Electronic Supplementary Information (ESI)

2D Green SPPS: Green solvents for on-resin removal of acid sensitive protecting groups and lactamization

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1. General information

All reagents, reactants and solvents were from standard suppliers of raw materials for peptide synthesis and were used as such. All HPLC analyses were carried out on an Agilent 1100 instrument using Waters XSelect CSH130 C18 2.5µ 4.6x150mm column, TFA/MeCN/H_2O (0.1:0:100, A), TFA/MeCN/H_2O (0.08:90:10, B) as buffers, 99% B over 10 min gradient, flow of 1.0 mL/min, detection at 220 nm and column temperature 45 °C. Peaks >0.10% were integrated in HPLC analyses of Ac-Nle-Asp-His-Phe-Arg-Trp-Lys-NH_2 and Ac-Nle-c[Asp-His-Phe-Arg-Trp-Lys]-NH_2. MS analyses were carried out an Agilent Q-TOF mass spectrometer, (Agilent, Santa Clara, CA, USA). The mass spectrometry system was operated in a positive mode using ESI, mass range 20-3200, mass accuracy at 0.02 u resolution up to 20000 ppm. The following source settings were used: gas temperature 300°C, gas flow 8 l/min, nebulizer 30psig, sheat gas tempearture 350°C and sheat gas flow 7.5 l/min. The determination of D-His content in crude Ac-Nle-Asp-His-Phe-Arg-Trp-Lys-NH_2 was carried out at C.A.T. GmbH & Co. (Tübingen, Germany). The sample was hydrolyzed using deuterated solvents (6 N D_2O/DCl) and after derivatization the enantiomeric purity was determined by GC-MS. The Ac-Nle-c[Asp-His-Phe-Arg-Trp-Lys]-NH_2 final product was isolated on a HETOSICC lyophilizer and the yield of the product is not corrected for the peptide content in the isolated material. Fmoc content determinations were carried out using a literature method[1] employing the above HPLC conditions (detection at 294 nm) for analyses. The price estimates for the SPPS and peptide precipitation solvents respectively were obtained by requesting quotations in December 2018 – January 2019 period with suppliers capable of delivering these materials in bulk quantities. The quotations were for 100 kg and 1000 kg annually and the lowest price obtained for each solvent is given in Figures S19 and S20 in section 6 of this ESI. For the assessment of swelling of PS/DVB resin in EtOAc, MeCN and EtOAc/MeCN (1:1) a 0.44M AMS (PS/DVB) resin was used. For the assessment of swelling in TFA a 0.96M AMS (PS/DVB) resin and a 1.30M NH_2 functionalized ChemMatrix resin were used.

2. Swelling of resins

1.0 g of each resin was swollen in a suitable amount of the appropriate solvent in a fritted syringe at room temperature. The syringe was sealed and shaken at room temperature for one hour after which the syringe with the swollen resin was allowed to stand at room temperature for one hour and the volume occupied by the swollen resin was determined. For the determinations of swelling in TFA the supernate liquid was removed by draining the syringe prior to the volume determinations.

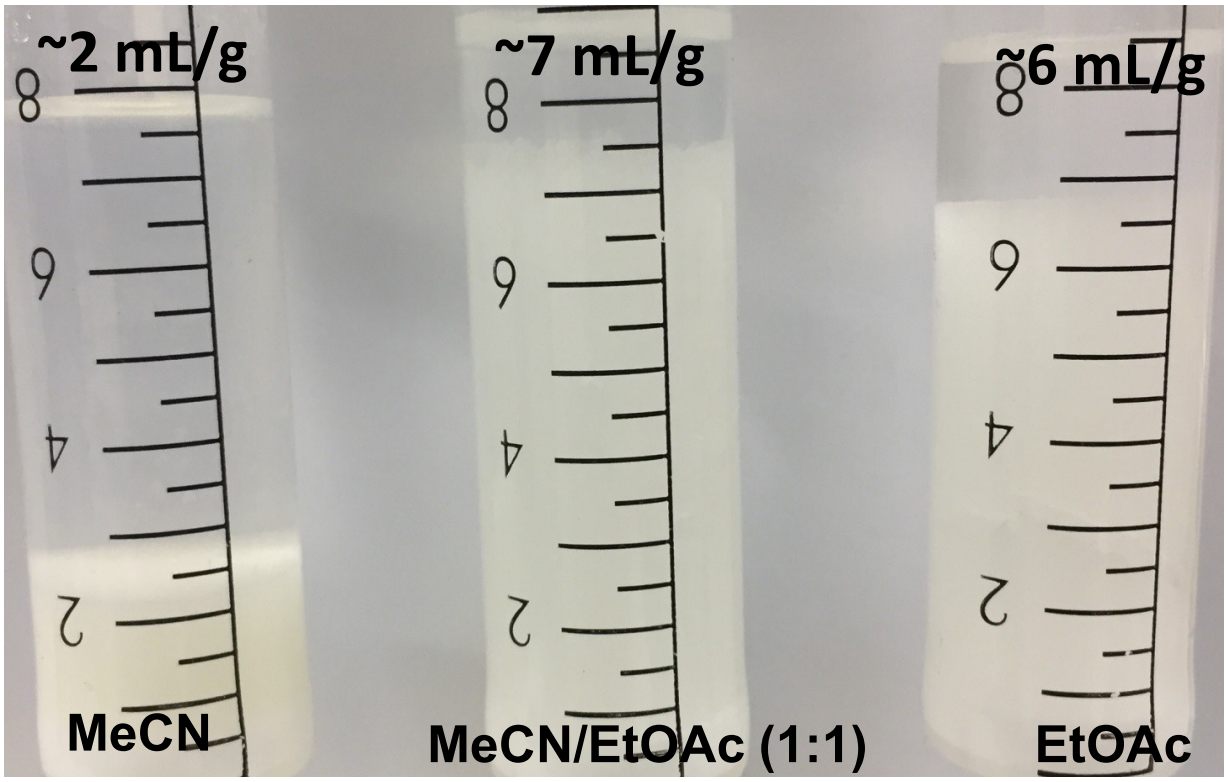


Figure S1. Swelling of a PS/DVB resin in MeCN, EtOAc and MeCN/EtOAc (1:1).

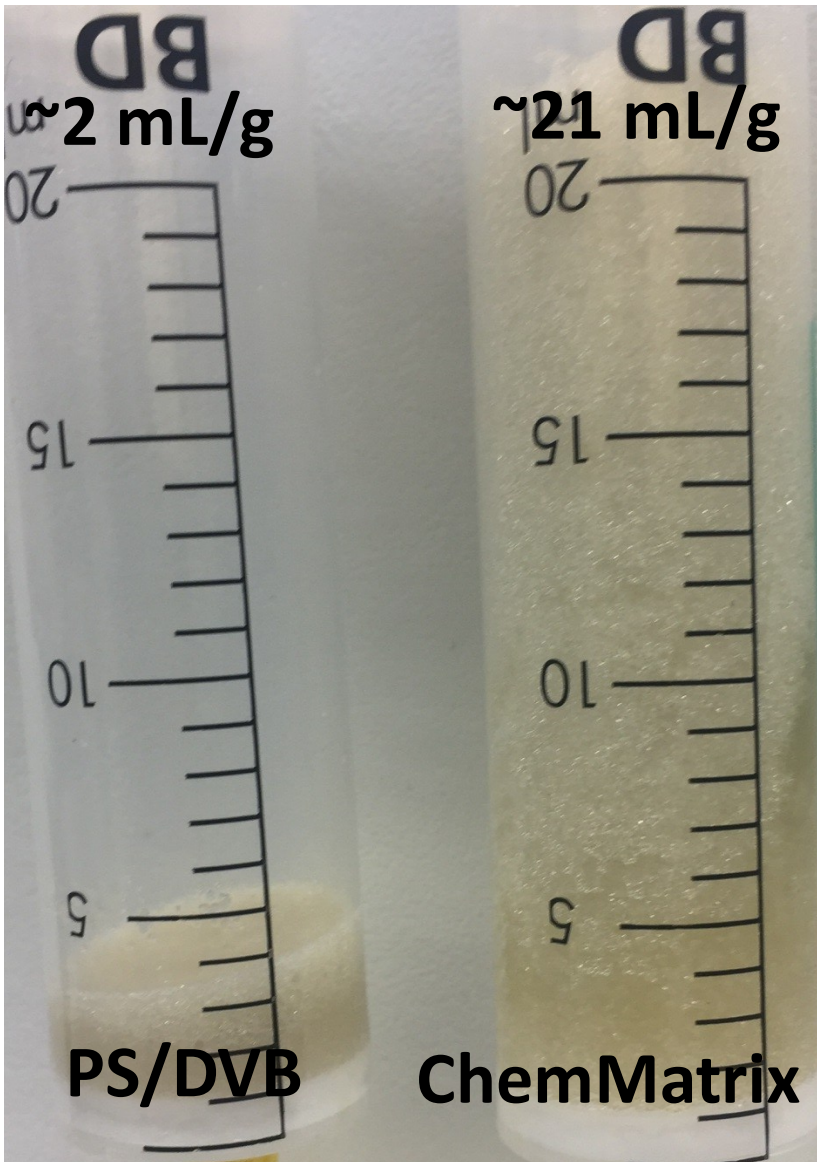


Figure S2. Swelling of a PS/DVB resin and a ChemMatrix resin in TFA.

3. Assessment of deblocking of Fmoc-AA(PG) RMG AMS resins

All requisite Fmoc-AA(PG) RMG AMS resins were prepared from the Fmoc-RMG AMS resin used in the synthesis of Ac-Nle-c[Asp-His-Phe-Arg-Trp-Lys]-NH_2 employing SPPS protocols described in Section 4 of this ESI. All PG removal experiments summarized in Tables 1 and 2 were carried out as follows: 100 mg of a Fmoc-AA(PG) RMG AMS resin was treated with 2 mL TFA/TIS/solvent and shaken in a fritted syringe at the given temperature for the given time. For the cases in which multiple PG removals were carried out after a PG removal treatment the resin was drained, washed three times with 5 mL of the reaction solvent (rt) and the next PG removal treatment was carried out. The extent of PG removal was determined by quantifying the amount of PG-H/PG-OH released into the supernate of a TFA/TIS (95:5) cleavage carried out after the completion of the PG removal and comparing the amount of the PG thus released to the amount of PG-H/PG-OH liberated into the supernate of a TFA/TIS (95:5) cleavage carried out using an untreated Fmoc-AA(PG) RMG AMS resin. The extent of Fmoc-AA-NH_2 leaching was determined by quantifying the amount of Fmoc-AA-NH_2 released into the supernate during the PG removal treatments and comparing it to the amount of Fmoc-AA-NH_2 released into the supernate during TFA/TIS (95:5) cleavage of untreated Fmoc-AA(PG) RMG resin. All PG removal and Fmoc-AA-NH_2 leaching determinations were carried out by taking out a 50 μL aliquot of a reaction supernate, adding it to 1.0 mL MeCN and carrying out a HPLC analysis using the method described in Section 1 of this ESI. The PG-H/PG-OH ratios in the supernates from the PG deblocking experiments varied for the different solvents examined, conceivably due to the differences in the contents of residual water in these solvents. An example of an analysis of PG removal/Fmoc-AA-NH_2 leaching is given below for the Table 1, entry 14 experiment (Figure S3).

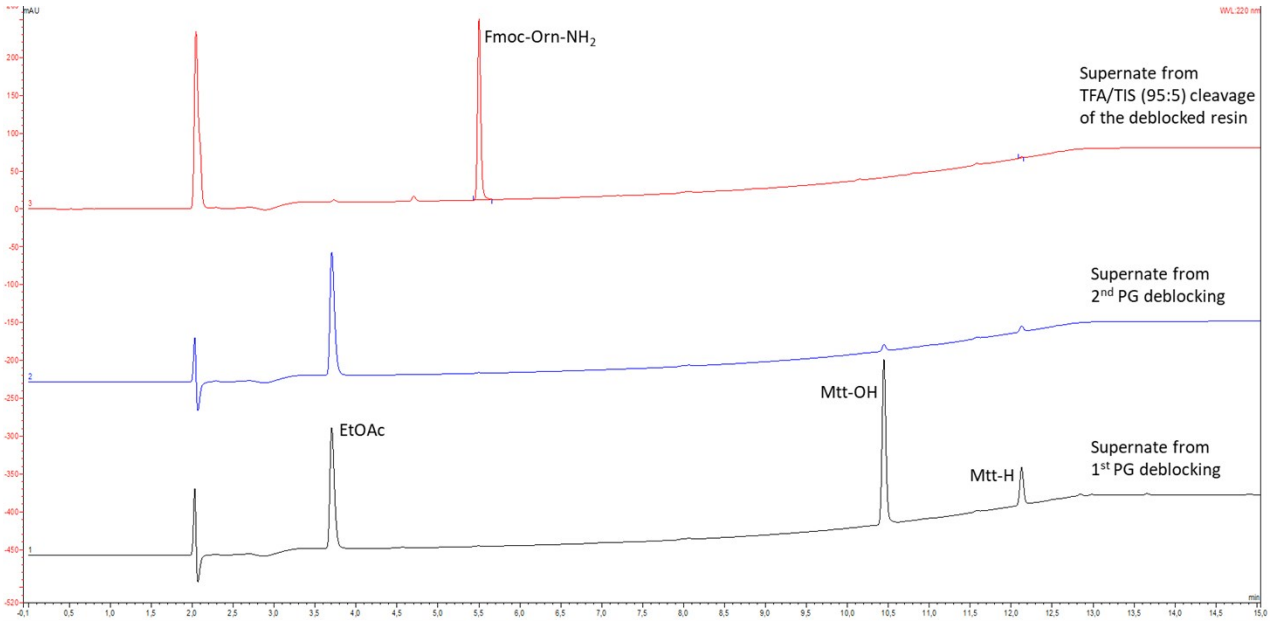


Figure S3. HPLC analysis of Table 1, entry 14 deblocking of Fmoc-Orn(Mtt)-RMG AMS resin. Content of Fmoc-Orn-NH_2 in supernates in 1st + 2nd deblockings: 0.09 mAu^{x}min (0.7%). Content of Mtt-H + Mtt-OH in the supernate from TFA/TIS (95:5) cleavage after Mtt deblocking: 0.04 mAu^{x}min (0.2%).

4. Synthesis of Ac-Nle-c[Asp-His-Phe-Arg-Trp-Lys]-NH_2

Synthesis of Fmoc RMG AMS resin.

The synthesis was carried out on an IKA® KS basic 130 shaking/heating apparatus employing a filter equipped 350 mL sealed reactor (Figure S4). Prior to the synthesis the temperature in the metal heating block of the apparatus was adjusted so that the required temperature of the solvent in the reactor was reached. The shaking frequency of the shaking plate of the apparatus was set to 320/min for all steps of the synthesis.

21.4 g of 0.44M AMS resin (9.42 mmol) was charged to the reactor. The resin was swollen in 200 mL NBP/EtOAc (1:1) for 1 h at rt and drained. Then, 5.2 g of Ramage linker (10.36 mmol), 1.5 g of Oxyma (10.36 mmol) and 150 mL of NBP/EtOAc (1:1) were added to the swollen resin, the reactor was sealed and the resulting slurry was shaken at 45 °C before 2.4 mL (15.5 mmol) of DIC was added. After 15 min an additional portion of DIC (2.4 mL, 15.5 mmol) was added and the reaction was shaken for 15 min upon which 5.9 mL AcOH (103 mmol) was added to cap any remaining free amino groups present and after 10 min of capping the reactor was drained. The drained polymer was washed with 3 x 150 mL NBP/EtOAc (1:9), 3 x 150 mL *i*-PrOH and dried to constant weight which gave 26.0 g of Fmoc-RMG AMS resin. Fmoc content of the resin per the method in Section 1 was 0.28 M i.e. 7.28 mmol of the resin was obtained.

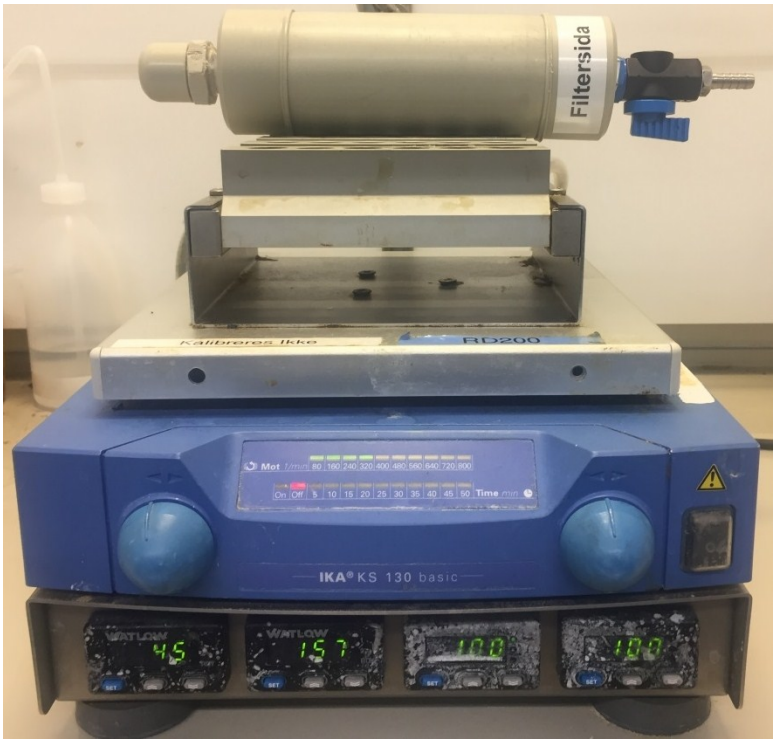


Figure S4. Sealed SPPS reactor on an IKA® KS basic 130 apparatus.

SPPS of Ac-Nle-Asp(O-2-PhiPr)-His(Trt)-Phe-Arg(Pbf)-Trp(Boc)-Lys(Mtt) RMG AMS resin.

The synthesis was carried out using the same reactor and shaking/heating device as was used in the synthesis of the Fmoc-RMG AMS resin above.

The SPPS commenced with 5.0 g of 0.28 M Fmoc-RMG AMS resin (1.4 mmol) which was swollen in NBP/EtOAc (1:9, 50 mL, 1 h at rt) and drained. The SPPS, depicted in Scheme 1, was then carried out at 45 °C throughout according to the general SPPS scheme as follows:

i. Fmoc removal, 2 x 15 min using 40 mL of 5% 4-MP in NBP/EtOAc (1:1). Per a previously reported protocol[2] in all Fmoc removals half of the base was added at the outset and the other half was added half way through the reaction (7.5 min).

ii. Wash after Fmoc removal, 4 x 40 mL of NBP/EtOAc (1:9), 5 min each.

iii. Coupling (30 min) using 2.0 equiv (2.8 mmol) of R-COOH/coupling additive/DIC (1:1:3). Per a previously reported protocol[2] in all couplings half of DIC was added at the outset and the other half was added half way through the reaction (15 min).

iv. Capping by adding 0.8 mL (14 mmol) of acetic acid (2 min).

v. Quench of the coupling/capping mixture with 2 mL EtOAc/*i*-PrOH (9:1), 2 min.

Eight coupling cycles were carried out according to the general protocol above, the following R-COOH reactants and coupling additives were employed:

Cycle 1, Lys^7 coupling: Fmoc-Lys(Mtt)-OH (1.75 g, 2.8 mmol) and Oxyma (0.40 g, 2.8 mmol)

Cycle 2, Trp^6 coupling: Fmoc-Trp(Boc)-OH (1.47 g, 2.8 mmol) and Oxyma (0.40 g, 2.8 mmol)

Cycle 3, Arg^5 coupling: Fmoc-Arg(Pbf)-OH (1.82 g, 2.8 mmol) and Oxyma (0.40 g, 2.8 mmol)

Cycle 4, Phe^4 coupling: Fmoc-Phe-OH (1.08 g, 2.8 mmol) and Oxyma (0.40 g, 2.8 mmol)

Cycle 5, His^3 coupling: Fmoc-His(Trt)-OH (1,74 g, 2.8 mmol), HONB (0.50 g, 2.8 mmol) and $HOBt^xH_2O$ (64 mg, 0,4 mmol). According to a Ninhydrin color test[3] at 25 min the coupling was not gone to full completion (weak positive color test) and the reaction was drained at 30 min upon which the coupling was repeated using the same reactants as in the 1st coupling and coupling time of 30 min. Upon the completion of the 2nd coupling the reaction was capped and the synthesis proceeded further per the standard protocol.

Cycle 6, Asp^2 coupling: Fmoc-Asp(O-2-Ph*i*Pr)-OH (1,33 g, 2.8 mmol) and Oxyma (0.40 g, 2.8 mmol)

Cycle 7, Nle^1 coupling: Fmoc-Nle-OH (0.99 g, 2.8 mmol) and Oxyma (0.40 g, 2.8 mmol)

Cycle 8, Ac^0 coupling: Acetic acid (160 μL, 2.8 mmol) and Oxyma (0.40 g, 2.8 mmol)

Upon the completion of the last step of the synthesis the reactor was drained and the resin was washed with 3 x 20 mL NBP/EtOAc (1:9) and 2 x 20 mL *i*PrOH followed be drying *en vacuo* to constant weight gave 7.6 g of resin **2**, which is 102% of the theoretically attainable amount based on the scale on the synthesis and molecular weight of the protected peptide attached to

the H-RMG AMS polymer. The amounts of NBP, EtOAc and 4-MP used throughout the synthesis are stated in Table S1.

Table S1. NBP, EtOAc and 4-MP used in SPPS of Ac-Nle-Asp(O-2-PhiPr)-His(Trt)-Phe-Arg(Pbf)-Trp(Boc)-Lys(Mtt) RMG AMS resin.

Process step	Total volume (mL)	EtOAc volume (mL)	NBP volume (mL)	4-MP volume (mL)
Swelling	50,0	5,0	45,0	
Lys^{7}, Fmoc removal	80,0	38,0	38,0	4,0
Lys^{7}, Wash	160,0	144,0	16,0	
Lys^{7}, Coupling	40,0	20,0	20,0	
Lys^{7}, Capping	0,8			
Lys^{7}, *i*- PrOH quench	2,0	1,8		
Trp^{6}, Fmoc removal	80,0	38,0	38,0	4,0
Trp^{6}, Wash	160,0	144,0	16,0	
Trp^{6}, Coupling	40,0	20,0	20,0	
Trp^{6}, Capping	0,8			
Trp^{6}, *i*- PrOH quench	2,0	1,8		
Arg^{5}, Fmoc removal	80,0	38,0	38,0	4,0
Arg^{5}, Wash	160,0	144,0	16,0	
Arg^{5}, Coupling	40,0	20,0	20,0	
Arg^{5}, Capping	0,8			
Arg^{5}, *i*- PrOH quench	2,0	1,8		
Phe^{4}, Fmoc removal	80,0	38,0	38,0	4,0
Phe^{4}, Wash	160,0	144,0	16,0	
Phe^{4}, Coupling	40,0	20,0	20,0	
Phe^{4}, Capping	0,8			
Phe^{4}, *i*- PrOH quench	2,0	1,8		
His^{3}, Fmoc removal	80,0	38,0	38,0	4,0
His^{3}, Wash	160,0	144,0	16,0	
His^{3}, Coupling	80,0	40,0	40,0	
His^{3}, Capping	0,8			
His^{3}, *i*- PrOH quench	2,0	1,8		
Asp^{2}, Fmoc removal	80,0	38,0	38,0	4,0
Asp^{2}, Wash	160,0	144,0	16,0	
Asp^{2}, Coupling	40,0	20,0	20,0	
Asp^{2}, Capping	0,8			
Asp^{2}, *i*- PrOH quench	2,0	1,8		
Nle^{1}, Fmoc removal	80,0	38,0	38,0	4,0
Nle^{1}, Wash	160,0	144,0	16,0	
Nle^{1}, Coupling	40,0	20,0	20,0	
Nle^{1}, Capping	0,8			
Nle^{1}, *i*- PrOH quench	2,0	1,8		
Ac^{0}, Fmoc removal	80,0	38,0	38,0	4,0
Ac^{0}, Wash	160,0	144,0	16,0	
Ac^{0}, Coupling	40,0	20,0	20,0	
Ac^{0}, Capping	0,8			
Ac^{0}, *i*- PrOH quench	2,0	1,8		
Final wash, (3 x NBP/EtOAc)	60	54	6	
Final wash, (2 x *i* -PrOH)	40			
Entire SPPS (mL)	**2452,4**	**1709,4**	**663,0**	**32,0**
Entire SPPS (%)		**69,7**	**27,0**	**0,9**

A test TFA/TIS/H_2O (90:5:5) cleavage of peptide resin **2** followed by a DEE precipitation afforded a sample of the linear Ac-Nle-Asp-His-Phe-Arg-Trp-Lys-NH_2 crude peptide which was analyzed by HPLC (Figures S5 and S6) and MS (Figure S7) per methods detailed in Section 1 of this ESI. A chiral GC-MS analysis per the method stated in section 1 (D-His analysis) revealed that the content of D-His in the crude material was 0.31% (Figure S12).

A test of PG removal from Ac-Nle-Asp(O-2-PhiPr)-His(Trt)-Phe-Arg(Pbf)-Trp(Boc)-Lys(Mtt) RMG AMS resin.

100 mg of the Ac-Nle-Asp(O-2-Ph*i*Pr)-His(Trt)-Phe-Arg(Pbf)-Trp(Boc)-Lys(Mtt) RMG AMS resin **2** was placed in a fritted syringe and treated with 2 mL 2% TFA/TIS in DCM and EtOAc/MeCN (1:1), respectively. The DCM containing syringe was shaken at rt for 1 h whereas the EtOAc/MeCN containing syringe was treated with the deprotection cocktail for 2 x 1 h at 45 °C. After the completion of the PG removal both syringes were drained and washed with the solvents used in the deprotections (3 x 5 mL), *i*-PrOH (3 x 5 mL) and dried en vacuo to constant weight. The dried resins were cleaved with 1 mL TFA/TIS/H_2O (90:5:5) for 2 h at rt, precipitated with ice cold DEE (2 x 30 mL) followed by drying the crude materials en vacuo to constant weight. The weights of the crude peptides thus obtained were 21 mg for the material stemming from the TFA/TIS in DCM mediated PG removal experiment and 24 mg for the material from the TFA/TIS in EtOAc/MeCN (1:1) induced deprotection. HPLC analyses of the Ac-Nle-Asp-His-Phe-Arg-Trp-Lys-NH_2 crudes from the two PG removal experiments are shown in Figures S13 and S14, respectively.

A test of solvents for crude peptide precipitation after a TFA cleavage of Ac-Nle-Asp(O-2-Ph*i*Pr)-His(Trt)-Phe-Arg(Pbf)-Trp(Boc)-Lys(Mtt) RMG AMS resin.

5 x 100 mg of the Ac-Nle-Asp(O-2-Ph*i*Pr)-His(Trt)-Phe-Arg(Pbf)-Trp(Boc)-Lys(Mtt) RMG AMS resin **2** was placed in five fritted syringe and each syringe was treated with 1 mL TFA/TIS/H_2O (90:5:5). The resulting five resin cleavage reactions were then shaken at rt for 2 h upon which the resulting crude peptides were precipitated using 2 x 30 mL of following ice cold solvents: i) DEE ii) CPME iii) 2-MeTHF iv) MTHP v) MTHP/*n*-heptane (1:4). The resulting crude peptides were dried en vacuo which afforded following amounts of the crude peptides: exp. i): 24 mg (91%), exp. ii): 21 mg (81%), exp. iii): 19 mg (72%), exp. iv): 20 mg (76%), exp. v): 24 mg (91%). HPLC analyses of the Ac-Nle-Asp-His-Phe-Arg-Trp-Lys-NH_2 crudes from the five precipitation solvent assessment experiments are shown in Figures S15 and S16, respectively.

Synthesis of Ac-Nle-c[Asp-His-Phe-Arg-Trp-Lys]-NH_2 from Ac-Nle-Asp(O-2-PhiPr)-His(Trt)-Phe-Arg(Pbf)-Trp(Boc)-Lys(Mtt) RMG AMS resin.

2.0 g (0.37 mmol) of resin **2** was placed in a fritted syringe and treated with 30 mL 2% TFA/TIS in EtOAc/MeCN (1:1) and the resulting slurry was shaken at 45 °C for 3 x 1 h using the IKA® KS basic 130 apparatus (Figure S4) employed in the SPPS of resin **2**. After each deblocking treatment the resin was washed with 3 x 20 mL EtOAc/MeCN (1:1) and after the final

deprotection the resin was free-based with two 5 min treatments using 5% DIEA (v/v) in 30 mL NBP/EtOAc (1:1) at rt. The free-based resin was then washed with 2 x 30 mL NBP/EtOAc (1:1), drained and cyclized for 16 h at rt using 2 equiv PyBOP/DIEA (1:2) in 20 mL NBP/EtOAc (1:1). The cyclized resin was washed with 2 x 30 mL NBP/EtOAc (1:1), 2 x 30 mL *i*-PrOH and dried *en vacuo* before cleaving the crude peptide off the resin for 2 h at rt using 20 mL TFA/TIS/H_2O (90:5:5). The crude peptide thus obtained was isolated by precipitation with 2 x 150 mL ice cold MTHP/*n*-heptane (1:4) and dried *en vacuo* to constant weight. An HPLC analysis of the crude peptide (Figures S5 and S8) revealed the purity of the cyclized material was ~90% and an MS analysis confirmed the expected molecular weight of the product (Figure S9). The crude material was dissolved in 200 mL 1% AcOH/10% MeCN, applied onto a 20 g pad of Amberchrom CG161M resin and eluted by 1% AcOH/30% MeCN. Most of MeCN was removed on a rotary evaporator under reduced pressure upon which the desalted material was isolated by lyophilization furnishing 255 mg (62%) of peptide **1** as a colorless solid. An HPLC analysis (Figures S5 and S10) revealed the purity of the desalted material was ~95% and an MS analysis confirmed the expected molecular weight of the product (Figure S11).

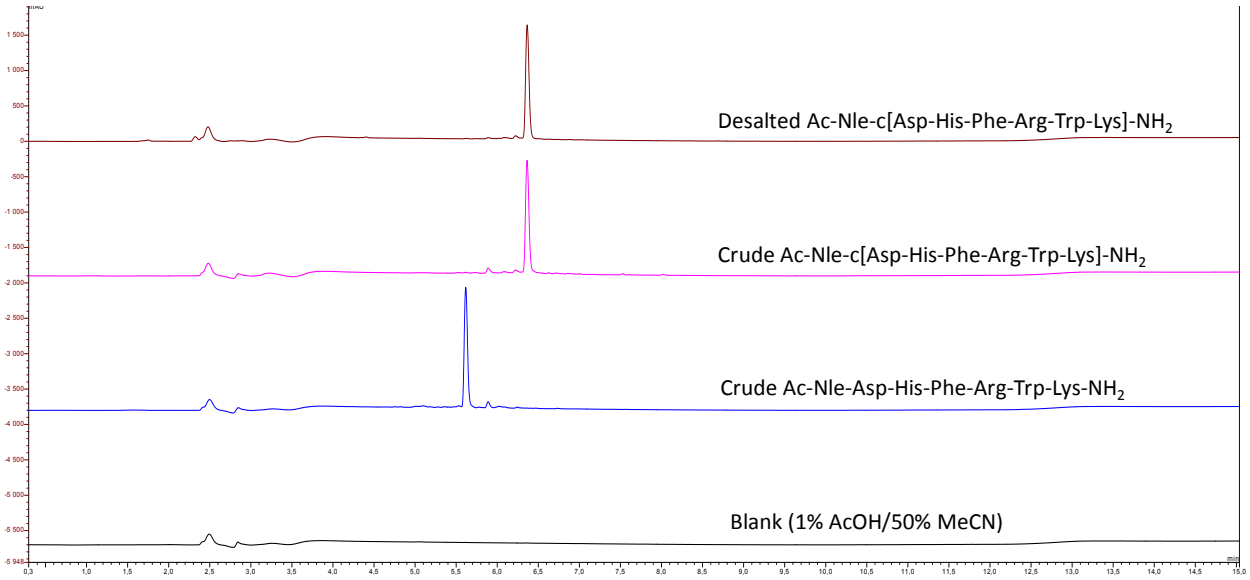
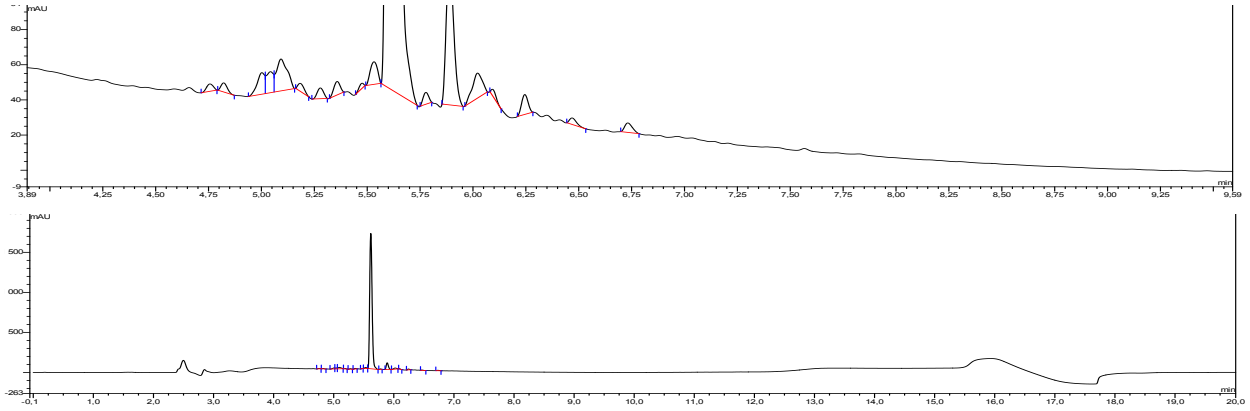


Figure S5. HPLC overlay of the linear crude, cyclized crude and desalted product **1**.



No.	Peakname	Ret.Time min	Area mAU*min	Amount	Type	Height mAU	Rel.Area %	Resolution
1	n.a.	4,755	0,1535	n.a.	BMB*	4,180	0,18	1,16
2	n.a.	4,823	0,1764	n.a.	BMB*	5,190	0,20	n.a.
3	n.a.	5,002	0,5026	n.a.	BM *	12,256	0,58	n.a.
4	n.a.	5,042	0,4579	n.a.	M *	11,966	0,53	n.a.
5	n.a.	5,092	1,0739	n.a.	MB*	18,157	1,23	n.a.
6	n.a.	5,187	0,1566	n.a.	BMB*	4,630	0,18	1,59
7	n.a.	5,278	0,2106	n.a.	BMB*	6,122	0,24	1,35
8	n.a.	5,357	0,2668	n.a.	BMB*	7,691	0,31	2,31
9	n.a.	5,472	0,0752	n.a.	BMB*	3,101	0,09	1,08
10	n.a.	5,532	0,5120	n.a.	BMb*	12,827	0,59	1,19
11	*n.a.*	*5,617*	*78,1263*	*n.a.*	*bMB**	*1689,584*	*89,85*	*2,57*
12	n.a.	5,777	0,2030	n.a.	BMB*	6,748	0,23	1,95
13	n.a.	5,890	3,3968	n.a.	BMB*	83,100	3,91	1,70
14	n.a.	6,020	0,7784	n.a.	BMB*	14,275	0,90	1,20
15	n.a.	6,098	0,0996	n.a.	BMB*	4,006	0,11	2,91
16	n.a.	6,245	0,3991	n.a.	BMB*	11,293	0,46	3,77
17	n.a.	6,470	0,1398	n.a.	BMB*	3,714	0,16	4,04
18	n.a.	6,733	0,2268	n.a.	BMB*	5,380	0,26	n.a.
Total:			**86,9551**	**0,0000**		**1904,222**	**100,00**	

Figure S6. HPLC analysis of the crude Ac-Nle-Asp-His-Phe-Arg-Trp-Lys-NH_2.

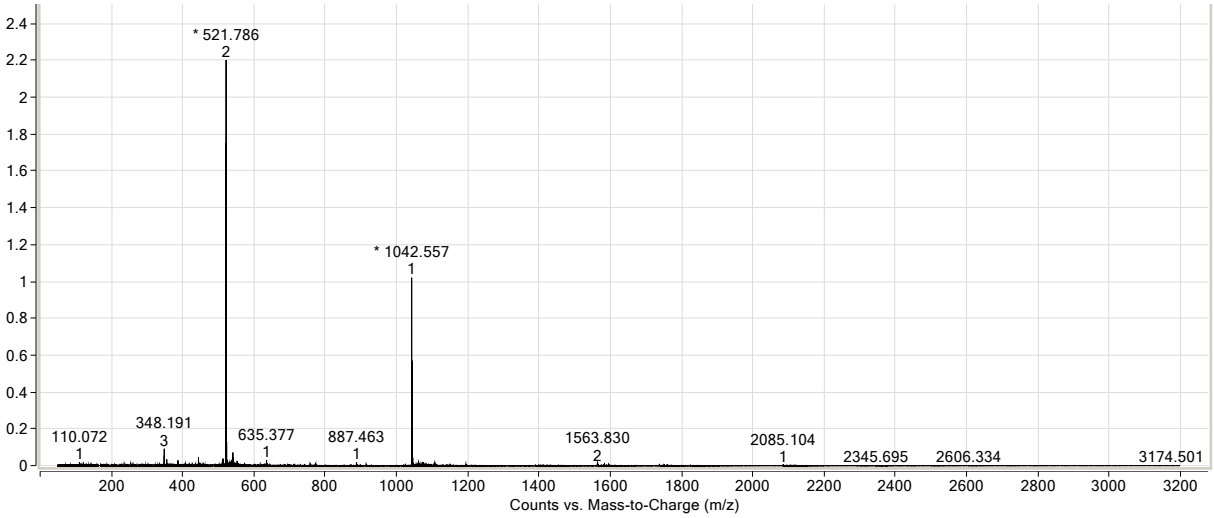
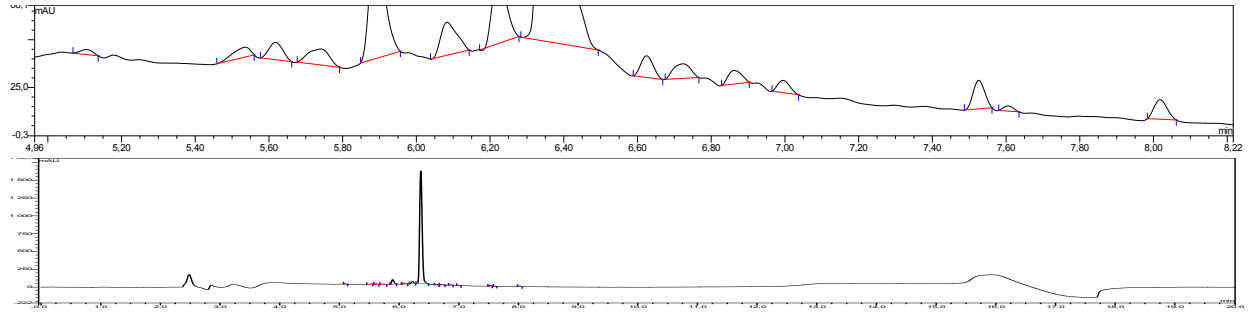


Figure S7. MS spectrum of the main peak in crude Ac-Nle-Asp-His-Phe-Arg-Trp-Lys-NH_2.



No.	Peakname	Ret.Time min	Area mAU*min	Amount	Type	Height mAU	Rel.Area %	Resolution
1	n.a.	5,107	0,1004	n.a.	BMB*	2,657	0,12	5,30
2	n.a.	5,532	0,2900	n.a.	BMB*	5,259	0,34	1,03
3	n.a.	5,618	0,3933	n.a.	BMB*	9,054	0,46	1,34
4	n.a.	5,743	0,5462	n.a.	BMB*	8,379	0,63	1,57
5	n.a.	5,890	3,0425	n.a.	BMB*	66,169	3,53	2,41
6	n.a.	6,082	0,8747	n.a.	BMB*	17,275	1,01	1,73
7	n.a.	6,222	1,5403	n.a.	BMB*	34,015	1,79	1,85
8	n.a.	6,363	77,0199	n.a.	BMB*	1582,074	89,30	3,68
9	n.a.	6,625	0,4426	n.a.	BMB*	11,357	0,51	1,23
10	n.a.	6,723	0,4153	n.a.	BMB*	7,624	0,48	1,64
11	n.a.	6,862	0,3043	n.a.	BMB*	7,113	0,35	1,93
12	n.a.	6,995	0,2423	n.a.	BMB*	6,410	0,28	8,61
13	n.a.	7,525	0,5390	n.a.	BMB*	14,986	0,62	1,44
14	n.a.	7,607	0,0843	n.a.	BMB*	2,688	0,10	6,76
15	n.a.	8,018	0,4153	n.a.	BMB*	10,243	0,48	n.a.
Total:			86,2506	0,0000		1785,302	100,00	

Figure S8. HPLC analysis of the crude Ac-Nle-c[Asp-His-Phe-Arg-Trp-Lys]-NH_2.

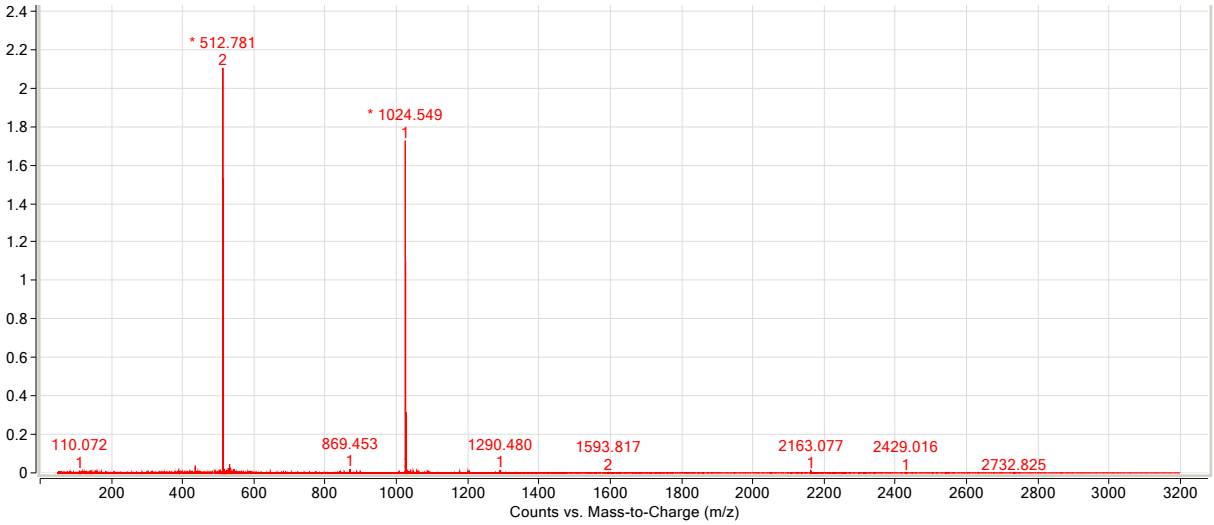
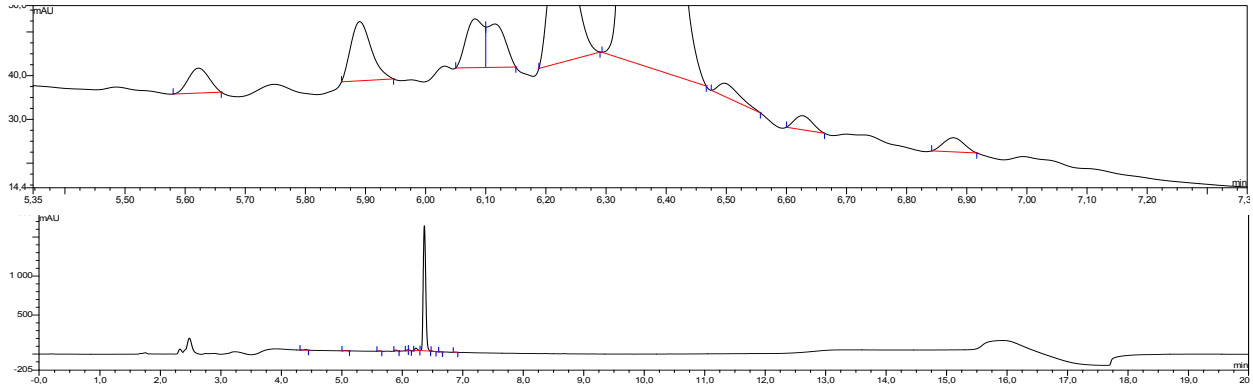


Figure S9. MS spectrum of the main peak in crude Ac-Nle-c[Asp-His-Phe-Arg-Trp-Lys]-NH_2.



No.	Peakname	Ret.Time min	Area mAU*min	Amount	Type	Height mAU	Rel.Area %	Resolution
1	n.a.	4,402	0,5000	n.a.	BMB*	10,538	0,61	7,42
2	n.a.	5,052	0,1508	n.a.	BMB*	2,285	0,18	6,48
3	n.a.	5,622	0,2252	n.a.	BMB*	5,687	0,28	4,15
4	n.a.	5,890	0,5349	n.a.	BMB*	13,544	0,65	n.a.
5	n.a.	6,082	0,3752	n.a.	BM *	11,153	0,46	n.a.
6	n.a.	6,115	0,3432	n.a.	MB*	9,918	0,42	n.a.
7	n.a.	6,223	1,6053	n.a.	BMB*	34,330	1,96	1,83
8	*n.a.*	*6,365*	*77,6628*	*n.a.*	*BMB**	*1602,015*	*94,98*	*1,90*
9	n.a.	6,502	0,1268	n.a.	BMB*	3,114	0,16	2,03
10	n.a.	6,628	0,1112	n.a.	BMB*	3,227	0,14	4,00
11	n.a.	6,878	0,1282	n.a.	BMB*	3,224	0,16	n.a.
Total:			**81,7636**	**0,0000**		**1699,036**	**100,00**	

Figure S10. HPLC analysis of the desalted Ac-Nle-c[Asp-His-Phe-Arg-Trp-Lys]-NH_2 product **1**.

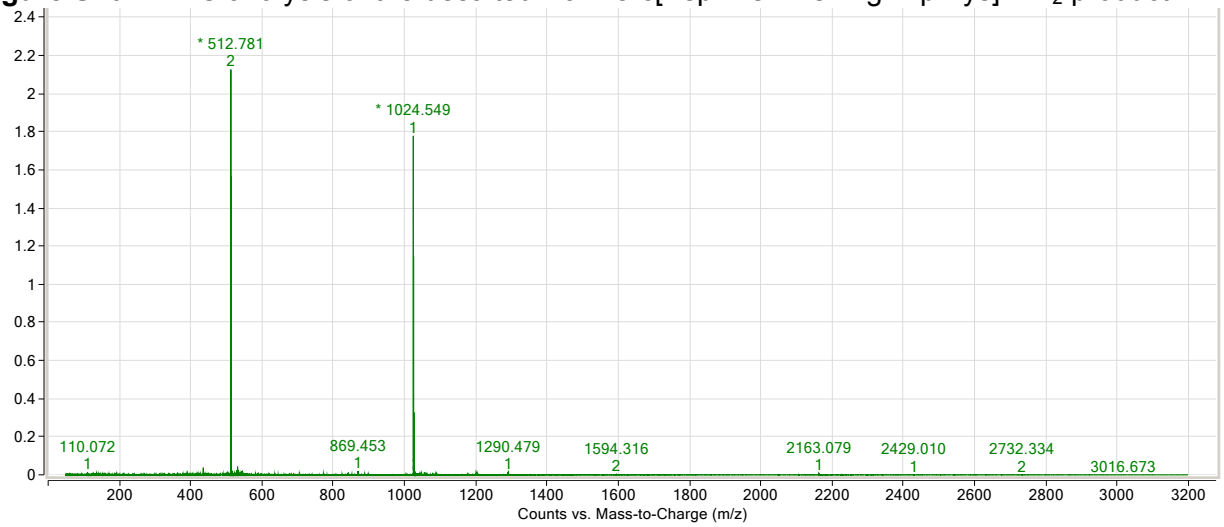
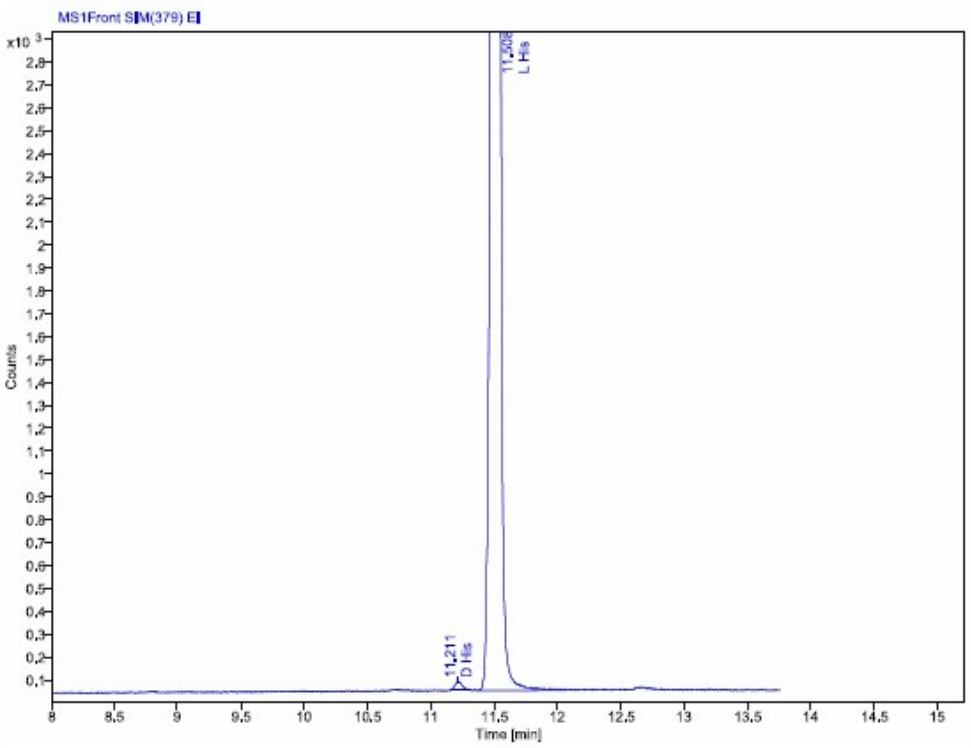


Figure S11. MS spectrum of the desalted product **1**.



Signal: MS1Front S|M(379) E|

RT [min]	Type	Width [min]	Area	Height	Area%	Name
11,211	MM m	0,07	149,2	35,7	0,31	D His
11,508	BB	0,71	47701,9	13360,0	99,69	L His

Figure S12. D-His content determination on the crude Ac-Nle-Asp-His-Phe-Arg-Trp-Lys-NH_2.

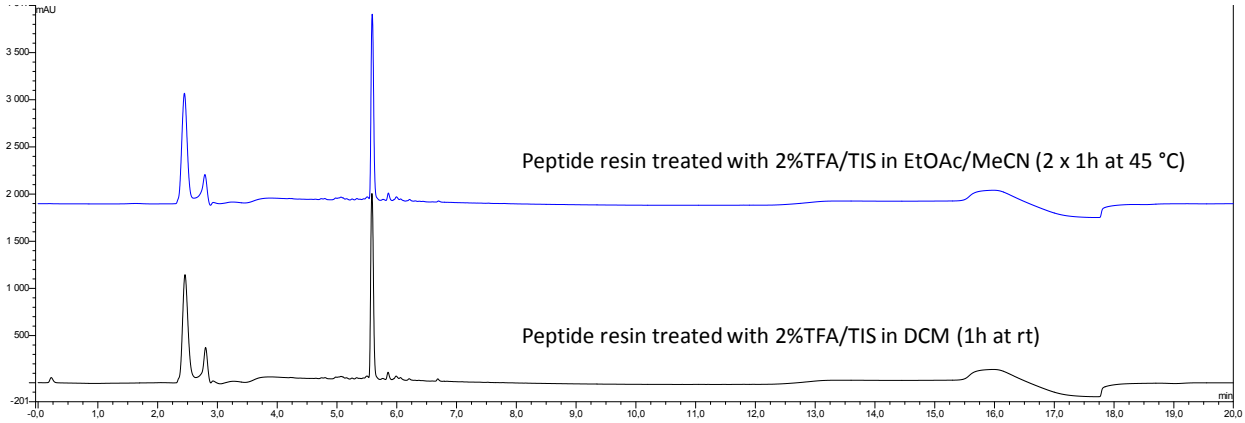


Figure S13. HPLC overlay of two Ac-Nle-Asp-His-Phe-Arg-Trp-Lys-NH_2 crudes from the assessment of solvents for PG removal from Ac-Nle-Asp(O-2-Ph*i*Pr)-His(Trt)-Phe-Arg(Pbf)-Trp(Boc)-Lys(Mtt) RMG AMS resin.

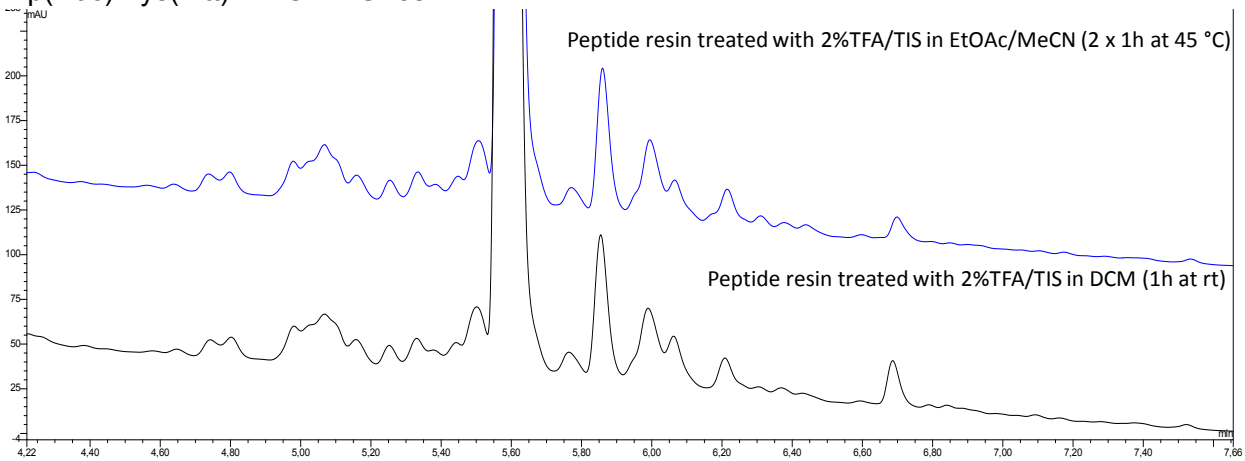


Figure S14. HPLC overlay (zoom-in) of two Ac-Nle-Asp-His-Phe-Arg-Trp-Lys-NH_2 crudes from the assessment of solvents for PG removal from Ac-Nle-Asp(O-2-Ph*i*Pr)-His(Trt)-Phe-Arg(Pbf)-Trp(Boc)-Lys(Mtt) RMG AMS resin.

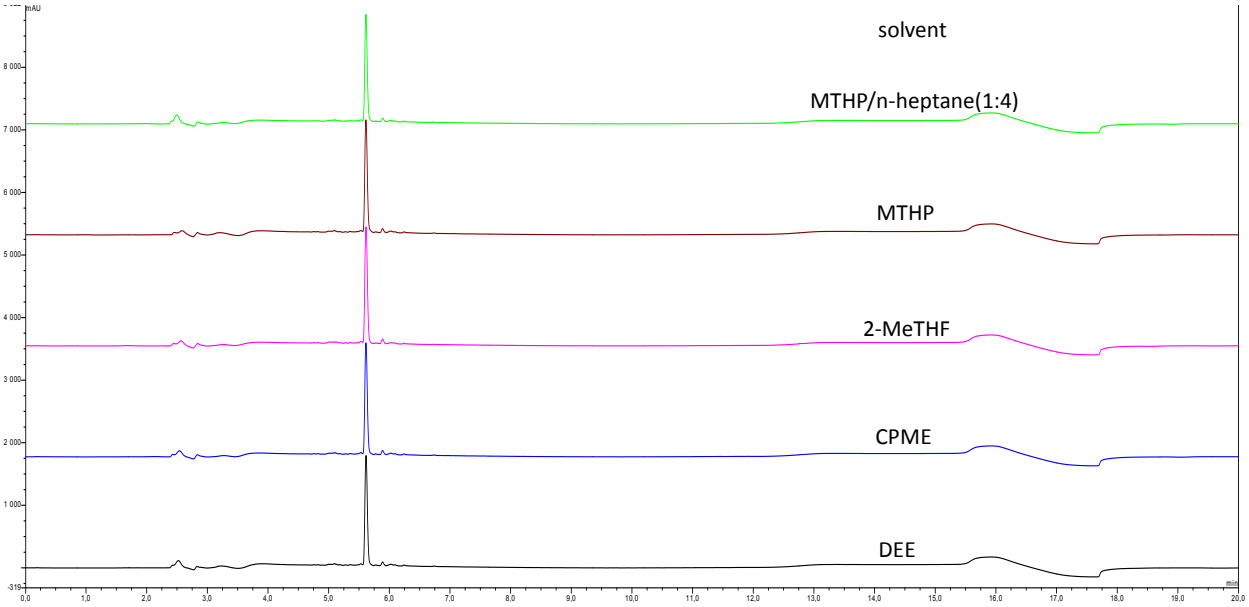


Figure S15. HPLC overlay of five Ac-Nle-Asp-His-Phe-Arg-Trp-Lys-NH_2 crudes from the assessment of solvents for precipitation after TFA cleavage of Ac-Nle-Asp(O-2-Ph*i*Pr)-His(Trt)-Phe-Arg(Pbf)-Trp(Boc)-Lys(Mtt) RMG AMS resin.

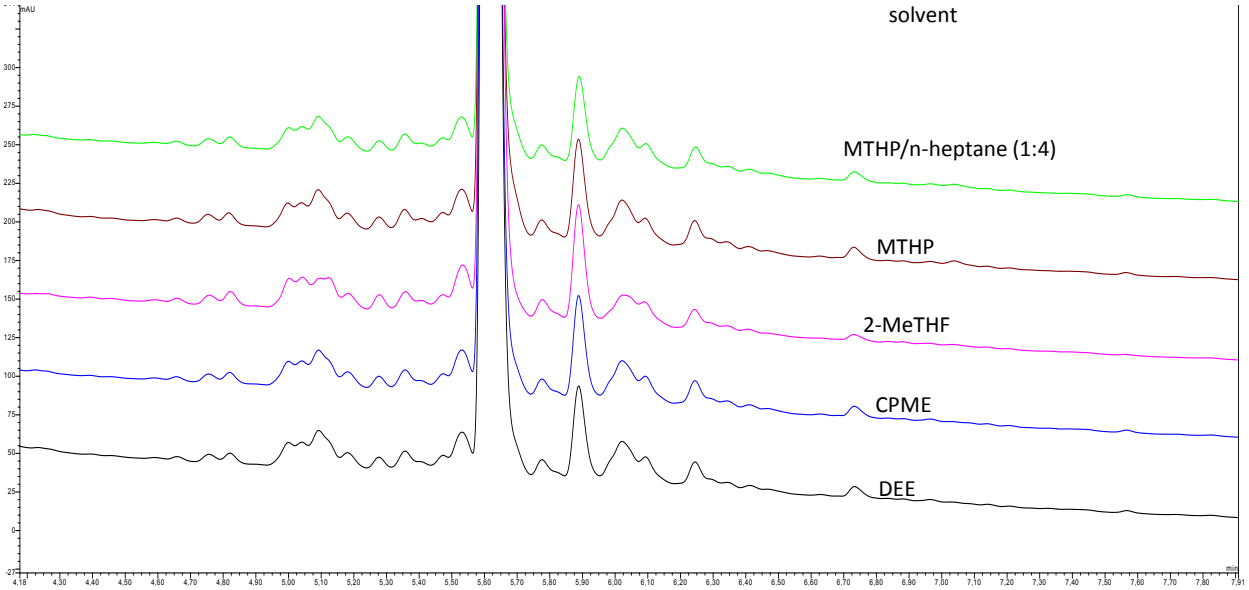


Figure S16. HPLC overlay (zoom-in) of five Ac-Nle-Asp-His-Phe-Arg-Trp-Lys-NH_2 crudes from the assessment of solvents for precipitation after TFA cleavage of Ac-Nle-Asp(O-2-Ph*i*Pr)-His(Trt)-Phe-Arg(Pbf)-Trp(Boc)-Lys(Mtt) RMG AMS resin.

5. Cleavages of Fmoc-Gly-Gly CTC resin

The Fmoc-Gly-Gly CTC resin (0.21 M) used as the substrate for the cleavage experiments herein was prepared from the corresponding H-Gly CTC resin by a coupling with 2 equiv Fmoc-Gly-OH/Oxyma/DIC (1:1:3) in NBP/EtOAc (1:1) according the protocol described in Section 4 of this ESI. The coupling was carried out at rt for 30 min after which the coupling mixture was capped with AcOH/DIC (3 equiv each) for 10 min, washed as described in Section 4 and dried to constant weight *en vacuo.*

3 x 100 mg (0.021 mmol each) of the Fmoc Gly-Gly CTC resin was placed in three fritted syringes and then 2 mL of the followings solvents was added: syringe 1, DCM; syringe 2, EtOAc/MeCN (1:1); syringe 3, 2-MeTHF. To all three syringes 1% v/v of TIS and TFA was added, syringes were sealed and allowed to shake at rt for 1 h.

Then, 50 μL of a supernate was taken out from each syringe and added to 1 mL MeCN. The samples of Fmoc-Gly-Gly-OH dipeptides thus obtained were analyzed by HPLC using the method described in Section 1 of this ESI (Figure S17). To determine the amounts and yields of Fmoc-Gly-Gly-OH released from each resin a sample of Fmoc-Gly-Gly-OH (7.4 mg, 0.021 mmol) dissolved in 2 mL TFA/TIS (95:5) was used as the reference. To distinguish between peaks stemming from compounds released from the resin and the solvent peaks HPLC analyses of the solvents used in the cleavage experiments were recorded (Figure S18).

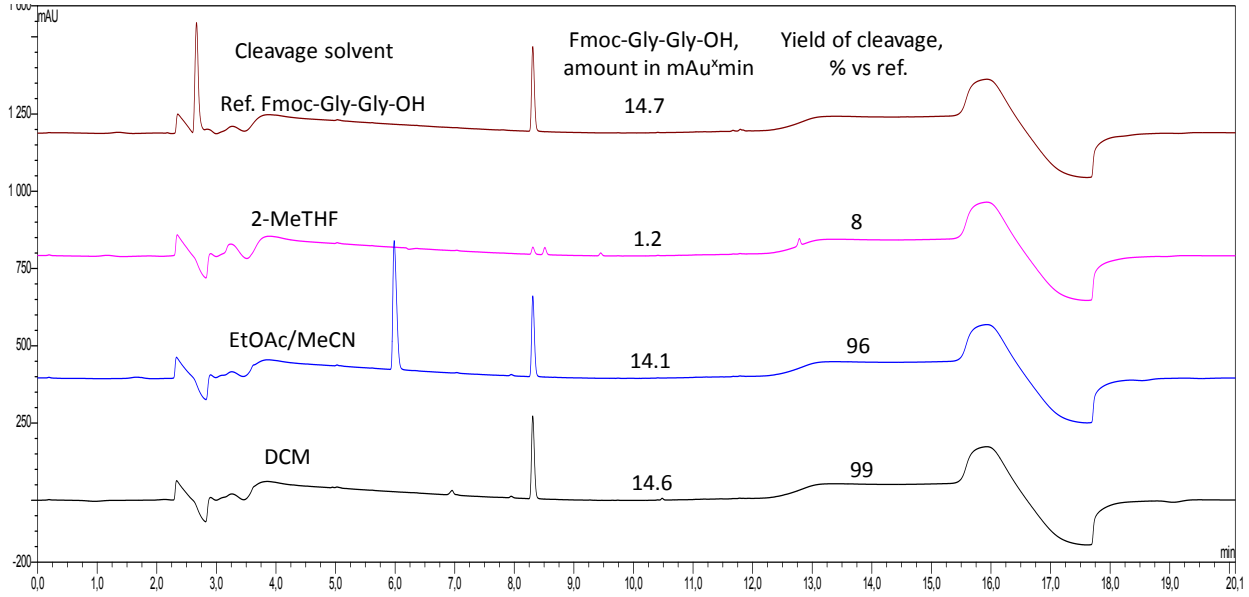


Figure S17. HPLC analyses of Fmoc-Gly-Gly-OH released from Fmoc-Gly-Gly CTC resin.

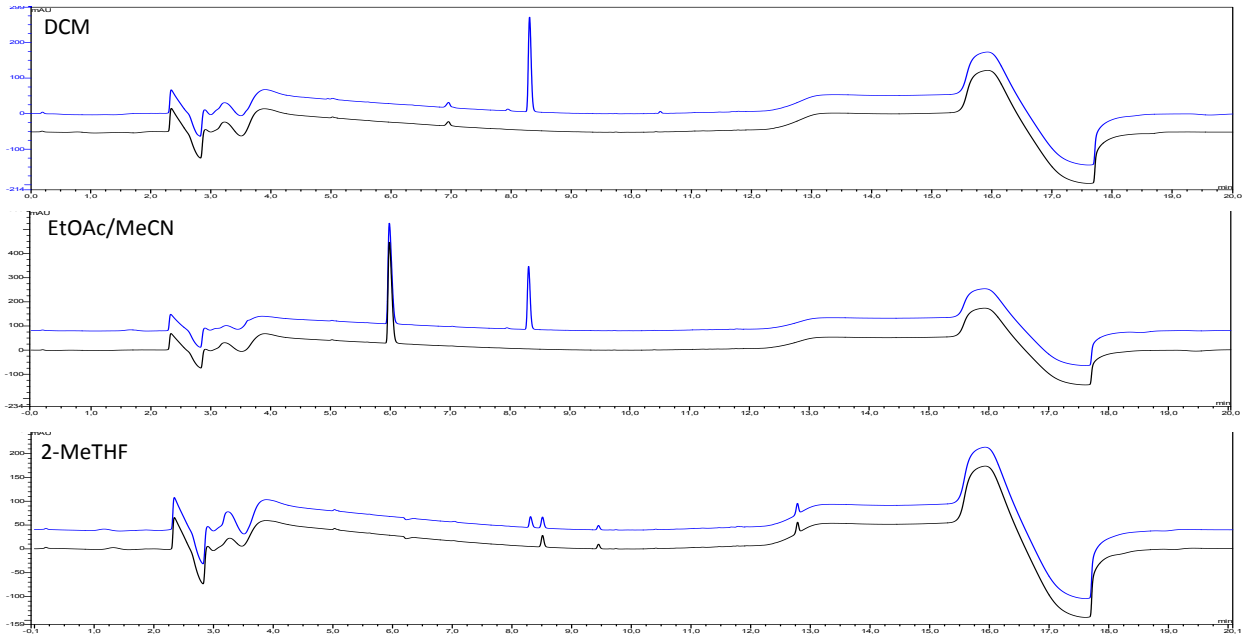


Figure S18. HPLC chromatograms of Fmoc-Gly-Gly-OH dipeptides vs the chromatograms of the solvents used in the cleavages of Fmoc-Gly-Gly CTC resin. HPLC chromatograms of dipeptides in blue, chromatograms of blank solvents in black.

6. Solvent pricing estimates

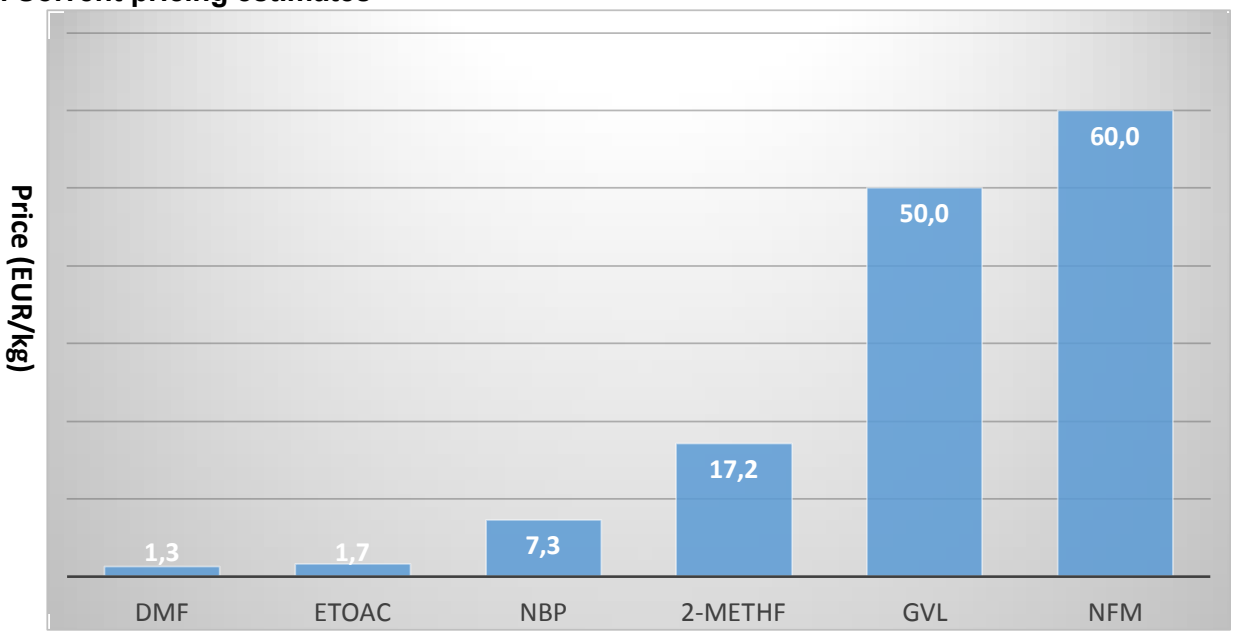


Figure S19. Estimated large scale pricing for DMF and greener solvents used in SPPS on PS/DVB based solid supports.

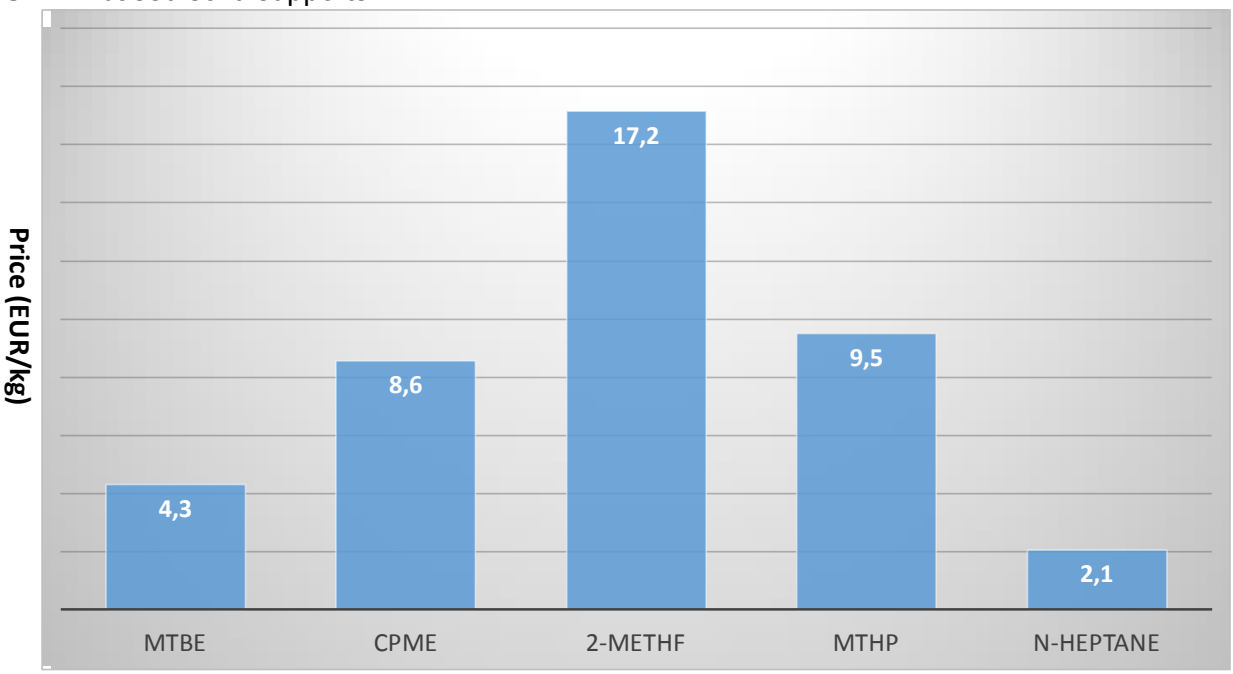


Figure S20. Estimated large scale pricing for MTBE and greener solvents used in precipitation of crude peptides after TFA cleavages.

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[1] M. Gude, J. Ryf, P. D. White, *Lett. Pep. Sci. 2003*, **9**, 203.

[2] J. Pawlas, *Proceedings of the 34th European Peptide Symposium and the 8th International Peptide Symposium,* Leipzig, Germany, *2016*, 60.

[3] E. Kaiser, R. L. Colescott, C. D: Bossinger, P. I. Cook, *Anal.Biochem. 1970*, **34**, 595.