SYNTHESIS OF TRITIUM LABELED PRAMIRACETAM

(CI-879; N-[2-[BIS(1-METHYLETHYL)AMINO]ETHYL]-2-OXO-1-PYRROLIDINEACETAMIDE)

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SUMMARY

Pramiracetam (CI-879; N-[2-[bis(1-methylethyl)amino]ethyl]-2-oxo-1-pyrrolidineacetamide) was radiolabeled with tritium. Catalytic reduction of ethyl 2,5-dihydro-2-oxo-1H-pyrrole-1-acetate in the presence of Pd/C gave ethyl 2-oxo-1-[3,4-^{3}H]pyrrolidineacetate. Treatment of the tritiated ester with N,N-bis(1-methylethyl)-1,2-ethanediamine gave pramiracetam, which was subsequently converted to the corresponding sulfate salt.

KEY WORDS: Pramiracetam sulfate, tritium, CI-879, N-[2-[bis(1-methylethyl)-amino]ethyl]-2-oxo-1-pyrrolidineacetamide sulfate, cognition activator.

Pramiracetam (CI-879; N-[2-[bis(1-methylethyl)amino]ethyl]-2-oxo-1-pyrrolidineacetamide), a new cognition activator drug, has been shown to exhibit learning and memory enhancement in animals.[1,2] Recently, we published the synthesis of carbon-14 labeled pramiracetam for distribution and metabolism studies.[3] Since the mechanism of action of this drug with regard to its cognitive effects is unknown, the radio-labeled pramiracetam with very high specific activity was desired for studying possible interactions of the drug with specific receptor systems. Accordingly, a special synthetic sequence was devised for the incorporation of high specific activity tritium into the molecule (Scheme I).

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0362-4803/86/040415–06$05.00

Received July 24, 1985
Revised October 11, 1985

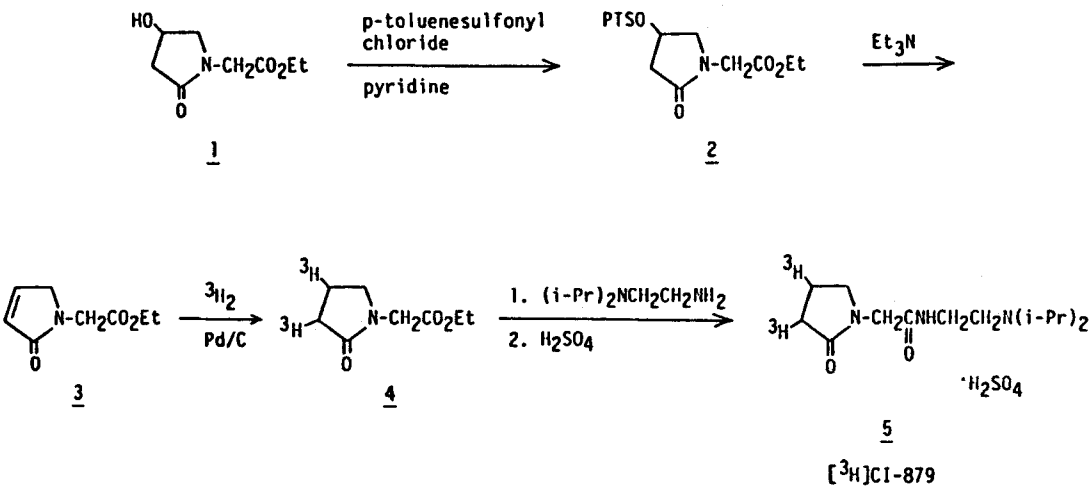
RESULTS AND DISCUSSION

From a radiochemical standpoint, it is usually desirable to introduce the radiolabel at a later stage of synthesis. Thus, the synthesis of the dehydro analog of pramiracetam as the precursor for tritiation was originally explored. However, attempts to dehydrate the 4-hydroxy analog of pramiracetam by conversion to the corresponding toluenesulfonate, bromide (with HBr), or chloride (with triphenylphosphine, carbon tetrachloride) were unsuccessful.

As a result, our efforts were turned to the preparation of ethyl 2,5-dihydro-2-oxo-1H-pyrrole-1-acetate (3). Treatment of ethyl 4-hydroxy-2-oxo-1-pyrrolidineacetate[4] (1) with toluenesulfonyl chloride produced the corresponding toluenesulfonate 2, which was then desulfonated with triethylamine to give the α,β-unsaturated lactam ester 3.

Incorporation of the tritium label into pramiracetam was accomplished by catalytic reduction of ethyl 2,5-dihydro-2-oxo-1H-pyrrole-1-acetate (3) with tritium gas in the presence of 20% Pd/C. The conditions used for this tritiation were developed using deuterium gas. The resulting double tritium labeled ester 4 was then treated with N,N-bis(1-methylethyl)-1,2-ethanediamine to give pramiracetam free base, which was subsequently converted to the corresponding sulfate salt.

Decoupled tritium nuclear magnetic resonance spectroscopy established unequivocably that the tritium labels were located in the C-3 and C-4 carbons as anticipated. A small percentage of tritium were also observed in the C-5 carbon, probably as a result of catalytic exchange between tritium gas and C-5 hydrogen under the reductive condition. The double tritium incorporation was also confirmed by the high specific activity determined-49.8 Ci/mmol. The specific activity represented approximately 86% tritium incorporation. This result indicated that there was possibly slow exchange between the tritium gas and the protic solvent (95% EtOH), but the rate of exchange was probably slower than that of reduction.



EXPERIMENTAL

^{1}H-NMR spectra were determined on a Bruker WH90 (90 MHz) spectrometer. Chemical shifts were reported in δ (ppm) downfield from tetramethylsilane. The ^{3}H-NMR spectrum of pramiracetam sulfate was provided to us from DuPont NEN Products on a Bruker WP200 spectrometer operating at 213.47 MHz. Infrared spectra were recorded on a Nicolet XL-1/3600 FT-IR spectrophotometer.

Mass spectra were obtained with a Finnigan Series 4000 G.C.-M.S. Melting points were determined with a Thomas Hoover capillary melting point apparatus and are uncorrected. Liquid scintillation counting was performed with a Packard 574 liquid scintillation counter using Beckman Ready-Solv MP scintillation cocktail.

Thin layer chromatography (TLC) was performed on Analtech silica gel plates (250 μ). Radiochemical purity determinations were performed by counting sections (5 mm) scraped from TLC plates and suspended in MeOH and cocktail before counting.

Column chromatography was carried out on Merck Kieselgel 60 (40-63nm). High pressure liquid chromatography (HPLC) analyses of the final product were

performed on a system consisting of an Alltech silica gel column (10 μ), an Altex 110A pump, and a DuPont UV detector (215 nm).

The tritiation condition using deuterium gas was developed in our laboratories, and the actual tritiation was performed by DuPont NEN Products (NEN) under contract with Warner-Lambert/Parke-Davis.

All authentic unlabeled intermediates and the final product were synthesized at Warner-Lambert/Parke-Davis.

Ethyl 4-[[(4-Methylphenyl)sulfonyl]oxy]-2-oxo-1-pyrrolidineacetate (2).

To a magnetically stirred solution of pyridine (30 mL) and ethyl 4-hydroxy-2-oxo-1-pyrrolidineacetate (1) (2.15 g, 11.5 mmol) was added 2.40 g (12.7 mmol) of p-toluenesulfonyl chloride. The solution was stirred overnight at room temperature. The excess pyridine was removed in vacuo. The residue was chromatographed on 120 g of silica gel using ethyl acetate as the eluant to afford 2.31 g (59%) of the toluenesulfonate 2 as colorless oil: ^{1}H NMR (90 MHz) ($CDCl_3$) δ7.73 (d, 2H, J = 8.1 Hz), 7.29 (d, 2H, J = 8.1 Hz), 5.0 - 5.2 (m, 1H), 3.5 - 4.4 (m, 6H), 2.45-2.65 (m, 2H), 2.43 (s, 3H), 1.24 (t, 3H, J = 6.3 Hz); IR (film) 2960, 1740, 1700, 1595 cm^{-1}.

Ethyl 2,5-Dihydro-2-oxo-1H-pyrrole-1-acetate (3).

A solution of the tosylate 2 (2.31 g, 6.77 mmol), triethylamine (20 mL), and tetrahydrofuran (20 mL) was magnetically stirred overnight at room temperature. The resulting precipitate was removed by filtration, and the remaining volatiles were removed in vacuo. Chromatography on 75 g of silica gel using ethyl acetate as the eluant afforded 1.12 g (98%) of the α, β-unsaturated lactam 3 as a colorless oil: ^{1}H NMR (90 MHz) ($CDCl_3$) δ7.18 (dt, 1H, J = 6.0, 1.5 Hz), 6.20 (dt, 1H, J = 6.0, 1.5 Hz), 4.0 - 4.3 (m, 4H), 4.23 (s,2H), 1.30 (t, 3H, J = 7.0 Hz); IR (film) 2950, 1720, 1665, 1200, 800 cm^{-1}.

Ethyl 2-Oxo-1-[3,4-$^2H_2$]pyrrolidineacetate.

To a 250 mL Erlenmeyer flask was added 100 mL of 95% ethanol, 600 mg

3.55 mmol) of α, β-unsaturated lactam 3, and 60 mg of Pearlman catalyst (20% Pd/C). The mixture was placed under a deuterium atmosphere and stirred for 3 h. The reaction mixture was filtered through Celite to remove the catalyst and evaporated in vacuo to give 570 mg (93%) of the dideuterated lactam, which was a single spot on TLC (silica gel, EtOAc, R_f = 0.5): ^1H NMR (90 MHz) ($CDCl_3$) δ 4.19 (q, 2H, J = 7.0 Hz), 4.05 (s, 2H), 3.50 (d, 2H, J = 6.8 Hz), 2.41 (bd, 1H, J = 7.9 Hz), 2.0 (m, 1H), 1.28 (t, 3H, J = 7.0 Hz); IR (film) 2990, 2200 weak, 1750, 1695 cm^{-1}; mass spectrum (m/e, rel intensity) 174 (46, M^+), 100 (100, $M^+ - CO_2Et$).

[3,4-$^3H_2$]Pramiracetam Sulfate N-[2-[Bis(1-methylethyl)amino]ethyl]-2-oxo-1-pyrrolidineacetamide(5).

The following conditions were used by NEN for the preparation of [3,4-$^3H_2$]pramiracetam sulfate: Ethyl 2,5-Dihydro-2-oxo-1H-pyrrole-1-acetate was reduced with carrier-free tritium gas in EtOH using 20% Pd/C for 2 h. After the reduction, the catalyst was filtered. The solvent was removed under reduced pressure, and 200 μl of N,N-bis(1-methylethyl)-1,2-ethanediamine was introduced. The resulting solution was heated at 95°-100°C for 24 h. The crude product was purified by preparative TLC (silica gel, $CH_2Cl_2/CH_3OH/NH_4OH/H_2O$ 60:40:1:20 using the lower phase of the binary system). The desired band was visualized by autoradiography, scraped, eluted with EtOH, and concentrated to give pramiracetam free base, which was subsequently converted to the corresponding sulfate salt using one equivalent of H_2SO_4: ^3H NMR ($CDCl_3$) (^1H-decoupled) δ 2.4 (d, J = 10.7 Hz), 2.1 (d, J = 10.7 Hz).

Due to high specific activity of the labeled compound, it underwent radiolytic decomposition after it was received from NEN. It was further purified by us by preparative TLC (silica gel, hexane/2-PrOH/NH_4OH 60:35:5). The radiochemical purity of the final product was greater than 98% by TLC (a. SiO_2, n-hexane/n-PrOH/NH_4OH 60:35:5, R_f = 0.36; b. SiO_2, CH_2Cl_2/MeOH/NH_4OH/H_2O 60:40:1:20, R_f = 0.71) and HPLC (silica gel 10 μ), heptane/2-PrOH/NH_4OH 60:35:5, flow rate 1 mL/min, R_t = 5 min).

ACKNOWLEDGEMENTS

We thank Dr. F. A. MacKellar and his staff of the Analytical and Physical Chemistry Section of Warner-Lambert/Parke-Davis for obtaining analytical and spectral data. We also acknowledge the assistance of DuPont NEN Produts in general and Mr. T. Kelly of NEN Custom Synthesis Group in particular for performing the tritium synthesis of pramiracetam.

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