Supporting Information

New TFA-Free Cleavage and Final Deprotection in Fmoc Solid-Phase Peptide Synthesis: Dilute HCl in Fluoro Alcohol

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Materials and Methods

Fmoc amino acids, Fmoc-Leu-Wang resin and Rink amide NovaPEG® resin were from Novabiochem® (UK). PAL-PEG-PS® resin and Fmoc-Gly-HMPA-PEG-PS® resin were from Applied Biosystems (USA). TentaGel® S Trt Fmoc-Ser(tBu) resin was received from Rapp Polymere Gmbh (Germany). 2-(7-Aza-1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), 2,2,2-trifluoroethanol (TFE) and 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) were from Fluorochem (UK). Dichloromethane, acetonitrile, methanol, isopropanol, diethyl ether and water (all HPLC grade) and conc. (*ca*. 37% w/v) hydrochloric acid were purchased from Fisher Scientific (UK). *N*,*N*-Dimethylformamide (DMF), 1-methylpyrrolidin-2-one (NMP), *N*,*N*-diisopropylethylamine (DIEA) and trifluoroacetic acid (TFA) (peptide synthesis grade), 1,4-dioxane and acetone (HPLC grade) as well as thioanisole, dimethyl sulfide, triisopropylsilane and piperidine were all obtained from Sigma-Aldrich (UK). All reagents and solvents were used as received. Room temperature (rt) refers to ambient temperature.

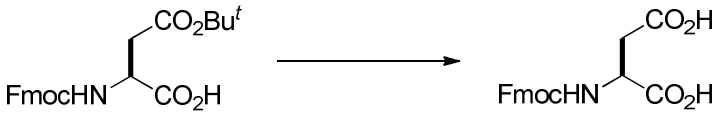
The individual compounds and reaction mixtures were analysed by reverse-phased (RP) HPLC on a Perkin-Elmer System 200 HPLC chromatography system at rt using a C18 Supelco column 250×22 mm and a linear gradient of 0-90% of acetonitrile/0.01 N aq HCl (Buffer B) in H_2O/0.01 N aq HCl (Buffer A)[1] in 30 min and a flow rate of 4 cm^3 min^{-1}. The appropriate fractions were collected and analyzed by ESI HRMS (Thermo Scientific LTQ Orbitrap XL) or MALDI-TOF (Perseptive BioSystems Voyager DE workstation, 10 mg cm^{-3} α-cyano-4-hydroxycinnamic acid in 50% aqueous acetonitrile containing 3% (v/v) TFA as a matrix) in a positive ion mode. Concentrations of amino acid deprotection samples were 2 mg cm^{-3}. Volume of the samples was 1 cm^3 each. The deprotection reactions were carried out at rt unless stated otherwise. All the amino acid derivatives tested except Fmoc-Cys(Trt)-OH were completely soluble in HFIP at 2 mg cm^{-3}.

Experimental Procedures

1. Removal of the *N*-trityl group from Fmoc-Asn(Trt)-OH

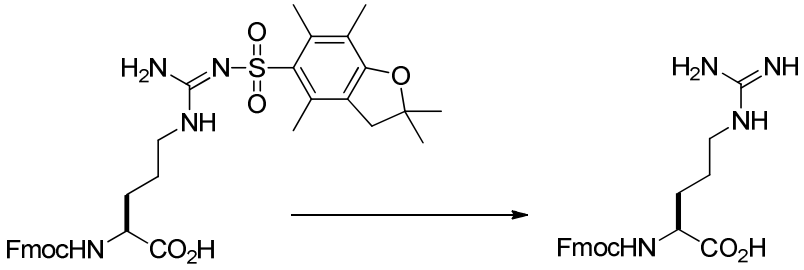
Two milligrams of the amino acid derivative were weighed into a screw-capped glass vial (1.5 cm^3), and 1 cm^3 of 0.1 N (*ca*. 0.37% w/v) hydrochloric acid in HFIP (10 μL of *ca*. 37% w/v aq HCl per 990 μL of HFIP) was added. The solution was left standing for the period of time indicated below and then analyzed by RP-HPLC. Judging by the appearance of intense yellow color of the trityl cation, the removal of the *N*-Trt group from Fmoc-Asn(Trt)-OH by 0.1 N HCl in HFIP was very fast. After 10 min of reaction, no trace of the starting material could be discerned in the HPLC trace (**Fig. S1**).

2. Removal of the *t*-butyl ester group from Fmoc-Asp(OtBu)-OH



The experiment was carried out as described above. We have observed clean *t*-butyl ester removal from Fmoc-Asp(OtBu)-OH by 0.1 N HCl in HFIP within 4 h at rt (**Fig. S2**).

3. Removal of the N^G-2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulphonyl (Pbf) group from Fmoc-Arg(Pbf)-OH



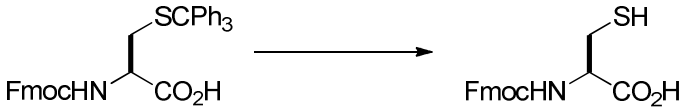
The experiments were carried out essentially as described above. Two milligrams of the amino acid derivative were weighed into a screw-capped glass vial (1.5 cm^3), and 1 cm^3 of either 0.1 N HCl in HFIP or 1 N HCl in HFIP or 0.1 N HCl in HFIP containing additional solvent was added. The nature and percentage of a solvent is indicated below. The solution was left standing for the period of time indicated below and then analyzed as described.

The Pbf group is one of the most difficult to remove acid-labile protecting groups employed in the Fmoc SPPS.[2] We have found that it could be removed from Fmoc-Arg(Pbf)-OH cleanly and quantitatively by 0.1 N HCl in HFIP within 3-4 h at rt (**Fig. S3**). If the concentration of HCl in HFIP was increased to 1 N, the removal was faster and was >98% complete within 30 min (**Fig. S4**).

Notably, hydrogen-bonding solvents such as DMF or NMP had a markedly negative effect on the rate of Pbf deprotection. Addition of just 10% (v/v) of any of the above solvents has led to complete suppression of the Pbf removal by 0.1 N HCl and HFIP within 1 h 10 min – 1 h 30 min (**Fig. S5**). However, in the presence of 20% (v/v) of a non-hydrogen-bonding solvent CH_2Cl_2 quantitative deprotection of Fmoc-Arg(Pbf)-OH by 0.1 N HCl and HFIP was achieved in 1 h 30 min (**Fig. S5**).

Even small quantities of hydrogen-bonding solvents had rather suppressive effect on the rate of Pbf removal. In the presence of just 5% (v/v) isopropanol a noticeable slowdown of the Pbf deprotection by 0.1 N HCl in HFIP has occurred while the increase in the percentage of the solvent to 10% has resulted in complete inhibition of the reaction (**Fig. S6**).

4. Removal of the *S*-trityl group from Fmoc-Cys(Trt)-OH with and without 1% (v/v) triisopropylsilane (TIS)



The experiments were carried out essentially as described above. Two milligrams of the amino acid derivative were weighed into a screw-capped glass vial (1.5 cm^3) and 1 cm^3 of either neat HFIP or

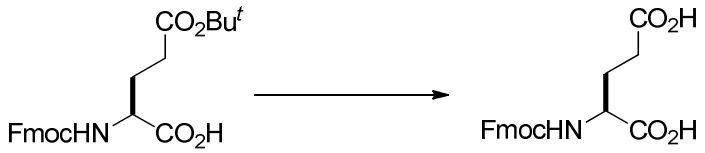
0.1 N HCl in HFIP or 0.1 N HCl in HFIP with 1% (v/v) TIS was added. The mixture was keft standing for the period of time indicated below and then analyzed as described.

The cysteine derivative was not completely soluble in HFIP at 2 mg cm^{-3}. The yellow color of the trityl cation appeared instantly when HFIP was added to solid Fmoc-Cys(Trt)-OH. Some deprotection even in the absence of any external acid was detected when the dispersion of Fmoc-Cys(Trt)-OH was kept for 30 min at rt (**Fig. S7**). With 0.1 N HCl in HFIP the removal of the *S*-trityl group was very rapid. Less than 1% of the starting material has remained after 5 min of reaction. Addition of 1% (v/v) TIS as a scavenger has led to quantitative deprotection after 35 min. The silane had no apparent effect on the deprotection rate.

5. Removal of the *N*-trityl group from Fmoc-Gln(Trt)-OH

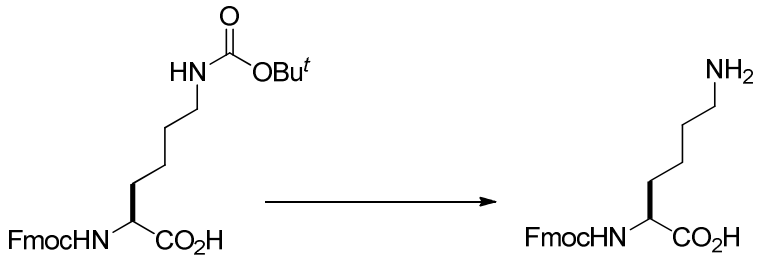
The experiment was carried out as described in entry **1**. The yellow colour of the trityl cation has appeared instantly on dissolution of the sample of Fmoc-Gln(Trt)-OH in 0.1 N HCl in HFIP. Deprotection of the *N*-Trt group was >99% complete after 15 min at ambient temperature (**Fig. S8**).

6. Removal of the *t*-butyl ester group from Fmoc-Glu(OtBu)-OH



The experiment was carried out as described in entry **1**. The *t*-butyl ester group was removed quantitatively from Fmoc-Glu(OtBu)-OH by 0.1 N HCl in HFIP within 4 h at rt (**Fig. S9**).

7. Removal of the Boc group from Fmoc-Lys(Boc)-OH



7a. Removal of the Boc group from Fmoc-Lys(Boc)-OH by neat HFIP at 60^{o}C.

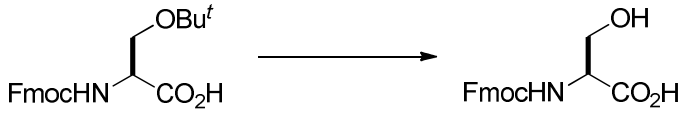
Two milligrams of the amino acid derivative were weighed into a screw-capped polypropylene tube with rubber O-ring (1.5 cm^3), 1 cm^3 of HFIP was added and the solution was kept in the oven at 60 ^{o}C for a period of time indicated below and then analyzed as described.

A removal of the Boc group from Boc-protected compounds in a fluoro alcohol solution at elevated temperature has been reported.[3] In our hands, the deprotection of Fmoc-Lys(Boc)-OH in neat HFIP was very sluggish, leading to less than 5% Boc cleavage after 4 h at 60 ^{o}C (**Fig. S10**).

7b. Removal of the Boc group from Fmoc-Lys(Boc)-OH by 0.1N HCl in HFIP at rt.

The experiment was carried out as described in entry **1**. We have observed clean and quantitative Boc removal from Fmoc-Lys(Boc)-OH by 0.1 N HCl in HFIP within 4 h at ambient temperature (**Fig. S11**).

8. Removal of the *t*-butyl ether group from Fmoc-Ser(tBu)-OH



8a. Removal of the *t*-butyl ether group from Fmoc-Ser(tBu)-OH by neat HFIP at 60^{o}C.

The experiment was carried out as described in entry **7a**. Deprotection of the *t*-butyl ether group from Fmoc-Ser(tBu)-OH in neat HFIP at 60 ^{o}C was very sluggish producing traces (>1%) of the deprotected compound after 4 h of reaction (**Fig. S12**).

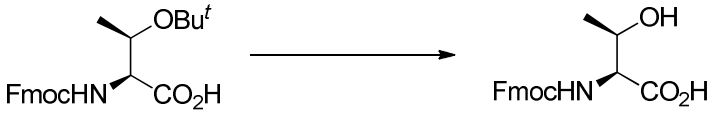
8b. Removal of the *t*-butyl ether group from Fmoc-Ser(tBu)-OH by 0.1N HCl at rt.

The experiments were carried out essentially as described in entry **1**. Two milligrams of the amino acid derivative were weighed into a screw-capped glass vial (1.5 cm^3), and 1 cm^3 of either 0.1 N HCl in HFIP or 0.1 N HCl in MeCN was added. The solution was left standing for the period of time indicated below and then analyzed as described.

We have observed clean *t*-butyl ether removal from Fmoc-Ser(tBu)-OH by 0.1 N HCl in HFIP within 4 h at rt (**Fig. S13**). Over 75% of the protecting group was cleaved after 5 min of reaction and after 1 h 30 min the conversion to the deprotected compound was over 95% complete (**Fig. S14**).

When a hydrogen-bonding solvent acetonitrile was substituted for HFIP for the deprotection of Fmoc-Ser(tBu)-OH with 0.1 N HCl, the reaction rate dropped down dramatically, producing *ca*. 3% *t*-butyl ether removal after 1 h (**Fig. S15**).

9. Removal of the *t*-butyl ether group from Fmoc-Thr(tBu)-OH



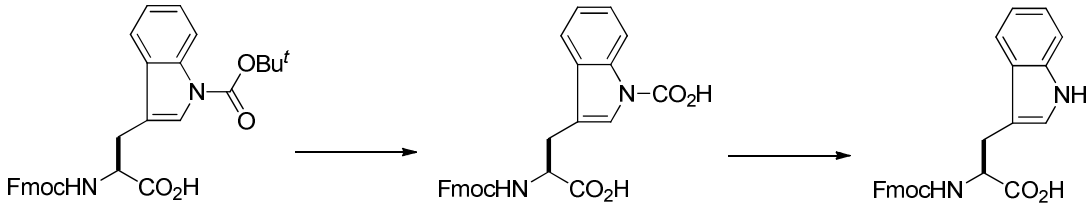
9a. Removal of the *t*-butyl ether group from Fmoc-Thr(tBu)-OH by neat HFIP at 60^{o}C.

The experiment was carried out as described in entry **7a**. Deprotection of the *t*-butyl ether group from Fmoc-Thr(tBu)-OH in neat HFIP at 60 ^{o}C was pretty sluggish producing *ca*. 2% of the deprotected compound after 4 h of reaction (**Fig. S16**).

9b. Removal of the *t*-butyl ether group from Fmoc-Thr(tBu)-OH by 0.1N HCl in HFIP at rt.

The experiment was carried out as described in entry **1**. The *t*-butyl ether group was removed cleanly from Fmoc-Thr(OtBu)-OH by 0.1 N HCl in HFIP within 4 h at rt (**Fig. S17**).

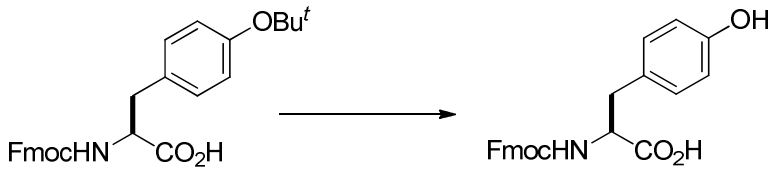
10. Removal of the N^{In}-Boc group from Fmoc-Trp(Boc)-OH



The experiment was carried out as described in entry **1**. The N^{In}-Boc group was removed quantitatively from Fmoc-Trp(Boc)-OH by 0.1 N HCl in HFIP within 4 h at ambient temperature (**Fig. S18**). Twin peaks of the deprotected compound may be explained by the persistence of the intermediate product Fmoc-tryptophane *N*-carboxylic acid in the buffer for the time of the HPLC experiment. In the ESI^+ HR-MS spectrum there is only one peak of the deprotected Fmoc-Trp-OH.

The deprotection of the Boc group from Trp was fast and quantitative with no starting material remaining after 30 min of reaction (**Fig. S19**). Notably, no side-products resulting from *t*-butylation of the indole ring of Trp were detected even after 4 h of reaction in 0.1 N HCl in HFIP at rt.

11. Removal of the *t*-butyl ether group from Fmoc-Tyr(tBu)-OH



11a. Removal of the *t*-butyl ether group from Fmoc-Tyr(tBu)-OH by neat HFIP at 60^{o}C.

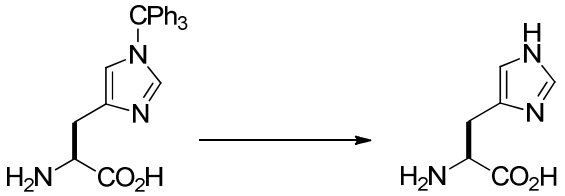
Two experiments were carried out essentially as described in entry **7a** except that the temperature was 60 ^{o}C in one case and 100 ^{o}C in the other. Removal of the *t*-butyl ether group from Fmoc-Tyr(tBu)-OH in neat HFIP at 60^{o}C was slow (**Fig. S20**). Prolonging reaction time was marginally successful: after 18 h of reaction at 60 ^{o}C less than 50% of the deprotected compound was produced together with *ca*. 2% of a side-product. Increasing the temperature to 100 ^{o}C led to a marginal improvement. At the same time the amount of the side-product has increased to 7% (**Fig. S20**, peak 3). As no encouraging results were obtained with the removal of the acid-labile protecting groups of other

amino acids (Lys, Ser and Thr) by neat HFIP as well, the idea of peptide deprotection by a fluoro alcohol at elevated temperature (60-100 ^{o}C) was abandoned.

11b. Removal of the *t*-butyl ether group from Fmoc-Tyr(tBu)-OH by 0.1N HCl in HFIP at rt.

The experiment was carried out as described in entry **1**. Removal of the *t*-butyl ether from Fmoc-Tyr(tBu)-OH was quantitative within 4 h of reaction with 0.1 N HCl in HFIP at rt (**Fig. S21**).

12. Removal of the N^{Im}-trityl group from H-His(Trt)-OH by 0.01N HCl in HFIP at rt.



The experiment was carried out essentially as described in entry **1** except that 0.01 N HCl in HFIP was used. The N^{Im}-trityl group on His is one of the most sensitive acid-labile protecting groups in the Fmoc SPPS being partially removed even under such mild conditions as HFIP – CH_2Cl_2 (1:4 v/v).[4] A quantitative removal of the N^{Im}-Trt group from H-His(Trt)-OH was achieved even by very dilute (0.01 N) HCl in HFIP within 30 min at rt (**Fig. S22**).

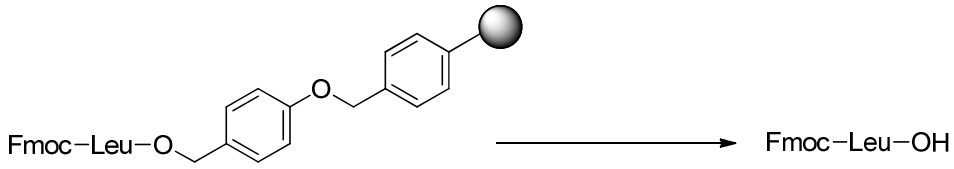
Table S1. MS data for amino acid derivatives.

#	Starting amino acid	Product	ES+ TOF HRMS, Da	
			Calc.	Obs.
1	Fmoc-Asn(Trt)-OH	Fmoc-Asn-OH	355.1294	355.1295
2	Fmoc-Asp(OtBu)-OH	Fmoc-Asp-OH	356.1134	356.1129
3	Fmoc-Arg(Pbf)-OH	Fmoc-Arg-OH	397.1876	397.1875
4	Fmoc-Cys(Trt)-OH	Fmoc-Cys-OH	344.0957	344.0973
5	Fmoc-Glu(OtBu)-OH	Fmoc-Glu-OH	370.1291	370.1275
6	Fmoc-Lys(Boc)-OH	Fmoc-Lys-OH	369.1814	369.1798
7	Fmoc-Ser(tBu)-OH	Fmoc-Ser-OH	328.1185	328.1168
8	Fmoc-Thr(tBu)-OH	Fmoc-Thr-OH	342.1341	342.1325
9	Fmoc-Trp(Boc)-OH	Fmoc-Trp-OH	427.1658	427.1652
10	Fmoc-Tyr(tBu)-OH	Fmoc-Tyr-OH	404.1498	404.1484

13. Cleavage of common acid-labile resin linkers by 0.1N HCl in the presence or absence of HFIP or a scavenger dimethylsulfide (DMS)

A sample of an appropriate resin (100 mg) was weighed into a 1.5 cm^3 Eppendorf polypropylene tube. A 1 cm^3 of a deprotection mixture consisting of 0.1 N HCl in an appropriate solvent or a mixture of solvents with or without a scavenger dimethylsulfide was added, and the reaction vessel was left standing with occasional agitation for 3 h at ambient temperature. Aliquots of the resin (*ca*. 20 mg) were withdrawn at the intervals of 15 min, 30 min, 1 h, 2 h and 3 h, washed on a sintered glass filter with DMF (3×1 cm^3), methanol (3×1 cm^3), CH_2Cl_2 (3×1 cm^3) and diethyl ether (3×1 cm^3), drained and dried *in vacuo* for a minimum of 1 h prior to analysis. A sample of a dried resin (*ca*. 5 mg) was weighed into a 1.5 cm^3 polypropylene tube and treated with 1 cm^3 of 20% (v/v) piperidine in DMF for a minimum of 10 min to cleave off the Fmoc group remaining on the resin. A 50-100 ul aliquot of the solution was then diluted to 1 cm^3 with 20% (v/v) piperidine in DMF and analysed in a UV-Vis spectrophotometer, and the absorbance value at the wavelength of 301 nm was recorded (ε_{301} = 8,000). The results were plotted against time (**Fig. S23**).

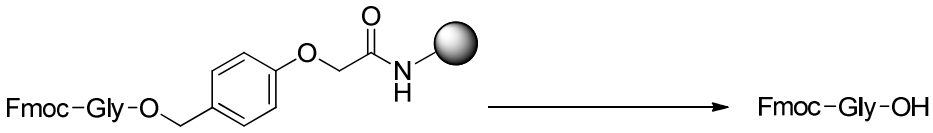
13a. Cleavage of Fmoc-Leu-Wang resin.



A sample of Fmoc-Leu-Wang resin with the initial loading of 0.958 mmol g^{-1} was treated with a mixture of 10 μL conc. (*ca*. 37% w/v) aq. HCl, 200 μL HFIP and 790 μL CH_2Cl_2. After 3 h of reaction at rt, the extent of cleavage was over 91%.

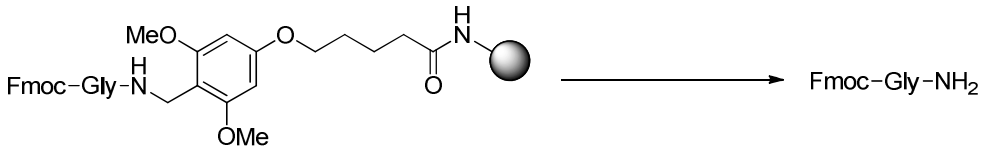
In contrast to the above, when isopropanol was substituted for HFIP in the cleavage mixture for Fmoc-Leu-Wang resin, no cleavage was detectable even after 72 h of reaction.

13b. Cleavage of Fmoc-Gly-HMPA-PEG-PS resin.



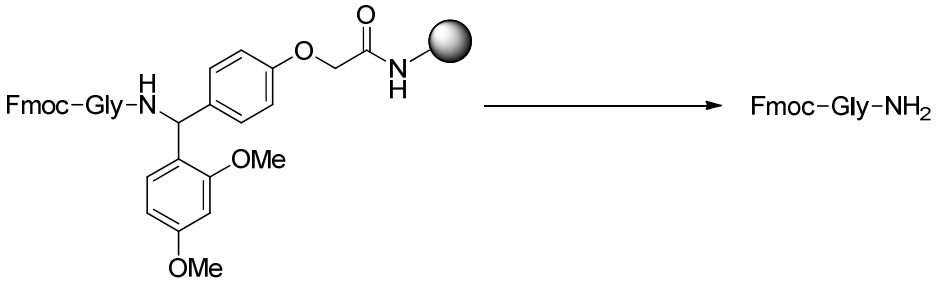
A sample of Fmoc-Gly-PEG-PS resin with the initial loading of 0.438 mmol g^{-1} was treated with a mixture of 10 μL conc. (*ca*. 37% w/v) aq HCl, 980 μL HFIP and 10 μL dimethyl sulfide (DMS) as a scavenger. After 3 h of reaction, the extent of cleavage was >84%.

13c. Cleavage of Fmoc-Gly-PAL-PEG-PS resin.



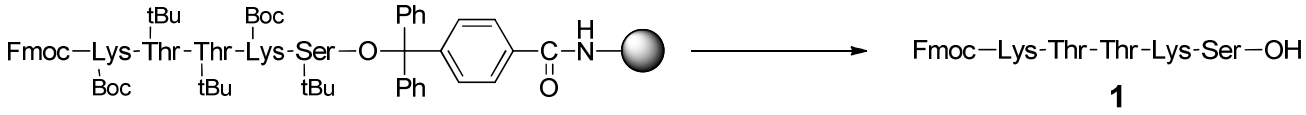
A sample of Fmoc-Gly-PAL-PEG-PS resin with the initial loading of 0.411 mmol g^{-1} was treated with a mixture of 10 μL conc. (*ca*. 37% w/v) aqueous HCl, 980 μL HFIP and 10 μL DMS. After 3 h of reaction, the extent of cleavage was 98%.

13d. Cleavage of Fmoc-Gly-Rink-NovaPEG resin.



A sample of Fmoc-Gly-Rink-NovaPEG® resin with the initial loading of 0.596 mmol g^{-1} was treated with a mixture of 10 μL conc. (*ca*. 37% w/v) aq HCl, 980 μL HFIP and 10 μL DMS. After 3 h of reaction, the extent of cleavage was >84%.

14. Synthesis of collagen-related peptides RCO-Lys-Thr-Thr-Lys-Ser-OH (1-7).



A peptide Fmoc-Lys-Thr-Thr-Lys-Ser-OH (**1**) was prepared by the Fmoc SPPS as described[5] on TentaGel® S Trt resin preloaded with Fmoc-Ser(tBu)-OH (0.24 mmol g^{-1}) at a 1.75 mmol scale (7 g of resin) using stepwise elongation protocol and five-fold excess of each of Fmoc-Thr(tBu)-OH and Fmoc-Lys(Boc)-OH. The Fmoc group removal was achieved by 20% (v/v) piperidine in DMF for 10 min before each coupling. HATU (4.75 equiv) with DIEA (10 equiv) in NMP were employed for activation of the Fmoc amino acids for 5 min at rt prior to addition to the Fmoc-deprotected resin. The resin was agitated by a gentle stream of N_2 for 1 h, then washed with NMP (25 cm^3) and DMF (50 cm^3), and the deprotection-coupling cycle was repeated until the last amino acid (Lys) was incorporated. After that the resin was washed with MeOH (50 cm^3), CH_2Cl_2 (50 cm^3) and Et_2O (25 cm^3) and dried *in vacuo*. A sample of pre-dried resin (0.6 g) was swollen in 0.1 N HCl in HFIP (5 cm^3) and left to cleave for 15 min at ambient temperature, washed twice by 0.1 N HCl in HFIP (5 cm^3), and the washings (*ca*. 15 cm^3) were collected into a 50 cm^3 QuickFit® round-bottom flask and left standing in the dark for 12 h at rt. After that the volatiles were removed on a rotary evaporator and a crude peptide Fmoc-KTTKS-OH was obtained as off-white solid foam and analysed by RP-HPLC (**Fig. S24**) and ESI HR-MS as described. This representative protocol was used to synthesize a series of related *N*-terminally modified peptides of a general structure RCO-KTTKS-OH (**Table S2**). No appreciable influence on the peptide yield or purity was observed in the experiments where deprotections were carried out using 0.1 N HCl in TFE at rt for 12 h (**5**) or 4 h (**6**), respectively.

Table S2. MS data for peptides.

#	Peptide	Formula	R =	$[M+H]^+$	
				Calc.	Observ.
1	Fmoc–KTTKS[a]	$C_{38}H_{56}N_7O_{11}$	Fm^bO–	786.4038	786.4032
2	Myristoyl–KTTKS[a]	$C_{37}H_{72}N_7O_{10}$	$C_{13}H_{27}$–	774.5341	774.5331
3	Palmitoyl–KTTKS[a]	$C_{39}H_{76}N_7O_{10}$	$C_{15}H_{31}$–	802.5654	802.5673
4	Stearoyl–KTTKS[a]	$C_{41}H_{80}N_7O_{10}$	$C_{17}H_{35}$–	830.5967	830.5968
5	Linoleyl–KTTKS[c]	$C_{41}H_{76}N_7O_{10}$	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7$–	826.56	826.16
6	Conjugated linoleyl–KTTKS[c]	$C_{41}H_{76}N_7O_{10}$	–[d]	826.56	825.65
7	10,12-Pentacosadiynoyl–KTTKS[c]	$C_{48}H_{86}N_7O_{10}$	$CH_3(CH_2)_{11}C{\equiv}C{-}C{\equiv}C(CH_2)_8$–	921.24	921.66

[a] ESI HRMS; [b] 9-fluorenylmethyl; [c] MALDI-TOF; [d] a mixture of *cis*- and *trans*-9,11- and -10,12-octadecadienoyl.

Figures

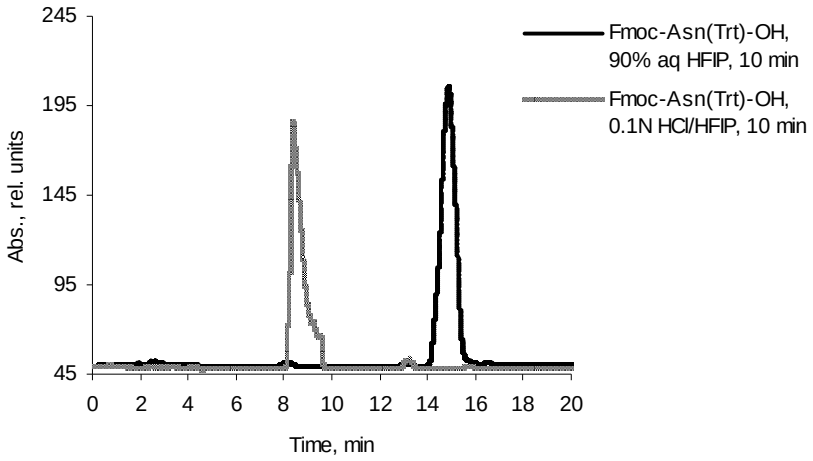


Fig. S1. Deprotection of Fmoc-Asn(Trt)-OH by 0.1 N HCl in HFIP.

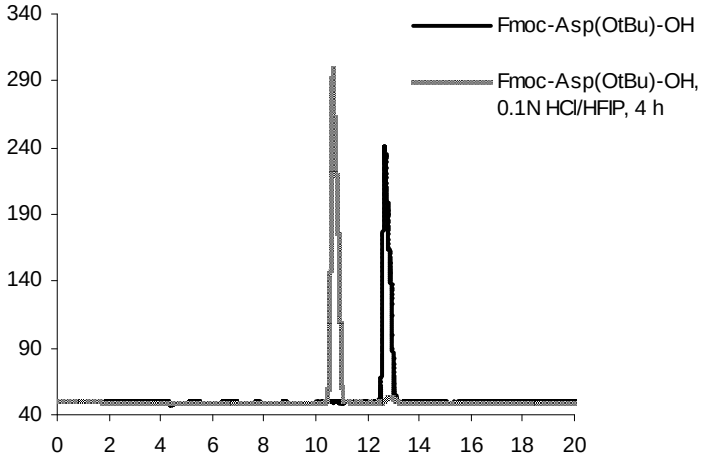


Fig. S2. Deprotection of Fmoc-Asp(OtBu)-OH by 0.1 N HCl in HFIP.

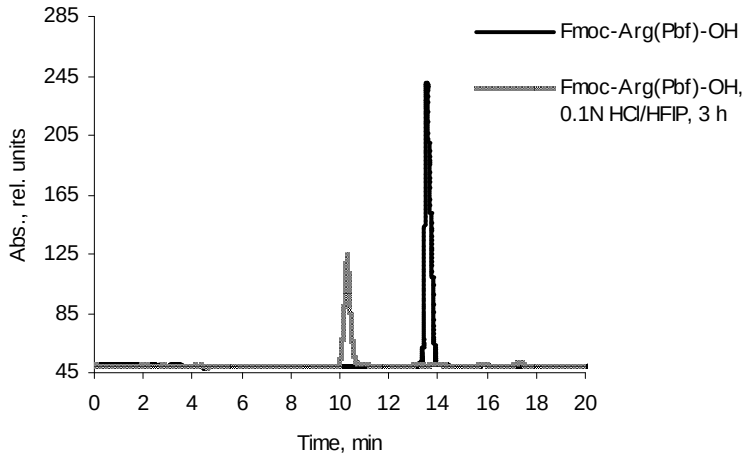


Fig. S3. Deprotection of Fmoc-Arg(Pbf)-OH by 0.1 N HCl in HFIP.

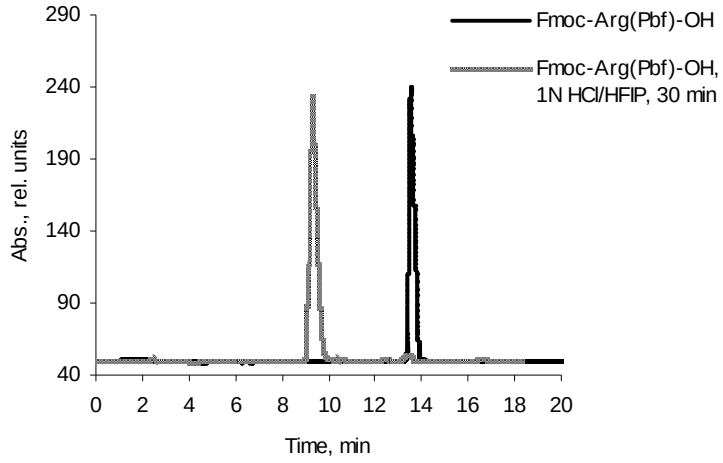


Fig. S4. Deprotection of Fmoc-Arg(Pbf)-OH by 1 N HCl in HFIP.

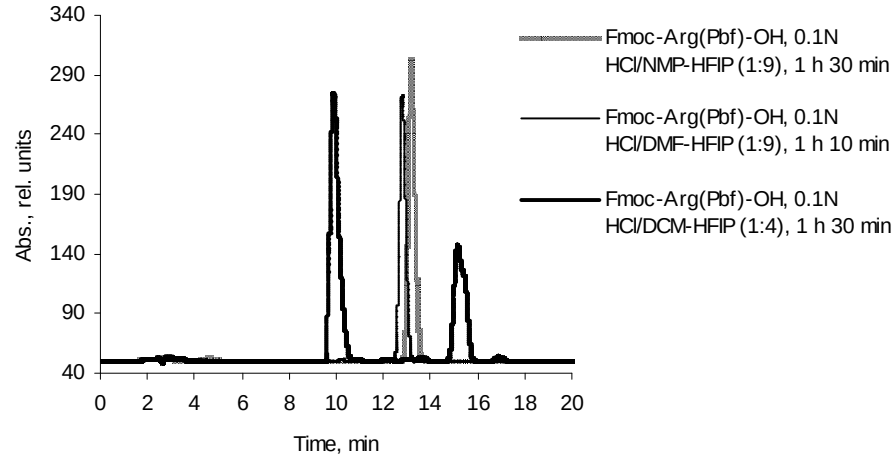


Fig. S5. Deprotection of Fmoc-Arg(Pbf)-OH by 0.1 N HCl in HFIP in the presence of a hydrogen-bonding solvent (DMF or NMP) or a non-hydrogen-bonding solvent (CH_2Cl_2).

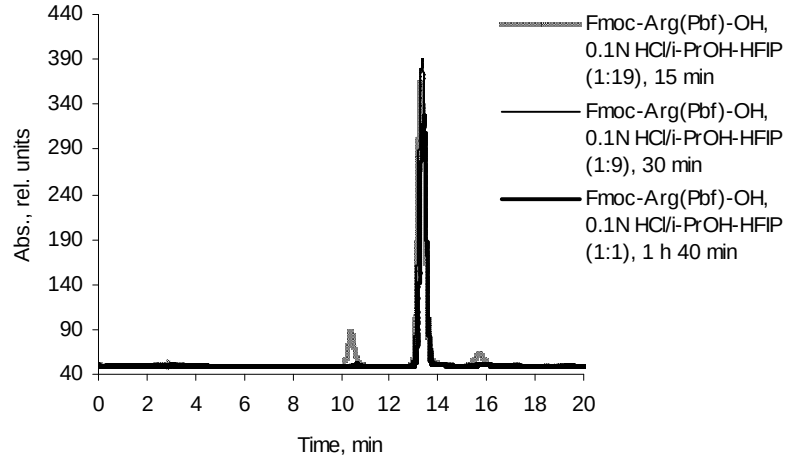


Fig. S6. Deprotection of Fmoc-Arg(Pbf)-OH by 0.1 N HCl in HFIP in the presence of increasing amounts of a hydrogen-bonding solvent isopropanol.

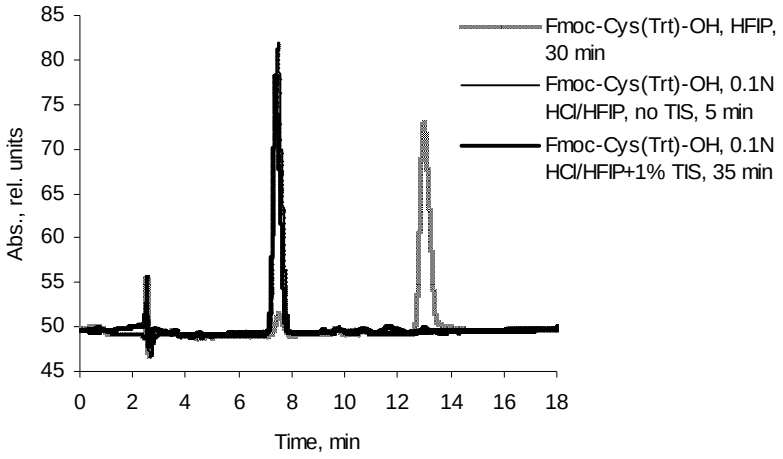


Fig. S7. Deprotection of Fmoc-Cys(Trt)-OH by 0.1 N HCl in HFIP with and without 1% (v/v) triisopropylsilane (TIS).

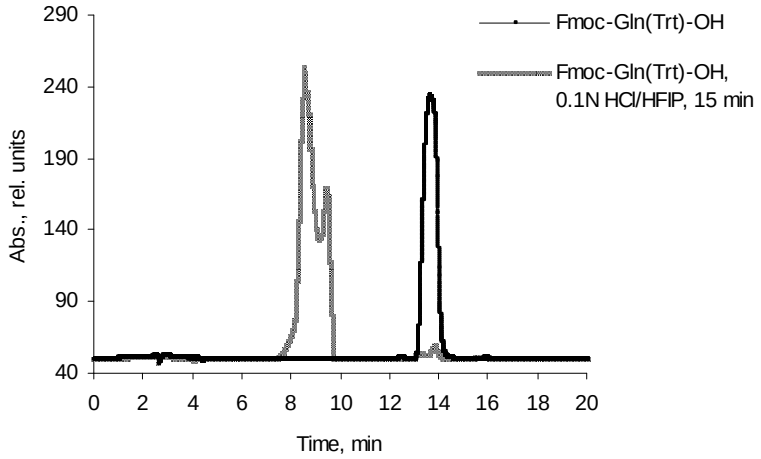


Fig. S8. Deprotection of Fmoc-Gln(Trt)-OH by 0.1 N HCl in HFIP.

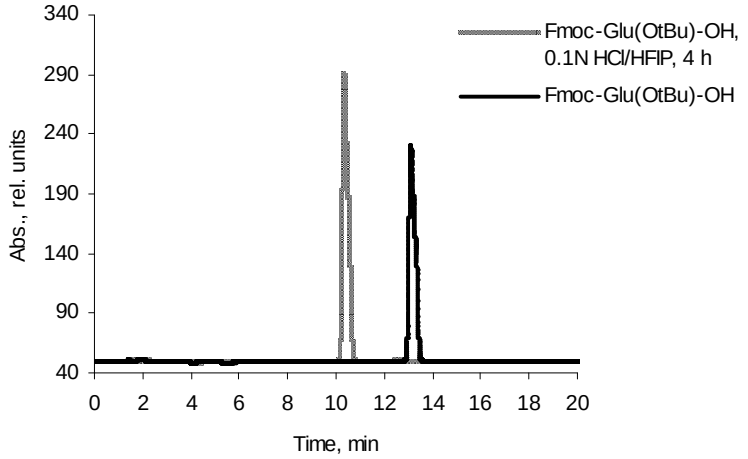


Fig. S9. Deprotection of Fmoc-Glu(OtBu)-OH by 0.1 N HCl in HFIP.

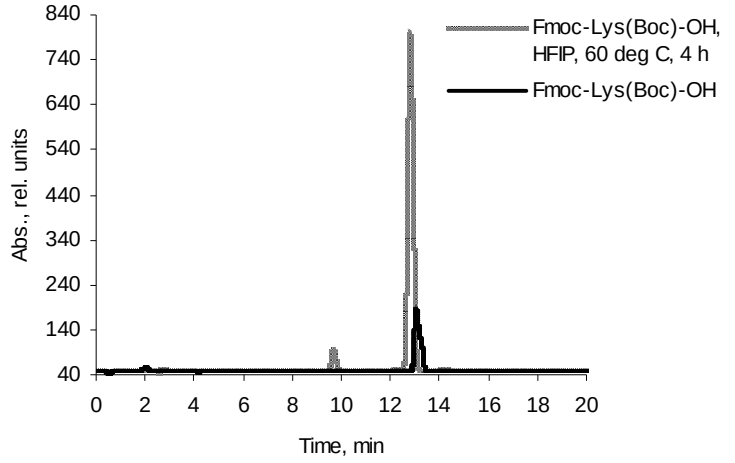


Fig. S10. Deprotection of Fmoc-Lys(Boc)-OH by HFIP at 60 ^{o}C.

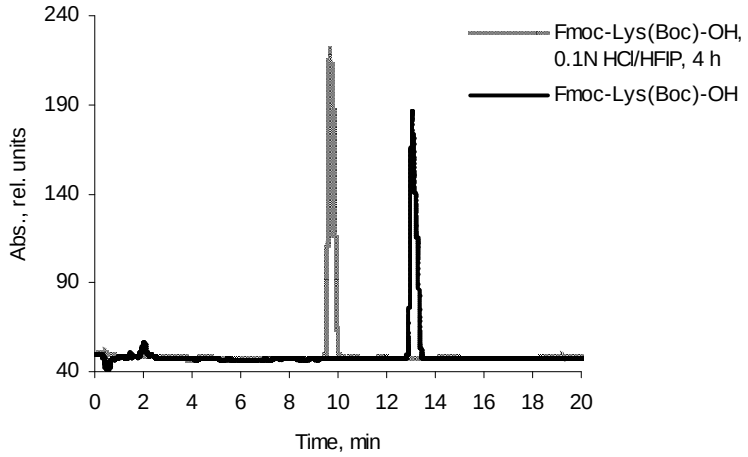


Fig. S11. Deprotection of Fmoc-Lys(Boc)-OH by 0.1 N HCl in HFIP.

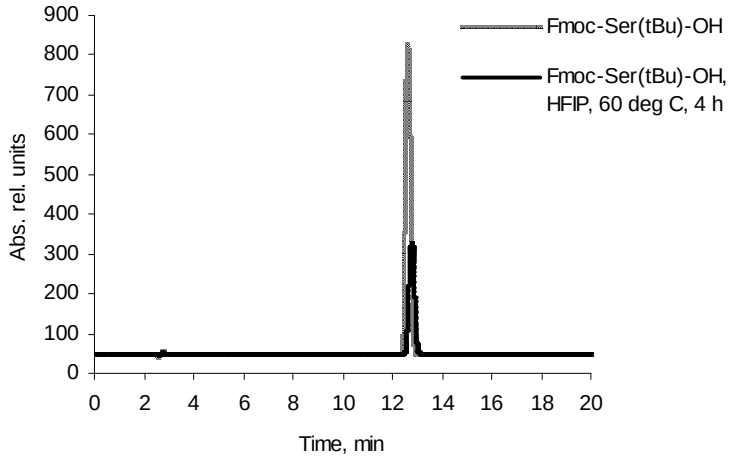


Fig. S12. Deprotection of Fmoc-Ser(tBu)-OH by HFIP at 60 ^{o}C.

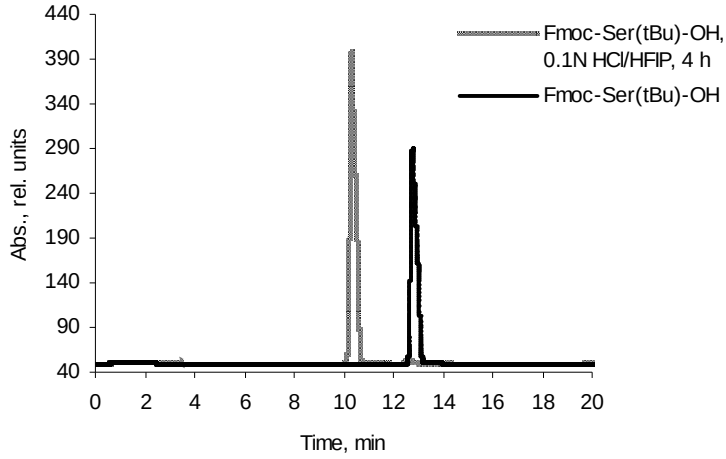


Fig. S13. Deprotection of Fmoc-Ser(tBu)-OH by 0.1 N HCl in HFIP.

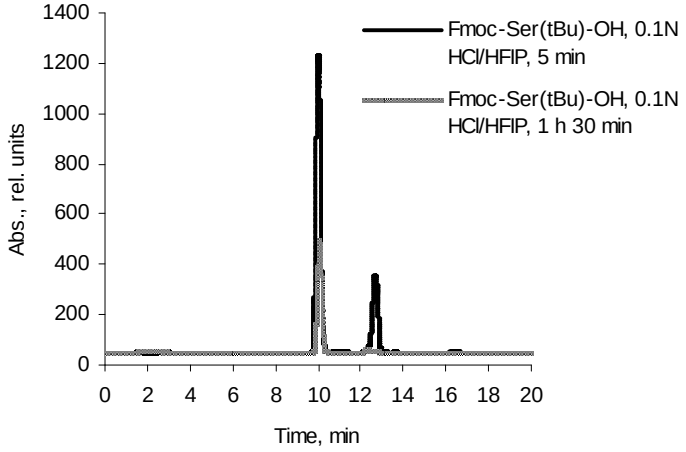


Fig. S14. The rate of deprotection of Fmoc-Ser(tBu)-OH by 0.1 N HCl in HFIP.

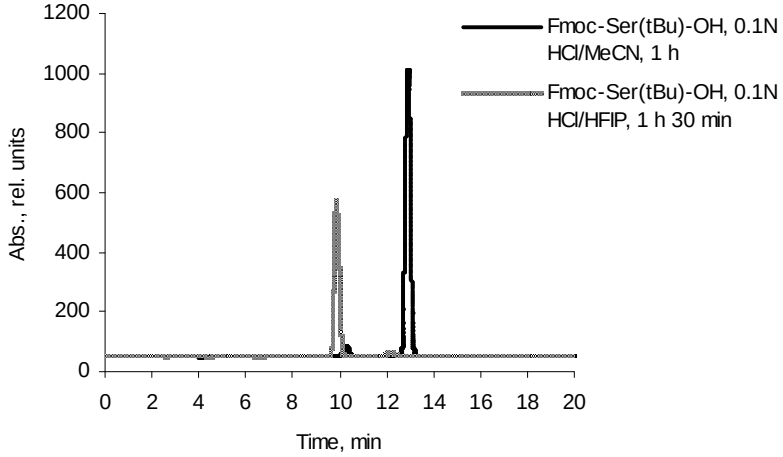


Fig. S15. Comparison of the rate of deprotection of Fmoc-Ser(tBu)-OH by 0.1 N HCl in HFIP or MeCN.

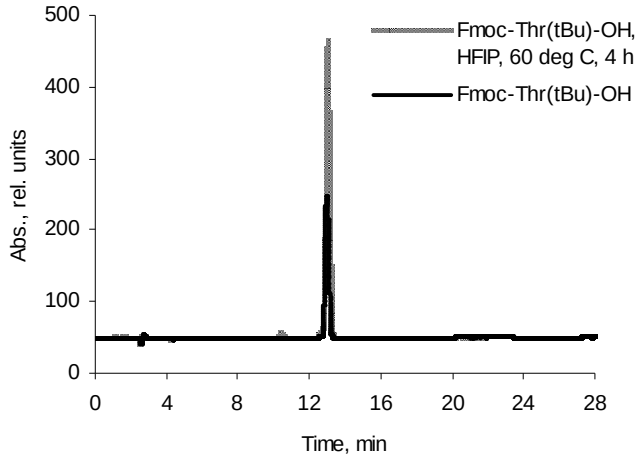


Fig. S16. Deprotection of Fmoc-Thr(tBu)-OH by HFIP at 60 ^{o}C.

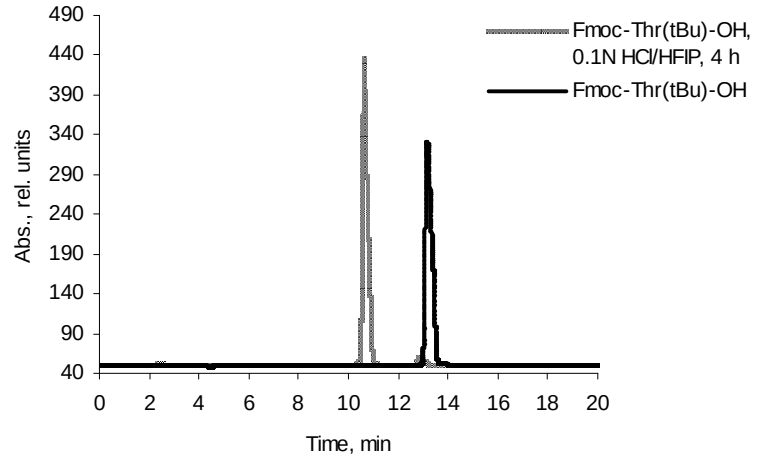


Fig. S17. Deprotection of Fmoc-Thr(tBu)-OH by 0.1 N HCl in HFIP.

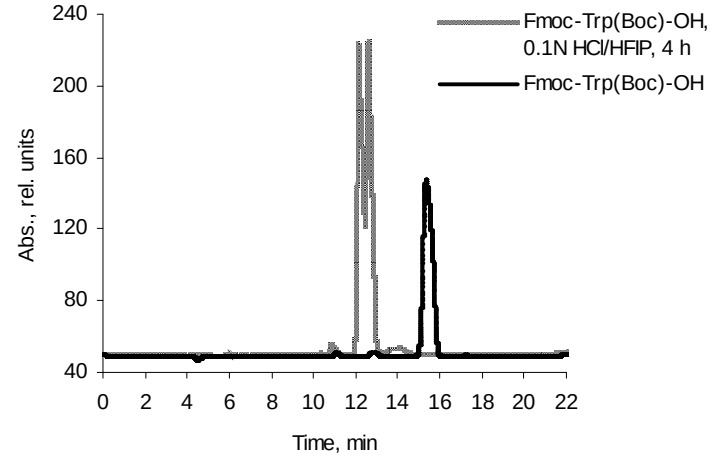


Fig. S18. Deprotection of Fmoc-Trp(Boc)-OH by 0.1 N HCl in HFIP.

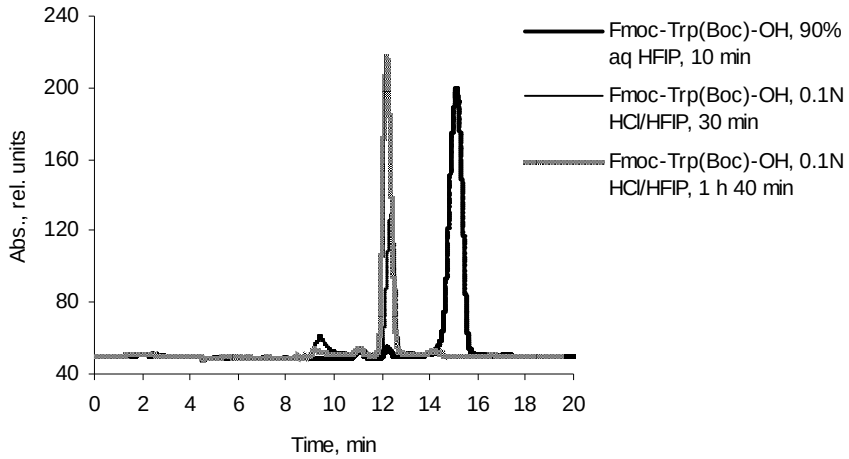


Fig. S19. The rate of deprotection of Fmoc-Trp(Boc)-OH by 0.1 N HCl in HFIP.

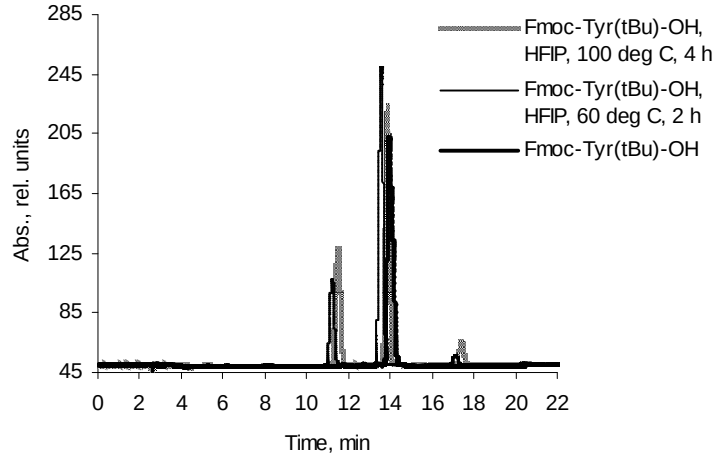


Fig. S20. Deprotection of Fmoc-Tyr(tBu)-OH by HFIP at elevated temperature.

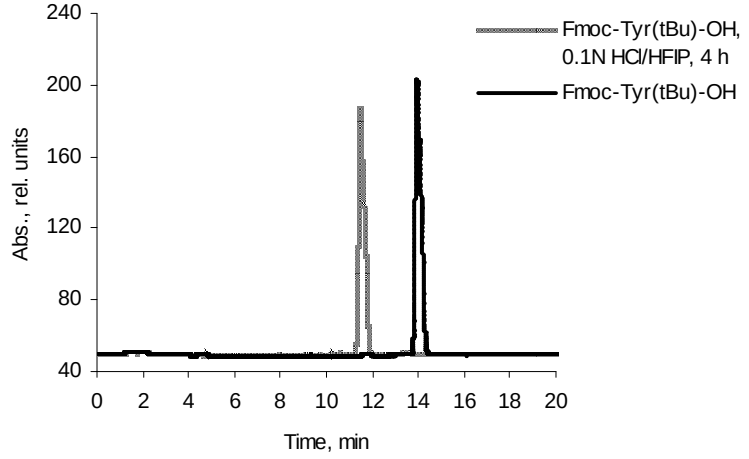


Fig. S21. Deprotection of Fmoc-Tyr(tBu)-OH by 0.1 N HCl in HFIP.

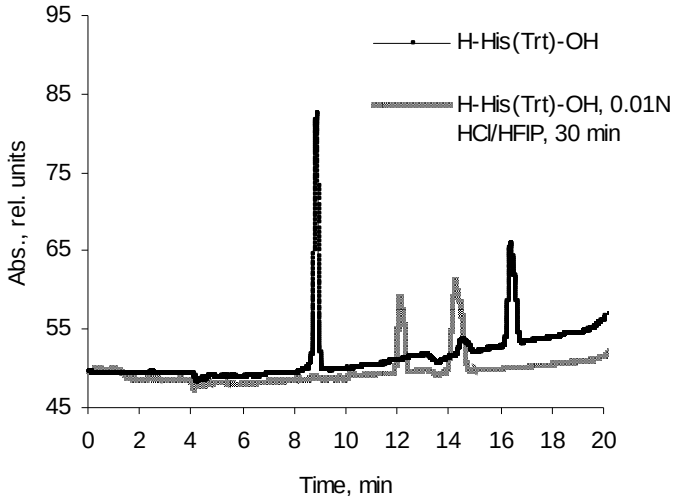
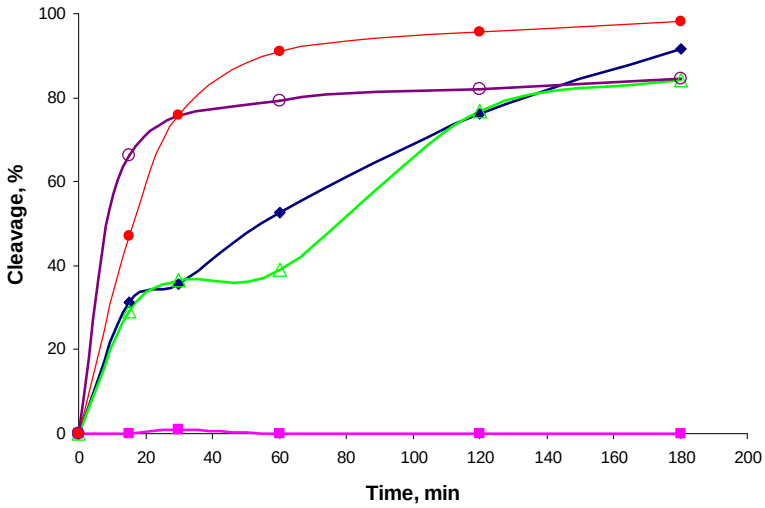


Fig. S22. Deprotection of H-His(Trt)-OH by 0.01 N HCl in HFIP.



(■) - Fmoc-Leu-Wang, 0.1 N HCl in Pr^{i}OH – CH_2Cl_2 (1:4 v/v)

(△) - Fmoc-Gly-HMPA-PEG-PS, 0.1 N HCl in HFIP + 1% DMS (v/v)

(◆) - Fmoc-Leu-Wang, 0.1 N HCl in HFIP – CH_2Cl_2 (1:4 v/v)

(○) - Fmoc-Gly-Rink-NovaPEG, 0.1 N HCl in HFIP + 1% DMS (v/v)

(●) - Fmoc-Gly-PAL-PEG-PS, 0.1 N HCl in HFIP + 1% DMS (v/v)

Fig. S23. Cleavage of common acid-labile supports by 0.1 N HCl in the presence of HFIP at rt.

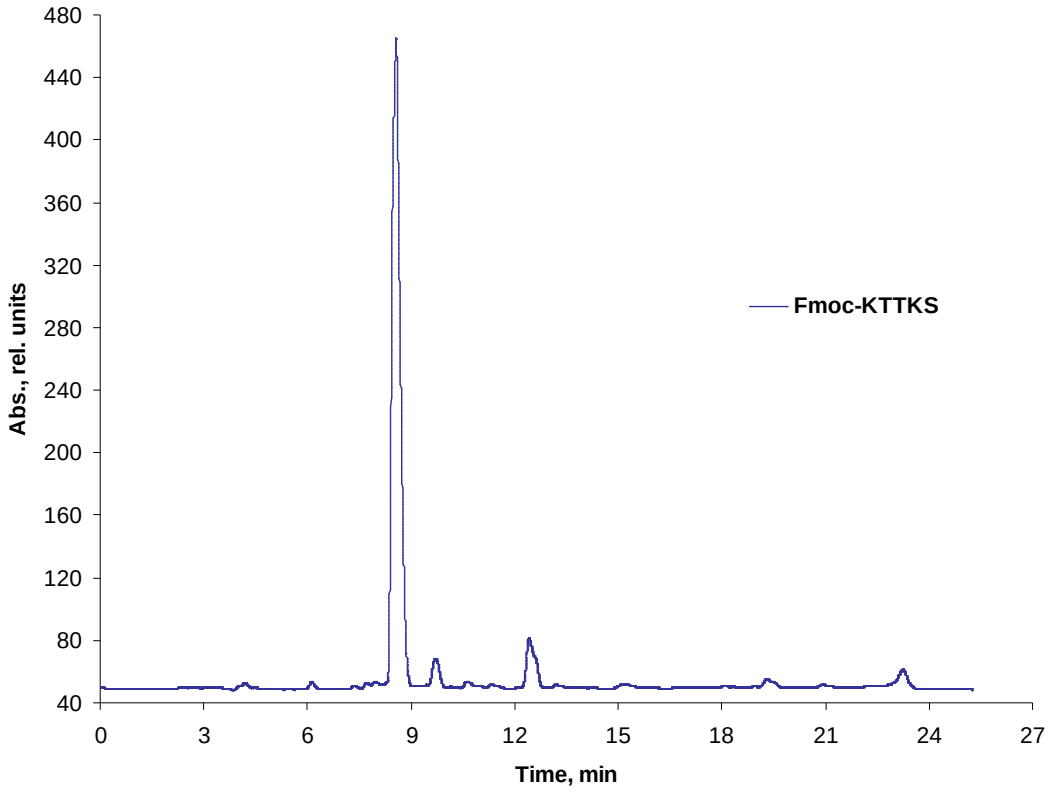


Fig. S24. RP-HPLC of a crude peptide Fmoc-Lys-Thr-Thr-Lys-Ser-OH.

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