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An Efficient Route to S-N-(9-Fluorenylmethoxycarbonyl)-4'-(1-azi-2,2,2-trifluoroethyl)phenylalanine.

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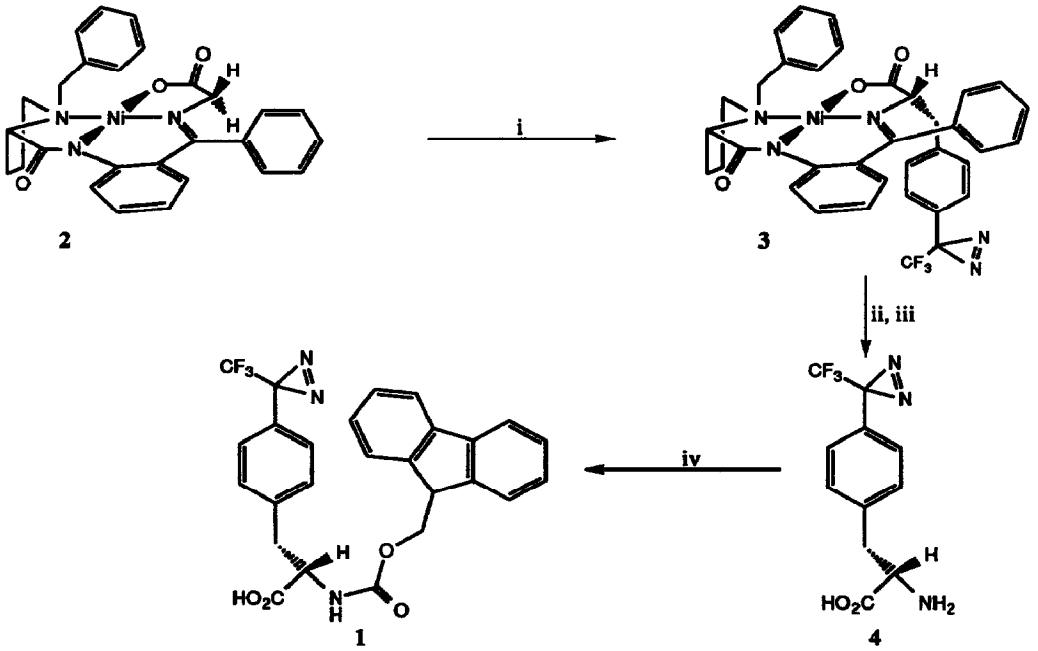
Abstract: An extremely efficient synthesis of optically pure photoactivatable phenylalanine derivative **1** is described. The key step involves a highly diastereoselective alkylation of a chiral glycine equivalent.

Photoaffinity labelling has become a powerful method in the study of biological structure and function. The most important class of photolabile group used to modify ligands is the chemically stable aryltrifluoromethyl-*3H*-diazirine which, upon photolysis, gives rise to carbenes which do not rearrange, and form chemically stable covalent cross-links with the receptor. As part of our studies on important biological receptors, we required access to peptides containing such moieties at precisely defined locations and identified phenylalanine derivative **1** as a useful system for incorporation into peptides *via* solid phase peptide synthesis.

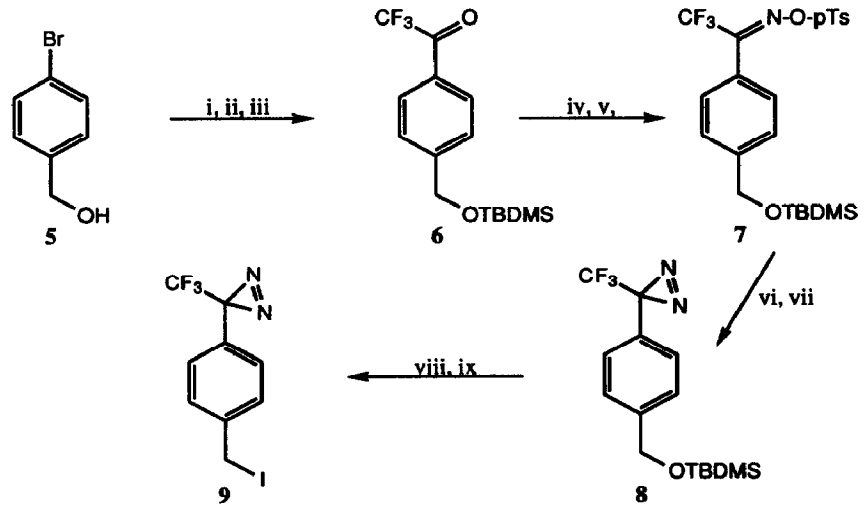


(i) **9**, NaOH, MeCN, 93%; (ii) aq. HCl, MeOH; (iii) Ion exchange, 95%; (iv) Fmoc-OSu [1], aq. Na_2CO_3, acetone, 79%

Scheme 1

Although syntheses of the corresponding amino acid of **1** have been reported[2,3] these involve the alkylation of a glycine derivative with a 4'-(1-azi-2,2,2-trifluoroethyl)benzyl halide under basic conditions, followed by subsequent, and rather wasteful, enzymatic resolution of the *N*-acyl amino acid, a route much adopted in the synthesis of optically active α-amino acids. We report here a highly efficient route which gives direct access to optically pure amino acid derivative **1**, and it's incorporation into peptides by solid-phase peptide synthesis.

An alternative method of synthesising amino acids with high enantioselectivity involves[4] the alkylation of the glycine moiety of the complex **2** (scheme 1)[5]. Although the required benzylation precursor for the complex **2**, 3-[α-Iodo-p-tolyl)-3-(trifluoromethyl)-*3H*-diazirine has been reported[2], in our hands this preparation proved wholly unsatisfactory, and a much improved procedure was developed (scheme 2)[5].



(i) TBDMSCl, imidazole, DMF, 93%; (ii) ^{n}BuLi, THF, -74 °C; (iii) Et_2NCOCF_3, 93%; (iv) $NH_2OH.HCl$, pyridine, EtOH, 90%; (v) pTsCl, DIPEA, DMAP, CH_2Cl_2, 97%; (vi) NH_3, Et_2O, 100%; (vii) Ag_2O, Et_2O, 95%; (viii) TBAF, THF, 95%; (ix) $CH_3P(OC_6H_5)_3I$, MeCN, 91%

Scheme 2

Thus the benzyl alcohol **5** was protected as the TBDMS ether and converted to the trifluoromethyl ketone **6** in high yield by reaction of the corresponding aryl lithium with N,N-diethyltrifluoroacetamide[6]. Attempts to synthesise the ketone **6** via reaction of the Grignard reagent with ethyl trifluoroacetate, TFA, TFAA, and N,N-diethyltrifluoroacetamide respectively all gave only low yields. The ketone **6** was converted to the corresponding oxime[2], and the p-toluenesulphonyl derivative **7** was readily prepared by reaction of the oxime with pTsCl at room temperature in the presence of DIPEA and catalytic amounts of DMAP[7]. Attempts to form the tosyl oxime by reaction in refluxing pyridine gave only poor yields of **7**. Reaction of **7** with excess ammonia at -78 °C [8,9] gave the diaziridine in quantitative yields, and oxidation of the diaziridine with Ag_2O provided the diazirine **8** in excellent yield. Deprotection of the alcohol with tetra-n-butyl ammonium fluoride rather than HCl/MeOH[2] was found to give much better yields, and benzyl iodide **9** was prepared using standard methods.

Alkylation of complex **2** was found to occur smoothly at room temperature with high yield (93%) and

high diastereoselectivity(>95%; no s,r diastereoisomer detected) using a modified procedure to that described in the literature[10]. Hydrolysis of the complex[11] occurred rapidly (10 min) in refluxing HCl/ MeOH, without destruction of the diazirine ring, to give the amino acid **4** after cation exchange chromatography. Attempts to synthesise **1** from **4** by reaction with Fmoc-Cl gave rise to unacceptable amounts of dipeptide formation; however, reaction with Fmoc-OSu enabled the Fmoc-amino acid **1** to be synthesised in good yield in the absence of dipeptides[12]. We have recently used this phenylalanine analogue to synthesise a number of medium-size photoactivatable peptides by solid-phase peptide synthesis[13]. The coupling efficiency of the photoactivatable analogue was found to be excellent (>98%), as was the stability of the diazirine throughout the synthesis and purification stages, where the absorbance maximum of the diazirine at 350 nm proved to be a useful aid to purification. Studies regarding the binding of these peptides to important biological receptors are in progress and will be reported elsewhere.

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REFERENCES AND NOTES

1. Abbreviations used: Fmoc, 9-fluorenylmethoxycarbonyl; TBDMS, *tert*-butyldimethylsilyl; DMF, dimethylformamide; THF, tetrahydrofuran; Ts, toluenesulphonyl; DIPEA, N,N-diisopropylethylamine; DMAP, 4-dimethylaminopyridine; TBAF, tetra-*n*-butylammonium fluoride, TFA, trifluoroacetic acid; TFAA, trifluoroacetic anhydride.

2. Shih, L. Y. and Bayley, H., *Analytical Biochem.*, **1983**, *144*, 132-141.

3. Nassal, M., *J. Amer. Chem. Soc.*, **1984**, *106*, 7540-7545.

4. Belokon, Y. *Janssen Chimica Acta*, **1992**, *10*, 4-12 (and references therein).

5. All compounds gave satisfactory data.

6. Procedure as follows: To a stirred solution of the silyl ether (24.94 g, 82.8 mmol) in THF (420 cm^3) at -74 °C (CO_2/Et_2O) was added dropwise ^{n}BuLi (1.6 M in hexane, 62 cm^3, 99.2 mmol) over a 1 h period. After stirring the mixture at the same temperature for 75 min, a solution of N,N-diethyltrifluoroacetamide (19.19 g, 113 mmol) in THF (60 cm^3) was added dropwise over 1 h. Following the addition the mixture was stirred at -74 °C for a further 75 min before the reaction was quenched with NH_4Cl (300 cm^3) without warming. After warming to room temperature, the mixture was diluted with Et_2O (500 cm^3) and washed with saturated aq. NH_4Cl (2 x 500 cm^3) and water (2 x 200 cm^3) before being dried ($MgSO_4$). Following removal of the drying agent, the solvent was removed *in vacuo* and the resulting pale yellow liquid distilled *in vacuo* to give trifluoromethyl ketone **6** (24.43 g, 93%) as a clear colourless liquid; b.p. 120-123 °C/0.7 mm; ν_{max}/cm^{-1} 1715 (CO); δ_H(300 MHz) 0.12 (6 H, s, Me), 0.96 (9 H, s, Bu^t), 4.83 (2 H, s, CH_2), 7.50 (2 H, d, *J* 8.4, Ar), 8.06 (2 H, d, *J* 7.7, Ar).

7. For example see: Hatanaka, Y., Nakayama, H., and Kanaoka, Y., *Heterocycles*, **1993**, *35*, 997-1004.

8. For example see: Baldwin, J. E., Jesudason, C. D., Moloney, M. G., Morgan, D. R., and Pratt, A. J., *Tetrahedron*, **1991**, *47*, 5603-5614.

9. For example see: Platz, M., Admasu, A. S., Kwiatkowski, S., Crocker, P. J., Imai, N., and

Watt, D. S., *Bioconjugate Chem.*, **1991**, *2*, 337-341.

10. Procedure as follows: The Ni(II) complex **2** was prepared as described previously[4]. To a stirred suspension of complex **2** (2.4390 g, 4.90 mmol) and finely powdered NaOH (0.85 g, 21.2 mmol) in MeCN (8 cm^3) at -20 °C under Ar, was added a solution of iodide **9** (1.2592 g, 3.86 mol) in MeCN (4 cm^3), in one portion. The mixture was warmed to room temperature and stirred for a further 20 h before being diluted with CH_2Cl_2 (200 cm^3) and washed with aq. AcOH (0.2 M; 125 cm^3) and water (2 x 100 cm^3). Following drying of the solution ($MgSO_4$) and removal of the drying agent, the solvent was removed *in vacuo* to give the crude product as a red oil. Purification by flash column chromatography (CH_2Cl_2:acetone; 5:1) gave pure Ni(II) complex **3** (2.4932 g, 93%) as a red solid m.p. 199.9 - 201.4 °C (dec.); ν_{max}/cm^{-1} 3450, 2860, 1630, 1335, 1160, 930, 750, 700 ; λ_{max} (EtOH) 404 (3.24), 320 (3.54), 248 (4.11); $[\alpha]_D$ (c = 0.030, EtOH) +10.74; δ_H(400 MHz) 1.65-1.75 (1 H, m, γH Pro), 1.84-1.91 (1 H, m, γH Pro), 2.20-2.28 (1 H, m, βH Pro), 2.31-2.42 (2 H, m, β and δH Pro), 2.82 (1 H, dd, *J* 6.1 and 10.2, βH Phe), 2.99-3.04 (2 H, m, βH Phe and δH Pro), 3.31 (1 H, dd, *J* 6.1 and 10.2, αH Pro), 3.46 (1 H, d, *J* 12.7, CH_2 Bzl), 4.26 (1 H, d, *J* 12.4, CH_2 Bzl), 4.29 (1 H, dd, *J* 4 and 5.7, αH Phe), 6.64-6.89 (3 H, m, Ar), 7.13-7.22 (6 H, m, Ar), 7.27-7.46 (4 H, m, Ar), 7.51-7.58 (2 H, m, Ar), 7.97-8.21 (3 H, m, Ar).

11. Procedure as follows: A solution of Ni(II) complex **3** (0.4154 g, 0.596 mmol) in MeOH (10 cm^3) and aq. HCl (1 M, 6 cm^3) was gently heated at reflux for 10 min. After removal of the solvent *in vacuo* the residue was diluted with water (20 cm^3) and extracted with CH_2Cl_2 (2 x 20 cm^3). The CH_2Cl_2 extracts were combined and washed with water (2 x 20 cm^3). All aqueous extracts were combined and adjusted to pH 2.5 with aq. NH_3 before being adsorbed onto a column of Dowex 50 (H^+) cation exchange resin. The amino acid **9** was eluted with 5% methanolic NH_3. Removal of the solvent *in vacuo* gave the crude product as a white solid which was recrystallised from MeOH/water to give pure amino acid **4** (0.1547 g, 95%) as a white solid; ν_{max}/cm^{-1} 3050, 2850, 1600, 1590, 1410, 1275, 940, 835, 705; λ_{max} (MeOH) 350 (2.05); $[\alpha]_D$ (c = 0.115, MeOH) -70.13; δ_H(300 MHz, CF_3COOD) 3.41-3.48 (1 H, m, βH), 3.64-3.71 (1 H, m, βH), 4.71-4.76 (1 H, m, αH), 7.30 (2 H, d, *J* 8.1, Ar), 7.42 (2 H, d, *J* 7.8, Ar).

12. Data for **1**: m.p. 119.2 - 120.5 °C; ν_{max}/cm^{-1} 3300 (NH), 1730, 1685, 935, 760, 740; λ_{max} (MeOH) 357 (2.45), 300 (3.78), 289 (3.72), 265 (4.30), 221 (4.47); $[\alpha]_D$ (c = 0.338, EtOH) +24.84; δ_H(400 MHz) 3.10 (1 H, dd, *J* 6.0 and 14.5, βH), 3.22 (1 H, dd, *J* 5.1 and 14.0, βH), 4.18-4.21 (1 H, m, CH Fmoc), 4.39 (1 H, dd, *J* 6.5 and 10.5, CH_2 Fmoc), 4.50 (1 H, dd, *J* 7.0 and 10.6, CH_2 Fmoc), 4.66-4.67 (1 H, m, αH), 5.17 (1 H, d, *J* 7.4, NH), 7.08 (2 H, d, *J* 8.0, Ar Phe), 7.13 (2 H, d, *J* 8.2, Ar Phe), 7.29-7.33 (2 H, m, Ar Fmoc), 7.41 (2 H, t, *J* 7.7, Ar Fmoc), 7.52-7.55 (2 H, m, Ar Fmoc), 7.78 (2 H, d, *J* 7.6, Ar Fmoc).

13. General procedure: Peptides were synthesised to a 30 μmol scale on Novasyn PR 500 amide resin with a Milligen 9050 peptide synthesiser using standard methodology for Fmoc amino acid derivatives. In order to couple **1** onto the growing peptide chain, the resin was removed from the machine following the Fmoc deprotection cycle and washed successively with 0.2M AcOH, MeOH, and CH_2Cl_2 before being dried *in vacuo* . The dried resin was suspended in a solution of **1** (29.9 mg, 60 μmol), and 1-hydroxybenzotriazole (8.2 mg, 61 μmol) in CH_2Cl_2 (2 cm^3) and DMF (0.25 cm^3) and the mixture stirred gently at room temperature under Ar for 15 min. After this period, N,N'-diisopropylcarbodiimide (9.5 μl, 61 μmol) was added and the mixture stirred for a further 3 h before the resin was collected at the pump, washed with CH_2Cl_2 and dried *in vacuo*. At all stages, precautions were taken to exclude light from mixtures containing the diazirine.

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