9-Amino-4,5-diazafluorene-9-carboxylic Acid (Daf), a New $C^{\alpha,\alpha}$-Disubstituted Glycine Containing a Spatially Constrained Bipyridine-Like Ligand for Transition Metals − Synthesis and Evaluation of Peptide-Coupling Conditions at its *C*- and *N*-Termini

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Acylation of the anion of *N*-benzyl-4,5-diazafluorene-9-methyleneamine with methyl or benzyl chloroformate, followed by acidic hydrolysis, resulted in 9-amino-4,5-diazafluorene-9-carboxylic acid methyl ester (H-Daf-OMe) and benzyl ester (H-Daf-OBzl), respectively. N^{α}-protection with Boc_2O at 60 °C gave Boc-Daf-OMe and Boc-Daf-OBzl, saponification or hydrogenolysis of which resulted in complete decarboxylation. However, hydrazinolysis of the ester function afforded Boc-Daf-$NHNH_2$, which was efficiently coupled with H-Ala-OMe by the acylazide method. Coupling of Boc-Ala-OH at the *N*-terminus of Daf could also be performed by the mixed anhydride method. However, coupling of the crowded Aib residue required the use of Boc-Aib-NCA. Daf, a new $C^{\alpha,\alpha}$-disubstituted glycine, is the first α-amino acid containing a rigid bipyridine ligand in a totally controlled spatial disposition relative to the C^{α} atom.

Introduction

The utility of peptide synthesis for the facile assembly of combinations of natural amino acids and artificial amino acids with functionalized side chains, permitting the construction of supramolecular devices and catalysts, has in the past few years been recognized as an important strategy.[1−3] Unnatural amino acids that can bind transition metals are especially interesting targets for the de novo design of metalloproteins,[4] as well as peptide-based electronic devices and molecular switches.[1−3,5] In this context, peptides containing a variety of 2,2′-bipyridine-type transition metal receptors,[6−8] whether covalently attached to their *C*- or *N*-termini, incorporated within the sequence as side chain-modified α-amino acids, or directly inserted into the main chain, have been extensively studied and shown to display supramolecular properties of templated self-organization, photoinduced intramolecular electron transfer and intramolecular luminescence quenching.[9−24] In this paper, we wish to report our detailed experimental procedures for the synthesis of terminally protected derivatives of 9-amino-4,5-diazafluorene-9-carboxylic acid (Daf) (Figure 1),[25] and an initial evaluation of their peptide coupling conditions.

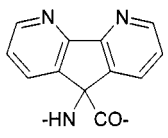

Figure 1. Structure of the 9-amino-4,5-diazafluorene-9-carboxylic acid residue (Daf).

Daf is characterized by a 4,5-diazafluorene architecture as a transition metal receptor[26−27] and presents new and interesting features: *(i)* the metal ligand site is in a *totally rigid and controlled spatial disposition* relative to the C^{α} atom of the amino acid, in contrast with previously described *flexible* amino acid metal receptors;[9−24] *(ii)* Daf can be inserted into the main chain of a peptide, not into a side chain, which should impose distinct conformational constraints on the backbone geometry,[24] *(iii)* it belongs to the class of $C^{\alpha,\alpha}$-disubstituted glycines, well-known for their strong conformational preferences and their very strong tendency to induce β-bends and $\alpha/3_{10}$-helices in peptides;[28−35] this would be expected to allow better control over the spatial organization of these metal receptors in peptide supramolecular architectures. In this context, we have previously designed the [20-C-6]-Bip residue, in which a crown ether effector is carried by an axially chiral $C^{\alpha,\alpha}$-disubstituted glycine.[36] We have also recently exploited one such scaffold for the synthesis of a peptide-based "rigid donor/rigid interchromophore spacer/rigid acceptor" system.[37]

Results and Discussion

For the synthesis of Daf, we considered a route involving acylation of the anion of *N*-benzyl-4,5-diazafluorene-9-methyleneamine as the key step. This method had been pro-

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posed by DuPriest et al.[38] for acylation of the delocalized anion of *N*-benzyl-fluorene-9-methyleneamine and was previously applied by us to the preparation of 9-aminofluorene-9-carboxylic acid (Afc) methyl ester.[39−40]

In this context, 4,5-diazafluoren-9-one **1** (Figure 2) was first prepared from phenanthroline,[26,41] and condensed with benzylamine. Surprisingly, the reaction did not proceed in the presence of BF_3 catalyst,[42] but did when use was made of $TiCl_4$ in CH_2Cl_2 at 0 °C − as in the case of the preparation of *N*-benzyl-fluorene-9-methyleneamine[38] − producing 4,5-diazafluorenone-benzyl-imine **2a** in 68% yield after crystallization from CH_2Cl_2/hexane. It was found to be very important to keep the reaction mixture at 0 °C, since at 25 °C a strong, blue-green colour sometimes developed, with unidentified side products then mostly being obtained after workup. In the same manner, condensation of **1** with benzhydrylamine produced the benzhydrylimine of 4,5-diazafluorenone **2b**, in 53% yield. When *p*-methoxybenzylamine was used, however, the corresponding imine could not be purified without extensive decomposition.

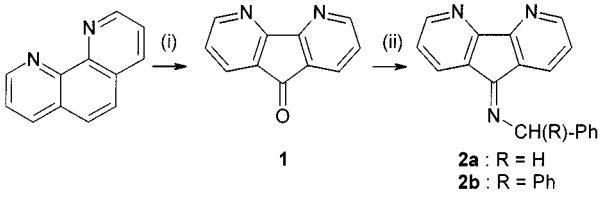

Figure 2. Preparation of *N*-benzyl- and *N*-benzhydryl-4,5-diazafluorene-9-methyleneamines **2a** and **2b** from phenanthroline. *(i)* $KMnO_4$; KOH/H_2O; 100 °C *(ii)* $PhCH_2-NH_2$ or Ph_2CH-NH_2; $TiCl_4$; CH_2Cl_2; 0 °C.

In the next key step, the delocalized anion obtained after abstraction of a benzylic proton of imine **2a** with the aid of NaHMDS (sodium hexamethyldisilazane) in THF was treated with methyl chloroformate (Figure 3). Acylation presumably occurred on both carbanionic sites, since both benzaldehyde and ketone **1** were present, in a ca. 2:1 ratio, after acidic hydrolysis of the reaction mixture. However, phenylglycine methyl ester resulting from acylation at the benzylic carbon could not be isolated. In several duplicate experiments the desired amino ester H-Daf-OMe (OMe, methoxy) **3a** was the only such compound obtained in a more or less constant yield of 25−30% after chromatography, while 9-benzoylamino-4,5-diazafluorene (**4**), resulting from oxidation of the anion of imine **2a**, was always present as the main side product. Such a relatively low yield, in comparison with that obtained in the related acylation of the anion of *N*-benzyl-fluorene-9-methyleneamine,[39−40] is probably the result of a decreased reactivity of the delocalized anion, stabilized by the strongly electron-withdrawing effect of the two nitrogens at the 4,5-positions. Attempts to improve the yield by varying the experimental conditions {temperature, reaction time, use of other base/solvent systems such as NaH/THF, NaH/DMSO, NaHMDS/DMSO or KHMDS (potassium hexamethyldisilazane)/toluene-THF} were unsuccessful. Treatment of imine **2b** under the same reaction conditions as **2a** (NaHMDS/THF, then ClCOOMe) did not give any amino ester **3a** after acidic hydrolysis, although benzophenone was isolated in 40% yield, together with ketone **1** (60%). Acylation of the delocalized anion of imine **2a** (treated with NaHMDS/THF) by benzyl chloroformate, which was also attempted in order to obtain a Daf ester function cleavable by hydrogenolysis (vide infra), gave H-Daf-OBzl (OBzl, benzyloxy) **3b** in a slightly higher yield (38%). Here, only traces of compound **4** were present and the main isolated side product was 9-amino-9-benzyl-4,5-diazafluorene **5** (11%), presumably originating from alkylation of the anion of **2a** by benzyl chloride present in the benzyl chloroformate as a contaminant.

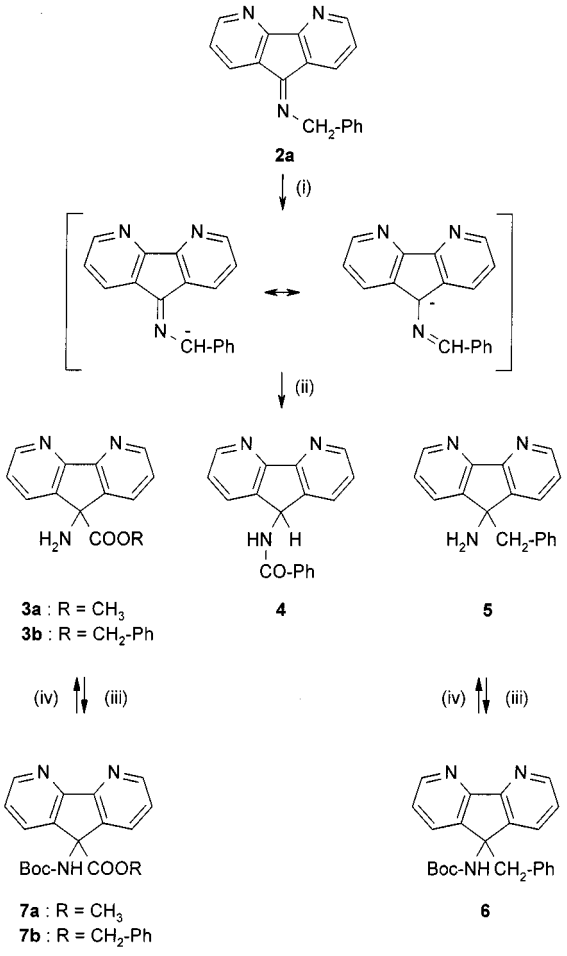

Figure 3. Synthesis of 9-amino-4,5-diazafluorene-9-carboxylic acid methyl and benzyl esters, and their *N*-Boc protected derivatives, from imine **2a**. *(i)* NaHMDS; THF; 0 °C *(ii)* (1) $ClCOOCH_3$ or $ClCOOCH_2Ph$; room temp. (2) 1 M HCl; room temp.; 2 h *(iii)* Boc_2O; CH_3CN; 60 °C *(iv)* TFA/CH_2Cl_2 1:1.

As expected, *N*-protection of the sterically hindered amine function of the amino esters **3a** and **3b** by a *tert*-butyloxycarbonyl (Boc) group required prolonged heating at 60 °C in the presence of a large excess of Boc_2O in acetonitrile[43−45] to furnish the fully protected amino acid residues Boc-Daf-OMe **7a** (73%) and Boc-Daf-OBzl **7b** (87%), respectively. Such treatment was also a convenient means for further purification of the Daf amino esters **3a** and **3b**, which could easily be recovered from chromato-

graphically purified **7a** and **7b** after N^{α}-deprotection in TFA (trifluoroacetic acid)/CH_2Cl_2 1:1. In the same manner, the amine **5** was acylated with Boc_2O to give **6** (80%) and recovered by acidic cleavage of the Boc protecting group.

The *C*-deprotection conditions of **7a** and **7b** were examined next. From previous studies we suspected that spontaneous decarboxylation of the desired N^{α}-protected free amino acid Boc-Daf-OH **8** (Figure 4) would be a problem, since the analogous 9-hydroxy-4,5-diazafluorene-9-carboxylic acid had been shown to decarboxylate at room temperature and had been impossible to isolate.[46] Decarboxylation had also previously been observed, but only to a certain extent, during saponification of the ester function of the 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.[39−40] Saponification of the ester function of **7a** in 1 N aqueous NaOH/MeOH at room temperature, followed by acidic hydrolysis of the reaction mixture, resulted in 9-*tert*-butyloxycarbonylamino-9-methoxy-4,5-diazafluorene **9** (43%) and ketone **1** (41%) as the only products, with no trace of the desired Boc-Daf-OH **8**. In a duplicate experiment, saponification of **7b** under similar experimental conditions produced compound **9** (13%), 9-*tert*-butyloxycarbonylamino-4,5-diazafluorene **10** (15%) and ketone **1** (21%) as the only products. The different product distributions in the two experiments are not surprising, since we have observed in control experiments that the decarboxylated product **10** is very easily oxidized to **1** (such decomposition occurs in $CDCl_3$ solution, albeit slowly). As it is reasonable to assume that the mechanism of formation of compound **9** involves decarboxylation of the COO^- function of the Boc-Daf-O^- Na^+ salt, followed by oxidation and reaction with methanol, it appears that all reaction products arise from decarboxylation of Boc-Daf-O^- Na^+, or Boc-Daf-OH, or both. The presence of a benzyl ester function in **7b** made its *C*-deprotection by hydrogenolysis under neutral conditions (Pd/C; MeOH) possible, but complete decarboxylation was again observed and compound **10** was obtained in 100% yield (crude).

As coupling of an amino acid residue at the *C*-terminus of Daf is a prerequisite for its incorporation into an internal position in a peptide fragment, we turned to the synthesis of the *N*-protected amino hydrazide of Daf, the key precursor for the exploitation of the acylazide method.[47] Interestingly, treatment of **7a** with a large excess of hydrazine hydrate in methanol at room temperature afforded Boc-Daf-$NHNH_2$ **11** in 91% yield, with only traces of the decarboxylation product **10**.

Conversion of the hydrazide **11** to the corresponding acylazide Boc-Daf-N_3 by the Honzl−Rudinger method[48] and in situ acylation of a large excess of alanine methyl ester (H-Ala-OMe) by the azide was successful in producing Boc-Daf-Ala-OMe **12** (Figure 5) in reasonable yield (64%), accompanied only by small quantities of the decarboxylation product **10** and ketone **1**. However, coupling of the hydrazide **11** with the more hindered H-Aib-OMe (Aib, α-aminoisobutyric acid) by the acylazide method under the same experimental conditions failed to give the desired Boc-Daf-Aib-OMe. Presumably, activation of Daf through its acylazide is not efficient enough to allow a fast acylation of Aib to compete with the decarboxylation reaction.

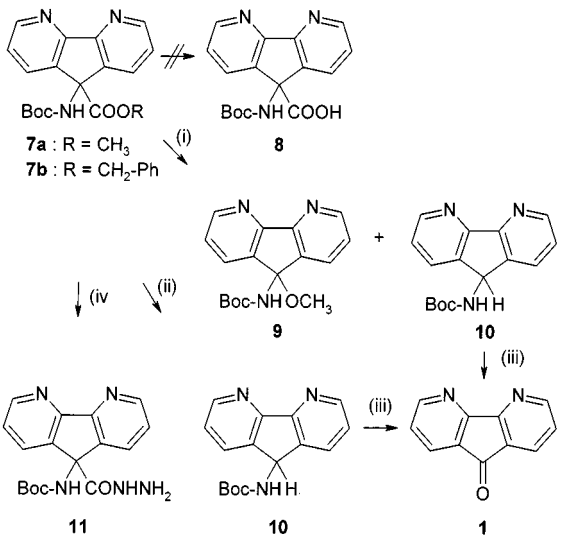

Figure 4. Hydrolysis, hydrogenolysis and hydrazinolysis of the ester functions of Boc-Daf-OMe **7a** or Boc-Daf-OBzl **7b**. *(i)* (1) 1 M NaOH; MeOH; room temp. (2) H^+ *(ii)* H_2/Pd-C; MeOH; room temp. *(iii)* spontaneous decomposition in $CDCl_3$ solution *(iv)* H_2NNH_2,H_2O; MeOH; room temp.

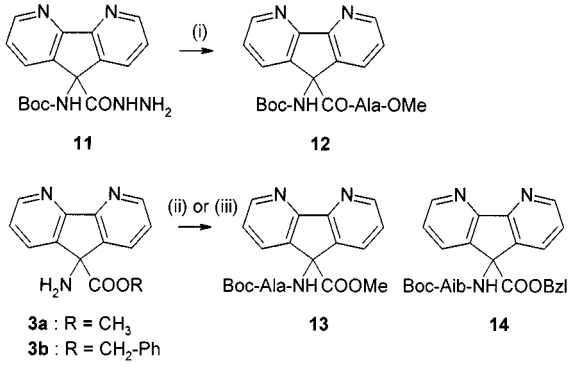

Figure 5. Coupling at the *C*- and *N*-termini of Daf. *(i)* (1) *i*Pr$(CH_2)_2$ONO; HCl; DMF; −40 °C (2) H-Ala-OMe·HCl; DIEA; DMF; −40 °C to room temp. *(ii)* (1) Boc-Ala-OH; NMM; EtOCOCl; THF; −10 °C (2) H-Daf-OMe; CH_2Cl_2; −10 °C to room temp. *(iii)* H-Daf-OBzl; Boc-Aib-NCA (excess); DIEA; THF; 60 °C.

For coupling of Ala at the *N*-terminus of Daf, we considered the mixed anhydride[49] and the symmetrical anhydride[50] methods, previously shown to be much more efficient than the EDC [*N*-ethyl-*N*′-(3-dimethylaminopropyl)-carbodiimide]/HOBt (1-hydroxy-1,2,3-benzotriazole) method[51] for the coupling of Ala at the *N*-terminus of $C^{\alpha,\alpha}$-diphenylglycine (Dph),[52] Afc[39−40] and other $C^{\alpha,\alpha}$-disubstituted glycines.[53−54] Therefore, an excess of the mixed anhydride Boc-Ala-OCOEt, prepared in situ, was used to check the acylation of **3a**, resulting without any particular difficulty in the desired dipeptide Boc-Ala-Daf-OMe **13**. In situ acylation of **3b** by Aib using an excess of the symmetrical anhydride $(Boc\text{-}Aib)_2O$ was also at-

tempted, but failed. However, efficient coupling of Aib at the *N*-terminus of Daf could be achieved with the aid of the protected *N*-carboxyanhydride Boc-Aib-NCA,[55] which reacted with **3b** in THF at 60 °C to furnish the dipeptide Boc-Aib-Daf-OBzl **14** in excellent yield (86%).

It is noteworthy that the ^{1}H NMR spectra of the Daf derivatives and peptides with the *N*-terminal Boc-Daf sequence (compounds **7a**, **7b**, **11**, **12**), in $CDCl_3$ at room temperature, displayed the same striking feature as previously observed for the Afc derivatives,[39−40] namely the *trans (anti)-cis (syn)* CO-NH isomerization of the urethane (carbamate) moiety, which resulted in broadened and split Boc CH_3 and Daf NH singlets. Such isomerization was not observed for the peptides **13** and **14**, with amide functions rather than carbamate moieties at the Daf *N*-termini.

Conclusions

This study has shown that Daf synthons, readily obtained (although in relatively low yield) by treatment of the delocalized anion of the *N*-benzyl Schiff base of 4,5-diazafluoren-9-one with methyl or benzyl chloroformate, may successfully be subjected to peptide coupling at both the *N*- and the *C*-termini. Because of the high instability of the protected amino acid Boc-Daf-OH, which spontaneously decarboxylated under both basic and neutral conditions and could not be isolated, coupling at the *C*-terminus could only be achieved by the acylazide method, using the hydrazide Boc-Daf-$NHNH_2$. This method was shown to be efficient for acylation of unhindered proteinogenic amino acids such as Ala, but failed for acylation of Aib. On the other hand, both Ala and Aib could be coupled in reasonable yield at the Daf *N*-terminus, the latter through its protected *N*-carboxyanhydride. Synthesis of Daf-rich peptides incorporating Ala, Gly and Aib and conformational analysis of these in solution is currently being investigated.[56] It will be of interest to compare the conformational behaviour of Daf peptides and Afc peptides, which − according to our previous results[39−40] and those of Lombardi et al.[57] − mostly adopt an extended (C_5) conformation with only a modest helical tendency. Possible conformational changes induced by metal complexation of Daf peptides will also be examined.

Experimental Section

General: Melting points were determined by means of a capillary tube immersed in an oil bath (Tottoli apparatus, Büchi) with a final temperature raise of 3 °C/min and are uncorrected. − ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 MHz and 75 MHz, respectively, the solvent ($CDCl_3$ or CD_3OD) being used as internal standard (δ = 7.27 or 3.31 for ^{1}H; δ = 77.00 or 49.00 for ^{13}C). Splitting patterns are abbreviated as follows: (s) singlet, (d) doublet, (t) triplet, (q) quadruplet, (m) multiplet. − The optical rotations were measured with an accuracy of 0.3%, in a 1 dm thermostatted cell. − Analytical TLC and preparative column chromatography were performed on F 254 Kieselgel and on Kieselgel 60 (0.040−0.063 mm) (Merck) respectively, with the following eluent systems: 2.5% MeOH/97.5% CH_2Cl_2 (I); 5% MeOH/95% CH_2Cl_2 (II); 7.5% MeOH/92.5% CH_2Cl_2 (III); 10% MeOH/90% CH_2Cl_2 (IV); 50% EtOAc (ethyl acetate)-50% CH_2Cl_2 (V). UV light (254 nm) was used for all compounds for viewing spots after thin layer chromatography (TLC).

4,5-Diazafluoren-9-one (1): According to the procedure of Henderson et al.,[26] a hot solution of $KMnO_4$ (64.5 g) in water (1 L) was added dropwise over ca. 2 h to a magnetically stirred, boiling solution of phenanthroline monohydrate (25 g) and KOH (13 g) in water (1.3 L). After addition was complete, the solution was refluxed for 1 h, filtered hot through paper and left at room temperature overnight. The yellow crystals were filtered, thoroughly washed with water and air dried. The crystals from five identical experiments were combined (yield 27.20 g) and dissolved in boiling water (2 L). The boiling, clear solution was concentrated to 1.5 L and left at room temperature overnight. The resulting crystals were filtered, washed with water and air dried (yield 24.13 g, 21%). M.p. 215−216 °C (ref.[26] M.p. 212−213 °C; ref.[41] M.p. 215−216 °C). R_f = 0.43 (II). − ^{1}H NMR ($CDCl_3$): δ = 8.79 [dd, J = 5.0 Hz and 1.2 Hz, 2 H, ArH^3H^6], 7.98 [dd, J = 7.5 Hz and 1.2 Hz, 2 H, ArH^1H^8], 7.35 [dd, J = 5.0 Hz and 7.5 Hz, 2 H, ArH^2H^7]. − ^{13}C NMR ($CDCl_3$): δ = 189.5 (C=O), 163.3, 155.1, 131.5, 129.3, 124.7 (C_{Ar}).

***N*-Benzyl-4,5-diazafluorene-9-methyleneamine (2a):** Benzylamine (8.2 mL; 75 mmol) was added to a magnetically stirred solution (under argon) of ketone **1** (3.64 g, 20 mmol) in CH_2Cl_2 (100 mL). The solution was cooled to 0 °C (ice-water bath) and a solution of $TiCl_4$ (1.4 mL; 12.7 mmol) in CH_2Cl_2 (15 mL) was added dropwise over ca. 0.5 h. The resulting pale yellow, milky suspension was stirred under argon at 0 °C for an additional hour, then rapidly filtered over Celite in a Büchner funnel (water aspirator), the precipitate being washed with CH_2Cl_2 (2 portions of 100 mL), then with Et_2O (diethyl ether) (2 portions of 100 mL). The filtrate, which contained a small amount of fluffy precipitate, was concentrated in vacuo at 40 °C to ca. 100 mL, filtered again through cotton wool, then diluted with hexane (50 mL) and concentrated to ca. 60 mL (removing most CH_2Cl_2), resulting in crystallization. The mixture was left in a refrigerator overnight, the crystals were filtered in a Büchner funnel, washed with several portions of hexane/CH_2Cl_2 4:1 (100 mL) and air dried, to give 3.69 g (68%) of pure imine **2a** as white crystals. M.p. 124−128 °C (decomp). R_f = 0.37 (II). − ^{1}H NMR ($CDCl_3$): δ = 8.78 and 8.76 [2 dd (dt-like), $J \approx$ 5.1 Hz and 1.4 Hz, 2 H, ArH^3H^6], 8.26 and 8.20 [2 dd, $J \approx$ 7.5 Hz and 1.2 Hz, 2 H, ArH^1H^8], 7.52 [m, 2 H, ArH (Ph)], 7.42 [m (t-like), 2 H, ArH^2H^7], 7.33 [m, 3 H, ArH (Ph)], 5.42 [s, 2 H, $PhCH_2N$]. − ^{13}C NMR ($CDCl_3$): δ = 161.5 (C=N), 159.1, 158.7, 152.6, 152.2, 139.2, 134.4, 130.0, 128.7, 127.6, 127.2, 126.7, 124.3, 123.5 (C_{Ar}), 57.3 ($PhCH_2N$). − $C_{18}H_{13}N_3$ (271.308): calcd. C 79.68, H 4.83, N 15.49; found C 79.52, H 4.85, N 15.26. More imine **2a** was present in the mother liquor (by TLC), but was very difficult to separate from unidentified side products either by further crystallization or by flash chromatography. Other experiments under identical experimental conditions gave yields of between 50% and 70% after crystallization. In initial experiments, in which the reaction mixture was allowed to warm to ca. 25 °C under argon for 1 h, a strong blue colour sometimes appeared and **2a**, which in this case was present in only a minor proportion relative to the side products, was isolated in much lower yield.

***N*-Benzhydryl-4,5-diazafluorene-9-methyleneamine (2b):** Benzhydrylamine (3.22 mL; 18.7 mmol) was added to a magnetically stirred solution of ketone **1** (0.91 g, 5 mmol) in CH_2Cl_2 (25 mL) under

argon. The solution was cooled to 0 °C (ice-water bath) and a solution of $TiCl_4$ (0.344 mL; 3.13 mmol) in CH_2Cl_2 (5 mL) was added dropwise over 1 h. The resulting pale yellow-green, milky suspension was stirred under argon at 25 °C for 45 min, then rapidly filtered over Celite in a Büchner funnel, the precipitate being washed with CH_2Cl_2 (2 portions of 25 mL) and then Et_2O (50 mL). The clear filtrate was concentrated to ca. 25 mL in vacuo at 40 °C and hexane (5 mL) was added in portions. Concentration in vacuo resulted in a turbid solution with no crystallization. More hexane (20 mL) was added and the resulting white precipitate was filtered out in a Büchner funnel, washed with several portions of hexane/CH_2Cl_2 3:1 (100 mL) and air dried, to give 2.23 g of a white powder. Dissolution of this solid in CH_2Cl_2 (75 mL) resulted in some decomposition, giving a turbid purple solution, which was stirred in the presence of activated charcoal and filtered through paper. The pale yellow solution obtained was concentrated to ca. 5 mL in vacuo at 40 °C, and Et_2O (50 mL) was added in portions. Crystallisation occurred at room temperature within 1 hour and was accelerated by addition of hexane (20 mL). The crystals were filtered, washed with several portions of hexane/Et_2O 1:2 (60 mL) and air dried (yield 0.798 g). More crystals were obtained after concentration of the mother liquor, to give a total of 0.917 g (53%) of pure imine **2b** as white crystals. M.p. 185−190 °C (dec.). R_f = 0.41 (II). − ^{1}H NMR ($CDCl_3$): δ = 8.76 [dd, J = 5.0 Hz and 1.5 Hz, 1 H] and 8.72 [dd, J = 5.0 Hz and 1.3 Hz, 1 H] [ArH^3H^6], 8.31 [dd, J = 7.5 Hz and 1.5 Hz, 1 H] and 8.20 [dd, J = 7.9 Hz and 1.3 Hz, 1 H] [ArH^1H^8], 7.50 [m (d-like), 4 H, ArH (Ph)], 7.36 [m (t-like), 5 H, ArH (Ph) and ArH^2H^7], 7.26 [m (t-like), 3 H, ArH (Ph)], 6.71 [s, 1 H, Ph_2CHN]. − ^{13}C NMR ($CDCl_3$): δ = 161.5 (C=N), 159.0, 157.6, 152.7, 152.1, 143.6, 134.6, 133.8, 130.4, 128.7, 127.3, 126.4, 126.7, 124.2, 123.4 (C_{Ar}), 69.4 (Ph_2CHN). − $C_{24}H_{17}N_3$ (347.400): calcd. C 82.97, H 4.93, N 12.10; found C 82.71, H 4.98, N 11.79.

Acylation of the Anions of the Imines 2a and 2b: A solution of **2a** (2.612 g, 9.64 mmol) in anhydrous THF (175 mL) was magnetically stirred under argon at 0 °C (ice/water bath) and a solution of 1 M NaHMDS in THF (21 mL; 21 mmol) was added by syringe. The resulting dark red-brown solution was stirred at room temperature for 1 h, then cooled to 0 °C. Then, 20 mL (33 mmol) of a solution of ClCOOMe (3 mL) in THF (20 mL), stirred under argon in the presence of a large excess of powdered K_2CO_3 for a few min immediately previously, was added by syringe. The resulting solution, which turned from dark red-brown to dark green within ca. 15 min, was stirred under argon at room temperature overnight, and cooled again to 0 °C. A solution of 1 M HCl (225 mL) was then added. The resulting solution was stirred for no more than 2 h at room temperature and rapidly extracted with CH_2Cl_2 (3 portions of 100 mL) and then with Et_2O (100 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated in vacuo at 40 °C, to give 1.72 g of crude neutral residue which showed two main UV-positive spots on analytical TLC (eluent II), corresponding to ketone **1** (R_f = 0.45) and benzaldehyde (R_f = 0.80) in a ratio of ca. 1:3.5 by ^{1}H NMR (other runs gave ratios **1**:benzaldehyde of ca. 1:2 to 1:3). The aqueous acidic phase was made basic by the addition of a large excess of $NaHCO_3$ (in portions) with magnetic stirring and extracted with CH_2Cl_2 (3 portions of 100 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated in vacuo at 40 °C, to give 1.56 g of crude basic residue which on analytical TLC (eluent II) showed a main UV-positive spot corresponding to the desired 9-amino-4,5-diazafluorene-9-carboxylic acid methyl ester **3a** and several minor spots corresponding to 9-benzoylamino-4,5-diazafluorene **4**, ketone **1**, and other unidentified products. This mixture was chromatographed on a 3 × 65 cm column of silica gel with eluent (II) to give: *(i)* 0.221 g of a dark brown, glassy oil which readily crystallised from acetonitrile/Et_2O, furnishing 0.073 g (2.6%) of pure **4** as white crystals from which an analytical sample (0.054 g) was obtained after a further crystallisation from methanol; *(ii)* 0.707 g (30.4%) of amino ester **3a**, pure by NMR, but contaminated by a dark brown greenish tar. Crystallisation from acetonitrile/Et_2O furnished an analytical sample as pale yellow-brown plates, but with a decreased yield. It was found more convenient to proceed directly to the next step (N^{α}-Boc protection), purify the resulting fully protected compound **7a** by chromatography and deprotect the amino group of **7a** to give back **3a** if necessary (vide infra).

Six duplicate experiments on similar scales (3−13 mmol of **2a**) gave 32%, 24%, 26%, 21%, 29% and 27% yields of **3a** after chromatography, always as a dark-brown glass (NMR pure) that slowly crystallized. Initial experiments on a smaller scale (0.25−0.5 mmol of **2a**) gave lower yields (ca. 7−10%), all of which were similar whether NaH/THF, NaH/DMSO or NaHMDS/DMSO were used as base/solvent systems. In the same manner, treatment of a solution of **2a** (0.271 g, 1 mmol) in THF (20 mL) with a solution of 0.5 M KHMDS in toluene (4.5 mL; 2.25 mmol), followed by addition of 2 mL (3.37 mmol) of a solution of ClCOOMe (0.75 mL)/THF (5 mL)/K_2CO_3, using the same experimental procedure and workup as above, gave 0.067 g (28%) of NMR-pure **3a** after chromatography.

Treatment of a solution of imine **2b** (0.174 g, 0.5 mmol) in THF (10 mL) with a solution of 1 M NaHMDS in THF (1 mL; 1 mmol), followed by addition of 1 mL (1.8 mmol) of a solution of ClCOOMe (0.7 mL)/THF (5 mL)/K_2CO_3, using the same experimental procedure and workup as above, followed by preparative TLC of the crude product, gave 0.036 g (40%) of benzophenone, 0.055 g (60%) of ketone **1** and several minor unidentified side products, but no trace of **3a**. Similar results were obtained with NaHMDS/DMSO as base/solvent system.

Treatment of a solution of imine **2a** (1.084 g, 4 mmol) in THF (75 mL) with a solution of 1 M NaHMDS in THF (10 mL; 10 mmol), followed by addition of 10 mL (14.7 mmol) of a solution of $ClCOOCH_2C_6H_5$ (8 mL; 50% in toluene)/THF (8 mL)/K_2CO_3, using the same experimental procedure and workup as above, gave after column chromatography of the basic extraction product on silica gel with eluent (I): *(i)* 0.479 g (37.6%) of NMR-pure 9-amino-4,5-diazafluorene-9-carboxylic acid benzyl ester **3b** as a brown-orange glass which slowly crystallized; and *(ii)* 0.123 g (11.2%) of NMR-pure 9-amino-9-benzyl-4,5-diazafluorene **5** as a yellow glass which slowly crystallized. Traces of compound **4** together with other unidentified impurities were observed by analytical TLC. As in the case of **3a**, it was found convenient to proceed directly to the N^{α}-Boc protection step of the obtained samples of **3b** and **5** for further chromatographic purification of the resulting compounds **7b** and **6**, respectively, with subsequent deprotection of the amino group to give back **3b** and **5** (vide infra).

N^{α}-Boc Protection: Boc_2O (0.534 g, 2.45 mmol) and CH_3CN (20 mL) were added to a sample of amino ester **3a** (0.266 g, 1.104 mmol), obtained as described above in 32% yield after chromatography, pure by NMR but contaminated with dark-brown tar (vide supra). The mixture was magnetically stirred on a water bath at 60 °C (complete dissolution required ca. 15 min), the reaction progress being monitored by analytical TLC. After 6 h, more Boc_2O (0.256 g, 1.17 mmol) was added and the solution was stirred at 60 °C for 16 h, then for a further 16 h after addition of a third quantity of Boc_2O (0.248 g, 1.02 mmol). The solution was evaporated to dryness in vacuo and the residue purified by chromato-

graphy on a 3 × 60 cm column of silica gel with eluent (I), to give 0.275 g (73%) of pure methyl 9-*tert*-butyloxycarbonylamino-4,5-diazafluorene-9-carboxylate (**7a**) as a white solid (overall yield from **2a**: 23%). Crystallisation of an aliquot from CH_2Cl_2/Et_2O gave an analytical sample as white crystals. Several other duplicate experiments gave similar yields.

In the same manner, the sample of NMR-pure amino ester **3b** (0.479 g, 1.51 mmol) obtained in 37.6% yield from **2a** as described above, was treated with Boc_2O (0.494 g, 2.26 mmol) in CH_3CN (25 mL). The solution was magnetically stirred at 60 °C for 6 h. More Boc_2O (0.494 g, 2.26 mmol) was then added and the solution was stirred at 60 °C for 17 h. More Boc_2O (0.329 g, 1.51 mmol) was once again added, and the solution was stirred at 60 °C for 24 h. The solution was evaporated to dryness in vacuo and the residue was chromatographed on a 2.3 × 52 cm column of silica gel with eluent (I), to give 0.548 g (87%) of pure benzyl 9-*tert*-butyloxycarbonylamino-4,5-diazafluorene-9-carboxylate (**7b**) as a white solid (overall yield from **2a**: 33%). Crystallisation from a highly concentrated CH_2Cl_2 solution with addition of Et_2O/hexane 1:1 gave an analytical sample (white crystals; 0.508 g).

Treatment of the amine **5** (0.143 g, 0.523 mmol) with Boc_2O (0.165 g, 0.75 mmol) in CH_3CN (10 mL) at room temperature for 22 h gave a mixture containing ca. 50% (by TLC) of the starting amine. More Boc_2O (0.165 g, 0.75 mmol) was added and the solution was stirred at 60 °C for 9 h. It was evaporated to dryness in vacuo and the residue was purified by chromatography on a 2.3 × 49 cm column of silica gel with eluent (I), to give 0.156 g (80%) of pure 9-*tert*-butyloxycarbonylamino-9-benzyl-4,5-diazafluorene (**6**) as a colourless glass. Crystallisation from Et_2O/hexane furnished an analytical sample (white crystals; 0.114 g).

N$^{\alpha}$-Boc Deprotection: Acidolysis of the Boc protecting group of compound **6** (0.096 g, 0.26 mmol) was accomplished by treatment with TFA (2 mL)/CH_2Cl_2 (2 mL) at room temperature for 4 h. The solution was evaporated to dryness in vacuo at 25 °C. The residue was dissolved in CH_2Cl_2, the solution was extracted with 5% $NaHCO_3$, dried over $MgSO_4$, filtered and evaporated in vacuo at 40 °C to give 0.061 g (87%) of pure amine **5** as a white solid. Recrystallisation from Et_2O gave an analytical sample.

Treatment of **7a** (0.034 g, 0.1 mmol) as above gave 0.017 g (72%) of pure **3a** as a pale yellow solid. In the same manner, **7b** (0.152 g, 0.36 mmol) gave 0.095 g (82%) of pure **3b** as a pale yellow solid. Recrystallization from Et_2O gave an analytical sample.

Methyl 9-Amino-4,5-diazafluorene-9-carboxylate (3a): Pale yellow crystals (CH_3CN/Et_2O). M.p. 156−160 °C. R_f = 0.18 (II). − ^{1}H NMR ($CDCl_3$): δ = 8.70 [dd, *J* = 4.9 Hz and 1.5 Hz, 2 H, ArH^3H^6], 7.91 [dd, *J* = 7.7 Hz and 1.5 Hz, 2 H, ArH^1H^8], 7.31 [dd, *J* = 4.8 Hz and 7.7 Hz, 2 H, ArH^2H^7], 3.58 [s, 3 H, OCH_3], 2.63 [s (broad), ≈3 H, NH_2]. − ^{13}C NMR ($CDCl_3$): δ = 172.2 (C=O), 157.9, 151.2, 142.0, 131.5, 123.7 (C_{Ar}), 65.6 (C$^{\alpha}$), 53.2 (OCH_3). − $C_{13}H_{11}N_3O_2$ (241.242): calcd. C 64.72, H 4.60, N 17.42; found C 64.49, H 4.59, N 17.46.

Benzyl 9-Amino-4,5-diazafluorene-9-carboxylate (3b): Pale yellow crystals (Et_2O). M.p. 125−127 °C. R_f = 0.24 (II). − ^{1}H NMR ($CDCl_3$): δ = 8.71 [dd, *J* = 4.9 Hz and 1.5 Hz, 2 H, ArH^3H^6], 7.84 [dd, *J* = 7.7 Hz and 1.5 Hz, 2 H, ArH^1H^8], 7.24 [dd, *J* = 4.9 Hz and 7.7 Hz, 2 H, ArH^2H^7], 7.22 [m, 3 H, ArH Bzl], 6.98 [m, 2 H, ArH Bzl], 5.03 [s, 2 H, OCH_2Ph], 2.41 [s (broad), ≈2 H, NH_2]. − ^{13}C NMR ($CDCl_3$): δ = 171.2 (C=O), 157.7, 150.9, 141.7, 134.6, 131.3, 128.2, 128.0, 127.3, 123.4 (C_{Ar}), 67.3 (OCH_2Ph), 65.6 (C$^{\alpha}$). − $C_{19}H_{15}N_3O_2$ (317.334): calcd. C 71.91, H 4.76, N 13.24; found C 71.89, H 4.82, N 13.24.

9-Benzoylamino-4,5-diazafluorene (4): White crystals (MeOH). M.p. 239−242 °C. R_f = 0.24 (II). − ^{1}H NMR ($CDCl_3$): δ = 8.58 [dd, *J* = 4.9 Hz and 1.3 Hz, 2 H, ArH^3H^6], 8.02 [m(d-like), 2 H, ArH Bz], 7.93 [dd, *J* = 7.6 Hz and 1.3 Hz, 2 H, ArH^1H^8], 7.53 [m, 3 H, ArH Bz], 7.21 [dd, *J* = 4.9 Hz and 7.6 Hz, 2 H, ArH^2H^7], 7.11 [d, *J* = 9.0 Hz, 1 H, NH], 6.47 [d, *J* = 8.7 Hz, 1 H, Ar_2CHN]. − ^{13}C NMR ($CDCl_3$): δ = 168.3 (C=O), 157.9, 150.8, 139.2, 133.4, 133.1, 132.1, 128.7, 127.4, 123.4 (C_{Ar}), 50.9 (Ar_2CHN). − ESI$^+$ MS; *m*/*z* (relative intensity): 597 (100) $[2M,Na]^+$; 575 (32) $[2M,H]^+$; 310 (25) $[M,Na]^+$; 288 (83) $[M,H]^+$. − $C_{18}H_{13}N_3O$ (287.308): calcd. C 75.24, H 4.56, N 14.63; found C 74.71, H 4.56, N 14.59.

9-Amino-9-benzyl-4,5-diazafluorene (5): White crystals (Et_2O). M.p. 171−173 °C. R_f = 0.18 (II). − ^{1}H NMR ($CDCl_3$): δ = 8.63 [dd, *J* = 5.0 Hz and 1.3 Hz, 2 H, ArH^3H^6], 7.61 [dd, *J* = 7.7 Hz and 1.5 Hz, 2 H, ArH^1H^8], 7.22 [dd, *J* = 5.0 Hz and 7.7 Hz, 2 H, ArH^2H^7], 7.18 [m, 3 H, ArH Bzl], 6.95 [m, 2 H, ArH Bzl], 3.16 [s, 2 H, CH_2 Bzl], 2.32 [s (broad), ≈2 H, NH_2]. − ^{13}C NMR ($CDCl_3$): δ = 156.8, 150.2, 145.0, 135.7, 131.5, 130.4, 127.8, 126.9, 122.9 (C_{Ar}), 61.9 (Ar_2CHN), 46.1 (CH_2 Bzl). − $C_{18}H_{15}N_3 \cdot 0.2\ H_2O$ (276.927): calcd. C 78.06, H 5.61, N 15.17; found C 78.25, H 5.59, N 15.03.

9-*tert*-Butyloxycarbonylamino-9-benzyl-4,5-diazafluorene (6): White crystals (Et_2O/hexane). M.p. 194−196 °C. R_f = 0.37 (II). − ^{1}H NMR ($CDCl_3$): δ = 8.65 [dd, *J* = 5.0 Hz and 1.5 Hz, 2 H, ArH^3H^6], 7.68 [m (broad), 2 H, ArH^1H^8], 7.22 [dd, *J* = 5.0 Hz and 7.7 Hz, 2 H, ArH^2H^7], 7.12 [m, 3 H, ArH Bzl], 6.80 [m, 2 H, ArH Bzl], ≈ 5.7 [s (very broad), 1 H, NH], 3.33 [s (broad), 2 H, CH_2 Bzl], ≈1.0 [s (very broad), 9 H, Boc CH_3]. − ^{13}C NMR ($CDCl_3$): δ = 157.4 (C_{Ar}), 154.7 (C=O Boc), 150.3, 143.0, 134.3, 131.4, 130.5, 127.9, 127.2, 122.7 (C_{Ar}), 80.2 (O−C Boc), 62.9 [Ar_2C(Bzl)N], 44.5 (CH_2 Bzl), 27.7 (CH_3 Boc). − $C_{23}H_{23}N_3O_2$ (373.438): calcd. C 73.97, H 6.21, N 11.25; found C 73.75, H 6.16, N 11.14.

Methyl 9-*tert*-Butyloxycarbonylamino-4,5-diazafluorene-9-carboxylate (7a): White crystals (CH_2Cl_2/Et_2O). M.p. 192−196 °C. R_f = 0.28 (II). − ^{1}H NMR ($CDCl_3$): δ = 8.76 [dd, *J* = 4.9 Hz and 1.5 Hz, 2 H, ArH^3H^6], 8.2−7.6 [m (very broad), 2 H, ArH^1H^8], 7.32 [dd, *J* = 4.9 Hz and 7.7 Hz, 2 H, ArH^2H^7], 6.25 and 5.76 [s (very broad), 1 H, NH from *anti* and *syn* conformers], 3.61 [s (broad), 3 H, OCH_3], 1.41 and 0.88 [s (very broad), 9 H, Boc CH_3 from *anti* and *syn* conformers]. − ^{13}C NMR ($CDCl_3$): δ = 170.0 (C=O Daf), 158.5 (C_{Ar}), 155.1 (C=O Boc), 151.4, 139.3, 132.4, 130.4, 123.7 (C_{Ar}), 81.0 (O−C Boc), 65.6 (C$^{\alpha}$), 53.6 (OCH_3), 28.0 (CH_3 Boc). − ESI$^+$ MS; *m*/*z* (relative intensity): 364 (97) $[M,Na]^+$; 342 (100) $[M,H]^+$. − $C_{18}H_{19}N_3O_4$ (341.356): calcd. C 63.33, H 5.61, N 12.31; found C 63.17, H 5.52, N 12.59.

Benzyl 9-*tert*-Butyloxycarbonylamino-4,5-diazafluorene-9-carboxylate (7b): White crystals (CH_2Cl_2/Et_2O/hexane). M.p. 159−161 °C. R_f = 0.30 (II). − ^{1}H NMR ($CDCl_3$): δ = 8.73 [d, *J* = 4.9 Hz, 2 H, ArH^3H^6], 8.2−7.6 [m (very broad), 2 H, ArH^1H^8], 7.26 [dd, *J* = 4.9 Hz and 7.7 Hz, 2 H, ArH^2H^7], 7.22 [m broad), 3 H, ArH Bzl], 7.02 [m (very broad), 2 H, ArH Bzl], 6.21 and 5.78 [s (very broad), 1 H, NH from *anti* and *syn* conformers], 5.05 [s (broad), 2 H, OCH_2Ph], 1.39 and 0.87 [s (very broad), 9 H, Boc CH_3 from *anti* and *syn* conformers]. − ^{13}C NMR ($CDCl_3$): δ = 168.9 (C=O Daf), 158.1 (C_{Ar}), 155.0 (C=O Boc), 151.0, 139.0, 134.4, 132.5, 130.3, 128.1, 127.4, 123.3 (C_{Ar}), 80.6 (O−C Boc), 67.8 (OCH_2Ph), 65.4 (C$^{\alpha}$), 27.8 (CH_3 Boc). − $C_{24}H_{23}N_3O_4 \cdot 0.2\ H_2O$ (421.051): calcd. C 68.46, H 5.60, N 9.98; found C 68.09, H 5.46, N 9.78.

Hydrolysis, Hydrogenolysis and Hydrazinolysis of the Ester Functions of (7a) and (7b). − (a) Hydrolysis: A solution of **7a** (0.015 g, 0.04 mmol) in MeOH (2 mL) and 1 N NaOH (0.06 mL) was stirred at room temperature for 20 h, then acidified by addition of an excess of 0.1 M HCl (25 mL) and rapidly extracted with CH_2Cl_2 (two portions of 25 mL). The CH_2Cl_2 solution was washed with water, dried over $MgSO_4$, filtered and evaporated in vacuo at 40 °C. The residue, which showed two spots on analytical TLC, was purified by chromatography on a preparative TLC plate of silica gel with eluent (II), to give 0.0054 g (43%) of pure 9-*tert*-butyloxycarbonylamino-9-methoxy-4,5-diazafluorene (**9**) and 0.0030 g (41%) of ketone **1**. In a duplicate experiment, a solution of **7b** (0.042 g, 0.1 mmol) in MeOH (5 mL) and 1 N NaOH (0.15 mL) was stirred at room temperature for 3 h, then acidified by addition of an excess of 0.5 M HCl (25 mL) and treated as above, to give **9** (0.0040 g, 13%), **1** (0.0038 g, 21%), 9-*tert*-butyloxycarbonylamino-4,5-diazafluorene **10** (0.0041 g, 15%) and another unidentified compound (0.0024 g), as the only products after preparative TLC on silica gel, again with no 9-*tert*-butyloxycarbonylamino-4,5-diazafluorene-9-carboxylic acid being detected. The *N*-Boc-protected amine **10** was found to decompose slowly in $CDCl_3$ solution, resulting in up to ca. 50% conversion into ketone **1** (by TLC and ^1H NMR) after 6 days at room temperature. Such decomposition also occurred when **10** was absorbed on silica gel for several days, although it could be purified on TLC plates of silica gel without significant problems.

(b) Hydrogenolysis: 10% Pd/C (0.050 g) was added to a solution of **7b** (0.090 g, 0.21 mmol) in MeOH (50 mL). The mixture was hydrogenated in a Parr apparatus at room temperature for 4 h, filtered through paper and evaporated in vacuo at 40 °C, to give crude **10** (0.065 g, 100%) as the single reaction product by TLC and NMR. Trituration in Et_2O/hexane gave an analytical sample as a white solid.

(c) Hydrazinolysis: Hydrazine hydrate (2.0 mL; 41.3 mmol) was added to a solution of **7a** (1.238 g, 3.63 mmol) in MeOH (85 mL). The solution was stirred at room temperature for 17 h, then evaporated in vacuo at 30 °C. The residue was repeatedly dissolved in MeOH and the solution evaporated in vacuo at 30 °C, until most of the excess of hydrazine was removed. The residue was then dissolved in eluent (II) (100 mL) and the solution purified by chromatography on a 3 × 40 cm column of silica gel with eluent (II), followed by (IV), to give 0.047 g (4.6%) of decarboxylation product **10** and 1.133 g (91.5%) of pure 9-*tert*-butyloxycarbonylamino-4,5-diazafluorene-9-carboxylic acid hydrazide **11** as a pale yellow solid. Duplicate runs gave isolated products **10**:**11** with similar yields (7%:88% and 2%:89%).

9-*tert*-Butyloxycarbonylamino-9-methoxy-4,5-diazafluorene (9): Pale yellow solid (crude). M.p. 137−141 °C. R_f = 0.25 (II). − ^1H NMR ($CDCl_3$): δ = 8.75 [dd, *J* = 4.9 Hz and 1.5 Hz, 2 H, ArH^3H^6], 8.09 [m (broad), 2 H, ArH^1H^8], 7.32 [dd, *J* = 4.9 Hz and 7.7 Hz, 2 H, ArH^2H^7], 5.56 [s, 1 H, NH], 2.99 [s, 3 H, OCH_3], 1.26 [s, 9 H, Boc CH_3]. − ^{13}C NMR ($CDCl_3$): δ = 158.3 (C_{Ar}), 153.4 (C=O Boc), 152.0, 138.4, 132.2, 123.8 (C_{Ar}), 89.6 (Ar_2CNO), 80.7 (O−C Boc), 51.9 (OCH_3), 28.0 (CH_3 Boc). − ESI^+ MS; *m*/*z* (relative intensity): 336 (100) $[M,Na]^+$; 314 (27) $[M,H]^+$. − $C_{17}H_{19}N_3O_3$ (313.346): calcd. C 65.16, H 6.11; found C 65.06, H 6.89.

9-*tert*-Butyloxycarbonylamino-4,5-diazafluorene (10): White solid (hexane/Et_2O). M.p. 161−166 °C. R_f = 0.22 (II); 0.15 (V). − ^1H NMR ($CDCl_3$): δ = 8.61 [d, *J* = 4.6 Hz, 2 H, ArH^3H^6], 7.89 [d, *J* = 7.6 Hz, 2 H, ArH^1H^8], 7.22 [dd, *J* = 4.8 Hz and 7.6 Hz, 2 H, ArH^2H^7], 5.81 [d, *J* = 9.2 Hz, 1 H, Ar_2CHN], 5.23 [d, *J* = 9.0 Hz, 1 H, NH], 1.49 [s, 9 H, Boc CH_3]. − ^1H NMR (CD_3OD): δ = 8.64 [d, *J* = 4.2 Hz, 2 H, ArH^3H^6], 8.03 [d, *J* = 7.5 Hz, 2 H, ArH^1H^8], 7.43 [dd, *J* = 4.9 Hz and 7.0 Hz, 2 H, ArH^2H^7], 5.79 [s, 1 H, Ar_2CHN], 1.51 [s, 9 H, Boc CH_3]. − ^{13}C NMR ($CDCl_3$): δ = 157.7 (C_{Ar}), 155.0 (C=O Boc), 150.7, 139.3, 132.7, 123.2 (C_{Ar}), 80.4 (O−C Boc), 52.1 (Ar_2CHN), 28.2 (CH_3 Boc). − ESI^+ MS; *m*/*z* (relative intensity): 306 (100) $[M,Na]^+$; 284 (19) $[M,H]^+$. − $C_{16}H_{17}N_3O_2$ (283.320): calcd. C 67.82, H 6.05, N 14.83; found C 67.91, H 6.25, N 14.67.

9-*tert*-Butyloxycarbonylamino-4,5-diazafluorene-9-carboxylic Acid Hydrazide (11): Pale yellow solid (crude). M.p. 228−230 °C. R_f = 0.08 (II). − ^1H NMR ($CDCl_3$): δ = 8.34 [d (broad), 2 H, ArH^3H^6], 8.20 [s (broad), 1 H, CONH hydrazide], 7.92 [d (broad), 2 H, ArH^1H^8], 7.23 [dd, *J* = 4.9 Hz and 7.7 Hz, 2 H, ArH^2H^7], 6.8−6.2 [s (very broad), 1 H, NH Daf], 3.97 [s (broad), 2 H, NH_2 hydrazide], 1.30 and 0.74 [s (very broad), 9 H, Boc CH_3 from *anti* and *syn* conformers]. − ^{13}C NMR ($CDCl_3$): δ = 168.1 (C=O Daf), 158.1 (C_{Ar}), 153.6 (C=O Boc), 151.1, ≈140, 131.5, 123.6 (C_{Ar}), 80.4 (O−C Boc), 65.4 (C^{α}), 27.9 (CH_3 Boc). − ESI^+ MS; *m*/*z* (relative intensity): 364 (100) $[M,Na]^+$; 342 (20) $[M,H]^+$. − $C_{17}H_{19}N_5O_3$ (341.362): calcd. C 59.81, H 5.61, N 20.52; found C 59.76, H 5.61, N 20.54.

Coupling at the *C*-Terminus of Daf: A suspension of Boc-Daf-$NHNH_2$ **11** (0.392 g, 1.15 mmol) in DMF (15 mL) was heated for a few min, until a clear solution was obtained. The solution was magnetically stirred under argon and cooled to ca. −50 ° C, the flask being sealed with a rubber septum. A solution of 3.8 M HCl in EtOAc (2.5 mL; 9.5 mmol) was then slowly added by syringe. The resulting clear pale yellow solution was stirred at −45 °C for 10 min and isoamyl nitrite (0.230 mL; 1.71 mmol) was added by syringe. The resulting solution was stirred at ca. −40 °C for 0.5 h, and a cold solution of HCl·H-Ala-OMe (1.606 g, 11.5 mmol) and diisopropylethylamine (DIEA) (4.2 mL; 24 mmol) in DMF (10 mL) was rapidly added by syringe. The resulting solution was stirred under argon, warming from −40 °C to room temperature overnight, and evaporated in vacuo at 40 °C. The pale yellow glassy residue was dissolved in EtOAc (100 mL) and H_2O (100 mL). The separated aqueous phase was extracted with EtOAc (100 mL). The organic phase was washed with H_2O (3 portions of 50 mL), dried over $MgSO_4$, filtered and evaporated in vacuo. The residue was dissolved in EtOAc. The solution was then concentrated in vacuo to a very small volume (ca. 1 mL), and Et_2O (ca. 15 mL) was added. Crystallisation occurred at room temperature. The white crystals of pure Boc-Daf-Ala-OMe **12** were filtered, thoroughly washed with Et_2O and air dried (yield: 0.168 g). More crystals were obtained from the filtrate after evaporation in vacuo, dissolving the residue in EtOAc, concentration of the solution to ca. 1 mL, dilution with Et_2O (ca. 15 mL) and standing at room temperature for 2 h and then in a refrigerator overnight. The clear supernatant solution was collected by pipette and the remaining crystals were triturated in 10 mL of hexane/Et_2O 4:1, filtered, washed with 50 mL of hexane/Et_2O 4:1, and air dried (yield: 0.096 g). The combined filtrates were evaporated to dryness in vacuo and the residue was purified by chromatography on a preparative TLC plate of silica gel with eluent (V) to give more **12** (0.040 g, total amount: 0.304 g, 64.1%) as well as compounds **10** (0.018 g, 5.5%) and **1** (0.028 g, 13.5%). A duplicate run gave slightly different yields of **12** (54.6%), **10** (29.5%) and **1** (3%).

The hydrazide **11** (0.085 g, 0.25 mmol) was also treated under the same experimental conditions and workup as above, except that HCl·H-Aib-OMe (0.154 g, 1 mmol) was used instead of HCl·H-Ala-OMe. Preparative TLC of the crude reaction product showed the presence of **1**, **10** and other unidentified compounds, but the

desired Boc-Daf-Aib-OMe could not be isolated from any of the chromatographic fractions.

Coupling at the *N*-Terminus of Daf: *N*-Methylmorpholine (NMM) (0.024 mL; 0.22 mmol) was added to a solution of Boc-Ala-OH (0.041 g, 0.21 mmol) in THF (1 mL), cooled to −10 °C (ice/salt bath). EtOCOCl (0.019 mL; 0.20 mmol) was then added. The reaction mixture was stirred under argon at −10 °C for 5 minutes and a solution of H-Daf-OMe **3a** (0.017 g, 0.071 mmol) in CH_2Cl_2 (1 mL) was added. The reaction mixture was stirred while warming from −10 °C to 0 °C over 1 h, then at room temperature for 5 h. EtOAc (100 mL) was added and the organic solution was successively washed with 0.5 M HCl (2 portions of 50 mL), H_2O (50 mL), 5% $NaHCO_3$ (2 portions of 50 mL) and H_2O (2 portions of 50 mL), dried over $MgSO_4$, filtered and evaporated in vacuo at 40 °C. The residue was purified on a preparative TLC plate of silica gel with eluent (IV), to give 0.015 g (52%) of pure Boc-Ala-Daf-OMe **13** as a pale yellow solid.

A solution of Boc-Aib-OH (0.0183 g, 0.09 mmol) and EDC (0.0086 g, 0.045 mmol) in CH_3CN (0.5 mL) was stirred at room temperature for 1 h and H-Daf-OBzl **3b** (0.0095 g, 0.03 mmol) was added. The mixture was heated at 60 °C for 18 h, then evaporated in vacuo. Analytical TLC of the crude reaction product showed only one UV-positive spot, corresponding to the starting amino ester **3b**, with no trace of the desired coupled product **14**.

Boc-Aib-NCA (0.897 g, 3.92 mmol) was added to a solution of H-Daf-OBzl **3b** (0.207 g, 0.65 mmol) and DIEA (0.115 mL; 0.65 mmol) in THF (3.7 mL). The solution was heated at 60 °C for 24 h, then concentrated in vacuo. The residue was dissolved in CH_2Cl_2, washed successively with 0.5 M HCl, H_2O, 5% $NaHCO_3$ and H_2O, dried over $MgSO_4$, filtered and evaporated in vacuo at 40 °C. The residue was purified by chromatography on a 1 × 35 cm column of silica gel with eluent (III), to give 0.281 g (86%) of Boc-Aib-Daf-OBzl **14** as a white solid, which was recrystallised from CH_2Cl_2/Et_2O to give colourless crystals.

Boc-Daf-Ala-OMe (12): White crystals ($EtOAc/Et_2O$). M.p. 197−200 °C. R_f = 0.22 (II); 0.08 (V). $[\alpha]_{589}^{25} = -43$, $[\alpha]_{578}^{25} = -47$, $[\alpha]_{546}^{25} = -58$, $[\alpha]_{436}^{25} = -119$, $[\alpha]_{365}^{25} = -277$ (c = 0.1; MeOH). − ^{1}H NMR ($CDCl_3$): δ = 8.38 [m (broad), 2 H, ArH^3H^6], 7.87 [m (broad), 2 H, ArH^1H^8], 7.27 and 7.25 [dd, J = 4.9 Hz and 7.7 Hz, 2 H, ArH^2H^7], 6.80 [m (very broad), 1 H, NH Ala], 6.72 [m (very broad), 1 H, NH Daf], 4.48 [dq, $J \approx$ 7.1 Hz and ≈ 7.1 Hz, 1 H, H^α Ala], 3.67 [s, 3 H, OCH_3], ≈ 1.4 and ≈ 0.70 [s (very broad), 9 H, Boc CH_3 from *anti* and *syn* conformers], 1.24 [d, J = 7.2 Hz, 3 H, CH_3 Ala]. − ^{13}C NMR ($CDCl_3$): δ = 172.6 and 167.8 (C=O Ala and Daf), 158.5, 158.3 (C_{Ar}), 153.3 (C=O Boc), 150.9, 141.9, 131.6, 130.9, 123.7, 123.6 (C_{Ar}), 80.1 (O−C Boc), 65.8 (C^α Daf), 52.4 (OCH_3), 49.2 (C^α Ala), 27.6 (broad; CH_3 Boc), 16.8 (CH_3 Ala). − ESI$^+$ MS; *m/z* (relative intensity): 435 (100) $[M,Na]^+$; 413 (27) $[M,H]^+$. − $C_{21}H_{24}N_4O_5$ (412.434): calcd. C 61.15, H 5.87, N 13.58; found C 60.76, H 5.79, N 13.55.

Boc-Ala-Daf-OMe (13): Pale yellow solid (crude). M.p. 187−190 °C. R_f = 0.23 (II); 0.43 (IV). − ^{1}H NMR ($CDCl_3$): δ = 8.76 [d, J = 4.4 Hz, 2 H, ArH^3H^6], 8.06 and 8.00 [d, J = 7.4 Hz, 2 H, ArH^1H^8], 7.77 [s (broad), 1 H, NH Daf], 7.27 [dd, J = 4.9 Hz and 7.5 Hz, 2 H, ArH^2H^7], 5.05 [d (broad), $J \approx$ 7.3 Hz, 1 H, NH Ala], 4.22 [dq, $J \approx$ 7.1 Hz and ≈ 7.1 Hz, 1 H, H^α Ala], 3.64 [s, 3 H, OCH_3], 1.41 [s, 9 H, Boc CH_3], 1.38 [d, J = 7.1 Hz, 3 H, CH_3 Ala]. − ^{13}C NMR ($CDCl_3$): δ = 173.1 and 169.4 (C=O Daf and Ala), 158.3, 158.2 (C_{Ar}), 155.8 (C=O Boc), 151.6, 151.5, 138.9, 138.7, 132.9, 132.5, 129.7, 123.8, 123.7 (C_{Ar}), 80.5 (O−C Boc), 65.2 (C^α Daf), 53.5 (OCH_3), 49.7 (C^α Ala), 28.2 (CH_3 Boc), 17.0 (CH_3 Ala). − ESI$^+$ MS; *m/z* (relative intensity): 435 (100) $[M,Na]^+$; 413 (96) $[M,H]^+$. − $C_{21}H_{24}N_4O_5$ (412.434): calcd. C 61.15, H 5.87; found C 61.31, H 6.11.

Boc-Aib-Daf-OBzl (14): White crystals (CH_2Cl_2/Et_2O). M.p. 189−190 °C. R_f = 0.33 (III). − ^{1}H NMR ($CDCl_3$): δ = 8.71 [d, J = 4.8 Hz, 2 H, ArH^3H^6], 8.11 [s (broad), 1 H, NH Daf], 7.97 [d, J = 7.4 Hz, 2 H, ArH^1H^8], 7.24 [m, 5 H, ArH^2H^7 and 3 ArH Bzl], 7.02 [m, 2 H, ArH Bzl], 5.06 [s, 2 H, OCH_2Ph], 4.94 (s, 1 H, NH Aib), 1.44 [s, 9 H, Boc CH_3], 1.34 [s, 6 H, Aib CH_3]. − ^{13}C NMR ($CDCl_3$): δ = 174.9 and 168.9 (C=O Aib and Daf), 158.3 (C_{Ar}), 154.9 (C=O Boc), 151.4, 138.9, 134.7, 132.5, 128.3, 128.5, 127.7, 123.6 (C_{Ar}), 80.5 (O−C Boc), 68.0 (OCH_2Ph), 65.4 (C^α Daf), 56.7 (C^α Aib), 28.3 (CH_3 Boc), 25.3 (CH_3 Aib). − $C_{28}H_{30}N_4O_5$ (502.552): calcd. C 66.91, H 6.01, N 11.15; found C 66.88, H 5.85, N 11.17.

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