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CONVENIENT SYNTHESIS OF N-TRITYL-O-ALKYL-L-HYDROXYAMINO ACIDS AND DERIVATIVES

APPLICATION TO THE SYNTHESIS OF RELATED PEPTIDES

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Abstract—The disodium salts of N-trityl-hydroxyamino acids **2** prepared *in situ* with NaH, in the presence of imidazole, were selectively alkylated with alkyl iodides to give N-trityl-O-alkyl-hydroxyamino acids **3**. Compounds **3** were readily converted to O-alkyl-hydroxyamino acids **5** or other intermediates useful for incorporation into a peptide chain. The applicability of these derivatives in the preparation of related peptides is illustrated by the synthesis of the protected analogues of enkephalin N-carbobenzoxy-tyrosyl-glycyl-glycyl-phenylalanyl-(O-ethyl) serine benzyl ester and N-carbobenzoxy-tyrosyl-glycyl-glycyl-phenylalanyl-(O-methyl) homoserine benzyl ester.

It is clearly shown in the case of methionine[5]-enkephalin that the exchange of the Met-side chain, which contains a S atom, by an isobutyl group produces quite active leucine[5]-enkephalin.[2] With this as background we became interested to replace S by a more electronegative element like O and provide experimental evidence whether and to what extent S in its γ-position affects the binding properties to the receptor(s) of enkephalin. It should be mentioned that the C-terminal heptapeptide of substance P becomes almost inactive when the S of the 11-Met residue is placed in β-position as in [(S-Et) Cys^{11}]-SP_{5-11}.[3] This communication describes a simple and convenient synthesis of O-alkyl-L-hydroxyamino acids and derivatives using the advantages of the N-trityl group for temporary amino protection and the preparation of the protected analogues of enkephalin N - carbobenzoxy-tyrosyl-glycyl-glycyl-phenylalanyl-O-ethyl) serine benzyl ester and N-carbobenzoxy-tryosyl-glycyl-glycyl-phenylalanyl-(O-methyl) homoserine benzyl ester.

Optically active derivatives of **5** have been prepared either by cumbersome resolution of their racemic mixtures[4] or by direct methylation of the N-t-Boc-amino acids L-Ser and L-Thr with sodium alkoxides and methyl iodide.[5] The latter method, however, does not proceed to completion, gives low yields and involves the danger of racemisation due to prolonged reaction time in strongly basic alkoxide solutions.

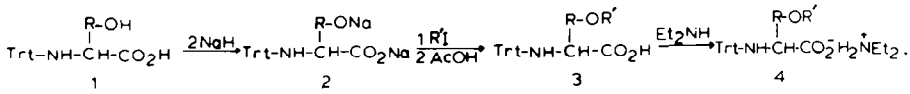
RESULTS AND DISCUSSION

The disadvantages met with the direct alkylation of N-t-Boc-protected hydroxyamino acids can be eliminated with the use of NaH as the base[6] and the bulky trityl moiety for amino protection. Thus, not only N-alkylation can be avoided but also the trityl group is easily removed[7] without affecting the so formed ether function.

Indeed treatment of **1** (Scheme 1) with NaH and a catalytic amount of imidazole in THF gives **2**, which are almost exclusively methylated or ethylated at the OH function to produce foamy **3** in good yields. The latter are easily converted to crystalline diethylammonium salts **4** for better characterisation (Table 1). However, when DMF was used as the solvent the reaction was proved to be partially selective due to concurrent carboxyl alkylation. Regardless of the solvent used for selective alkylation, care should be taken to avoid unreacted starting material, which is very difficult to separate. On the contrary other byproducts, e.g. dialkylated derivatives, can be readily removed on workup.

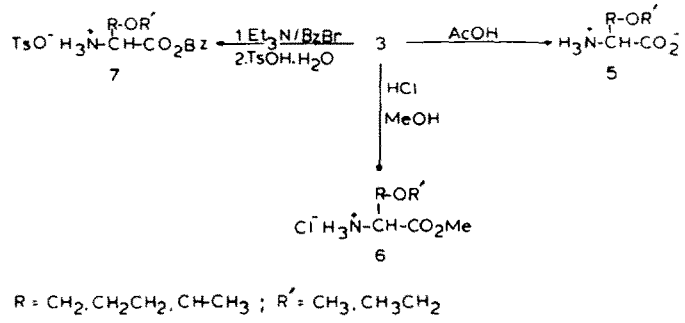
Compounds **3** can be easily converted to **5** or other derivatives as illustrated in Scheme 2. Thus, treatment of **3** with dry HCl in methanol at room temperature afforded the methyl esters **6** in high yields. Reaction of **3** with Et_3N and benzyl bromide in acetone produced the corresponding benzyl esters, which were isolated as the *p*-toluenesulphonates **7**. On the other hand, **3** gave excellent yields of **5** upon standing at room temperature with 10% acetic acid solution in ethanol.

As the next step, the incorporation of the benzyl esters of (O-ethyl)serine and (O-methyl)homoserine into a peptide chain was investigated. Thus, coupling of the *p*-toluenesulphonates of the above compounds with N-t-butyloxycarbonylphenylalanine by the mixed anhydride method[8] gave N-t-Boc-Phe-(O-Et)Ser-OBzl and N-t-Boc-Phe-(O-Me)Hser-OBzl in good yield. The N-protecting group was removed by treatment with 50% CF_3COOH/CH_2Cl_2 and the resulting dipeptide esters trifluoroacetates were condensed with N-Trt-Gly-Gly[7] by the DCC/HOBt method.[9] The trityl group was removed by treatment with 1N HCl/AcOH and the tetrapeptide benzyl esters hydrochlorides were coupled with N-Z-Tyr-ONp to afford N-Z-Tyr-Gly-Gly-Phe-(O-Et) Ser-OBzl and N-Z-Tyr-Gly-Gly-Phe-(OMe) Hser-OBzl in



R = CH_2, CH_2CH_2, $CH{-}CH_3$; R' = CH_3, CH_3CH_2

Scheme 1.



Scheme 2.

Table 1. Yields and physical data of N-Trt-O-alkyl-L-hydroxyamino acid diethylammonium salts

O-Alkylhydroxy-amino acid[a]	Molecular formula	Yield %	Mp [°C]	$[\alpha]_D^{25}$ [b]	C [%] Found (Calcd)	H [%] Found (Calcd)	N [%] Found (Calcd)
Ser[Me][c]	$C_{27}H_{34}N_2O_3$	75	150-2	-10.8°	74.33 (74.62)	7.75 (7.89)	6.20 (6.45)
Hser[Me][d]	$C_{28}H_{36}N_2O_3$	80	135-6	+10.8°	74.82 (74.96)	8.14 (8.09)	6.14 (6.25)
Thr[Me][d]	$C_{28}H_{36}N_2O_3$	72	163-5	+31.0°	74.88 (74.96)	7.99 (8.09)	6.19 (6.25)
Hyp[Me][e]	$C_{29}H_{36}N_2O_3$	66	139-41	-15.1°[f]	75.40 (75.62)	7.66 (7.88)	6.12 (6.08)
Ser[Et][e]	$C_{28}H_{36}N_2O_3$	80	130-2	-9.3°	74.77 (74.96)	8.10 (8.09)	6.32 (6.25)
Hser[Et][d]	$C_{29}H_{38}N_2O_3$	65	120-1	+12.1°	75.18 (75.29)	8.13 (8.28)	6.11 (6.06)
Thr[Et][d]	$C_{29}H_{38}N_2O_3$	73	167-8	+27.9°	75.33 (75.29)	8.19 (8.28)	6.01 (6.06)
Hyp[Et][e]	$C_{30}H_{38}N_2O_3$	71	153-5	-12.4°[g]	75.84 (75.91)	8.12 (8.07)	5.85 (5.90)

[a] Infra-red spectra of all compounds showed characteristic absorptions at 2800-2200, 1640-1550, 1130-1110, 750 and 700 cm^{-1}; [b] Optical rotations were recorded for 2% concentrations in MeOH, unless otherwise stated; [c] Recrystallised from ethyl acetate-petroleum ether (b.p.60-80°); [d] Recrystallised from acetone-petroleum ether (b.p. 60-80°); [e] Recrystallised from acetone-hexane; [f] c 3.05 (MeOH); [g] c 3.55 (MeOH);

Table 2. Yields and physical data of O-alkyl-L-hydroxyamino acids

O-Alkyl-L-hydroxyamino acid	Yield [%]	Mp (dec.) [°C]	$[\alpha]_D^{25}$	c	Solvent
Thr[Me][a]	90	213-5	-37.2°	0.9	H_2O
Hser[Me][b]	94	252	+18.2°	2	1N HCl
Ser[Et][c]	93	243-5	-9.6°	2	H_2O
Hser[Et][d]	87	260-1	-13.7°	2.5	H_2O

[a] Lit. (13) Mp 213-4°C, $[\alpha]_D^{22}$ -37.0° (c 0.9, H_2O); [b] Lit. (14) Mp 251-3°C, $[\alpha]_D$ +18.5° (c 2, 1N HCl); [c] $C_5H_{11}NO_3$ (133.15): Calcd C 45.10 H 8.33 N 10.52, Found C 44.97 H 8.41 N 10.43; [d] Lit.(15) Mp 262°C, $[\alpha]_D^{30}$ -14° (c 2.5, H_2O).

Table 3. Yields and physical data of O-alkyl-L-hydroxyamino acid methyl and benzyl esters

Compound	Molecular formula	Mp. °C	$[\alpha]_D^{25}$ (c 2 MeOH)	C% Found (Calcd)	H% Found (Calcd)	N% Found (Calcd)	Yield (%)
HCl.(O-Et)Ser-OMe	$C_6H_{14}ClNO_3$	170–172 (dec)	−2.8° [a]	39.00 (39.24)	7.70 (7.68)	7.53 (7.63)	86 [b]
HCl.(O-Me)HSer-OMe	$C_6H_{14}ClNO_3$	oil	+12.0°				92
Tos.(O-Et)Ser-OBzl	$C_{19}H_{25}NO_6S$	104–106	−12.5°	57.60 (57.70)	6.40 (6.37)	3.39 (3.54)	79 [c]
Tos.(O-Me)Hser-OBzl	$C_{19}H_{25}NO_6S$	126	−7.8°	57.58 (57.70)	6.35 (6.37)	3.60 (3.54)	82 [c]
Tos.(O-Me)Thr-OBzl	$C_{19}H_{25}NO_6S$	136–137	−26.7°	57.63 (57.70)	6.41 (6.37)	3.65 (3.54)	88 [c]

[a] c 5, H_2O; [b] Recrystallisation from MeOH–Et_2O; [c] Recrystallisation from i-PrOH–Et_2O.

crystalline form and high yield. Both pentapeptide derivatives behaved as single, homogeneous compounds, when checked by TLC in several solvent systems. Furthermore, they gave the expected amino acid values following acid hydrolysis. The biological properties of the deprotected derivatives [(O-Et)Ser^5]- and [(O-Me)Hser^5]-enkphalin will be reported in a comparative study with additional enkephalin analogues.

EXPERIMENTAL

Capillary m.ps were taken on a Büchi SMP-20 apparatus and are uncorrected. Optical rotations were determined with a Carl-Zeiss precision polarimeter (0.005°). IR spectra were recorded as Nujol mulls, on a Perkin-Elmer 457 grating spectrophotometer. Elemental analyses were performed by the Laboratory of Microanalysis of the National Hellenic Research Foundation, Athens, Greece. Solvents used for the alkylation reactions were dried and purified according to standard procedures.[10] Analytical TLC was performed on Merck Kieselgel 60 F_{254} films (0.20 mm layer thickness) precoated on aluminium foils. Solvent systems used were the following: A, 1-BuOHAcOHwater (4:1:1), organic phase); B, 1-BuOH–pyridine–water (20:10:1); C, 1-BuOH–AcOH–pyridine–water (30:6:20:24); D, MeOH–$CHCl_3$ (8:2); E, 1-BuOH–AcOH–water (4:1:5), upper phase); F $CHCl_3$–MeOH (7:3). Spots were visualised with UV light at 254 nm, with ninhydrin and chlorine–tolidine reagent. Alkylations were run under a dry N_2. An 80% NaH oil dispersion (Merck) was routinely freed off oil by washing with dry hexane prior its use.

The N-tritylated Ser and Thr[11,12] were liberated from their diethylammonium salts by washing an EtOAc soln of the appropriate salt with 5% citric acid, precooled at 0°, and brine. The organic layer, after being dried ($MgSO_4$), was evaporated to dryness and the resulting residue was left to remain *in vacuo*, over KOH, overnight. the N-trityl-hydroxyproline[11,12] being especially sensitive to acids, was used directly for alkylation as the diethylammonium salt. Side products, thus formed, were easily removed on workup. Finally, the N-trityl-homoserine diethylammonium salt[11,12] was dissolved in 1N NaOH and neutralised carefully with 50% AcOH at 0°. The resulting acid was extracted with Et_2O and the organic phase was washed well with brine and dried ($MgSO_4$). Evaporation of the solvent *in vacuo* at room temp left N-trityl-homoserine as a foam, with no sign of lactonisation.

N-Trt-O-alkyl-hydroxyamino acid diethylammonium salts (4)

General procedure. To a suspension of 3.75 g (125 mmol) of NaH (80% oil dispersion) and 0.175 g (2.5 mmol) imidazole in 45 ml THF, a soln of N-Trt-hydroxyamino acid (12 mmol) in 25 ml THF was added with stirring, at −15°, in 15 min. After 45 min at that temp, 7.6 ml (95 mmol) EtI or 1.6 ml (25.5 mmol) MeI were added and the mixture was stirred at −5° for 2 hr. Then additional 1.5 g NaH and 15 ml EtI or 2 ml MeI were added and stirring was continued until completion of the reaction (1–24 h; checked by TLC). The resulting mixture was subsequently diluted, at 0°, with 200 ml H_2O and extracted with Et_2O (2 × 40 ml). The cooled aqueous phase was then neutralised by dropwise addition of glacial AcOH and extracted with Et_2O (2 × 50 ml). Organic layers were combined, washed with 5% citric acid and brine, dried ($MgSO_4$) and concentrated *in vacuo* to about 30 ml. Addition of 1.5 ml Et_2NH afforded after standing for 1 day at room temp, crystalline **4**, which was collected, washed with Et_2O and recrystallised (Table 1).

O-Alkyl-hydroxyamino acids (5)

General procedure. A portion of **3** (20 mmol) was dissolved in 30 ml 10% AcOH in EtOH and the resulting soln was kept at room temp for 1 day. The precipitated crystalline **5** was filtered off, washed with EtOH and Et_2O and recrystallised from water–EtOH (Table 2). Compounds **5** showed the expected characteristic IR bands at 3200–2300, 1670–1550, 1150 and 1100 cm^{-1}.

O-Alkyl-hydroxyamino acid methyl ester hydrochlorides (6)

A soln of **3** (10 mmol) in 35 ml dry MeOH was saturated with dry HCl and kept at room temp for 6 hr. The solvent was subsequently removed *in vacuo* and the oily residue left, upon addition of dry Et_2O and refrigeration overnight, afforded **6** (Table 3); IR: 3300–2300, 1750, 1230, 1110 and 740 cm^{-1}.

O-Alkyl-hydroxyamino acid benzyl ester p-toluenesulphonates (7)

A mixture of **3** (10 mmol), 2.8 ml (20 mmol) Et_3N and 2.4 ml (20 mmol) benzyl bromide in 25 ml acetone was stirred at room temp for 1 day. Then excess $EtNH_2$ (30 mmol) was added while cooling. The resulting mixture was allowed to reach room temp and stirred for an additional 30 min. The solvent and excess $EtNH_2$ were subsequently evaporated *in vacuo* and the residue was partitioned between Et_2O and 5% citric acid. The organic phase was washed with water, dried ($MgSO_4$) and evaporated to afford an oily residue. This residue together with 1.9 g (10 mmol) p-toluenesulphonic acid monohydrate were dissolved in the minimum volume of i-PrOH and the resulting soln was warmed to 60° for 5 min. Dilution with Et_2O and standing at room temp overnight afforded crystalline **7** (Table 3) which after recrystallisation from i-PrOH–Et_2O showed characteristic IR bands at 3200–2500, 1750, 1210, 1165 and 1120 cm^{-1}.

N-t-Butyloxycarbonyl-phenylalanyl-(O-ethyl) serine benzyl ester

N-t-Boc-phenylalanine (0.79 g, 3 mmol) and N-methyl-morpholine (0.3 g, 3 mmol) were dissolved in THF (9 ml) and cooled to −20°. Isobutyl chloroformate (0.39 ml, 3 mmol) was added followed, after stirring at −15° for 10 min, by a cold soln of (O-ethyl)serine benzyl ester *p*-toluenesulphonate (1.48 g,

3.6 mmol) and N-methyl-morpholine (0.36 g, 3.6 mmol) in THF (8 ml). After stirring at −5° for 3 hr and at 20° for 1 hr, the mixture was diluted with EtOAc (100 ml) and extracted with several portions of 2M citric acid, water, at 5% $NaHCO_3$aq and water. The organic layer was dried over Na_2SO_4 and the solvent removed *in vacuo* to leave an oil which was crystallised from EtOAc–petroleum ether: yield 1.25 g (85%); m.p. 78–79°; $[\alpha]_D^{25}$ −15.9° (*c*1, MeOH); TLC: $R_{f(E)}$0.91; $R_{f(F)}$0.89.

N-t-Butyloxycarbonyl-phenylalanyl-(O-methyl)homoserine benzyl ester

This compound was prepared in a manner similar to that used in the synthesis of N-t-Boc-Phe-(O-Et)Ser-OBzl: yield 1.26 g (84%); m.p. 80–82°; $[\alpha]_D^{25}$ −24.7° (*c*1, MeOH); TLC: $R_{f(E)}$0.90, $R_{f(F)}$0.87.

N-Trityl-glycyl-glycyl-phenylalanyl-(O-ethyl) serine benzyl ester

N-t-Boc-Phe-(O-Et) Ser-OBzl (0.97 g, 2 mmol) was dissolved in a mixture of CF_3COOH (4 ml) and CH_2Cl_2 (4 ml). After 45 min at 20°. the solvents were removed *in vacuo*. The evaporation repeated with the addition of MeOH and after precipitation with ether and drying *in vacuo* over KOH-pellets, the trifluoroacetate salt [m.p. 153–155°; $[\alpha]_D^{25}$ − 13.8° (*c*1 MeOH)] was dissolved in DMF (4 ml), neutralised with N-methyl-morpholine and cooled to −5° (soln A). To a chilled soln of 0.75 g (2 mmol) N-Trt-Gly-Gly–OH (7) and 1-hydroxy-benzotriazole (0.52 g, 4 mmol) in DMF (4 ml) was added N,N'-dicyclohexylcarbodiimide (0.412 g, 2 mmol). The mixture was kept for 15 min at −4° and another 20 min at room temp and then mixed with soln A. After 15 hr at room temp (progress of the coupling reaction was followed by TLC and the ninhydrin test) the mixture was filtered from the precipitated N,N-dicyclohexylurea and the solvent evaporated *in vacuo*. The remaining residue was taken up with EtOAc, washed with 2% citric acid, water, 5% $NaHCO_3$ aq and water and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the residue dried *in vacuo* to give the foamy product: yield 0.95 g (64%); m.p. 83–85°; $[\alpha]_D^{25}$ − 13.5° (*c*1, MeOH); TLC: $R_{f(E)}$0.92; $R_{f(F)}$0.85.

N-Trityl-glycyl-glycyl-phenylalanine-(O-methyl)homoserine benzyl ester

A sample (1 g, 2 mmol) N-t-Boc-Phe-(O-Me)Hser-OBzl was deprotected with 10% CF_3COOH/CH_2Cl_2 and the trifluoroacetate salt [m.p. 100–104°; $[\alpha]_D^{25}$ − 8.7° (*c* 0.75, MeOH)] was neutralised (N-methyl-morpholine) and coupled with N-Trt-Gly-Gly by the DCC/HOBt method as above. After the same workup the foamy product (1.2 g, 81%) had m.p. 88–91° and $[\alpha]_D^{25}$ − 23° (*c*1, MeOH); TLC $R_{f(E)}$ 0.90; $R_{f(F)}$ 0.84.

N-Carbobenzoxy-tyrosyl-glycyl-glycyl-phenylalanyl-(O-ethyl)-serine benzyl ester

A sample of N-Trt-Gly-Gly-Phe-(O-Et)Ser-OBzl (0.745 g, 1 mmol) was dissolved in 4 ml 1N HCl/AcOH. After 1 h the solvent was evaporated under reduced pressure; the residue was triturated to a solid with ether, collected by filtration, washed with ether and dried *in vacuo*. The hygroscopic solid was dissolved in DMF (5 ml), the pH of the soln was adjusted to 7.5 with Et_3N and N-carbobenzoxy-tyrosine *p*-nitrophenyl ester (0.50 g, 1 mmol + 20% excess) was added. After 48 hr at room temp, the solvent was removed under reduced pressure and the residue solidified by addition of water. The solid was filtered off, washed copiously with 5% $NaHCO_3$ aq, water, 10% citric acid and water, and dried over P_2O_5. The product was recrystallised from EtOAc-ether: Yield 600 mg (75%); m.p. 179–181°; $[\alpha]_D^{25}$ − 12.6° (*c* 1, MeOH); TLC: $R_{f(E)}$ 0.87 $R_{f(F)}$ 0.84; Amino acid analysis gave the following molar ratios: Tyr, 0.98; Gly, 1.95; Phe, 1.05; Ser, 1.01.

N-Carbobenzoxy-tyrosyl-glycyl-glycyl-phenylalanyl-(O-methyl) homoserine benzyl ester

N-Trt-Gly-Gly-Phe-(O-Me)Hser-OBzl (0.759, 1 mmol) was deprotected, neutralised and coupled with Z-Tyr-ONp in a manner similar to that used in the synthesis of the pentapeptide with (O-Et) Ser, yield 600 mg (74%); m.p. 109–111°; $[\alpha]_D^{25}$ − 21.4° (*c*1, MeOH); TLC: $R_{f(E)}$ 0.82; $R_{f(F)}$ 0.79; Amino acid analysis gave the following molar ratios: Tyr, 0.95; Gly, 2.06; Phe, 1.07; Hser, 0.92.

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