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SYNTHESIS OF N-BUTYLOXYCARBONYLALANYL-1,4-DIOXA-7-AZASPIRO[4,4]NONANE-8(S)-CARBOXYLIC ACID ETHYL ESTER

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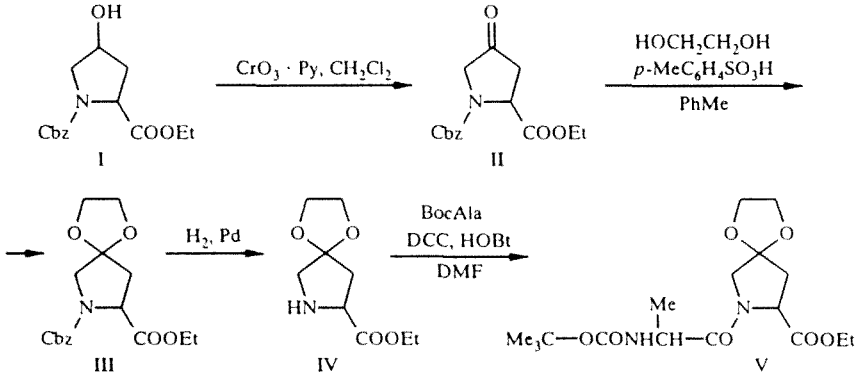
A four-step method has been developed for the synthesis of N-butyloxycarbonylalanyl-1,4-dioxa-7-azaspiro-[4,4]nonane-8(S)-carboxylic acid ethyl ester through the formation of N-benzyloxycarbonyl-1,4-dioxa-7-aza-spiro[4,4]nonane-8(S)-carboxylic acid ethyl ester.

There has been considerable interest in the last decade in the synthesis and study of the properties of peptides containing residues of unnatural amino acids [1-3]. These compounds are distinguished by a higher stability towards enzymic degradation compared to peptides consisting only of residues of natural amino acids.

The dipeptide alanylproline and especially its derivatives are inhibitors of angiotensin-converting enzyme. Extremely active antihypertensive preparations are known in this series of compounds which are widely used at the present time for the treatment of hypertension and cardiac imperfections (enalapril, lisinopril, etc.).

One of the most fruitful routes in the search for new antihypertensive agents is the replacement of the proline residue in alanylproline by a residue of an unnatural amino acid (quinopril, spiropril, etc.). 1,1-Dioxa-7-aza-spiro[4,4]nonane-8(S)-carboxylic acid may serve as such as residue. It has its own value but may also be used for the synthesis of derivatives of dehydroproline and 4-ketoproline [4].

The synthesis of N-butyloxycarbonylalanyl-1,4-dioxa-7-azaspiro[4,4]nonane-8(S)-carboxylic acid ethyl ester (V) was carried out by the following scheme.



The ethyl ester of N-Cbz-4-hydroxyproline (I) was used as starting material and was oxidized using $CrO_3 \cdot Py$ to the ethyl ester of N-Cbz-4-ketoproline (II). The ethyl ester of N-benzyloxycarbonyl-1,4-dioxa-7-azaspiro[4,4]nonane-8(S)-carboxylic acid (III) was formed by reacting the ketoproline (II) with ethylene glycol in the presence of toluene-p-sulfonic acid under azeotropic distillation conditions. The base (IV) obtained by the hydrogenolysis of compound (III) was condensed with t-butyl-oxycarbonylalanine by the dicyclohexylcarbodiimide (DCC) method and led to the dipeptide (V). The final product was isolated chromatographically.

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EXPERIMENTAL

Cyclohexylammonium salt of N-Cbz-hydroxyproline (Reanal, Hungary) was used in the investigation. Esterification of N-Cbz-hydroxyproline (after removing CHA) was effected with absolute ethanol in the presence of thionyl chloride by the known procedure of [5]. The purity of ester (I) was 98%. N-Cbz-4-ketoproline ethyl ester was synthesized by the method of [6].

The content of N-Cbz-hydroxyproline, of N-Cbz-4-ketoproline ethyl ester, and also the purity of the desired product were determined by HPLC on a Dupont 850 chromatograph. The column used (4.6 × 100 mm) was packed with Silasorb C_{18} reversed-phase sorbent. The eluent for N-Cbz-hydroxyproline and its ethyl ester was a system containing 15% CH_3CN and 85% 0.2 M ammonium acetate, and for N-Cbz-4-ketoproline 40% CH_3CN and 60% 0.1 M pH 2.5 phosphate buffer. Detection was with a UV spectrophotometer at a wavelength of 220 nm.

The amounts of N-Cbz-1,4-dioxa-7-azaspiro[4,4]nonane-8(S)-carboxylic acid ethyl ester, of the unsubstituted amino acid ethyl ester (IV), and of the dipeptide, viz. N-butyloxycarbonylalanyl-1,4-dioxa-7-azaspiro[4,4]nonane-8(S)-carboxylic acid ethyl ester, were determined using the reversed-phase mentioned.

A system consisting of 40% CH_3CN and 60% 0.2 M ammonium acetate, at λ = 230 nm was used for N-Cbz-1,4-dioxa-7-azaspiro[4,4]nonane-8(S)-carboxylic acid ethyl ester and for the unsubstituted amino acid ester. For the dipeptide N-butyloxycarbonylalanyl-1,4-dioxa-7-aza-spiro[4,4]nonane-8(S)-carboxylic acid ethyl ester, the eluent was 50% CH_3CN and 50% 0.2 M ammonium acetate, λ = 225 nm.

The PMR spectra were taken on a Bruker WH 90/DS spectrometer in $CDCl_3$ solution, internal standard was TMS.

N-Benzyloxycarbonyl-1,4-dioxa-7-azaspiro[4,4]nonane-8(S)-carboxylic Acid Ethyl Ester (III). The method of [4] was used as a basis. A substance (2.07 g) containing N-benzyloxycarbonyl-4-ketoproline ethyl ester (1.8 g, 6.17 mmole), with ethyleneglycol (7.3 g, 117.6 mmole), and freshly distilled dry toluene (65 ml) was placed in a three-necked flask fitted with a mechanical stirrer, a reflux condenser, and a Dean and Stark adapter. The reaction mixture was heated in an oil bath to 120°C and maintained at this temperature for 17.5 h, distilling off water azeotropically. Toluene containing water was removed in small portions from the Dean and Stark adapter during the reaction and the reaction mixture was made up with portions of dry toluene. After cooling, the reaction mixture was poured onto crushed ice. The mixture was extracted with ethyl acetate (3 × 100 ml). The extracts were combined and dried over anhydrous Na_2SO_4. The solid was filtered off and washed with ethyl acetate. The solution was evaporated on a rotary evaporator. Crude material (1.09 g) containing 37% desired product was obtained. This was purified on a column of silica gel (eluent hexane–ethyl acetate, 4:1, 2 liter). A colorless oil (0.65 g, 77% purity) was obtained after evaporation. PMR spectrum ($CDCl_3$): 7.4-7.5 (5H, m, Ph), 5.18* (2H, d, CH_2–Ph), 5.07* (2H, d, CH_2–Ph), 4.43-4.53 (1H, m, α-Pro), 4.23* (2H, q, O–CH_2–CH_3), 4.08* (2H, m, O–CH_2CH_3), 3.88-3.99 (4H, m, O–CH_2CH_2–O), 3.56-3.64 (2H, d d, δ-Pro), 2.36-2.48 and 2.18-2.27 (2H, m, β-Pro), 1.25* (3H, t, CH_3), 1.13* ppm (3H, t, CH_3).

1,4-Dioxa-7-azaspiro[4,4]nonane-8(S)-carboxylic Acid Ethyl Ester (IV). A substance (0.54 g) containing N-benzyloxycarbonyl-1,4-dioxa-7-azaspiro[4,4]nonane-8(S)-carboxylic acid ethyl ester (III) (0.42 g, 1.25 mmole) was dissolved in methanol (30 ml) and placed in a two-necked flask fitted with a magnetic stirrer and a bubbler for introducing hydrogen. Acetic acid (3 ml) and distilled water (0.6 ml) were added. Freshly prepared palladium black was added to the solution and the mixture was hydrogenated until disappearance of the starting material (~5 h). The catalyst was filtered off, washed with methanol (30 ml), and the filtrate evaporated to dryness on a rotary evaporator. An oil (0.24 g, 75% purity) was obtained which was dried in a desiccator over P_2O_5. The yield of 1,4-dioxa-7-azaspiro[4,4]nonane-8(S)-carboxylic acid ethyl ester acetate salt was 55.4%.

N-Butyloxycarbonylalanyl-1,4-dioxa-7-aza-spiro[4,4]nonane-8(S)-carboxylic Acid Ethyl Ester (V). The acetate salt of 1,4-dioxa-7-azaspiro[4,4]nonane-8(S)-carboxylic acid ethyl ester (0.24 g, 0.918 mmole) was placed in a round-bottomed flask (100 ml volume) fitted with a magnetic stirrer and a dropping funnel, and was dissolved in previously purified dry dimethylformamide (2.5 ml). Then Boc-Ala (0.173 g) dissolved in dimethylformamide (2 ml) was added dropwise. The solution was cooled to −5°C and triethanolamine (0.128 ml, 0.848 mmole) was added dropwise with stirring. The mixture was cooled to −35°C and a solution of hydroxybenzotriazole (0.14 g) in dimethyl-formamide (0.5 ml) was added dropwise. The solution was cooled to −60°C and DCC (0.14 g) dissolved in dimethyl-formamide (0.8 ml) was added. The mixture was maintained at this temperature for 1 h 15 min then the cooling was reduced. The reaction mixture was stirred at room temperature for 5 h and left in the refrigerator for 2 d. The precipitate of dicyclohexylurea was filtered off and washed with dimethylformamide (3 × 0.5 ml). Water (21 ml) was added to the filtrate and the mixture extracted with ethyl acetate (4 × 10 ml). The ethyl

acetate layer was washed sequentially with 5% $NaHCO_3$ solution (15 ml), saturated NaCl solution (11 ml), and with water (11 ml). The solution was dried over anhydrous Na_2SO_4, which was filtered off, and the filtrate evaporated under reduced pressure. The residue was dried in a desiccator over P_2O_5. A dense oil (0.035 g: 8.3%) with a purity of 85% (by HPLC) was obtained. PMR spectrum ($CDCl_3$): 5.30-5.40 (1H, br s, NH-Ala), 4.64 (1H, d d, α-Pro), 4.33-4.42 (1H, m, α-Ala), 4.18 (2H, q, $COCH_2$), 3.90-4.00 (4H, m, CH_2-O), 3.75 (1H, d, δ-Pro), 3.60 (1H, d, δ-Pro), 2.35 (1H, d d, β-Pro), 2.18 (1H, d d, β-Pro), 1.51 (9H, s, CH_3), 1.31 (3H, d, CH_3Ala), 1.21 ppm (3H, t, CH_2CH_3).

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