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A novel, base-labile fluorous amine protecting group: synthesis and use as a tag in the purification of synthetic peptides

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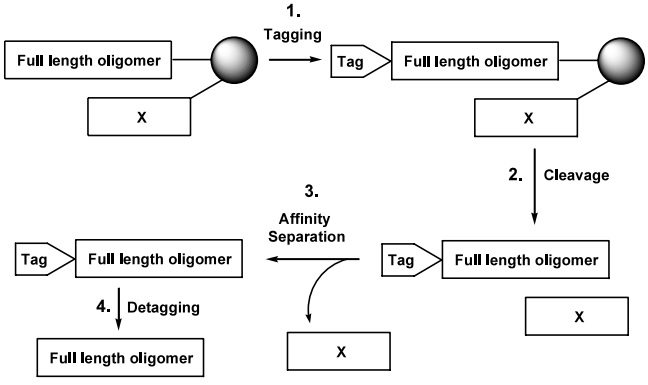
We dedicate this paper to Professor G. I. Tesser on the occasion of his 75th birthday.

Abstract—A new, base-labile fluorous tag based on the Msc amine protecting group was synthesized. Its use in the purification of synthetic peptides by fluorous HPLC or fluorous SPE was demonstrated.

The use of fluorous techniques[1] for the separation of reaction mixtures has found wide attraction in synthetic organic disciplines in recent years.[2] An important development comprises the so-called 'light-fluorous' strategy, developed by Curran and co-workers,[1a,3] in which compounds differing in fluorine content are separated by chromatography using a fluorous stationary phase. For this purpose, an array of fluorinated handles have become available in recent years, including fluorous versions of the Z,[4] Boc,[5] *t*-Bu,[6] Bn,[7] THP,[8] acyl-based,[9] silyl-based,[10] and alkoxyethylether[11] protecting groups.

The application of fluorous handles promises to be a valuable asset in stepwise solid-phase synthesis procedures. Target molecules, the purification of which from final product mixtures is hampered by the occurrence of closely eluting impurities, can be effectively isolated with the aid of fluorous-based separation. Two different approaches can be distinguished, that is, attachment of an appropriate fluorous protecting group to either final products (tagging, see Scheme 1) or to intermediate unreacted species (capping). The success of fluorous capping in solid-phase chemistry was demonstrated by Seeberger et al.,[10b,c] who were able to separate the desired products from the fluorous impurities after the solid-phase assembly of oligosaccharides. We have recently disclosed the first example of the use of a fluorous tag, being a fluorinated derivative of the Z-protecting group (FZ-tag), in the purification of oligopeptides obtained through solid-phase peptide synthesis (SPPS).[4a] However, although the acid-lability of the FZ-tag could be tuned by the introduction of additional substituents, the application of the FZ-tag in Fmoc-based SPPS proved to be restricted. It requires the use of amino acids with highly acid-labile protected



Scheme 1. Schematic representation of tagging strategy. On-resin tagging of the desired product (1) yields a mixture after cleavage (2) from which the desired product can be selectively obtained through affinity separation (3). X = capped incomplete sequences.

Keywords: fluorous; Msc protecting group; synthetic peptides; purification.

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side-chain groups, and of a highly acid-labile linker. We reasoned that a base-labile fluorous protecting group would be an attractive alternative tag for standard Fmoc-based SPPS, with Boc/*t*-Bu side-chain protections and standard linker-resin systems such as Wang and Rink amide resins. Accordingly, we set out to design, synthesize and apply of a fluorous version of the methylsulfonylethoxycarbonyl (Msc, Fig. 1)[12] protecting group for amines, introduced by Tesser and coworkers nearly 30 years ago.[13] The new fluorous protecting group was named FMsc, in analogy with FZ.[4a]

The FMscCl tagging reagent **5** was readily synthesized as follows (Scheme 2). In the first step, commercially available [1*H*,1*H*,2*H*,2*H*]-perfluorodecyl iodide **2** was substituted with 2-mercaptoethanol to give **3**. After oxidation of thioether **3** to the corresponding sulfone **4** (30% peracetic acid in acetic acid), target FMscCl **5** was obtained by chloroformylation of **4**, in an excellent overall yield of 88%.[14]

With reagent **5** in hand, the ease and efficiency of introduction and removal of the FMsc tag **1** was explored. Pure model peptide **6** was treated with FMscCl **5** (5 equiv.) and DiPEA (10 equiv.) in DMF to give the corresponding tagged peptide FMsc-**6**, as corroborated by LCMS. Treatment of FMsc-**6** with 2% aq. NH_3 solution for 15 min quantitatively furnished starting peptide **6**.

On the basis of these results we turned our attention to the application of the FMsc tag in the purification of peptides **7**–**9** (Table 1) obtained through SPPS. These peptides are difficult to isolate in pure form using reversed-phase HPLC.[15,16] For instance, the assembly

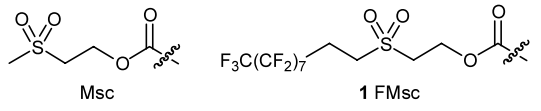
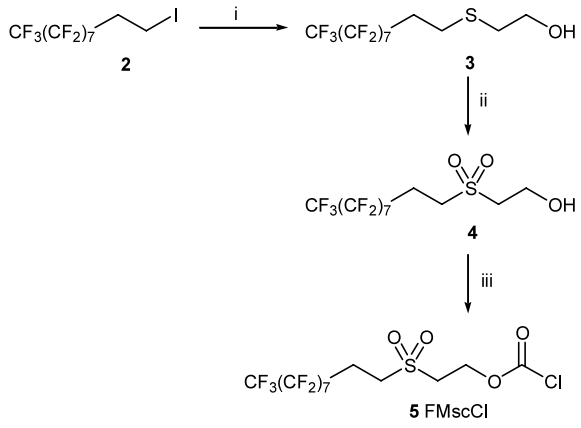


Figure 1. Msc protecting group and the FMsc tag **1**.



Scheme 2. *Reagents and conditions*: (i) 2-mercaptoethanol, NaOH, *t*-BuOH, reflux (91%); (ii) AcOOH (30% in AcOH), H_2O, 97%; (iii) phosgene (20% in toluene), THF, quant.

Table 1. Synthetic peptides

	Sequence
6	GEPKPAGamide
7	GCCSLPPCALNNPDYCamide
8	RQIKIWFQNRRMKWKKamide
9	LSELDDRADALQAGFSQFESSAAKLKRKYWWKNLK

of 35-mer **9** by a standard SPPS protocol suffered from incomplete couplings leading to a tedious isolation procedure, as shown by the HPLC pattern of crude **9** (Fig. 2A).

Peptides **7**–**9** were synthesized using standard Fmoc-based SPPS with either HCTU (**7** and **8**) or BOP/HOBt (**9**) as condensing agents and starting from Rink amide (**7**, **8**) and Wang resins (**9**). In the latter case, the first amino acid was condensed with the resin using DIC and DMAP. Each condensation step was followed by capping (Ac_2O, DiPEA, HOBt) of the residual unreacted amines. After completion of the synthesis of the respective oligopeptide sequences and removal of the final Fmoc group, the resin-bound peptides were tagged with FMscCl/DiPEA (5 and 10 equiv., respectively) in DMF. The peptides were then cleaved from the solid support with concomitant liberation of the side-chain functionalities using TFA/TIS/H_2O (95/2.5/2.5, v/v/v).

At this stage, we turned our attention to the purification of tagged 35-mer **9**. As can be seen (Fig. 2B), full length, FMsc-tagged **9** eluted significantly later from a fluorous HPLC (FHPLC) column than non-tagged impurities. Semi-preparative FHPLC with a TFE/H_2O gradient afforded FMsc-**9** in 99% purity (Fig. 2C). Detagging (2% aq. NH_3, Fig. 2D) finally afforded target peptide **9** in 21% overall yield (based on initial loading of the resin). Tagged peptides **7** and **8** were purified with FHPLC and detagged with equal

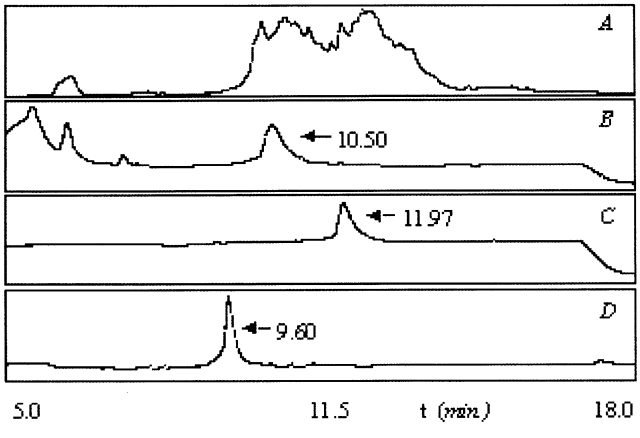


Figure 2. HPLC chromatograms (5–18 min, 214 nm) of 35-mer **9**. **A**. Crude **9**. **B**. Crude FMsc-**9**. **C**. FHPLC-purified FMsc-**9**. **D**. Pure **9** after detagging. HPLC methods:[17] **2A** and **2D**: method X, **2B** and **2C**: method Y. Peaks with correct mass are indicated with arrows. Shift of retention time is due to different sample composition.

Table 2. Data on (de)tagged peptides-FHPLC

Peptide	Yield (%)	Purity (%)	$[M_{tagged}+H]^{+a}$	$[M_{detagged}+H]^{+a}$
7	37	98	2252.0	1669.0
8	10	94	2828.8	2246.6
9	21	99	2343.0[b]	4099.121[c]

[a] ESI-MS.
[b] $[M+2H]^{2+}$.
[c] HRMS (calcd 4099.128).

efficiency (Table 2, purity determined through integration of LC peaks).

In an alternative approach, we investigated whether FMsc-tagged peptides **7**–**9** could be purified by fluorous silica gel extraction.[3,18] As an example, crude FMsc-**7** was applied to a fluorous solid-phase extraction (FSPE) cartridge. With the aim of removing non-tagged impurities, the cartridge was eluted first with H_2O and then with MeOH/H_2O 1/1 (v/v)[19] Subsequent elution of the cartridge with MeOH afforded FMsc-**7** in a significantly enhanced purity after detagging (59% yield, 91% purity, Table 3). Although the purity of the final detagged peptide is somewhat lower than is observed after FHPLC, the ease of execution and potential scale ($\sim$50 mg crude FMsc-**7** could be applied to the FSPE cartridge in one single run) makes this purification procedure an attractive alternative.

In a similar way, FMsc-tagged **8** was purified and detagged (7% yield, 72% purity, Table 3). In this case, the use of a mixture of TFE/H_2O 1/1 (v/v)[19] proved to be more effective for eluting the fluorous peptide. In contrast, purification of FMsc-**9** on a FSPE cartridge failed, arguably due to the relative low fluorine content (only 7% by weight compared to 14% in **7** and 11% in **8**).

Table 3. Data on (de)tagged peptides-FSPE

Peptide	Crude material (mg)	Detagged yield (%)	Purity (%)	Eluents[19]
7	45.9	59	91	MeOH/H_2O
8	17.2	7	72	TFE/H_2O

In summary, we have demonstrated the development and application of a new fluorous FMsc protecting group **1**. Application of FMscCl **5** in Fmoc-based SPPS allows the generation of full-length peptides equipped with a base-labile fluorous tag. These peptides were purified by either FHPLC or FSPE, after which the tag is easily and quantitatively removed. We are currently evaluating the full potential of the FMsc protecting group in both SPPS and organic synthesis.

Acknowledgements

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14. ESI-MS data: **3** $[M+Na]^+$ 547.1, **4** $[M+Na]^+$ 579.1, **5** was solvolysed in MeOH to give the methyl carbonate $[M+Na]^+$ 637.1. NMR data of **5**: ^1H NMR ($CDCl_3$): δ (ppm)

4.82–4.71 (m, 2H, H1), 3.51–3.41 (m, 2H, H2), 3.41–3.26 (m, 2H, H3), 2.86–2.53 (m, 2H, H4); ^{13}C NMR (acetone-d_6): δ (ppm) 120.3–110.6 ($8\times CF_x$), 65.4 (C1), 51.8 (C2), 45.9 (C3), 24.8, 24.4, 24.0 (t, C4).

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16. In our laboratory, synthesis of **8** was found to be troublesome.
17. HPLC methods: X. Alltech Alltima C18 column (150× 4.6 mm), 5→90% MeCN/H_2O+0.05% TFA in 17 min at 1.0 mL/min. Y. Keystone Scientific Operations FluoPhase WP column (100×4.6 mm), 20→100% TFE/H_2O+0.05% TFA in 13 min at 1.5 mL/min. UV Detection in both methods at 214 nm.
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19. All eluents used in FSPE contained 0.05% TFA.