Mio- and Dio-Fmoc – Two Modified Fmoc Protecting Groups Promoting Solubility

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Dedicated to Professor Louis A. Carpino

Abstract: Two novel Fmoc-derived protecting groups, Mio-Fmoc and Dio-Fmoc, were developed, which cause dramatically enhanced solubility of the corresponding derivatives in most organic solvents. Furthermore, the suitability of these groups in solid-phase peptide synthesis was highlighted.

Key words: protecting groups, solid-phase synthesis, solubility, peptides

Protecting groups are of fundamental importance in modern synthetic organic chemistry. The successful total synthesis of a huge number of natural products is mainly based on proper employment of these groups. Undoubtedly one of the most frequently used protecting groups for amines is the Fmoc group, developed by Carpino more than 30 years ago.[1] The utilization of the Fmoc group instead of the originally used Boc group[2] has revolutionized solid-phase peptide synthesis (SPPS) and to this day most SPPS rely on the Fmoc strategy.[3] In contrast to the vast dissemination in SPPS, the Fmoc group has only found a limited use in solution synthesis, principally for two reasons: Fmoc derivatives are often poorly soluble in many organic solvents and the cleavage of the Fmoc group generates non-volatile byproducts (dibenzofulvene and its adducts with secondary amines). The solubility of Fmoc-protected proteinogenic amino acids is mostly satisfactory but appropriate derivatives of larger molecules (e.g. peptidomimetics) are often scarcely soluble.

It seems reasonable to remedy the solubility problem by the introduction of bulky groups onto the fluorene skeleton. In 2000 Stigers et al. reported the development of the 2,7-di-*tert*-butyl-Fmoc group (Dtb-Fmoc) and its properties.[4,5] The solubility of Dtb-Fmoc derivatives is considerably enhanced in many solvents compared with the appropriate Fmoc compounds. Unfortunately, the cleavage rate of Dtb-Fmoc groups is dramatically diminished by the *tert*-butyl groups. In the same year Carpino reported the 2,7-bis(trimethylsilyl)-Fmoc (Bts-Fmoc) group which solved this problem.[6] The cleavage times of Bts-Fmoc derivatives differ only marginally from those bearing the Fmoc group. The disadvantage of the Bts-Fmoc group is the relatively extensive preparation of the reagent (6 steps from fluorene to Bts-Fmoc chloride) and that it is not commercially available so far.

In connection with one of our current research projects, the development of molecular rods with an oligospiroketal architecture, such as **1** (Figure 1), we required a protecting group for the terminal piperidine moieties, which is compatible with a Fmoc-based SPPS strategy. Not surprisingly, the use of the parent Fmoc group produced extremely poorly soluble solids preventing any further usability. The problem could not be circumvented with the Dtb-Fmoc group, which provided only moderate enhancement of the solubility. This situation led us to develop two new Fmoc-derived protecting groups with substantially higher solubility and straightforward synthetic accessibility. Herein, we report the synthesis of the 2-(2-ethylhexyl)-fluorene-9-methoxycarbonyl ('monoisooctyl-Fmoc', Mio-Fmoc) group, the 2,7-bis(2-ethylhexyl)-fluorene-9-methoxycarbonyl ('diisooctyl-Fmoc', Dio-Fmoc) group, the solubility of various derivatives, as well as the successful employment in SPPS.

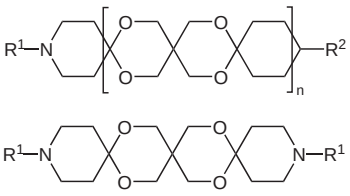

Figure 1 Oligospiroketals **1** and trispiranes **2**

It is well known that the introduction of a 2-ethylhexyl group ('isooctyl' group) often causes dramatically enhanced solubility of the appropriate derivatives.[7] Therefore, we decided to use this residue for our new Fmoc groups.

Our synthesis started with the Friedel–Crafts acylation of fluorene **3** with 2-ethylhexanoyl chloride. Primarily we planned to introduce two acyl groups into the 2- and 7-position of the fluorene skeleton simultaneously. In the literature several examples of two-fold acylation of fluorene have been reported.[8] Surprisingly, we found that under typical previously described conditions [RCOCl (2 equiv), $AlCl_3$ (2 equiv), CS_2, reflux] only one acyl group is installed giving 2-(2-ethylhexanoyl)fluorene **4a** in quantitative yield. The same product could also be obtained with one equivalent of the acid chloride and cata-

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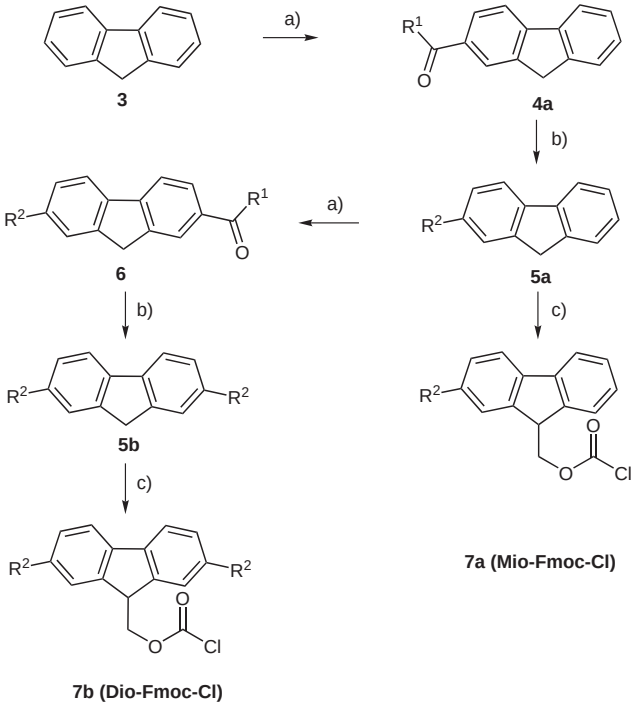
lyst using dichloromethane as solvent in 86% yield. The reduction of the keto group with $LiAlH_4/AlCl_3$ afforded 2-(2-ethylhexyl)fluorene (2-isooctyl-fluorene) **5a**. In contrast to **4a** the fluorene skeleton of **5a** is donor-substituted allowing a second acylation to give the ketone **6**, which was reduced with $LiAlH_4/AlCl_3$ as well.

Finally, the chlorocarbonyloxymethyl moiety was introduced in both **5a** and **5b** in three steps, consisting of carboxylation, reduction of the carboxylic acid with $BH_3{\cdot}Me_2S$, and treatment with an excess of phosgene (Scheme 1).[9]

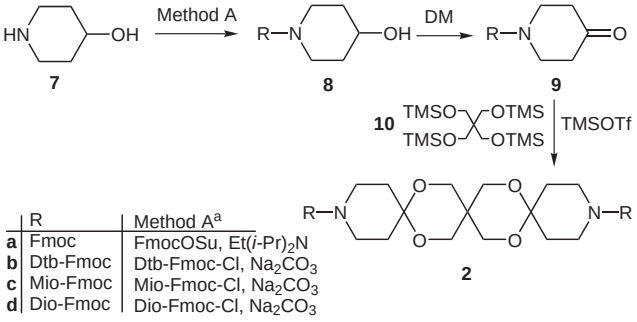
To prove the suitability of the new protecting groups, we prepared four trispiranes **2a**–**d** bearing Mio-Fmoc and Dio-Fmoc groups as well as, for comparison, Dtb-Fmoc, and the unsubstituted Fmoc groups.



Scheme 1 Synthesis of Mio-Fmoc-Cl **7a** and Dio-Fmoc-Cl **7b**. *Reagents and conditions*: a) R^1COCl (1 equiv), $AlCl_3$, CH_2Cl_2; b) $LiAlH_4$, $AlCl_3$, Et_2O; c) i) BuLi, THF, ii) CO_2, iii) $BH_3{\cdot}Me_2S$, iv) $COCl_2$. R^1 = hept-3-yl, R^2 = 2-ethylhexyl ('isooctyl').

The synthesis of the trispiranes is outlined in Scheme 2. 4-Hydroxypiperidine was acylated with Fmoc-OSu or with the chloroformates (Dtb-Fmoc-Cl, **7a**, **7b**) giving the urethanes **8** in very good yields. After oxidation of the hydroxy group with Dess–Martin periodinane[10] the resulting ketones **9** were treated with the tetrakis(trimethylsilyl)ether of pentaerythritol **10**[11] in the presence of catalytic amounts of TMSOTf according to the procedure described by Noyori[12] to afford the trispiranes **2** (Scheme 2).

The solubility of compounds **2** in MeOH and Et_2O is summarized in Table 1. To our delight, **2c** and **2d**, bearing the new Fmoc groups, show dramatically improved solubility, whereas the Dtb-Fmoc (**2b**) group showed only a marginal improvement. An exact value for the solubility of **2c** and **2d** in Et_2O could not be determined because with decreasing amounts of solvent these compounds form highly viscous solutions containing more than 100 gL^{-1} of the solute.



	R	Method A[a]
a	Fmoc	FmocOSu, Et(*i*-Pr)$_2$N
b	Dtb-Fmoc	Dtb-Fmoc-Cl, Na_2CO_3
c	Mio-Fmoc	Mio-Fmoc-Cl, Na_2CO_3
d	Dio-Fmoc	Dio-Fmoc-Cl, Na_2CO_3

Scheme 2 Synthesis of trispiranes **2**. (DM = Dess–Martin periodinane).

Table 1 Solubility and Polarity of Compounds **2**

Compound	R	MeOH[a]	Et_2O[a]	R_f[b]
2a	Fmoc	<0.1	0.53	0.32
2b	Dtb-Fmoc	0.36	2.26	0.41
2c	Mio-Fmoc	13.53	> 100	0.37
2d	Dio-Fmoc	3.23	> 100	0.78

[a] Solubility in gL^{-1}.
[b] CH_2Cl_2–MeOH (100:4).

To assess the usability of Mio-Fmoc and Dio-Fmoc groups in SPPS we protected the dipeptide H-GlyGly-OH with these groups and coupled the products with Wang resin pre-loaded with glycine. For comparison we used also the commercially available Fmoc-GlyGly-OH as well as Dtb-Fmoc-GlyGly-OH. The coupling yields and the average times for cleavage of the protecting groups are collected in Table 2. We found that coupling yields of dipeptides with solubility-enhanced Fmoc groups are comparable with those of Fmoc-GlyGly-OH. The Dio-Fmoc group has cleavage times similar to the Dtb-Fmoc group, but the Mio-Fmoc group is cleaved at twice the speed of the Dtb-Fmoc group.

Finally, we investigated the influence of Fmoc substituents on the polarity of the corresponding compounds on the basis of R_f values. Whereas the Mio-Fmoc derivatives have polarities similar to Dtb-Fmoc derivatives, the Dio-Fmoc group decreases the polarity remarkably (Tables 1 and 2). This should be particularly useful for the purification of Dio-Fmoc bearing products and for the separation of desired products from Dio-Fmoc bearing by-products (or corresponding conversion products), for example, by flash column chromatography.

Table 2 Coupling, Cleavage, and Polarity of R-GlyGly-OH[13]

R	Coupling yield (%)[a]	Cleavage (min)[b]	R_f[c]	R_f[d]
Fmoc	65	10	0.36	0.48
Dtb-Fmoc	78	40	0.60	0.66
Mio-Fmoc	61	20	0.50	0.51
Dio-Fmoc	62	50	0.70	0.80

[a] TBTU/HOBt, H-Gly-Wang resin.
[b] 20% piperidine in DMF, average time until complete cleavage in min.
[c] R-GlyGly-OH, EtOAc–PE–HCOOH (30:10:1).
[d] Piperidino-dibenzo-fulvene adduct, CH_2Cl_2–MeOH (10:1).

In summary, we reported the syntheses and properties of two novel protecting groups, Mio-Fmoc and Dio-Fmoc, which cause dramatically enhanced solubility of appropriate derivatives in most organic solvents. Furthermore, we have shown that the structural modifications of the Fmoc group only marginally influence their suitability in solid-phase peptide synthesis.

Acknowledgment

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(13) **Friedel–Crafts Acylation of Fluorenes; General Procedure A**
To a stirred mixture of fluorene (**3** or **5a**, 60.2 mmol) and $AlCl_3$ (8.8 g, 66.0 mmol, 1.1 equiv) in anhyd CH_2Cl_2 (200 mL) 2-ethylhexanoyl chloride (11 mL) was added at 0 °C. After stirring overnight, ice and concd HCl were poured into the mixture until all the solids had been dissolved. The phases were separated, the aqueous phase was extracted twice with dichloromethane, the combined organic phases were dried, and concentrated. The residue was purified by flash chromatography (silica).
2-(2-Ethylhexanoyl)fluorene (4a)
Flash chromatography (PE–CH_2Cl_2, 1:1) gave 15.1 g of **4a** (51.7 mmol, 86%) as a yellow solid; R_f 0.5 (PE–CH_2Cl_2, 1:1). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.81–0.93 (m, 6 H), 1.19–1.35 (m, 4 H), 1.46–1.64 (m, 2 H), 1.73–1.88 (m, 2 H), 3.36–3.45 (m, 1 H), 3.92 (s, 2 H), 7.25 (dd, ^3J = 6.8 Hz, ^4J = 1.0 Hz, 1 H), 7.36 (dt, ^3J = 7.4 Hz, ^4J = 1.5 Hz, 2 H), 7.80 (d, ^3J = 7.6 Hz, 2 H), 7.99 (dd, ^3J = 8.1 Hz, ^4J = 1.6 Hz, 1 H), 8.13 (d, ^4J = 0.8 Hz, 1 H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 12.0, 13.9, 22.9, 25.6, 29.8, 31.9, 36.9, 47.7, 119.7, 125.2, 127.0, 127.5, 127.9, 136.3, 140.5, 143.3, 144.5, 146.1, 204.5.
2-(2-Ethylhexanoyl)-7-(2-ethylhexyl)fluorene (6)
Flash chromatography (PE–EtOAc, 10:1) gave 6.81 g of **6** (16.83 mmol, 95%) as a yellow oil; R_f 0.5 (PE–EtOAc, 20:1). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.77–0.89 (m, 12 H), 1.17–1.31 (m, 12 H), 1.39–1.60 (m, 3 H), 1.68–1.83 (m, 2 H), 2.49–2.61 (m, 2 H), 3.31–3.40 (m, 1 H), 3.86 (s, 2 H), 7.13 (dd, ^3J = 7.9 Hz, ^4J = 1.2 Hz, 1 H), 7.30 (s, 1 H), 7.67 (d, ^3J = 7.8 Hz, 1 H), 7.93 (dd, ^3J = 8.1 Hz, ^4J = 1.5 Hz, 1 H), 8.07 (s, 1 H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 10.8, 12.0, 13.9, 14.1, 22.9, 23.0, 25.4, 25.6, 28.8, 29.8, 31.9, 32.3, 36.8, 40.4, 41.3, 47.6, 119.3, 120.4, 124.8, 126.0, 127.5, 128.1, 135.9, 138.1, 142.3, 143.3, 144.6, 146.4, 204.5.
Reduction of 2-Ethylhexanoyl Groups; General Procedure B
$AlCl_3$ (12 g, 90.0 mmol, 1.7 equiv) was placed in a 500-mL round-bottom flask and anhyd Et_2O (300 mL) was added cautiously dropwise with external cooling with an ice bath and stirring. A solution of **4a** (51.7 mmol) or **6** in anhyd Et_2O (100 mL) was added dropwise. After stirring for 30 min $LiAlH_4$ (3.42 g, 90.1 mmol, 1.7 equiv) was added portionwise. The mixture was refluxed for 90 min and then allowed to cool to r.t. The excess of $LiAlH_4$ was destroyed with EtOAc and then dil. HCl was added until gas evolution ceased. The phases were separated, the organic phase was dried with $MgSO_4$, and evaporated. The residue was purified by flash chromatography (silica).
2-(2-Ethylhexyl)fluorene (5a)
Flash chromatography (PE–CH_2Cl_2, 100:3) gave 8.49 g of **5a** (30.5 mmol, 59%) as a yellow solid; R_f 0.4 (PE–EtOAc, 100:3). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.99–1.09 (m, 6 H), 1.36–1.51 (m, 8 H), 1.69–1.80 (m, 1 H), 2.68–2.80 (m, 2 H), 3.98 (s, 2 H), 7.29 (d, ^3J = 7.8 Hz, 1 H), 7.39 (dt, ^3J = 7.4 Hz, ^4J = 1.2 Hz, 1 H), 7.45 (s, 1 H), 7.48 (t, ^3J = 6.9 Hz, 1 H), 7.64 (d, ^3J = 7.4 Hz, 1 H), 7.81 (d, ^3J = 7.8 Hz, 1 H), 7.87 (d, ^3J = 7.4 Hz, 1 H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 10.8, 14.2, 23.1, 25.4, 28.9, 32.4, 36.8, 40.3, 41.3, 119.4, 119.5, 124.9, 125.8, 126.2, 126.6, 127.8, 139.2, 140.7, 141.8, 143.1, 143.3.
2,7-Bis(2-ethylhexyl)fluorene (5b)
Flash chromatography (PE) gave 4.47 g of **5b** (11.44 mmol, 68%) as a colorless oil; R_f 0.6 (PE). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.85–0.93 (m, 12 H), 1.29–1.38 (m, 16 H), 1.62–1.64 (m, 2 H), 2.55–2.67 (m, 4 H), 3.85 (s, 2 H), 7.15 (d, ^3J = 7.7 Hz, 2 H), 7.32 (s, 2 H), 7.65 (d, ^3J = 7.7 Hz, 2 H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 10.8, 14.2, 23.1, 25.4, 28.9, 32.3, 36.7, 40.3, 41.4, 119.0, 125.8, 127.7, 139.4, 140.1, 143.2.

Fluorene-9-carboxylic Acids; General Procedure C1

Fluorene **5a** or **5b** (19.4 mmol) was dissolved in anhyd THF (30 mL) and cooled to –78 °C. *n*-BuLi (1.6 M, hexane; 15 mL, 24 mmol, 1.2 equiv) was added dropwise and then the solution was stirred for 1 h. A stream of carbon dioxide, obtained by evaporation of dry ice was passed through a drying jar, into the solution for 30 min, while warming to r.t. The mixture was acidified with dil. HCl to pH 1 and extracted with Et_2O. The solvent was evaporated and the residue was purified by flash chromatography.

Fluorene-9-methanols; General Procedure C2

Substituted fluorene-9-carboxylic acid (17.24 mmol) was dissolved in anhyd THF (100 mL) and then $BH_3{\cdot}Me_2S$ (3.5 mL, 30.9 mmol, 2.1 equiv) was added dropwise at 0 °C. After warming to r.t. the solution was stirred for 2 h; crushed ice and an aq solution of tartaric acid were added cautiously until gas evolution ceased. The mixture was extracted several times with Et_2O, the organic phases were washed twice with a sat. aq solution of $NaHCO_3$, dried, and evaporated. The residue was purified by flash chromatography giving the fluorene-9-methanols.

Fluorene-9-methyl Chloroformates; General Procedure C3

Fluorene-9-methanol (4.18 mmol) was stirred with a solution of phosgene (10% in toluene, 7.79 mmol, 1.9 equiv) in a sealed flask overnight. The solvent was removed in vacuo and the residue was purified by flash chromatography.

2-(2-Ethylhexyl)fluorene-9-methyl Chloroformate (Mio-Fmoc-Cl) (7a)

Instead of flash chromatography the residue of C1 was purified by dissolution in an aq solution of K_2CO_3 and extracted several times with Et_2O. After acidification with dil. HCl to pH 1 the product was extracted with CH_2Cl_2. The solution was dried and evaporated giving the pure fluorene-9-carboxylic acid. Yields: C1, 90%; C2, 55%; C3, 70%; R_f 0.6 (PE–EtOAc, 100:2). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.85–0.89 (m, 6 H), 1.20–1.34 (m, 8 H), 1.56–1.62 (m, 1 H), 2.52–2.64 (m, 2 H), 4.23 (t, ^3J = 7.6 Hz, 1 H), 4.44–4.57 (m, 2 H), 7.18 (d, ^3J = 7.8 Hz, 1 H), 7.27 (dt, ^3J = 7.4 Hz, ^4J = 1.0 Hz, 1 H), 7.33 (s, 1 H), 7.37 (t, ^3J = 7.4 Hz, 1 H), 7.53 (d, ^3J = 7.5 Hz, 1 H), 7.63 (d, ^3J = 7.8 Hz, 1 H), 7.70 (d, ^3J = 7.5 Hz, 1 H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 10.8, 14.1, 23.0, 25.4, 28.8, 32.3, 40.3, 41.3, 46.0, 73.6, 119.8, 119.9, 125.0, 125.8, 126.8, 128.1, 129.2, 138.8, 141.4, 141.5, 142.4, 150.7.

2,7-Bis(2-ethylhexyl)fluorene-9-methyl Chloroformate (Dio-Fmoc-Cl) (7b)

Yields: C1, 89%; C2, 60%; C3, 97%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.85–0.90 (m, 12 H), 1.26–1.35 (m, 16 H), 1.54–1.62 (m, 2 H), 2.53–2.66 (m, 4 H), 4.23 (t, ^3J = 7.6 Hz, 1 H), 4.52 (d, ^3J = 7.7 Hz, 2 H), 7.18 (d, ^3J = 7.8 Hz, 2 H), 7.33 (s, 2 H), 7.61 (d, ^3J = 7.8 Hz, 2 H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 10.8, 14.1, 23.0, 25.4, 28.8, 32.3, 40.3, 41.3, 45.9, 73.8, 119.5, 125.8, 129.2, 139.0, 141.0, 142.4, 150.7.

Solid-Phase Peptide Synthesis

The assembly of the peptides was performed in a mechanical shaker with a fritted glass reactor. Wang resin[16] (1 mmol/g, 200–400 mesh) is used as the solid support which is preloaded with Fmoc-Gly-OH.[17] The preloaded resins (50 mg, loading 0.89 mmol/g) were swollen in DMF (0.5 mL) for 5 min, and than the excess solvent was removed by filtration. The Fmoc protecting group was removed by treating twice with 20% piperidine in DMF (5 min and 15 min) and then the resin was washed 5 times with DMF. The coupling step was performed by the Fmoc strategy using TBTU/HOBt[18] as activation reagents. In a typical experiment, each Fmoc-dipeptide (Table 2) was introduced by a common coupling (60 min) using a two-fold excess of the dipeptide, activation of reagents, and a three-fold excess of DIPEA in DMF (0.5 mL). After washing five times with DMF and twice with CH_2Cl_2 the resin was dried in high vacuum. The substitution levels of the loaded resins were determined spectrophotometrically by Fmoc cleavage.[3]

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