

Synthesis of Terminally Protected 9-Amino-4,5-diazafluorene-9-carboxylic acid, the First Rigid, Transition-Metal Receptor, C^{α,α}-Disubstituted Glycine

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Abstract : *N*-*tert*-Butyloxycarbonyl-9-amino-4,5-diazafluorene-9-carboxylic acid methyl ester, Boc-Daf-OMe, the first C^{α,α}-disubstituted glycine containing a rigid bipyridine ligand in a totally controlled spatial situation relative to the C^α atom of the amino acid, and a potential building block for the synthesis of peptide supramolecular devices, has been synthesized by acylation of the anion of *N*-benzyl-4,5-diazafluorene-9-methylenamine, followed by *N*-protection. Hydrazinolysis of the ester function afforded the hydrazide Boc-Daf-NHNH₂, a key precursor for the acylazide coupling method.
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It is now well established that peptides rich in C^{α,α}-disubstituted α-aminoacids have strong conformational preferences with a very high tendency to fold into β-bends and α / ₃₁₀-helices or, in some cases, to adopt a fully-extended (C₅) conformation.¹ Therefore, the design of building blocks based on such aminoacids could allow the construction of peptide supramolecular architectures in which two or more effectors, located in the side chains, interact in a well defined and controlled spatial organization.² We have recently exploited one of such scaffolds for the synthesis of a peptide-based system of rigid donor-rigid interchromophore spacer-rigid acceptor.³

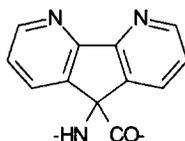


Figure 1. Structure of the 9-amino-4,5-diazafluorene-9-carboxylic acid residue (Daf).

In this connection, some of us have also designed the [20-C-6]-Bip residue in which a crown-ether effector is carried by an axially chiral C^{α,α}-disubstituted α-aminoacid.⁴ In this letter we wish to present the synthesis of the terminally protected 9-amino-4,5-diazafluorene-9-carboxylic acid residue (Daf) (Fig.1), characterized by a rigid 2,2'-bipyridine ligand.

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The ability of both 2,2'-bipyridine and 4,5-diazafluorene architectures to complex a variety of transition metals is well documented.^{5,6} In addition, supramolecular properties have indeed been observed by Imperiali and coworkers⁷ and other groups⁸ in metal complexes of peptides incorporating α -amino acid residues containing various kinds of 2,2'-bipyridine-type *flexible* receptors. However, Daf represents the first example in which the location of the 2,2'-bipyridine receptor is in a *totally rigid and controlled spatial situation* relative to the C $^{\alpha}$ atom of the amino acid.

For the synthesis of Daf we chose to extend a route proposed by DuPriest *et al.*⁹, involving acylation of the anion of N-benzyl-fluorene-9-methylenamine as the key step, previously applied by us with success for the preparation of the protected 9-aminofluorene-9-carboxylic acid (Afc).¹⁰

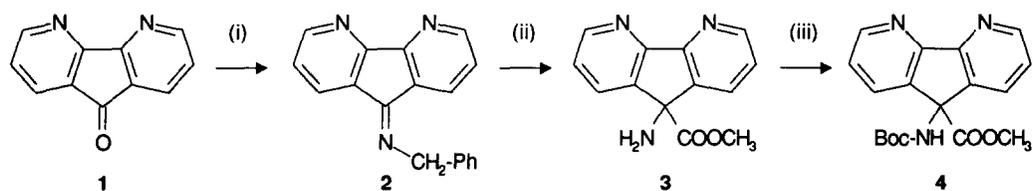


Figure 2. Synthetic scheme for the preparation of Boc-Daf-OMe (4) : (i) Ph-CH₂-NH₂ ; TiCl₄ ; CH₂Cl₂ ; 0 °C (ii) (1) NaHMDS ; THF ; 0 °C (2) ClCOOCH₃ ; r.t. (3) 1 M HCl ; r.t. ; 2 h (iii) Boc₂O ; CH₃CN ; 60 °C.

4,5-Diazafluoren-9-one **1** (Fig. 2) was first prepared by oxidation of phenanthroline,^{6a} and treated with benzylamine and TiCl₄ in CH₂Cl₂ at 0 °C,⁹ to give 4,5-diazafluorenone-benzyl-imine **2**¹¹ in 50-60 % yield after crystallization from CH₂Cl₂/hexane.¹² The imine **2** was then treated with an excess (2 equivalents) of sodium hexamethyldisilazane in THF at 0 °C. The resulting dark red-brown aza-allylic anion was stirred at room temperature for 1 hour, cooled to 0° C, and treated with a THF solution containing a large excess of methyl chloroformate, just previously stirred for a few minutes in the presence of K₂CO₃.⁹ The reaction mixture was stirred at room temperature overnight, cooled to 0° C and hydrolyzed by 1 M aqueous HCl at room temperature for 2 hours, to afford the amino ester **3**¹¹ in 32 % yield after chromatography.¹⁴ Such relatively low yield, compared to the related acylation of the anion of N-benzyl-fluorene-9-methylenamine,¹⁰ probably results from a decreased reactivity of the delocalized anion, stabilized by the strong electron withdrawing effect of the two nitrogens at the 4,5-positions. For the same reason, the lowered nucleophilicity of the amino function of **3** could be responsible for its difficult acylation by Boc₂O in acetonitrile,¹⁵ which required a prolonged heating at 60 °C to give the protected aminoester **4**¹¹ in 73 % yield after chromatography.

Another consequence of the electron withdrawing effect of the two nitrogens was the spontaneous decarboxylation of the sodium salt of the N-protected aminoacid Boc-Daf-OH (**5**) (Fig. 3): saponification of the ester function of **4** in 1 M NaOH/ MeOH at room temperature, followed by neutralization of the reaction mixture, resulted in compound **6**¹¹ as the only product. Although the mechanism of formation of **6** is not yet

clear at this stage, it is reasonable to assume that it would involve decarboxylation of the COO^- function of the Boc-Daf- $\text{O}^- \text{Na}^+$ salt. Decarboxylation was previously observed, but only to some extent, during saponification of the ester function of the related related 9-*tert*-butyloxycarbonyl-aminofluorene-9-carboxylic acid methyl ester, Boc-Afc-OMe, as well as during coupling of the N-protected amino acid Boc-Afc-OH.¹⁰ The complete decarboxylation observed in the present diaza series is not surprising, since the analogous 9-hydroxy-4,5-diazafluorene-9-carboxylic acid is known to decarboxylate at room temperature.¹⁶

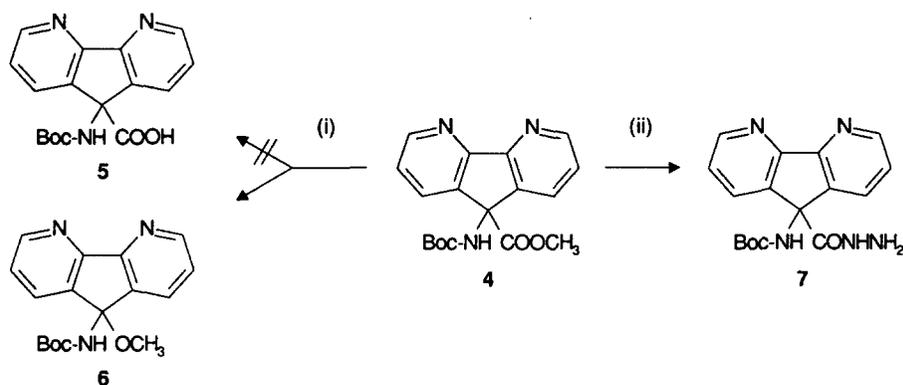


Figure 3. Saponification and hydrazinolysis of the ester function of Boc-Daf-OMe (4) : (i) (1) 1 M NaOH ; MeOH ; r.t. 12 h (2) H^+ (ii) $\text{H}_2\text{NNH}_2, \text{H}_2\text{O}$; MeOH ; r.t. 17 h.

As for coupling of an amino acid residue at the C-terminus of Daf, a prerequisite for its incorporation in an internal position of a peptide fragment, we synthesized the N-protected amino hydrazide 7, the key precursor for the exploitation of the acylazide method.^{17,18} Indeed, treatment of 4 by a large excess of hydrazine hydrate in methanol at room temperature afforded Boc-Daf-NHNH₂ (7)¹¹ in 91 % yield after chromatography with only traces of the decarboxylation product.

In conclusion, the Daf synthons made available from the present study can be subjected to peptide coupling at both N- and C- termini. The synthesis of transition-metal complexes, as well as the conformational analysis of Daf-rich peptides, will be soon performed. According to our recent results¹⁰ and those of Yamada *et al.*,¹⁹ peptides incorporating the parent 9-aminofluorene-9-carboxylic acid (Afc) mostly adopt an extended (C_5) conformation, but a modest helical tendency is also observed. It will be of interest to compare the conformational behaviour of both Afc and Daf peptides, and to examine whether conformational changes can be induced by metal complexation of Daf peptides.

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