Pergamon

Tetrahedron Letters 43 (2002) 7809–7812

TETRAHEDRON LETTERS

Use of benzyloxycarbonyl (Z)-based fluorophilic tagging reagents in the purification of synthetic peptides

Dmitri V. Filippov,[a] Dirk J. van Zoelen,[a] Steven P. Oldfield,[a] Gijs A. van der Marel,[a] Herman S. Overkleeft,[a] Jan W. Drijfhout[b] and Jacques H. van Boom[a,*]

[a]*Leiden Institute of Chemistry, Leiden University, PO Box 9502, 2300 RA Leiden, The Netherlands*
[b]*Department of Immunohematology and Bloodtransfusion, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands*

Received 23 July 2002; accepted 16 August 2002

Abstract—Three novel fluorous reagents (i.e. **1**–**3**), derived from benzyl chloroformate (Z-Cl), have been synthesized and used for the tagging of peptides prepared following a Fmoc-based solid-phase approach. It is shown that the implementation of a benzyloxycarbonyl (Z)-based fluorophilic tag facilitates purification of peptides using fluorous reverse phase chromatography.

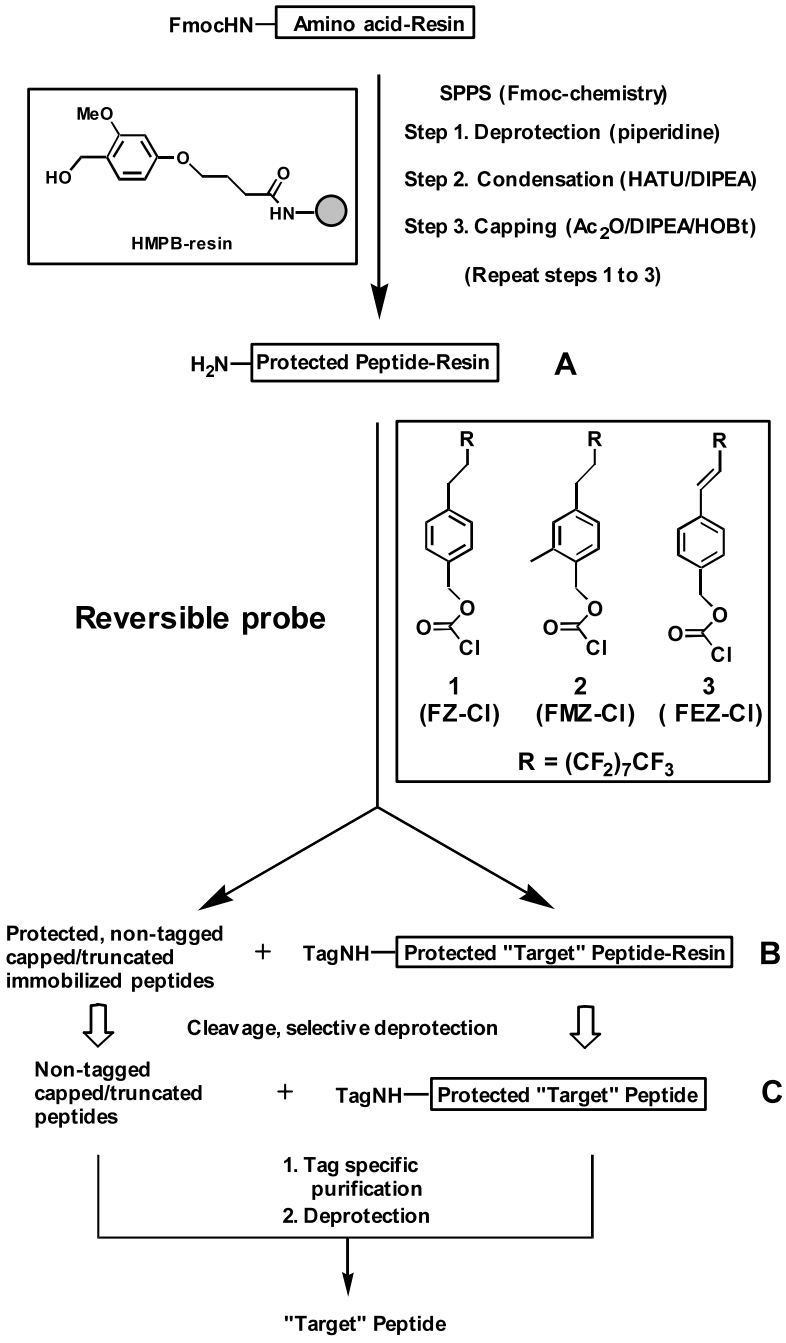
Stepwise solid-phase peptide synthesis (SPPS), originally devised[1] by Merrifield, has been improved over the past years by the introduction of automation and new chemical methodologies[2] (i.e. protective groups, resin supports as well as condensing and capping agents). Despite these advances it became evident that the occurrence of unwanted truncated and deletion sequences could not be prevented. The latter is mainly due to the fact that the efficacy of a condensation step strongly depends on the secondary structure of the growing peptide chain and, to a lesser extent, on the nature of the incoming amino acid residue. Consequently, routine SPPS of polypeptides may lead to the accumulation of impurities which can only be removed by a laborious and time-consuming purification procedure. It has been reported[3] that the separation of chromatographically similar impurities can be facilitated by the incorporation of a reversible chromatographic tag at the H_2N-terminus of an immobilized target peptide obtained in the final stage (see stage A in Scheme 1) of a stepwise SPPS protocol. Cleavage of the tagged peptide from the solid support (see transition of stage B into C in Scheme 1) will afford, after a tag defined specific purification procedure and subsequent removal of the tag, the target peptide.

It was envisaged that the implementation of a reversible benzyloxycarbonyl (Z)-based probe derivatized with a suitable fluorophilic tail would be a powerful tool in the purification by fluorous chromatography[4] of target sequences obtained via a stepwise SPPS approach. Here we present a convenient route for the synthesis of three Z-based fluorine-tagged reagents [i.e. **1** (FZ-Cl), **2** (FMZ-Cl) and **3** (FEZ-Cl) in Scheme 1] and the potential usefulness of **1** (FZ-Cl) in the purification of synthetic peptides as exemplified here for the basic docosameric peptide **19** and the hydrophobic nonadecamer **20**.

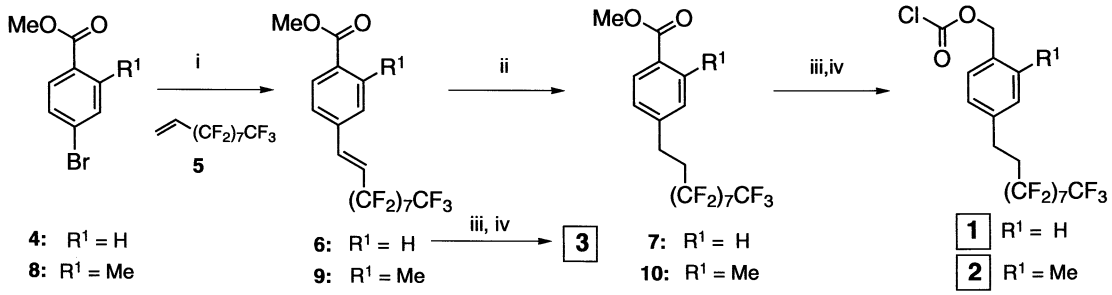
The route of the synthesis of the three fluorophilic reagents **1**–**3** is presented in Scheme 2 and is based on a Heck cross-coupling of 4-bromobenzoic acid methyl ester[5] (**4**) with commercially available 1H,1H,2H-perfluoro-1-decene (**5**). Thus, reaction of **4** with **5** under the influence of the Herrmann–Beller catalyst[6] gave the perfluoro-*E*-alkene derivative **6**. Reduction of **6** with $LiAlH_4$ led, after treatment of the resulting benzyl alcohol derivative with phosgene, to the isolation of **3** (FEZ-Cl) in an overall yield of 65%. On the other hand, hydrogenation of **6** followed by reduction of **7** with $LiAlH_4$, and subsequent subjection of the benzyl alcohol derivative to phosgene, gave **1** (FZ-Cl) in a yield of 57% over the three steps. The *ortho*-methyl substituted fluorophilic reagent **2** (FMZ-Cl) was also readily prepared by subjecting the known[7] 2-methyl-4-bromobenzoic acid methyl ester (**8**) to the three-step sequence of events, as mentioned for the conversion of **4** into **1** (FZ-Cl).[8]

* Corresponding author. Tel.: +31 71 5274274; fax: +31 71 5274307; e-mail: j.boom@chem.leidenuniv.nl

0040-4039/02/$ - see front matter
PII: S0040-4039(02)01723-9



Scheme 1.



Scheme 2. *Reagents and conditions*: (i) **5**, Herrmann–Beller catalyst, NaOAc, DMF, 125°C, 14 h (72%); (ii) Pd/C 10%, H_2, 3 bar, 3 h (88%); (iii) $LiAlH_4$, Et_2O, 0°C, 2 h (90%); (iv) 20% phosgene in toluene, THF, 3 h (100%).

Having the Z-based fluorophilic tagging reagents **1**–**3** in hand, we first explored the use of **1** (FZ-Cl) in the preparation of the heptameric peptide **14** (see entry 4 in Table 1). To this end, the MBHA-resin containing the very acid labile HMPB linker (see Scheme 1) was selected as the solid support. Sequential elongation of the resin, loaded with glycine (0.5 mmol/g), was performed by repeating (6×) the three-step event, as depicted in Scheme 1, to give the immobilized and protected peptide **11** (entry 1, Table 1). With the intention to enhance the possible occurrence of unwanted impurities the condensation step 1, involving activation of the incoming individual Fmoc-protected amino acids with the powerful reagent HATU, was limited in all cases throughout this study to 5 min and not repeated. Tagging of the NH_2-terminus of peptide **11** with **1** (FZ-Cl) in the presence of DIPEA proceeded smoothly to give **12** (entry 2) in a quantitative yield, as gauged by the TNBS test.[9] Treatment of **12** with trifluoroacetic acid (1%) in DCM for 20 min at 20°C gave, as evidenced by HPLC–MS analysis, the expected[10] FZ-tagged and partially protected[11] peptide **13** (entry 3), which could be readily purified (see Fig. 1) by Fluophase™ column chromatography.[12] At this stage, the FZ-tagged partially *t*-Bu-protected peptide **13** was treated with TFA/H_2O (98/2) and the progress of the deblocking was monitored by HPLC analysis. It turned out that roughly 90% of **13** had been converted, after 6 h at 20°C, into the target peptide **14** (entry 4). Interestingly, subjection of the corresponding Z-protected peptide to the same acidic conditions showed, after 7.5 h at 20°C, the presence (40%) of the peptide **14**, indicating that the Z-group is more acid stable than the FZ-group. Moreover, on the basis of the additional qualitative results obtained in subjecting the FMZ- and FEZ-tagged peptides **15** and **16** (see entries 5 and 6, respectively) to the same acidic conditions, the following order of decreasing acid stability was established: FEZ>Z>FZ>FMZ. The latter order of acid lability was also in line with the observation that both FMZ- and FZ-protecting groups could be quantitatively removed with neat TFA in 2 and 9 h, respectively. In contrast, unmasking of the FEZ-group[13] could be effected most conveniently within 2 h using TFMSA (10%) in TFA.

Table 1. Sequences of the immobilized and non-immobilized peptides

1 H-Gly-Glu(**O*t*-Bu**)-Pro-Lys(**Mtt**)-Pro-Ala-Gly-resin (**11**)
2 **FZ**-Gly-Glu(**O*t*-Bu**)-Pro-Lys(**Mtt**)-Pro-Ala-Gly-resin (**12**)
3 **FZ**-Gly-Glu(**O*t*-Bu**)-Pro-Lys-Pro-Ala-Gly-OH [**13**, $(M+H)^+$ 1291]
4 H-Gly-Glu-Pro-Lys-Pro-Ala-Gly-OH [**14**, $(M+H)^+$ 655]
5 **FMZ**-Gly-Glu(**O*t*-Bu**)-Pro-Lys-Pro-Ala-Gly-OH [**15**, $(M+H)^+$ 1305]
6 **FEZ**-Gly-Glu(**O*t*-Bu**)-Pro-Lys-Pro-Ala-Gly-OH [**16**, $(M+H)^+$ 1289]
7 **FZ**-Gly-Ala-Tyr(***t*-Bu**)-Lys(**Mtt**)-Gly-Leu-Pro-Ala-Lys(**Mtt**)-Lys(**Mtt**)-Pro-Thr(***t*-Bu**)-Ala-Pro-Thr(***t*-Bu**)-Ile-Glu(**O*t*-Bu**)-Gly-Ala-Lys(**Mtt**)-Lys(**Mtt**)-Gly-resin (**17**)
8 **FZ**-Gly-Ala-Tyr(***t*-Bu**)-Lys-Gly-Leu-Pro-Ala-Lys-Lys-Pro-Thr(***t*-Bu**)-Ala-Pro-Thr(***t*-Bu**)-Ile-Glu(**O*t*-Bu**)-Gly-Ala-Lys-Lys-Gly-OH [**18**, $(M+H)^+$ 2990]
9 H-Gly-Ala-Tyr-Lys-Gly-Leu-Pro-Ala-Lys-Lys-Pro-Thr-Ala-Pro-Thr-Ile-Glu-Gly-Ala-Lys-Lys-Gly-OH [**19**, $(M+H)^+$ 2185]
10 H-Gly-Val-Trp-Pro-Leu-Phe-Leu-Leu-Leu-Leu-Ala-Leu-Pro-Pro-Lys-Ala-Tyr-Ala-Gly-OH [**20**, $(M+H)^+$ 2040]

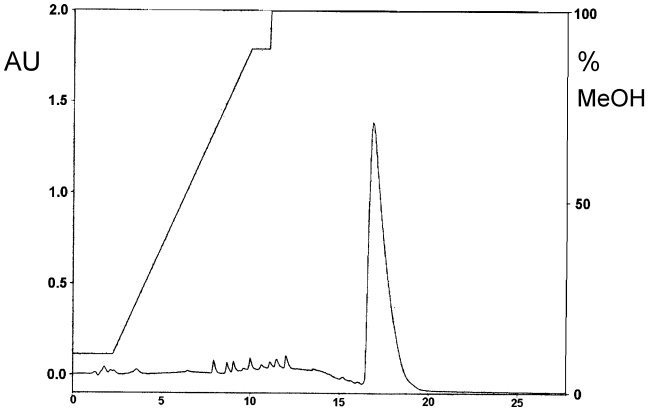


Figure 1. Chromatogram obtained by eluting crude FZ-tagged **13** (1 mg), loaded on a Fluophase™ column, with a gradient of MeOH in 0.1% aq. TFA.

The usefulness of our approach was demonstrated first in the purification of the basic docosameric peptide **19** (entry 9) using the reagent **1** (FZ-Cl) for the introduction of the reversible fluorophilic tag. Accordingly, cleavage of the FZ-tagged and immobilized peptide **17** (entry 7), prepared according to the SPPS protocol in Scheme 1, from the solid support under mild acidic conditions (1% TFA in DCM) gave peptide **18** (entry 8), which was purified via Fluophase™ column chromatography (Fig. 2) by applying a gradient of trifluoroethanol in aqueous TFA buffer.[12] HPLC–MS analysis of detagged peptide **19** (entry 9), resulting from prolonged acid treatment (98% TFA, 9 h) of peptide **18**, revealed the presence of one product with the expected mass (see entry 9). Apart from this, it was also gratifying to establish that the hydrophobic peptide **20** (entry 10) could be isolated in a homogeneous form and satisfactory yield (35%) following the same protocol.

The results presented in this paper clearly show that the reversible fluorophilic FZ-tag is a promising new asset[14] in the purification of peptides prepared according to a routine SPPS methodology. In addition, the use of the relatively acid labile FMZ-tag will lead to a substantial decrease in time required for its complete removal. It is also not excluded that the readily accessible reagents **1** (FZ-Cl), **2** (FMZ-Cl) and **3** (FEZ-Cl) may find application as fluorous tags in synthetic organic chemistry. The scope of our Z-based reversible tagging methodology is at present under investigation.[15]

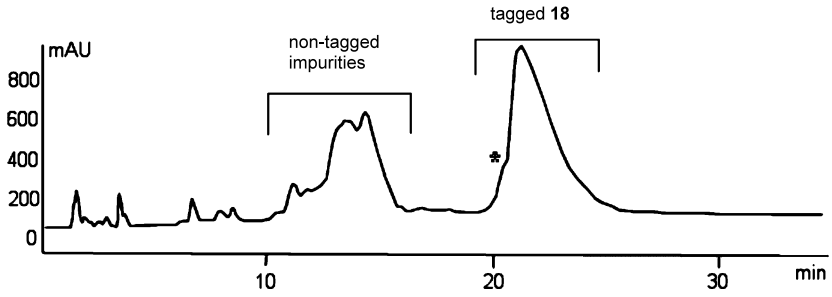
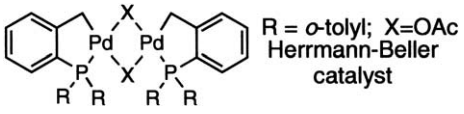


Figure 2. Chromatogram obtained by eluting crude FZ-tagged **18** (0.5 mg), loaded on a Fluophase™ column, with a gradient of trifluoroethanol in 0.05% aq. TFA. MS analysis showed that the product eluted as a broad peak at 21 min is peptide **18**. The small 'shoulder' indicated with an asterisk is due to partial loss of a *t*-Bu protecting group in **18**.

References

1. Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149–2154.
2. (a) Kent, S. B. H. *Annu. Rev. Biochem.* **1988**, *57*, 957–989; (b) Fields, G. B.; Noble, R. L. *Int. J. Peptide Protein Res.* **1990**, *35*, 161–214.
3. (a) Merrifield, R. B.; Bach, E. B. *J. Org. Chem.* **1978**, *43*, 4808–4816; (b) Funakoshi, S.; Fukuda, H.; Fujii, N. *J. Chromatogr.* **1993**, *638*, 21–27; (c) Ball, H. L. *Int. J. Peptide Protein Res.* **1996**, *48*, 31–47.
4. (a) Luo, Z.; Zhang, Q.; Oderaotoshi, Y.; Curran, D. P. *Science* **2001**, *291*, 1766–1769; (b) Luo, Z.; Williams, J.; Read, R. W.; Curran, D. P. *J. Org. Chem.* **2001**, *66*, 4261–4266; (c) Curran, D. P.; Luo, Z. *J. Am. Chem. Soc.* **1999**, *121*, 9069–9072.
5. Chen, W. P.; Xu, L. J.; Xiao, J. L. *Tetrahedron Lett.* **2001**, *42*, 4275–4278.
6. Herrmann, W. A.; Brossmer, C.; Reisinger, C. P.; Reiermeier, T. H.; Ofele, K.; Beller, M. *Chem. Eur. J.* **1997**, *3*, 1357–1364.



7. Keuning, K. J.; Evenhuis, N. *Recl. Trav. Chim. Pays-Bas* **1935**, *54*, 73–75.
8. Selected analytical data: **1**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.31 (m 4H); 5.27 (s 2H); 2.98–2.90 (m 2H); 2.25–2.43 (m 2H). ^{13}C NMR (50.1 MHz, $CDCl_3$) δ 150.5 (C=O); 140.6 (CH); 131.9 (C_q); 129.5 (CH); 128.8 (CH); 73.1 (CH_2); 32.75; (m CH_2); 26.20 (br. CH_2). ESI MS (m/z) 537 $(M-CO_2-Cl^-)^+$.
2: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.32 (d, $J=9$ Hz, 1H); 7.11 (m 2H); 5.31 (s 2H); 2.90 (m 2H); 2.38 (s, m 5H). ^{13}C NMR (50.1 MHz, $CDCl_3$) δ 150.2 (C=O); 140.86 (C_q); 130.89 (CH); 130.71 (CH); 129.49 (C_q); 126.21 (CH); 71.63 (CH_2); 32.3 (CH_2); 26.14 (CH_2); 18.84 (Me).
3: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.47 (m 4H); 7.27 (dt, $J=16.1$ Hz ^{4}J (H, F) $=2.2$ Hz, 1H); 6.25 (dt, $J=16.1$ Hz ^{3}J (H,F) 12.1 Hz, 1H); 5.31 (s 2H). ^{13}C NMR (50.1 MHz, $CDCl_3$) δ 150.8 (C=O); 138.9 (t, ^{3}J (C, F) $=9.9$ Hz, CH); 135.4 (C_q); 134.6 (C_q); 129.3 (CH); 128.0 (CH); 115.6 (t, $^{2}J=23$ Hz, CH); 72.5 (CH_2).
10: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.88 (d, $J=9$ Hz, 1H); 7.08 (m 2H); 3.89 (s 3H); 2.96 (m 2H); 2.60 (s 3H); 2.37 (m 2H). Anal. calcd for $C_{19}H_{13}F_{17}O_2$: C, 38.27; H, 2.20. Found: C, 37.99; H, 2.45.
9. The TNBS test (Hancock, W. S.; Battersby, J. E. *Anal. Biochem.* **1976**, *71*, 260–264) was used for the visualization (red colour) of free amino groups. The more common Kaiser test (Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595–598), which requires heating, gave false positive results due to concomitant cleavage of the methyltrityl (Mtt) group under the applied conditions and ensuing reaction between ninhydrin and the ε-NH_2 group of lysine.
10. The Mtt-group (Aletras, A.; Barlos, K.; Gatos, D.; Koutsogianni, S.; Mamos, P. *Int. J. Peptide Protein Res.* **1995**, *45*, 488–496), which can be cleaved with 1% TFA, was used for lysine side chain protection to offset potential solubility problems inherent to partially protected oligopeptides.
11. A typical procedure for the tagging of peptides: Fmoc-deprotected peptide resin **11** (27 mg, 12 μmol) was swollen in NMP and suspended in 0.3 mL of the same solvent, FZ-Cl (**1**) (31 mg, 50 μmol) was added followed by DIPEA (15 μL, 85 μmol) and the suspension was shaken for 1 h until a negative TNBS-test was obtained. The resin was washed with NMP and DCM. Subsequently, the peptide was cleaved by washing of the resin with 1% TFA in DCM (20 mL in 10 portions for 20 min) and the bright yellow solution obtained was treated with triisopropylsilane (250 μL) until the colour disappeared. Extraction of the organic phase with water and lyophilizing of the water layer furnished tagged peptide **13** (TFA-salt, 10 mg, 60%). ESI MS 1291.5 $(M+H)^+$.
12. Fluorous HPLC was performed on a Fluophase™ column (100 mm×4.6 mm, part. size 5 μm, pore size 300 Å, Keystone, USA) eluting (1.5 mL/min) peptide **13** first with 80:10:10 MeOH/H_2O/1% aq. TFA over 2 CV (column volumes), then with a gradient up to 90:10 MeOH/1% aq. TFA over 7 CV and finally with MeOH for 10 CV. In the case of peptide **18** a gradient of trifluoroethanol from 25% to 90% in 0.05% aq. TFA over 13 CV was applied.
13. No degradation of FEZ-tag could be detected by RP HPLC analysis after treatment of peptide **16** with TFA/H_2O (98/2) for 1 h at 20°C.
14. For a recent example of a heavy-fluorous *Z*-based protection, see: Schwinn, D.; Bannwarth, W. *Helv. Chim. Acta* **2002**, *85*, 255–264.
15. Preliminary experiments indicated that a Z-based fluoroalkylated tag containing two fluorophilic tails was readily accessible via the Heck cross-coupling procedure.