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ALKYLATION OF AZAGLYCINE AMIDE CONTAINING PEPTIDES

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Abstract: Azaglycine dipeptides are alkylated at the amide nitrogen with alcohols under mild conditions by using the redox condensation system triphenylphosphine / diethyl azodicarboxylate.

Various methods are known in the literature to alkylate the phenolic function of tyrosine, the Mitsunobu procedure[1] is often the method of choice, occurring with high yield and without racemization.[2] Alkylating Z-Tyr-Pro-AzaGly-NH_2 with *i*PrOH using the afore mentioned reaction, the major product, isolated with 80% yield was Z-Tyr(*i*Pr)-Pro-N_B(*i*Pr)-NH_A-$CONH_2$ ($[\alpha]_D^{22}$ = -23.5, c = 1 in AcOH). The regiospecificity of this reaction was demonstrated as follows: Z-Phe-AzaGly-NH_2 was alkylated with methanol using the Mitsunobu methodology to produce in 40% yield Z-Phe-N_B(Me)-N_AH-$CONH_2$. The two regioisomers Z-Phe-N(Me)-NH-$CONH_2$ and Z-Phe-NH-N(Me)-$CONH_2$ (= Z-Phe-AzaAla-NH_2) were prepared by two different methods according to a literature procedure[3]. Examination of the 250 MHz NMR spectrum of these compounds clearly indicates that the Mitsunobu alkylation occurs regioselectively at the amide bond[4].

We thought that alkylation of the amide bond of an azaamino acid moiety could be of valuable interest for the introduction of hydrophobic, lipophilic groups in a peptide chain. We therefore decided to investigate the general application of this method. The starting products (X-Phe-AzaGly-NH_2, X= Boc or Fmoc) were synthesized using the DCC/HOBt method[5]. The alkylation reactions (Scheme) were conducted in THF using 3 eq. of the Mitsunobu complex and various alcohols (See Table and experimental procedure[6]).

$$\text{X-NH-Phe-CO-NH-NH-CONH}_2 \xrightarrow[\text{ROH, THF}]{\text{PPh}_3\text{, DEAD}} \text{X-NH-Phe-CO-N(R)-NH-CONH}_2$$

Scheme: X = Z (benzyloxycarbonyl protecting group), Fmoc (9-fluorenylmethoxycarbonyl protecting group) or Boc (tert-butyloxycarbonyl protecting group)

This reaction occurs selectively at the amide bond to give only the desired alkylated peptide. TLC analysis of the reaction mixture showed only the alkylated compound, unreacted starting materials and PPh_3O formed during the reaction; no other products were detected.

Additionally we attempted to use PBu_3 instead of PPh_3 as part of the complex. The reaction occurs in modest yield with Boc-Phe-AzaGly-NH_2 and when using Fmoc as an N-protecting group some deprotection was observed. In these reactions the peptide is often contaminated with PPh_3O formed during the reaction, and careful chromatography is necessary to separate it completely. In cases where separation of PPh_3O was not possible, deprotection of the crude peptide at the N-terminal end was carried out followed by purification of the hydrochloride by aqueous/organic extraction.

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Table: Alkylation of X-Phe-N(R)-NH-$CONH_2$[a] via Mitsunobu reaction

Number	R	X	Yield(%)	$[\alpha]_D$ [22b]	Yield(%)[c] of Deprotection (X= HCl)	$[\alpha]_D$ [22b]
1	Methyl	Fmoc	36	+ 27	80	+ 23
2	Ethyl	Fmoc	not isolated	/	56[d]	+ 62,5
3	isopropyl	Fmoc	not isolated	/	55[d]	not done
4	(2-Pyridyl)-CH_2	Boc	35[e]	+ 8,9	84	not done
5	(2-Naphthyl)-CH_2	Boc	20[e]	+ 40,1	90	not done

a: All new compounds gave correct elemental analyses and satisfactory spectra (NMR, Fab MS). b: c= 1 in AcOH.c: Fmoc Deprotection was performed by using a mixture of Et_2NH in DMF[7] and Boc deprotection by using a mixture of HCl in dioxane.d: Yield of alkylation and deprotection. e: PBu_3 used instead of PPh_3.

In conclusion we have shown that alkylation of the amide bond of an azaglycine residue in a peptide can be easily performed by using the Mitsunobu procedure. These alkylated azaglycine derivatives were deprotected using standard methodology (See Table).

References and Notes.

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2. Barlos, K.; Lampropoulou, M.; Papaioannou, V.; Ptrianakou, S.; *Liebigs Ann. Chem.* **1986**, 1407.
3. Dutta, A.S.; Morley, J.S.; *J. Chem. Soc. Perkin Trans 1* **1975**, 1712.
4. Alkylation using the Mitsunobu procedure occurs at the more acidic proton of the substrate to alkylate.[1] The N_BH proton of an azaamino acid residue is more acidic than a "normal" NH proton in a peptide bond (Gray, C.J.; Ireson, J.C.; Parker, R.C.; *Tetrahedron* **1977**, *33*, 739).These results are therefore as expected.
5. König, W.; Geiger, R.; *Chem. Ber.* **1970**, *103*, 788. Fmoc-Phe-AzaGly-NH_2 ($[\alpha]_D^{22}$ = -16,2; c=1 in AcOH) and Boc-Phe-AzaGly-NH_2 ($[\alpha]_D^{22}$ = +2,2; c=1 in AcOH) were obtained in 81% and 57% yield, respectively.
6. **Experimental Procedure of 2.**To a suspension of Fmoc-Phe-AzaGly-NH_2 (4g/9 mmol) in 50ml abs. THF was added successively: EtOH (4,23ml/8eq.), PPh_3 (4,72g/2eq.) and DEAD (2,83ml/2eq.). After 15 min stirring PPh_3 (1,18g/0,5eq.) and DEAD (0,70ml/0,5eq.) were added. This procedure was repeated again after 15min. After 4h the solution was evaporated under reduced pressure.The crude reaction medium containing a mixture of alkylated peptide, unreacted starting material, residual PPh_3, PPh_3O and DEAD complex was partially purified by chromatography on silica gel (1 CH_2Cl_2 / AcOEt: 9/1 and 2 CH_2Cl_2 / MeOH: 9,5/0,5). The fractions containing the peptide (contaminated with a little PPh_3O) were collected, evaporated under reduced pressure. The residue was triturated in hexane, filtered and dried. The peptide was deprotected in a typical manner using Et_2NH (10eq.) in DMF.[7] After evaporation of the solution, trituration of the residue with ether, the peptide was suspended in water, acidified to pH 6,4 with 1N HCl , extracted 3 times with AcOEt to eliminate residual PPh_3O. The water phase was then lyophilized to afford the pure alkylated peptide. Yield: 56%
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