DOI: 10.1002/ejoc.201000682

(9*H*-Fluoren-9-yl)methanesulfonyl (Fms): An Amino Protecting Group Complementary to Fmoc

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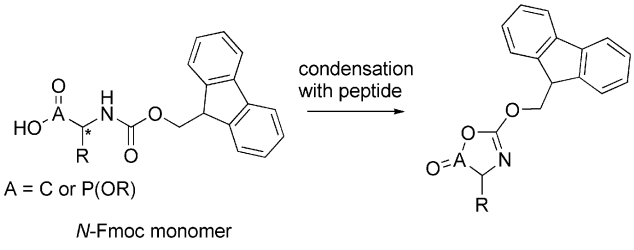
Keywords: Protecting groups / Sulfonamides / Phosphorus / Peptides

A sulfonamide-based protecting group (PG), (9*H*-fluoren-9-yl)methanesulfonyl (Fms), which can be used in a similar way to the well-established Fmoc PG, was developed. The advantages of this new PG were demonstrated in the successful formation of a phosphonamide between an *N*-Fms-protected α-phosphonoalanine monoester and secondary alkylamines, including (*R*)-2-phenylethylamine, (*S*)-phenylalanine *tert*-butyl ester (H-Phe-O*t*Bu), H-Pro-Gly-O*t*Bu, and H-Phe-Phe-O*t*Bu, without formation of oxazaphospholine, which is a serious problem associated with the Fmoc PG. The success should pave the way to the solid-phase synthesis of unnatural peptides substituted with α-amino phosphonic acid (AP) at essentially any arbitrary position without significant modification of the Fmoc-based chemistry that has been accumulated since Carpino's report in 1970. The *N*-Fms-AP monomer would attract much attention in the field of peptide mimetics.

Introduction

Carpino's Fmoc [(9*H*-fluoren-9-yl)methyloxycarbonyl] group is established as a useful protecting group (PG) for amines.[1] The Fmoc PG is easily detached, typically by treatment with 5–20% piperidine in DMF, although it has high stability toward $HOCOCF_3$, HCl, HBr, and tertiary amines.[1b,2] Its secondary amine lability, in addition to its high acid-resistant properties, enhances its utility, particularly as a temporary PG in the synthesis of peptides in both the solid and solution phases.[1c] Fmoc removal, followed by condensation with an *N*-Fmoc α-amino acid monomer, smoothly elongates the peptide chain, but as shown in Scheme 1, the activated monomer ester is sometimes cleaved by nucleophilic attack of the carbamate C=O oxygen atom onto A = O to give an oxazolone derivative.[3]



Scheme 1.

This pathway becomes more significant in the synthesis of α-amino phosphonic acid containing peptides, probably because the high affinity of oxygen for phosphorus facilitates this cyclization, causing difficulty in the attachment of α-substituted α-aminophosphonic acid onto the N-terminus of peptides.[4] This paper communicates the development of a new non-problematic amino PG that is removable under the same conditions as those used for Fmoc cleavage, which will enable utilization of the Fmoc-based synthetic chemistry that has been accumulated since 1970.[1a]

Results and Discussion

Our target PG is the (9*H*-fluoren-9-yl)methanesulfonyl (Fms) group, which protects amines RR′NH (**1**) as sulfonamide **2** by reaction with FmsCl. The expected characteristics of **2** include (1) lower nucleophilicity of the sulfonamide S=O group owing to the smaller contribution of N(+)=S–O(–), as compared to N(+)=C–O(–), thereby avoiding the above-mentioned cyclization problem;[5] (2) weaker metal-coordinating ability of the sulfonamide group as compared with carbamates, thereby increasing the applicability of Fms-protected compounds in metal-catalyzed reactions; and (3) the potential possibility of piperidine-assisted β-elimination of sulfurous acid, thereby generating deprotected amine **1**, together with SO_2 and 9-methylene-9*H*-fluorene or its piperidine adduct.[1a,1b] FmsCl was easily synthesized on a >30-g scale from (9*H*-fluoren-9-yl)methanol (**3**) through chlorination ($SOCl_2$, 80 °C, 4 h; 80% yield), sulfonylation of **4** (Na_2SO_3, acetone/H_2O, 90 °C, 8 h; 98% yield), and chlorination of **5** (PCl_5/$POCl_3$, 25 °C, 18 h; 88% yield).[6] The structure was confirmed by X-ray crystallo-

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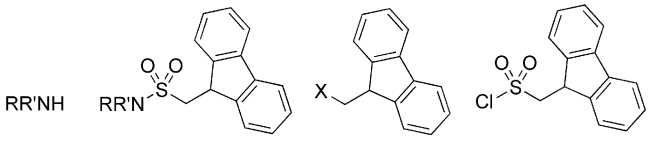
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201000682.

graphic analysis. We found that FmsCl can be stored in a general vial at room temperature for at least six months without significant decomposition.



a: R = $C_6H_5CH_2CH_2$, R' = H
b: R = *c*-C_6H_{11}, R' = H
c: R = $C_6H_5CH_2C(CH_3)_2$, R' = H
d: R = C_6H_5, R' = H
e: R = $C_6H_5CH_2CH_2$, R' = CH_3

Simple amines **1a**–**e**, in addition to various α-amino esters (vide infra), were quantitatively protected with an isolated yield of 80–95% by using FmsCl {[**1**] = 200 mM, [FmsCl] = 300 mM, [*N*,*N*-diisopropylethylamine (DIEA)] = 400–600 mM, CH_2Cl_2, 0–30 °C, 3–8 h}.[6] CH_2Cl_2, $CHCl_3$, THF, toluene, and CH_3CN were the solvents of choice for use with sterically demanding tertiary amines such as DIEA and pentamethylpiperidine. Using DMF solvent or other bases, such as triethylamine, pyridine, *N*-methylimidazole, imidazole, triazole, pyrazine, and K_2CO_3, caused decomposition of FmsCl into 9-methylene-9*H*-fluorene, decreasing the yield of **2**. Schötten–Baumann conditions were not applicable to the protection of α-amino acids.

Table 1 summarizes the results of Fms removal from **2a**–**e** to give **1a**–**e**. Simple primary alkylamine **1a** was quantitatively deprotected from **2a** within 1 min under the standard conditions for removal of Fmoc {[**2a**] = 200 mM, [piperidine] = 1000 mM, DMF, 25 °C; Table 1, Entry 1}. CH_3OH, dioxane, THF, CH_2Cl_2, and toluene could also be used, although the reactivity was lowered by one to two orders of magnitude (Table 1, Entries 2–6). Even with a 1.25-mol amount of piperidine, the deprotection proceeded smoothly without the formation of *N*-fluorenylmethylated **1a** (Table 1, Entry 8).[7] The substrate concentration could be varied from 20 to 1000 mM (Table 1, Entries 9 and 10). Not only primary alkyl primary amine **1a** but also secondary and tertiary alkyl primary amines **1b** and **1c** were deprotected from **2b** and **2c** under the standard conditions (Table 1, Entries 11 and 12). In a similar way, the Fms group in Fms-protected arylamine **2d** and secondary amine **2e** was smoothly removed (Table 1, Entries 13 and 14).

The general applicability to *N*-Fms-protected α-amino esters is demonstrated in Table 2.[6] Under the standard conditions described above, Fms-Gly-O*t*Bu[8] was quantitatively converted into H-Gly-O*t*Bu within 10 min. α-Substituted α-amino carboxylic esters, such as H-Ala-O*t*Bu, H-Leu-O*t*Bu, H-Phe-O*t*Bu, H-Val-O*t*Bu, H-Phg-O*t*Bu, and H-Pro-O*t*Bu, were also completely deprotected and isolated in 78–87% yield after converting the corresponding *N*-benzoyl derivative. Lack of racemization was confirmed by using H-Phg-O*t*Bu: the *S*/*R* enantiomer ratio of 99:1 did not change during the protection/deprotection process.

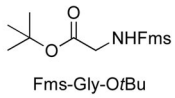
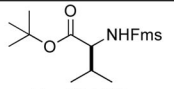
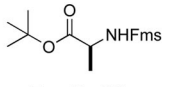
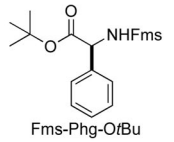
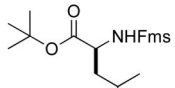
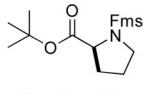
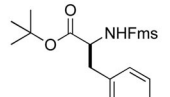
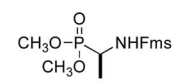
Table 1. Deprotection of amines **1a**–**e** from *N*-Fms-protected compounds **2a**–**e**.[a]

Entry	*N*-Fms-amine	[Sub.] / mM	[Piperidine] / mM	Solvent	*t* / min	% Conv.[b]
1	**2a**	200	1000	DMF	<1	>99 (96)
2	**2a**	200	1000	CH_3OH	60	>99 (91)
3	**2a**	200	1000	dioxane	30	98 (90)
4	**2a**	200	1000	THF	60	>99 (91)
5	**2a**	200	1000	CH_2Cl_2	30	>99 (94)
6	**2a**	200	1000	toluene	30	>99 (88)
7	**2a**	200	500	DMF	<1	>99
8	**2a**	200	250	DMF	<1	>99
9	**2a**	20	100	DMF	5	>99
10	**2a**	1000	5000	DMF	<1	>99
11	**2b**	200	1000	DMF	<1	>99 (91)
12	**2c**	200	1000	DMF	<1	>99 (79)
13	**2d**	200	1000	DMF	<1	>99 (87)
14	**2e**	200	1000	DMF	<1	>99 (84)

[a] Reactions were carried out at 25 °C on a 0.2–0.5-mmol scale. [b] Determined by ^1H NMR spectroscopic analysis of the reaction mixture after addition of an equimolar amount of $HOCOCF_3$ for piperidine.[6] The value in parentheses is the isolated yield after benzoylation [2.5 equiv. of C_6H_5COCl, 2.5 equiv. of $N(C_2H_5)_3$, 25 °C, 8 h], followed by silica gel column chromatography.

Removal of the Fms group from Fms-Phe-OH, as well as the α-amino phosphonic ester Fms-AlaP(OCH_3)-OCH_3,[8] could be efficiently attained.

Table 2. Removal of the Fms protecting group from α-amino esters.[a]

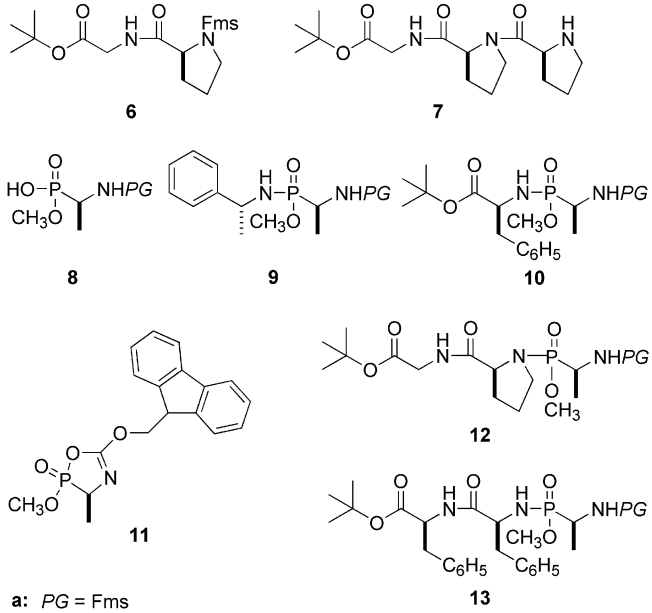
Entry	*N*-Fms-α-amino ester	% Yield[b]	Entry	*N*-Fms-α-amino ester	% Yield[b]
1	 Fms-Gly-O*t*Bu	84	5	 Fms-Val-O*t*Bu	80
2	 Fms-Ala-O*t*Bu	83	6	 Fms-Phg-O*t*Bu	78[c]
3	 Fms-Leu-O*t*Bu	80	7	 Fms-Pro-O*t*Bu	87
4	 Fms-Phe-O*t*Bu	83	8	 Fms-AlaP(OCH_3)-OCH_3	81

[a] Reactions were carried out on a 0.06–0.28-mmol scale in DMF at 25 °C for 10 min with *N*-Fms-protected α-amino esters (200 mM) and piperidine (1000 mM) unless otherwise specified. In all cases, the conversion was >99%. [b] Isolated yield after benzoylation [2.5 equiv. of C_6H_5COCl, 2.5 equiv. of $N(C_2H_5)_3$, 25 °C, 8 h] followed by silica gel column chromatography. In all cases, the conversion was >99%. [c] No racemization of H-Phg-O*t*Bu was confirmed by HPLC analysis [CROWNPAK CR, 200-nm detection, $NaClO_4$–$HClO_4$ buffer (pH 2.0)] after converting into H-Phg-OH ($HOCOCF_3$, 25 °C, 1 h).[6]

The base stability of Fms-Phe-O*t*Bu was compared with that of Fmoc-Phe-O*t*Bu under the conditions of [Fms- or Fmoc-protected amine] = 25 mM, [amine] = 25 mM, $CDCl_3$, 3 h, and 25 °C, giving H-Phe-O*t*Bu: piperidine [20 (Fms) vs. 2.2% (Fmoc)], piperazine (22 vs. 2.3%), morpholine (0.8 vs. <0.1%), dicyclohexylamine (3.8 vs. <0.1%), diethylamine (6.2 vs. <0.1%), dimethylaminopyridine (9.9 vs. 0.9%), DIEA (<0.1 vs. <0.1%), pentamethylpiperidine (<0.1 vs. <0.1%), triethylamine (19 vs. 0.8%), and proton sponge (<0.1 vs. <0.1%).[6] We found that the Fms PG is more labile than the Fmoc PG, which increases the efficiency of Fms removal from the Fms-protected amines. This tendency is, however, disadvantageous for chemoselective protection and deprotection; thus, selection of an appropriate amine is recommended in the case of chemical transformations requiring bases. Both the Fms and Fmoc PGs were stable under typical acidic conditions ($HOCOCF_3$, neat, 25 °C, 24 h; 6 M HCl, CH_3OH, 25 °C, 24 h).[1a,2,6] Fmoc-Gly-OH is known to be labile under hydrogenolysis conditions (1 atm H_2, 10 mol-% Pd/C, 1:4 $HOCOCH_3/CH_3OH$, 25 °C, 24 h).[9] Indeed, under these conditions, 35% of Fmoc-Phe-OH was converted into H-Phe-OH, together with (9*H*-fluoren-9-yl)methane. These conditions exerted no effect on Fms-Gly-OH or Fms-Phe-OH:[6] even at 100 atm of H_2, Fms-Phe-OH remained intact.

The Fms PG was confirmed to have the same efficiency as the Fmoc PG in carboxylic acid based peptide synthesis by liquid-phase synthesis of the simple tripeptide H-Pro-Pro-Gly-OH, a collagen protein unit tripeptide.[6,10] H-Gly-O*t*Bu was combined with Fms-Pro-OH by the benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) method (CH_2Cl_2, 25 °C, 8 h), giving Fms-Pro-Gly-O*t*Bu (**6**). The Fms PG of the dipeptide was chemoselectively removed by treatment with piperidine (5 mol) in DMF. The PyBOP condensation of H-Pro-Gly-O*t*Bu with Fms-Pro-OH, followed by N-Fms removal, afforded **7** in 85% total yield from **6**. Treatment of **7** with $HOCOCF_3$ at 25 °C for 1 h quantitatively yielded H-Pro-Pro-Gly-OH.

The utility of the Fms PG in the potential synthesis of α-amino phosphonic acid containing peptides was confirmed by the successful condensation of Fms-AlaP(OCH_3)-OH (**8a**)[8] with secondary alkylamines by using a newly developed phosphonamidation system consisting of 1-hydroxy-7-azabenzotriazole (HOAt), DIEA, and *N*,*N*′-diisopropylcarbodiimide (DIC).[6] Reaction of **8a** with (*R*)-phenylethylamine ([**8a**] = [amine] = 100 mM, CH_3CN, [HOAt] = [DIEA] = [DIC] = 200 mM, 25 °C, 3 h)[11] afforded desired product **9a** [^{31}P NMR ($CDCl_3$): δ = 29 ppm] in 82% isolated yield. Under the same conditions, by contrast, Fmoc-AlaP(OCH_3)-OH (**8b**) gave **9b** in less than 20% yield, and the main product in the reaction mixture was oxazaphospholine derivative **11** [^{31}P NMR (CD_3CN): δ = 17 ppm].[12] Using H-Phe-O*t*Bu in a condensation reaction with Fms-AlaP(OCH_3)-OH gave the dipeptide Fms-AlaP(OCH_3)-Phe-O*t*Bu (**10a**) in 90% isolated yield, the Fms group of which was then quantitatively removed to give **10c** ([**10a**] = 40 mM, [piperidine] = 200 mM, $CDCl_3$, 25 °C, 1 h). The N-terminus of the dipeptides, H-Pro-Gly-O*t*Bu and H-Phe-Phe-O*t*Bu, could also be successfully condensed with Fms-AlaP(OCH_3)-OH to give, after Fms removal, H-AlaP(OCH_3)-Pro-Gly-O*t*Bu (**12c**) and H-AlaP(OCH_3)-Phe-Phe-O*t*Bu (**13c**) in 91 and 89% isolated yield, respectively.



a: *PG* = Fms
b: *PG* = Fmoc
c: *PG* = H

Conclusions

We have developed Fms, a new sulfonamide-based PG[2,13] for the protection of various amines including primary, secondary, and tertiary alkyl primary and secondary amines, α-amino carboxylic esters, and α-amino phosphonic esters. Smooth and quantitative removal of the Fms PG can be attained under essentially identical conditions to those used for Fmoc removal. The substitutability between Fmoc and Fms has been demonstrated in the synthesis of a tripeptide. The advantages of Fms over Fmoc have been clearly shown by the successful dehydrative condensation of an *N*-Fms-protected α-amino phosphoalanine monoester with an α-substituted α-amino carboxylic ester and amide, giving a phosphonamide-based peptide. Phosphonopeptide chemistry, which attracts much attention from researchers interested in mimetics of peptides and nucleotides, has developed mainly through the use of phosphonic acid terminal peptides and residue-phosphorylated peptides.[11,14] The successful synthesis of an α-amino phosphonic acid N-terminal peptide demonstrated herein should provide a potential tool for the diverse synthesis of α-amino phosphonic acid containing peptides, which is our ongoing project.[15]

Experimental Section

Fms Protection: A 20-mL Young's type Schlenk flask containing a Teflon-coated magnetic stirring bar was charged with 2-phenylethylamine (**1a**; 126 μL, 1.00 mmol) and CH_2Cl_2 (5.0 mL). The whole mixture was cooled to 0 °C in an ice bath, and then DIEA

(330 μL, 2.00 mmol) and FmsCl (418 mg, 1.50 mmol) were introduced. The ice bath was removed, and the flask was then immersed into a 30 °C water bath. After stirring the colorless solution for 3 h, water (2.0 mL) was added. The aqueous layer was extracted with CH_2Cl_2 (2 × 2.0 mL). The combined organic layer was washed with brine (2.0 mL) and dried with Na_2SO_4. Filtration followed by evaporation under a reduced pressure gave a yellow oil (580 mg), which was then subjected to silica gel column chromatography (25 g, CH_2Cl_2) to give **2a** (345 mg, 95% yield) as a white solid.

Fms Removal: A 10-mL Young's type Schlenk flask containing a Teflon-coated magnetic stirring bar was charged with 1-(9*H*-fluoren-9-yl)-*N*-phenethylmethanesulfonamide (**2a**; 182 mg, 0.500 mmol), mesitylene (60.1 mg, 0.500 mmol, internal standard), and DMF (2.50 mL). To this was added piperidine (247 μL, 2.50 mmol), and the clear solution was stirred at 25 °C for 1 min. Because of difficulty in efficient separation of **1a** from piperidine and the piperidine adduct, the isolated yield of **1a** was indirectly determined to be 96% after benzoylation followed by silica gel column chromatography.

Supporting Information (see footnote on the first page of this article): Details of the preparation of FmsCl, procedures for protection/deprotection, stability examination, synthesis of a tripeptide, and condensation between *N*-Fms-protected α-phosphonoalanine monoester and secondary alkyl primary amines.

Acknowledgments

This work was aided by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan (No. 25E07B212).

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[5] For the theoretical calculation of the double bond character of N–C(O) and N–S(O) in *N*-methoxycarbonyl- and *N*-methylsulfonyl-protected pyrrole derivatives, see: I. Chataigner, C. Panel, H. Gérard, S. R. Piettre, *Chem. Commun.* **2007**, 3288–3290. The sulfonamide N atom has higher sp^3 hybridization, resulting in higher aromatic character and in a smaller N–S rotation barrier.

[6] For details see the Supporting Information.

[7] *N*-Fluorenylmethylation sometimes occurs in the removal of Fmoc PG during peptide synthesis. See ref.[1]

[8] α-Amino carboxylic acid abbreviation follows the IUPAC rule. The *N*-PG and ester PG are denoted as a prefix and a suffix, respectively. α-Amino phosphonic acid is denoted by the addition of "P" at the end of the three-letter abbreviation, e.g., H-AlaP(OH)-OH for 1-aminoethylphosphonic acid.

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[15] Other carbamate-type PGs such as Nsc having a high interchangeablity with Fmoc could be extended to the corresponding sulfonamide-based PGs in a similar way to the present chemistry.

Received: May 12, 2010
Published Online: June 23, 2010

