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Efficient Loading of Sulfonamide Safety-Catch Linkers by Fmoc Amino Acid Fluorides

Raffaele Ingenito,* Dijana Drežnjak, Stefan Guffler, and Holger Wenschuh

Jerini AG, Rudower Chaussee 29, 12489 Berlin, Germany

ingenito@jerini.com

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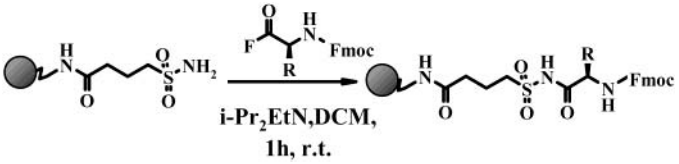
ABSTRACT



Fmoc-protected amino acid fluorides were found to be excellent reagents for the acylation of sulfonamide safety-catch linkers (SCL) suitable for the subsequent preparation of peptide C-terminal thioesters. High loadings were obtained on different types of resins with low levels of epimerization.

The efficient acylation of sulfonamide type linkers[1] by means of Fmoc-protected amino acids with minimal epimerization at the chiral α-carbon is critical for the successful Fmoc-based solid-phase synthesis of thioesters by the safety-catch linker strategy.[2]

Recently, it was reported that the reactivity of the sulfonamide function is comparable to that of an alcohol.[3] Accordingly, it was demonstrated that acylation of solid-support-bound alkanesulfonamide safety-catch linkers with Fmoc amino acids proceeds best under conditions previously described for effective acylation of alcohols (PyBOP, DIEA, DCM, or $CHCl_3$, −20 °C).[4] However, long reaction times (8 or 16 h) and careful selection of reaction conditions were needed in order to obtain adequate loadings.[3]

These results prompted a systematic study on the potential application of Fmoc-protected amino acid fluorides,[5] recently demonstrated to be highly reactive reagents for the acylation of very hindered amino acids and hydroxy functions on solid supports,[6,7] to the acylation of the sulfonamide safety-catch linker (SCL).

Initially, model studies were carried out on the acylation of a commercial 4-sulfamylbutyryl SCL-PS resin (loading: 1.12 mmol/g). Fmoc-Ser(*t*Bu)-OH, known to racemize readily under conditions[8] commonly used for solid-phase peptide synthesis, was used as a sensitive model system. The acid fluoride was synthesized via cyanuric fluoride[5] and used in the presence of various bases (DMAP, DIEA, NMI, TMP, and DABCO) and solvent systems (DMF, DCM, and $CHCl_3$).

The highest acylation yields were obtained when DCM was used as solvent (for 2 equiv of DIEA; 0.5 M final concentration; reaction time 60 min; DCM 86%, $CHCl_3$ 44%, DMF 38%).[9] Therefore DCM was chosen as the solvent of choice, and the efficiency of loading was examined in the presence of varying amounts of different bases. It was found that DMAP and DIEA (2 and 3 equiv) gave the highest loadings (Table 1). Racemization was tested for all bases (1.5, 2, or 3 equiv) via assembly of the dipeptide L-Ser-Phe-OMe and HPLC-based comparison of the peak area with

(1) Backes, J. A.; Virgilio, A. A.; Ellman, J. A. *J. Am. Chem. Soc*. **1996**, *118*, 3055.
(2) Ingenito, R.; Bianchi, E.; Fattori, D.; Pessi, A. *J. Am. Chem. Soc*. **1999**, *121*, 11369.
(3) Backes, B. J.; Ellman J. A. *J. Org. Chem*. **1999**, *64*, 2322.
(4) Kim, M. H.; Patel, D. V. *Tetrahedron Lett*. **1994**, *35*, 5603. See however: Coste, J.; Campagne, J.-M. *Tetrahedron Lett*. **1995**, *36*, 4253.
(5) Carpino, L. A.; Sadat-Aalaee, D.; Chao, H. G.; DeSelms, R. H. *J. Am. Chem. Soc*. **1990**, *112*, 9651.
(6) Wenschuh, H.; Beyermann, M.; Krause, E.; Brudel, M.; Winter, R.; Schümann, M.; Carpino, L. A.; Bienert, M. *J. Org. Chem*. **1994**, *59*, 3275.
(7) Granitza, D.; Beyermann, M.; Wenschuh, H.; Haber, H.; Carpino, L. A.; Truran, G. A.; Bienert, M. *J. Chem. Soc., Chem. Commun*. **1995**, 2223.
(8) Di Fenza, A.; Tancredi, M.; Galoppini, C.; Rovero, P. *Tetrahedron Lett*. **1998**, *39*, 8529.
(9) In general, loadings were determined by cleavage of the Fmoc group from a defined amount of resin using 20% piperidine in DMF for 20 min and UV measurement of the resulting piperidine−dibenzofulvene adduct at 301 nm.

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Table 1. Anchoring via Fmoc-Ser(*t*Bu)-F to Sulfonamide SCL-PS and Racemization Values Obtained in the Presence of Various Bases[a]

		equiv of base							
		0.5	1	1.5		2		3	
entry	base	yield %	yield %	yield %	D,L %	yield %	D,L %	yield %	D,L %
1	no base	0.2	0.2	0.2	nd	0.2	nd	0.2	nd
2	DMAP	32	50.5	96.8	14.7	99.3	17.5	100	22.6
3	DIEA	28.8	40	53.6	<0.1	86.4	0.5	99.2	1.3
4	NMI	4.6	28.1	35.3	2	55.6	4.1	65.4	6.6
5	TMP	0.2	0.2	2	nd	6	nd	10	nd
6	DABCO	6	40.5	51.2	11.4	60.4	25.4	68.1	34.2

[a] 3 equiv of Fmoc-Ser(*t*Bu)-F; coupling concentration 0.5 M in DCM; reaction time 60 min.

the corresponding control peptide D-Ser-Phe-OMe[10] (Table 1 and Figure 1). It was found that 2 equiv of DIEA gave the lowest level of racemization (0.5%) with a high acylation yield (86%).

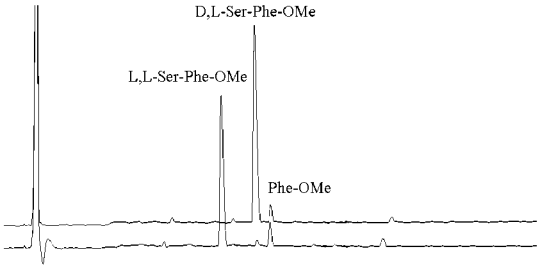

Figure 1. RP−HPLC separation of dipeptides L-Ser-Phe- OMe and D-Ser-Phe-OMe obtained from loading of the SCL with Fmoc-Ser(tBu)-F and subsequent nucleophilic displacement with NH_2−Phe-OMe.

To establish the general applicability of the method, all accessible amino acid fluorides were synthesized according to known procedures via cyanuric fluoride[5] or DAST[11] and used for acylation of the SCL-PS resin (Table 2). It was found that in most cases high loadings could be obtained in short reaction times (1 h). Only in the case of Pro was double acylation needed to obtain a satisfactory yield. Loss of configuration was determined for selected cases (Cys, Leu, Glu) in addition to the standard serine system by means of HPLC evaluation of the relative amounts of the D,L- and L,L-diastereomers of the dipeptides Xxx-Phe-OMe. D,L-Forms were found to be low in all cases: Cys-Phe-OMe (3.7%),[12] Leu-Phe-OMe (<0.1%), Glu-Phe-OMe (0.1%).

Table 2. Coupling of Fmoc Amino Acid Fluorides[a] to Sulfonamide SCL-PS Resin and Sulfonamide SCL-TG Resin (Champion I and NovaSynTG)

	yield, %		
Fmoc-AA-F	PS 60 min	Champion I 60 min	NovaSynTG 60 min
Ala	85	85	83
Asn	67	70	72
Asp	80	100	100
Cys	93	96	94
Gln	100	100	89
Glu	65	61	63
Gly	100	100	100
His	70	69	67
Ile	67	75	73
Leu	85	81	90
Lys	73	81	80
Met	67	98	87
Phe	100	100	100
Pro	66[b]	75[b]	69[b]
Ser	86	87	86
Thr	80	86	82
Trp	100	100	89
Tyr	100	100	95
Val	95	94	92

[a] 3 equiv of Fmoc amino acid fluoride; 2 equiv of DIEA; coupling concentration 0.5 M in DCM for PS resin and 0.25 M in DCM for PEG-based resins. [b] Double coupling.

Finally, to explore the described method for other types of resins, the 3-carboxypropane sulfonamide SCL was attached to commercially available amino functionalized PEG-based resins such as the NovaSynTG resin (loading 0.44 mmol/g) and the Champion I resin (loading 0.34 mmol/g) using DIC/HOBt activation followed by loading of the Fmoc amino acid fluorides as described above. As shown in Table 2. the loadings were similar to those obtained for the PS resin. In conclusion, it was found that Fmoc amino acid fluorides are highly effective reagents for the acylation of sulfonamide safety-catch linker resins. This generally applicable approach enables high loadings using short reaction times with low levels of racemization.

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Supporting Information Available: Experimental procedures and HPLC data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Resins loaded with the corresponding amino acid were treated with 20% piperidine (2 × 6 min) for Fmoc removal, washed with DMF, and capped with Boc anhydride. After washing with DMF and NMP, resins where activated with iodoacetonitrile followed by nucleophilic displacement of the amino acid with NH_2-Phe-OMe to provide the dipeptides. After workup and final deprotection of side chains, the unpurified dipeptides were directly analyzed by reversed-phase HPLC and LC-MS to determine the percentage of racemization in the initial coupling step. The D,L-epimers of Ser-Phe-OMe, Cys-Phe-OMe, Glu-Phe-OMe, and Leu-Phe-OMe were prepared as HPLC standards using the same procedure.

(11) Kaduk, C.; Wenschuh, H.; Beyermann, M.; Forner, K.; Carpino, L. A.; Bienert, M. *Lett. Pep. Sci.* **1995**, *2*, 285.

(12) Relative to the pronounced sensitivity of Cys under ordinary solid-phase synthesis conditions (see Han, Y.; Albericio, F.; Barany, G. *J. Org. Chem.* **1997**, *62*, 4307 and references therein) the loss of configuration detected in the present work appears to be satisfactory.