penicillamine) is readily available, other dialkylcysteines have been more difficult to obtain. A report of a general synthesis for dialkylcysteine derivatives by Stanfield et al. has appeared recently.[7] Their synthesis involves the addition of sulfur to an α,β-dehydro amino acid derivative using phosphorus pentasulfide, hydrolysis of the resulting thiazoline to the free mercapto amino acid, and sequential protection of the mercapto and amino groups. Protection of the mercapto group requires a sodium/liquid ammonia reaction.

A simpler and more direct approach is the introduction of the protected mercaptan directly by Michael addition of a sulfur nucleophile to the dehydro amino acid derivative. It has been shown, for example, that benzyl mercaptan will undergo Michael addition to oxazolones of α,β-dehydro amino acids to yield *S*-benzyl-β-alkylcysteines upon hydrolysis.[8] We have previously used the Michael addition of *p*-methylbenzyl mercaptan to β,β-dialkyl-

(1) Meraldi, J.-P.; Hruby, V. J.; Brewster, A. I. R. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 1373.
(2) Schulz, H.; du Vigneaud, V. J. *J. Med. Chem.* **1966**, *9*, 647.
(3) Chan, W. Y.; Fear, R.; du Vigneaud, V. *Endocrinology (Baltimore)* **1967**, *81*, 1267.
(4) Sawyer, W. H.; Pang, P. K. T.; Seto, J.; McEnroe, M.; Lammek, B.; Manning, M. *Science (Washington, D.C.)* **1981**, *212*, 49.
(5) Yim, N. C. F.; Moore, M. L.; Huffman, W. F.; Bryan, H. G.; Chang, H.-L.; Kinter, L. B.; Edwards, R.; Stassen, F. L.; Schmidt, D.; Heckman, G. *J. Med. Chem.* **1986**, *29*, 2425.
(6) Mosberg, H. I.; Hurst, R.; Hruby, V. J.; Gee, Y.; Yamamura, H. I.; Galligan, J. J.; Burks, T. F. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 5871.
(7) Stanfield, C. F.; Cody, W. L.; Ruby, V. J. *J. Org. Chem.* **1986**, *51*, 5153.
(8) Carter, H. E.; Stevens, C. M.; Ney, L. F. *J. Biol. Chem.* **1941**, *139*, 247.

0022-3263/88/1953-4605$01.50/0