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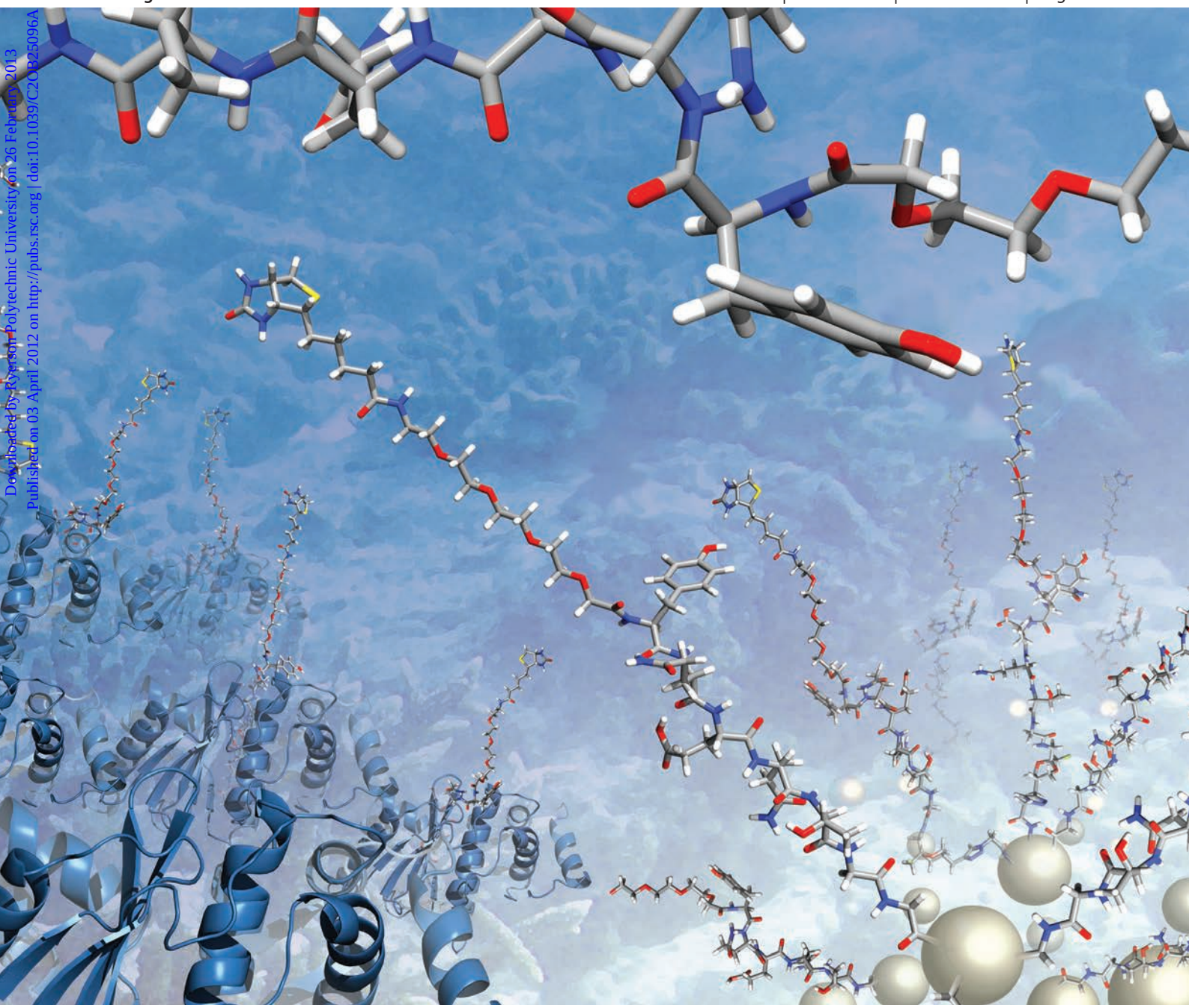

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PAPER

A general solid phase method for the synthesis of sequence independent peptidyl-fluoromethyl ketones†

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We present here a new, general, solid phase strategy for the synthesis of sequence independent peptidyl-fluoromethyl ketones using standard Fmoc peptide chemistry. Our method is based on the synthesis of bifunctional linkers which allows the incorporation of amino acid fluoromethyl ketone unit at the *C*-terminal end of peptide sequences. Application of this approach for the synthesis of activity based probes for SENPs is also described.

Introduction

Fluoromethyl ketones (FMKs) and, more often, peptidyl-fluoromethyl ketones (PFMKs) have emerged as important targets for bioorganic and medicinal chemistry. Many of these compounds have been designed as activity based probes for cysteine proteases[1] or potential drugs for treatment of certain diseases.[2] In addition, the FMK electrophilic group has been used to prepare selective activity based probes for cathepsin B[3] and G,[4] caspases,[5] calpain I,[6] SENPs[7] or *N*-glycanase.[8] Despite the number of the PFMKs reported is continuously growing, a simple, general and efficient/atom economy method to prepare FMK-based peptides is still lacking. Their synthesis is usually performed by solution coupling of the carboxy-terminated peptide sequences with the corresponding fluoroalcohol[4,6,9] followed by the alcohol oxidation with $K_2Cr_2O_7$,[10] using a modified Pfitzner–Moffat procedure[11] or Dess–Martin reagent[4,6,9a,12] to provide the expected peptidyl-fluoromethyl ketones. However, the solution coupling reaction is limited[13] to peptide sequences that do not contain cross-reacting side-chain groups (*e.g.* amino, carboxy, *etc.*) or require orthogonal protection of the side chains of amino acids (thus increasing costs and reducing yield of the final compound); moreover, a major drawback is the low solubility of the fully protected peptide, as well as the low reaction rate and racemisation of the C-terminal amino acid that may occur during the coupling reaction. Lastly, the structure of sensitive residues, in particular Cys, Met, Trp and Tyr, may be easily altered during the final oxidation process, yielding a great series of byproducts. Consequently, such routes cannot be generally applicable and it is necessary to identify a general, sequence independent, solid phase method for the synthesis of PFMKs. Such a general and smooth method would also open the access to peptide library synthesis, a field relatively less explored in the chemistry of FMKs. To our knowledge synthetic supported solid phase strategies are known for acyloxymethyl,[14] aminomethyl and thiomethyl ketones.[15] However, this method yields very often low-purity peptides (especially when long sequences are prepared). To address the above mentioned issues, we present here a new strategy to prepare PFMKs using a general, sequence independent, Fmoc-based solid phase synthetic method.

Results and discussion

Beside the difficulties encountered in the obtaining of PFMKs, the synthesis of FMKs themselves is challenging. Unlike the trifluoromethyl ketones for which mild and efficient preparation methods have been developed,[16] the synthesis of mono α-fluorinated methyl ketone derivatives is less accessible.[6a,16a,17] The direct conversion of the acids into the corresponding FMKs is usually reported, using magnesium fluoromalonate,[18] although the preparation of fluoromalonates is tedious and difficult. Reaction of allene epoxides with tetrabutylammonium fluoride trihydrate in THF[19] or dediazonative hydrofluorination of diazoketones[20] constitutes alternative strategies which proved efficient for small scale synthesis, but rather inconvenient for large scale preparations. Considering the importance of the aspartic acid fluoromethyl ketone (D-FMK) as well as the glycine fluoromethyl ketone (G-FMK) units as reactive groups in the structure of various activity based probes for caspases[1,5] and sentrin proteases (SENPs)[7] respectively, we turned our sights to

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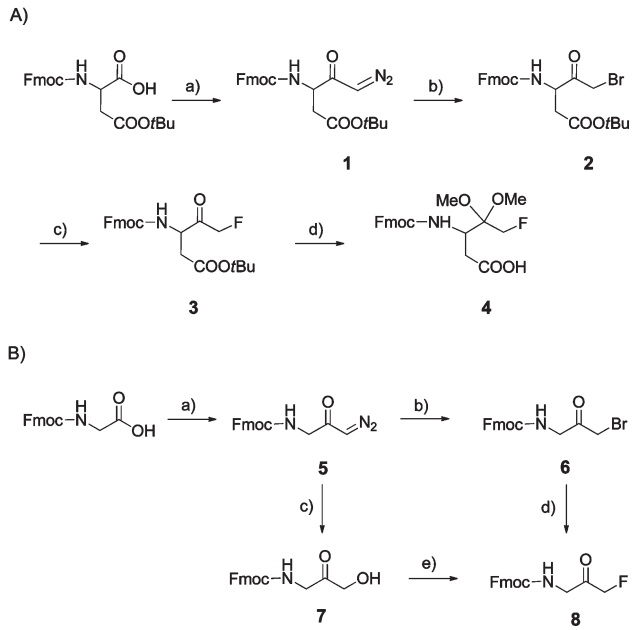
†Electronic supplementary information (ESI) available: Experimental details for synthesis of diols **9** and **10** as well as HPLC chromatograms and MS (ES^+) spectra of peptide products are given in Supporting Information file. See DOI: 10.1039/c2ob25096a



Scheme 1 Synthesis of the FMKs used in this work. Reagents and conditions: A: (a) i: *iso*-butylchloroformate, *N*-methylmorpholine, THF, −10 °C, 30 min; ii: CH_2N_2/Et_2O (*in situ*), 0 °C to r.t., 3 h, 84%; (b) HBr/HOAc (33 wt%)/water, THF, 0 °C, 15 min, quantitative; (c) TBAF, PTSA, THF, 4 h, reflux, 49%; (d) methanol, PTSA, toluene, 60 °C, 5 h, 77%. B: (a) i: *iso*-butylchloroformate; *N*-methylmorpholine, THF, −10 °C, 30 min; ii: CH_2N_2/Et_2O (*in situ*), 0 °C to r.t., 3h, 96%; (b) HBr/HOAc (33 wt%)/water, THF, 0 °C, 10 min, quantitative; (c) H_2SO_4 0.25 M, 1,4-dioxane, 90 °C, 2 h, 69%; (d) TBAF, PTSA, THF, 4 h, reflux; (e) perfluoro-1-butanesulfonyl fluoride (PBSF), $Et_3N \times 3HF$, Et_3N, acetonitrile, −30 °C to r.t., 3 h, 61%.

the synthesis of these compounds and their incorporation into peptide sequences as *C*-terminal functionalities.

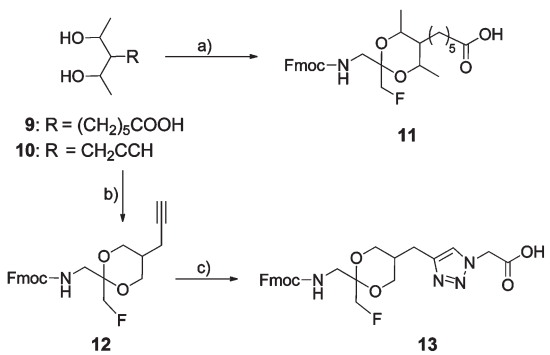
The synthetic routes to prepare the Fmoc protected D-FMK **3** and G-FMK **8** are described in Scheme 1. Briefly, Fmoc-Asp (O*t*Bu)-OH was transformed into the diazoketone derivative **1** in a two step process involving the activation of the carboxylic acid followed by reaction with diazomethane. Compound **1** was treated with HBr/HOAc to give the bromomethyl ketone **2** which was then reacted with tetra-*n*-butylammonium fluoride (TBAF) in presence of *p*-toluenesulfonic acid (PTSA)[21] to yield the fluoromethyl ketone **3**. In order to incorporate the D-FMK unit in peptide sequences, the keto group in **3** was protected as dimethylketal. Notably, the acetalization step affected the *t*-butyl ester, compound **4** being prepared with a 77% yield (Scheme 1A). Similarly, G-FMK **8** was initially attempted starting from Fmoc-Gly-OH through diazoketone **5** and bromomethyl ketone **6** (Scheme 1B). However, the separation of G-FMK **8** obtained by this method proved to be difficult. Therefore, we chose to transform the diazoketone **5** into the hydroxymethyl ketone **7** by treatment with aqueous H_2SO_4 0.25 M,[22] followed by fluorination of **7** with perfluoro-1-butanesulfonyl fluoride (PBSA) and $Et_3N \times 3HF$ complex[23] to give the target G-FMK **8** in good yield (61%).

Ketal deprotection studies conducted on **4** and monitored by TLC showed that complete hydrolysis occurs in less than 30 min at room temperature using the acidic cleavage conditions usually encountered for solid phase peptide synthesis (SPPS), namely TFA/H_2O, 95 : 5.

With FMKs **3** and **8** in our hands, we proceeded to their incorporation in peptide sequences. In the particular case of the D-FMK, the attachment to the resin can be easily performed by means of the side chain carboxylic group of compound **4**, and peptide elongation can be carried out by standard Fmoc-SPPS protocols. However, incorporation of FMKs derived from other amino acids, such as G-FMK **7**, at *C*-terminal of various peptide sequences require a different approach as a result of their reverse backbone polarity. Therefore, we focused on finding a bifunctional linker that contain: (i) a group that is able to react with the keto functionality of the FMK, is stable under the standard SPPS conditions and can be easily removed under the acidic conditions used for the peptide cleavage from the solid support; and (ii) a group which ensures the attachment of the linker to the solid support.

To do so, we initially considered a linker that contained a 1,3-diol unit useful for the ketone protection (which can be easily deprotected in acidic medium) and a carboxylic functionality required to anchor the FMK onto the resin. In the design of our linker, we took in consideration that the introduction of a carboxyl group in the structure of the 1,3-dioxane-protected FMK could influence the acid catalyzed deprotection reaction since it is known that the presence of a second withdrawing group (COOH in our case) on the dioxane ring drastically reduce the hydrolysis rate.[24]

Therefore, we based our synthesis on compound **9** (Scheme 2) which has a long aliphatic chain to counterbalance the inducing effect (−I) of the carboxyl. Moreover, the two methyl groups in positions 4 and 6 of 1,3-dioxane ring in compound **11** favour the hydrolysis reaction. Compound **9** was obtained in a three step process. First, acetylacetone was treated with ethyl-6-bromohexanoate in presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as base to give ethyl 7-acetyl-8-oxononanoate (compound **S1** in ESI†) which was further reduced with sodium borohydride and the ester group was hydrolysed to yield **9** as a mixture of diastereoisomers (see ESI† for details about the



Scheme 2 Synthesis of the protected G-FMKs. Reagents and conditions: (a) i: trimethylsilyl chloride (TMSCl), Et_3N, dry Et_2O, (**S3** in ESI†); ii: **8**, trimethylsilyl trifluoromethanesulfonate (TMSOTf), −27 °C, dry DCM, 44%; (b) i: TMSCl, Et_3N, dry Et_2O, 91% (**S4** in ESI†); ii: **8**, TMSOTf, dry DCM, −27 °C, 81%; (c) N_3CH_2COOH, Tris-[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA), $CuSO_4 \cdot 5H_2O$, ascorbic acid, H_2O/*t*-BuOH (1 : 1), 10 h, 93%.

synthesis of **9**). Compound **9** was then transformed into its *O*-silylated derivative (**S3** in ESI†) which was further reacted with **8**, using trimethylsilyl trifluoromethanesulfonate (TMSOTf) as catalyst, to afford the protected fluoromethyl ketone **11**, in 44% yield after workup, as a complex mixture of diastereoisomers as inferred from ^{1}H and ^{19}F NMR spectra.

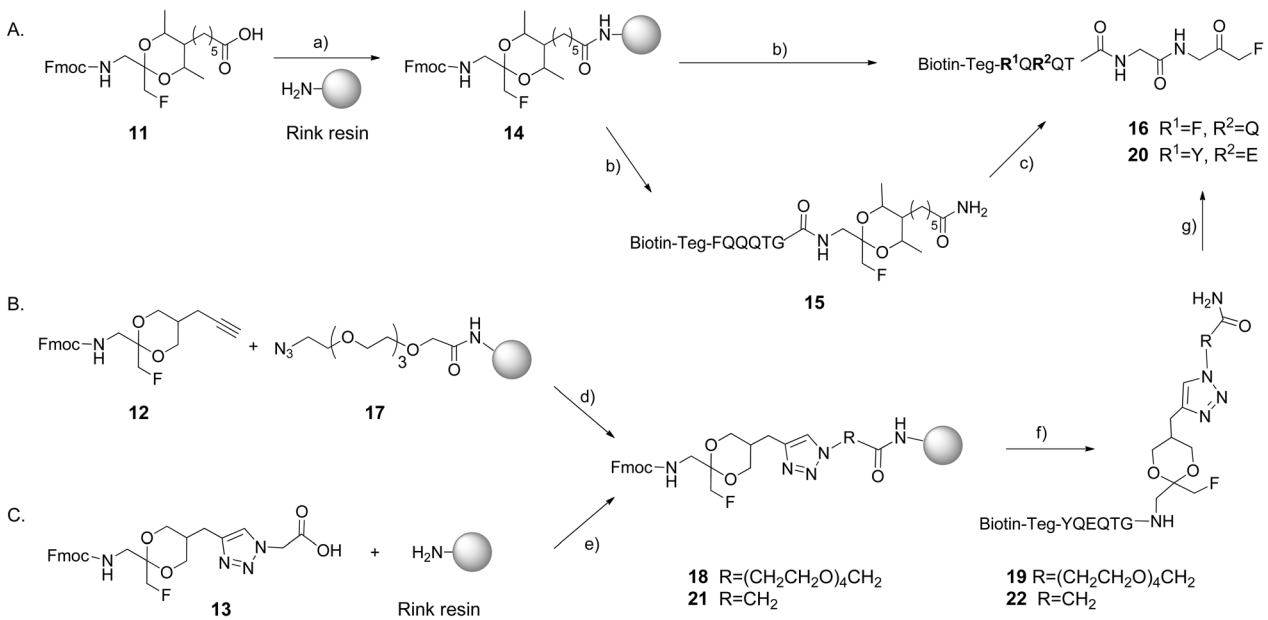
Next, we studied the deprotection of **11** to the parent G-FMK **8** under acidic cleavage conditions, as well as its stability in the SPPS conditions. Initial deprotection tests using a mixture of TFA/H_2O 95 : 5 showed partial cleavage of the dioxane with the formation of the FMK **8**. Fortunately, complete deprotection could be achieved with HCl 37%.

Since all diastereoisomers are hydrolysed to compound **8**, it was of no great advantage to separate them. Very importantly, no fluorine displacement was observed during the cleavage conditions or in the presence of nucleophilic piperidine (used for Fmoc deprotection during peptide synthesis) when compound **11** was treated with DMF/piperidine 4 : 1 for 12 hours at room temperature.

The linker utility was demonstrated by the synthesis of the peptidyl-fluoromethyl ketone **16** (Biotin-Teg-FQQQTG-**8**, Teg = tetraethylenglycol based spacer[25]) which has been recently shown to act as an activity based probe for SENPs.[7] In order to obtain peptide **16**, starting from Rink resin, dioxane **11** and the required amino acids were introduced by standard solid phase peptide chemistry using *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium hexafluorophosphate (HBTU)/*N*-hydroxybenzotriazole (HOBt) as coupling agents. After biotin insertion, the peptide was cleaved from the resin concomitant with the removal of the side chain protecting groups using a mixture of TFA/TIS(triisopropylsilane)/H_2O 95 : 2.5 : 2.5 for 3 hours at room temperature (Scheme 3A). After precipitation, isolation and purification by preparative reversed phase HPLC, a mixture of diastereomers of the 1,3-dioxane protected peptide **15** as well as the expected peptidyl-fluoromethyl ketone **16** were obtained (Scheme 3A). All diastereoisomers **15** that resisted the action of the cleavage mixture were completely hydrolysed to **16** using concentrated HCl (37%) (see ESI†).

Despite the successful synthesis of **16**, the low yield in preparation of **11** and the large number of the resulted diastereoisomers constitute important drawbacks of this method. To overcome these limitations, we envisioned another strategy that makes use of the copper catalysed *click chemistry* coupling reaction. Thus, we prepared the acetal **12** (Scheme 2) which can be either directly attached to an azide modified resin (Scheme 3B) or further functionalized with a carboxyl group containing substrate to yield **13** (Scheme 2) which can be directly attached to a commercial resin (Scheme 3C). Compared to the synthesis of **11**, preparation of 1,3-dioxane **12** involves fewer steps and occurs with higher yields. Initial deprotection tests performed in TFA/TIS/H_2O (95 : 2.5 : 2.5) unexpectedly leaved dioxane **13** intact. However, concentrated HCl (37%) could easily release FMK **8**.

Compounds **12** and **13** were further used in the synthesis of the peptide sequence **20** (Biotin-Teg-YQEQTG-**8**), derived from the sequence of SUMO1 (small ubiquitin-like modifier – the natural substrate of SENPs), which is expected to act as an activity based probe for SENPs. It is to be noted that compound **20** cannot be obtained by direct solution coupling (without protection of Asp and Tyr side chain groups) or by using oxidative methods. Firstly, alkyne **12** was attached to the azide functionalized Rink resin **17**, by *click chemistry* reaction (Scheme 3B). Peptide elongation using standard Fmoc SPPS followed by cleavage from the resin (TFA/TIS/H_2O: 95 : 2.5 : 2.5) gave peptide **19** as indicated by ES-MS spectra. Biotin-Teg-FYQETG-**8**



Scheme 3 Solid phase synthesis of PFMKs: A: Peptide sequence: Biotin-Teg-FQQQTG-**8** (**16**) (a) SPPS using Fmoc strategy: i: Attachment of **11** : **11**/HBTU/HOBt/DIPEA (1 equiv/1 equiv/1 equiv/3 equiv), DMF, r.t., 3 h; peptide elongation procedure: ii: deprotection: 20% piperidine in DMF, 2 × 10 min; iii: coupling: *N*-Fmoc-amino acid/HBTU/HOBt/DIPEA (4 equiv/4 equiv/4 equiv/12 equiv), DMF, r.t., 1 h; (b) peptide cleavage: TFA/TIS/H_2O (95 : 2.5 : 2.5) (**15**, **16**); (c) HCl 37%, r.t., 2 h, (**16**). B: (d) TBTA, $CuSO_4 \cdot 5H_2O$, ascorbic acid, H_2O/*t*-BuOH (1 : 1), 20 h (**18**); C: (e) **13**/HBTU/HOBt/DIPEA (1 equiv/1 equiv/1 equiv/3 equiv), DMF, r.t., 3 h, (**21**); (f) i: SPPS in the same conditions as above (route A), ii: TFA/TIS/H_2O (95 : 2.5 : 2.5), (**19**, **22**); (g) HCl 37%, r.t., 2 h, Biotin-Teg-YQEQTG-**8** (**20**).

peptide **20** could be obtained by hydrolysis of **19** with concentrated HCl.

The same peptide **20** was prepared by direct attachment of the dioxane **13** onto a commercial Rink resin followed by peptide elongation, cleavage with TFA/TIS/H_2O and hydrolysis of the intermediate 1,3-dioxane protected peptide **22** with HCl 37% (Scheme 3C).

Conclusions

We described a new, general and efficient solid phase synthetic method for the preparation of peptidyl-fluoromethyl ketones using standard Fmoc peptide chemistry as well as a synthetic strategy to obtain glycine and aspartic acid fluoromethyl ketones. This approach allows the facile introduction of amino acid based fluoromethyl ketones at *C*-terminal end of any peptide sequence. The utility of the solid phase method has been demonstrated by the synthesis of the PFMK **16**, which is known to act as an activity based probe for SENPs, and by obtaining a new PFMK **20**, which cannot be synthesised through previously reported methods and that is also expected to behave as an activity based probe for SENPs.

Current efforts are focused on the synthesis of PFMK libraries and study of the biological properties of the synthesized peptides.

Experimental section

General experimental information

All air and/or water sensitive reactions were performed in anhydrous solvents and under a positive pressure of argon. Dry tetrahydrofurane (THF) and diethyl ether were obtained by distillation under argon over sodium and benzophenone, while CH_2Cl_2 was distilled over CaH_2. All other solvents, reagents and resins were purchased from commercial suppliers and used without further purification. The NMR spectra were recorded at r.t. on JEOL-Delta 400, Bruker DPX-500, Bruker DPX-360, Bruker Advanced-300 or Bruker DPX-250 spectrometers. Chemical shifts (δ) are reported in parts per million (ppm) values using residual solvent peak as internal reference. Multiplicities are abbreviated as follows: br-broad; s-singlet; d-doublet; t-triplet; q-quadruplet; and m-multiplet. The mass spectra were obtained by electrospray ionization (ES) technique using a ThermoFinnigan LCQ instrument. High resolution EI mass spectra were measured on a Finnigan MAT 95S spectrometer while analyses using ESI or APCI ionization techniques were recorded on ThermoScientific LTQ-FT and LTQ XL spectrometers. Thin layer chromatography (TLC) was performed on silica gel coated aluminium F_{254} plates from Merck. All plates were visualized by UV irradiation at 254 nm and/or staining with potassium permanganate. Preparative column chromatography was performed on silica Merck 230. Reversed phase HPLC analyses and purifications of the peptide products were carried out on a VARIAN Pro Star 210 system, equipped with UV detection, using an analytical C18 VYDAC (300 Å, 4.6 mm × 150 mm, 5 μm) or a preparative C18 VYDAC (300 Å, 20 mm × 250 mm, 10 μm) columns, respectively and eluting with gradient mixtures of water (containing 0.1% TFA) and acetonitrile. The retention time (R_t) is given in minutes with the gradient in percentage of acetonitrile.

General method for the synthesis of diazoketones 1 and 5

A modification of a described procedure[14] was followed: to a solution of *N*-Fmoc-protected amino acid [(Fmoc-Gly-OH or Fmoc-Asp(O*t*Bu)-OH), 5 mmol, 1 equiv) in dry THF (25 mL), were sequentially added *N*-methylmorpholine (6.25 mmol, 1.25 equiv) and *iso*butyl chloroformate (5.75 mmol, 1.15 equiv) at −10 °C. The reaction was stirred at −10 °C for 30 min. A solution of diazomethane in diethyl ether (15–20 mmol, generated *in situ* from α-nitrosomethylurea[26] and dried over potassium hydroxide) was added to the reaction mixture at 0 °C. The reaction was allowed to warm to room temperature over a period of 3 hours and then quenched with water (10 mL) and acetic acid (0.5 mL). The reaction mixture was diluted with ethyl acetate (50 mL) and washed with water, brine and saturated aqueous sodium bicarbonate. The organic layer was dried over $MgSO_4$ and the solvents were removed at the rotary evaporator. Crude diazoketones **1** and **5** were purified by column chromatography (silica gel, ethyl acetate/pentane = 2 : 3, R_f = 0.3 for **1** and ethyl acetate/pentane = 1 : 1, R_f = 0.35 for **5**) to give **1** (1.83 g, 84%) and **5** (1.54 g, 96%), respectively.

***tert*-Butyl 3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-5-diazo-4-oxopentanoate (1).** All analytical data for **1** were found to be identical with those of the ref. 14. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.44 (s, 9H, *t*-Bu), 2.60 (dd, ^2J = 16.8 Hz, ^3J = 4.9 Hz, 1H, CH*H*COO*t*Bu), 2.91 (dd, ^2J = 16.8 Hz, ^3J = 4.9 Hz, 1H, C*H*HCOO*t*Bu), 4.21 [t, ^3J = 6.4 Hz, 1H, C*H* (Fmoc)] 4.22–4.93 [m, 2H, CH_2(Fmoc)], 4.60–4.64 (m, 1H, C*H*-NH), 5.34 (s, 1H, C*H*N_2), 5.51 (br. s, 1H, N*H*), 7.32 [t, ^3J = 7.6 Hz, 2H, H_{Ar}(Fmoc)], 7.39 [t, ^3J = 7.6 Hz, 2H, H_{Ar}(Fmoc)], 7.59 [d, ^3J = 7.6 Hz, 2H, H_{Ar}(Fmoc)], 7.77 [d, ^3J = 7.6 Hz, 2H, H_{Ar}(Fmoc)]; ^{13}C-NMR (101 MHz, $CDCl_3$) δ (ppm): 28.2, 36.9, 47.5, 66.8, 82.0, 120.2, 124.0, 125.2, 127.3, 127.9, 141.5, 143.7, 143.9, 156.0, 170.8, 192.7.

(9*H*-Fluoren-9-yl)methyl (3-diazo-2-oxopropyl)carbamate (5). ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 3.95 (s, 2H, CH_2NH), 4.22 [t, ^3J = 6.6 Hz, 1H, C*H*(Fmoc)], 4.45 [d, ^3J = 6.6 Hz, 2H, CH_2(Fmoc)], 5.27 (s, 1H, C*H*N_2), 5.51 (br. s, 1H, N*H*), 7.31 [t, ^3J = 7.2 Hz, 2H, H_{Ar}(Fmoc)], 7.40 [t, ^3J = 7.2 Hz, 2H, H_{Ar}(Fmoc)], 7.60 [d, ^3J = 7.2 Hz, 2H, H_{Ar}(Fmoc)], 7.76 [d, ^3J = 7.2 Hz, 2H, H_{Ar}(Fmoc)]; ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 43.7, 53.7, 67.3, 120.1, 124.9, 127.0, 127.8, 141.5, 143.9, 156.3, 156.7, 191.0; HRMS (ES^+) calcd for $C_{18}H_{15}N_3NaO_3$ $[M + Na]^+$: 344.1006; found: 344.1006.

General method for the synthesis of bromomethyl ketones 2 and 6

A previously reported procedure[14] was followed: a solution of diazoketone **1** (or **5**) (2 mmol) in THF (15 mL) was cooled to 0 °C and a solution of HBr 33% in acetic acid and water (1 : 2), (6 mL) was added dropwise. After the evolution of gas stopped (10–15 min), the reaction mixture was diluted with ethyl acetate and washed with water, brine and saturated aqueous sodium bicarbonate. The organic phase was dried over $MgSO_4$ and the

solvents were removed under vacuum. Bromomethyl ketones **2** and **6** were obtained as yellow (**2**) or white (**6**) solids in quantitative yields and were used further without any purification.

***tert*-Butyl 3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-5-bromo-4-oxopentanoate (2).** ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.42 (s, 9H, *t*-Bu), 2.72 (dd, ^{2}J = 17.0 Hz, ^{3}J = 4.8 Hz, 1H, CH*H*COO*t*Bu), 2.93 (dd, ^{2}J = 17.0 Hz, ^{3}J = 4.8 Hz, 1H, CH*H*COO*t*Bu), 4.06 (s, 2H, CH_2Br), 4.22 [t, ^{3}J = 6.4 Hz, 1H, C*H*(Fmoc)], 4.43 [dd, ^{2}J = 10.6 Hz, ^{3}J = 6.4 Hz, 1H. C*H*H-(Fmoc)], 4.57 [dd, ^{2}J = 10.6 Hz, ^{3}J = 6.4 Hz, 1H. CH*H*(Fmoc)], 4.63–4.76 (m, 1H, C*H*-NH), 5.80 (d, ^{3}J = 7.9, 1H, N*H*), 7.31 [t, ^{3}J = 7.5 Hz, 2H, H_{Ar}(Fmoc)], 7.41 [t, ^{3}J = 7.5 Hz, 2H, H_{Ar}(Fmoc)], 7.58 [d, ^{3}J = 7.5 Hz, 2H, H_{Ar}(Fmoc)], 7.75 [d, ^{3}J = 7.6 Hz, 2H, H_{Ar}(Fmoc)]; ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 28.0, 31.2, 36.7, 47.3, 54.2, 67.0, 82.5, 120.1, 124.9, 125.0, 127.1, 127.9, 141.4, 143.5, 155.9, 170.3, 203.0; HRMS (ES^+) calcd for $C_{24}H_{26}BrNNaO_5$ $[M + Na]^+$: 510.0887; found: 510.0887.

(9*H*-Fluoren-9-yl)methyl (3-bromo-2-oxopropyl)carbamate (6). ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 3.92 (s, 2H, CH_2Br), 4.23 [t, ^{3}J = 6.8 Hz, 1H, C*H*(Fmoc)], 4.35 (d, ^{3}J = 5.1 Hz, 2H, NH–CH_2–CO), 4.43 [d, ^{3}J = 6.8 Hz, 2H, CH_2(Fmoc)], 5.40 (s, 1H, N*H*), 7.33 [t, ^{3}J = 7.4 Hz, 2H, H_{Ar}(Fmoc)], 7.41 [t, ^{3}J = 7.4 Hz, 2H, H_{Ar}(Fmoc)], 7.60 [d, ^{3}J = 7.4 Hz, 2H, H_{Ar}(Fmoc)], 7.77 [d, ^{3}J = 7.4 Hz, 2H, H_{Ar}(Fmoc)]; ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 31.1, 47.2, 48.6, 67.4, 120.2, 125.2, 127.2, 127.9, 141.5, 143.8, 156.3, 198.0; HRMS (ES^+) calcd for $C_{18}H_{16}BrNNaO_3$ $[M + Na]^+$: 396.0206; found: 396.0206.

***tert*-Butyl 3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-5-fluoro-4-oxopentanoate (3).** To a stirred solution of **2** (300 mg, 0.616 mmol, 1 equiv) and *p*-toluenesulfonic acid (318 mg, 1.84 mmol, 3 equiv) in THF (5 mL) was added dropwise a solution of tetra-*n*-butylammonium fluoride (TBAF, 964 mg, 3.69 mmol, 6 equiv) in THF (5 mL) and the reaction mixture was heated to reflux. The reaction was monitored by TLC. After 4 hours the solvent was evaporated and the residue was dissolved in ethyl acetate (30 mL), washed with water (2 × 20 mL) and dried over $MgSO_4$. Crude **3** was purified by column chromatography (silica gel, ethyl acetate/pentane = 1 : 4, R_f = 0.33) to give **3** (130 mg, 49%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.43 (s, 9H, *t*-Bu), 2.74 (dd, ^{2}J = 16.9 Hz, ^{3}J = 4.4 Hz, 1H, CH*H*COO*t*Bu), 2.98 (dd, ^{2}J = 16.9 Hz, ^{3}J = 4.4 Hz, 1H, C*H*HCOO*t*Bu), 4.22 [t, ^{3}J = 6.4 Hz, 1H, C*H*(Fmoc)], 4.43 [dd, ^{2}J = 10.5 Hz, ^{3}J = 6.8 Hz, 1H. C*H*H(Fmoc)], 4.54 [dd, ^{2}J = 10.5 Hz, ^{3}J = 6.8 Hz, 1H. CH*H*(Fmoc)], 4.58–4.67 (m, 1H, C*H*-NH), 4.98 (dd, $^{2(H,F)}J$ = 48.3 Hz, $^{2(H,H)}J$ = 15.7 Hz, 1H, C*H*HF), 5.10 (dd, $^{2(H,F)}J$ = 48.1 Hz, $^{2(H,H)}J$ = 15.7 Hz, 1H, CH*H*F), 5.74 (d, ^{3}J = 8.5 Hz, 1H, N*H*), 7.32 [t, ^{3}J = 7.5 Hz, 2H, H_{Ar}(Fmoc)], 7.41 [t, ^{3}J = 7.5 Hz, 2H, H_{Ar}(Fmoc)], 7.58 [d, ^{3}J = 7.5 Hz, 2H, H_{Ar}(Fmoc)], 7.75 [d, ^{3}J = 7.5 Hz, 2H, H_{Ar}(Fmoc)]; ^{19}F-NMR (376 MHz, $CDCl_3$) δ (ppm): 170.89 (t (overlapped dd), $^{2(H,F)}J$ = $^{2(H,F)}J'$ = 48.8 Hz); ^{13}C-NMR (101 MHz, $CDCl_3$) δ (ppm): 28.1, 36.8, 47.4, 57.4, 67.2, 82.5, 84.3 (d, $^{1(F,C)}J$ = 183.3), 120.2, 125.0, 127.2, 128.0, 141.5, 143.7, 156.0, 170.4, 202.9 (d, $^{3(F,C)}J$ = 18.2 Hz); HRMS (ES^+) calcd for $C_{24}H_{26}FNO_5K$ $[M + K]^+$: 466.1427; found: 466.1418.

3-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-5-fluoro-4,4-dimethoxypentanoic acid (4). To a solution of **3** (100 mg, 0.23 mmol, 1 equiv) in toluene (10 mL), methanol (1 mL) and *p*-toluenesulfonic acid (4 mg, 0.023 mmol, 0.1 equiv) were added. The reaction mixture was heated at 60 °C for 5 hours and the reaction was monitored by TLC. After cooling to room temperature the reaction mixture was diluted with toluene (10 mL), washed with an aqueous solution of sodium acetate 5% (15 mL), brine (15 mL) and water (15 mL). Crude **4** was purified by column chromatography (silica gel, ethyl acetate/pentane = 1 : 3, R_f = 0.15) to yield **4** (74 mg, 77%). ^{1}H-NMR (360 MHz, $CDCl_3$) δ (ppm): 2.86 (dd, ^{2}J = 17.3 Hz, ^{3}J = 4.0 Hz, 1H, CH*H*COOH), 3.07 (dd, ^{2}J = 17.3 Hz, ^{3}J = 4.0 Hz, 1H, C*H*HCOOH), 3.70 (s, 6H, OCH_3), 4.21 [t, ^{3}J = 6.5 Hz, 1H, C*H*(Fmoc)], 4.45 [dd, ^{2}J = 10.5 Hz, ^{3}J = 6.5 Hz, 1H C*H*H(Fmoc)], 4.55 [dd, ^{2}J = 10.5 Hz, ^{3}J = 6.5 Hz, 1H CH*H*(Fmoc)], 4.61–4.77 (m, 1H, C*H*–NH), 4.98 (dd, $^{2(H,F)}J$ = 44.9 Hz, $^{2(H,H)}J$ = 14.4 Hz, 1H, C*H*HF), 5.10 (dd, $^{2(H,F)}J$ = 44.7 Hz, $^{2(H,H)}J$ = 14.4 Hz, 1H, CH*H*F), 5.73 (d, ^{3}J = 7.8 Hz, 1H, N*H*), 7.33 [t, ^{3}J = 7.3 Hz, 2H, H_{Ar}(Fmoc)], 7.42 [t, ^{3}J = 7.3 Hz, 2H, H_{Ar}(Fmoc)], 7.58 [d, ^{3}J = 7.3 Hz, 2H, H_{Ar}(Fmoc)], 7.77 [d, ^{3}J = 7.3 Hz, 2H, H_{Ar}(Fmoc)]; ^{19}F-NMR (376 MHz, $CDCl_3$) δ (ppm): 170.92 [t (overlapped dd), $^{2(H,F)}J$ = $^{2(H,F)}J'$ = 44.8 Hz]; ^{13}C-NMR (90 MHz, $CDCl_3$) δ (ppm): 35.4, 47.4, 52.42, 54.2, 67.2, 84.2 (d, $^{1(F,C)}J$ = 183.8), 120.2, 125.0, 125.1, 127.2, 128.0, 141.8, 143.5, 156.0, 171.7; HRMS (ES^+) calcd for $C_{22}H_{24}FNO_6K$ $[M + K]^+$: 456.1219; found: 456.1218.

(9*H*-Fluoren-9-yl)methyl (3-hydroxy-2-oxopropyl)carbamate (7). A solution of diazoketone **5** (1.7 g, 5.29 mmol) in 1,4-dioxane (5 mL) was added to a solution of aqueous H_2SO_4 0.25 M (22 mL) in 1,4-dioxane (10 mL) and the reaction mixture was heated at 90 °C until the evolution of gas stopped (2 hours). The reaction mixture was cooled to room temperature, basified with solid sodium bicarbonate and extracted with ethyl acetate (3 × 50 mL). Combined organic phases were washed successively with aqueous sodium bicarbonate (50 mL), water (2 × 50 mL) and brine (2 × 50 mL), dried over $MgSO_4$, concentrated *in vacuo* and purified by column chromatography (silica gel, ethyl acetate/pentane = 1 : 1; R_f = 0.49) to give **7** as white solid (1.14 g, 69%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 2.83 (br. s, 1H, OH), 4.14 (br. s, 2H, CH_2OH), 4.23 [t, ^{3}J = 6.3 Hz, 1H, C*H*(Fmoc)], 4.34 (br. s, 2H, NH–CH_2–CO), 4.44 [d, ^{3}J = 6.3 Hz, 2H, CH_2(Fmoc)], 5.39 (br. s, 1H, N*H*), 7.32 [t, ^{3}J = 7.4 Hz, 2H, H_{Ar}(Fmoc)], 7.41 [t, ^{3}J = 7.4 Hz, 2H, H_{Ar}(Fmoc)], 7.59 [d, ^{3}J = 7.4 Hz, 2H, H_{Ar}(Fmoc)], 7.77 [d, ^{3}J = 7.4 Hz, 2H, H_{Ar}(Fmoc)]; ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 47.2, 47.7, 66.7, 67.3, 120.1, 125.1, 127.2, 127.9, 141.4, 143.8, 156.5, 205.5; HRMS (ES^+) calcd for $C_{18}H_{17}NNaO_4$ $[M + Na]^+$: 334.1050; found: 334.1049.

(9*H*-Fluoren-9-yl)methyl (3-fluoro-2-oxopropyl)carbamate (8). Hydroxy ketone **7** (850 mg, 2.73 mmol, 1 equiv) was placed in a dried flask and purged twice with argon. Dry acetonitrile (25 mL) was added and the clear solution was cooled to −30 °C. To this solution perfluoro-1-butanesulfonyl fluoride (PBSF, 0.98 mL; 5.46 mmol, 2 equiv), triethylamine (1.52 mL, 10.92 mmol, 4 equiv) and Et_3N × 3 HF complex (0.89 mL, 5.46 mmol, 2 equiv) were sequentially added. The reaction was

allowed to warm to room temperature over a period of 3 hours, when TLC showed complete conversion of the substrate **7**. The solvent was evaporated under vacuum and the solid residue was purified by column chromatography (silica gel, ethyl acetate/pentane = 1 : 3, R_f = 0.40) to give **8** as a white solid (0.52 g, 61%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 4.24 [t, ^3J = 6.4 Hz, 1H, C*H*(Fmoc)], 4.33 (d, 2H, ^3J = 3.6 Hz, NH-CH_2-CO), 4.43 [d, ^3J = 6.4 Hz, 2H, CH_2(Fmoc)], 4.94 (d, $^{2(H,F)}J$ = 47.2 Hz, 2H, CH_2F), 5.42 (br. s, 1H, N*H*), 7.33 [t, ^3J = 7.4 Hz, 2H, H_{Ar}(Fmoc)], 7.41 [t, ^3J = 7.4 Hz, 2H, H_{Ar}(Fmoc)], 7.61 [d, ^3J = 7.4 Hz, 2H, H_{Ar}(Fmoc)], 7.77 [d, ^3J = 7.4 Hz, 2H, H_{Ar}(Fmoc)]; ^{19}F-NMR (376 MHz, $CDCl_3$) δ (ppm): 169.0 (t, $^{2(H,F)}J$ = 47 Hz); ^{13}C-NMR (101 MHz, $CDCl_3$) δ (ppm): 47.2, 48.2, 67.3, 84.61 (d, $^{C,F}J$ = 183.0 Hz), 120.1, 125.1, 127.2, 127.8, 141.4, 143.8, 156.3, 202.3; HRMS (ES^+) calcd for $C_{18}H_{16}FNNaO_3$ $[M + Na]^+$: 336.1006; found: 336.1007.

6-(2-(((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)methyl)-2-(fluoromethyl)-4,6-dimethyl-1,3-dioxan-5-yl)hexanoic acid (11). A solution of G-FMK (**8**) (83 mg, 0.26 mmol, 1 equiv) in dry DCM (5 mL) was added to a stirred solution of the silylated alcohol (**S3**) in DCM (15 mL), under argon. Compound **S3** (see ESI†) was obtained *in situ* from diol **9** (240 mg, 1.10 mmol). The solution was cooled to −27 °C and TMSOTf (20 µL) was added; the reaction mixture was allowed to warm to room temperature and was stirred for 12 hours. An additional amount of TMSOTf (40 µL) was added and the reaction mixture was allowed to stir for another 14 hours until TLC showed complete conversion. The crude of reaction was washed with water, the organic phase dried over anhydrous $MgSO_4$ and concentrated in vacuum. The residue was purified by column chromatography (silica gel, ethyl acetate/pentane = 1 : 1 (R_f = 0.60) to afford **11** as mixture of diastereoisomers (59 mg, 44%). ^{1}H-NMR (400 MHz, CD_3OD) δ (ppm): 0.87–1.59 (overlapped signals, aliphatic H), 2.20–2.28 (overlapped signals, aliphatic H), 4.07–4.99 (overlapped signals, aliphatic H), 7.29 [t, ^3J = 7.4 Hz, 2H, H_{Ar}(Fmoc)], 7.37 [t, ^3J = 7.4 Hz, 2H, H_{Ar}(Fmoc)], 7.64 [d, ^3J = 7.4 Hz, 2H, H_{Ar}(Fmoc)], 7.78 [d, ^3J = 7.4 Hz, 2H, H_{Ar}(Fmoc)]; ^{19}F-NMR (376 MHz, $CDCl_3$) δ (ppm): 166.66 (t, $^{2(H,F)}J$ = 47.0 Hz, CH_2F- diastereoisomer 1), 167.79 (t, $^{2(H,F)}J$ = 46.8 Hz, CH_2F- diastereoisomer 2), 168.49 (t, $^{2(H,F)}J$ = 47.9 Hz, CH_2F- diastereoisomer 3), 168.56 (t, $^{2(H,F)}J$ = 47.4 Hz, CH_2F- diastereoisomer 4), 169.06 ppm (t, $^{2(H,F)}J$ = 46.8 Hz, CH_2F- diastereoisomer 5); ^{13}C-NMR (101 MHz, $CDCl_3$) δ (ppm): 13.95, 16.49, 18.58, 19.40, 20.82, 21.60, 24.69, 26.80, 29.52, 33.91, 46.03, 47.13, 60.55, 66.13, 66.73, 67.53, 69.96, 82.5–86.4 (overlapped d), 119.87, 124.94, 126.98, 127.62, 141.23, 143.79, 156.90, 174.60; MS (ES^+): *m*/*z*: 514.3 $[M + H]^+$, 536.6 $[M + Na]^+$, 1049.4 $[2M + Na]^+$, 1065.4 $[2M + K]^+$; HRMS (ES^+) calcd for $C_{29}H_{36}FNNaO_6$ $[M + H]^+$: 536.2419; found: 536.2414.

(9*H*-Fluoren-9-yl)methyl((2-(fluoromethyl)-5-(prop-2-yn-1-yl)-1,3-dioxan-2-yl)methyl) carbamate (12). A solution of G-FMK (**8**) (100 mg, 0.319 mmol, 1 equiv) in dry DCM (5 mL) was added to a stirred solution of silylated diol **S3** (154 mg, 0.638 mmol, 2 equiv) in dry DCM (15 mL) under argon. The solution was cooled to −27 °C and TMSOTf (10 µL) was added. The reaction mixture was allowed to warm to room temperature and stirred for 48 h with TLC monitoring. After that it was washed twice with water (20 mL) and the organic phase dried over anhydrous $MgSO_4$ and concentrated in vacuum. The residue was loaded on a chromatographic column (silica gel, ethyl acetate/pentane = 1 : 2, R_f = 0.62) to afford **12** as a mixture of two diastereoisomers (*cis* and *trans*) in a ratio of 1 : 1 as determined from the ^{1}H-NMR spectrum (105 mg, 81%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.30–1.35 (overlapped peaks, 2H, C*H*CH_2CCH *cis* and *trans*); 2.03 (t, ^4J = 2.5 Hz, 2H, CH_2CC*H* *cis* and *trans*), 2.28 (dd, ^3J = 6.9 Hz, ^4J = 2.5 Hz, 2H, CHCH_2CCH *cis* or *trans*), 2.34 (dd, ^3J = 7.3 Hz, ^4J = 2.5 Hz, 2H, CHCH_2CCH *cis* or *trans*), 3.57 (overlapped peaks, 4H, NHCH_2 *cis* and *trans*), 3.82 (dd, ^2J = 11.7 Hz, ^3J = 7.6 Hz, 2H, H_{eq} *cis* or *trans*), 3.88 (dd, ^2J = 11.7 Hz, ^3J = 6.4 Hz, 2H, H_{eq} *cis* or *trans*), 4.10 (td, ^2J = 11.7 Hz, ^3J = 4.0 Hz, 4H, H_{ax} *cis* and *trans*), 4.24 [t, ^3J = 7.0 Hz, 2H, C*H*(Fmoc) *cis* and *trans*], 4.37 (d, $^{2(H,F)}J$ = 47.0 Hz, 2H, CH_2F *cis* or *trans*), 4.43 [d, ^3J = 7.0 Hz, 4H, CH_2(Fmoc) *cis* and *trans*], 4.47 (d, $^{2(H,F)}J$ = 47.5 Hz, 2H, CH_2F *cis* or *trans*), 5.00 (br. s, 2H, N*H* *cis* and *trans*), 7.32 [t, ^3J = 7.4 Hz, 4H, H_{Ar}(Fmoc) *cis* and *trans*], 7.40 [t, ^3J = 7.4 Hz, 4H, H_{Ar}(Fmoc) *cis* and *trans*], 7.60 [d, ^3J = 7.4 Hz, 4H, H_{Ar}(Fmoc) *cis* and *trans*], 7.77 [d, ^3J = 7.4 Hz, 4H, H_{Ar}(Fmoc) *cis* and *trans*]; ^{19}F-NMR (376 MHz, $CDCl_3$) δ (ppm): 169.44 ppm (overlapped t, CH_2F); ^{13}C-NMR (63 MHz, $CDCl_3$) δ (ppm): 18.4, 18.6, 21.2, 21.5, 32.8, 33.1, 34.3, 41.7, 42.7, 47.4, 60.5, 64.0, 64.2, 67.1, 70.5, 70.6, 79.2–82.8 (5 peaks overlapped d, CH_2F *cis* and *trans* and C*C*H), 96.8, 120.1, 125.2, 127.2, 127.8, 141.5, 144.1, 156.7; MS (ES^+) *m*/*z*: 410.2 $[M + H]^+$, 432.5 $[M + Na]^+$, 634.0 $[(3M + H + K)/2]^{2+}$; HRMS (ES^+) calcd for $C_{24}H_{24}FNNaO_4$ $[M + Na]^+$: 432.1582; found: 432.1579.

2-(4-((2-(((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)methyl)-2-(fluoromethyl)-1,3-dioxan-5-yl)methyl)-1*H*-1,2,3-triazol-1-yl)-acetic acid (13). To a solution of azidoacetic acid (39 mg, 0.388 mmol, 2 equiv) and alkyne **12** (53 mg, 0.129 mmol, 1 equiv) in H_2O/*t*-BuOH (1 : 1, 10 mL), tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) (3.42 mg, 6.47 µmol, 5 mol %), $CuSO_4 \cdot 5H_2O$ (1.62 mg, 6.47 µmol, 5 mol%) and a fresh solution of aqueous ascorbic acid (3.42 mg, 19.42 µmol, 15 mol%) were added. The resulted solution was stirred at room temperature under argon atmosphere for 10 hours until TLC showed the completion of the reaction. The mixture was diluted with ethyl acetate (15 mL), washed with water (2 × 15 mL) and dried over anhydrous $MgSO_4$. The organic phase was evaporated to dryness and the residue was washed with diethyl ether to remove the unreacted azidoacetic acid. The expected dioxane **13** (*cis* and *trans* isomers) precipitated as a white solid (61 mg, 93%). ^{1}H-NMR (500 MHz, MeOD) δ (ppm): 2.00–2.22 (overlapped peaks, C*H*CH_2-triazol *cis* and *trans*), 2.77 (d, ^3J = 6.7 Hz, 1H, CH_2-triazol *cis* or *trans*), 2.85 (d, ^3J = 7.1 Hz, 1H, CH_2-triazol *cis* or *trans*), 3.70–3.80 (overlapped peaks, 4H, H_{eq} *cis* and *trans*), 3.90 (br. s, 2H, NHCH_2 *cis* and *trans*), 4.01–4.06 (overlapped peaks, 4H, H_{ax} *cis* and *trans*), 4.23 [t, ^3J = 6.8 Hz, 2H, C*H*(Fmoc) *cis* and *trans*] 4.38 [d, ^3J = 6.8 Hz, 2H, CH_2(Fmoc) *cis* or *trans*], 4.41 [d, ^3J = 6.8 Hz, 2H, CH_2(Fmoc) *cis* or *trans*], 4.43 (d, $^{2(H,F)}J$ = 47.0 Hz, 2H, CH_2F *cis* or *trans*), 4.50 (d, $^{2(H,F)}J$ = 47.0 Hz, 2H, CH_2F *cis* or *trans*), 5.18 (s, 4H, CH_2COOH, *cis* and *trans*), 5.66 (br. s, 2H, N*H* *cis* and *trans*), 7.26–7.33 [overlapped peaks, 5H, H_{Ar}(Fmoc) *cis* and *trans* and

C*H*(triazol)], 7.34–7.41 [overlapped peaks, 4H, H_{Ar}(Fmoc) *cis* and *trans*], 7.67 [d, ^{3}J = 7.4 Hz, 4H, H_{Ar}(Fmoc) *cis* and *trans*], 7.78–7.81 [overlapped peaks, 4H, H_{Ar}(Fmoc) *cis* and *trans*]; ^{19}F-NMR (376 MHz, $CDCl_3$) δ (ppm): 169.08–169.57 (overlapped peaks, CH_2F, *cis* and *trans*); ^{13}C NMR (63 MHz, $CDCl_3$): 24.2, 24.8, 33.4, 39.1, 40.6, 47.2, 48.0, 64.1, 64.3, 65.4, 66.6, 79.3 (d, $^{1(F,C)}J$ = 167.5 Hz), 80.1 (d, $^{1(F,C)}J$ = 170.0 Hz) 112.8, 119.7, 119.9, 124.9, 126.7, 127.4, 141.0, 143.7, 156.7, 176.2; MS (ES^+) *m/z*: 511.4 $[M + H]^+$, 1021.0 $[2M + H]^+$, 1043.3 $[2M + Na]^+$, 1059.4 $[2M + K]^+$; HRMS (ES^+) calcd for $C_{26}H_{28}FN_4O_6$ $[M + H]^+$: 511.1987; found: 511.1986.

General solid phase peptide synthesis procedure

Unless otherwise stated, each building block was introduced using *N*-Fmoc-amino acid/HBTU/HOBt/DIPEA (4 equiv/4 equiv/4 equiv/12 equiv with respect to the resin loading reported by the supplier) in DMF (approximately 0.2 M) for 1–2 h at room temperature. Fmoc protecting groups were removed with piperidine/DMF 1 : 4 (2 × 10 min). The peptide was cleaved from the resin using 1 mL of cleavage cocktail (95% TFA, 2.5% H_2O, 2.5% TIS) and allowed to shake for 4 hours. Ice cold ether (40 mL) was used to precipitate the product. The resin was washed with TFA (1.5 mL) and the solution was collected separately in ice cold ether. The solid was centrifuged, separated from ether and dissolved in a minimal amount of acetonitrile/water (0.1% TFA). The product was analyzed on a C18 reversed phase high performance liquid chromatography (HPLC) column using a gradient of water (0.1% TFA)/acetonitrile and purified on a corresponding preparative C18 column, using the same gradient. Fractions containing the product were pooled, frozen and lyophilized to dryness.

Preparation of resin 14

Compound **11** (30 mg, 0.058 mmol, 1 equiv) was attached to Rink resin (83 mg, 0.7 mmol g^{-1} resin loading) using HBTU (21.9 mg, 0.058 mmol, 1 equiv)/HOBt (7.8 mg, 0.058 mmol, 1 equiv) as coupling reagents. The unreacted resin amino groups were then blocked by acetylation with acetic anhydride (10 equiv). The resin was washed with DMF and used in further step.

Preparation of Biotin-Teg-FQQQTG-11 (15) and Biotin-Teg-FQQQTG-8 (16)

Functionalized resin **14** was further elongated using standard SPPS procedure, as described above. After the TFA-mediated peptide cleavage (TFA/TIS/H_2O (95 : 2.5 : 2.5) a mixture of **15** and **16** was obtained. The peptides were separated by reversed phase HPLC R_t = 9.6–12.5 min (different diastereoisomers) for **15** and R_t = 9.5 min for **16**, using the following gradient: 20% CH_3CN, followed by a slope to 40% CH_3CN within 30 min. A solution of HCl 10 N (1 mL) was added to a solution of Biotin-Teg-FQQQTG-**11** (**15**) (4 mg/0.5 mL H_2O) and stirred at room temperature for 3 hours. The resulted crude peptide **16** was purified by RP-C18-HPLC, R_t = 13.6 min, using the following elution gradient: 2 min with 12% CH_3CN, followed by a slope to 40% CH_3CN within 16 min (yield 6 mg in total, 8%). Alternatively the crude mixture of peptides **15** and **16** was hydrolysed using HCl 10 N (1 mL) to get peptide **16** which was purified by RP-C18-HPLC using the above mentioned method. Biotin-Teg-FQQQTG-**11** (**15**): MS (ES^+) *m/z*: 1439.4 $[M + H]^+$, 1461.7 $[M + Na]^+$; Biotin-Teg-FQQQTG-**8** (**16**): (6 mg, 8% yield); MS (ES^+) *m/z*: 1240.5 $[M + H]^+$, 1262.6 $[M + Na]^+$.

Preparation of resin 18

N_3-Teg-COOH (147 mg, 0.53 mmol, 9.2 equiv) was linked on Rink resin (82 mg, 0.7 mmol g^{-1} resin loading) using HBTU/HOBt as coupling reagents. The unreacted sites were blocked with acetic anhydride (10 equiv) and the resin was washed with DMF to give resin **17**. Compound **12** (37 mg, 0.090 mmol, 1.5 equiv) was dissolved in a mixture H_2O/*t*-BuOH 1 : 1 (2 mL) and added over the resin under nitrogen atmosphere. To this mixture, TBTA (2.39 mg, 4 μmol), $CuSO_4 \cdot 5H_2O$ (1.12 mg, 4 μmol) and fresh aqueous solution of ascorbic acid (2.68 mg, 0.013 mmol) were added. The mixture was shaken at room temperature for 10 hours. *t*-BuOH (2 mL) and an additional amount of fresh solution of ascorbic acid (2.68 mg, 0.013 mmol) were added and mixture was stirred for another 10 hours. The resin **18** was then washed with *t*-BuOH and DMF and kept wet until it was used in the next step.

Preparation of resin 21

Compound **13** (35 mg, 0.68 mmol, 1 equiv) was attached to Rink resin (96 mg, 0.7 mmol g^{-1} resin loading) using HBTU/HOBt as coupling reagents. The reaction was shaken for 3 h, the resin was washed with DMF and kept wet until it was used in the next step (functionalized resin after loading approx. 23%).

Preparation of peptides 19 and 22

The functionalized resins **18** and **21** were elongated according to general SPPS procedure to afford after the TFA-mediated cleavage from the resin, the crude peptides **19** and **22**, which were purified by reversed phase HPLC, as it follows **19**: R_t = 10.6 min and R_t = 12.6 min (two diastereoisomers), RP-C18, gradient: 2 min with 12% CH_3CN, followed by a slope to 30% CH_3CN within 16 min; MS (ES^+) *m/z*: 815.8 $[(M + 2H)/2]^{2+}$, 1629.4 $[M + H]^+$, 1651.6 $[M + Na]^+$. **22**: R_t = 10.0 min and R_t = 10.9 min (two diastereoisomers), RP-C18, gradient: 2 min with 12% CH_3CN, followed by a slope to 50% CH_3CN within 16 min; MS (ES^+) *m/z*: 727.6 $[M + 2H]^{2+}$, 1453.5 $[M + H]^+$, 1475.5 $[M + Na]^+$.

Preparation of Biotin-Teg-YQEQTG-8 (20)

To a solution of peptide **19** (or peptide **22**) dissolved in water (0.5 mL) HCl 10 N (1.5 mL) was added, and the reaction mixture was stirred at room temperature for 2 hours until complete conversion of **19** (or **22**) as inferred from HPLC. The solution was frozen, lyophilized and the crude **20** was purified by RP-C18-HPLC, R_t = 7.7 min. Gradient: 2 min with 12% CH_3CN, followed by a slope to 30% CH_3CN within 16 min

(yield: 12% starting from **19**, and 14% starting from **22**, respectively). MS (ES^+) *m/z*: 1257.4 $[M + H]^+$, 1279.4 $[M + Na]^+$.

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Notes and references

1 (*a*) B. F. Cravatt, A. T. Wright and J. W. Kozarich, *Annu. Rev. Biochem.*, 2008, **77**, 383–414; (*b*) M. J. Evans and B. F. Cravatt, *Chem. Rev.*, 2006, **106**, 3279–3301; (*c*) J. C. Powers, J. L. Asgian, O. D. Ekici and K. E. James, *Chem. Rev.*, 2002, **102**, 4639–4750.

2 (*a*) R. E. Esser, R. A. Angelo, M. D. Murphey, L. M. Watts, L. P. Thornburg, J. T. Palmer, J. W. Talhouk and R. E. Smith, *Arthritis Rheum.*, 1994, **37**, 236–247; (*b*) R. E. Esser, L. M. Watts, R. A. Angelo, L. P. Thornburg, J. J. Prior and J. T. Palmer, *J. Rheumatol.*, 1993, **20**, 1176–1183.

3 (*a*) D. Rasnick, *Anal. Biochem.*, 1985, **149**, 461–465; (*b*) C. P. Lawrence, A. Kadioglu, A. L. Yang, W. R. Coward and S. C. Chow, *J. Immunol.*, 2006, **177**, 3827–3836; (*c*) N. K. Ahmed, L. A. Martin, L. M. Watts, J. Palmer, L. Thornburg, J. Prior and R. E. Esser, *Biochem. Pharmacol.*, 1992, **44**, 1201–1207.

4 N. P. Peet, J. P. Burkhart, M. R. Angelastro, E. L. Giroux, S. Mehdi, P. Bey, M. Kolb, B. Neises and D. Schirlin, *J. Med. Chem.*, 1990, **33**, 394–407.

5 (*a*) C. J. Van Noorden, *Acta Histochem.*, 2001, **103**, 241–251; (*b*) Y. Zhao, T. L. Brown, H. Kohler and S. Muller, *Apoptosis*, 2003, **8**, 631–637.

6 (*a*) S. Chatterjee, K. Josef, G. Wells, M. Iqbal, R. Bihovsky, J. P. Mallamo, M. A. Ator, D. Bozyczko-Coyne, S. Mallya, S. Senadhi and R. Siman, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 1237–1240; (*b*) S. Chatterjee, M. A. Ator, D. Bozyczko-Coyne, K. Josef, G. Wells, R. Tripathy, M. Iqbal, R. Bihovsky, S. E. Senadhi, S. Mallya, T. M. O'Kane, B. A. McKenna, R. Siman and J. P. Mallamo, *J. Med. Chem.*, 1997, **40**, 3820–3828.

7 C. Dobrotă, D. Fasci, N. D. Hădade, G.-D. Roiban, C. Pop, V. M. Meier, I. Dumitru, M. Matache, G. S. Salvesen and D. P. Funeriu, *ChemBioChem*, 2012, **13**, 80–84.

8 M. D. Witte, C. V. Descals, S. V. P. de Lavoir, B. I. Florea, G. A. Van der Marel and H. S. Overkleeft, *Org. Biomol. Chem.*, 2007, **5**, 3690–3697.

9 (*a*) H. Z. Zhang, H. Zhang, W. Kemnitzer, B. Tseng, J. Cinatl Jr., M. Michaelis, H. W. Doerr and S. X. Cai, *J. Med. Chem.*, 2006, **49**, 1198–1201; (*b*) L. Revesz, L. C. Briswalter, R. Heng, A. Leutwiler, R. Mueller and H.-J. Wuethrich, *Tetrahedron Lett.*, 1994, **35**, 9693–9696.

10 B. Imperiali and R. H. Abeles, *Tetrahedron Lett.*, 1986, **27**, 135–138.

11 K. Fearon, A. Spaltenstein, P. B. Hopkins and M. H. Gelb, *J. Med. Chem.*, 1987, **30**, 1617–1622.

12 P. D. Edwards, J. A. Schwartz, M. M. Stein, D. A. Trainor and R. A. Wildonger, *U. S. Patent*, 5 194 588, 1993.

13 F. Guzmán, S. Barberis and A. Illanes, *Electron. J. Biotechnol.*, 2007, **10**, 279–314.

14 D. Kato, K. M. Boatright, A. B. Berger, T. Nazif, G. Blum, C. Ryan, K. A. Chehade, G. S. Salvesen and M. Bogyo, *Nat. Chem. Biol.*, 2005, **1**, 33–38.

15 (*a*) W. J. Wood, L. Huang and J. A. Ellman, *J. Comb. Chem.*, 2003, **5**, 869–880; (*b*) A. Lee, L. Huang and J. A. Ellman, *J. Am. Chem. Soc.*, 1999, **121**, 9907–9914.

16 (*a*) S. Rozen and R. Filler, *Tetrahedron*, 1985, **41**, 1111–1153; (*b*) R. J. Linderman and D. M. Graves, *Tetrahedron Lett.*, 1987, **28**, 4259–4262; (*c*) Y. Hanzawa, A. Yamada and Y. Kobayashi, *Tetrahedron Lett.*, 1985, **26**, 2881–2884.

17 D. E. McClure, B. H. Arison, J. H. Jones and J. J. Baldwin, *J. Org. Chem.*, 1981, **46**, 2431–2434.

18 J. T. Palmer, *U. S. Patent*, 5 101 068, 1992.

19 M. M. Kabat, *J. Fluorine Chem.*, 1989, **42**, 435–439.

20 G. A. Olah and J. Welch, *Synthesis*, 1974, 896–497.

21 T. Matsumoto, M. Ohsaki, F. Matsuda and S. Terashima, *Tetrahedron Lett.*, 1987, **28**, 4419–4422.

22 J. Haddow, C. J. Suckling and H. C. S. Wood, *J. Chem. Soc., Perkin Trans. 1*, 1989, 1297–1304.

23 D. Zhou, K. E. Carlson, J. A. Katzenellenbogen and M. J. Welch, *J. Med. Chem.*, 2006, **49**, 4737–4744.

24 P. G. M. Wuts and T. W. Greene, *Greene's Protective Groups in Organic Synthesis*, John Wiley & Sons, New Jersey, 2007.

25 N. D. Bogdan, M. Matache, V. M. Meier, C. Dobrota, I. Dumitru, G. D. Roiban and D. P. Funeriu, *Chem.–Eur. J.*, 2010, **16**, 2170–2180.

26 (*a*) F. Arndt, *Org. Synth.*, 1943, **Coll. Vol. 2**, 165–167; (*b*) F. Arndt, *Org. Synth.*, 1943, **Coll. Vol. 2**, 461–463.