Contribution of the Trityl Group to Magnetic Asymmetry in *N*-Trityl Amino Acid Benzyl Esters

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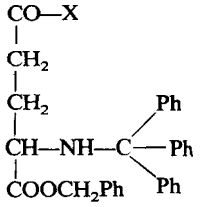
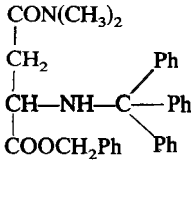
The magnetic nonequivalence of the *O*-methylene protons of certain *N*-trityl amino acid benzyl esters is reported. It is shown that magnetic asymmetry arises from hindered internal rotation of the α-benzyl group. The degree of proximity of the bulky *N*-trityl to the α-benzyl group, and consequently the degree of free rotation of the latter, is dependent on the nature of the amino acid side-chain R.

RESULTS AND DISCUSSION

A recent paper reported the synthesis of N^5-dialkyl-L-glutamine and N^4-dialkyl-L-asparagine derivatives.[1,2,†] Interestingly enough, two of these, namely α-benzyl N^2-trityl-(N^5-dimethyl)-L-glutaminate (**1**) and α-benzyl N^2-trityl-(N^4-dimethyl)-L-asparaginate (**2**), appeared to display magnetic asymmetry and we felt it worth while to study their NMR patterns in more detail. Indeed, the NMR spectra of **1** and **2** revealed that their two α-methylene benzylic protons are magnetically non-equivalent. Thus, compound **1**, in $CDCl_3$, showed an AB quartet centred at $\delta = 4.6$ ppm with a coupling constant of $J = 12$ Hz and a chemical shift difference $\Delta\nu = 0.30$ ppm, while **2** showed an AB pattern at $\delta = 4.8$ ppm, ($J = 12$ Hz), but a smaller difference in the chemical shift ($\Delta\nu = 0.08$ ppm). Similarly, dibenzyl N^2-trityl-L-glutamate[3] (**3**) exhibited an AB pattern centred at $\delta = 4.5$ ppm, ($J = 12$ Hz, $\Delta\nu = 0.22$ ppm) for the α-methylene benzylic protons and an A_2 pattern at lower field, $\delta = 5.2$ ppm, for the γ-methylene benzylic protons. Consequently, α-benzyl N^2-trityl-L-glutamate[4] (**4**) displayed an AB pattern which was centred at $\delta = 4.55$ ppm ($J = 12$ Hz, $\Delta\nu = 0.22$ ppm) and, in contrast to the dibenzyl ester, no singlet at lower field. In DMSO-d_6, **1**, **3** and **4** showed the same chemical shift difference for the α-methylene benzylic protons as in $CDCl_3$. Compound **2** appeared to have a slightly different $\Delta\nu$ value in DMSO-d_6;[5] we believe this to be due to the difficulty of calculating accurately a very small chemical shift difference with a Varian A-60 spectrometer. That in our case the non-equivalence is not due to the asymmetrically-substituted carbon atom[6–10,§] was shown, firstly, by NMR spectra run at different temperatures. The AB quartet of **1** in DMSO-d_6 was converted to an A_2 singlet at approximately 160 °C, and thus it seems reasonable to postulate that at high temperature the geminal protons become equivalent through free rotation in the molecule.



1 X=$N(CH_3)_2$
3 X=OCH_2Ph
4 X=OH



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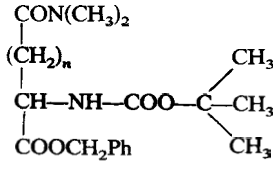

5 $n = 2$
6 $n = 1$

As the temperature studies indicated that restricted rotation was the major factor contributing to the non-equivalence, it was tempting to assume that the rotation of the α-benzyl group is largely influenced by the highly anisotropic and bulky trityl group. This assumption was supported by considering molecular models, and most importantly, by replacing the *N*-trityl with the *N*-*t*-butyloxycarbonyl group. Indeed α-benzyl N^2-*tert*-butyloxycarbonyl-(N^5-dimethyl)-L-glutaminate (**5**) and its L-asparaginate analogue (**6**) exhibited a sharp singlet for the α-CH_2 benzylic protons at $\delta \simeq 5.1$ ppm, which apparently indicates that free rotation is restored. It should be added that the signal of the α-CH_2 benzylic protons observed in the region of 4.5–4.8 ppm shifted to lower field (5.1–5.2 ppm) when the trityl group was replaced with the *tert*-butyloxycarbonyl group in all the trityl derivatives

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† All the studied optically-active amino acids are of the L-configuration. Abbreviations follow the recommendations of the IUPAC-IUB commission on biochemical nomenclature, see Ref. 1.
§ One of the referees has suggested that the non-equivalence of the α-benzylic protons might be due to the inherent asymmetry in these molecules, but we believe from the evidence cited that this is not the case.

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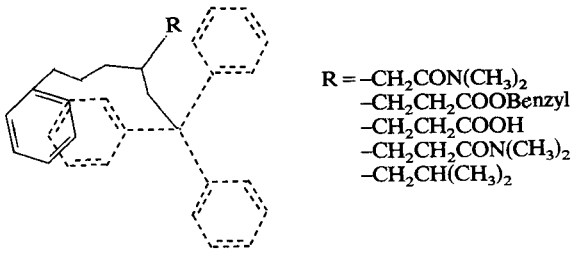
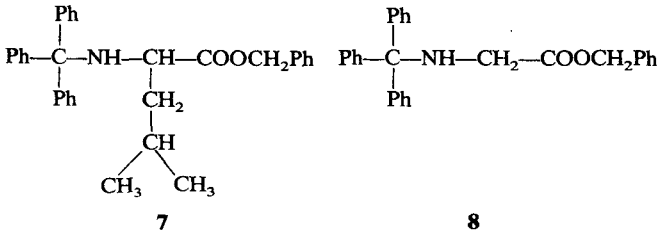


Figure 1. Possible stacking arrangement of phenyl and benzyl groups in *N*-trityl amino acid benzyl esters.

discussed. The AB pattern of the CH_2 geminal α-benzylic protons of these derivatives suggests that these protons are differently shielded in the magnetic zone of the anisotropic trityl group. These findings are in line with the hypothesis that once the *N*-trityl group is introduced, the α-benzyl group is forced preferentially into an orientation which allows π^*–π^* interaction with a vicinal aromatic ring of the trityl group (Fig. 1).

Evidence for the stacking of aromatic rings in peptides containing tyrosine and phenylalanine has been reported.[11,12] In our case the degree of proximity of the *N*-trityl to the α-benzyl group, and consequently the degree of free rotation of the latter, should be dependent on the nature of the substituent R which points away from the cluster of aromatic rings. Supporting evidence in accord with this expectation was obtained when the NMR spectra of N^2-trityl-L-leucine benzyl ester[13] (**7**) and N^2-tritylglycine benzyl ester (**8**) were compared. Thus, **7** exhibited an AB quartet centred at $\delta = 4.5$ ppm ($J = 12$ Hz, $\Delta\nu = 0.24$ ppm), while **8** displayed an A_2 singlet at lower field $\delta =$ 5.0 ppm). [It is well known that *N*-trityl amino acid esters, with the exception of glycine derivatives, are difficult to hydrolyze due to the steric hindrance exercised by the bulky trityl group, while *N*-trityl dipeptide esters hydrolyze readily (see Ref. 19).]

These findings again indicate that in *N*-trityl amino acid benzyl esters a bulky substituent R is a determining factor in the existence of a preferred conformation of the benzyl group with respect to the trityl group for observable asymmetry. On the other hand, *N*-trityl dipeptide esters, e.g. *N*-tritylglycyl-L-leucine benzyl ester (**9**), gave no sign of magnetic asymmetry and the CH_2 geminal protons of the benzyl group appeared as a singlet at $\delta = 5.05$ ppm.



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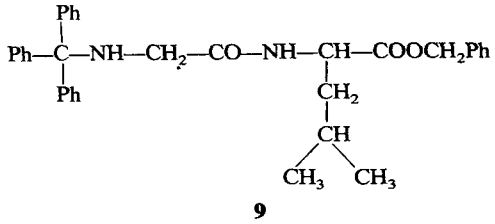

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EXPERIMENTAL

Melting points were taken on a Buchi SMP-20 capillary melting point apparatus and are uncorrected. Microanalyses were performed by the Laboratory of Microanalysis of the National Hellenic Research Foundation, Athens, Greece. NMR spectra were obtained with a Varian A-60 spectrometer, in $CDCl_3$ and DMSO-d_6, using samples 10±2% by volume in solute. Chemical shifts are reported in δ units using tetramethylsilane as the internal standard. Thin-layer chromatography (TLC) was carried out on silica gel Si F chromatographic sheets with the following solvent systems: (a) benzene–ethanol (9:1), and (b) *n*-butanol–acetic acid–water (4:1:1), and developed by UV and chloridine–tolidine reagent.

α-Benzyl N²-tert-butyloxycarbonyl-(N⁵-dimethyl)-L-glutaminate† (**5**). To a solution of α-benzyl N^2-Boc-L-glutamate[14] (3.37 g, 10 mmol) in THF (20 ml), cooled to −10 °C, were added triethylamine (1.01 g 10 mmol) and ethyl chlorocarbonate (1.08 g, 10 mmol). After 3 min a solution of dimethylamine hydrochloride (2.44 g, 200%) in 10 ml of THF–H_2O (6:4) was neutralized with triethylamine (3.03 g, 30 mmol) and added immediately with vigorous shaking. Half an hour later the solvent was evaporated to dryness and the residue was taken up in CH_2Cl_2 (100 ml). This solution was washed with 3×70 ml of 5% $NaHCO_3$, then with water and then dried over Na_2SO_4. After removal of the solvent the residue was crystallized by the addition of light petroleum. Recrystallization from ethyl acetate–light petroleum afforded 3.03 g (83%) of product; mp 99–100 °C; $[a]_D^{24}$ −27.5 °C (c = 1, MeOH); NMR (main absorptions) ($CDCl_3$) δ 1.45 (s, 9H, $C(CH_3)_3$), 2.9 (d, 6H, $N(CH_3)_2$), 5.15 (s, 2H, $ArCH_2$), 5.9 (d, $J = 9$ Hz, 1H, NH, D_2O exchangeable), 7.3 (s, 5H, C_6H_5).

Anal. Calcd for $C_{19}H_{28}O_5N_2$: C, 62.63; H, 7.69; N, 7.69. Found C, 62.27; H, 7.58; N, 7.47.

α-Benzyl N²-tert-butyloxycarbonyl-(N⁴-dimethyl)-L-asparaginate (**6**). This compound was prepared in a similar manner to the L-glutaminate analogue. The oily product failed to crystallize after several trials, but was found to be homogeneous according to TLC, R_F(a) 0.8, R_F(b) 0.92: NMR (main absorptions) ($CDCl_3$) δ 1.45 (s, 9H, $(CH_3)_3C$), 2.9 (d, 6H, $N(CH_3)_2$), 5.1 (s, 2H, $ArCH_2$), 5.9 (d, 1H, NHCO), 7.35 (s, 5H, C_6H_5).

Anal. Calcd for $C_{18}H_{26}O_5N_2$: C, 61.71; H, 7.42; N, 8.0. Found C, 61.32; H, 7.54; N, 8.30.

N-Tritylglycyl-L-leucine benzyl ester (**9**). Coupling of *N*-tritylglycine[13] (1.58 g, 5 mmol) with L-leucine benzyl ester *p*-toluenesulphonate[15] (1.96 g, 5 mmol) by the mixed-anhydride procedure,[16] afforded the desired

† This compound was prepared by Mr Th. Tsegenidis of this laboratory.

product in oily form; yield 1.56 g (60%); NMR (main absorptions) ($CDCl_3$) δ 1.0 (br. d, 6H, $C(CH_3)_2$), 5.05 (s, 2H, $ArCH_2$), 7.2 (br. signal, 20H, aromatic protons).

A sample of the oily product was subjected to catalytic hydrogenation[13] over PdO. The dipeptide thus obtained, glycyl-L-leucine, had mp 223–225 °C, $[a]_D^{20}$ −35.3 °C, in accord with the literature.[17,18]

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