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THE SYNTHESIS OF A LIGHT-SWITCHABLE AMINO ACID FOR INCLUSION INTO CONFORMATIONALLY MOBILE PEPTIDES

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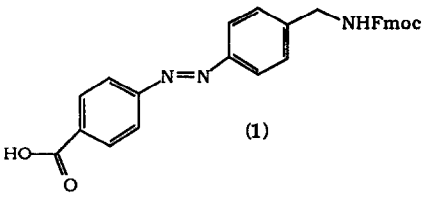
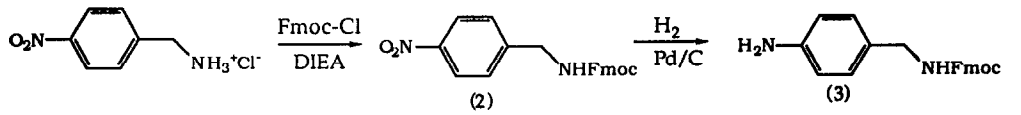
Abstract: An efficient synthesis of protected amino acid **1**, containing a light-switchable azabenzene moiety and suitable for incorporation into conformationally mobile linear and cyclic peptides, is described.

The ability to regulate the conformation of bioactive molecules, a key feature in allosteric processes such as signal transduction and feedback inhibition, has generally been found only in complex protein systems.[1] Small organic molecules and polymers with the potential for conformational switching have been designed to mimic the activity of these larger systems, such as metal ion binding and transport.[2] However, little work has been done in incorporating allosteric control into truly biologically active systems. This paper describes the synthesis of protected amino acid **1** containing the light-switchable azabenzene moiety which is suitable for incorporation into conformationally mobile linear and cyclic bioactive peptides.



(1)

Retrosynthetically, **1** was dissected at the diazene bond to generate a left-hand fragment and a right-hand fragment. The synthesis of the right-hand fragment was initiated by treatment of a suspension of 4-nitrobenzylamine hydrochloride (5.3 mmol) in CH_2Cl_2 (100 mL) with 9-fluorenylmethyl chloroformate (Fmoc-Cl) (5.3 mmol) and diisopropylethyl amine (DIEA) (26.5 mmol) for 2 hrs at room temperature to afford a 92% yield of the desired 2 (Scheme 1), which was treated with H_2 and Pd/C (5% by weight) in MeOH for 3 hrs at room temperature to provide aniline-derivative **3** in 80% yield.

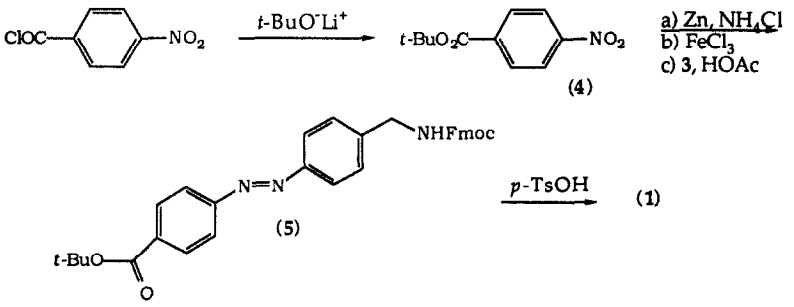


(2) (3)

Scheme 1. Synthesis of the right fragment of **1**.

The left-hand fragment of **1** was prepared by treating 4-nitrobenzoyl chloride (10.7 mmol) in THF (20 ml) with lithium-*t*-butoxide (16.1 mmol) for 1 hr at 0°C, followed by 12 hrs at room temperature, to provide *t*-butyl-4-nitrobenzoate (4) in 60% yield (Scheme 2). Treatment of **4** (6.2 mmol) in 2-methoxyethanol (30 mL) with zinc (18.6 mmol) and NH_4Cl (10 mmol) produced the hydroxylamine, which was oxidized with $FeCl_3$

(15.5 mmol) in H_2O/EtOH (5:1) for 3 hrs at 0°C to generate the nitroso-derivative.[3] This compound (5.2 mmol) was isolated and treated directly with **3** (2.6 mmol) in acetic acid (40 mL) for 24 hr at room temperature to provide diazene **5** in 93% yield. Treatment of **5** (3 mmol) in refluxing benzene (40 mL) with *p*-TsOH (0.1 mmol) for 1 hr provided the desired final product (**1**) in 93% yield.[4]



Scheme 2. Synthesis of amino acid **1**.

That **1** was compatible with peptide synthesis was confirmed by preparing linear peptide **6** by a solid phase procedure using the Merrifield resin modified with a *p*-alkoxybenzylalcohol linker.[5] Fluorenylmethyloxycarbonyl (Fmoc) was used as the semipermanent amine protecting group, and amino acid couplings were performed via the hydroxybenzotriazole (HOBT) esters. The peptide was cleaved from the resin with trifluoroacetic acid (TFA), and purified by HPLC. The peptide sequence was confirmed by mass spectrometry and amino acid analysis.

(6) H_2N-Gly-(**1**)-Gly-Asn-Pro-Gly-Gly-CO_2H

In conclusion, we have developed an efficient synthesis of light-switchable amino acid **1**, and demonstrated the compatibility of this amino acid in an Fmoc-based, solid phase peptide synthesis. The ability of the azabenzene moiety to undergo a light-promoted trans-cis isomerization should have a unique effect on the conformation of biologically active peptides containing **1**, and we are currently exploring this with cyclic peptides.

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References and Notes:

1. Perutz, M. F. *Q. Rev. Biophys.* **1989**, *22*, 139-236, and references cited within.
2. a) Tabushi, I. *Pure Appl. Chem.***1988**, *60*, 581-6. b) Rebek, J. *Acc. Chem. Res.* **1984**, *17*, 258-64. c) Kumar, G. S.; Neckers, D. C. *Chem. Rev.* **1989**, *89*, 1915-1925.
3. The methyl and ethyl esters of 4-nitrosobenzoic acid were unstable.
4. Spectral data for **1**: ^{1}H NMR (DMSO-d_6, 200 MHz) δ 3.4(b, 1H), 4.3(m, 3H), 4.42(d, J=6.6 Hz, 2H), 7.4(m, 6H), 7.73(d, J=6.7 Hz, 2H), 7.9(m, 7H), 8.19(d, J=8.5 Hz, 2H). MS (FAB, DTT/DTE matrix): (M+H) 478.3 (cald); 478.2 (found).
5. Wang, S. S. *J. Am. Chem Soc.* **1973**, *95*, 1328.

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