Electronic Supplementary Information (ESI)

*Re*Green SPPS: enabling circular chemistry in environmentally sensible solid-phase peptide synthesis

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1. General information

All HPLC analyses were carried out on an Agilent 1100 or a Waters Alliance instruments. MS analyses were carried out an Agilent Q-TOF mass spectrometer, (Agilent, Santa Clara, CA, USA). All LC-MS analyses were performed on a tandem liquid chromatography mass spectrometry system consisting of an Agilent 1290, 1200 bar system with DAD, connected to an Agilent quadrupole time-of-flight (Q-TOF) mass spectrometer. The mass spectrometry system was operated in a positive mode using sheath gas electrospray ionization (ESI), mass range 20-3200, mass accuracy at 0.02 u, resolution up to 20000 ppm. The following source settings were used: gas temp 300 ^{o}C, gas flow 8 l/min, nebulizer 30psig, sheath gas temperature 350 ^{o}C and sheath gas flow 7.5 l/min. Analytical separations were achieved using a Waters Acquity UPLC instrument. All small scale SPPS experiments were carried out on a Chemspeed PSW1100 synthesizer. The 10 mmol SPPS of Aib-ACP was carried out using a previously described[1] SPPS apparatus.

2. SPPS development

2.1. Assessment of a model Fmoc deprotection

As the substrate for the model Fmoc removal 0.66M Fmoc-RAM AMS resin was used throughout where RAM = Rink amide (Knorr) linker and AMS = aminomethyl PS/DVB(1%) resin. PS = polystyrene, DVB = divinylbenzene. For all Fmoc removals reaction time was 5 min and 5% (v/v) 4-methylpiperidine (4-MP) was used as the base throughout. Fmoc removal conversions were determined by measuring the residual Fmoc content on the resins after completion of the Fmoc removals using an Fmoc content determination method[2] and a employing previously reported HPLC conditions as the analytical method.[1] 0.44M AMS, PS/DVB(1%) resin was used for all swelling determinations employing a previously reported protocol.[1] The impact of solvent and temperature on the outcome of the Fmoc removal was examined and the result of this investigation is summarized in Table S1 where the color legend is as follows: in red, conventional solvent (DMF); in green, green solvents; in yellow, satisfactory Fmoc removal conversions (>90%).

Table S1. Assessment of Fmoc removal on Fmoc-RAM AMS resin using 5% 4-MP/solvent.

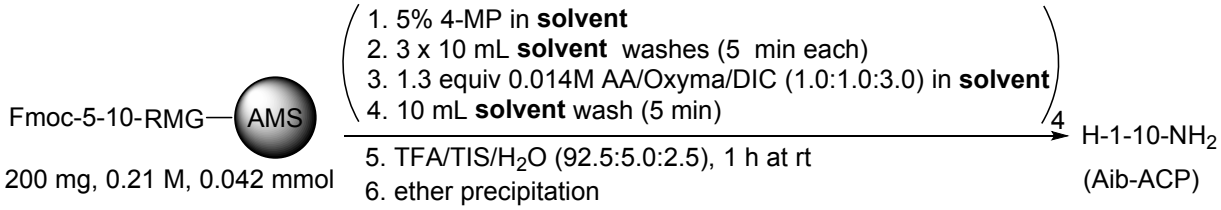
Entry	Solvent	Conversion (%)		Swelling (mL/g)
		25 °C	50 °C	
1	DMF	90.5	99.3	5.8
2	EtOAc	5.9	36.7	5.6
3	2% DMPU/EtOAc	8.6	65.7	6.0
4	10% DMPU/EtOAc	18.2	84.0	6.2
5	50% DMPU/EtOAc	62.8	98.4	7.4
6	2% DMSO/EtOAc	13.7	62.0	5.2
7	10% DMSO/EtOAc	28.9	91.1	5.6
8	50% DMSO/EtOAc	91.9	99.1	4.5

In keeping with a previous report[3] poor Fmoc removal kinetics in EtOAc were observed (entry 2). Nevertheless, upon adding a polar cosolvent and/or increasing the reaction temperature suitable rates of Fmoc removal could be attained (entries 3 – 8). It is worth noting that DMSO/EtOAc (entries 6 – 8) exhibited higher rates of Fmoc removals than the higher swelling DMPU/EtOAc did (entries 3 – 5).

2.2. Assessment of the Val[1]–Aib[4] part of Aib-ACP SPPS

The assessment of Val[1]-Aib[4] part of the synthesis was carried out using Fmoc-Ile[5]-Asp(O*t*Bu)[6]-Tyr(*t*Bu)[7]-Ile[8]-Asn(Trt)[9]-Gly[10]-RMG AMS resin (Fmoc-5-10 resin) where RMG = Ramage (tricyclic amide) linker.[4] The Fmoc-5-10 starting resin was prepared from 0.44 M AMS (PS/1%DVB) resin using standard Fmoc SPPS methodologies.[5]

Table S2. Assessment of Val[1]-Aib[4] part of Aib-ACP SPPS[1]



Entry	Temp (°C)	Solvent			Fmoc removal time (min)	Coupling time (min)	HPLC purity (%)[3]	
		Fmoc removals (1)	Couplings (3)	Washes (2 & 4)			Aib-ACP	des-Aib
1	40	50%DMPU/EtOAc	50%DMPU/EtOAc	DMF	15	25	37.8	46.1
2	40	20%DMPU/EtOAc	50%DMPU/EtOAc	DMF	15	25	37.1	45.7
3	40	50%DMPU/EtOAc	20%DMPU/EtOAc	DMF	15	25	51.1	32.2
4	40	20%DMPU/EtOAc	20%DMPU/EtOAc	DMF	15	25	50.1	33.1
5	50	50%DMPU/EtOAc	50%DMPU/EtOAc	DMF	15	25	45.2	38.5
6	50	20%DMPU/EtOAc	50%DMPU/EtOAc	DMF	15	25	40.9	40.9
7	50	50%DMPU/EtOAc	20%DMPU/EtOAc	DMF	15	25	55.2	26.8
8	50	20%DMPU/EtOAc	20%DMPU/EtOAc	DMF	15	25	51.9	29.6
9	55	20%DMPU/EtOAc	20%DMPU/EtOAc	DMF	2 x 15	25	73.1	6.3
10	55	20%DMPU/EtOAc	20%DMPU/EtOAc	DMF	3 x 15	25	74.7	6.1
11	55	20%DMPU/EtOAc	20%DMPU/EtOAc	DMF	2 x 15	2 x 25	77.6	1.6
12	55	20%DMPU/EtOAc	20%DMPU/EtOAc	DMF	3 x 15	2 x 25	76.4	1.9
13	50	10%DMPU/EtOAc	10%DMPU/EtOAc	DMF	2 x 15	25[2]	79.3	2.5
14	50	10%DMPU/EtOAc	10%DMPU/EtOAc	10%DMPU/EtOAc	2 x 15	25[2]	67.8	4.6
15	50	10%DMPU/EtOAc	10%DMPU/EtOAc	2%DMPU/EtOAc	2 x 15	25[2]	70.7	4.3
16	50	10%DMPU/EtOAc	10%DMPU/EtOAc	EtOAc	2 x 15	25[2]	73.3	3.3
17	55	10%DMPU/EtOAc	10%DMPU/EtOAc	2%DMPU/EtOAc	2 x 15	25[2]	69.8	4.7
18	55	10%DMPU/EtOAc	10%DMPU/EtOAc	2%DMSO/EtOAc	2 x 15	25[2]	73.2	3.1
19	55	10%DMSO/EtOAc	10%DMSO/EtOAc	2%DMPU/EtOAc	2 x 15	25[2]	78.8	2.3
20	55	10%DMSO/EtOAc	10%DMSO/EtOAc	2%DMSO/EtOAc	2 x 15	25[2]	79.6	2.3

[1] In red, conventional solvent (DMF); in green, green solvents; in yellow, satisfactory HPLC purities of the product (>75%); 33.3% of DIC was added at the outset and the remaining 66.6% at t= 15 min; Fmoc-AA-OH derivatives were used for Aib[4]-Gln[2] couplings, Boc-Val-OH was used for Val[1] coupling; All final Boc-1-10 resins were *i*-PrOH washed (2 x 5 mL) and dried to constant weight *en vacuo* before proceeding to TFA cleavage (step 5). [2]Only Aib[4] and Aib[3] couplings were re-coupled (25 min). [3]HPLC analyses were carried out using Kinetex C18 column (30 °C, 50x4.6mm, 2.6um), TFA/H_2O (0.1:100, **A**), and TFA/MeCN (0.1:100, **B**) mobile phases, gradient of 40% B over 15 min and flow rate of 1.0 ml min^{-1}.

Following the assessment of temperature and solvent composition in a model Fmoc removal (section 2.1. of this ESI) the assessment of the Val[1]-Aib[4] part of the Aib-ACP was carried out

using conditions that were suitable for the Fmoc removal (Table S1, entry 5, 50 °C) as a starting point. The effect of temperature, solvents, as well as extent of Fmoc deprotections and couplings were investigated, for a summary of the results see Table S2. To maximize the usage of the easily recyclable EtOAc solvent the aim was to find conditions in which minimal amounts of a polar aprotic cosolvent could be used without compromising the efficiency of the chemistries involved. The strategy was to i) examine green solvents for the chemical steps while keeping DMF as the wash solvent ii) green the whole SPPS process upon identifying suitable green steps for the couplings and Fmoc removal. Thus, using EtOAc/DMPU as the solvent system the effect of temperature as well as the content of cosolvent was examined (entries 1 – 8). While content of DMPU in Fmoc removals seemed to have only a marginal effect on the purity (entries 1, 3, 5, 7 vs 2, 4, 6, 8) decreasing the content of DMPU in couplings and increasing the temperature were both beneficial (entries 1, 2, 5, 6 vs 3, 4, 7, 8). Further, using 20% DMPU/EtOAc for all chemical steps the extent of both couplings and Fmoc removals was examined at slightly increased temperature (55 °C, entries 9 – 12). While purities of the product for 2 x 15 min and 3 x 15 min Fmoc removals respectively were quite comparable (entries 9 and 11 vs 10 and 12) extending couplings from 1 x 25 min to 2 x 25 min resulted in an appreciable purity increase accompanied by a marked decrease in the content of the major deletion Aib (des Aib) impurity (entries 9 and 10 vs 11 and 12). An attempt was made to decrease DMPU content even further (10%) which worked well with DMF as the wash solvent (79.3% product purity, entry 13) while resorting to green solvents for washes resulted in a purity drop to 68 – 73% (entries 14 – 16). In an attempt to improve the efficiency of the solvent washes the temperature was raised from 50 to 55 °C while also examining EtOAc/DMPU vs EtOAc/DMSO as SPPS solvents (entries 17 – 20). We determined that 10% DMSO/EtOAc as the solvent for the chemistry (entries 19 and 20) worked better than the corresponding 10% DMPU/EtOAc syntheses did (entries 17 and 18). In fact, the experiment using 10%DMSO/EtOAc for chemical steps and 2% DMSO/EtOAc for solvent washes gave the highest purity of all conditions examined (entry 20, 79.6%). Based on the assessment of Val^{1}–Aib^{4} SPPS delineated in Table S2 the entry 20 conditions were employed in a scale-up synthesis of the whole Aib-ACP model peptide (See section 3 of this ESI and Scheme 1 of the main article).

3. Scale-up SPPS of Aib-ACP

Table S3. Overview of AAs and coupling agents used in the SPPS of Aib-ACP

AA nr	Cas nr	AA	MW	mmol AA	equiv AA	g AA	g Oxyma	Total g DIC
Gly^{10}	29022-11-5	Fmoc-Gly-OH	297,31	13,00	1,30	4,07	1,85	4,10
Asn^{9}	132388-59-1	Fmoc-Asn(Trt)-OH	596,67	13,00	1,30	8,16	1,85	4,10
Ile^{8}	71989-23-6	Fmoc-Ile-OH	354,41	13,00	1,30	4,85	1,85	4,10
Tyr^{7}	71989-38-3	Fmoc-Tyr(*t* Bu)-OH	459,53	13,00	1,30	6,29	1,85	4,10
Asp^{6}	71989-14-5	Fmoc-Asp(OtBu)	411,45	13,00	1,30	5,63	1,85	4,10
Ile^{5}	71989-23-6	Fmoc-Ile-OH	354,41	13,00	1,30	4,85	1,85	4,10
Aib^{4}	94744-50-0	Fmoc-Aib-OH	325,36	13,00	1,30	4,45	1,85	4,10
Aib^{3}	94744-50-0	Fmoc-Aib-OH	325,36	10,00	1,00	3,42	1,42	3,16
re-Aib^{3}	94744-50-0	Fmoc-Aib-OH	325,36	10,00	1,00	3,42	1,42	3,16
Gln^{2}	132327-80-1	Fmoc-Gln(Trt)-OH	610,72	13,00	1,30	8,36	1,85	4,10
Val^{1}	13734-41-3	Boc-Val-OH	217,30	13,00	1,30	2,97	1,85	4,10
					SUM	53,51	17,62	39,12

Table S4. Overview of solvents and 4-MP used in the SPPS of Aib-ACP

Process step	Total volume (mL)	EtOAc volume (mL)	DMSO volume (mL)	4-MP volume (mL)
Swelling	200,0	180,0	20,0	
Gly^{10}, Fmoc removal 1	202,0	180,0	20,0	2,0
Gly^{10}, Fmoc removal 2	210,0	180,0	20,0	10,0
Gly^{10}, Wash	800,0	784,0	16,0	
Gly^{10}, rinse	100,0	90,0	10,0	
Gly^{10}, Coupling	200,0	180,0	20,0	
Gly^{10}, Wash	200,0	196,0	4,0	
Asn^{9}, rinse	100,0	90,0	10,0	
Asn^{9}, Fmoc removal 1	202,0	180,0	20,0	2,0
Asn^{9}, Fmoc removal 2	210,0	180,0	20,0	10,0
Asn^{9}, Wash	800,0	784,0	16,0	
Asn^{9}, rinse	100,0	90,0	10,0	
Asn^{9}, Coupling	200,0	180,0	20,0	
Asn^{9}, Wash	200,0	196,0	4,0	
Ile^{8}, rinse	100,0	90,0	10,0	
Ile^{8}, Fmoc removal 1	202,0	180,0	20,0	2,0
Ile^{8}, Fmoc removal 2	210,0	180,0	20,0	10,0
Ile^{8}, Wash	800,0	784,0	16,0	
Ile^{8}, rinse	100,0	90,0	10,0	
Ile^{8}, Coupling	200,0	180,0	20,0	
Ile^{8}, Wash	200,0	196,0	4,0	
Tyr^{7}, rinse	100,0	90,0	10,0	
Tyr^{7}, Fmoc removal 1	202,0	180,0	20,0	2,0
Tyr^{7}, Fmoc removal 2	210,0	180,0	20,0	10,0
Tyr^{7}, Wash	800,0	784,0	16,0	
Tyr^{7}, rinse	100,0	90,0	10,0	
Tyr^{7}, Coupling	200,0	180,0	20,0	
Tyr^{7}, Wash	200,0	196,0	4,0	
Asp^{6}, rinse	100,0	90,0	10,0	
Asp^{6}, Fmoc removal 1	202,0	180,0	20,0	2,0
Asp^{6}, Fmoc removal 2	210,0	180,0	20,0	10,0
Asp^{6}, Wash	800,0	784,0	16,0	
Asp^{6}, rinse	100,0	90,0	10,0	
Asp^{6}, Coupling	200,0	180,0	20,0	
Asp^{6}, Wash	200,0	196,0	4,0	
Ile^{5}, rinse	100,0	90,0	10,0	
Ile^{5}, Fmoc removal 1	202,0	180,0	20,0	2,0
Ile^{5}, Fmoc removal 2	210,0	180,0	20,0	10,0
Ile^{5}, Wash	800,0	784,0	16,0	
Ile^{5}, rinse	100,0	90,0	10,0	
Ile^{5}, Coupling	200,0	180,0	20,0	
Ile^{5}, Wash	200,0	196,0	4,0	
Aib^{4}, rinse	100,0	90,0	10,0	
Aib^{4}, Fmoc removal 1	202,0	180,0	20,0	2,0
Aib^{4}, Fmoc removal 2	210,0	180,0	20,0	10,0
Aib^{4}, Fmoc removal 3	210,0	180,0	20,0	10,0
Aib^{4}, Wash	800,0	784,0	16,0	
Aib^{4}, rinse	100,0	90,0	10,0	
Aib^{4}, Coupling	200,0	180,0	20,0	
Aib^{4}, Wash	200,0	196,0	4,0	
Aib^{3}, rinse	100,0	90,0	10,0	
Aib^{3}, Fmoc removal 1	202,0	180,0	20,0	2,0
Aib^{3}, Fmoc removal 2	210,0	180,0	20,0	10,0
Aib^{3}, Fmoc removal 3	210,0	180,0	20,0	10,0
Aib^{3}, Wash	800,0	784,0	16,0	
Aib^{3}, rinse	100,0	90,0	10,0	
Aib^{3}, Coupling	200,0	180,0	20,0	
Aib^{3}, $Wash^{1}$	150,0	135,0	15,0	
Aib^{3}, $Wash\ 2^{1}$	150,0	135,0	15,0	
Aib^{3}, Re-coupling	200,0	180,0	20,0	
Aib^{3}, Wash	200,0	196,0	4,0	
Gln^{2}, rinse	100,0	90,0	10,0	
Gln^{2}, Fmoc removal 1	202,0	180,0	20,0	2,0
Gln^{2}, Fmoc removal 2	210,0	180,0	20,0	10,0
Gln^{2}, Fmoc removal 3	210,0	180,0	20,0	10,0
Gln^{2}, Wash	800,0	784,0	16,0	
Gln^{2}, rinse	100,0	90,0	10,0	
Gln^{2}, Coupling	200,0	180,0	20,0	
Gln^{2}, Wash	200,0	196,0	4,0	
Val^{1}, rinse	100,0	90,0	10,0	
Val^{1}, Fmoc removal 1	202,0	180,0	20,0	2,0
Va^{1}, Fmoc removal 2	210,0	180,0	20,0	10,0
Val^{1}, Fmoc removal 3	210,0	180,0	20,0	10,0
Val^{1}, Wash	800,0	784,0	16,0	
Val^{1}, rinse	100,0	90,0	10,0	
Val^{1}, Coupling	200,0	180,0	20,0	
Val^{1}, Wash	200,0	196,0	4,0	
Final wash, (3 xDMSO/EtOAc)	600	588	12	
Entire SPPS (mL)	**20160,0**	**18848,0**	**1152,0**	**160,0**
Entire SPPS (%)		**93,49**	**5,71**	**0,79**

[1]0.1M Oxyma was used in this wash

4. Solubility of AAs in DMSO/EtOAc

Table S5. Solubility of a series of standard Fmoc-AA-OH derivatives in DMSO/EtOAc (1:9)

No	AA	Cas nr.	Solubility at 0.1M/rt
1	Fmoc-His(Trt)-OH	109425 - 51 - 6	0.0833 M[1]
2	Fmoc-Arg(Pbf)-OH	154445-77-9	yes
3	Fmoc-Lys(Boc)-OH	71989-26-9	yes
4	Fmoc-Ile-OH	71989-23-6	yes
5	Fmoc-Phe-OH	35661-40-6	yes
6	Fmoc-Leu-OH	35661-60-0	yes
7	Fmoc-Trp(Boc)-OH	143824-78-6	yes
8	Fmoc-Ala-OHxH_2O	207291-76-7	yes
9	Fmoc-Met-OH	71989-28-1	yes
10	Fmoc-Pro-OH	71989-31-6	yes
11	Fmoc-Cys(Trt)-OH	103213-32-7	yes
12	Fmoc-Asn(Trt)-OH	132388-59-1	yes
13	Fmoc-Val-OH	68858-20-8	yes
14	Fmoc-Gly-OH	29022-11-5	yes
15	Fmoc-Ser(*t*- Bu)-OH	71989-33-8	yes
16	Fmoc-Gln(Trt)-OH	132327-80-1	yes
17	Fmoc-Tyr(*t*- Bu)-OH	71989-38-3	yes
18	Fmoc-Asp(O-*t* Bu)-OH	71989-14-5	yes
19	Fmoc-Glu(O-*t* Bu)-OH	204251-24-1	yes
20	Fmoc-Thr(*t*- Bu)-OH	71989-35-0	yes

[1]Soluble at 0.1M at 30 °C, also soluble in DMSO/EtOAc (2:8) at rt.

5. LC-HRMS analysis of Aib-ACP

Experimental conditions: column: Waters peptide CSH C18, 2.1x150mm, 1.7um, 130Å; column temperature: 30°C; injection volume: 2 µL, sampler temperature: 10°C; MS mode: positive 50-3200; DAD: 220 nm; data rate: 2.5Hz; detector cell: standard cell 1uL; flow: 0.25 ml/min; jet weaver: v100 mixer; mobile phase A: 0.1 % TFA in water, mobile phase B: 0.10 % TFA in MeCN. Gradient (Time(min), %B): 0, 1; 1, 1; 30, 95; 32, 95; 32,1, 1; 35, 1.

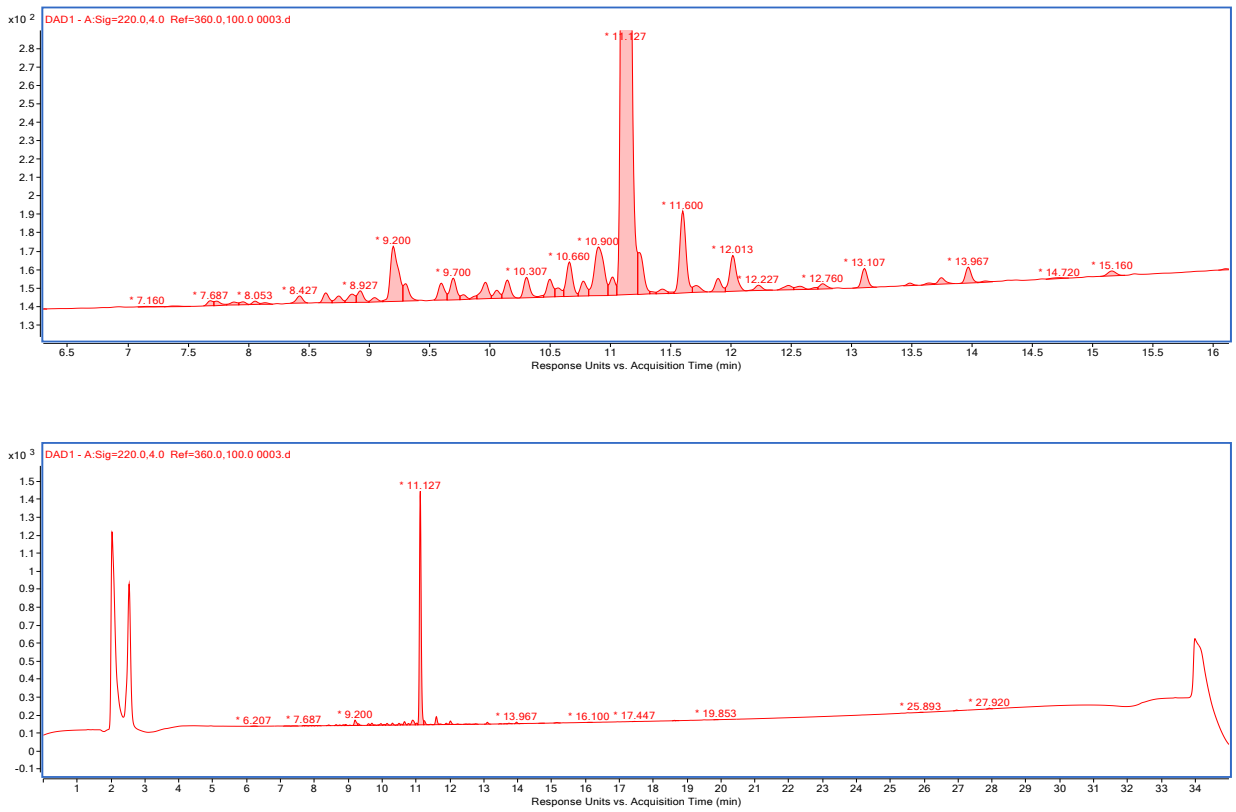


Figure S1. LC-HRMS analysis of crude product from 10 mmol Aib-ACP synthesis (Section 3 of this ESI)

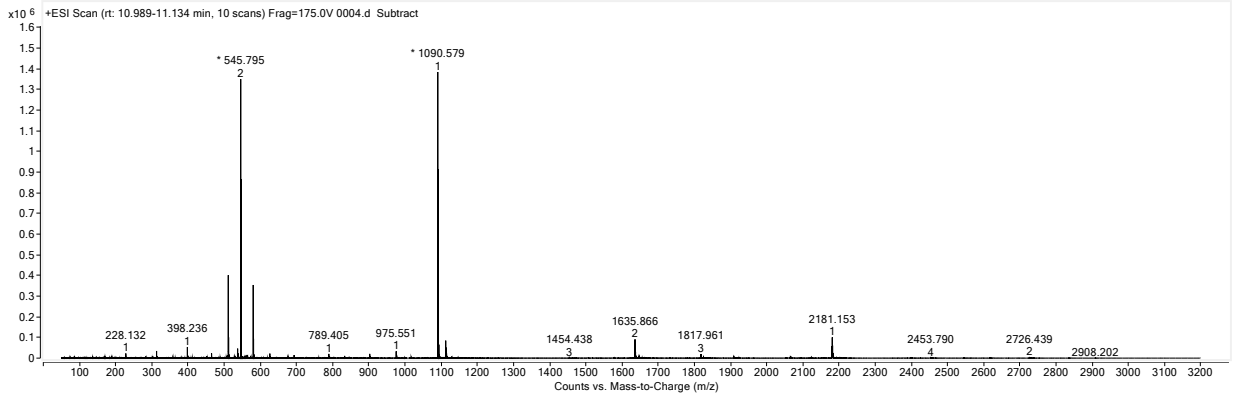


Figure S2. MS spectrum of the target peptide (m/z 1090.5897)

Table S6. Area% for integrated peaks and MS identity for Aib-ACP.

Peak	Rt	Area	most(m+z)/z	z	decon.	diff	RRT	Area %
1	6,21	9,330			0,0000	-1089,5820	0,562	0,16
2	7,16	2,740			0,0000	-1089,5820	0,648	0,05
3	7,37	2,870			0,0000	-1089,5820	0,667	0,05
4	7,69	9,270			0,0000	-1089,5820	0,695	0,15
5	7,73	8,910			0,0000	-1089,5820	0,700	0,15
6	7,88	6,840			0,0000	-1089,5820	0,713	0,11
7	7,95	5,320			0,0000	-1089,5820	0,720	0,09
8	8,05	5,620			0,0000	-1089,5820	0,729	0,09
9	8,14	3,490			0,0000	-1089,5820	0,736	0,06
10	8,43	14,180			0,0000	-1089,5820	0,762	0,24
11	8,64	18,060			0,0000	-1089,5820	0,782	0,30
12	8,75	14,160			0,0000	-1089,5820	0,791	0,24
13	8,86	18,870			0,0000	-1089,5820	0,802	0,31
14	8,93	21,330			0,0000	-1089,5820	0,808	0,36
15	9,05	8,870			0,0000	-1089,5820	0,819	0,15
16	9,2	140,28			0,0000	-1089,5820	0,832	2,34
17	9,3	30,7			0,0000	-1089,5820	0,841	0,51
18	9,6	33,87			0,0000	-1089,5820	0,869	0,56
19	9,7	42,94			0,0000	-1089,5820	0,878	0,72
20	9,78	8,21			0,0000	-1089,5820	0,885	0,14
21	9,893	4,41			0,0000	-1089,5820	0,895	0,07
22	9,967	36,19			0,0000	-1089,5820	0,902	0,6
23	10,06	15,57			0,0000	-1089,5820	0,910	0,26
24	10,147	34,14			0,0000	-1089,5820	0,918	0,57
25	10,307	38,7			0,0000	-1089,5820	0,933	0,65
26	10,447	2,61			0,0000	-1089,5820	0,945	0,04
27	10,5	33,97			0,0000	-1089,5820	0,950	0,57
28	10,567	17,94			0,0000	-1089,5820	0,956	0,3
29	10,66	67,18			0,0000	-1089,5820	0,964	1,12
30	10,773	28,84			0,0000	-1089,5820	0,975	0,48
31	10,9	147,11			0,0000	-1089,5820	0,986	2,45
32	11,02	33,57			0,0000	-1089,5820	0,997	0,56
33	11,127	4551,61	1090,579	1	1089,5717	-0,0103	1,007	75,87
34	11,24	70,49			0,0000	-1089,5820	1,017	1,17
35	11,327	3,36			0,0000	-1089,5820	1,025	0,06
36	11,433	10,4			0,0000	-1089,5820	1,034	0,17
37	11,48	2,17			0,0000	-1089,5820	1,039	0,04
38	11,6	162,4			0,0000	-1089,5820	1,049	2,71
39	11,713	15,33			0,0000	-1089,5820	1,060	0,26
40	11,893	27,82			0,0000	-1089,5820	1,076	0,46
41	12,013	76,83			0,0000	-1089,5820	1,087	1,28
42	12,227	12,04			0,0000	-1089,5820	1,106	0,2
43	12,473	11,75			0,0000	-1089,5820	1,128	0,2
44	12,573	8,44			0,0000	-1089,5820	1,138	0,14
45	12,72	3,28			0,0000	-1089,5820	1,151	0,05
46	12,76	11,56			0,0000	-1089,5820	1,154	0,19
47	13,107	36,18			0,0000	-1089,5820	1,186	0,6
48	13,48	4,5			0,0000	-1089,5820	1,220	0,08
49	13,64	4,05			0,0000	-1089,5820	1,234	0,07
50	13,747	13,98			0,0000	-1089,5820	1,244	0,23
51	13,967	29,96			0,0000	-1089,5820	1,264	0,5
52	14,107	3,26			0,0000	-1089,5820	1,276	0,05
53	14,72	3,88			0,0000	-1089,5820	1,332	0,06
54	15,16	12,92			0,0000	-1089,5820	1,372	0,22
55	16,1	2,99			0,0000	-1089,5820	1,457	0,05
56	17,447	2,9			0,0000	-1089,5820	1,578	0,05
57	18,627	7,99			0,0000	-1089,5820	1,685	0,13
58	18,66	3,71			0,0000	-1089,5820	1,688	0,06
59	19,853	2,29			0,0000	-1089,5820	1,796	0,04
60	25,893	4,33			0,0000	-1089,5820	2,343	0,07
61	26,953	14,42			0,0000	-1089,5820	2,439	0,24
62	27,92	18,55			0,0000	-1089,5820	2,526	0,31

6. Recycling of Aib-ACP SPPS waste stream

The SPPS waste stream as well as EtOAc, DMSO and Oxyma which were recovered were analyzed employing the following HPLC method: column: Waters XSelect CSH130 C18 2.5 µm 4.6x150mm; detection wavelength: 220 nm, column temperature: 30 °C; injection volume: 5 µL; sampler temperature:10°C; flow: 0.5 ml/min; mobile phase A: 0.1 % TFA in water, mobile phase B: 0.08 % TFA in 90% MeCN/10 %water. Gradient (Time(min), %B): 0, 0; 40, 100; 54, 100; 55, 0; 62, 0. HPLC overlay for the waste stream and the isolated EtOAc, DMSO and Oxyma is shown in Fig. S3 while HPLC overlay for the recycled EtOAc, DMSO and Oxyma vs the virgin counterparts is shown in Fig. S4 of this ESI.

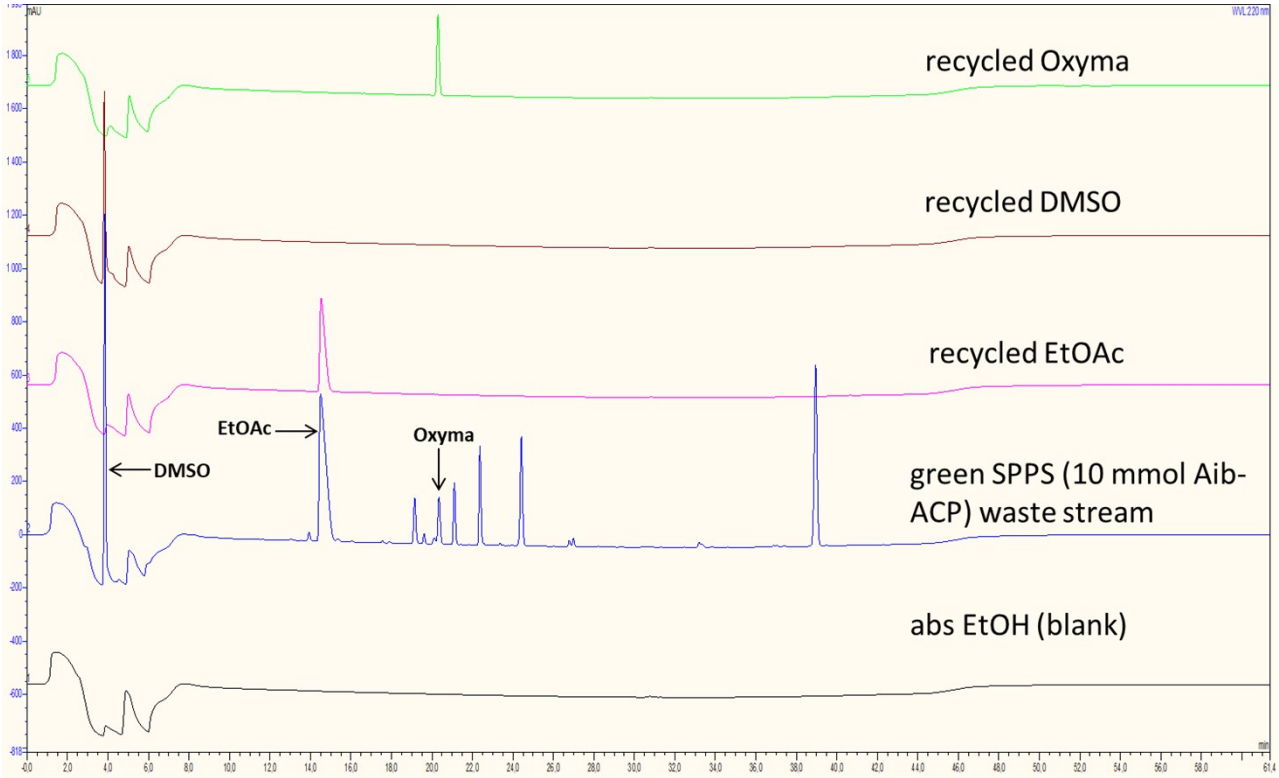


Figure S3. HPLC overlay for green SPPS waste stream and recycled EtOAc, DMSO and Oxyma. EtOH was used as sample solvent for all analyses.

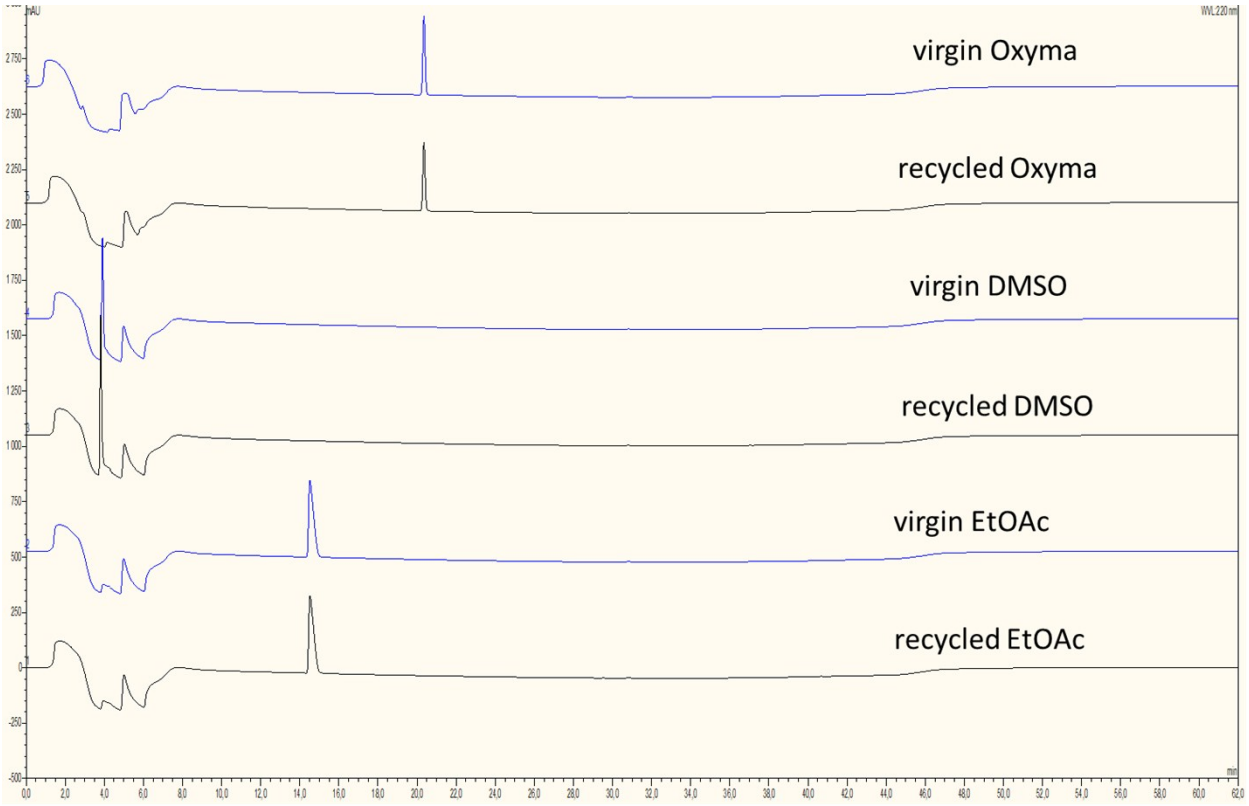


Figure S4. HPLC overlay for recycled and virgin EtOAc, DMSO and Oxyma. EtOH was used as sample solvent for all analyses.

7. Assessment of environmental impact and cost of Aib-ACP SPPS

In this section, results pertaining to the environmental assessment of Aib-ACP SPPS are summarized. Four cases of Aib-ACP SPPS are compared:

i) conventional, DMF as solvents throughout

ii) Green SPPS employing NBP/EtOAc[6]

iii) Green SPPS herein employing DMSO/EtOAc

iv) *Re*Green SPPS herein employing DMSO/EtOAc and recycling of EtOAc (86 %) and DMSO (70%). Oxyma recycling is not considered herein albeit aspects of recycling this coupling agent are discussed in the main article.

For cases ii) – iv) the NBP/EtOAc and DMSO/EtOAc ratios in couplings and Fmoc removals is considered to be 1:9 whereas NBP/EtOAc and DMSO/EtOAc ratios in solvent washes is considered to be 1:49. In all four cases the total SPPS solvent consumption is considered to be the same, see Table S4 in this ESI.

In all four cases the Aib-ACP is considered to be carried out according to the protocol depicted in Scheme 1 and described in detail in the Materials & Methods section the only difference between the four cases being the solvent of the synthesis and employment of recycling in case iv). For cases ii) – iv) the content of solvent 1 (NBP or DMSO) in the waste stream is considered to be 6% and content of EtOac in the waste stream is considered to be 94%.

The scale of the Aib-ACP SPPS is considered to be 1 mol in all four cases.

The amount of crude Aib-ACP obtained is considered to be the same in all four cases (974 g) and is based on the amount of crude Aib-ACP isolated using the DMSO/EtOAc SPPS herein.[7]

The amounts of TFA and scavengers used in the cleavage of Aib-ACP peptide resin and antisolvent used in crude peptide isolation are included in the calculation of cEF together with all the SPPS raw materials employed (see Table S7). Thus, for the cleavage of Aib-ACP peptide resin (5124 g) on 1 mol scale following amounts of the reagents for cleavage and crude peptide precipitation would be used: i) TFA, 23.70 L (35.31 kg); ii) TIS, 1.28 L (0.98 kg); iii) water, 0.64 L (0.64 kg); iv) diethyl ether, 153.71 L (109.14 kg). The total amount of reagents used in the cleavage of peptide resin and crude peptide precipitation was 191.65 kg.

Based on quotations from solvent vendors approved for GMP manufacturing (January 2019) the large scale prices of the solvents used herein were taken to be as follows: DMF, 1.35 EUR/kg;[1] EtOAc, 1.65 EUR/kg; [1] NBP, 7.35 EUR/kg; [1] DMSO, 9.08 EUR/kg (Fisher).

Based on quotations from providers of industrial solvent recycling (October 2017) the price of recycling were considered as follows: DMSO, 1.0 EUR/kg; EtOAc, 0.4 EUR/kg.

Based on the pricing of industrial waste disposal (August 2019) the price of disposing SPPS waste by incineration was considered to be 0.21 EUR/kg (Stena Recycling Malmö).

Table S7. Total amounts of raw materials for Aib-ACP SPPS carried out on 1 mol scale

Raw material	Starting resin	∑ AAs	Oxyma	DIC	AcOH	4-MP	TFA cleavage	Total
Amount (kg)	3,70	5,35	1,76	6,44	0,60	13,40	191,64	222,89

Table S8. Total amounts and cost of solvents for Aib-ACP SPPS carried out on 1 mol scale

Solvent attributes	SPPS solvent			
	i) (DMF)	ii) (NBP/EtOAc)	iii) (DMSO/EtOAc)	iv) (DMSO/EtOAc) + recycling
Solvent 1 (L)	2016,0	121,0	121,0	36,3
Solvent 2 (L)	n.a.	1895,0	1895,0	265,3
∑ Solvents (L)	2016,0	2016,0	2016,0	301,6
Solvent 1 (kg)	1903,1	116,3	133,1	39,9[a]
Solvent 2 (kg)	n.a.	1709,3	1709,3	239,3[b]
∑ Solvents (kg)	1903,1	1825,6	1842,4	279,2
Solvent 1 (EUR)	2569,2	854,8	1098,7	329,6
Solvent 2 (EUR)	n.a.	2820,3	2820,3	437,8
∑ solvents (EUR)	2569,2	3675,1	3919	767,4

[a]considering that 70% of DMSO was recovered and reused, cost of recycling is 93,2 EUR; [b]considering that 86% of EtOAc was recovered and reused, cost of recycling is 588,0 EUR.

8. Test coupling using virgin vs recycled starting materials

To assess the performance of EtOAc, DMSO and Oxyma recycled from the waste stream from 10 mmol Aib-ACP SPPS (see section 6 of this ESI) a test SPPS coupling was carried out. For this SPPS performance test the Aib^4 coupling of the Aib-ACP SPPS was used employing H-5-10-RMG AMS intermediate resin taken out during the 10 mmol Aib-ACP synthesis as the starting resin. Two head-to-head experiments were carried out employing:

i) recycled EtOAc, DMSO and Oxyma

ii) virgin EtOAc, DMSO and Oxyma

The set-up for both experiments was identical as in the 10 mmol Aib-ACP SPPS (see section 3 of this ESI) using 100 mg (0.20 mmol/g, 0.02 mmol) of the starting resin. Thus the resin was swollen in 2 mL DMSO/EtOAc (1:9) for 30 min and drained upon which the coupling was carried out using 1.3 equiv (0.026 mmol) of Fmoc-Aib-OH/Oxyma/DIC (1.0:1.0:2.5) at 50 °C for 30 min. 40% of DIC was added at the outset and the remaining 60% at t= 15 min. At t=30 min both resins were drained, washed (3 x 5 mL DMSO/EtOAc (1:9) and 3 x 5 mL *i*-PrOH) and dried to constant weight *in vacuo* yielding 106 mg (99.8% of theory) in both cases. Resulting dry Fmoc-4-10-RMG AMS resins were cleaved with 1.0 mL TFA/TIS/H_2O (92.5:5.0:2.5) at rt for 2 h using Et_2O as precipitation solvent. A sample of H-5-10-RMG AMS resin was cleaved under identical conditions as well to obtain a sample of crude H-5-10-NH_2 peptide as a reference. Next, HPLC analyses for all three peptides were caried out (see Fig. S5) for which the HPLC method employed in section 6 of this ESI was used. These analyses revealed that the Aib^4 couplings using recycled and virgin EtOAc, DMSO and Oxyma as starting materials respectively proceeded in >99% conversion and in essentially undistinguinshable manner. This proved that the recycled EtOAc, DMSO and Oxyma starting materials can be employed in SPPS again without any adverse effects.

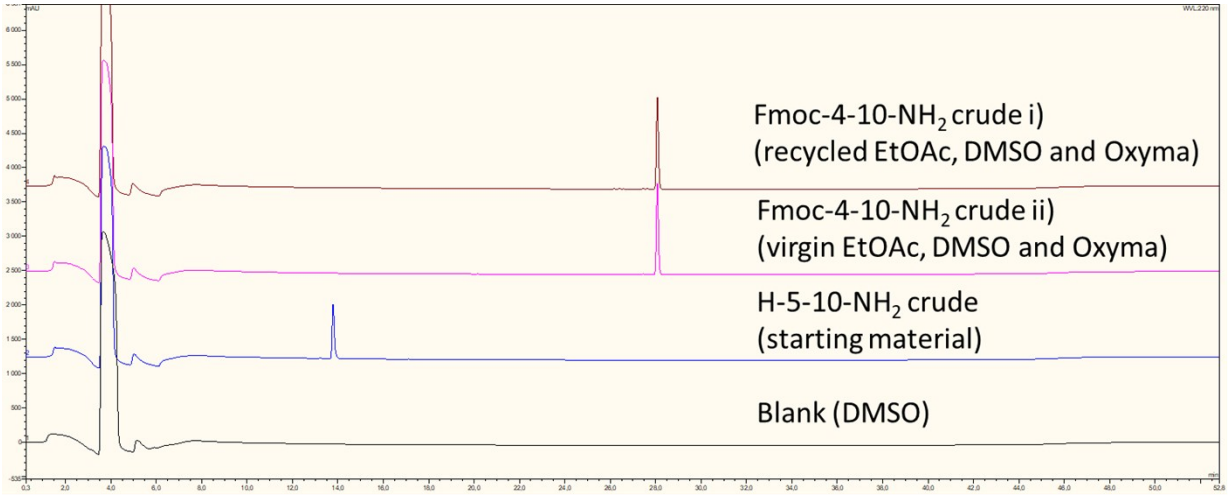


Figure S5. HPLC overlay for crude Fmoc-4-10-NH_2 prepared using recycled and virgin EtOAc, DMSO and Oxyma in the Aib[4] coupling examined. HPLC chromatogram of H-5-10-NH_2 crude (starting material) is shown as well, DMSO was used as sample solvent for all analyses.

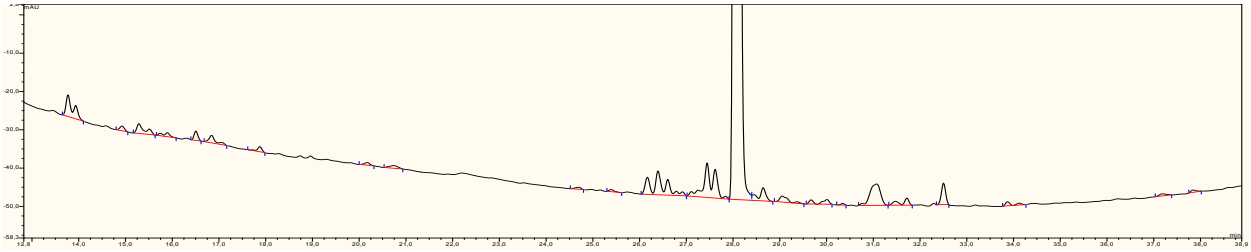


Figure S6. HPLC analysis for crude Fmoc-4-10-NH_2 prepared using virgin EtOAc, DMSO and Oxyma in the Aib[4] coupling examined.

Table S9. Area% for integrated peaks.

No.	Peakname	Ret.Time min	Area mAU*min	Amount	Type	Height mAU	Rel.Area %	Resolution
1	4	13,767	0,5654	n.a.	BM *	5,646	0,37	0,98
2	n.a.	13,933	0,3944	n.a.	MB*	3,494	0,26	5,63
3	n.a.	14,933	0,1402	n.a.	BMB*	1,236	0,09	1,83
4	n.a.	15,283	0,5019	n.a.	BMB*	2,548	0,33	2,12
5	n.a.	15,900	0,1786	n.a.	BMB*	1,045	0,12	2,24
6	n.a.	16,500	0,2261	n.a.	BMB*	2,403	0,15	2,06
7	n.a.	16,850	0,3222	n.a.	BMB*	1,957	0,21	5,95
8	n.a.	17,883	0,1490	n.a.	BMB*	1,328	0,10	12,23
9	n.a.	20,183	0,0908	n.a.	BMB*	0,671	0,06	1,96
10	n.a.	20,750	0,1468	n.a.	BMB*	0,707	0,10	13,10
11	n.a.	24,700	0,0728	n.a.	BMB*	0,518	0,05	3,31
12	n.a.	25,400	0,0670	n.a.	BMB*	0,557	0,04	2,71
13	n.a.	26,400	1,8742	n.a.	BMB*	6,185	1,23	2,89
14	n.a.	27,450	2,2639	n.a.	BMb*	9,001	1,49	3,63
15	*n.a.*	*28,067*	*141,0102*	*n.a.*	*bM **	*1328,518*	*92,77*	*3,29*
16	n.a.	28,650	0,5918	n.a.	MB*	3,425	0,39	1,57
17	n.a.	29,050	0,3376	n.a.	BMB*	1,573	0,22	1,83
18	n.a.	30,017	0,2940	n.a.	BMB*	1,222	0,19	0,76
19	n.a.	30,350	0,0344	n.a.	BMB*	0,431	0,02	2,56
20	n.a.	31,067	1,4608	n.a.	BMB*	5,581	0,96	1,35
21	n.a.	31,717	0,3394	n.a.	BMB*	1,843	0,22	2,21
22	n.a.	32,500	0,5886	n.a.	BMB*	5,626	0,39	8,06
23	n.a.	33,867	0,1736	n.a.	BMB*	1,068	0,11	14,56
24	n.a.	37,183	0,0946	n.a.	BMB*	0,542	0,06	2,67
25	n.a.	37,833	0,0745	n.a.	BMB*	0,591	0,05	n.a.
Total:			**151,9926**	**0,0000**		**1387,716**	**100,00**	

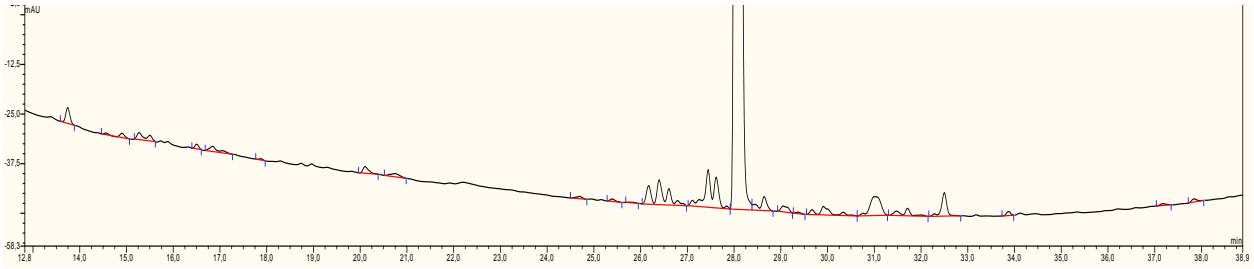


Figure S7. HPLC analysis for crude Fmoc-4-10-NH_2 prepared using recycled EtOAc, DMSO and Oxyma in the Aib^4 coupling examined.

Table S10. Area% for integrated peaks.

No.	Peakname	Ret.Time min	Area mAU*min	Amount	Type	Height mAU	Rel.Area %	Resolution
1	4	13,750	0,4072	n.a.	BMB*	3,982	0,27	6,72
2	n.a.	14,917	0,1562	n.a.	BMB*	1,064	0,10	1,78
3	n.a.	15,267	0,3967	n.a.	BMB*	1,771	0,26	6,87
4	n.a.	16,500	0,1090	n.a.	BMB*	1,189	0,07	1,81
5	n.a.	16,850	0,2863	n.a.	BMB*	1,316	0,19	5,42
6	n.a.	17,883	0,0283	n.a.	BMB*	0,322	0,02	12,34
7	n.a.	20,100	0,2320	n.a.	BMB*	1,680	0,15	2,16
8	n.a.	20,750	0,1837	n.a.	BMB*	0,815	0,12	12,45
9	n.a.	24,700	0,0869	n.a.	BMB*	0,583	0,06	3,21
10	n.a.	25,400	0,0800	n.a.	BMB*	0,643	0,05	1,86
11	n.a.	25,833	0,0228	n.a.	BMB*	0,152	0,01	1,36
12	n.a.	26,400	1,8586	n.a.	BMB*	6,268	1,21	2,88
13	n.a.	27,450	2,3649	n.a.	BMb*	9,492	1,54	3,73
14	*n.a.*	*28,083*	*142,7778*	*n.a.*	*bM **	*1336,617*	*93,09*	*3,19*
15	n.a.	28,650	0,6157	n.a.	MB*	3,500	0,40	1,57
16	n.a.	29,050	0,2736	n.a.	BMB*	1,484	0,18	1,19
17	n.a.	29,367	0,0456	n.a.	BMB*	0,364	0,03	1,25
18	n.a.	29,917	0,6368	n.a.	BMb*	2,140	0,42	1,96
19	n.a.	31,000	1,3110	n.a.	bMB*	4,759	0,85	1,46
20	n.a.	31,717	0,4309	n.a.	BMB*	1,899	0,28	2,15
21	n.a.	32,500	0,7829	n.a.	BMB*	5,925	0,51	7,74
22	n.a.	33,867	0,1078	n.a.	BMB*	1,037	0,07	16,07
23	n.a.	37,167	0,0670	n.a.	BMB*	0,449	0,04	2,98
24	n.a.	37,833	0,1097	n.a.	BMB*	0,816	0,07	n.a.
Total:			**153,3710**	**0,0000**		**1388,267**	**100,00**	

9. Fmoc-Cys(Trt)-OH stability in DMF and DMSO/EtOAc

To examine the stability of oxidation prone AAs during SPPS using DMSO/EtOAc as a synthesis solvent a stability assessment of an oxidation sensitive AA derivative was carried out. Cys was chosen as the oxidation prone AA and stability of its derivative Fmoc-Cys(Trt)-OH was examined in both DMF and DMSO/EtOAc (1:9). 0.1M stock solutions of Fmoc-Cys(Trt)-OH in both solvents were prepared and stability of the AA was determined after 48 h at rt. Four stability experiments was carried out for each solvent: i) 0.1M Fmoc-Cys(Trt)-OH in the solvent of the experiment ii) 0.1M Fmoc-Cys(Trt)-OH/Oxyma in the solvent of the experiment iii) 0.1M Fmoc-Cys(Trt)-OH/Oxyma+10 mol% of DTT as antioxidant in the solvent of the experiment iv) 0.1M Fmoc-Cys(Trt)-OH/Oxyma+10 mol% of DITU as antioxidant in the solvent of the experiment (Fig. S8). For all experiments HPLC analyses were carried out at t=0 h and t = 48 h by taking out 50 μL of the reaction mixture and diluting these aliquots with 1.0 mL MeCN. Following HPLC method was used for all analyses: column: Waters XSelect CSH130 C18 2.5μm 4.6x150mm; column temperature: 45°C; injection volume: 10 μL; sampler temperature:10°C; flow: 1.0 ml/min; mobile phase A: 0.1 % TFA in water, mobile phase B: 0.08 % TFA in 90% MeCN/10 %water. Gradient (Time(min), %B): 0, 1; 10, 100; 13, 100; 14, 100; 19, 1; 20, 1.

The Fig. S8 HPLC analyses revealed that in DMSO/EtOAc (1:9) Fmoc-Cys(Trt)-OH was fully stable, irrespective of presence of Oxyma and DTT or DITU as antioxidants. On the other hand, in DMF Fmoc-Cys(Trt)-OH was not stable. The instability of the AA was conceivably due to a limited stability of the Fmoc group in DMF, as evidenced by the presence of a large dibenzofulvene (DBF) peak (rt ~9 min) formed from the Fmoc group.[2] Further, Fmoc-Cys(Trt)-OH/Oxyma in DMF was unstable as well, although the means of the breakdown of the AA was not pertaining to the instability of the Fmoc group, as evidenced by the complete absence of the DBF peak in the chromatogram. To understand the mode of the breakdown of Fmoc-Cys(Trt)-OH/Oxyma in DMF an LC-HRMS analysis was carried out (section 10 of this ESI). Based on this LC-HRMS elucidation, a mechanistic proposal for the breakdown of Fmoc-Cys(Trt)-OH/Oxyma in DMF was put forth (section 11 of this ESI). Finally, Fmoc-Cys(Trt)-OH/Oxyma in DMF in the presence of 10 mol% DTT or 10 mol% DITU was fully stable, indicating that the AA breakdown which occurred with Fmoc-Cys(Trt)-OH/Oxyma in DMF was conceivably of oxidative nature, and was fully inhibited by the presence of DTT or DITU acting as antioxidants. For a further discussion see section 11 of this ESI.

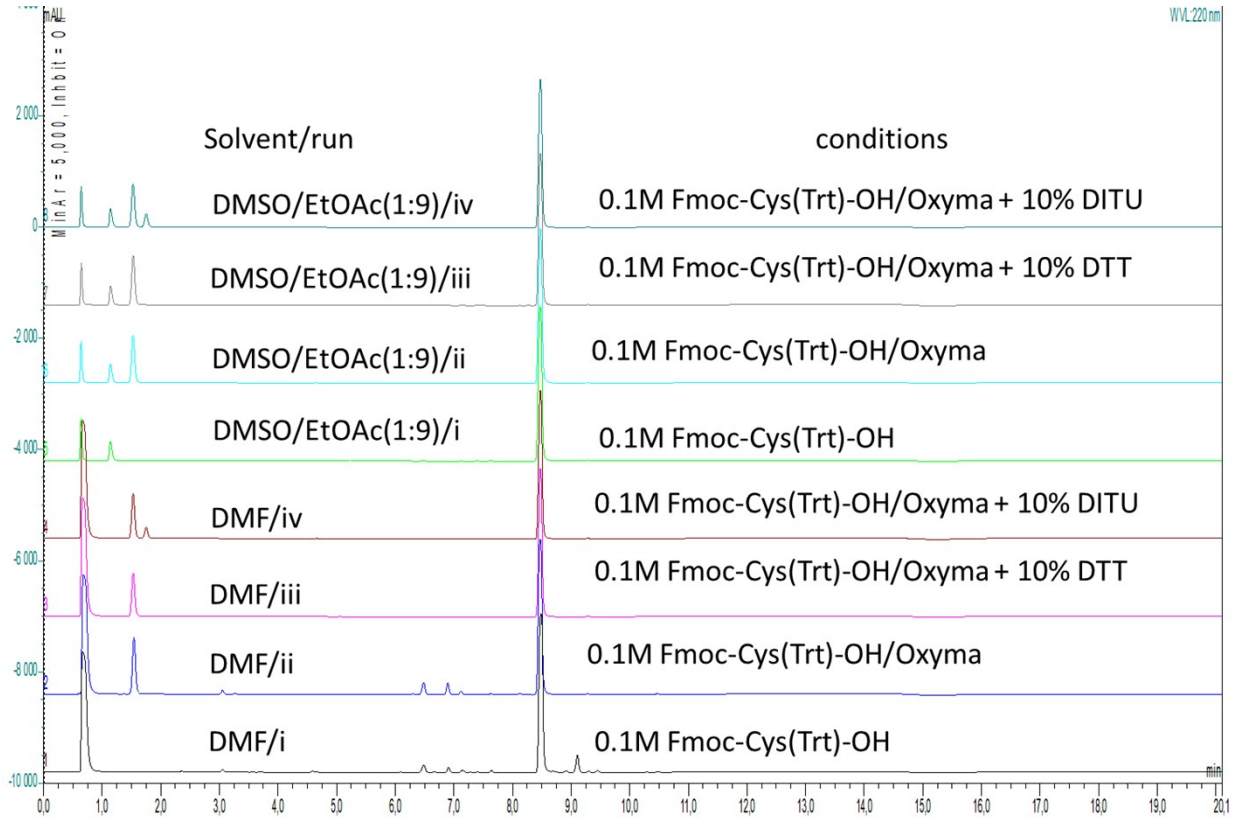


Figure S8. HPLC overlay for the assessment of stability of 0.1M Fmoc-Cys(Trt)-OH in DMF and DMSO/EtOAc (1:9) respectively after 48 h at rt.

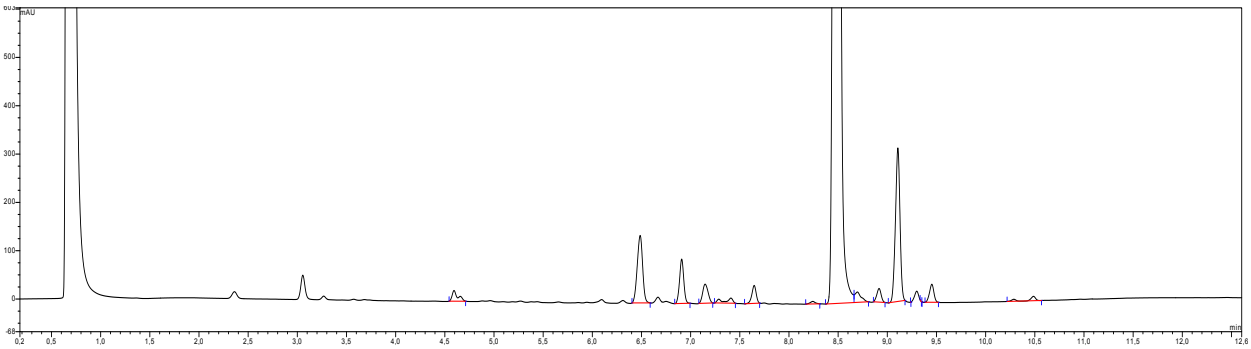


Figure S9. HPLC assessment of stability of 0.1M Fmoc-Cys(Trt)-OH in DMF/run i after 48 h at rt.

Table S11. Area% for integrated peaks.

No.	Peakname	Ret.Time min	Area mAU*min	Amount	Type	Height mAU	Rel.Area %	Resolution
1	n.a.	4,593	1,4754	n.a.	BMB*	22,153	0,55	21,76
2	n.a.	6,487	8,8565	n.a.	BMB*	139,685	3,31	4,74
3	n.a.	6,908	4,5467	n.a.	BMB*	91,873	1,70	2,63
4	n.a.	7,147	2,5066	n.a.	BMB*	39,370	0,94	1,40
5	n.a.	7,410	1,0215	n.a.	BMB*	10,512	0,38	1,35
6	n.a.	7,647	1,8760	n.a.	BMB*	37,100	0,70	7,08
7	n.a.	8,242	0,3092	n.a.	BMB*	5,238	0,12	2,24
8	*n.a.*	*8,485*	*224,3080*	*n.a.*	*BM **	*2853,740*	*83,84*	*n.a.*
9	n.a.	8,695	1,5872	n.a.	MB*	21,426	0,59	n.a.
10	n.a.	8,917	1,4343	n.a.	BMB*	28,201	0,54	2,30
11	n.a.	9,107	17,0183	n.a.	BMB*	317,671	6,36	2,94
12	n.a.	9,238	0,0001	n.a.	BMB*	0,027	0,00	9,47
13	n.a.	9,340	0,0041	n.a.	BMB*	0,466	0,00	n.a.
14	n.a.	9,353	0,0000	n.a.	BMB*	0,000	0,00	n.a.
15	n.a.	9,453	1,9378	n.a.	BMB*	37,310	0,72	12,59
16	n.a.	10,487	0,6606	n.a.	BMB*	8,999	0,25	n.a.
Total:			**267,5422**	**0,0000**		**3613,774**	**100,00**	

Figure S10. HPLC assessment of stability of 0.1M Fmoc-Cys(Trt)-OH in DMF/run ii after 48 h at rt.

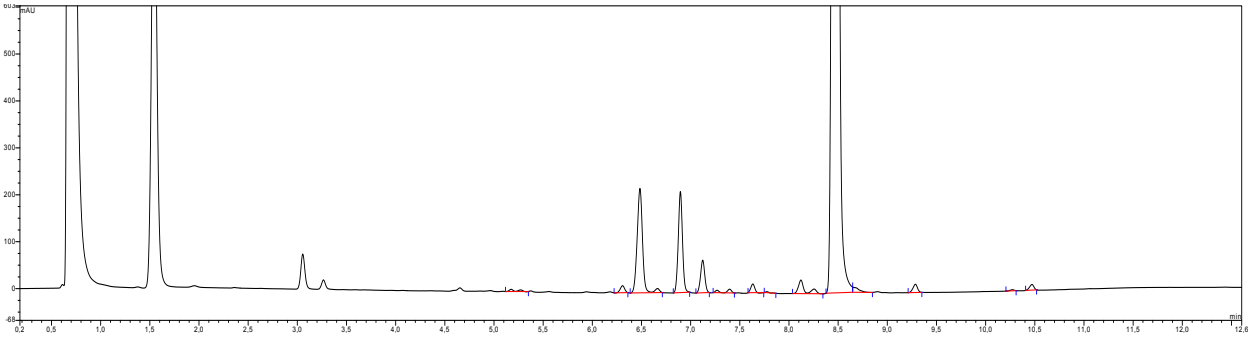


Table S12. Area% for integrated peaks.

No.	Peakname	Ret.Time min	Area mAU*min	Amount	Type	Height mAU	Rel.Area %	Resolution
1	n.a.	5,175	0,3568	n.a.	BMB*	4,682	0,15	14,73
2	n.a.	6,307	0,7609	n.a.	BMB*	14,931	0,31	2,00
3	n.a.	6,485	14,1663	n.a.	BMB*	222,570	5,77	4,76
4	n.a.	6,895	10,6596	n.a.	BMB*	215,402	4,34	2,97
5	n.a.	7,123	3,4271	n.a.	BMB*	69,521	1,40	1,56
6	n.a.	7,397	0,5741	n.a.	BMB*	7,897	0,23	1,35
7	n.a.	7,632	0,7973	n.a.	BMB*	18,402	0,32	2,19
8	n.a.	7,777	0,0744	n.a.	BMB*	2,033	0,03	4,82
9	n.a.	8,122	2,2290	n.a.	BMB*	28,893	0,91	3,40
10	*n.a.*	*8,473*	*210,1288*	*n.a.*	*BM **	*2799,385*	*85,56*	*n.a.*
11	n.a.	8,650	0,7622	n.a.	MB*	10,828	0,31	n.a.
12	n.a.	9,285	0,9383	n.a.	BMB*	17,608	0,38	12,23
13	n.a.	10,272	0,1143	n.a.	BMB*	2,403	0,05	2,47
14	n.a.	10,470	0,6143	n.a.	BMB*	11,963	0,25	n.a.
Total:			**245,6034**	**0,0000**		**3426,518**	**100,00**	

Figure S11. HPLC assessment of stability of 0.1M Fmoc-Cys(Trt)-OH in DMF/run iii after 48 h at rt.

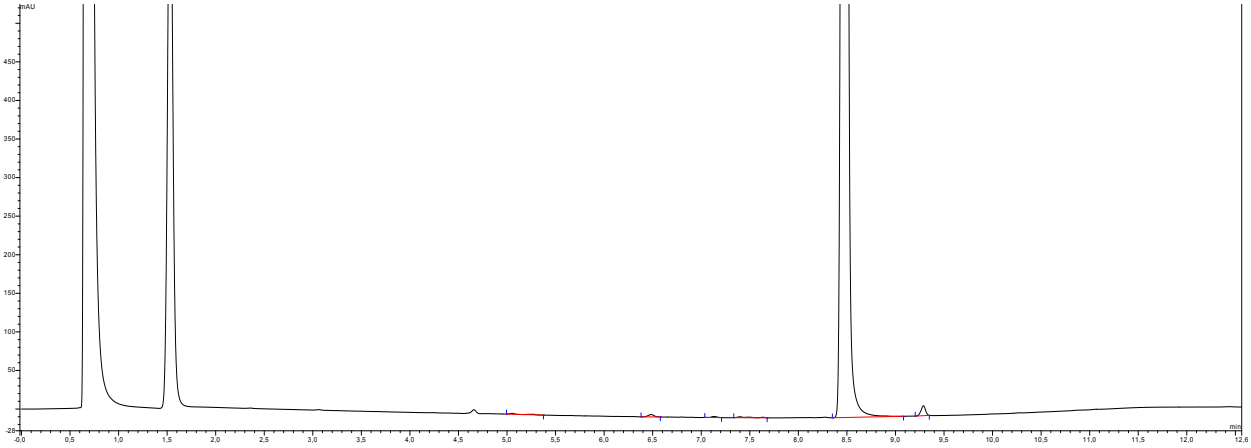


Table S13. Area% for integrated peaks.

No.	Peakname	Ret.Time min	Area mAU*min	Amount	Type	Height mAU	Rel.Area %	Resolution
1	n.a.	5,057	0,1070	n.a.	BMB*	1,124	0,06	15,32
2	n.a.	6,485	0,2160	n.a.	BMB*	3,049	0,12	6,14
3	n.a.	7,132	0,0842	n.a.	BMB*	1,391	0,05	2,82
4	n.a.	7,400	0,1669	n.a.	BMB*	1,232	0,09	10,59
5	*n.a.*	*8,477*	*185,6891*	*n.a.*	*BMB**	*2668,064*	*99,32*	*8,26*
6	n.a.	9,288	0,6879	n.a.	BMB*	12,850	0,37	n.a.
Total:			**186,9513**	**0,0000**		**2687,709**	**100,00**	

Figure S12. HPLC assessment of stability of 0.1M Fmoc-Cys(Trt)-OH in DMF/run iv after 48 h at rt.

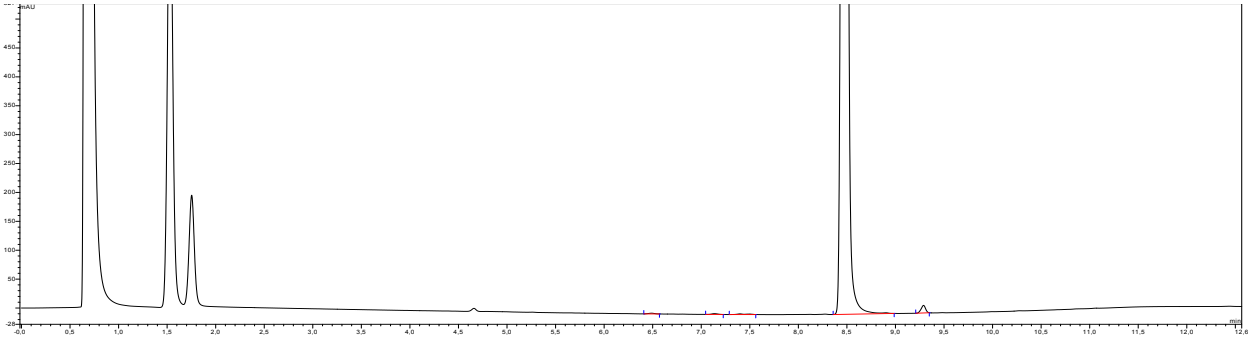


Table S14. Area% for integrated peaks.

No.	Peakname	Ret.Time min	Area mAU*min	Amount	Type	Height mAU	Rel.Area %	Resolution
1	n.a.	6,488	0,0875	n.a.	BMB*	1,229	0,05	5,70
2	n.a.	7,137	0,0962	n.a.	BMB*	1,359	0,05	2,47
3	n.a.	7,400	0,1333	n.a.	BMB*	1,166	0,07	10,15
4	*n.a.*	*8,477*	*187,5465*	*n.a.*	*BMB**	*2676,504*	*99,46*	*8,26*
5	n.a.	9,288	0,6983	n.a.	BMB*	13,099	0,37	n.a.
Total:			**188,5619**	**0,0000**		**2693,356**	**100,00**	

Figure S13. HPLC assessment of stability of 0.1M Fmoc-Cys(Trt)-OH in DMSO/EtOAc (1:9)/run i after 48 h at rt.

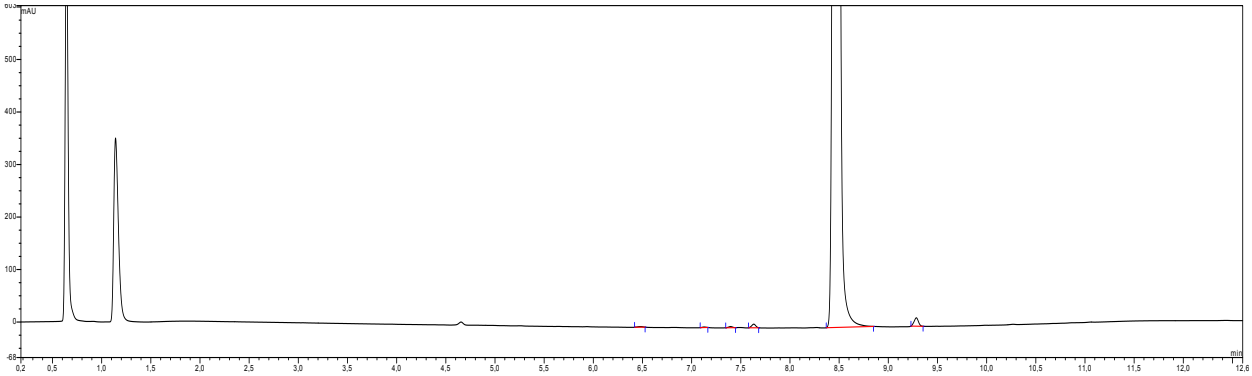


Table S15. Area% for integrated peaks.

No.	Peakname	Ret.Time min	Area mAU*min	Amount	Type	Height mAU	Rel.Area %	Resolution
1	n.a.	6,478	0,0694	n.a.	BMB*	1,158	0,03	7,30
2	n.a.	7,125	0,0456	n.a.	BMB*	1,042	0,02	3,64
3	n.a.	7,397	0,0976	n.a.	BMB*	2,132	0,05	3,10
4	n.a.	7,632	0,3278	n.a.	BMB*	6,877	0,16	8,56
5	*n.a.*	*8,473*	*206,2607*	*n.a.*	*BMB**	*2779,921*	*99,35*	*8,06*
6	n.a.	9,285	0,8132	n.a.	BMB*	15,901	0,39	n.a.
Total:			**207,6143**	**0,0000**		**2807,031**	**100,00**	

Figure S14. HPLC assessment of stability of 0.1M Fmoc-Cys(Trt)-OH in DMSO/EtOAc (1:9)/run ii after 48 h at rt.

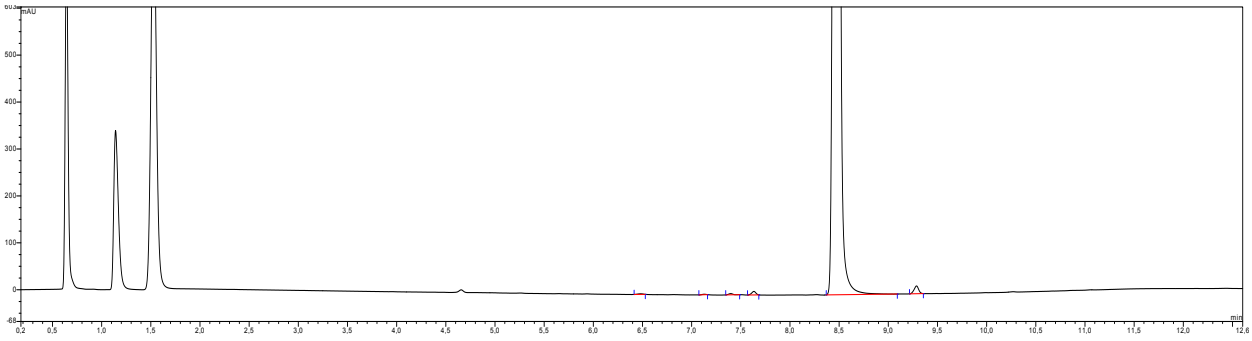


Table S16. Area% for integrated peaks.

No.	Peakname	Ret.Time min	Area mAU*min	Amount	Type	Height mAU	Rel.Area %	Resolution
1	n.a.	6,480	0,0752	n.a.	BMB*	1,222	0,04	7,31
2	n.a.	7,127	0,0437	n.a.	BMB*	1,012	0,02	3,66
3	n.a.	7,397	0,1205	n.a.	BMB*	2,588	0,06	3,08
4	n.a.	7,633	0,3650	n.a.	BMB*	7,474	0,17	8,49
5	*n.a.*	*8,475*	*207,8852*	*n.a.*	*BMB**	*2781,354*	*99,29*	*7,94*
6	n.a.	9,287	0,8857	n.a.	BMB*	16,515	0,42	n.a.
Total:			**209,3754**	**0,0000**		**2810,165**	**100,00**	

Figure S15. HPLC assessment of stability of 0.1M Fmoc-Cys(Trt)-OH in DMSO/EtOAc (1:9)/run iii after 48 h at rt.

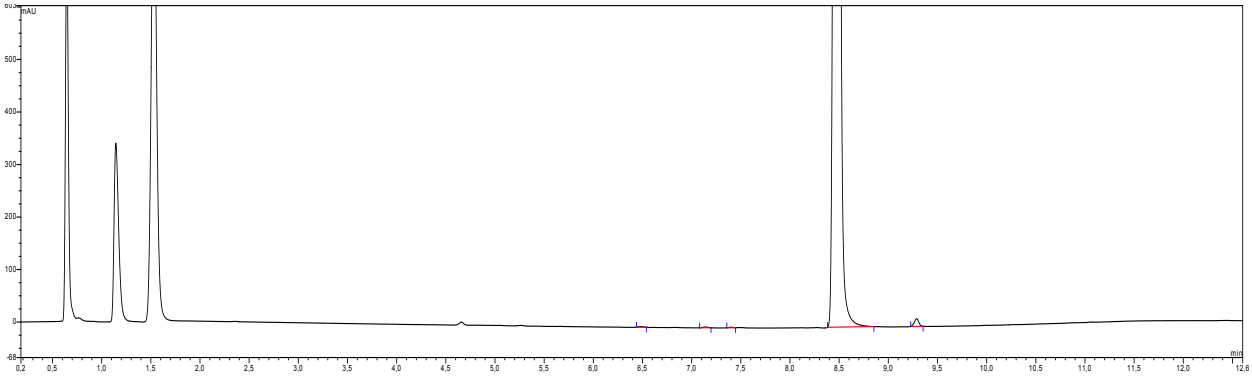


Table S17. Area% for integrated peaks.

No.	Peakname	Ret.Time min	Area mAU*min	Amount	Type	Height mAU	Rel.Area %	Resolution
1	n.a.	6,490	0,0647	n.a.	BMB*	1,195	0,03	6,67
2	n.a.	7,135	0,0852	n.a.	BMB*	1,401	0,04	3,07
3	n.a.	7,398	0,0368	n.a.	BMB*	0,881	0,02	11,64
4	*n.a.*	*8,478*	*197,9148*	*n.a.*	*BMB**	*2738,511*	*99,52*	*8,12*
5	n.a.	9,288	0,7669	n.a.	BMB*	14,683	0,39	n.a.
Total:			**198,8685**	**0,0000**		**2756,671**	**100,00**	

Figure S16. HPLC assessment of stability of 0.1M Fmoc-Cys(Trt)-OH in DMSO/EtOAc (1:9)/run iv after 48 h at rt.

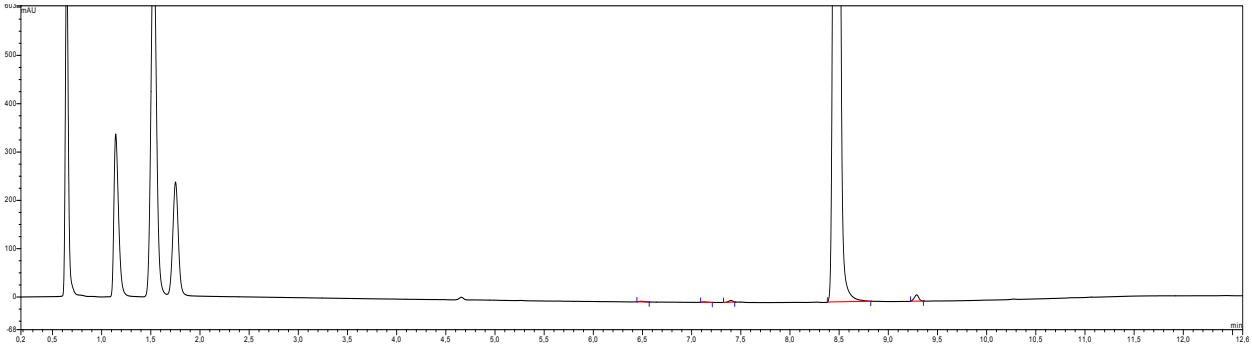


Table S18. Area% for integrated peaks.

No.	Peakname	Ret.Time min	Area mAU*min	Amount	Type	Height mAU	Rel.Area %	Resolution
1	n.a.	6,490	0,0601	n.a.	BMB*	1,061	0,03	7,28
2	n.a.	7,130	0,0411	n.a.	BMB*	0,781	0,02	3,40
3	n.a.	7,398	0,1603	n.a.	BMB*	3,508	0,09	11,62
4	*n.a.*	*8,477*	*185,2393*	*n.a.*	*BMB**	*2672,267*	*99,49*	*8,34*
5	n.a.	9,288	0,6825	n.a.	BMB*	12,978	0,37	n.a.
Total:			**186,1833**	**0,0000**		**2690,595**	**100,00**	

10. LC-HRMS of products of degradation of Fmoc-Cys(Trt)-OH/Oxyma in DMF

LC-HRMS analysis of 0.1M Fmoc-Cys(Trt)-OH/Oxyma in DMF after 48 h at rt was carried out to gain understanding of the Fmoc-Cys(Trt)-OH breakdown under these conditions. MS spectra and tentative structural assignments for the observed breakdown products are provided in Figures S17 – S29. Experimental conditions for this LC-HRMS analysis were the same as described in section 5 of this ESI.

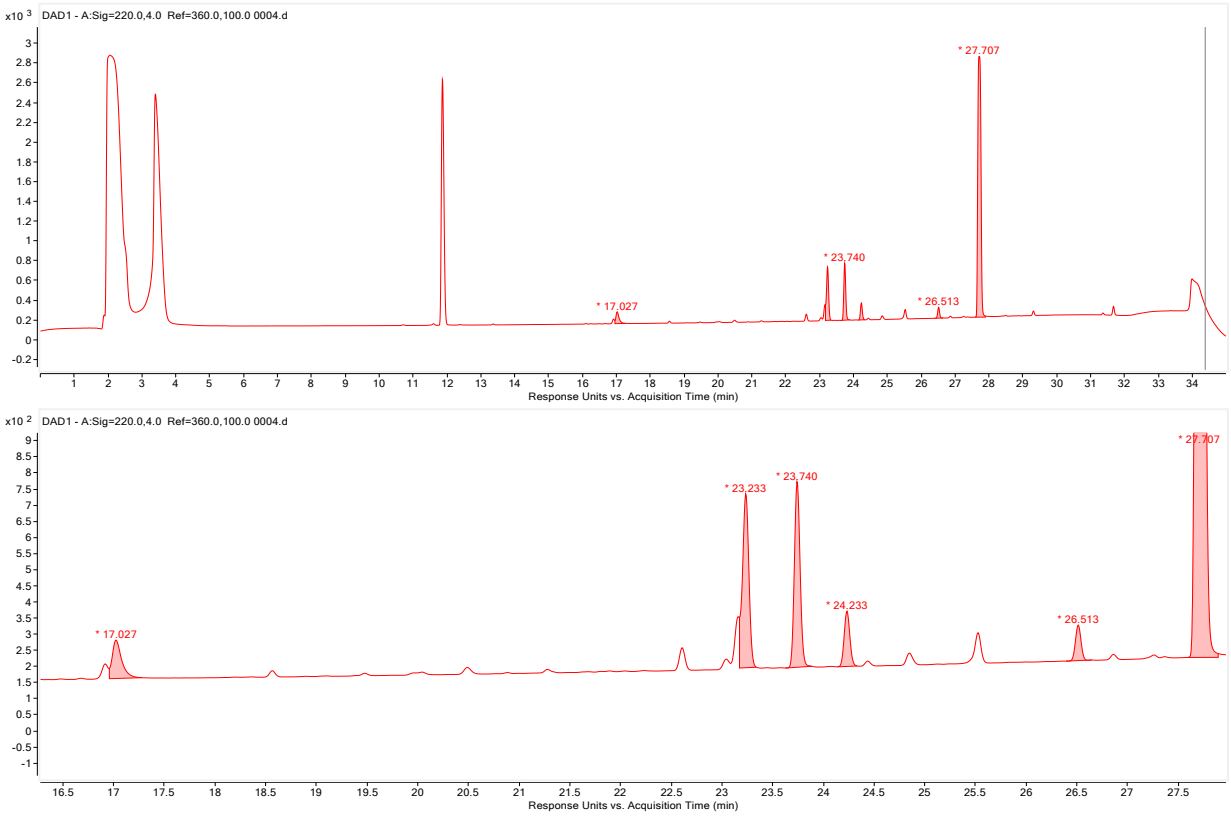


Figure S17. LC-HRMS analysis of Fmoc-Cys(Trt)-OH/Oxyma in DMF after 48 h at rt (Section 8, Fig. S6)

Table S19. Area% for integrated peaks and tentative MS identities for Fmoc-Cys(Trt)-OH and degradants in Fmoc-Cys(Trt)-OH/Oxyma in DMF after 48 h at rt.

Peak	Rt	Area	(m+z)/z	z	decon.	RRT	Area %	identity
1	17,03	767,230	392,0804	1	391,0731	0,615	3,26	Fmoc-Cys(SO_3H)-OH
2	23,23	2349,930	547,1080	1	547,1080	0,839	9,98	Trt-2 x Oxyma adduct (detected as Na salt)
3	23,74	2258,370	717,1570	1	716,1497	0,857	9,59	Fmoc-Cys(SO_2)-Cys-Fmoc
4	24,23	667,280	685,1670	1	684,1597	0,875	2,83	(Fmoc-Cys-OH)$_2$
5	26,51	418,750	407,1370	1	407,1370	0,957	1,78	Trt- Oxyma adduct (detected as Na salt)
6	27,71	17084,650	608,1870	1	608,1870	1,000	72,56	Fmoc-Cys(Trt)-OH (detected as Na salt)

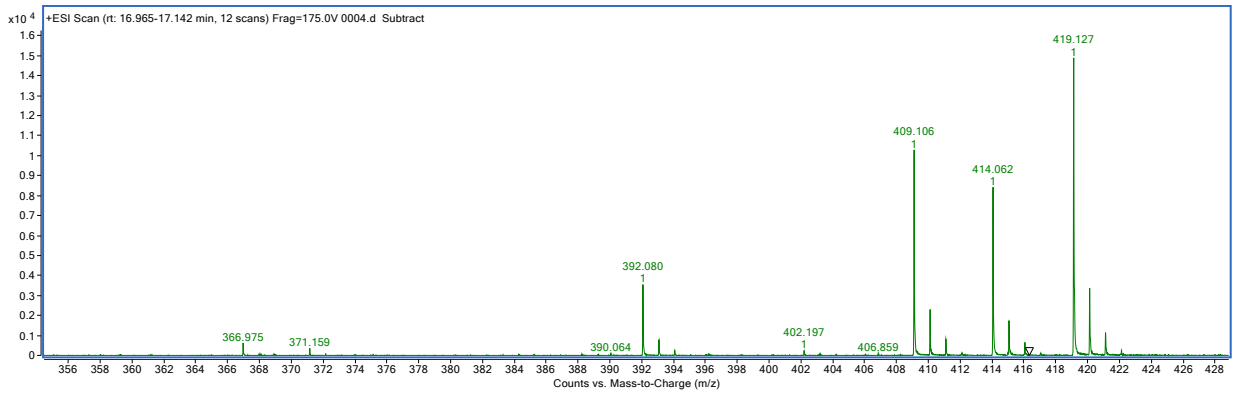
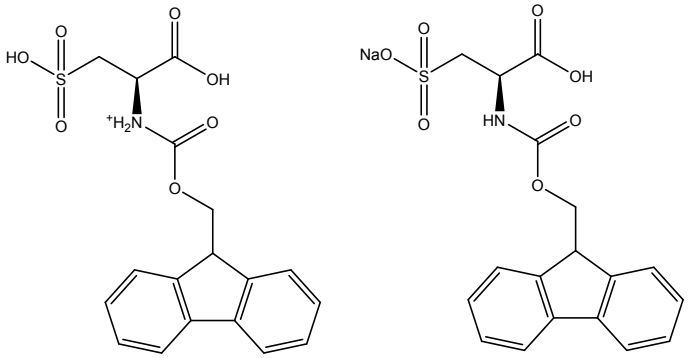


Figure S18. MS spectrum for Fmoc-Cys(SO_3H)-OH



Chemical Formula: $C_{18}H_{18}NO_7S^+$
Exact Mass: 392,0798
Molecular Weight: 392,4015

Chemical Formula: $C_{18}H_{17}NNaO_7S$
Exact Mass: 414,0623
Molecular Weight: 414,3838

Figure S19. Chemical structure and molecular weight for Fmoc-Cys(SO_3H)-OH

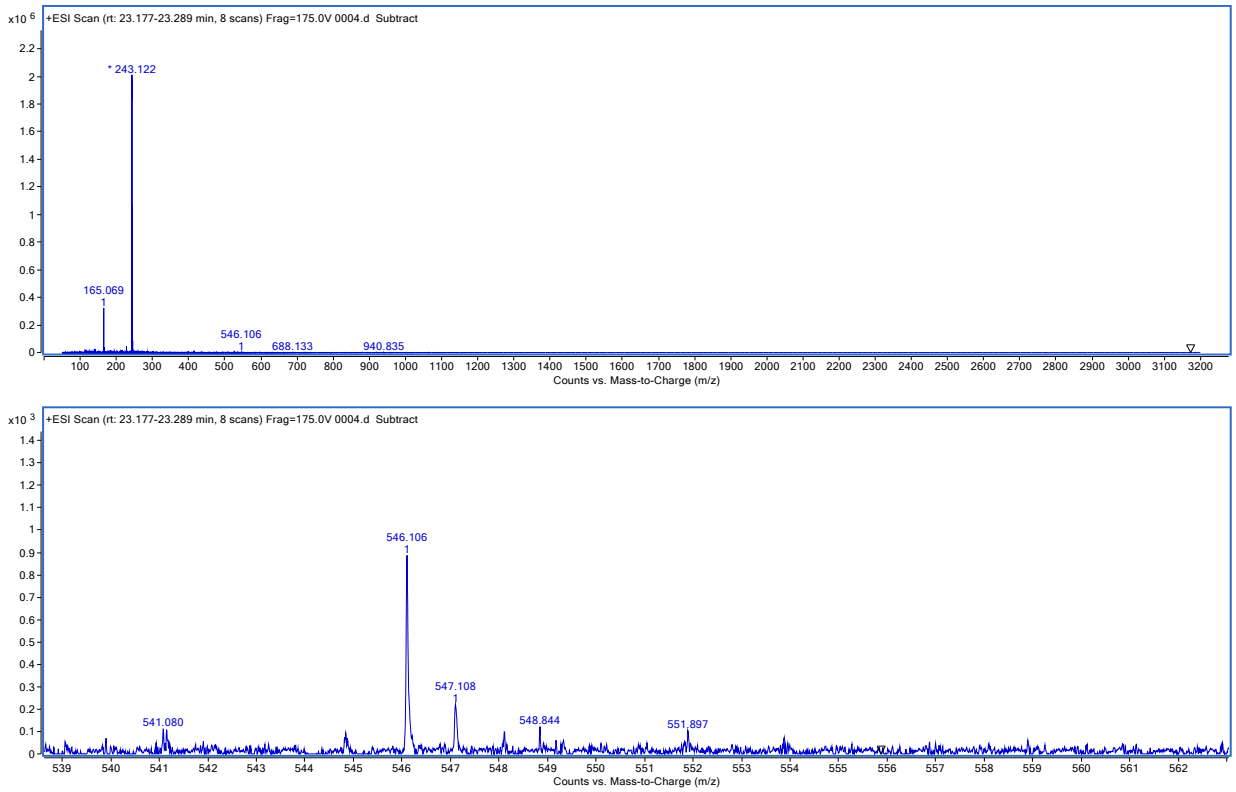
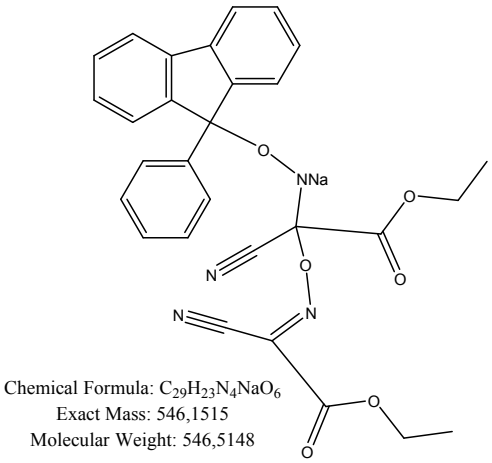


Figure S20. MS spectrum for Trt-2 x Oxyma adduct[8]



Chemical Formula: $C_{29}H_{23}N_4NaO_6$
Exact Mass: 546,1515
Molecular Weight: 546,5148

Figure S21. Chemical structure and molecular weight for Trt-2 x Oxyma adduct

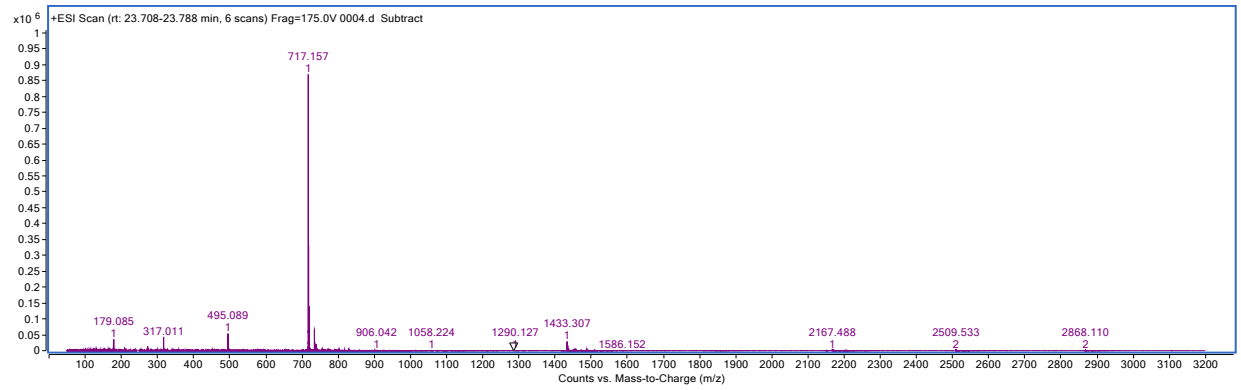
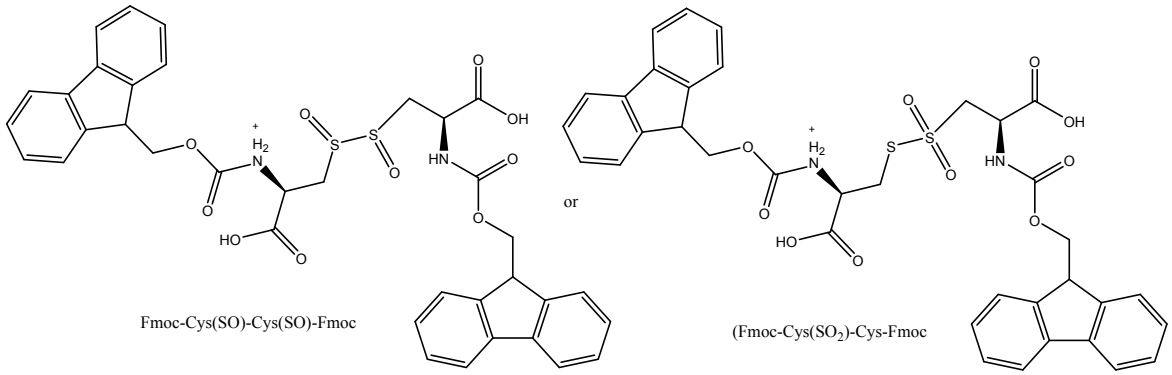


Figure S22. MS spectrum for Fmoc-Cys(SO_2)-Cys-Fmoc or Fmoc-Cys(SO)-Cys(SO)-Fmoc



Fmoc-Cys(SO)-Cys(SO)-Fmoc

Chemical Formula: $C_{36}H_{33}N_2O_{10}S_2^+$
Exact Mass: 717,1571
Molecular Weight: 717,7835

(Fmoc-Cys(SO_2)-Cys-Fmoc

Chemical Formula: $C_{36}H_{33}N_2O_{10}S_2^+$
Exact Mass: 717,1571
Molecular Weight: 717,7835

Figure S23. Chemical structure and molecular weight for Fmoc-Cys(SO_2)-Cys-Fmoc and Fmoc-Cys(SO)-Cys(SO)-Fmoc

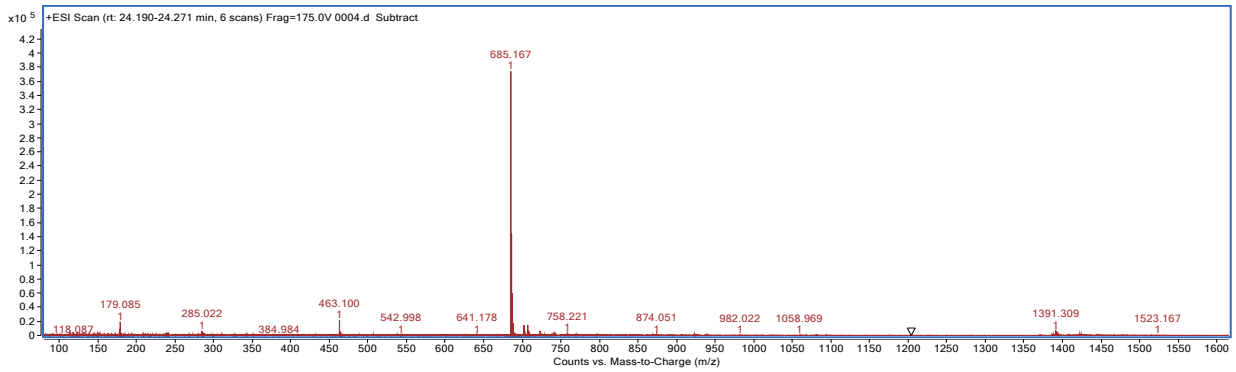
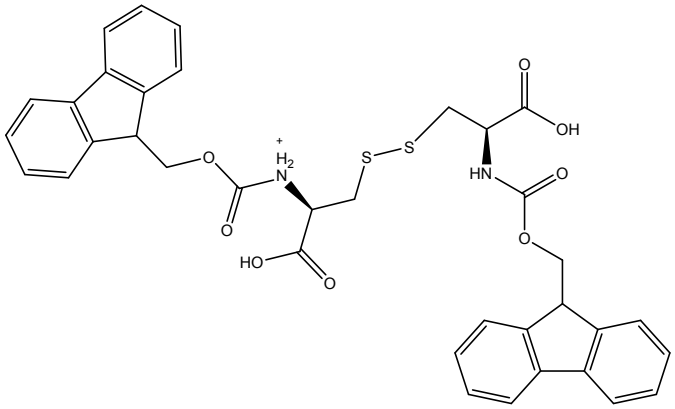


Figure S24. MS spectrum for $(Fmoc\text{-}Cys\text{-}OH)_2$



Chemical Formula: $C_{36}H_{33}N_2O_8S_2^+$
Exact Mass: 685,1673
Molecular Weight: 685,7855

Figure S25. Chemical structure and molecular weight for $(Fmoc\text{-}Cys\text{-}OH)_2$

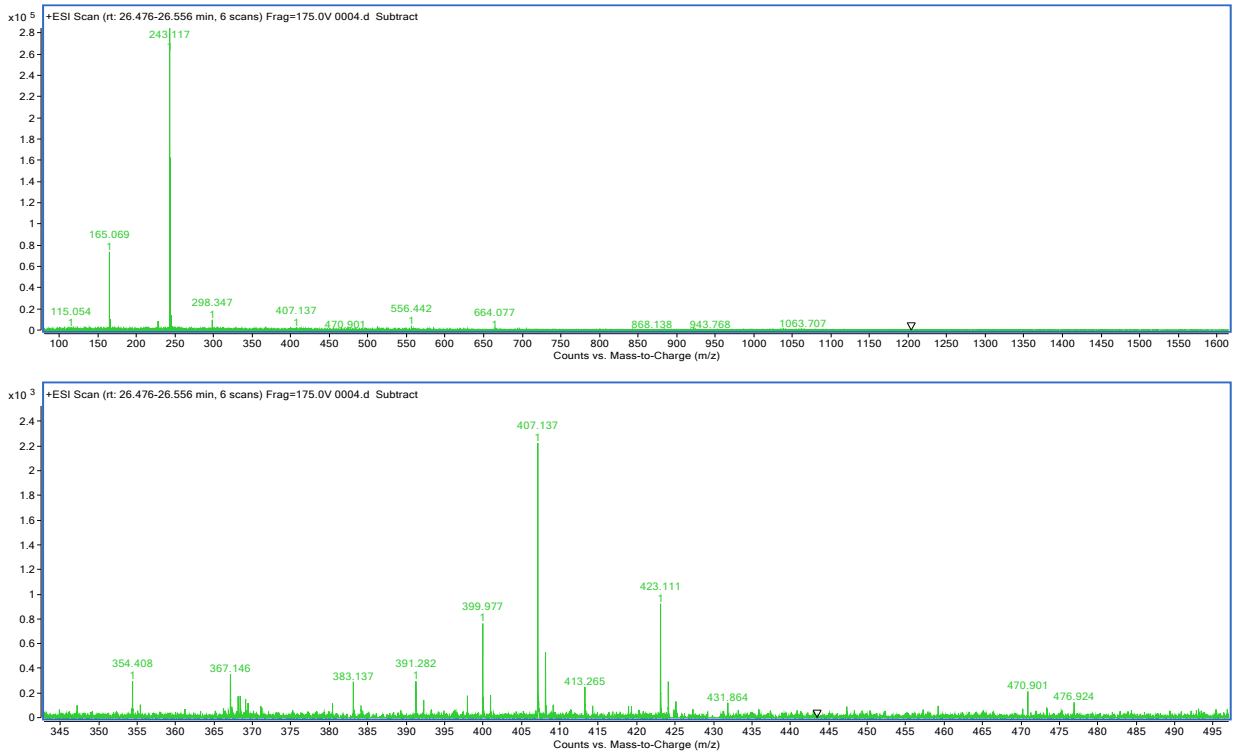
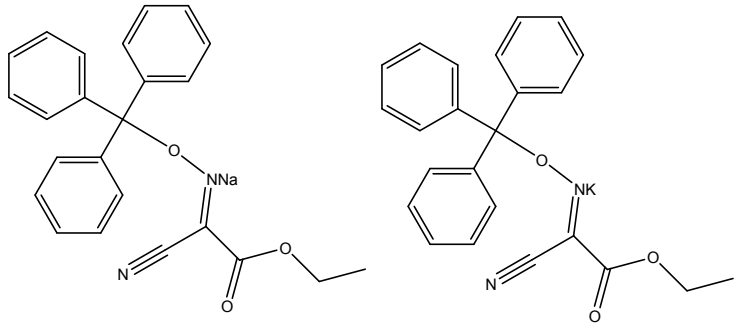


Figure S26. MS spectrum for Trt-Oxyma adduct



Chemical Formula: $C_{24}H_{20}N_2NaO_3$
Exact Mass: 407,1372
Molecular Weight: 407,4248

Chemical Formula: $C_{24}H_{20}KN_2O_3$
Exact Mass: 423,1111
Molecular Weight: 423,5333

Figure S27. Chemical structure and molecular weight for Trt-Oxyma adduct

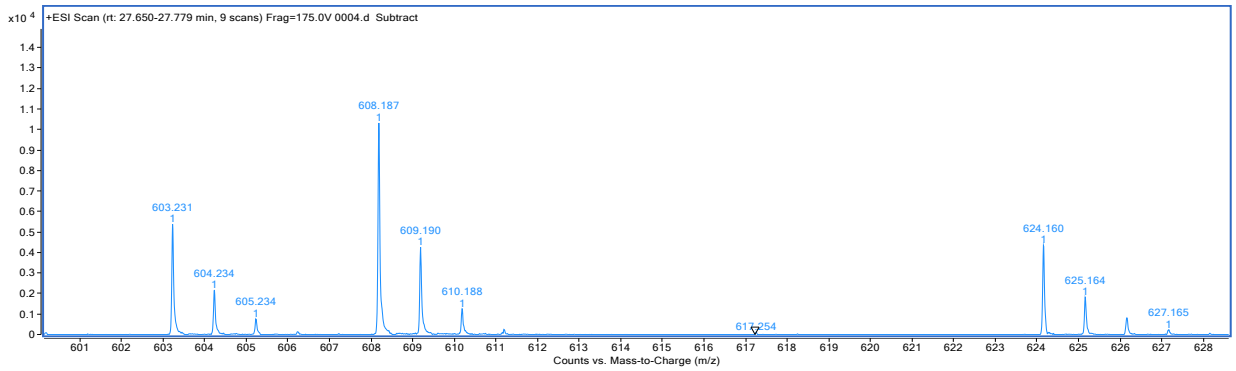
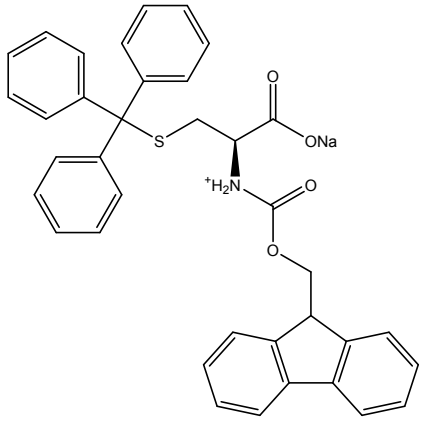


Figure S28. MS spectrum for Fmoc-Cys(Trt)-OH



Chemical Formula: $C_{37}H_{31}NNaO_4S^+$
Exact Mass: 608,1866
Molecular Weight: 608,7072

Figure S29. Chemical structure and molecular weight for Fmoc-Cys(Trt)-OH

11. Proposed mechanism of degradation of Fmoc-Cys(Trt)-OH/Oxyma in DMF

A tentative mechanistic proposal for the degradation of Fmoc-Cys(Trt)-OH in the presence of Oxyma in DMF is put forth. This putative mechanism is based on i) degradation studies of thiol substituted acids carried out herein (sections 8 and 15 of this ESI) ii) LC-HMRS assessment of the Fmoc-Cys(Trt)-OH degraded in DMF in the presence of Oxyma (section 9 of this ESI) iii) prior art cited herein.

The proposed first step of the observed Fmoc-Cys(Trt)-OH degradation is the conversion of Oxyma to the corresponding N-oxyl radical which can be caused for example by the presence of peroxides[9] and/or traces of metals/metal ions[10] in the solvent (Fig. S20). Subsequently, the N-oxyl radical abstracts an electron from a Fmoc-Cys(Trt)-OH thiol lone pair in a similar manner BTNO radical (formed from HOBtxH_2O) abstracts an electron from a lone pair on the nitrogen of dimethylaniline.[7] The resulting Fmoc-Cys$^{\bullet}$(Trt)-OH radical then loses a Trt^+ cation to form Fmoc-Cys$^{\bullet}$-OH thiyl radical. We propose that this degradation mechanism is far more likely than a scenario in which Oxyma (pKa 4.6) acts as an acid and cleaves the Cys-Trt sulfur-carbond bond to form Fmoc-Cys$^-$OH anion, which then gets oxidized. The reasons against Oxyma cleaving the Cys-Trt linkage are as follows:

i) upon addition of 10 mol% DTT or DITU (acting as a radical oxygen species scavenger) no Fmoc-Cys(Trt)-OH degradation occurs even though DTT or DITU is added in a substoichiometric amount compared to Oxyma, not impacting the ability of Oxyma to act as an acid. We propose that it is more likely that DTT or DITU suppresses the formation of the N-oxyl radical, which in turns prevents the attack of the radical on Fmoc-Cys(Trt)-OH and its degradation;

ii) Cl-HOBt (pKa 3.35), which is far more acidic than Oxyma does not cause a degradation of Fmoc-Cys(Trt)-OH (see section 15 of this ESI) rendering it unlikely that an acid induced Cys-Trt cleavage initiates the observed Fmoc-Cys(Trt)-OH degradation.

Further, we propose that the the Fmoc-Cys$^{\bullet}$-OH thiyl radical formed from Fmoc-Cys(Trt)-OH reacts with oxygen to form Fmoc-Cys(S-OO$^{\bullet}$)-OH peroxyl radical, which can rearrange to Fmoc-Cys((S=O)O$^{\bullet}$)-OH sulphonyl radical[11] which is in turn oxidized to the stable Fmoc-cysteic acid (Fmoc-Cys(SO_3H)-OH) observed by LC-HRMS in the crude product from the reaction between Fmoc-Cys(Trt)-OH and Oxyma in DMF (see section 9 of this ESI). Moreover, in the presence of Fmoc-Cys(H)-OH/ Fmoc-Cys$^{\bullet}$-OH the thiyl and and sulphonyl radicals respectively can readily dimerize to form disulfide ((Fmoc-Cys-OH)$_2$) and disulfinate((Fmoc-Cys(SO)-Cys(SO)-Fmoc)/thiosulfonate (Fmoc-Cys(SO_2)-Cys-Fmoc) dimers,[12] which were also observed in the

crude product from the reaction between Fmoc-Cys(Trt)-OH and Oxyma in DMF (see section 9 of this ESI). Finally, it is worth noting that the Trt moiety cleaved from Fmoc-Cys(Trt)-OH was not observed as the common Trt-H or Trt-OH but as adducts with one and two molecules of Oxyma instead.

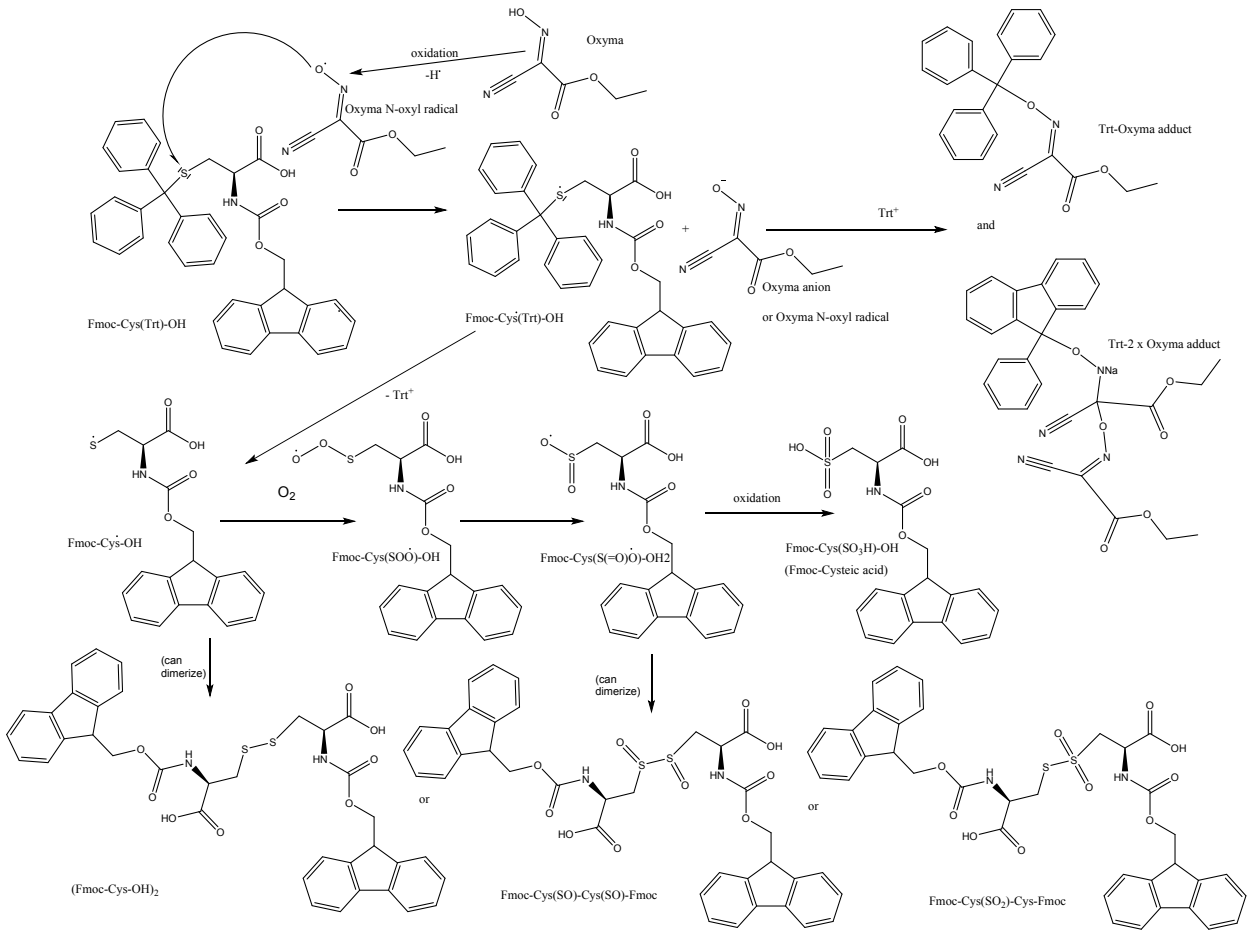


Figure S30. A tentative mechanism for the oxidation of Fmoc-Cys(Trt)-OH in DMF in the presence of Oxyma

12. HPLC analyses of Fmoc-C(H)$_6$-NH$_2$ crudes

HPLC analyses of Fmoc-C(H)$_6$-NH$_2$ crudes prepared according to the protocols described in Materials & Methods section of the main article were carried out in DMSO which was the most suitable solvent for these peptides from solubility standpoint. 100 g/L DITU was added to suppress any unwanted side reactions prior to performing the analyses. The conditions for these HPLC analyses were the same as described in section 5 of this ESI.

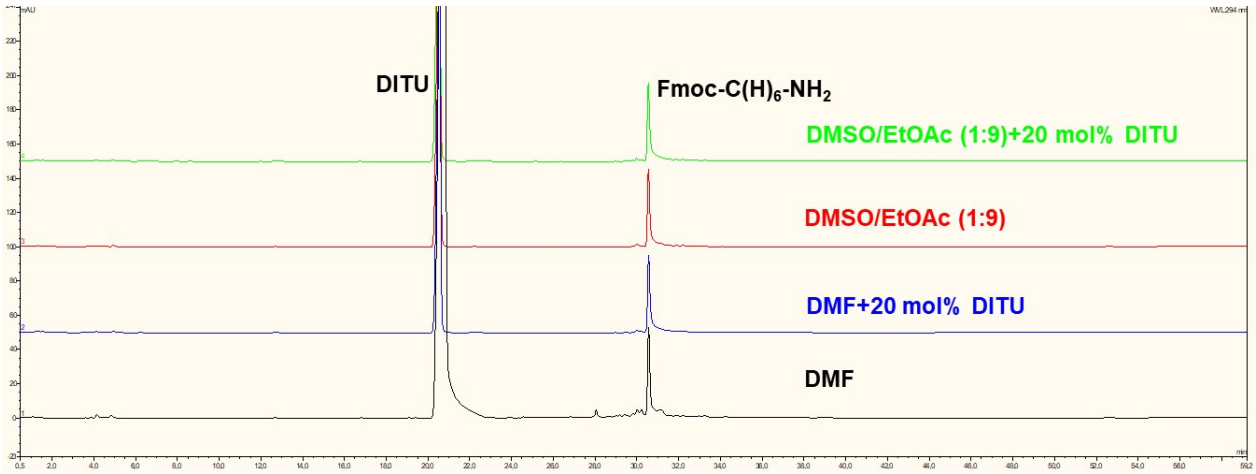


Figure S31. HPLC overlay for Fmoc-C(H)$_6$-NH$_2$ crudes prepared using DMF and DMSO/EtOAc (1:9) in couplings, ±20 mol% DITU.

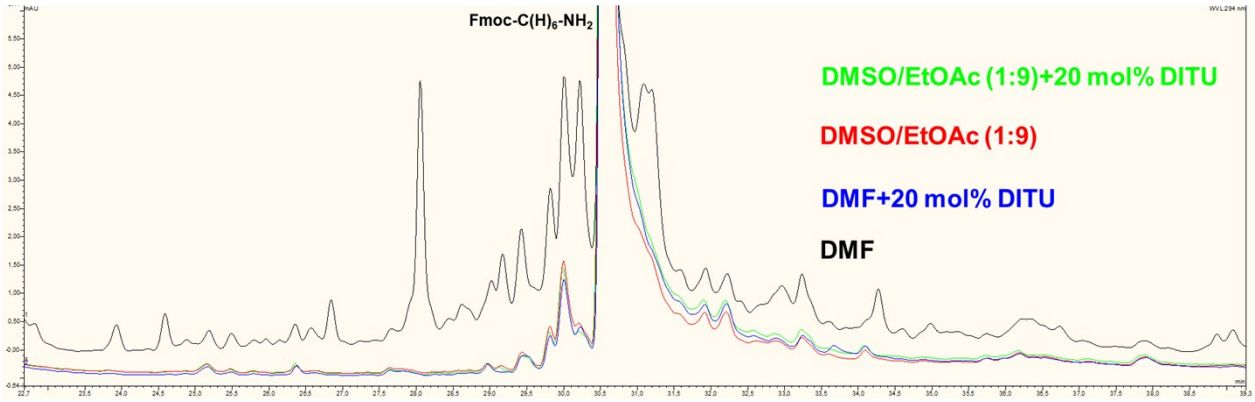


Figure S32. HPLC overlay (zoom-in) for Fmoc-C(H)$_6$-NH$_2$ crudes prepared using DMF and DMSO/EtOAc (1:9) in couplings, ±20 mol% DITU.

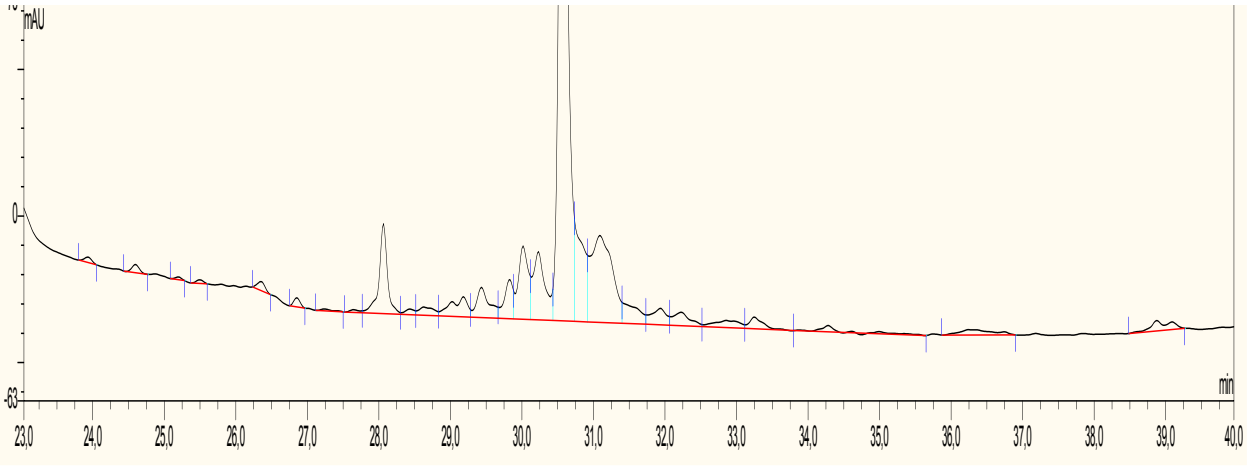


Figure S33. HPLC analysis for Fmoc-C(H)$_6$-NH$_2$ crude prepared using DMF in couplings.

Table S20. Area% for integrated peaks.

No.	Peakname	Ret.Time min	Area mAU*min	Amount	Type	Height mAU	Rel.Area %	Resolution
1	n.a.	23,933	0,2189	n.a.	BMB*	1,843	0,26	3,44
2	n.a.	24,600	0,3338	n.a.	BMB*	2,808	0,40	3,38
3	n.a.	25,200	0,0948	n.a.	BMB*	0,967	0,11	1,69
4	n.a.	25,500	0,1348	n.a.	BMB*	1,194	0,16	4,32
5	n.a.	26,367	0,3885	n.a.	BMB*	3,086	0,46	2,54
6	n.a.	26,850	0,3172	n.a.	BMB*	3,070	0,38	2,02
7	n.a.	27,250	0,1099	n.a.	BMB*	0,631	0,13	n.a.
8	n.a.	27,650	0,1403	n.a.	BM *	0,919	0,17	n.a.
9	n.a.	28,067	4,1133	n.a.	M *	30,709	4,89	n.a.
10	n.a.	28,433	0,2897	n.a.	M *	1,898	0,34	n.a.
11	n.a.	28,633	0,6889	n.a.	M *	2,783	0,82	n.a.
12	n.a.	29,183	1,8072	n.a.	M *	6,851	2,15	0,65
13	n.a.	29,433	2,1476	n.a.	M *	10,346	2,56	n.a.
14	n.a.	29,833	1,8238	n.a.	M *	13,332	2,17	n.a.
15	n.a.	30,017	4,0028	n.a.	M *	25,008	4,76	n.a.
16	n.a.	30,233	4,6948	n.a.	M *	23,280	5,59	n.a.
17	*n.a.*	*30,567*	*38,3652*	*n.a.*	*M **	*286,414*	*45,65*	*n.a.*
18	n.a.	30,750	5,0182	n.a.	M *	31,969	5,97	n.a.
19	n.a.	31,083	10,3862	n.a.	M *	29,572	12,36	n.a.
20	n.a.	31,417	1,7848	n.a.	M *	6,814	2,12	n.a.
21	n.a.	31,933	1,3613	n.a.	M *	5,609	1,62	n.a.
22	n.a.	32,233	1,2400	n.a.	M *	4,568	1,48	n.a.
23	n.a.	32,850	0,9877	n.a.	M *	2,405	1,18	0,70
24	n.a.	33,250	0,8665	n.a.	Mb*	3,971	1,03	3,44
25	n.a.	34,283	0,6634	n.a.	bMB*	2,188	0,79	3,59
26	n.a.	36,250	0,9976	n.a.	BMB*	1,813	1,19	4,69
27	n.a.	38,867	1,0665	n.a.	BMB*	3,522	1,27	n.a.
Total:			**84,0438**	**0,0000**		**507,570**	**100,00**	

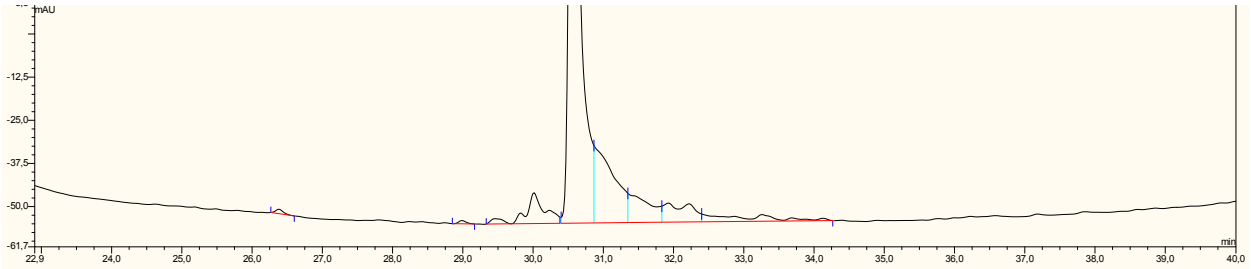


Figure S34. HPLC analysis for Fmoc-C$(H)_6$-NH_2 crude prepared using DMF +20 mol% DITU in couplings.

No.	Peakname	Ret.Time min	Area mAU*min	Amount	Type	Height mAU	Rel.Area %	Resolution
1	n.a.	26,383	0,1608	n.a.	BMB*	1,292	0,29	13,49
2	n.a.	28,983	0,1163	n.a.	BMB*	0,945	0,21	4,36
3	n.a.	30,017	2,8379	n.a.	BM *	8,906	5,12	2,26
4	*n.a.*	*30,567*	*37,7560*	*n.a.*	*M ***	*248,347*	*68,15*	*n.a.*
5	n.a.	30,883	7,3441	n.a.	M *	21,835	13,26	n.a.
6	n.a.	31,367	2,9544	n.a.	M *	8,126	5,33	n.a.
7	n.a.	31,933	2,4388	n.a.	M *	5,519	4,40	n.a.
8	n.a.	32,417	1,7897	n.a.	MB*	2,159	3,23	n.a.
Total:			**55,3978**	**0,0000**		**297,129**	**100,00**	

Table S21. Area% for integrated peaks.

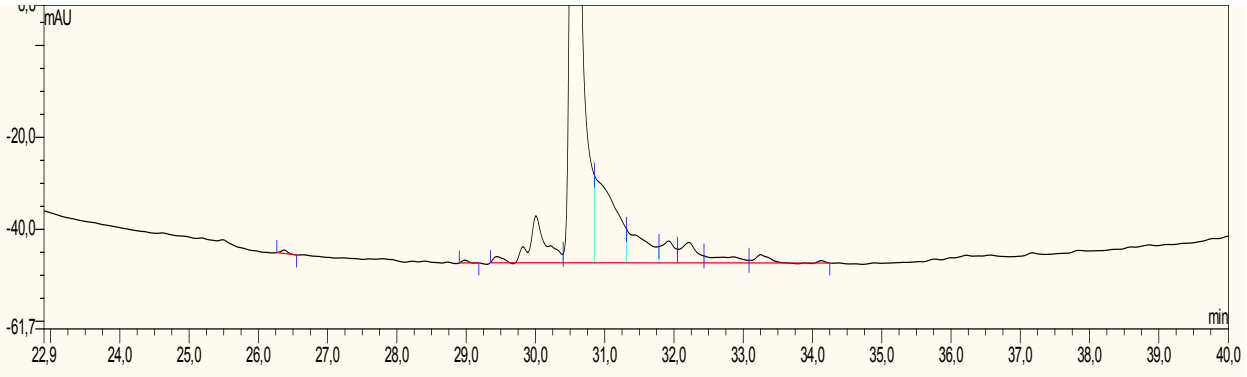


Figure S35. HPLC analysis for Fmoc-C(H)$_6$-NH$_2$ crude prepared using DMSO/EtOAc (1:9) in couplings.

Table S22. Area% for integrated peaks.

No.	Peakname	Ret.Time min	Area mAU*min	Amount	Type	Height mAU	Rel.Area %	Resolution
1	n.a.	26,383	0,0769	n.a.	BMB*	0,727	0,15	15,35
2	n.a.	28,983	0,0610	n.a.	BMB*	0,613	0,12	4,62
3	n.a.	30,000	3,0093	n.a.	M *	10,184	5,87	2,39
4	*n.a.*	*30,567*	*36,0544*	*n.a.*	*M ***	*248,558*	*70,27*	*n.a.*
5	n.a.	30,867	6,3600	n.a.	M *	18,520	12,40	n.a.
6	n.a.	31,333	2,3304	n.a.	M *	6,849	4,54	n.a.
7	n.a.	31,917	1,0475	n.a.	M *	4,743	2,04	n.a.
8	n.a.	32,217	1,1819	n.a.	M *	4,450	2,30	n.a.
9	n.a.	32,450	0,7212	n.a.	M *	1,425	1,41	n.a.
10	n.a.	33,250	0,4628	n.a.	MB*	1,797	0,90	n.a.
Total:			**51,3056**	**0,0000**		**297,866**	**100,00**	

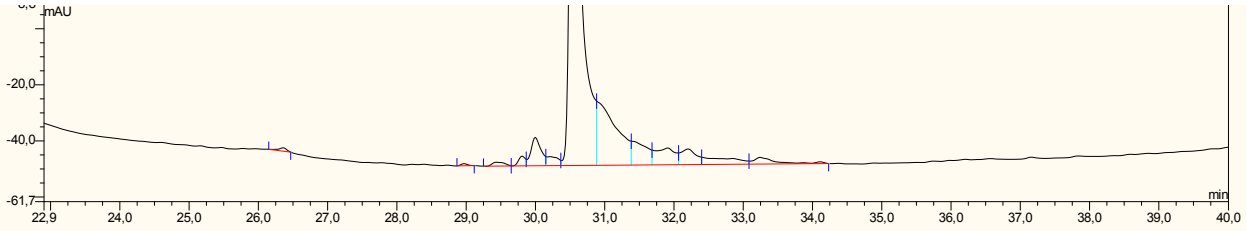


Figure S36. HPLC analysis for Fmoc-C$(H)_6$-NH_2 crude prepared using DMSO/EtOAc (1:9) +20 mol% DITU in couplings.

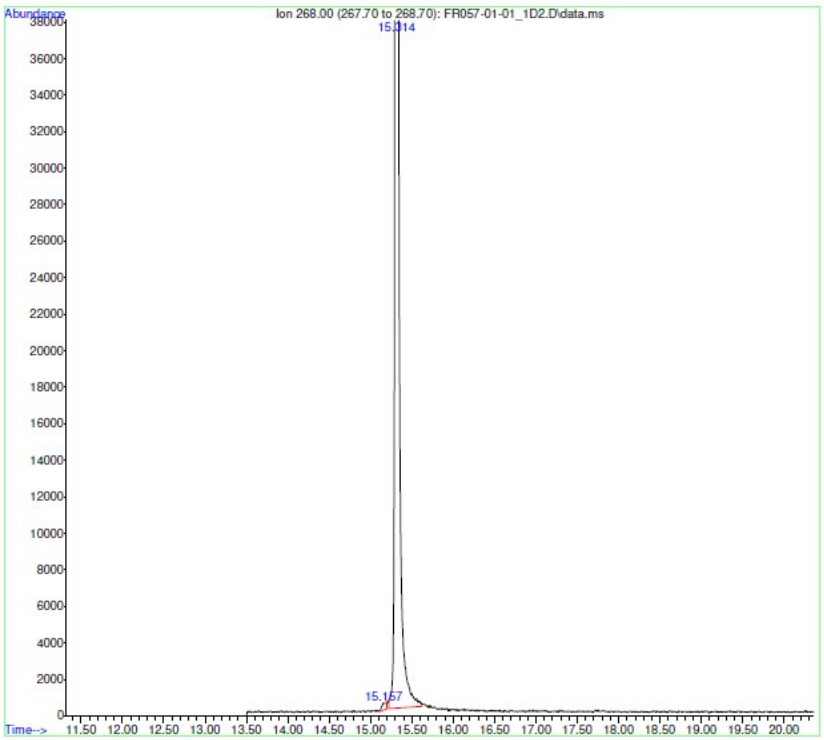
Table S23. Area% for integrated peaks.

No.	Peakname	Ret.Time min	Area mAU*min	Amount	Type	Height mAU	Rel.Area %	Resolution
1	n.a.	26,367	0,1604	n.a.	BMB*	1,206	0,28	13,99
2	n.a.	28,967	0,0811	n.a.	BMB*	0,751	0,14	1,75
3	n.a.	29,433	0,2804	n.a.	BM *	1,369	0,49	n.a.
4	n.a.	29,800	0,3799	n.a.	M *	3,448	0,66	n.a.
5	n.a.	30,000	1,7113	n.a.	M *	10,127	2,96	n.a.
6	n.a.	30,217	0,6194	n.a.	M *	3,216	1,07	n.a.
7	*n.a.*	*30,550*	*39,2187*	*n.a.*	*M *	*248,318*	*67,89*	*n.a.*
8	n.a.	30,900	7,5648	n.a.	M *	22,615	13,10	n.a.
9	n.a.	31,400	2,1302	n.a.	M *	8,464	3,69	n.a.
10	n.a.	31,917	1,9657	n.a.	M *	5,969	3,40	n.a.
11	n.a.	32,200	1,4338	n.a.	M *	5,578	2,48	n.a.
12	n.a.	32,417	1,3089	n.a.	M *	2,516	2,27	n.a.
13	n.a.	33,233	0,9118	n.a.	MB*	2,376	1,58	n.a.
Total:			**57,7665**	**0,0000**		**315,953**	**100,00**	

13. Chiral GC-MS analyses of Fmoc-C(H)$_6$-NH$_2$ crudes

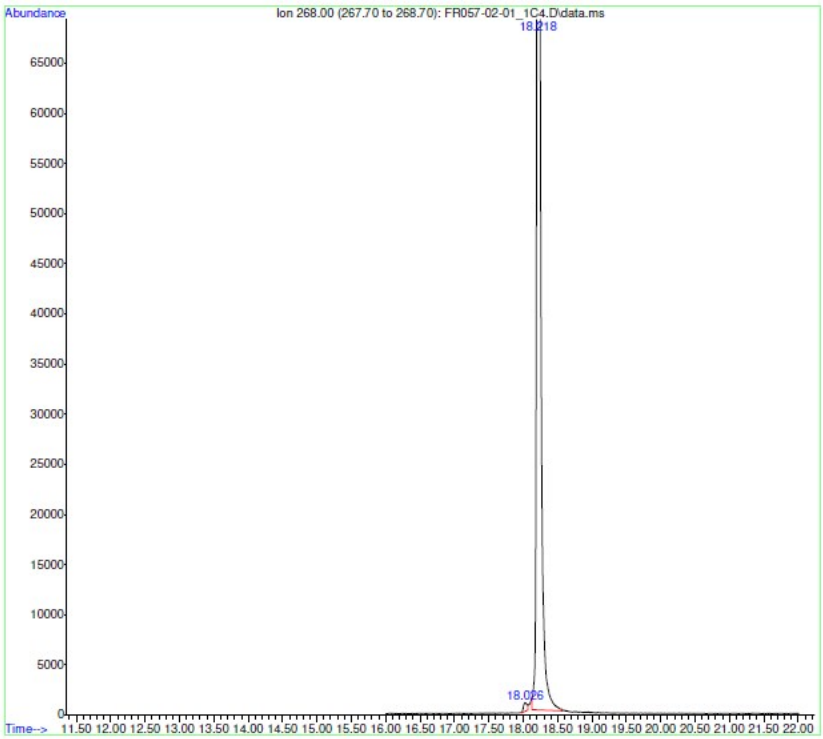
The determination of D-Cys content in Fmoc-Cys(H)$_6$-NH$_2$ crudes was carried out at C.A.T. GmbH & Co. (Tübingen, Germany). The samples were hydrolyzed using deuterated solvents (6 N D_2O/DCl) and after derivatization the enantiomeric purity was determined by GC-MS.

13.1 DMF as solvent for couplings (no DITU)



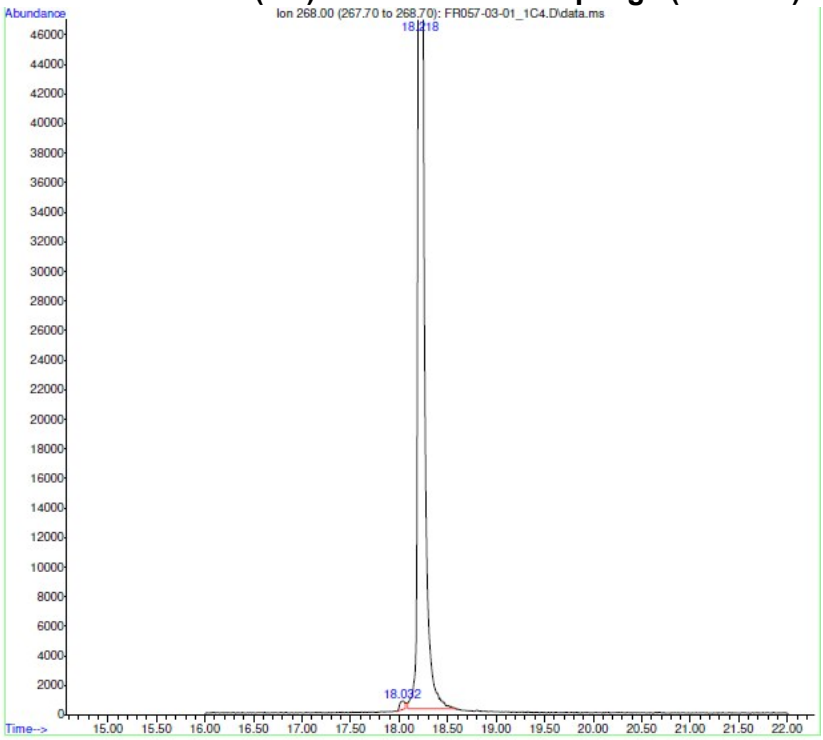
peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total	
1	15.157	1816	1823	1827	M3	402	15530	0.47%	0.466%	D Cys
2	15.314	1830	1841	1877	M2	138673	3320596	100.00%	99.534%	L Cys

13.2 DMF as solvent for couplings (20 mol% DITU)



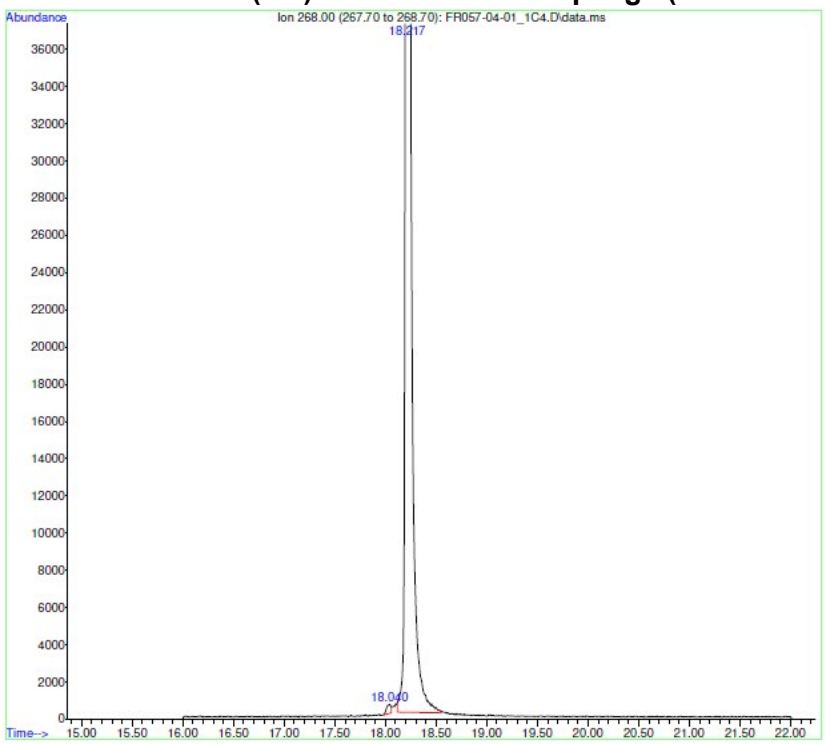
peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total	
1	18.026	2250	2256	2261	M	890	32115	0.46%	0.458%	D Cys
2	18.218	2268	2279	2321	M2	262539	6985469	100.00%	99.542%	L Cys

13.3 DMSO/EtOAc (1:9) as solvent for couplings (no DlTU)



peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total	
1	18.032	2251	2257	2261	M	592	19257	0.39%	0.388%	D Cys
2	18.218	2264	2279	2321	M2	176004	4937693	100.00%	99.612%	L Cys

13.4 DMSO/EtOAc (1:9) as solvent for couplings (20 mol% DITU)



peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total	
1	18.040	2251	2258	2260	M	488	15601	0.42%	0.423%	D Cys
2	18.217	2267	2279	2318	M2	130024	3673860	100.00%	99.577%	L Cys

14. LC-HRMS analysis of Fmoc-C(H)$_6$-NH$_2$ from SPPS using DMF in couplings

The crude Fmoc-Cys(H)$_6$-NH$_2$ prepared using DMF in couplings (no DITU) was analyzed by LC-HRMS using following conditions:

Experimental conditions: column: Waters peptide CSH C18, 4.6x150mm, 2.5um, temperature: 30°C; injection volume: 5 µL, sampler temperature: 5°C; MS mode: positive 50-3200; DAD: 220 nm; data rate: 5.0Hz; detector cell: standard cell 1uL; flow: 0.5 ml/min; jet weaver: v100 mixer; mobile phase A: 0.1 % TFA in water, mobile phase B: 0.08 % TFA in water/MeCN (1:9). Gradient (Time(min), %B): 0, 0; 1, 0; 40, 100; 46, 100; 47, 0; 50, 0.

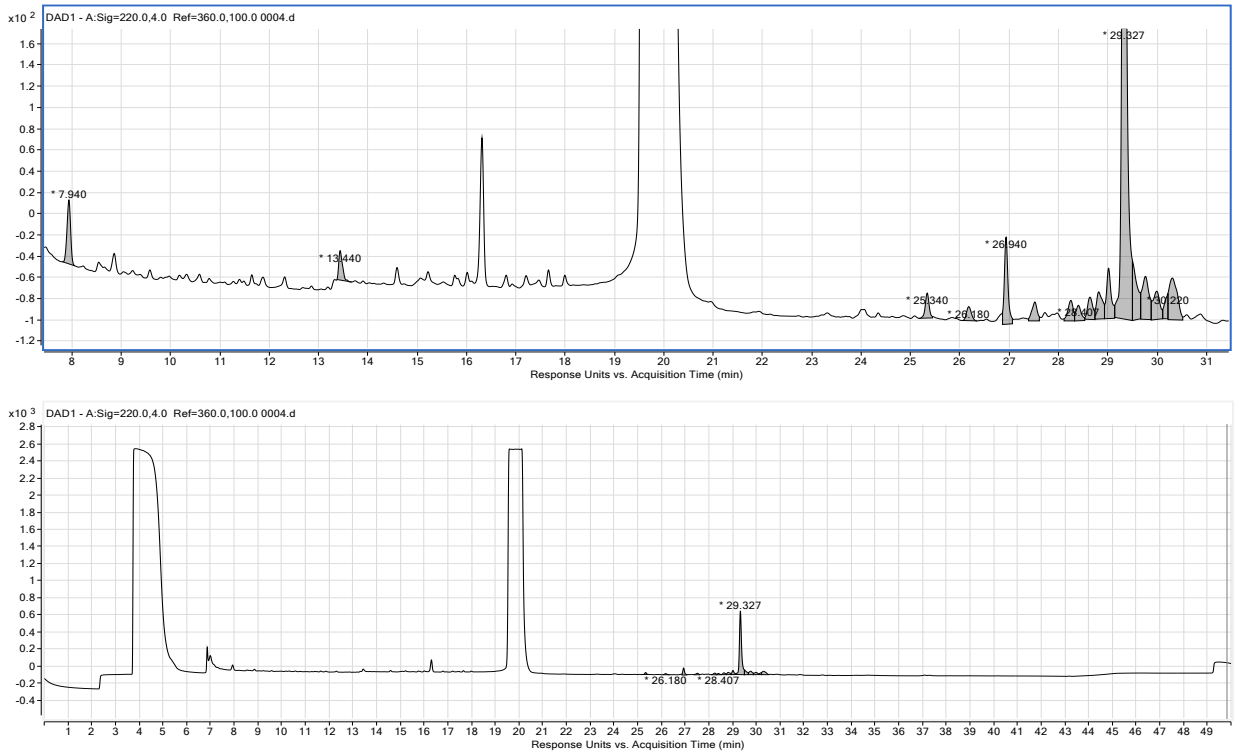


Figure S37. LC-HRMS analysis of Fmoc-Cys(H)$_6$-NH$_2$ prepared using DMF in couplings (no DITU).The peak at ~20 min is DITU which was added to the crude material to prevent non-synthesis related oxidative breakdown of the Fmoc-Cys(H)$_6$-NH$_2$ prior to the MS analysis.

Table S24. Area% for integrated peaks and tentative MS identities for Fmoc-Cys$(H)_6$-NH_2 and byproduct from Fmoc-Cys$(H)_6$-NH_2 SPPS using DMF (no DITU) in couplings.

Peak	Rt	Area	(m+z)/z	z	decon.	diff	RRT	Area %	identity
1	7,94	305,48	115,124	1	114,1167	-743,0333	0,271	3,61	non-peptide
2	13,44	142,68	398,154	1	397,1467	-460,0033	0,458	1,69	non-peptide
3	25,34	127,61	896,133	1	895,1257	37,9757	0,864	1,51	+[K]
4	26,18	90,46	982,257	1	981,2497	124,0997	0,893	1,07	oxidized byproduct
5	26,94	421,26	915,425	1	914,4177	57,2677	0,919	4,98	oxidized byproduct
6	27,52	133,62	933,294	1	932,2867	75,1367	0,938	1,58	oxidized byproduct
7	28,253	142,08	872,135	1	871,1277	13,9777	0,963	1,68	oxidized byproduct
8	28,407	106,68	981,295	1	980,2877	123,1377	0,969	1,26	oxidized byproduct
9	28,64	156,17	1325,253	1	1324,2457	467,0957	0,977	1,85	oxidized byproduct
10	28,813	216,93	856,14	1	855,1327	-2,0173	0,982	2,56	Ox. 2SH to cyclic
11	29,013	316,53	755,146	1	754,1387	-103,0113	0,989	3,74	Des Cys(H)
12	29,327	4722,84	858,156	1	857,1487	-0,0013	1,000	55,83	Product, Fmoc-(1-6)-NH_2
13	29,5	360,41			0,0000	-857,1500	1,006	4,26	
14	29,767	389,22			0,0000	-857,1500	1,015	4,6	
15	29,993	263,27			0,0000	-857,1500	1,023	3,11	
16	30,22	109,33						1,29	
17	30,307	454,18						5,37	

15. Assessment of stability of thiol substituted acids under different conditions

Following the observation that Fmoc-Cys(Trt)-OH/Oxyma was stable in DMSO/EtOAc (1:9) but not in DMF (section 9 of this ESI) a stability study was carried out to increase understanding of what parameters govern the instability of protected thiol functionalized carboxylic acids. Following parameters were examined: i) effect of protecting groups ii) effect of solvent iii) effect of coupling agent. All examined thiol substituted acids and coupling agents were 0.1M and were allowed to stand for 3 days at rt after which 50 µL aliquots of the reaction mixtures were taken out, diluted by 1.0 mL MeCN and analyzed by HPLC using the method described in section 9 of this ESI.

15.1. Assessment of stability of thiol substituted acids examining the effect of protecting groups

Following **thiol substituted acids** were examined: Fmoc-Cys(Trt)-OH, Fmoc-Cys(Mmt)-OH, Fmoc-Cys(Acm), Fmoc-Cys(*t*-Bu)-OH, Fmoc-Cys(S*t*-Bu)-OH, Fmoc-Cys(DPM)-OH,[13] Fmoc-Cys(THP)-OH[14] and Mpa(Trt)-OH. All **thiol substituted acids** were tested in the presence of 0.1M Oxyma and were examined in DMF and DMSO/EtOAc (1:9), see Figs S38 – S45. While in DMSO/EtOAc (1:9) all **thiol substituted acids** were fully stable in DMF Fmoc-Cys(Trt)-OH, Fmoc-Cys(Mmt)-OH and Mpa(Trt)-OH were unstable.

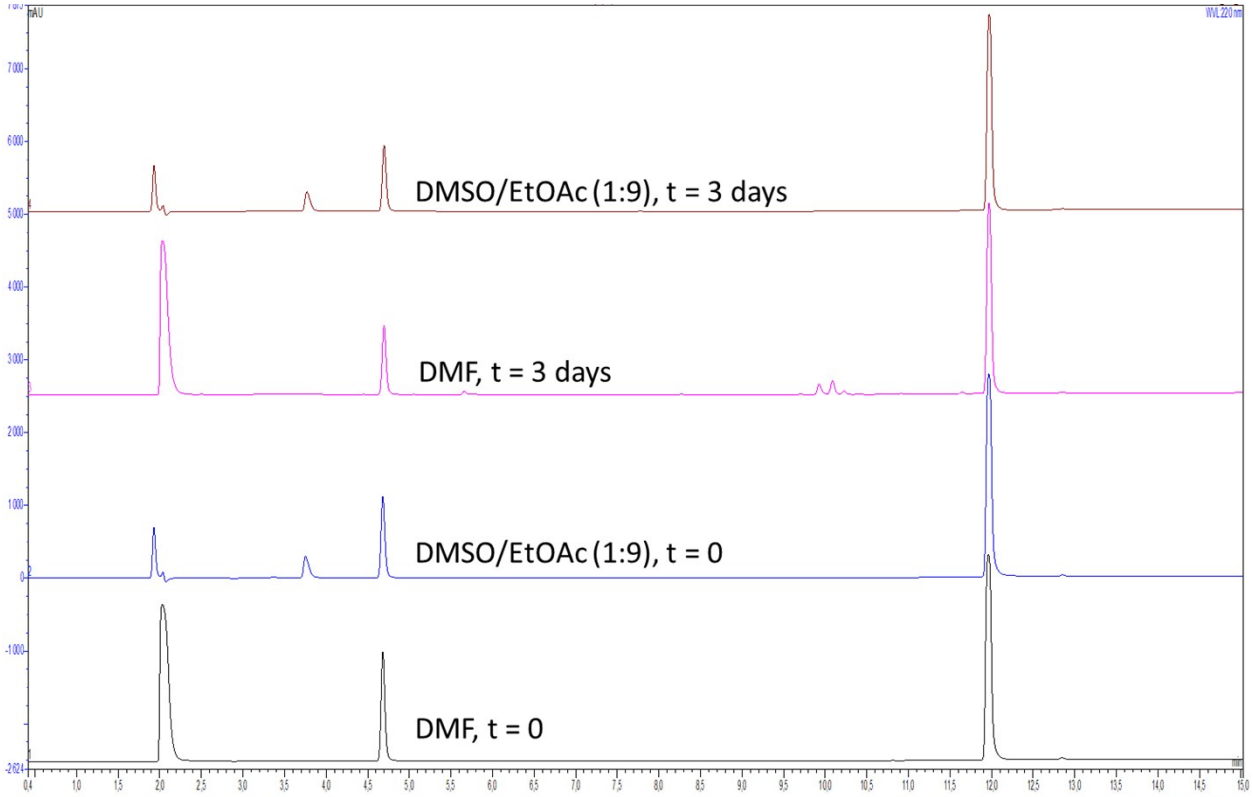


Figure S38. Stability of 0.1M Fmoc-Cys(Trt)-OH/Oxyma in DMF and DMSO/EtOAc after 3 days at rt

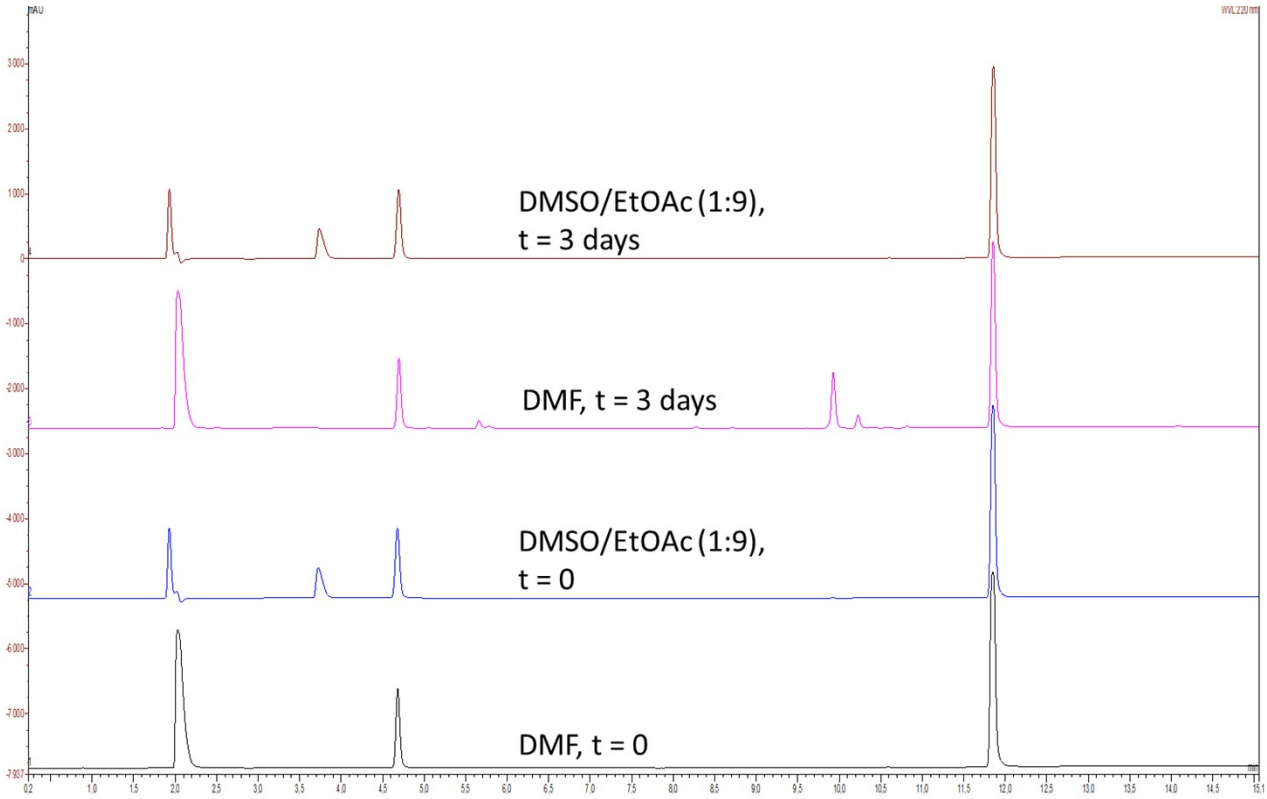


Figure S39. Stability of 0.1M Fmoc-Cys(Mmt)-OH/Oxyma in DMF and DMSO/EtOAc after 3 days at rt

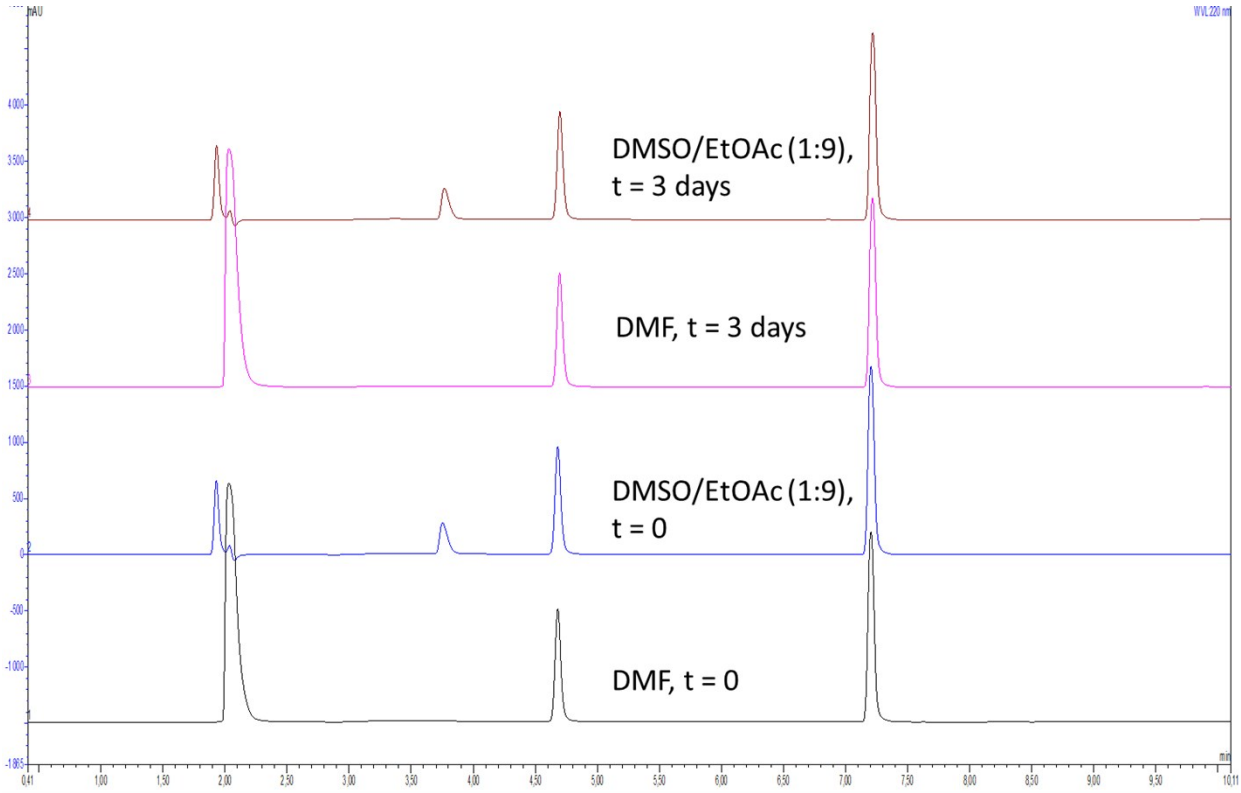


Figure S40. Stability of 0.1M Fmoc-Cys(Acm)-OH/Oxyma in DMF and DMSO/EtOAc after 3 days at rt

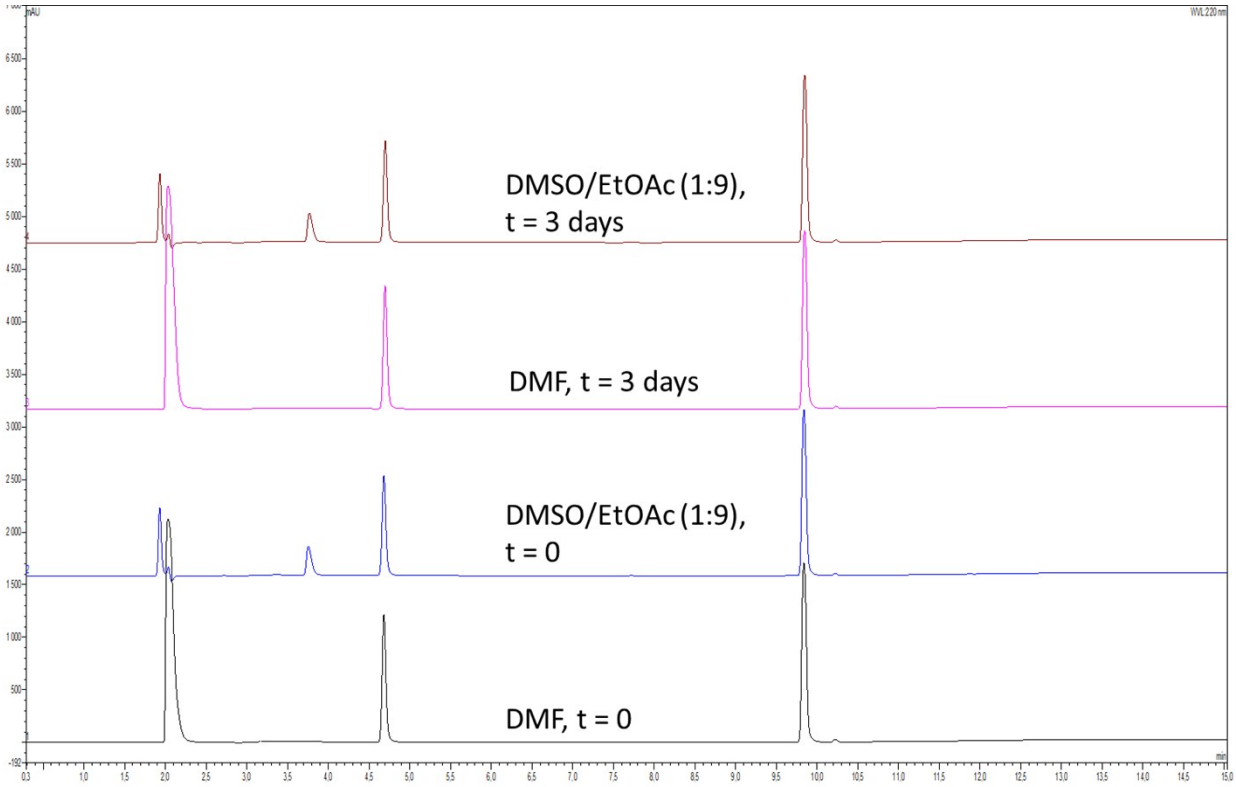


Figure S41. Stability of 0.1M Fmoc-Cys(*t*-Bu)-OH/Oxyma in DMF and DMSO/EtOAc after 3 days at rt

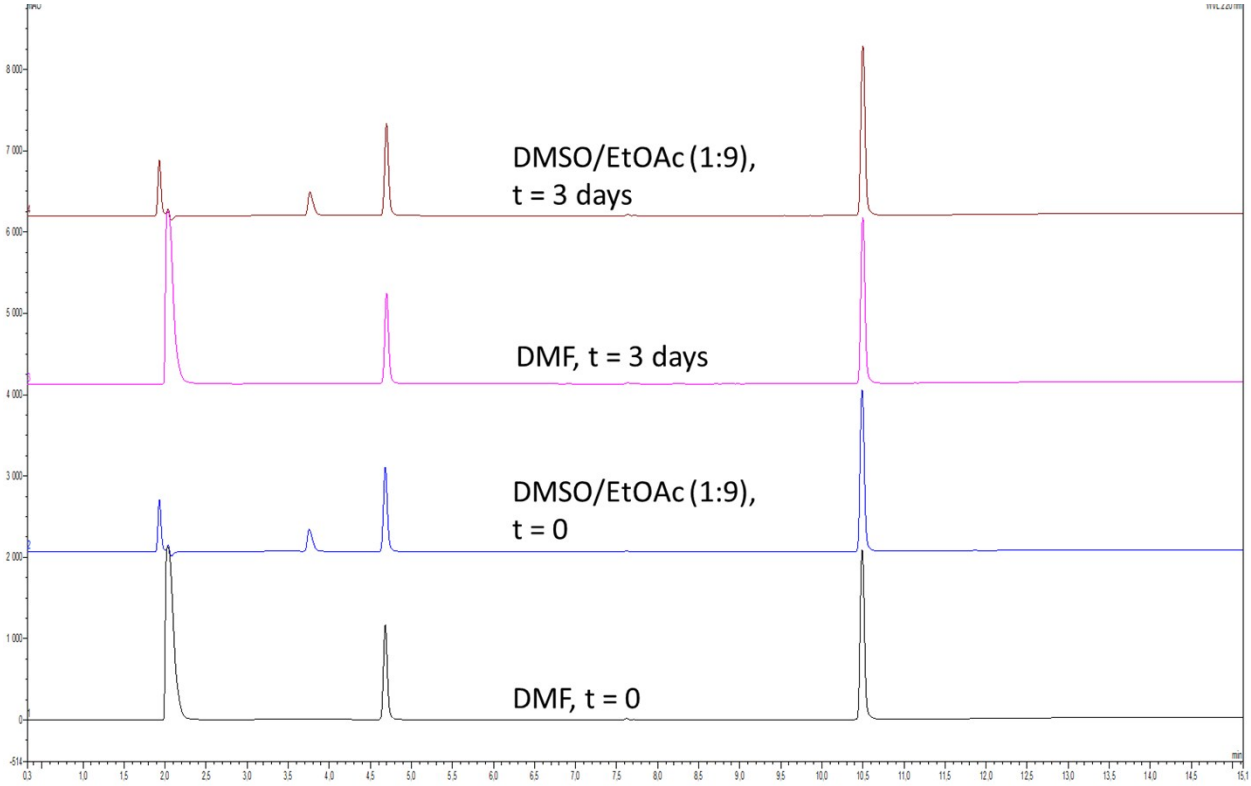


Figure S42. Stability of 0.1M Fmoc-Cys(S*t*-Bu)-OH/Oxyma in DMF and DMSO/EtOAc after 3 days at rt

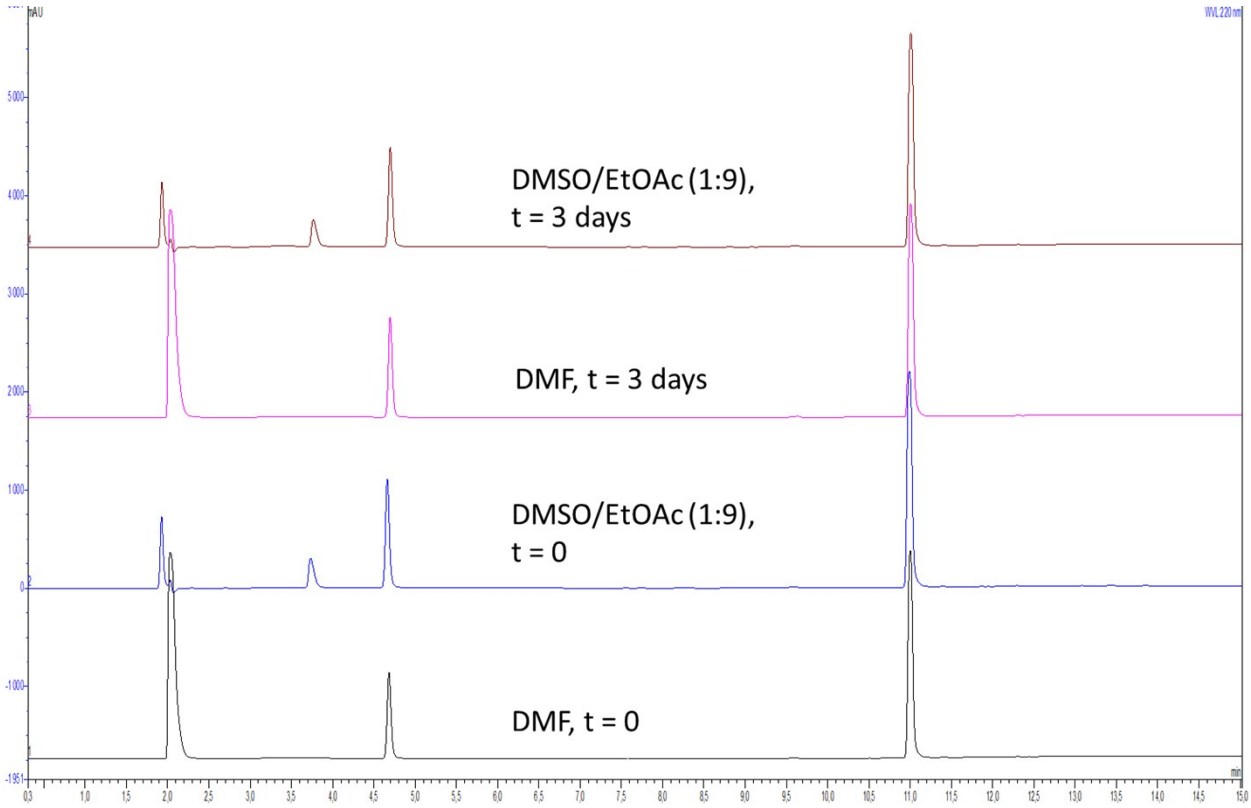


Figure S43. Stability of 0.1M Fmoc-Cys(DPM)-OH/Oxyma in DMF and DMSO/EtOAc after 3 days at rt

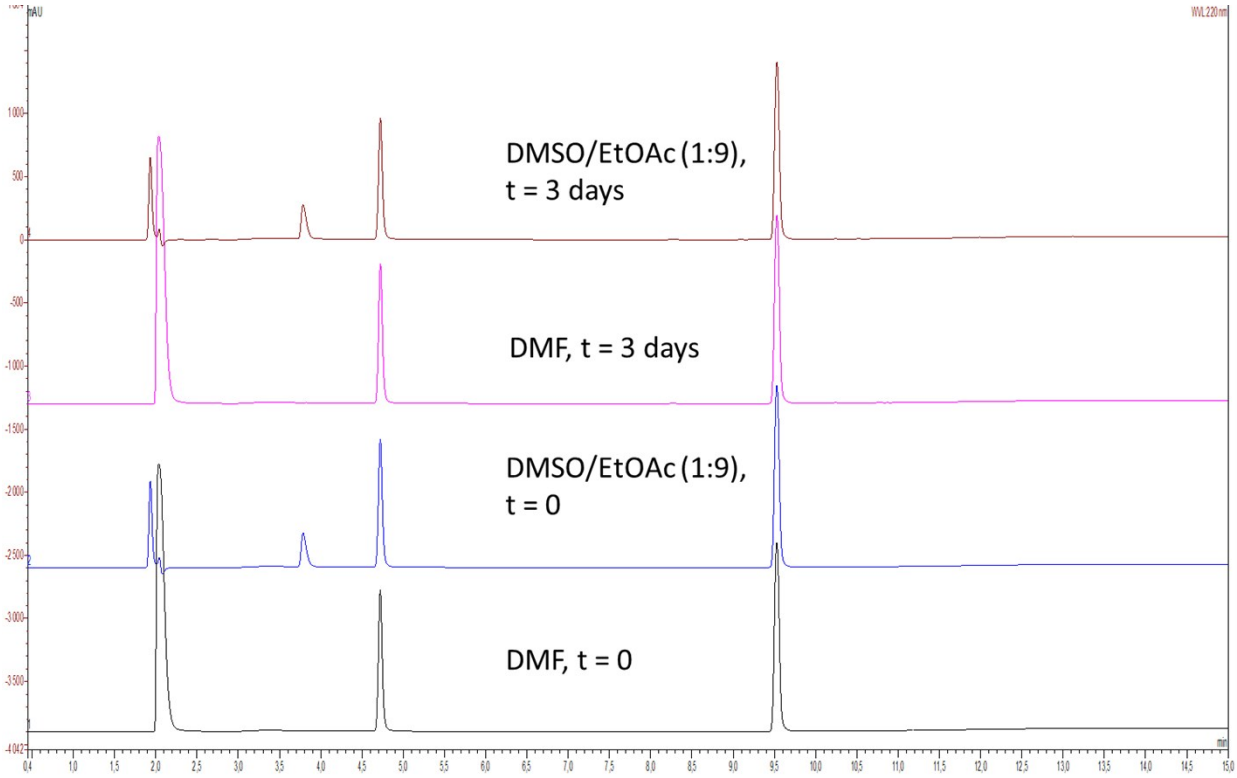


Figure S44. Stability of 0.1M Fmoc-Cys(THP)-OH/Oxyma in DMF and DMSO/EtOAc after 3 days at rt

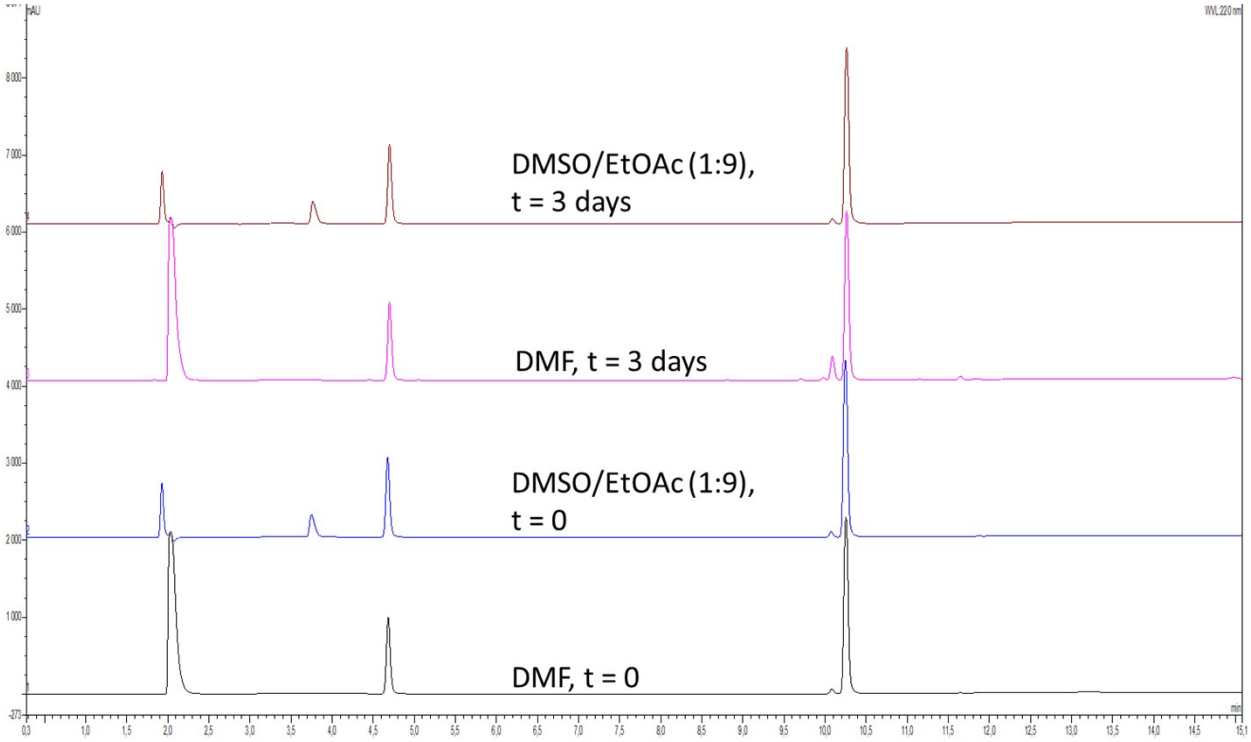


Figure S45. Stability of 0.1M Mpa(Trt)-OH/Oxyma in DMF and DMSO/EtOAc after 3 days at rt

15.2. Assessment of stability of thiol substituted acids examining the effect of solvent

Following solvents were examined: N-methylpyrrolidone (NMP), N-ethylpyrrolidone (NEP), N-butylpyrrolidone (NBP), 1,3-Dimethyl-2-imidazolidinone (DMIU), N,N′-Dimethylpropyleneurea (DMPU), dimethylacetamide (DMAC), N-formylmorpholine (NFM), different batches dimethylformamide (DMF), N,N-Dimethylformamide dimethyl acetal (DMACE), diethylformamide (DEF), N-formylpyrrolidone (NFP). Stability of 0.1M Fmoc-Cys(Trt)-OH/Oxyma after 3 days at rt in the solvents above was examined, see Figs S46 – S52. These experiments that revealed that Fmoc-Cys(Trt)-OH was fully stable in all solvents with the exception of dimethyl acetal DMACE and formamides DMF, DEF and NFP. In stark contrast to the instability in DMF, DEF and NFP the AA was fully stable in the formamide NFM. It ought to be stated that at this time it is not known whether the role of formamide solvents in causing breakdown of Fmoc-Cys(Trt) in the presence of Oxyma is due to the solvent intrinsically, or due to trace amounts of extraneous materials (peroxides, metals, etc.) causing the breakdown. It is also worth noting that while an addition of 10 mol% DITU completely inhibited the breakdown of Fmoc-Cys(Trt)-OH/Oxyma in DMF the addition of 10 mol% of the base N-methylmorpholine (NMM) did not prevent the breakdown of the AA. Finally, excluding light from the test reactions did not prevent the breakdown of Fmoc-Cys(Trt)-OH.

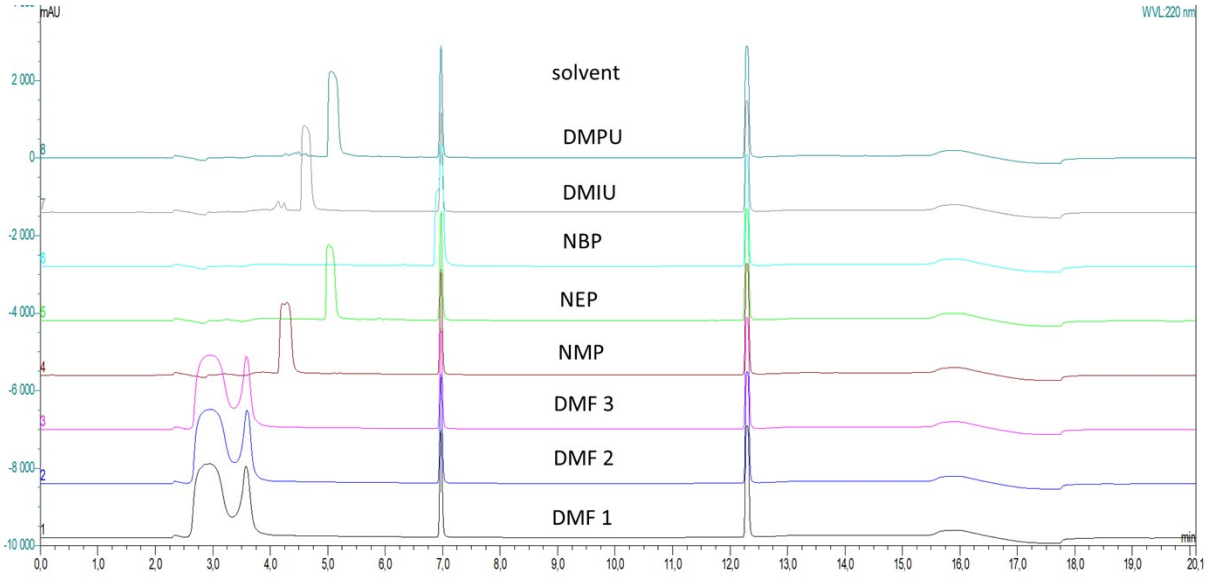


Figure S46. Stability of 0.1M Fmoc-Cys(Trt)-OH/Oxyma in different solvents at t=0

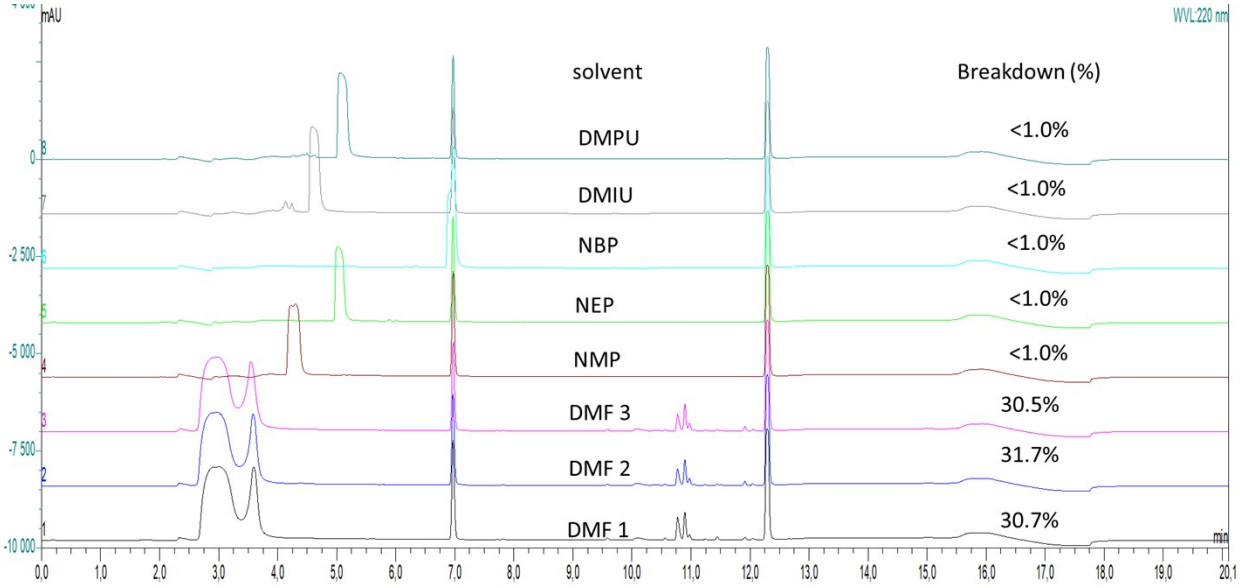


Figure S47. Stability of 0.1M Fmoc-Cys(Trt)-OH/Oxyma in different solvents after 3 days at rt

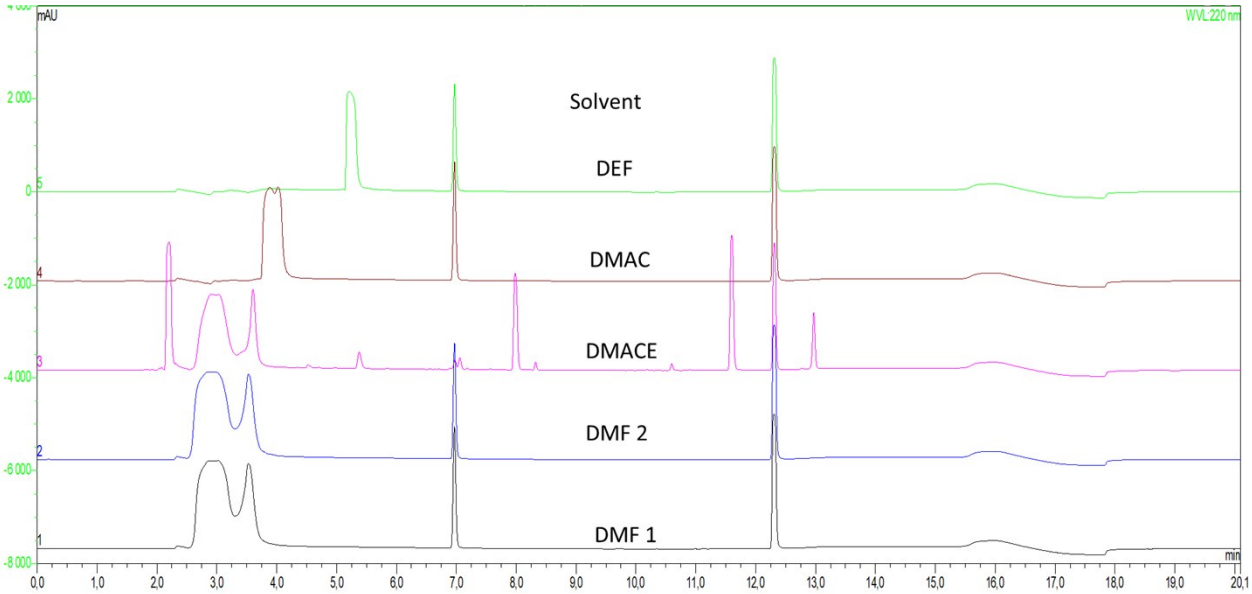


Figure S48. Stability of 0.1M Fmoc-Cys(Trt)-OH/Oxyma in different solvents at t=0

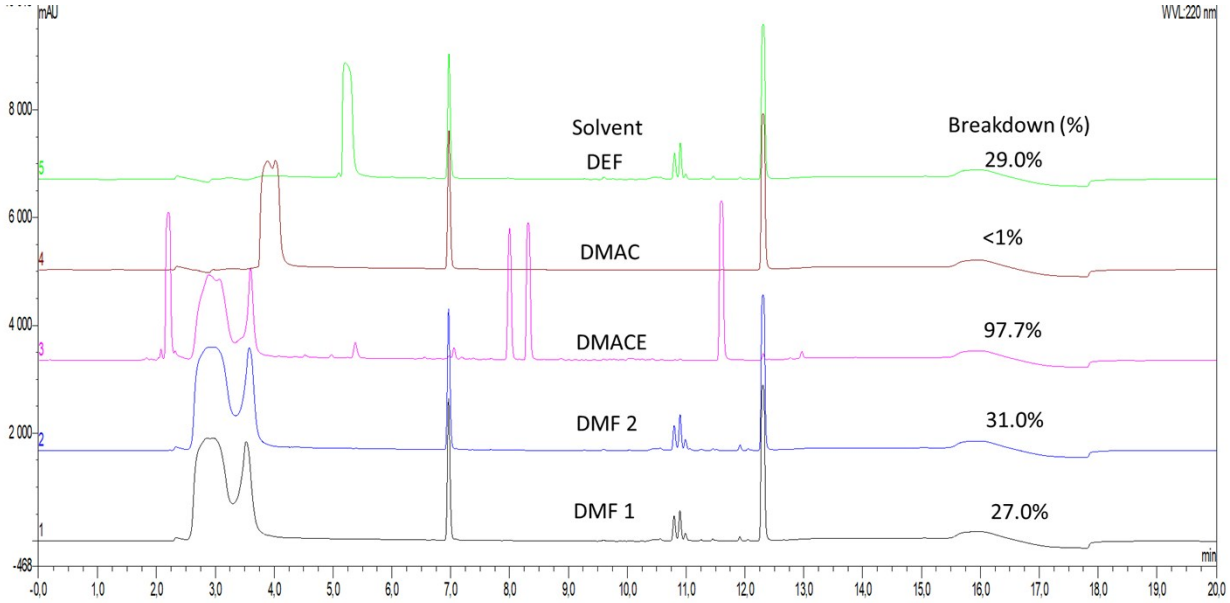


Figure S49. Stability of 0.1M Fmoc-Cys(Trt)-OH/Oxyma in different solvents after 3 days at rt

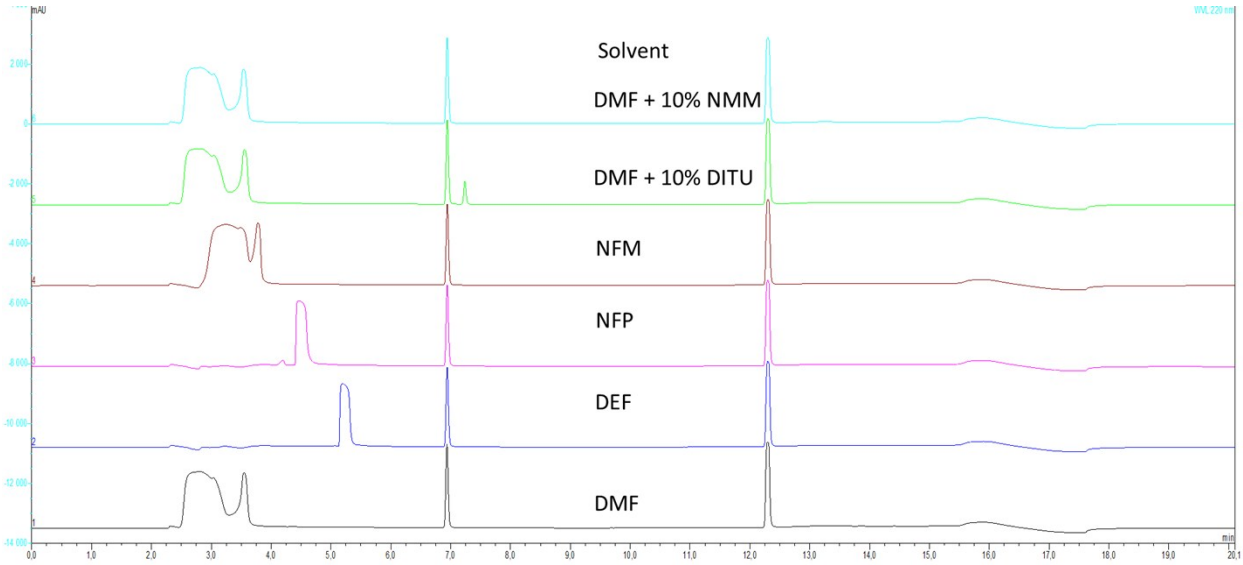


Figure S50. Stability of 0.1M Fmoc-Cys(Trt)-OH/Oxyma in different solvents at t=0

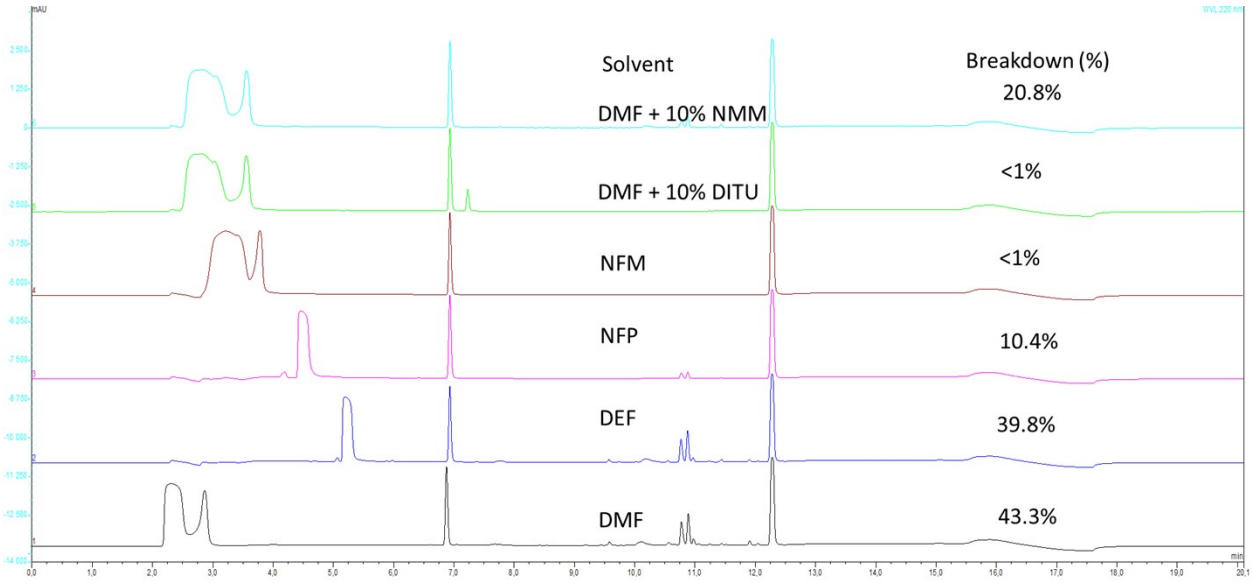


Figure S51. Stability of 0.1M Fmoc-Cys(Trt)-OH/Oxyma in different solvents after 3 days at rt

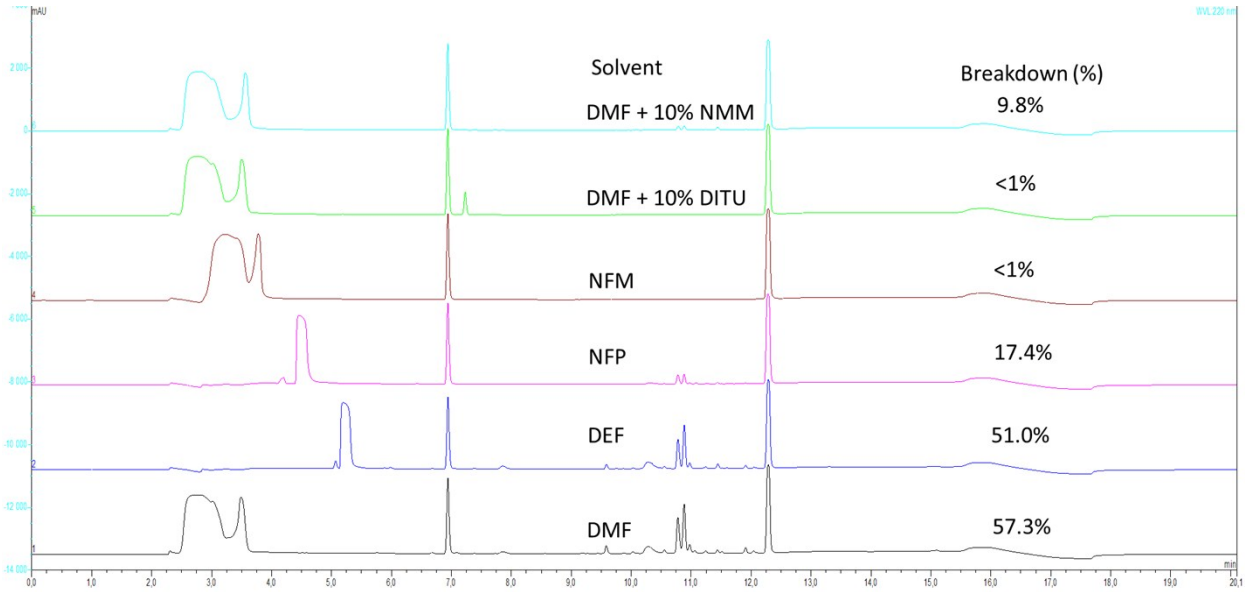


Figure S52. Stability of 0.1M Fmoc-Cys(Trt)-OH/Oxyma in different solvents after 3 days at rt in the absence of light

15.3. Assessment of stability of thiol substituted acids examining the effect of coupling agent

Following coupling agents were examined: 6-chloro-1-hydroxybenzotriazole (Cl-HOBt), 5-(hydroxyimino)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione(Oxyma-B), 2-mercaptobenzothiazole (2-MBT), ethyl cyanohydroxyiminoacetate (Oxyma), 1-hydroxybenzotriazole hydrate ($HOBtxH_2O$), 3-hydroxy-1,2,3-benzotriazin-4-one (HOOBt) and N-hydroxy-5-norbornene-2,3-dicarboximide (HONB). Stability of 0.1M Fmoc-Cys(Trt)-OH/coupling agent after 3 days at rt in DMF and DMSO/EtOAc (1:9) was examined, see Figs S53 – S59. These experiments revealed that Fmoc-Cys(Trt)-OH in DMSO/EtOAc (1:9) was fully stable in the presence of all coupling agents examined. On the other hand, in DMF the AA was not stable in the presence of Oxyma, $HOBtxH_2O$, HOOBt and HONB. In that sense it is worth noting that while the pKa 4.6 coupling agents $HOBtxH_2O$ and Oxyma caused breakdown of Fmoc-Cys(Trt)-OH in DMF in the presence of the more acidic Cl-HOBt (pKa 3.35) the AA was fully stable. Furthermore, in the presence of the more basic HONB (pKa ~6) the AA was unstable while in the presence of the thiol tethered 2-MBT (pKa 7.0) the AA was fully stable. In other words the stability of Fmoc-Cys(Trt)-OH in DMF is not a simple function of pKa of the coupling agent present and further studies will be aimed at examining the role of coupling agents on stability of oxidation prone AAs.

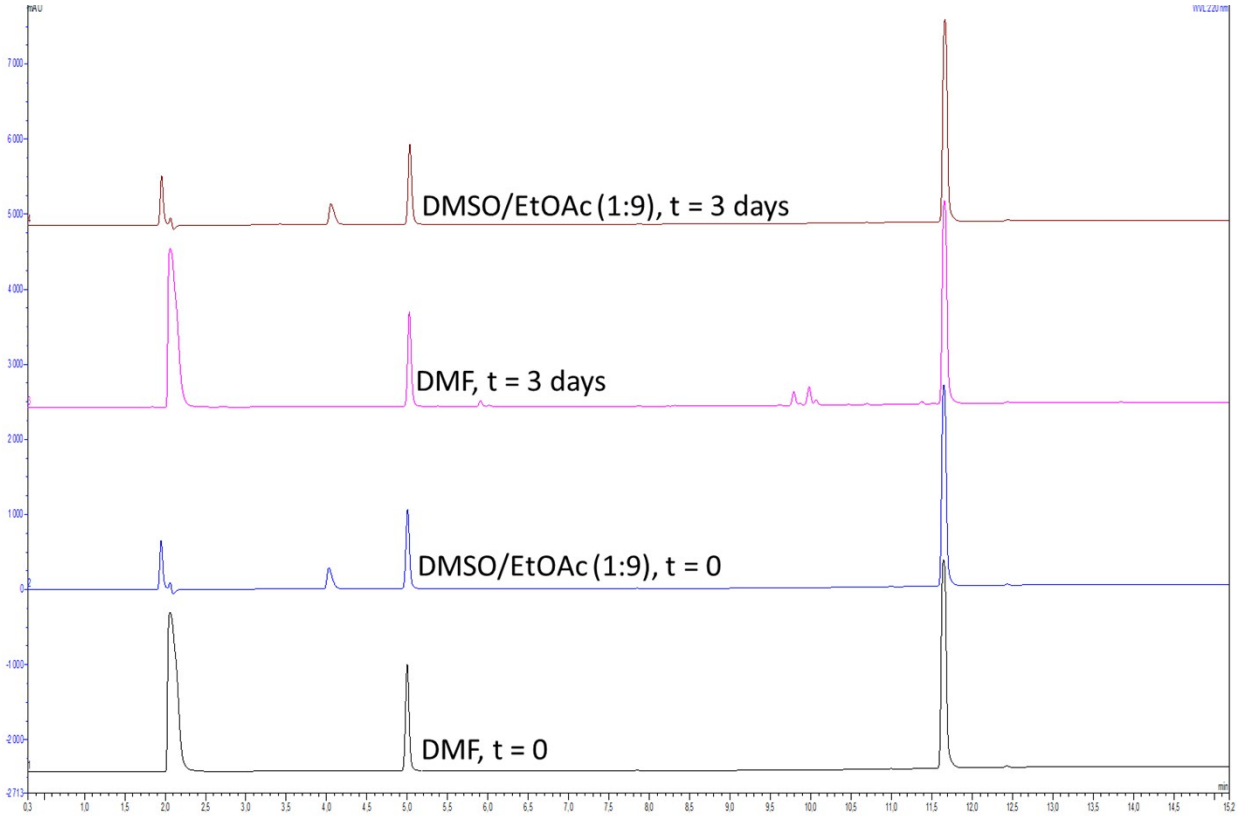


Figure S53. Stability of 0.1M Fmoc-Cys(Trt)-OH/Oxyma in DMF and DMSO/EtOAc (1:9)

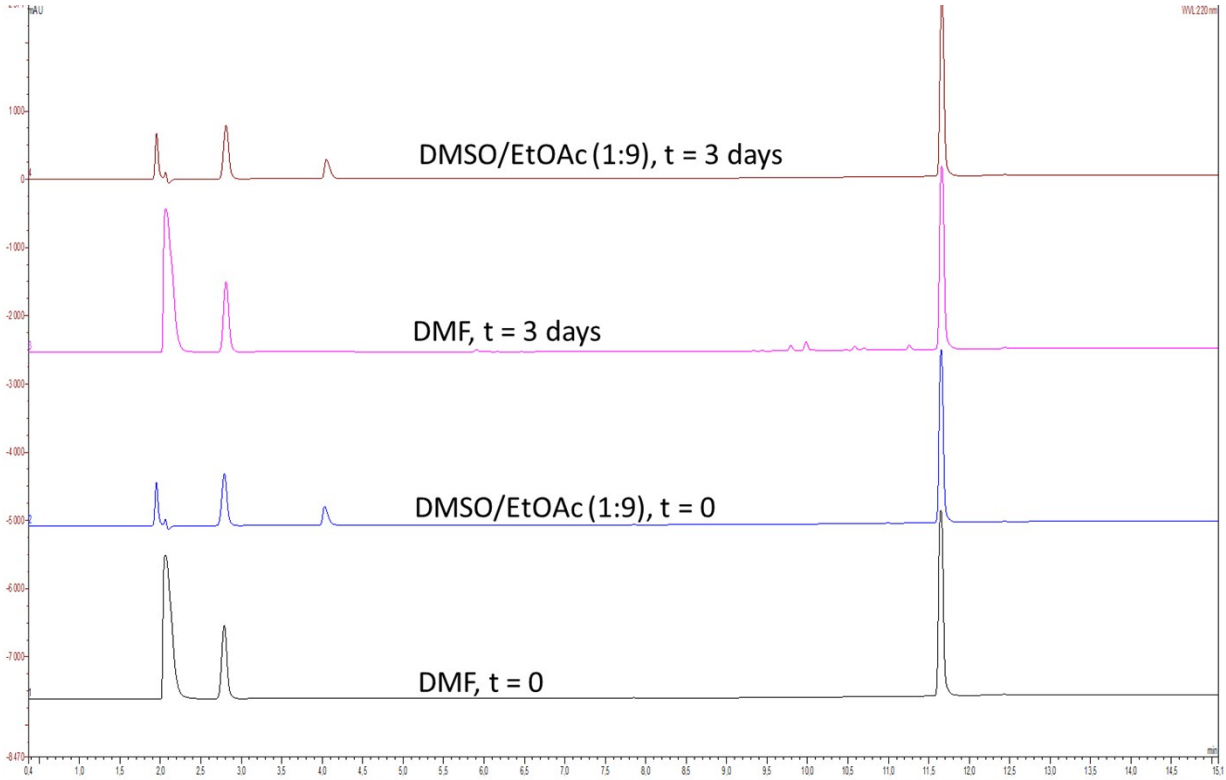


Figure S54. Stability of 0.1M Fmoc-Cys(Trt)-OH/HOBtxH_2O in DMF and DMSO/EtOAc (1:9)

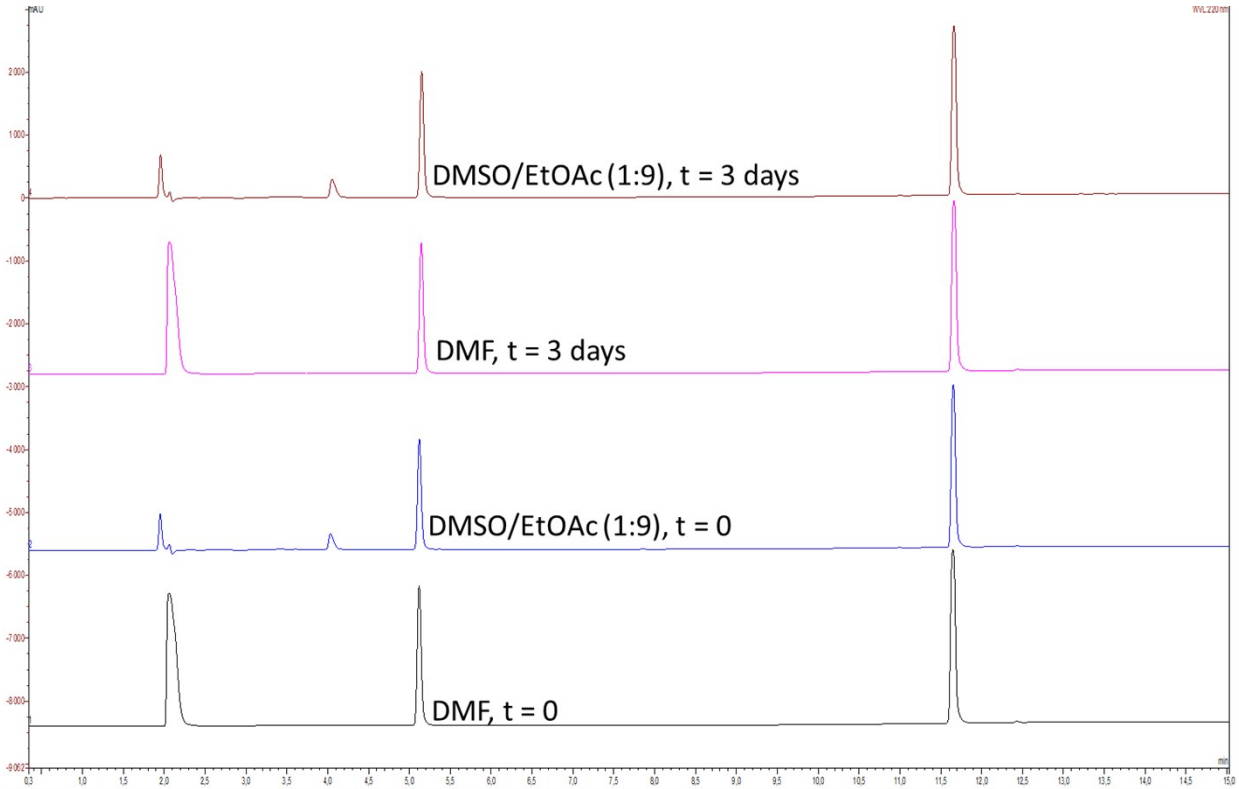


Figure S55. Stability of 0.1M Fmoc-Cys(Trt)-OH/Cl-HOBt in DMF and DMSO/EtOAc (1:9)

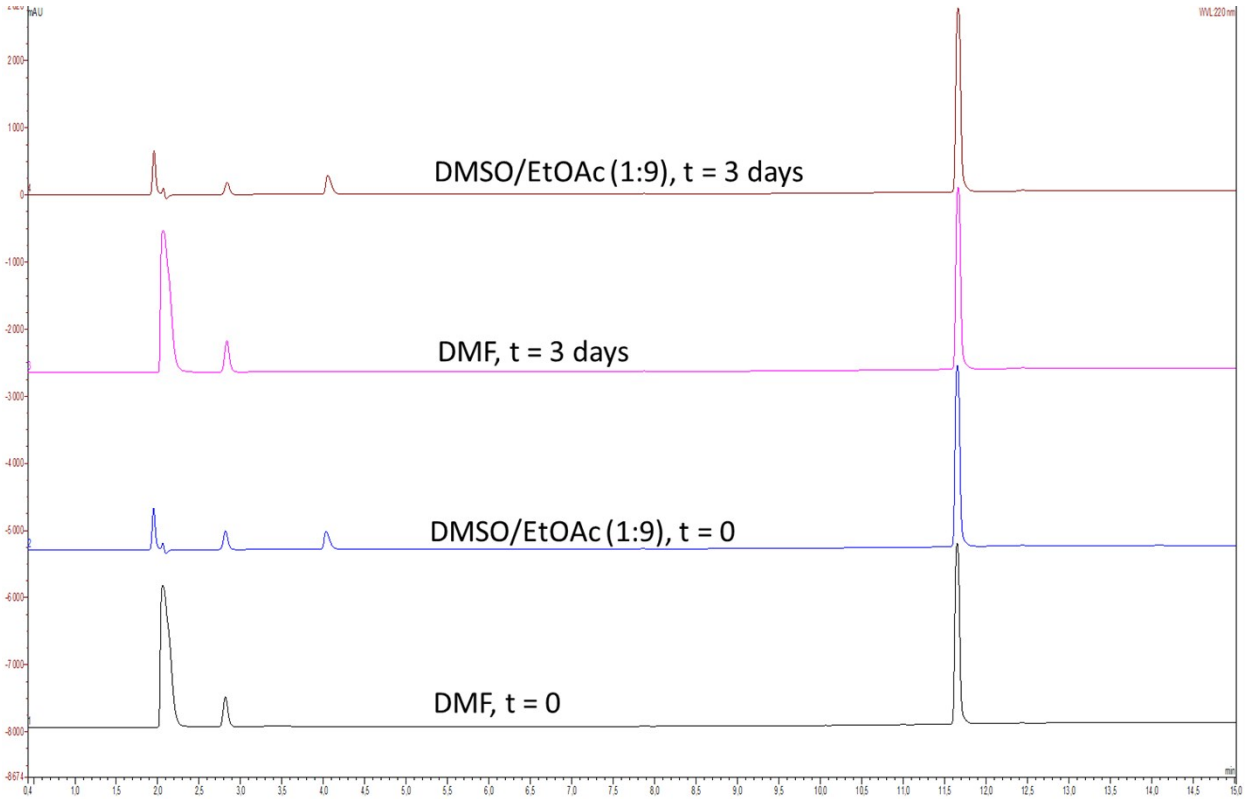


Figure S56. Stability of 0.1M Fmoc-Cys(Trt)-OH/Oxyma-B in DMF and DMSO/EtOAc (1:9)

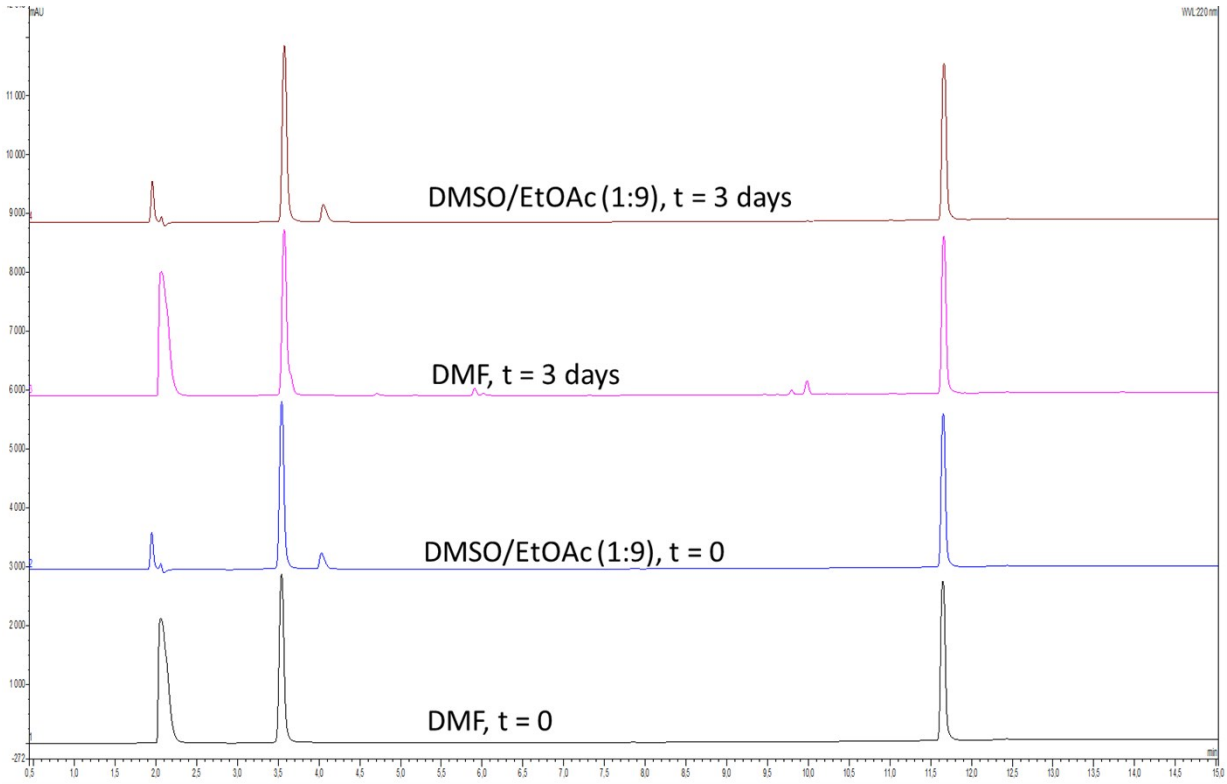


Figure S57. Stability of 0.1M Fmoc-Cys(Trt)-OH/HOOBt in DMF and DMSO/EtOAc (1:9)

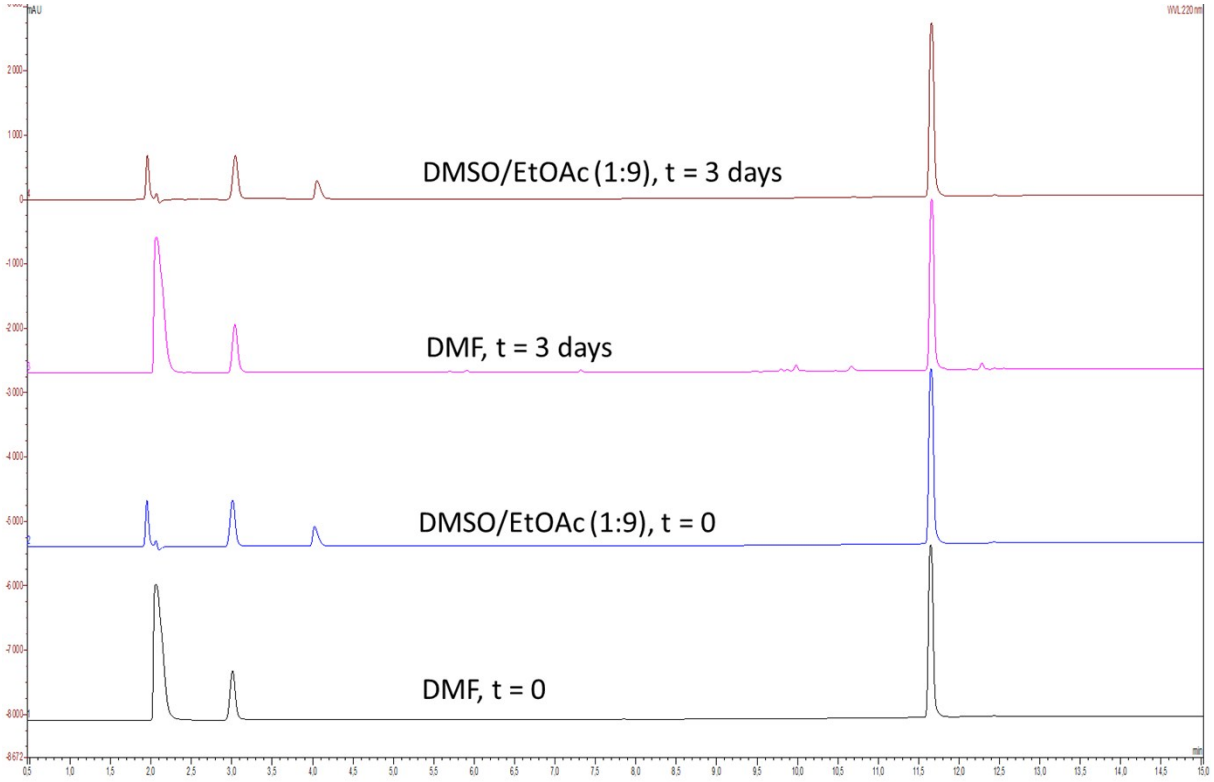


Figure S58. Stability of 0.1M Fmoc-Cys(Trt)-OH/HONB in DMF and DMSO/EtOAc (1:9)

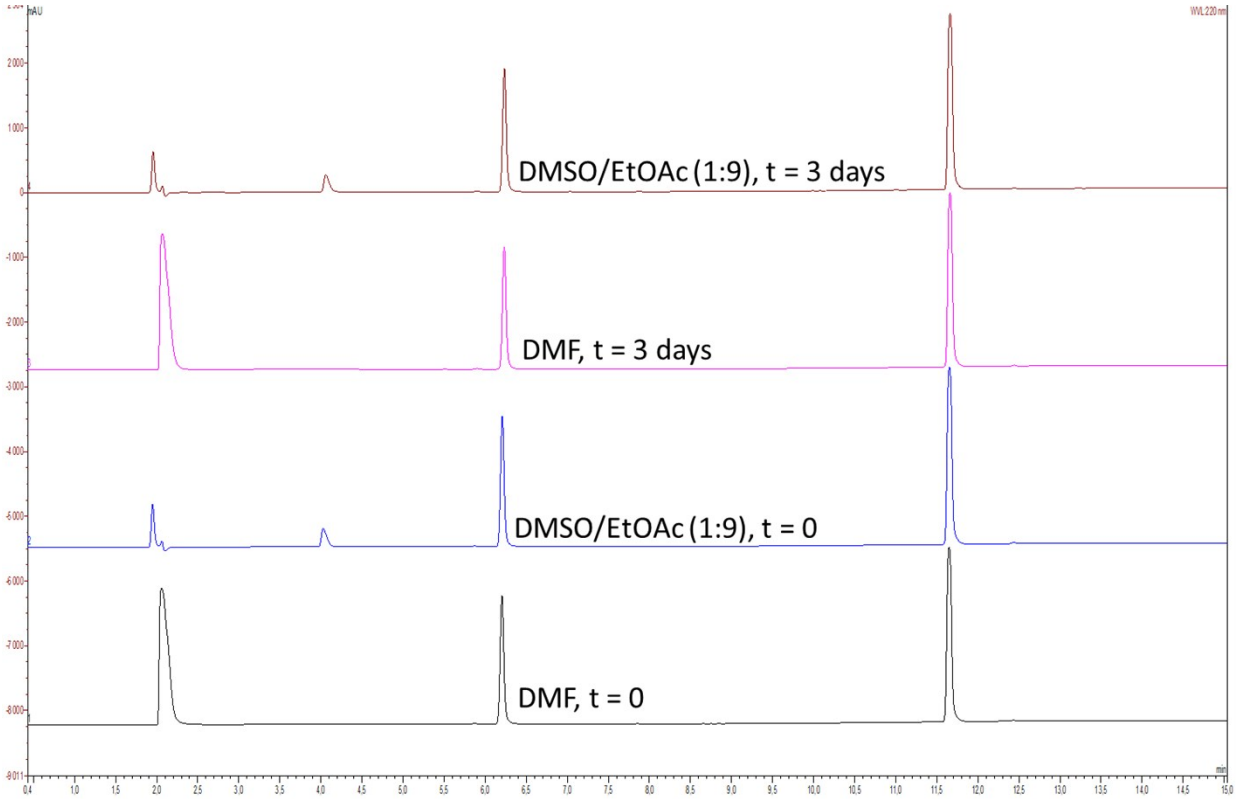


Figure S59. Stability of 0.1M Fmoc-Cys(Trt)-OH/2-MBT in DMF and DMSO/EtOAc (1:9)

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[7] In the test cleavages of 0.5 g of Aib-ACP peptide resin 95 mg of the crude peptide were obtained. If all of the isolated peptide resin (51.24 g) was cleaved, 9.74 g of the crude Aib-ACP would be isolated. If the scale of the synthesis was 1 mol (scale-up factor 100) 974 g of the crude peptide would be obtained.

[8] We propose that the initial reaction between the Oxyma radical and the trityl moiety is accompanied by a cyclization to afford a 9-phenylfluorene system: the cyclization of trityl cations to form 9-phenylfluorenes is well known, see for example D. Llloyd, D. J. Walton, J.-P. Declercq, G. Germain and M. van Meerssche, *J. Chem. Res., Synop*. 1979, **7**, 249. The 9-phenylfluorene-Oxyma adduct thus obtained is then attacked by Oxyma N-oxyl radical, for reactions between N-oxyl radicals and double bond based frameworks see: I. B. Krylov, S. A. Paveliev, B. N. Shelimov, B.V. Lokshin, I. A. Garbuzova, V. A. Tafeenko, V. V. Chernyshev, A. S. Budnikov, G. I. Nikishina and A. O. Terent'ev, *Org. Chem. Front.,* 2017, **4**, 1947.

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