NEW COMPOUNDS

Synthesis of Some Steroidal Esters of α-Amino Acids

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A synthetic approach for the preparation of aminoacyl, aminopeptyl, or aminodepsipeptyl steroidal esters is described.

In view of the importance of steroid nitrogen mustards in which the active agent is linked to the steroid by a bond easily cleaved by enzymatic action (*1–3*), it was decided to use steroidal esters of amino acids as a biologically acceptable platform of transporting the alkylating agents to the tumor site.

In the present work we report the synthesis of steroidal esters of α-amino acids, which can also be used as intermediates in the synthesis of steroidal peptides or depsipeptides. The esterification of amino acids with the hydroxy group of the steroid molecule in the C-3 and C-17 positions has been chosen.

For the preparation of the protected amino acyl steroidal esters, the same synthetic approaches as those given for the synthesis of depsipeptides of the peptolides type can be applied (*4*).

The formation of the ester bond requires a higher degree of activation of the carboxyl component. Two methods of coupling for the ester formation have been used, namely, the imidazolide method (*5*) and the 8-hydroxyquinoline ester method (*6*, *7*). *tert*-Butoxycarbonylamino acids were coupled with the free hydroxyl of the steroids according to the Merrifield procedures (*8*) for the first method and according to Stewart (*9*) for the second method.

Furthermore it was shown that it is possible to synthesize *tert*-butoxycarbonyldidepsipeptyl esters of steroids by using the imidazolide method. The protected depsipeptide was synthesized in a similar way (*8*), by condensation of Boc-L-Pro-OH with the L-lactic acid benzyl or diphenylmethyl ester, following debenzylation by catalytic hydrogenation.

Experimental Section

Melting points were determined on a Büchi melting point apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer 521 spectrophotometer in solid-phase potassium bromide, NMR spectra were determined with a Varian Associates A-60 instrument using deuteriochloroform as a solvent and tetramethylsilane as the internal standard. Optical rotation was measured photoelectrically with a Perkin-Elmer Model P-141 instrument. Elemental analyses (C, H, N) were performed by Dr. H. Mantzos of the Analytical Laboratory of the National Research Foundation. Analytical results obtained for those elements were within ±0.4% of the theoretical values. Amino and hydroxy acid residues are of the L configuration. Abbreviations used in this paper are Boc = *tert*-butoxycarbonyl and H-Lac-OH = lactic acid.

Preparation of the Aminoacyl Steroidal Esters. Method A. To a stirred solution of 6.5 mmol of Boc-aminoacyl component in 10 mL of dichloromethane at 0 °C was added 6.5 mmol of carbonyldiimidazole (CDI) in 10 mL of dichloromethane. After 20 min of agitation, a solution of 5 mmol of steroid in 10 mL of dichloromethane was added, and the mixture was stirred at 0 °C for 1 h. Then, it was allowed to stay at room temperature for 4 days. After that time the solvent was removed under reduced pressure, and the residue was dissolved in ethyl acetate. The solution was washed with water, a cold solution of 1 N H_2SO_4, water, saturated sodium bicarbonate, and water. After being dried over sodium sulfate and elimination of the solvent under reduced pressure, the residue was triturated with petroleum ether (60–80 °C) and the solid filtered off and recrystallized from ethyl acetate–petroleum ether to give aminoacyl steroidal ester. The prepared compounds were homogeneous on TLC, in the following solvent systems: ethyl acetate/petroleum ether (2:1), chloroform/methanol (1:1), and toluene/pyridine/acetic acid (80:10:1).

Yields and physical constants are summarized in Table I.

Method B. (a) N-tert-Butoxycarbonyl-L-alanine-8-hydroxyquinoline (Boc-Ala-OQ). To a stirred solution of 1.51 g (8 mmol) of Boc-alanine and 1.28 g (8.8 mmol) of 8-hydroxyquinoline in 25 mL of dichloromethane at −15 °C was added 1.65 g (8 mmol) of dicyclohexylcarbodiimide (DCC). After the addition, the mixture was stirred at −10 °C for 3 h, and at room temperature for 10 h. Then 2–3 drops of 50% acetic acid was added, and the *N,N'*-dicyclohexylurea formed was filtered off. The filtrate was evaporated under reduced pressure, and the residue was dissolved in ethyl acetate. The organic layer was washed with water, cold 0.5 N H_2SO_4, water, saturated sodium bicarbonate, and water. After drying of the mixture over sodium sulfate the solvent was removed under reduced pressure and the residue was crystallized from ethyl acetate–petroleum ether to give Boc-Ala-OQ in 68% yield: mp 79–81 °C; $[a]_D^{24}$ −54.8 (c = 1, $CHCl_3$); IR ν_{max} 1770, 1700, 1680 cm^{-1} (CO); NMR δ 8 (8 aromatic protons, m), 5.42 (NH), 4.83 (1 H, CH_3CH, q), 1.75 (3 H, CH_3–CH), d), 1.52 [9 H, $C(CH_3)_3$].

(b) Boc-aminoacyl* Steroidal *Esters. To a solution of Boc-Ala-OQ (5 mmol) and steroid (5 mmol) in 20 mL of dichloromethane was added imidazole (50 mmol). The mixture was stirred at room temperature for 20 h. Then the solvent was evaporated and the residue was dissolved in ethyl acetate, and the products were isolated as method A.

Removal of the N-tert-Butoxycarbonyl Group. With CF_3-COOH. One millimole of Boc-aminoacyl steroidal ester was dissolved in a mixture of 3 mL of dichloromethane and 3.5 mL

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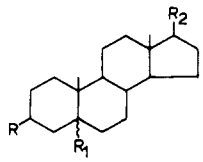
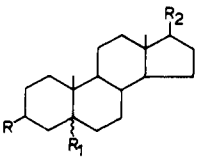
Table I. Boc-aminoacyl and Boc-aminoacylhydroxy Acid Steroidal Esters



no.	R	R_1	R_2	mp, °C	method % yield	$[\alpha]_D^t$	formula	IR ν(CO), cm^{-1}
I	Boc-Ala-O-	5α	=O	163-164	A, 78 B, 80	+46.8 (t = 18) c, 1 ($CHCl_3$)	$C_{27}H_{43}NO_5$	1750, 1720, 1700
II	Boc-Ala-O-	Δ^5	=O	178-180	A, 81 B, 76	−9.9 (t = 22) c, 1 ($CHCl_3$)	$C_{27}H_{41}NO_5$	1750, 1720, 1700, 1690, 1670
III	Boc-Pro-Lac-O	5α	=O	171-172	A, 75	−25.3 (t = 16) c, 1 ($CHCl_3$)	$C_{32}H_{49}NO_7$	1750, 1730, 1710
IV	Boc-Pro-Lac-O-	Δ^5	O=	192-193	A, 70	−78.2 (t = 16) c, 1 ($CHCl_3$)	$C_{32}H_{47}NO_7$	1760, 1730, 1700
V	Boc-Ala-O-	5α	$(CH_3)\overset{\vert}{C}H(CH_2)_3CH(CH_3)_2$	112-114	A, 92 B, 87	+6.0 (t = 20) c, 1 ($CHCl_3$)	$C_{35}H_{61}NO_4$	1720, 1700
VI	Boc-Ala-O-	Δ^5	$(CH_3)\overset{\vert}{C}H(CH_2)_3CH(CH_3)_2$	109-110	A, 88 B, 86	−36.7 (t = 22) c, 1 ($CHCl_3$)	$C_{35}H_{56}NO_4$	1710, 1690, 1670
VII	Boc-Gly-O-	Δ^5	$(CH_3)\overset{\vert}{C}H(CH_2)_3CH(CH_3)_2$	114-115	A, 83 B, 78	−31.2 (t = 23) c, 1 ($CHCl_3$)	$C_{34}H_{57}NO_4$	1750, 1720, 1670
VIII	Boc-Ala-O-	Δ^5	$-COCH_3$	172-173	A, 82 B, 78	+5.4 (t = 20) c, 1 ($CHCl_3$)	$C_{29}H_{45}NO_5$	1730, 1710, 1690
IX	=O	5α	Boc-Ala-O-	149-151	A, 86 B, 80	+11.9 (t = 16) c, 1 ($CHCl_3$)	$C_{27}H_{43}NO_5$	1740, 1720, 1700

Table II. Salts of Aminoacyl Steroidal Esters



no.	R	R_1	R_2	mp, °C	$[\alpha]_D^t$	formula
Ia	H-Ala-O-, CF_3COOH	5α	=O	168-173 dec	+55.2 (t = 16) c, 1 (CH_3OH)	$C_{24}H_{36}NO_5F_3$
IIa	H-Ala-O-, CF_3COOH	Δ^5	=O	140-145	+11.8 (t = 22) c, 1 (CH_3OH)	$C_{24}H_{34}NO_5F_3$
IIIa	H-Pro-Lac-O-, HOOCCOOH	5α	=O	196-197	+4.4 (t = 16) c, 1 (CH_3OH)	$C_{29}H_{43}NO_9$
IIIb	H-Pro-Lac-O-, HCl	5α	=O	198-20	+2.5 (t = 17) c, 1 (CH_3OH)	$C_{27}H_{42}NO_5CL$
IVa	H-Pro-Lac-O-, HOOCCOOH	Δ^5	=O	187-189		$C_{29}H_{41}NO_9$
IVb	H-Pro-Lac-O-, HCl	Δ^5	=O	212-214 dec	−43.7 (t = 21) c, 1 (CH_3OH)	$C_{27}H_{40}NO_5Cl$
Va	H-Ala-O-, CF_3COOH	5α	$(CH_3)\overset{\vert}{C}H(CH_2)_3CH(CH_3)_2$	174-186 dec	+10.2 (t = 26) c, 1 (N-Me-2-pyrrolidone)	$C_{32}H_{54}NO_4F_3$
Vb	H-Ala-O-, HCl	5α	$(CH_3)\overset{\vert}{C}H(CH_2)_3CH(CH_3)_2$	237-248 dec	+36 (t = 20) c, 1 (CH_3OH)	$C_{30}H_{54}NO_2CL$
VIa	H-Ala-O-, CF_3COOH	Δ^5	$(CH_3)\overset{\vert}{C}H(CH_2)_3CH(CH_3)_2$	167-175 dec	−29.2 (t = 26) c, 1 (N-Me-2-pyrrolidone)	$C_{32}H_{52}NO_4F_3$
VIIa	H-Gly-O-, CF_3COOH	Δ^5	$(CH_3)\overset{\vert}{C}H(CH_2)_3CH(CH_3)_2$	176-179 dec	−28.5 (t = 26) c, 1 (N-Me-2-pyrrolidone)	$C_{31}H_{50}NO_4F_3$
VIIa	H-Ala-O-, CF_3COOH	Δ^5	$-COCH_3$	178-182 dec	−53.3 (t = 24) c, 1 (CH_3OH)	$C_{26}H_{38}NO_5F_3$
IXa	=O	5α	H-Ala-O-, CF_3COOH	170-172 dec	+12.3 (t = 19) c, 1 (CH_3OH)	$C_{24}H_{36}NO_5F_3$

of trifluoroacetic acid at 0 °C with occasional shaking. After 30 min at room temperature, the solvent was removed under reduced pressure at 30 °C. The oily residue was treated with absolute ether, and the precipitate which formed was collected by filtration. Then, it was dried under vacuum over P_2O_5 and KOH and recrystallized from ethyl acetate containing small amount of methanol.

With HCl. The Boc-aminoacyl steroidal ester was treated with 4 N HCl in dioxane at room temperature for 30 min. The prepared compounds were isolated as in the method before.

Formation of Oxalate Salts. HCl (1 mmol) and CF_3COOH (1 mmol) salts were dissolved in a mixture of cold 1 M potassium bicarbonate and ethyl acetate. The aqueous phase was extracted several times with ethyl acetate dried over sodium sulfate, and part of the solvent was removed under reduced pressure. To this it was added 1.1 mmol of oxalic acid dissolved in 3 mL of methanol. The precipitate was recrystallized from methanol. The yields and physical constants are reported in Table II (TLC; 2-BuOH/3% NH_3 (10/4)).

tert-Butoxycarbonyl-L-prolyl-L-lactic Acid Diphenylmethyl Ester. As described in method A, equimolar amounts of Boc-proline, carbonylimidazole (7 mmol), and L-lactic acid diphenylmethyl ester (6 mmol) are reacted to give 82% yield of the desired ester in crystalline form, recrystallized from ethanol-water: mp 81-83 °C; $[\alpha]_D^{26}$ -82.4 (c = 1, $CHCl_3$).

L-Lactic Acid Diphenylmethyl Ester. This ester was prepared in 87% yield according to a previously reported method (*10*), recrystallized from ethyl acetate-petroleum ether (60-80 °C): mp 80-81 °C; $[\alpha]_D^{26}$ -18.1 (c = 1, $CH_3COOC_2H_5$).

N-tert-Butoxycarbonyl-L-prolyl-L-lactic Acid. The Boc-didepsipeptide was obtained by catalytic hydrogenation over 10% Pd/C of the *tert*-butoxycarbonyl-L-prolyl-L-lactic acid diphenylmethyl ester.

One millimole of the ester in ca. 40 mL of a mixture of 2-propanol-methanol (40:5) was hydrogenated at room temperature for 6 h. After filtration of the catalyst the solvent was evaporated under reduced pressure and the crude acid was dissolved in aqueous sodium bicarbonate, extracted with ethyl acetate, acidified with cold 1 N H_2SO_4, and extracted with ethyl acetate. After drying of the product over sodium sulfate, the solvent was removed and the residue was recrystallized from a mixture of ethyl acetate-petroleum ether to give the desired acid in 83% yield: mp 111-112 °C; $[\alpha]_D^{16}$ -78.3 (c= 1, $CHCl_3$).

N-tert-Butoxycarbonyl-L-alanylalanine 17β-Hydroxy-5α-androstan-3-one Ester. Boc-Ala-OH (1.2 mmol) and H-Ala-steroid (1 mmol) (liberated from the trifluoroacetate salt IXa) were dissolved in 10 mL of dichloromethane. The solution was cooled at ca. -20 °C, and 1.3 mmol of dicyclohexylcarbodiimide was added. The mixture was stirred overnight. The precipitate of *N,N'*-dicyclohexyurea was filtered off and the solvent was evaporated. The residue was dissolved in ethyl acetate and washed with water, sodium bicarbonate, and 1 N H_2SO_4. After drying of the product over sodium sulfate, the solvent was evaporated and the desired compound was isolated in 73% yield: mp 115-117 °C (ethyl acetate-petroleum ether); $[\alpha]_D^{31}$ 3.1 (c = 1, $CHCl_3$); IR ν_{max} 1740, 1720, 1690, 1660 cm^{-1} (CO); TLC $CHCl_3/CH_3OH$ (1:1), benzene/acetonitrile (1:1).

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Synthetic Precursors of Benz[*a*]anthracenes. 3,9- and 3,10-Dimethoxybenz[*a*]anthracene-7,12-diones

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A one-step cycloaddition of 3-methoxystyrene to 6-methoxy-1,4-naphthoquinone under oxidative conditions afforded two new compounds, 3,9-dimethoxybenz[*a*]anthracene-7,12-dione and 3,10-dimethoxybenz[*a*]anthracene-7,12-dione. One- or two-step procedures were used to convert these diones to the respective dimethoxybenz[*a*]anthracenes and dimethoxy-7,12-dimethylbenz[*a*]anthracenes. The new compound 3,10-diacetoxy-7,12-dimethylbenz[*a*]anthracene was also prepared. This method facilitates entry into these diols of benz[*a*]anthracene and the potent carcinogen 7,12-dimethylbenz[*a*]anthracene and therefore provides synthetic access to possible metabolites.

Recent work by Morreal and Bronstein (*1*) detailed a six-step preparation of the 3,9-diol of 7,12-dimethylbenz[*a*]anthracene. In separate work, Morreal and Alks (*2*) prepared the 3,9-diol of benz[*a*]anthracene in eight steps by using a Stobbe condensation.

In this report we describe the novel one-step preparation of the new compounds 3,9-dimethoxybenz[*a*]anthracene-7,12-dione (**1**) and 3,10-dimethoxybenz[*a*]anthracene-7,12-dione (**2**) from which the respective dimethoxybenz[*a*]anthracenes and dimethoxy-7,12-dimethylbenz[*a*]anthracenes were easily prepared from known general methods. A mixture of compounds **1** and **2** was obtained by the Diels-Alder addition of 3-methoxystyrene (*3*) to 6-methoxy-1,4-naphthoquinone (*4*) (prepared from 6-hydroxy-1,4-naphthoquinone (*5*)) in the presence of chloranil and trichloroacetic acid (*6*). After oxidative workup, column chromatography yielded the individual isomers. Reduction of **1** and **2** with zinc in pyridine/acetic acid (*7*) gave 3,9-dimethoxybenz[*a*]anthracene (**3**) and 3,10-dimethoxybenz[*a*]anthracene (**4**). By use of the classical Grignard method of Sandin and Fieser (*8*), diones **1** and **2** were converted to 3,9-dimethoxy-7,12-dimethylbenz[*a*]anthracene (**5**) and 3,10-dimethoxy-7,12-dimethylbenz[*a*]anthracene (**6**). From compound **6** the diacetate **7** was prepared by demethylation with BBr_3 (*1*) and acetylation of the product by an acetic anhydride/sodium acetate reagent.

Experimental Section

All melting points were determined by using a Fisher-Johns hot-stage apparatus and are uncorrected. Low-resolution mass spectra were taken on a Finnegan 3300 mass spectrometer equipped with a Finnegan 6000 data system. High-resolution mass spectra were obtain from a VG Micromass ZAB-2F mass spectrometer equipped with a VG 2000 data system. Magnetic resonance spectra were taken on a Varian XL-100 spectrometer using $CDCl_3$ (0.5% Me_4Si) as solvent, while IR spectra were obtained on a Perkin-Elmer 467 spectrophotometer as KBr

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