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SYNTHESIS AND CONFORMATION OF N-TRITYL DIPEPTIDE DERIVATIVES[1]

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Abstract—N-Tritylamino acids activated with DCC/HOBt, were coupled with various amino acid derivatives without racemization. The trityl group was split off quantitatively in 10% CCl_3COOH monohydrate or $CH_2ClCOOH$ in CH_2Cl_2. Under these conditions detritylation of N-Trt-Trp-Gly-NH_2 proceeds without formation of an oxindole derivative and side alkylation products, even in the absence of a scavenger. Dipeptide derivatives **1** and **2** exhibited magnetic asymmetry, attributed to steric factors.

The trityl group is an important protecting group in peptide chemistry[2] and it has been chosen for special purposes because it can be removed selectively in the presence of other protecting groups including Bpoc.[3] However, the synthesis of N-tritylamino acids was quite difficult and the complications met with them during the coupling step have limited the widespread use of the N-trityl moiety as a temporary amino protecting group. It is now well established that the preparation of N-tritylamino acids can be effected by "one pot" synthesis via their silyl esters in high yields.[4] The second step that leads to a highly peptide bond formation using N-tritylamino acids as the acylating agent, concerns the main subject of this investigation.

RESULTS AND DISCUSSION

Taking into consideration the bulkiness of the trityl group, it is understandable that mixed anhydrides of tritylamino acids direct the coupling step with amino acid or peptide esters toward carbamate rather than peptide bond formation. That steric hindrance does play a role here is shown by the ready coupling of tritylglycine with amino acid and peptide esters; the lack of a large side chain (R) in glycine permits nucleophilic attack on the carbonyl function by the amino component.[5] Resort to other methods of coupling like the dicyclohexylcarbodiimide, the phosphorazo or the chloride, give very low yields.[6,7]

It is interesting to note that when tritylamino acids were preactivated with N,N'-dicyclohexylcarbodiimide mediated with 1-hydroxy-benzotriazole[8-10] the coupling reaction proceeded smoothly with high yields. Apparently, the mechanism of the coupling step involving the nucleophilic attack of the carbonyl group of the tritylamino acid adducts by the amino component, is not influenced by the steric effect exercized by the proximity of the bulky N-trityl and side R groups of the acylating agent. Most specifically trityl derivatives of phenylalanine, leucine, proline and tryptophane were coupled with high yield (65–85%). Characteristically, trityl–phenylalanyl–leucine benzyl ester was obtained in 83% against 43% by the standard DCC method. Even succinimide active esters of trityl–phenylalanine and trityl–proline upon coupling with glycine benzyl ester failed to produce the corresponding dipeptide derivatives in more than 40% yield over the same period of reaction time.

Emphasis should be placed upon the synthesis of trityl–asparaginyl–glycine ethyl ester. When equimolar amounts of the N-trityl acylating agent and the amino component were used, the dipeptide derivative was obtained in 50% yield and indicated the absence of nitrile formation by IR checking. Also, N-trityl–leucyl–methionine amide, prepared in a high yield, was detritylated with HCl/CH_3COOH in the absence of a scavenger to afford homogeneous leucyl–methionine amide hydrochloride.[11] In our hands the deprotection of Boc–leucyl–methionine amide with HCl/CH_3COOH or CF_3COOH, even in the presence of a scavenger, proceeds with the formation of impurities which are difficult to separate.

During the course of this work, it was observed that the trityl group splits off quantitatively in 10% CCl_3COOH monohydrate or $CH_2ClCOOH$ containing the equivalent amount of water, in CH_2Cl_2 at room temperature. Under these conditions other acid-sensitive protecting groups (Boc and Npys[12]) are not affected. More important, detritylation of N-trityl–tryptophane and derivatives proceeds with no formation of oxindole derivative or side alkylation byproducts, even in the absence of a scavenger. The latter may be attributed to the bulkiness and/or stability of $(C_6H_5)_3C^+$.

These findings permit the combination of the trityl moiety with other protecting groups for the synthesis of complex polypeptides either by classical solution techniques or by the solid phase methodology. The recent preparation of Leu[5]-enkephalin by application of N-tritylamino acids in solid phase synthesis points out that the trityl method may become a standardized method with considerable promise.[13]

The IR spectra of **1**, **2**, **3** and **4** (Chart 1) in $CHCl_3$ solution indicate an equilibrium mixture with the cis conformer strongly predominating (intense absorption band at 3400 cm^{-1}).

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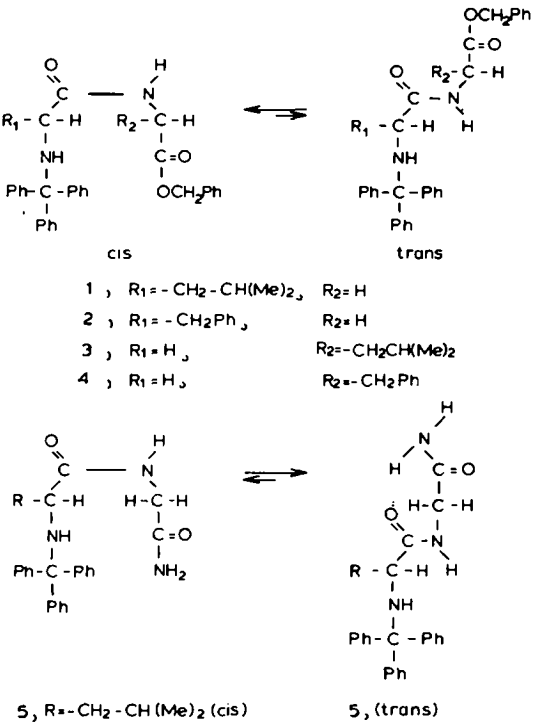

Chart 1.

The ^{1}H-NMR spectra of **1** and **2** reveal that the two CH_2 glycine protons are distinctly non-equivalent. Thus compound **1** in $CDCl_3 + D_2O$ showed an AB quartet centred at $\delta = 3.6$ ppm with a coupling constant $J = 16$ Hz and chemical shift difference $\Delta\nu = 0.06$ ppm, while **2** showed an AB pattern centred at $\delta = 3.6$ ppm ($J = 16$ Hz), but a bigger difference in the chemical shift ($\Delta\nu = 0.12$ ppm). In DMSO-$d_6 + D_2O$, both compounds **1** and **2** showed different chemical shift differences. Thus, **1** showed $\Delta\nu = 0.11$ ppm, while **2** showed $\Delta\nu = 0.04$ ppm which could suggest a change in the population of several different conformers or a change in solvent effect on chemical shifts on the conformers.

Replacement of the trityl group by Boc or carbobenzoxy groups, resulted in an A_2 singlet, (accidental equivalence) at lower field ($\delta = 4.1$ ppm), suggesting a strong anisotropic effect of the trityl group on the CH_2 protons. Rotations of groups or anisotropic effects in one amino acid usually affect the next amino acid, when the common amide bond is in a cis conformation. We believe that the non-equivalence is due to the asymmetric location of the CH_2 protons in the anisotropic field of the trityl group, provided a cis amide bond conformation is present.

Higher temperature studies of **1** in DMSO-$d_6 + D_2O$ did not substantially change the AB pattern. At 160°, the AB pattern showed slightly smaller $\Delta\nu$ difference ($\Delta\nu = 0.08$ ppm). Compound **2** showed an even smaller chemical shift difference ($\Delta\nu = 0.02$ ppm) for the CH_2 glycine protons at 160° and the pattern still remained as a clear AB pattern.

These studies could suggest that either the Boltzmann populations of different conformers each with a cis amide bond conformation or the populations of the major (cis) and minor (trans?) conformers (less likely) become more equal at higher temperatures. Alternatively, the observed closing of the AB pattern at higher temperatures could also suggest a steric effect of the bulky and highly anisotropic trityl group on the CH_2 glycine protons, a fact which is only compatible with a cis amide bond conformation.

The discrepancy of compounds **3** and **4** in showing no asymmetry in spite of the presence of the trityl group and the prevailing cis conformation of the amide bond, could be due to local symmetry felt by the CH_2 protons of glycine. Examination of molecular models indicates that both the CH_2 protons in **3** and **4** enter into the shielding zone of the trityl group ($\delta = 3.0$ ppm) and are not sterically hindered. This would allow a rotational freedom for the two CH_2 protons, also helping to make them effectively equivalent.

N-Trityl–leucyl–glycine amide (**5**), in contrast to compound **1**, shows a conspicuously large difference in chemical shift between the resonances of the trans and cis carboxamide protons of the glycine amide moiety ($\delta = 5.6$ and 6.2 ppm). This could be attributed to hydrogen bonding between the trans amide proton with the CO of the leucyl residue to form a 7-membered ring, which is favored only in the case of a trans amide bond conformation.[14] The CH_2 glycine protons of the deuterated compound **5** appear as an A_2 singlet, thus indicating that in a trans orientation of the amide bond the CH_2 protons are not affected by the bulky groups (N–trityl and side chain R). Moreover, the IR spectrum of **5** shows absorption bands indicating a cis/trans equilibrium directed towards the trans conformer.

EXPERIMENTAL

Melting points were determined in open capillary tubes, on a Buchi SMP-20 apparatus, and are reported uncorrected. Thin layer chromatograms were done on silica gel plates with sample loads of 30–50 μg. The following solvent systems were used and allowed to ascend for 13–15 cm: A, 1-butanol–acetic acid–pyridine–water (15:3:10:12, v/v); B, 1-butanol–acetic acid–water (4:1:1, v/v); C, 1-butanol–acetic acid–water (4:1:5, upper phase); D, benzene–ethanol (7:3, v/v); E, ethyl acetate–hexane (8:2, v/v); F, ethyl acetate–hexane (2:1, v/v), The compounds were visualized by reaction with ninhydrin or chlorine followed by tolidine solution.[15] Optical rotations were measured in a Carl Zeiss precision polarimeter (0.005°). Elemental analyse were done by the Microanalytical Laboratory at the National Hellenic Research Foundation and data fall within ±0.4% of the theory. ^{1}H NMR spectra were obtained with a Varian XL–200 MHz spectrometer in $CDCl_2$ or DMSO–D_6. Chemical shifts are reported in δ units using tetramethylsilane as the internal standard. Infrared spectra were recorded with a Perkin–Elmer 457 grating spectrophotometer.

N-Trityl-dipeptide benzyl Esters. General procedure of coupling by the DCC/HOBt method. To a chilled soln of N-Trt-amino acid (2.5 mmol) and 1-hydroxybenzotriazole (5 mmol) in DMF (5 ml) was added N,N′-dicyclohexylcarbodi-imide (2.5 mmol). The mixture was kept for 15 min at 0° and another 15 min at room temp and then mixed with 2.5 mmol of amino acid benzyl ester toluene-p-sulfonate and triethylamine (2.5 mmol) in DMF (7 ml) precooled at 0°. After 12 h at room temp the reaction mixture was filtered from the precipitated N,N′-dicyclohexylurea and the solvent evaporated *in vacuo*. The remaining residue was taken up with ethyl acetate, washed with 2% citric acid, 10% Li_2CO_3 solution and water and dried over Na_2SO_4. The solvent was evaporated under

vacuum and the residue crystallized upon addition of petroleum ether. The yields (after recrystallization), the physical properties and other analytical data for the N-trityl-dipeptide benzyl esters are given in Table 1.

Preparation of free dipeptides. A soln of 2 mmol of trityl-peptide benzyl ester in 30 ml of AcOH–H_2O (85:15 v/v) was heated for 5 min in a water-bath. The resulting mixture was left to attain room temperature and subsequently 2-propanol was added until a clear solution was obtained. The soln was hydrogenated over 10% Pd on charcoal (1 g). After 3 h the catalyst was filtered off and the solvent evaporated *in vacuo*. Complete removal was ensured by the addition of a few ml of ethanol and repetition of the evaporation *in vacuo*. Upon addition of aceton the free dipeptide precipitated. The yields after the purification process, as well as the physical properties and other analytical data are given in Table 2.

N-Trityl-asparaginyl-glycine ethyl ester. This compound was prepared from N-Trt-Asn (5 mmol) and glycine ethyl ester hydrochloride according to the general procedure of coupling by the DCC/HOBt method. Recrystallization from ether–petroleum ether gave 1.15 g (50%); m.p. 178–180°; $[\alpha]_D^{25} - 66.4°$ (c 5,6 $CHCl_3$). Reported[5]; yield 22% m.p. 182°; $[\alpha]_D^{25} - 67.9°$ (c 5.7, $CHCl_3$). IR (KBr) of the product showed no absorption at 2260 cm^{-1} characteristic for the –C≡N group of β-cyanoalanine.

N-Trityl-leucyl-methionine methyl ester. This compound was prepared from N-Trt-Leu (1 mmol) and methionine methyl ester in a similar manner to that described for N-Trt-Asn-Gly-OEt. Recrystallization from ether-petroleum ether gave 0.44 g (85%); m.p. 104°; $[\alpha]_D^{25} - 18°$ (c 0.5, $CHCl_3$); TLC: Rf_D 0.87, Rf_E 0.62.

N-Trityl-leucyl-methionine amide. A methanolic soln (40 ml) of 0.4 g (0.77 mmol) of N-Trt-Leu-Met-OMe saturated with dry ammonia, was permitted to remain for 3 days at room temperature. Then the solvent was removed *in vacuo* and the resulting product solidified with petroleum ether; yield 0.37 g (97%); m.p. 173–176°. Recrystallization from AcOEt-petroleum ether raised the m.p. up to 179–180°; $[\alpha]_D^{25} - 47°$ (c 1, $CHCl_3$); TLC: Rf_D 0.85, Rf_E 0.13. Detritylation of the above product with 1N HCl/CH_3COOH for 45 min, in the absence of a scavenger afforded homogeneous leucyl-methionine amide hydrochloride; yield 95%; m.p. 111–112°; $[\alpha]_D^{25} + 8.7°$ (c 1, H_2O).

Table 1. Physical properties on N-Trityl-dipeptide benzyl esters

Compound	%Yield	mp, °C	$[\alpha]_D^{25}$	Elemental Analysis				TLC[a]			
				Formula		%C	H	N	Rf_A	Rf_D	Rf_E	Rf_F
Trt-Phe-Gly-OBzl	87	142-145[b]	-8.1°(c 1, $CHCl_3$)	$C_{37}H_{34}N_2O_3$	Calcd	80.12	6.18	5.05	0.81	0.84		0.47
					Found	79.80	6.40	5.19				
Trt-Phe-Leu-OBzl	83	oil[c]							0.87	0.85		
Trt-Pro-Gly-OBzl	68	194-196[d]	-94°(c 1,DMF)	$C_{33}H_{32}N_2O_3$	Calcd	78.54	6.39	5.55		0.82	0.37	
					Found	77.88	5.98	5.20				
Trt-Pro-Leu-OBzl	81	50-55[e]	-65.8°(c 1,$CHCl_3$)	$C_{37}H_{40}N_2O_3$	Calcd	79.25	7.19	4.99		0.88	0.61	
					Found	78.95	7.05	5.05				
Trt-Leu-Gly-OBzl	65	125-127[f]	+20°(c 0.5,$CHCl_3$)	$C_{34}H_{36}N_2O_3$	Calcd	78.43	6.97	5.38		0.87		0.85
					Found	77.84	5.18	5.09				
Trt-Trp-Gly-OBzl[g]	65	190-193[f]	-11°(c 1,$CHCl_3$)	$C_{39}H_{35}N_3O_3$	Calcd	78.92	5.90	7.08	0.92	0.82		0.67
					Found	78.10	5.70	6.99				
Trt-Gly-Leu-OBzl[h]	70	89-90[i]	+7°(c 1,$CHCl_3$)	$C_{34}H_{36}N_2O_3$	Calcd	78.43	6.97	5.38		0.87		0.82
					Found	78.10	7.20	5.42				
Trt-Gly-Phe-OBzl[h]	48	108-109[i]	+10.4°(c 1,$CHCl_3$)	$C_{37}H_{34}N_2O_3$	Calcd	80.12	6.18	5.05		0.88		0.78
					Found	79.39	5.72	5.03				

[a]Only single spots were detected for loads of at least 50 μg. Letters in parentheses indicate solvents system given in Experimental Section. [b]After recrystallization from methanol. [c]Attemps to crystallize it, failed. [d]After recrystallization from ethanol. [e]Upon standing in the desiccator. [f]After recrystallization from AcOEt-petroleum ether (1:1 v/v). [g]This compound was prepared from Trt-Trp-OH and H-Gly-OBzl with coupling time 24 h. [h]By the mixed anhydrides method the yield was 35%. [i]After recrystallization from ether-petroleum ether (1:1 v/v).

Table 2. Physical properties of free dipeptides

Compound	% Yield	mp, °C	$[\alpha]_D^{25}$	TLC[a]		mp, °C(lit)	$[\alpha]_D^{20}$(lit)
				Rf_A	Rf_B		
H-Phe-Gly-OH	98	242-243[b]	+92°(c 0.25, H_2O)	0.45	0.50	249-250(17)	+95°(c 0.3,H_2O)(17)
H-Phe-Leu-OH	97	237-240[b]	+1.8°(c 0.25, 0.3N HCl)	0.70	0.75	258-260(18)	+4.8°(c 0.3,0.3N HCl)(18)
H-Pro-Gly-OH	94	218-220[c]	-60°(c 0.25, 0.1N HCl)	0.25	0.17	230-232(19)	
H-Pro-Leu-OH	85	250-251[d]	-80°(c 0.25, H_2O)	0.48	0.43	249.5-250.5(20)	-79°(c 0.25, H_2O)(20)

[a] Only single spots were detected for loads of at least 50 μg. Letters in parentheses indicate solvents system given in Experimental Section. [b]After recrystallization from 2-propanol-water (1:2 v/v). [c]This compound was purified by column chromatography on Sephadex G-15 using AcOH(5%) as the eluent. [d]After recrystallization from DMF-water (1:3 v/v).

N-Trityl-leucyl-glycine amide. This compound was prepared from N-Trt-Leu (3 mmol) and glycine amide hydrochloride (4 mmol) according to the general procedure of coupling by the DCC (3.3 mmol)/HOBt (6 mmol) method. Recrystallization from methanol gave 1 g (78%); m.p. 210–213°; $[\alpha]_D^{25}$ −12.1° (c 1, DMF); TLC: $Rf_{(AcOEt)}$ 0.30, Rf_E 0.14; IR ($CHCl_3$) 3500, 3460, 3420, 3340, 3200, 1680, 1665 cm^{-1}; NMR ($CDCl_3$), δ 0.9 (br, d, 6H, $(CH_3)_2C$), 3.6 (s, 2H, CH_2), 5.6 (s, 1H, cis $CONH_2$), 6.2 (s, 1H, trans $CONH_2$). This compound was also received in 95% yield by ammonolysis of N-Trt-Leu-Gly-OMe, which was obtained as an oil from N-Trt-Leu and HCL.Gly-OMe, according to the general procedure of coupling by the DCC/HOBt method. Detritylation of the above product (0.65 g, 1.5 mmol) with toluene-p-sulfonic acid hydrate (0.313 g, 1.65 mmol) in acetone (3 min boiling under reflux) afforded leucyl-glycine amide toluene-p- sulfonate which was recrystallized from 2-propanol-ether. Yield 91% m.p. 146–148°; $[\alpha]_D^{25}$ +26.2 (c 1, DMF); TLC $Rf_{(A)}$ 0.52, $Rf_{(C)}$ 0.20.

N-Trityl-tryptophyl-glycine amide. Prepared from N-Trt-Trp (2.5 mmol) and glycine amide, hydrochloride (2 mmol) according to the general procedure of coupling by the DCC (2.5 mmol)/HOBt (5 mmol) method. The product was recrystallized from ethyl acetate-petroleum ether (1:2); yield 0.70 g (70%); m.p. 217–224°(dec); $[\alpha]_D^{25}$ − 18.1° (c 1, DMF); TLC, $Rf_{(A)}$ 0.85, $Rf_{(C)}$ 0.80.

N-Trt-proline succinimide ester. To a chilled soln of Trt-Pro (0.71 g, 2 mmol) and N-hydroxysuccinimide (0.23 g, 2 mmol) in dimethoxyethane (10 ml), N,N'-dicyclohexylcarbodi-imide (0.44 g, 2.2 mmol) was added. The mixture was kept at 4° for 20 h. The N,N'-dicyclohexylurea, which separated out was filtered off and washed with dimethoxyethane. The combined filtrates were evaporated to dryness leaving an oil, which was crystallized from 1-propanol. Recrystallization from 1-propanol gave 0.65 g (71%); m.p. 161–163°; $[\alpha]_D^{25}$ −112.8° (c 1, AcOEt); TLC: Rf_A 0.86, Rf_D 0.92, Rf_E 0.34; (Found C, 73.64; H, 5.85; N, 6.04 $C_{28}H_{26}N_2O_4$: C, 73.99; H, 5.77; N, 6.16%.)

N-Trityl-phenylalanine succinimide ester. This compound was prepared in a manner similar to that used for the synthesis of Trt-Pro-OSu: yield 69% m.p. 94–98°; $[\alpha]_D^{25}$ −64.4° (c 1, AcOEt); TLC: Rf_D 0.95; Rf_E 0.31; (Found C, 75.86; H, 5.64; N, 5.13 calc for $C_{32}H_{28}N_2O_4$: C, 76, 17; H, 5.59; N, 5.55%.)

N-Trityl-phenylalanyl-glycine benzyl ester. Glycine benzyl ester toluene-p-sulphonate (3 mmol) was dissolved in DMF (10 ml), neutralized with triethylamine and allowed to react with N-Trt-Phe-OSu (3.3 mmol). After 2 days at room temperature (progress of the coupling reaction was followed by TLC and the ninhydrin test) solvent was removed under reduced pressure and the remaining oily residue was worked up as in the general procedure of coupling by the DCC/HOBt method. Recrystallization from methanol gave the dipeptide derivative in 38% yield. This compound had identical physical properties and other analytical data to those of the same dipeptide, obtained by the DCC/HOBt coupling.

Similarly, succinimide active ester of N-Trt-Pro upon coupling with glycine benzyl ester toluene-p-sulphonate gave the corresponding dipeptide derivative in 39% yield.

Detritylation of N-Trityl-tryptophyl-glycine amide. N-Trt-Trp-Gly-NH_2 (0.125 g, 0.25 mmol) was suspended in 2-propanol (2 ml) and $CCl_3COOH \cdot H_2O$ (90 mg, 100% excess) was added. The mixture was heated for 3 min on the steam bath, then chilled, and ether was added. The product was recrystallized from 2-propanol-ether. Yield 90 mg (85%); m.p. 125–128°; TLC $Rf_{(A)}$ 0.60; $Rf_{(C)}$ 0.38. An aqueous solution of the product gave an absorption spectrum with a maximum at 279 mμ (ϵ_{mol} 5950), and a minimum at 244 mμ (ϵ_{mol} 2150). No oxindole derivative was detected according to earlier reports.[16,17]

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