Supplementary Information

Secondary Amine Selective Petasis (SASP) Bioconjugation

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Supplementary figures and text:

General methods. Unless otherwise noted, the chemicals and solvents used were of analytical grade and were used as received from commercial sources. All commercial materials (Aldrich, Fluka, Nova) were used without further purification. All solvents were reagent grade or HPLC grade (Fisher). Aldolase from rabbit muscle, creatine phosphokinase from rabbit muscle (Type I, salt-free, lyophilized powder), cytochrome c from equine heart, myoglobin from equine heart (lyophilized powder), were purchased from Sigma Aldrich. All reactions were performed under air in round bottom flask. Yields refer to chromatographically pure compounds; % yield were obtained by comparison of HPLC peak areas of products and starting material. HPLC and MS were used to monitor the reaction progress. Analytical TLC was performed on EM Reagent 0.25-mm silica gel plates with visualization by UV irradiation at 254 nm. Purifications by column chromatography were performed using EM silica gel 60 (230–400 mesh). The eluting system for each purification was determined by TLC analysis. Chromatography solvents were used without distillation. All of the organic solvents were removed under reduced pressure using a rotary evaporator. Water (double distilled H_2O), was purified using a Millipore MilliQ water purification system. Centrifugations were performed with an Eppendorf Mini Spin Plus (Eppendorf, Hauppauge, NY).

Instrumentation and sample analysis. *NMR*. ^{1}H and ^{13}C spectra were acquired at 25 °C in DMSO-d_6 using an Agilent DD2 (600 MHz) spectrometer with a 3-mm He triple resonance (HCN) cryoprobe. All ^{1}H NMR chemical shifts (δ) were referenced relative to the residual DMSO-d_5 peak at 2.50 ppm or internal tetramethylsilane (TMS) at 0.00 ppm. ^{13}C NMR chemical shifts were referenced to DMSO-d_6 at 39.52 ppm. ^{13}C NMR spectra were proton decoupled. NMR spectral data are reported as chemical shift (multiplicity, coupling constants (J), integration). Multiplicity is reported as follows: singlet (s), broad singlet (br s), doublet (d), doublet of doublets (dd), doublet of triplets (td), triplet (t) and multiplet (m). Coupling constant (J) in hertz (Hz).

LC/MS. Mass spectrometry was performed using an Agilent 1100 high performance liquid chromatograph coupled to an Agilent MSD VL mass spectrometer.

HRMS and *MS/MS*. High resolution MS data were acquired on a Q-ToF mass spectrometer using positive polarity electrospray ionization (+ESI). Tandem MS experiments were performed using collision induced dissociation (CID) with N_2 as the collision gas.

HPLC. Semi-preparative chromatography was performed on Beckman Coulter equipped with System Gold 168 detector and 125P solvent module HPLC with a 10 mm C-18 reversed-phase column. All separations involved a mobile phase of 0.1% FA (v/v) in water (solvent A) and 0.1% FA (v/v) in acetonitrile (solvent B). The semi-preparative HPLC method use a linear gradient of 0–80% acetonitrile in 0.1% aqueous FA over 30 min at room temperature with a flow rate of 3.0 mL min^{-1}. The eluent was monitored by absorbance at 220 nm and 254 nm unless otherwise noted.

Analytical HPLC. Analytical HPLC chromatography (HPLC) was performed on an Agilent 1100 series HPLC equipped with a 4.6 mm C-18 reversed-phase column. All separations involved mobile phase of 0.1% FA (v/v) in water (solvent A) and 0.1% FA (v/v) in acetonitrile (solvent B). Peptide compositions were evaluated by analytical reverse phase HPLC using a gradient of 0.1% FA in acetonitrile versus 0.1% FA in water. Analytical HPLC method use a linear gradient of 0-60% or 0-80% 0.1% FA (v/v) acetonitrile in 0.1% aqueous FA over 30 min at room temperature with a flow rate of 1.0 mL min^{-1}. The eluent was monitored by absorbance at 220 nm unless otherwise noted.

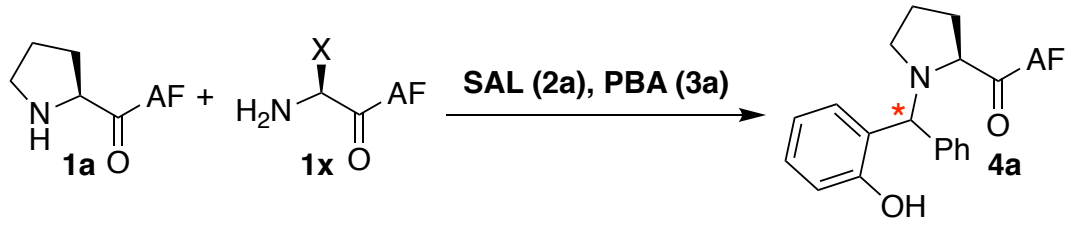
Gel analysis. For protein analysis, sodium dodecyl sulfate-PAGE (SDS-PAGE) was carried out on a Mini-Protean apparatus (Bio-Rad, Hercules, CA), using a 10-20% precast linear gradient polyacrylamide gel (Bio-Rad). Gels were run for 80 min at 120 V to separate the bands. Commercially available markers (Bio-Rad) were applied to at least one lane of each gel for assignment of apparent molecular masses. Visualization of protein bands was accomplished by staining with Coomassie Brilliant Blue R-250 (Bio-Rad).

Peptide library synthesis. **General procedure for solid-phase peptide synthesis**.[1] Peptides were

synthesized manually on a 0.25 mm scale using Rink amide resins. Fmoc–group was deprotected using 20% piperidine–DMF for 20 min to obtain a deprotected peptide-resin. The side chain protecting groups used were: Asn(Trt), Cys(Trt), Asp(tBu), Glu(tBu), His(Trt), Lys(Boc), Gln(Trt), Arg(Pbf), Ser(tBu), Thr(tBu), Trp(Boc), Tyr(tBu). Fmoc-protected amino acids (1.25 mm) were sequentially coupled on the resin using HBTU (1.25 mm) and DIEA (1.25 mm) for 2 h at room temperature. Peptides were synthesized using standard protocols. Side chain deprotection and the peptide was cleaved from the resin using a cocktail of 95:2.5:2.5, trifluoroacetic acid:triisopropylsilane:water for 2 h. The resin was removed by filtration and the resulting solution was concentrated. The oily residue was triturated with diethyl ether to obtain a white suspension. The resulting solid was purified by reverse-phase HPLC with a gradient of H_2O/CH_3CN with 0.1% FA. The organic solvent was removed on a vacuum centrifuge, and the remaining water was removed by lyophilization.

Peptide modification. **General method for the modification of peptides with SASP reaction using aldehydes and boronic acid in one pot.** All aldehydes and boronic acids were purchased from Sigma Aldrich. To a 2 mg peptide XAF **1** (12-17 mM) in 0.4 mL of 25 mM phosphate buffer (pH 7.3): DMF (4:1) was added SAL **2a** (3 equiv., 36 mM-51 mM) and PBA **3a**(4 equiv., 48 mM-68 mM). The reaction was stirred at room temperature for 4-24 h. The reaction was analyzed by MS and purified by HPLC to obtain white solid. HPLC: 0.1% FA (v/v) in water (solvent A): 0.1% FA (v/v) acetonitrile (solvent B); gradient 0-60 % or 0-80 %, depending on nature of peptides, 0.1% FA (v/v) acetonitrile in 25 min, flow rate = 1.0 mL/min, detection wavelength 220 nm.

General method for the verification of the chemoselective nature of SASP reaction. To mixture of peptides PAF **1a** (2 mg, 15 mM) and XAF **1** (2 mg, 12-17 mM) in 0.4 mL of 25 mM phosphate buffer (pH 7.3): DMF (4:1) was added SAL **2a** (3 equiv., 36 mM-51 mM) and PBA **3a** (4 equiv., 48 mM-68 mM). The solution was stirred at room temperature for 24 h. The reaction was analyzed by LC/MS. LC: 0.1% FA (v/v) in water (solvent A): 0.1% FA (v/v) acetonitrile (solvent B); gradient 0-60 %, 0.1% FA (v/v) acetonitrile in 25 min, flow rate = 1.0 mL/min, detection wavelength 220 nm.



Reaction #	Peptide sequence	XAF conversion (%)	PAF conversion (%)
1	PAF	-	70
2	AAF+PAF	0	72
3	EAF + PAF	0	61
4	RAF +PAF	0	68
5	VFAF +PAF	0	68
6	NAF + PAF	0	89
7	DAF + PAF	0	68
8	SAF + PAF	0	83
9	WAF + PAF	0	88
10	YAF + PAF	0	69
11	LAF + PAF	0	79
12	KAF + PAF	0	64
13	MAF + PAF	0	40

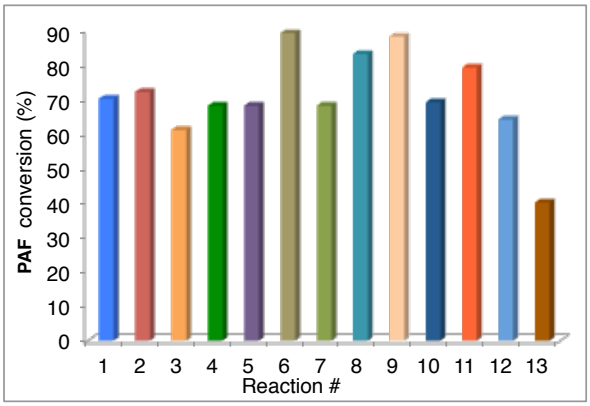
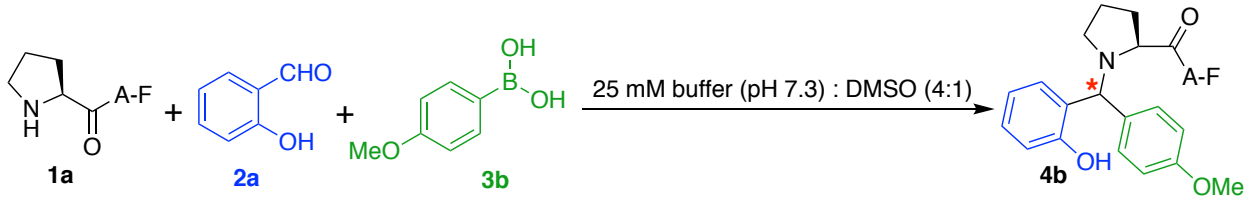
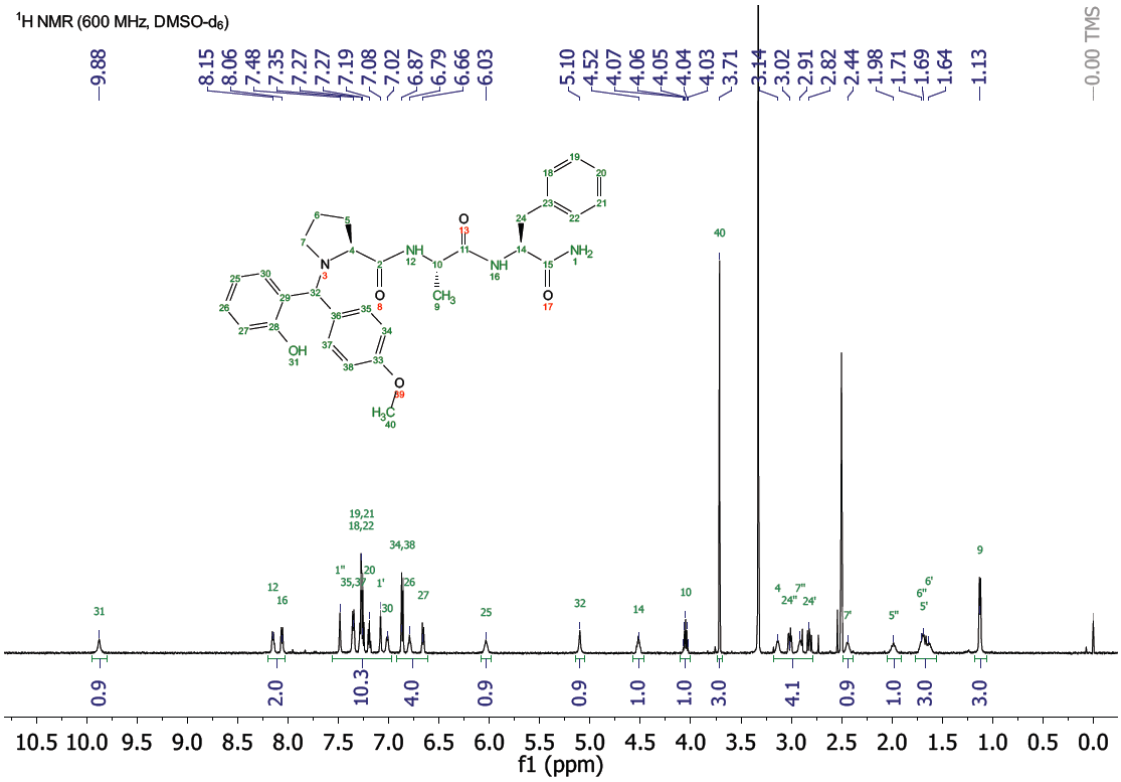


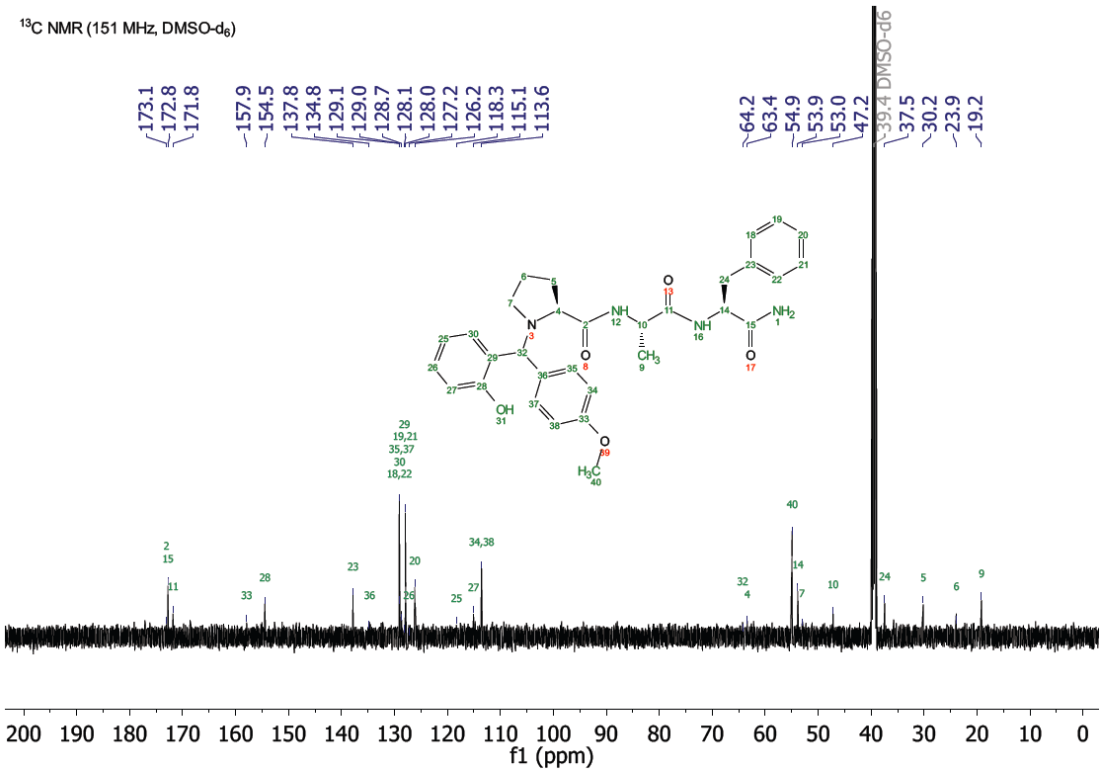
Fig. S1 Verification of chemoselectivity of the SASP reaction for secondary amines. Reactions with a mixture of two peptides PAF **1a** and XAF **1** (X = Ala, Glu, Arg, Val, Asn, Asp, Ser, Trp, Tyr, Leu, Lys, and Met) in one pot. The reaction showed Petasis product **4a** corresponding to peptide PAF only. Reaction conditions: PAF **1a** (2 mg, 15 mM), XAF **1** (2 mg, 12-17 mM), SAL **2a** (3 equiv., 36 mM-51 mM) and PBA **3a** (4 equiv., 48 mM-68 mM) in 0.4 mL of 25 mM phosphate buffer (pH 7.3): DMF (4:1) at RT.

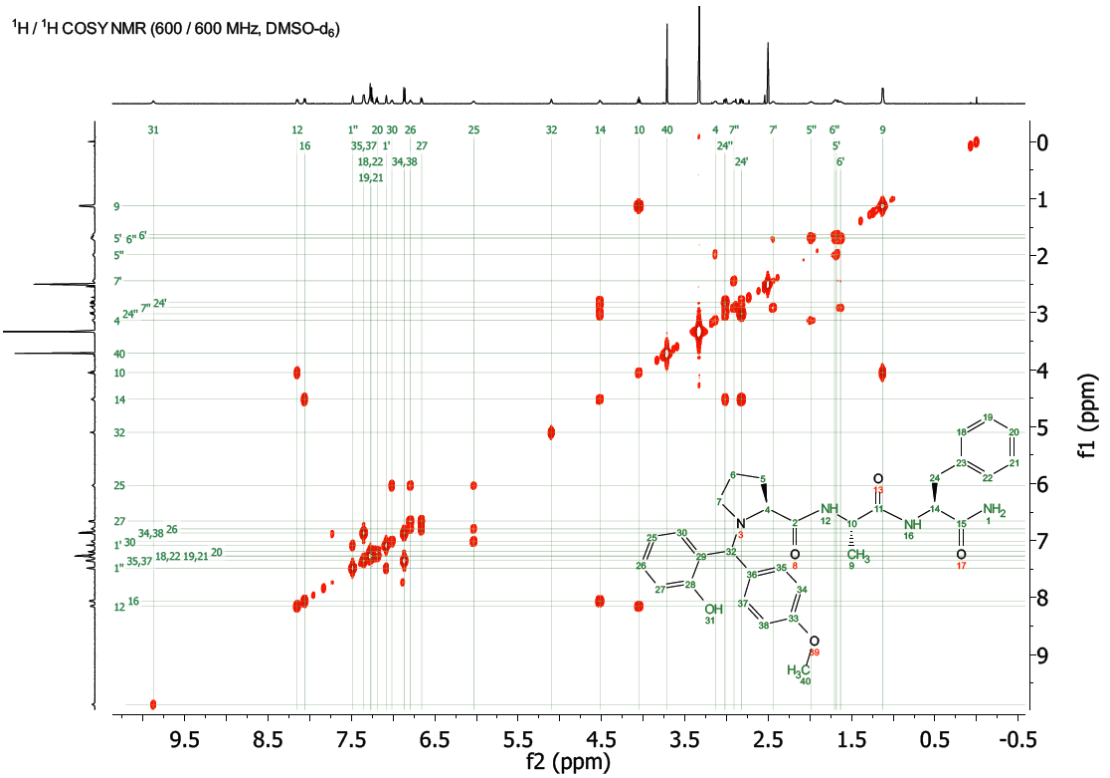


Synthesis of small molecules. L-Pro-L-Ala-L-Phe SASP product 4b with SAL and PMB. To a solution of L-Pro-L-Ala-L-Phe **PAF 1a** (8 mg, 0.024 mmol) in 25 mM phosphate buffer (pH 7.3): DMSO (4:1) (1 mL) was added SAL **2a** (8.72 mg, 0.072 mmol) and PMB **3b** (14.5 mg, 0.096 mmol). The resulting solution was stirred at room temperature. After 16 h, the reaction was concentrated by lyophilization. The resulting material was purified by HPLC to afford the product **4b** as a single diastereomer. HPLC: 0.1% FA (v/v) in water (solvent A): 0.1% FA (v/v) acetonitrile (solvent B); gradient 0-60 %, 0.1% FA (v/v) acetonitrile in 25 min, flow rate = 1.0 mL/min, detection wavelength 220 nm.

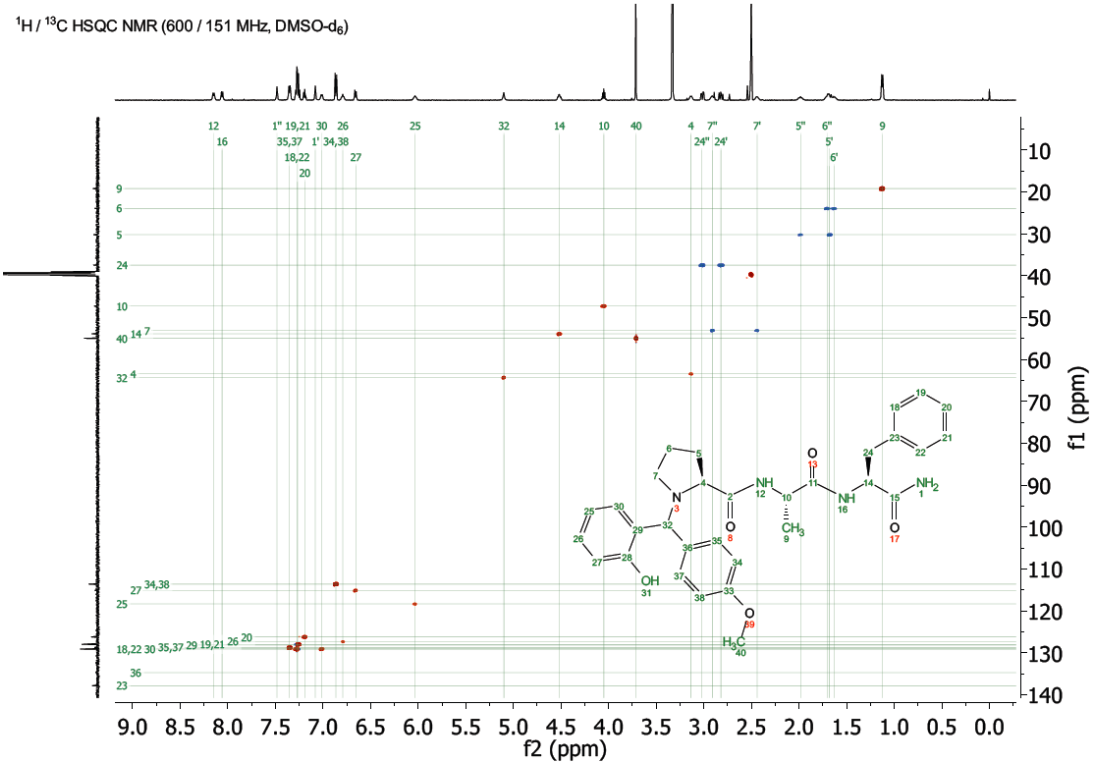
Single diastereomer: ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.98 (s,1H), 8.15 (d, *J* = 7.7 Hz, 1H), 8.06 (d, *J* = 8.2 Hz, 1H), 7.48 (s, 1H), 7.35 (d, *J* = 8.2 Hz, 2H), 7.28 (m, 2H), 7.26 (m, 2H), 7.19 (t, *J* = 6.9 Hz, 1H), 7.08 (s, 1H), 7.02 (d, *J* = 7.4 Hz,1H), 6.87 (d, *J* = 8.2 Hz, 2H), 6.79 (d, *J* = 7.7 Hz,1H), 6.66 (t, *J* = 7.7 Hz,1H), 6.03 (brs,1H), 5.10 (s,1H), 4.52 (m,1H), 4.05 (p, *J* = 7.0 Hz,1H), 3.71 (s, 3H), 3.14 (m,1H), 3.02 (dd, *J* = 13.8, 4.8 Hz,1H), 2.91 (m,1H), 2.82 (dd, *J* = 13.8, 9.6 Hz,1H), 2.44 (m,1H), 1.98 (m,1H),1.69 (m,1H), 1.67 (m, 2H), 1.13 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (151 MHz, DMSO-d_6): 173.1, 172.8, 171.8, 157.9, 154.5, 137.8, 134.8, 129.1,129.0, 128.7, 128.1, 128.0, 127.2, 126.2, 118.3, 115.1,113.6, 64.2, 63.4, 54.9, 53.9, 53.0, 47.2, 37.5, 30.2, 23.9, 19.2. See Supplementary Figure 2 for ^{1}H, ^{13}C, COSY, HSQC, HMBC, TOCSY and ROESY NMR spectra.



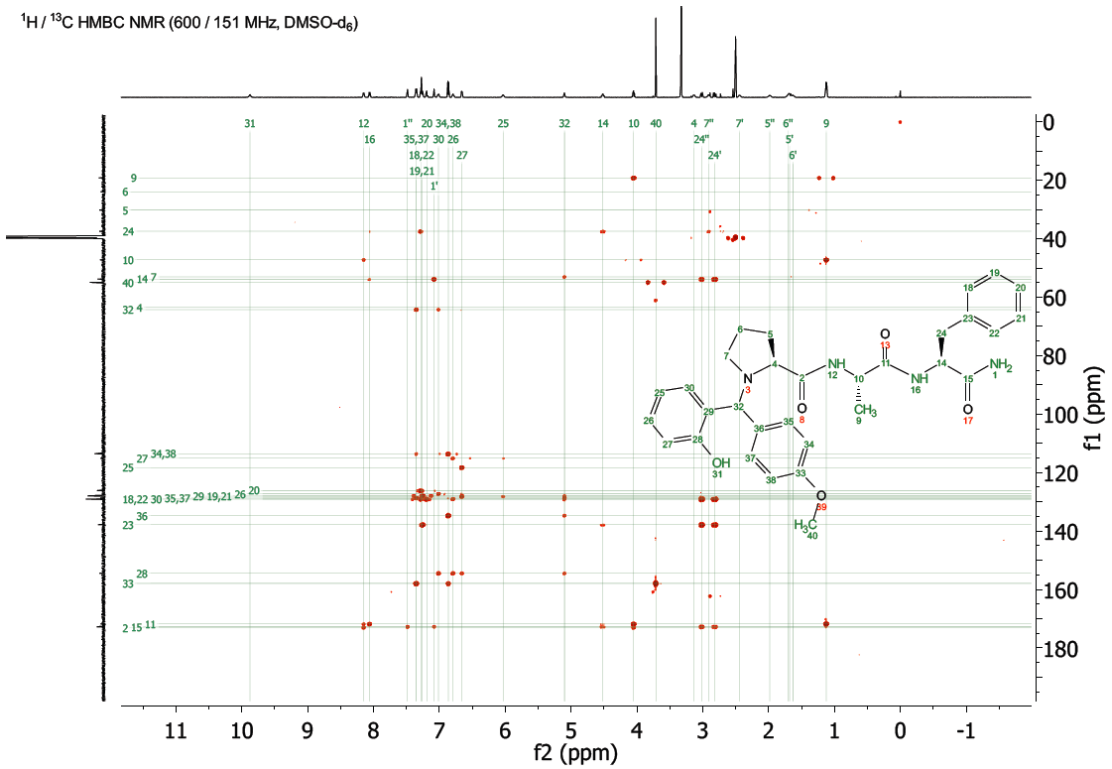

13C NMR (151 MHz, DMSO-d6)
173.1
172.8
171.8
157.9
154.5
137.8
134.8
129.1
129.0
128.7
128.1
128.0
127.2
126.2
118.3
115.1
113.6
64.2
63.4
54.9
53.9
53.0
47.2
39.4 DMSO-d6
37.5
30.2
23.9
19.2
f1 (ppm)


1H / 1H COSY NMR (600 / 600 MHz, DMSO-d6)
f2 (ppm)
f1 (ppm)

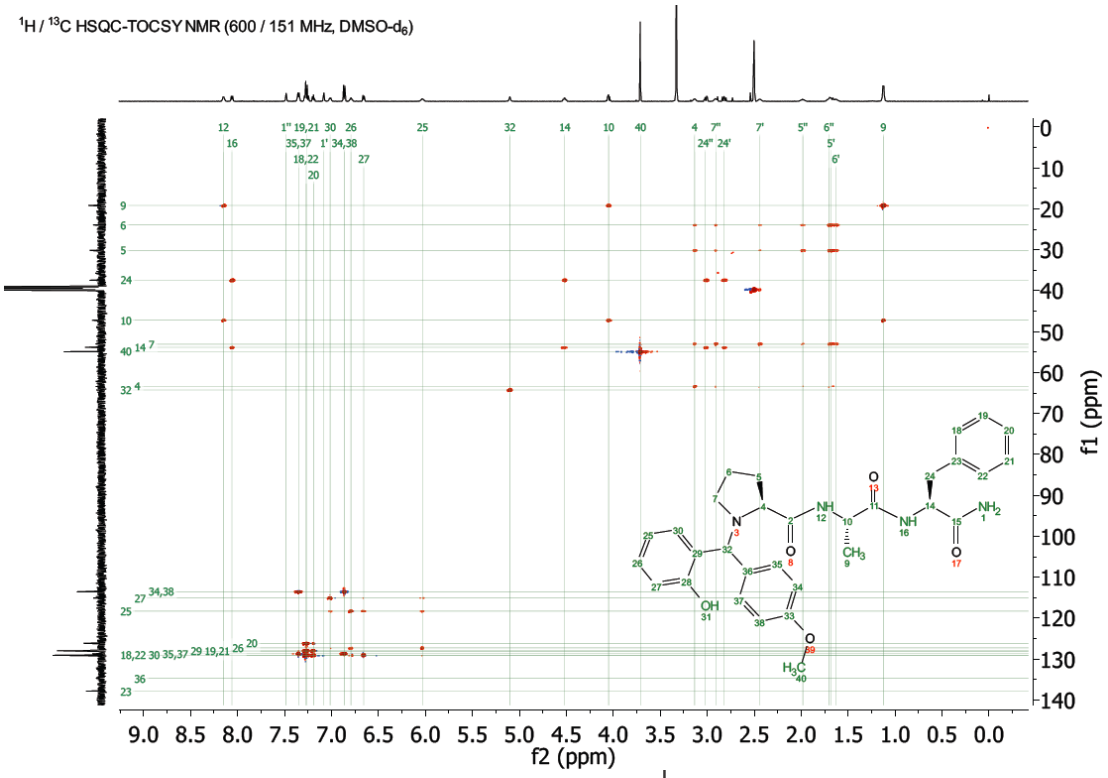
^{1}H / ^{13}C HSQC NMR (600 / 151 MHz, DMSO-d_6)



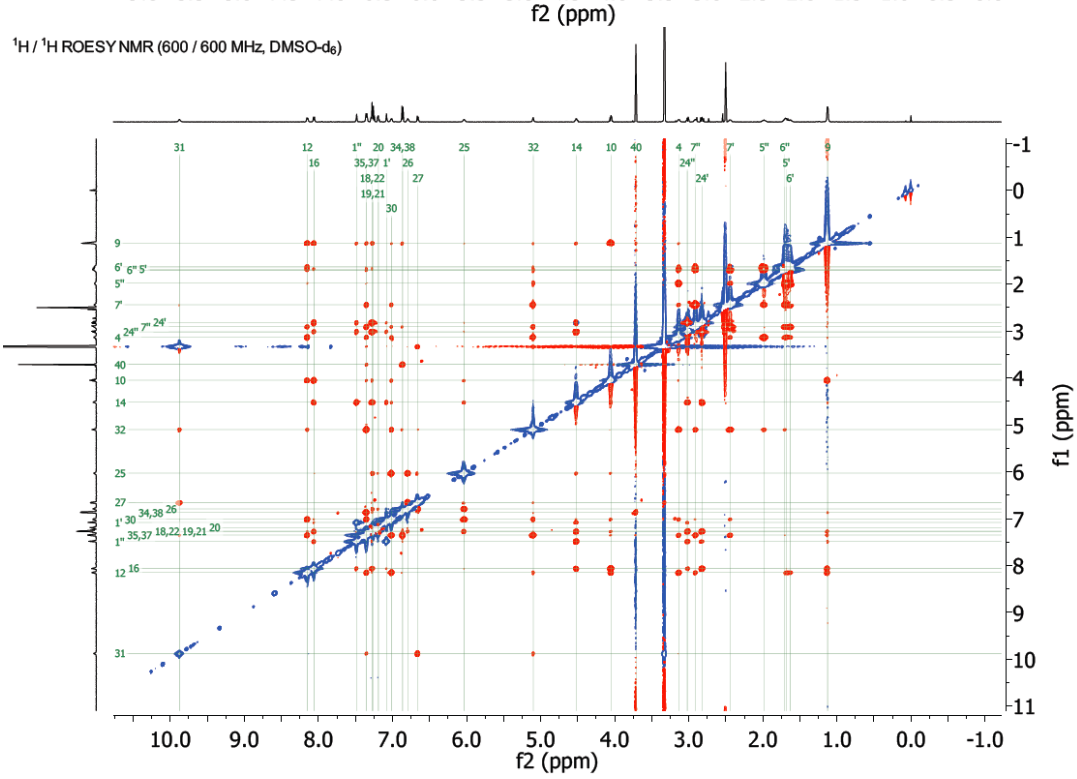
^{1}H / ^{13}C HMBC NMR (600 / 151 MHz, DMSO-d_6)

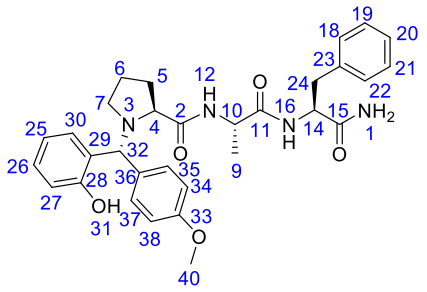


^{1}H / ^{13}C HSQC-TOCSY NMR (600 / 151 MHz, DMSO-d_6)



^{1}H / ^{1}H ROESY NMR (600 / 600 MHz, DMSO-d_6)





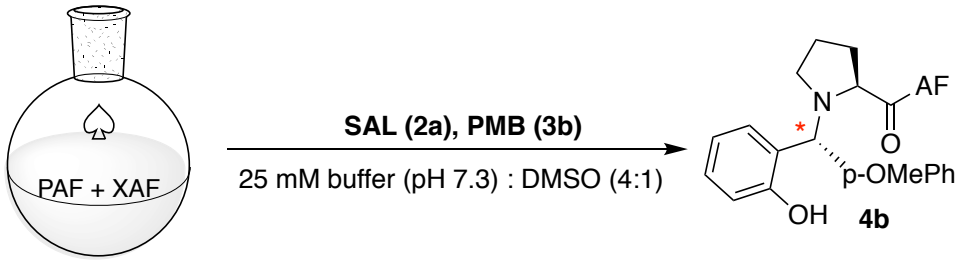
PAF-NH_2 + 4-methoxyphenylboronic acid + salicylaldehyde

^{1}H NMR data[a] for 4b				
Atom No.	Chemical Shift[b] (ppm)	Multiplicity[c]	Coupling Constant (Hz)	Integration
1’	7.08	s	N/A	1
1’’	7.48	s	N/A	1
4	3.14	m	N/A	1
5’	1.65-1.72	m	N/A	1
5’’	1.98	m	N/A	1
6	1.60-1.74	m	N/A	2
7’	2.44	m	N/A	1
7’’	2.91	m	N/A	1
9	1.13	d	6.8	3
10	4.05	p	7.0	1
12	8.15	d	7.7	1
14	4.49-4.55	m	N/A	1
16	8.06	d	8.2	1
18, 22	7.27-7.29	m	N/A	2
19, 21	7.24-7.27	m	N/A	2
20	7.19	t	6.9	1
24’	2.82	dd	13.8, 9.6	1
24’’	3.02	dd	13.8, 4.8	1
25	6.03	br s	N/A	1
26	6.66	t	7.7	1
27	6.79	d	7.7	1
30	7.02	d	7.4	1
31	9.98	s	N/A	1
32	5.10	s	N/A	1
34, 38	6.87	d	8.2	2
35, 37	7.35	d	8.2	2
40	3.71	s	N/A	3

a: Recorded at 600 MHz in DMSO-d_6 on a Varian spectrometer
b: Chemical shifts referenced to TMS at 0.00 ppm
c: s=singlet, d=doublet, t=triplet, p=pentet, dd=doublet of doublets, br=broad, m=multiplet

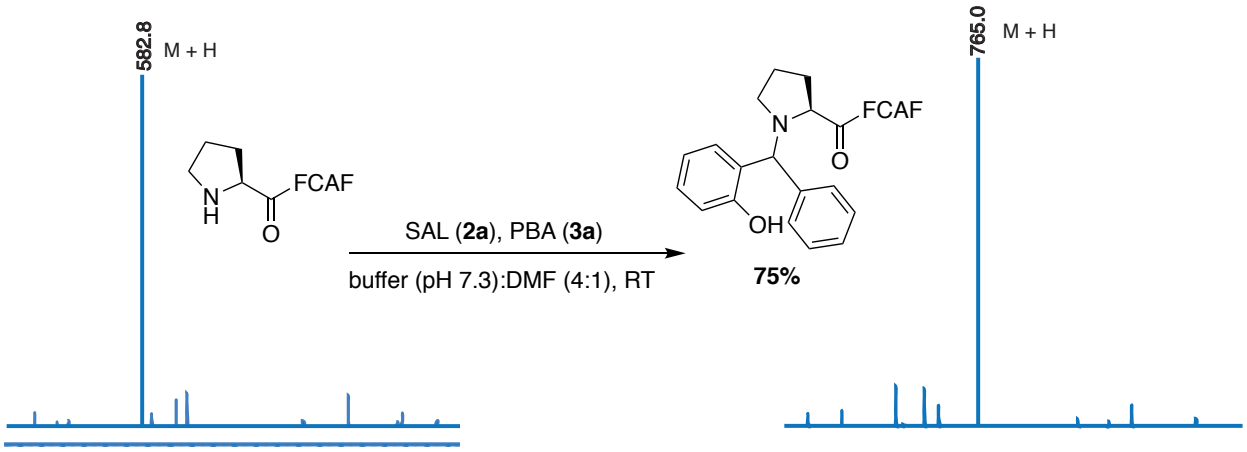
^{13}C NMR data[a] for 4b	
Atom No.	Chemical Shift[b] (ppm)
2	173.1
4	63.4
5	30.2
6	23.9
7	53.0
9	19.2
10	47.2
11	171.8
14	53.9
15	172.8
18, 22	129.1
19, 21	128.0
20	126.2
23	137.8
24	37.5
25	118.3
26	127.2
27	115.1
28	154.5
29	128.1
30	129.0
32	64.2
33	157.9
34, 38	113.6
35, 37	128.7
36	134.8
40	54.9
[a:] Recorded at 151 MHz in DMSO-d_6 on a Varian spectrometer [b:] Chemical shifts referenced to DMSO-d_6 at 39.45 ppm	

Fig. S2. SASP product 4b characterization. ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, HSQC-TOCSY and ROESY NMR of the product **4b**.



Reaction #	Peptide	XAF Conv. (%)	PAF Conv. (%)
1	PAF	-	90
2	EAF+PAF	0	81
3	KAF+PAF	0	88
4	TAF + PAF	0	91
5	WAF +PAF	0	93
6	YAF +PAF	0	84
7	LAF + PAF	0	93
8	HAF + PAF	0	60

Fig. S3 Chemoselectivity of the SASP reaction for secondary amines with reactive PMB 2b under physiological conditions. Reactions with a mixture of two peptides PAF **1a** and XAF **1** (X = Ala, Glu, Arg, Val, Asn, Asp, Ser, Trp, Tyr, Leu, Lys, and Met), in one pot. The reaction showed Petasis product **4b** corresponding to peptide PAF only. Reaction conditions: PAF **1a** (2 mg, 15 mM), XAF **1** (2 mg, 12-17 mM), SAL **2a** (3 equiv., 36 mM-51 mM) and PMB **3b** (4 equiv., 48 mM-68 mM) in 0.4 mL of 25 mM phosphate buffer (pH 7.3): DMSO (4:1) was stirred at RT for 4 h.



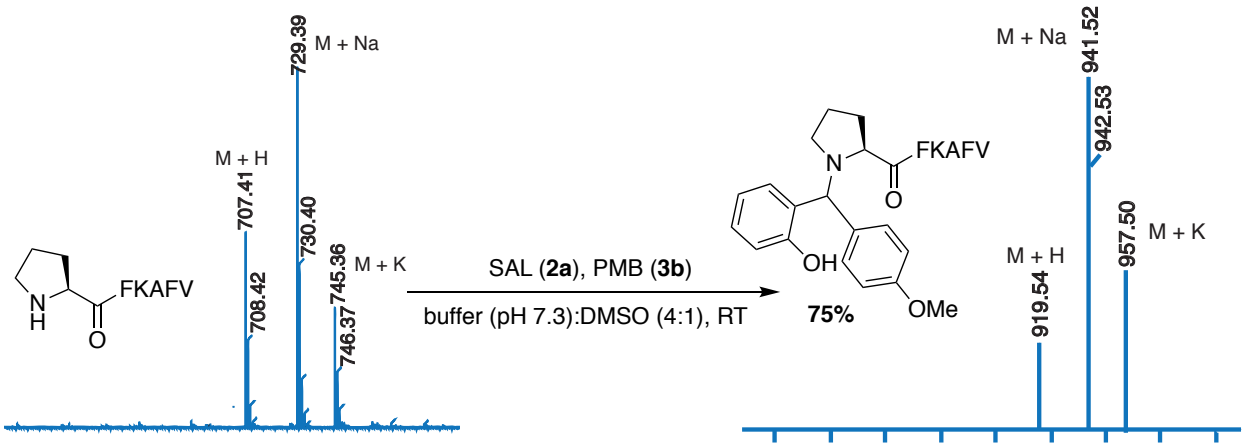


Fig. S4. Method for testing the compatibility of SASP with free cysteines and lysines. To a peptide **PACAF** (2 mg, 6.87 mM) in 0.5 mL solution of 25 mM phosphate buffer (pH 7.3): DMF (4:1) was added SAL **2a** (3 equiv., 20.4 mM), PBA **3a** (4 equiv., 27.5 mM). The solution was stirred at room temperature for 4 h. To a peptide **PFKAFV** (2 mg, 5.6 mM) in 0.5 mL solution of 25 mM phosphate buffer (pH 7.3): DMSO (4:1) was added SAL **2a** (3 equiv., 16.8 mM), PMB **3b** (4 equiv., 22.4 mM). The solution was stirred at room temperature for 4 h. The reactions were analyzed by LC/MS. LC: 0.1% FA (v/v) in water (solvent A): 0.1% FA (v/v) acetonitrile (solvent B); gradient 0-60 %, 0.1% FA (v/v) acetonitrile in 25 min, flow rate = 1.0 mL/min, detection wavelength 220 nm.

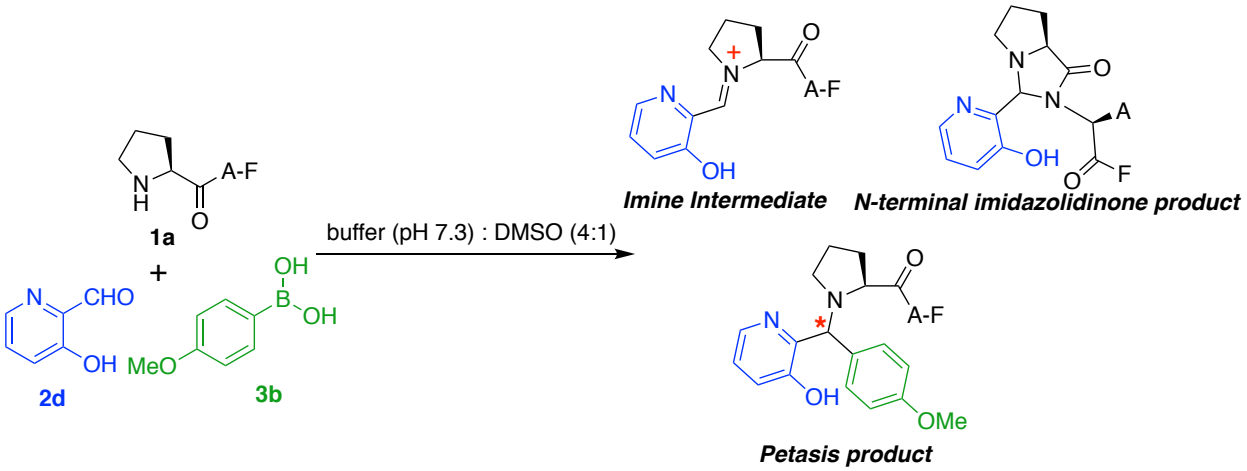


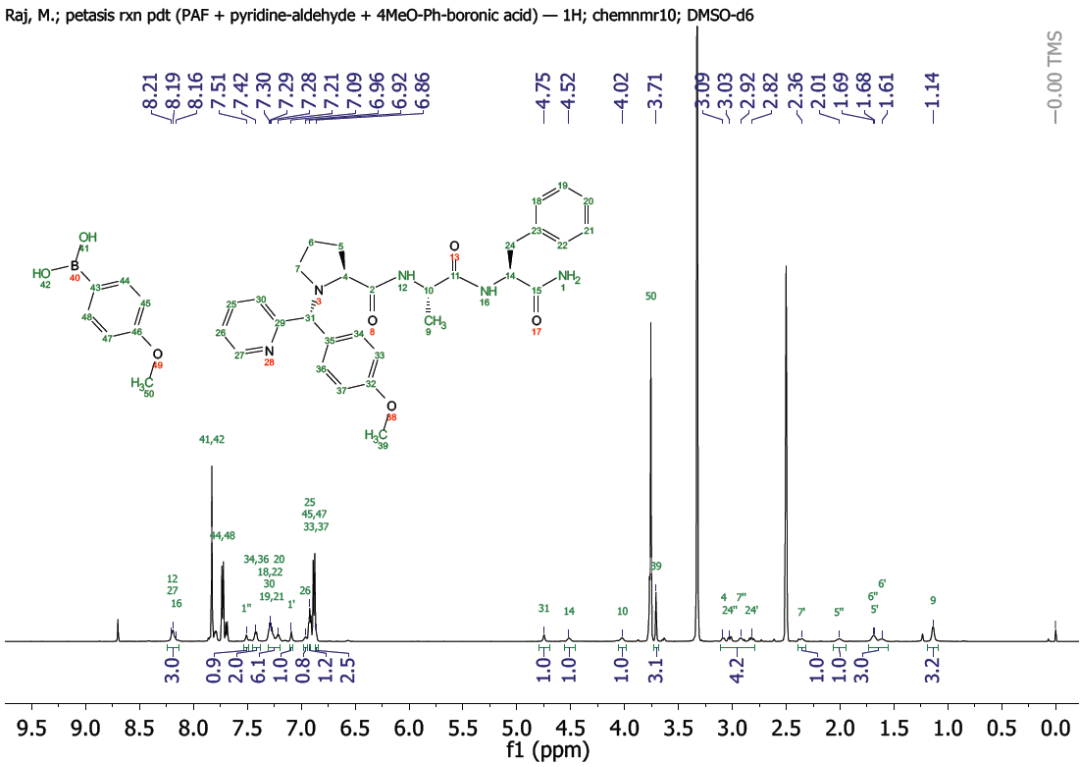
Table S1. Optimization of the reaction conditions for the Petasis product.

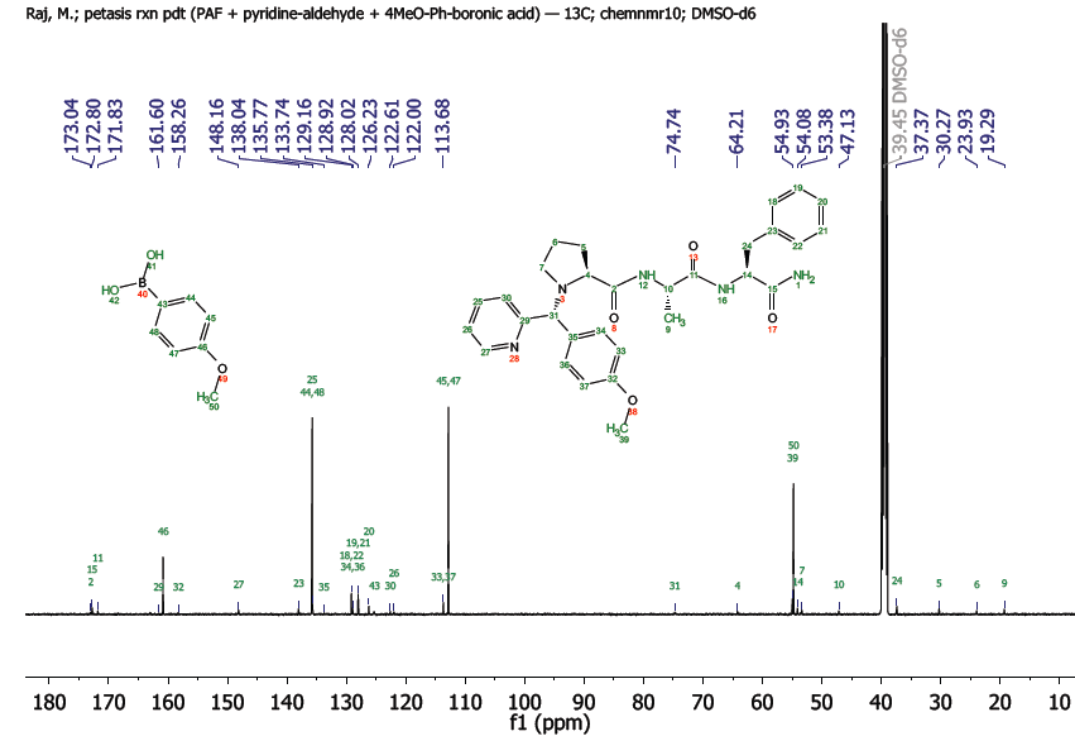
Aldehyde **2d**	Boronic acid **3b**	Imine intermediate	N-terminal imidazolidinone product	Petasis product
3 equiv.	4 equiv.	14 %	32 %	53 %
1.5 equiv	4 equiv.	16 %	6 %	77 %
1.5 equiv.	8 equiv.	13 %	5 %	81 %

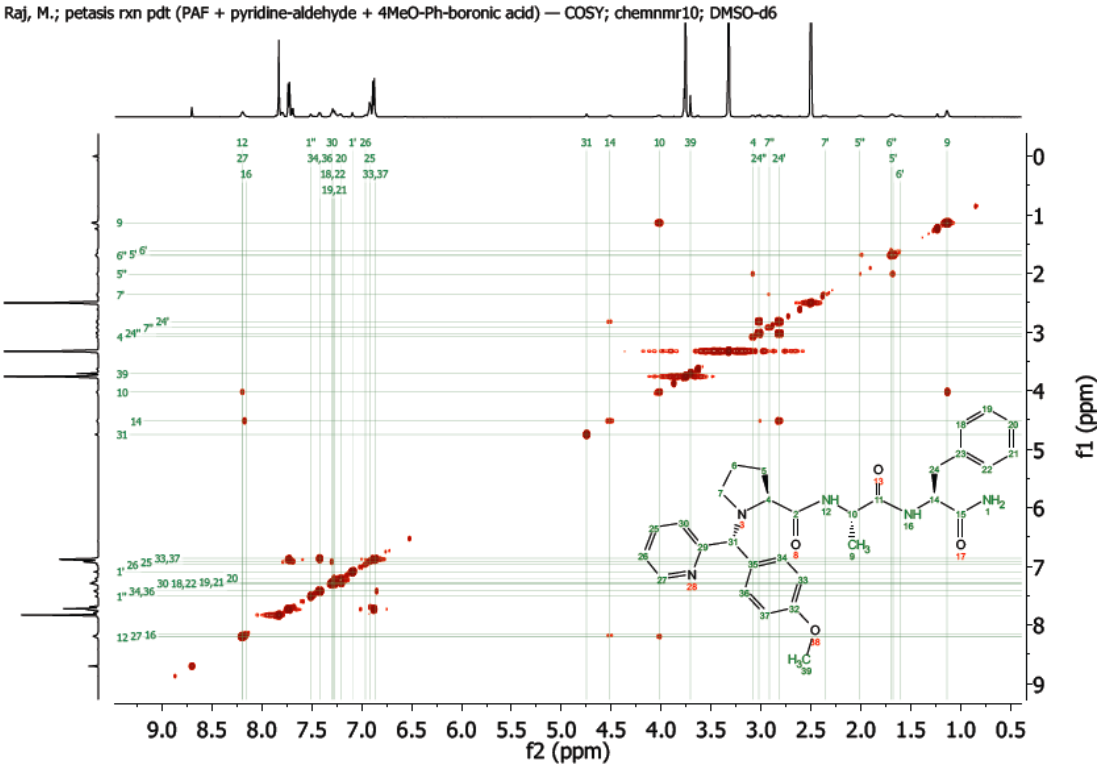
Reaction conditions: 2 mg of peptide PAF **1a**, aldehyde **2d** (1.5-3 equiv.), PMB **3b** (4-8 equiv.) in 0.4 mL of 25 mM phosphate buffer (pH 7.3): DMSO (4:1) was stirred for 4 h at 37 ^{o}C.

Synthesis of small molecules. PAF SASP product 4c with 2PCA and PMB. To a solution of PAF **1a** (8 mg, 0.024 mmol) in 25 mM phosphate buffer pH 7.3: DMSO (4:1) (1 mL) was added 2PCA **2b** (3.85 mg, 0.036 mmol) and PMB **3b** (29.0 mg, 0.192 mmol). The resulting solution was stirred at room temperature. After 16 h, the reaction was concentrated by lyophilization. The resulting material was purified by HPLC to afford the product **4c** as a single diastereomer. HPLC: 0.1% FA (v/v) in water (solvent A): 0.1% FA (v/v) acetonitrile (solvent B); gradient 0-60 %, 0.1% FA (v/v) acetonitrile in 25 min, flow rate = 1.0 mL/min, detection wavelength 220 nm.

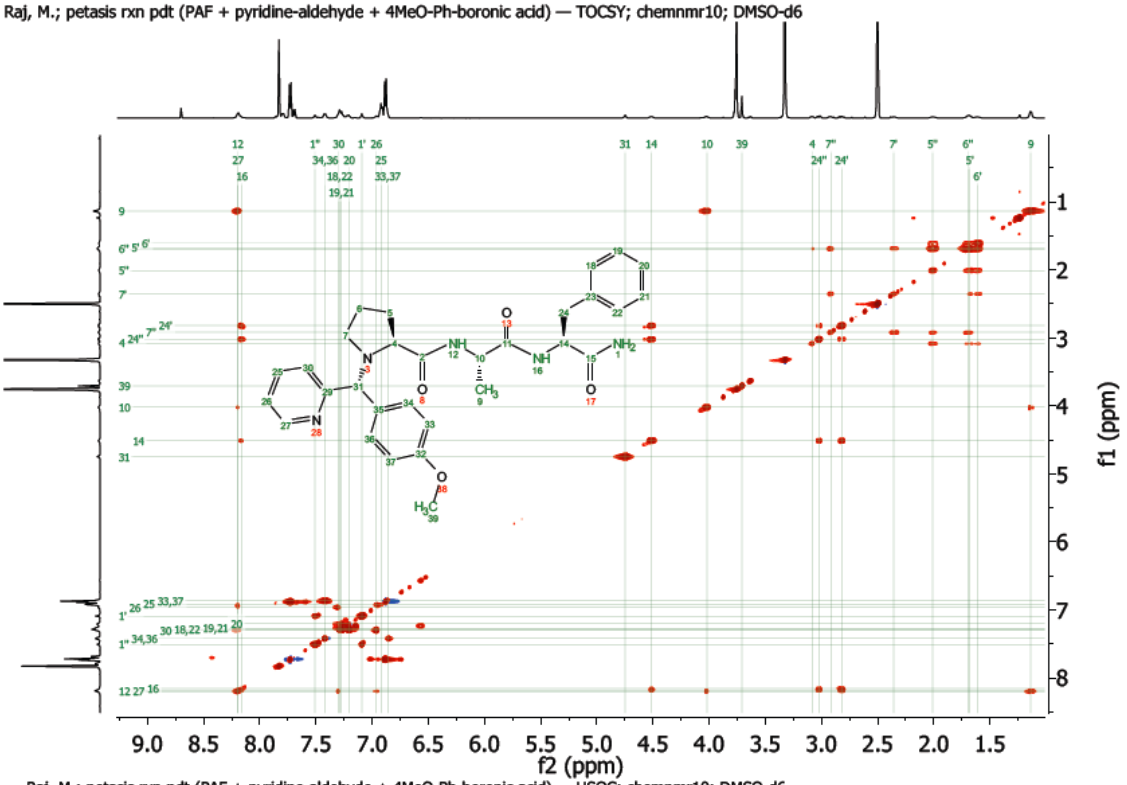
Single diastereomer ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.20 (m, 1H), 8.18 (m, 1H), 8.16 (m, 1H), 7.51 (s, 1H), 7.42 (d, *J* = 8.1 Hz, 2H), 7.30 (m, 1H), 7.09 (s, 1H), 7.29 (m, 2H), 7.27 (m, 2H), 7.21 (m, 1H), 6.96 (m, 1H), 6.92 (m, 1H), 6.86 (m, 2H), 4.75 (s, 1H), 4.52 (br s, 1H), 4.02 (m, 1H), 3.71 (s, 3H), 3.09 (d, *J* = 8.8 Hz, 1H), 3.03 (dd, *J* = 13.6, 4.2 Hz, 1H), 2.92 (m, 1H), 2.82 (dd, *J* = 13.6, 9.8 Hz, 1H), 2.36 (m, 1H), 2.01 (m, 1H), 1.70 (m, 1H), 1.68 (m, 1H), 1.61 (m, 1H), 1.14 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (151 MHz, DMSO-d_6): 173.0, 172.8, 171.8, 161.6, 158.3, 148.2, 138.0, 135.8, 133.7, 129.2, 128.9, 128.0, 126.2, 122.6, 122.0, 113.7, 74.7, 64.2, 54.9, 54.1, 53.4, 47.1, 37.4, 30.3, 23.9, 19.3. See below for ^{1}H, ^{13}C, COSY, HSQC, HMBC, TOCSY and ROESY NMR spectra.




Raj, M.; petasis rxn pdt (PAF + pyridine-aldehyde + 4MeO-Ph-boronic acid) — 13C; chemnmr10; DMSO-d6
173.04
172.80
171.83
161.60
158.26
148.16
138.04
135.77
133.74
129.16
128.92
128.02
126.23
122.61
122.00
113.68
74.74
64.21
54.93
54.08
53.38
47.13
39.45 DMSO-d6
37.37
30.27
23.93
19.29
f1 (ppm)


Raj, M.; petasis rxn pdt (PAF + pyridine-aldehyde + 4MeO-Ph-boronic acid) — COSY; chemnmr10; DMSO-d6
f2 (ppm)
f1 (ppm)

Raj, M.; petasis rxn pdt (PAF + pyridine-aldehyde + 4MeO-Ph-boronic acid) — TOCSY; chemnmr10; DMSO-d6



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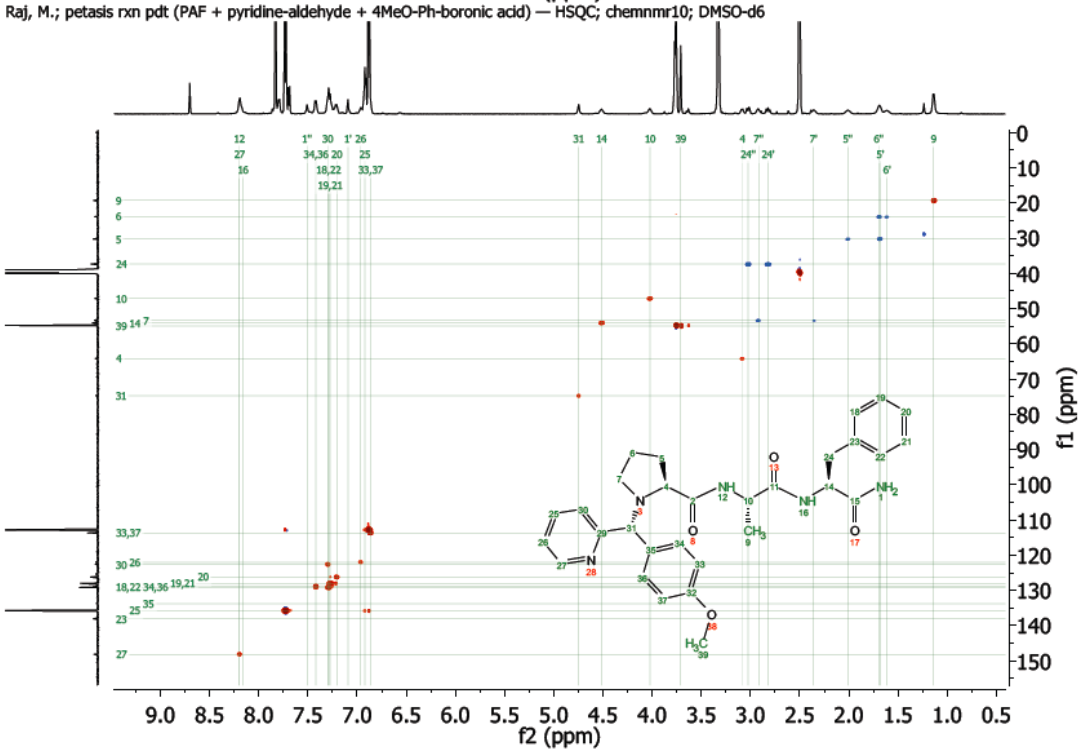

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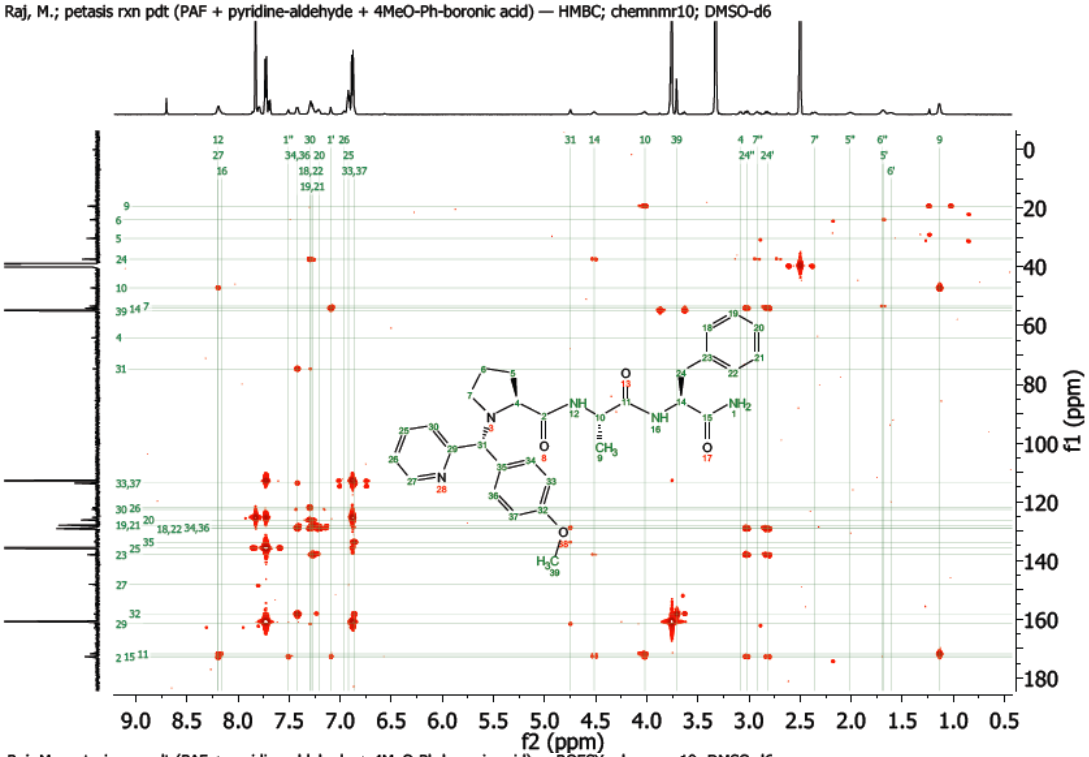
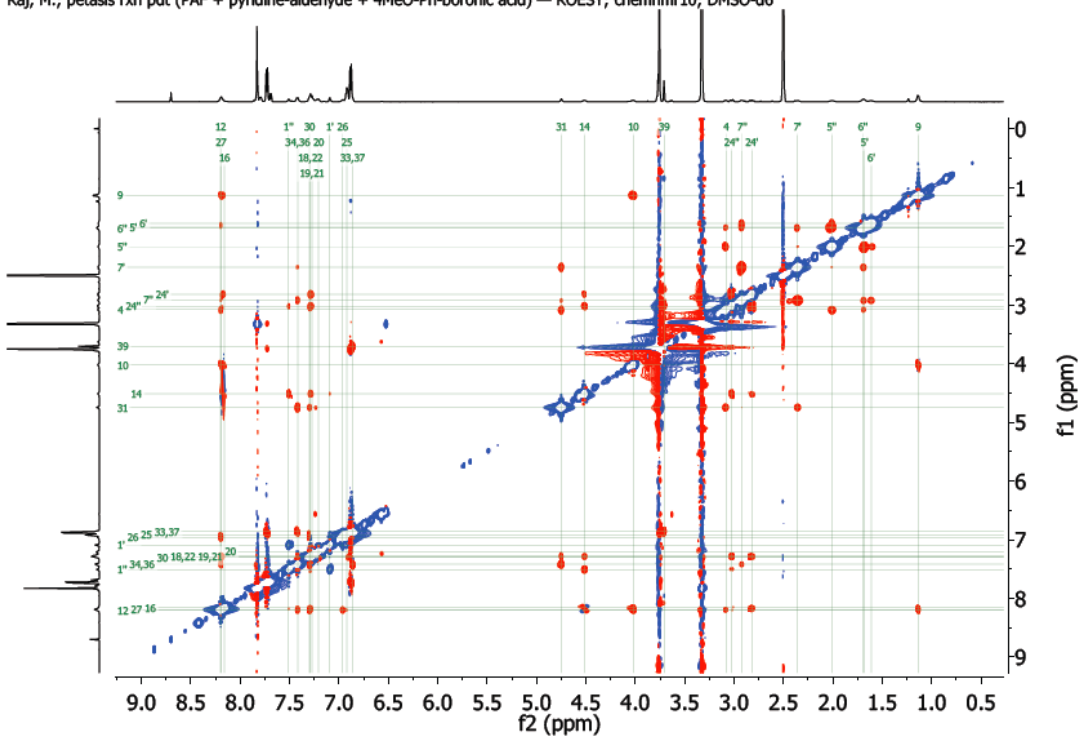
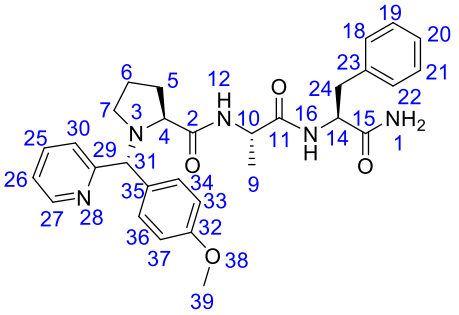


Raj, M.; petasis rxn pdt (PAF + pyridine-aldehyde + 4MeO-Ph-boronic acid) — ROESY; chemnmr10; DMSO-d6





PAF-NH_2 + 4-methoxyphenylboronic acid + picolinaldehyde (PCA)

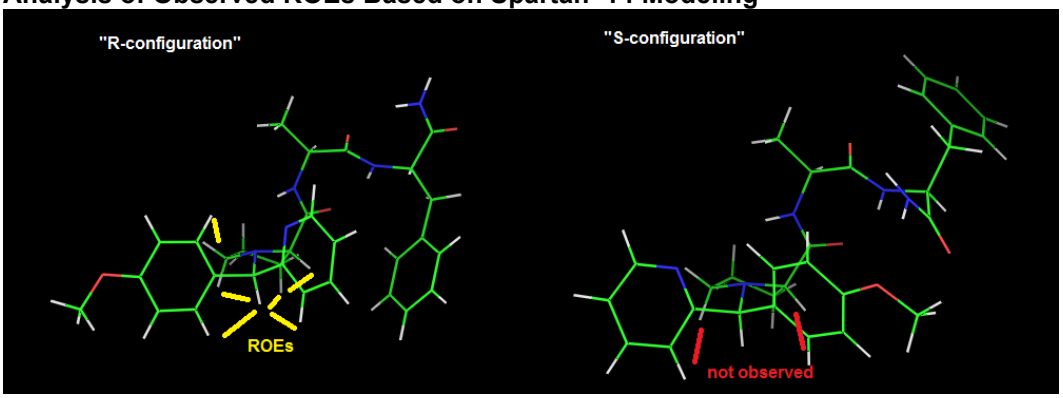
^{1}H NMR data[a] for 4c				
Atom No.	Chemical Shift[b] (ppm)	Multiplicity[c]	Coupling Constant (Hz)	Integration
1’	7.09	s	N/A	1
1’’	7.51	s	N/A	1
4	3.09	d	8.8	1
5’	1.66-1.70	m	N/A	1
5’’	2.01	m	N/A	1
6’	1.61	m	N/A	1
6’’	1.67-1.73	m	N/A	1
7’	2.36	m	N/A	1
7’’	2.92	m	N/A	1
9	1.14	d	6.8	3
10	4.02	m	N/A	1
12	8.19-8.21	m	N/A	1
14	4.52	br s	N/A	1
16	8.13-8.18	m	N/A	1
18, 22	7.28-7.30	m	N/A	2
19, 21	7.26-7.28	m	N/A	2
20	7.19-7.23	m	N/A	1
24’	2.82	dd	13.6, 9.8	1
24’’	3.03	dd	13.6, 4.2	1
25	6.91-6.93	m	N/A	1
26	6.96	m	N/A	1
27	8.17-8.20	m	N/A	1
30	7.29-7.31	m	N/A	1
31	4.75	s	N/A	1
33, 37	6.84-6.88	m	N/A	2
34, 36	7.42	d	8.1	2
39	3.71	s	N/A	3

a: Recorded at 600 MHz in DMSO-d_6 on a Bruker AVANCE III HD spectrometer
b: Chemical shifts referenced to TMS at 0.00 ppm
c: s=singlet, d=doublet, dd=doublet of doublets, br=broad, m=multiplet

^{13}C NMR data[a] for 4c	
Atom No.	Chemical Shift[b] (ppm)
2	173.0
4	64.2
5	30.3
6	23.9
7	53.4
9	19.3
10	47.1
11	171.8
14	54.1
15	172.8
18, 22	129.2
19, 21	128.0
20	126.2
23	138.0
24	37.4
25	135.8
26	122.0
27	148.2
29	161.6
30	122.6
31	74.7
32	158.3
33, 37	113.7
34, 36	128.9
35	133.7
39	54.9
[a:] Recorded at 151 MHz in DMSO-d_6 on a Bruker AVANCE III HD spectrometer [b:] Chemical shifts referenced to DMSO-d_6 at 39.45 ppm	

Method for analysis of diastereoselectivity of SASP reaction and determination of the absolute configuration of the SASP product. Product 4c obtained by the reaction of peptide PAF with 2PCA, and PMB. The relative stereochemistry of SASP product **4c** was determined by through-space correlations (i.e., ROEs) from the ROESY spectrum and then verified by computational chemistry predictions of the NMR chemical shifts as mentioned above. Using the DP4+ method, the *R*-configuration was predicted to be the correct stereoisomer with >99% probability.[2] See below for details.

Analysis of Observed ROEs Based on Spartan '14 Modeling



Comparison of ROEs from ROESY spectrum with conformers from a MMFF94s molecular mechanics Monte Carlo search in Spartan '14 are consistent with the *R* stereoconfiguration. Key ROEs are shown above.

Fig. S5. SASP product 4c characterization. ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, TOCSY and ROESY NMR of the product **4c**. The reaction afforded a single diastereoisomer with (de > 99%). Comparison of ROEs from ROESY spectrum with conformers from a MMFF94s molecular mechanics Monte Carlo search in Spartan '14 are consistent with the *R* stereo configuration. Key ROEs are shown above. DP4+ calculations based on experimental ^{13}C and ^{1}H NMR chemical shifts are consistent with the formation of only one diastereoisomer (de >99%).

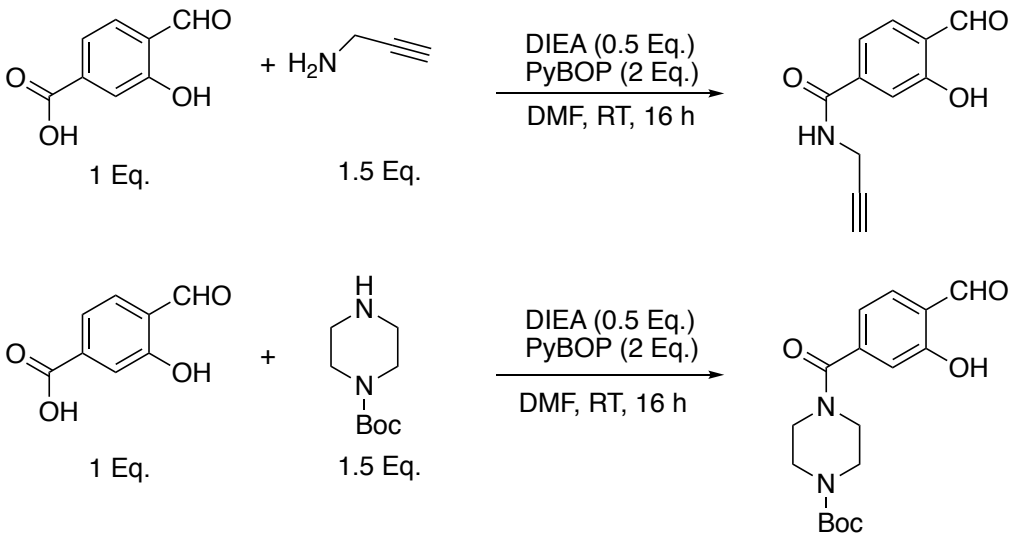


Fig. S6. General method for the synthesis of water-soluble analogs of SAL. To a mixture of 4-formyl-3-hydroxybenzoic acid, DIEA, PyBOP in DMF was added propargyl amine or 1-Boc-piperazine dropwise over 5 mins and reaction mixture was stirred at room temperature for 16 h. Reaction mixture was diluted with ethyl acetate and extracted with water followed by washing of organic layer with brine solution thrice. Combined ethyl acetate layers were dried with sodium sulfate and volatiles were removed under reduced pressure. Crude reaction mixture was purified over silica gel using 30 % ethyl acetate and hexane as an eluent to generate pure derivatives of 4-formyl-3-hydroxybenzoicacid.

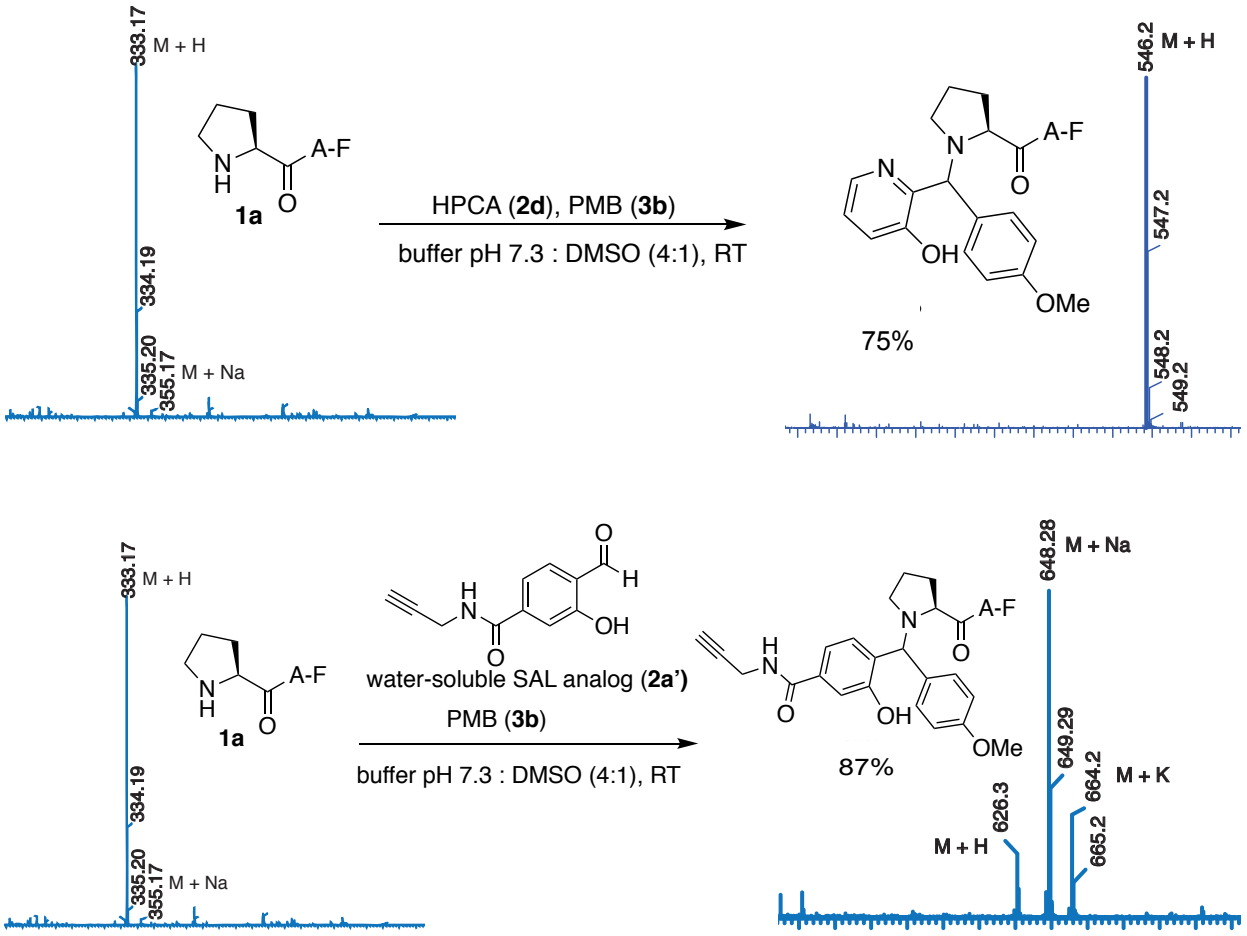


Fig. S7. (a) HRMS of reaction of peptide PAF **1a**, HPCA **2d** and PMB **3b**. (b) HRMS of the reaction of peptide PAF **1a**, water-soluble SAL analog **2a'** and PMB **3b.**

Stability of Petasis bioconjugate. Method for determining the hydrolytic stability of Petasis product 4b and 4c under different pH conditions. To a Petasis product **4b or 4c** (1 μM), 0.5 mL of 25 mM phosphate buffer (pH ranging from 3.5 to 9.5): ACN (9:1) was added and the resulting solution was stirred at room temperature. The stability of the products **4b and 4c** were monitored by injecting samples in the HPLC after regular intervals 1 h, 12 h and 24 h. % Conversion to the degraded product was determined by calculating areas under the peak in HPLC. HPLC: 0.1% FA (v/v) in water (solvent A): 0.1% FA (v/v) acetonitrile (solvent B); gradient 0-60 %, 0.1% FA (v/v) acetonitrile in 25 min, flow rate = 1.0 mL/min, detection wavelength 220 nm. The characterization of the degraded product of **4b** was analyzed by MS; see below for details.

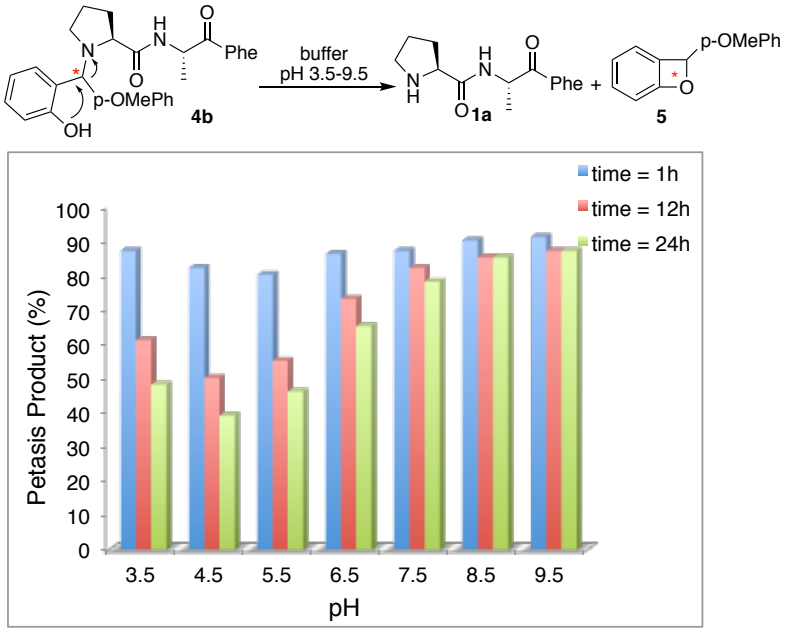


Fig. S8. Stability studies of Petasis product 4b under different pH conditions. Reaction conditions: Petasis product **4b** (1 µM) in 25 mM phosphate buffer at different pH ranging from 3.5 to 10.5 at room temperature. The reactions were monitored by injecting the sample in HPLC/MS after regular intervals of time 1h, 12h and 24h. 50% hydrolysis was observed at very low pH (3.5 to 5.5) probably due to the protonation of the proline at low pH. We are currently trying to figure out the nature of the hydrolysis product **5** by carrying out reaction at high scale and by isolating it.

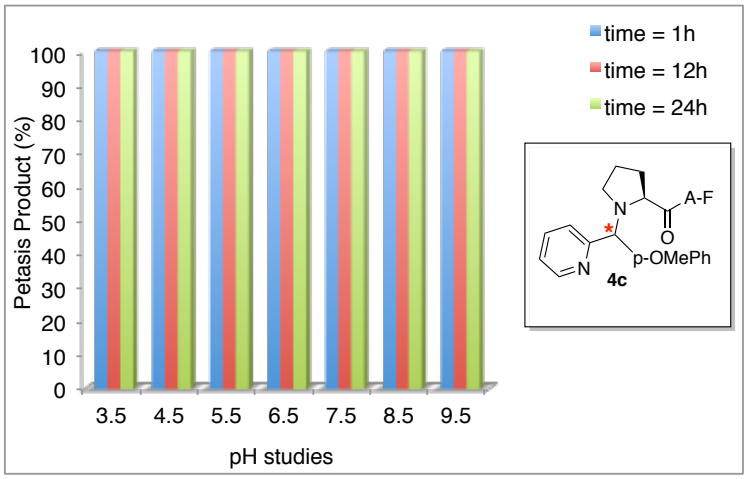


Fig. S9. Stability studies of Petasis product 4c under different pH conditions. Reaction conditions: Petasis product **4c** (1 uM) in 25 mM phosphate buffer at different pH ranging from 3.5 to 10.5 at room temperature. The reaction was monitored by injecting the sample in HPLC/MS after regular intervals of time 1 h, 12 h and 24 h. No degradation of Petasis product **4c** was observed even upto 24 h in different pH conditions

Rate of SASP reaction. **Method for testing the effect of pH on the rate of the SASP reaction.** To a solution of **PAF 1a** (2 mg, 12 mM) in 0.5 mL of 25 mM phosphate buffer (pH ranging from 5.5 to 9.5): DMSO (4:1) was added SAL **2a** (1.5 equiv., 18 mM) and PMB **3b** (8 equiv., 96 mM). The resulting

solution was stirred at room temperature for 2h. The reaction was quenched by diluting with water and by freezing at -80 °C. The samples were concentrated by lyophilization, analyzed by MS and monitored by injecting reaction in HPLC after regular intervals of time 15 min, 30 min, 1 h and 2 h. % Conversion to the Petasis product **4b** was determined by calculating areas under the peak in HPLC. HPLC: 0.1% FA (v/v) in water (solvent A): 0.1% FA (v/v) acetonitrile (solvent B); gradient 0-60 %, 0.1% FA (v/v) acetonitrile in 25 min, flow rate = 1.0 mL/min, detection wavelength 220 nm.

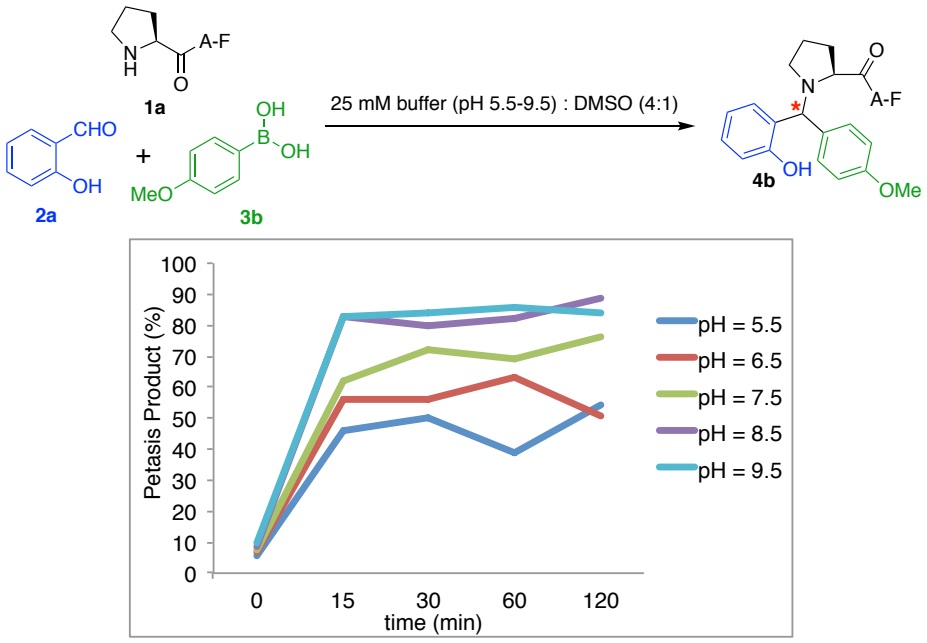
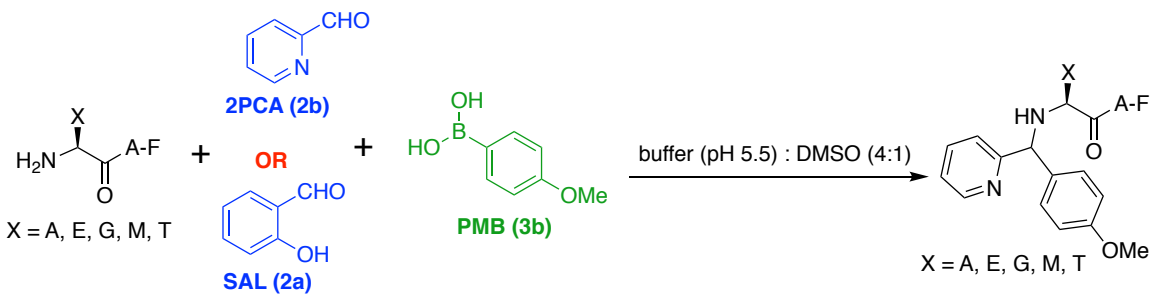


Fig. S10. Effect of pH on the rate of the SASP reaction. Reaction conditions: PAF **1a** (2 mg, 12 mM), SAL **2a** (18 mM) and PMB **3b** (96 mM) in 0.5 mL of 25 mM phosphate buffer: DMSO (4:1) in different pH conditions ranging from 5.5 to 9.5 and at room temperature. % Conversion to the Petasis product **4b** was determined by injecting the sample in HPLC after regular intervals of time and analysis by MS.



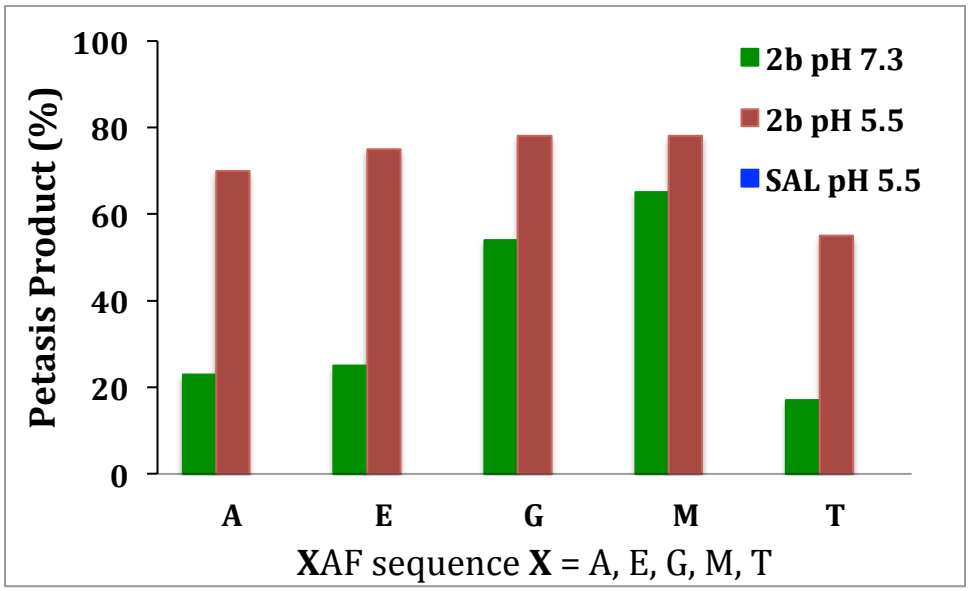
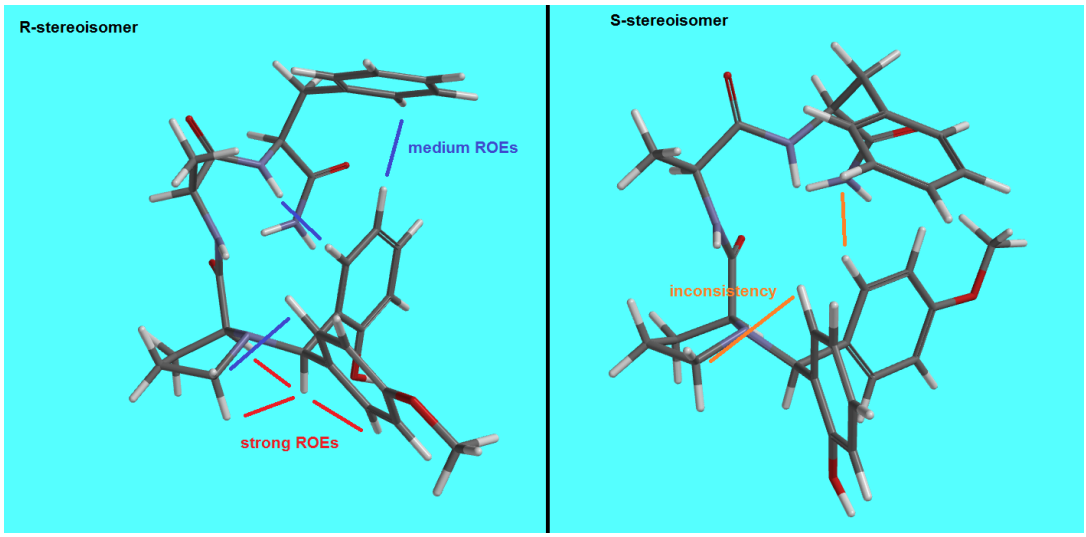


Fig. S11. Effect of pH on the Petasis reaction with N-terminal primary peptides. Reaction conditions: XAF **1a (**2 mg, 12 mM), X = A, E, G, M and T, SAL **2a/2PCA 2b** (18 mM) and PMB **3b** (96 mM) in 0.5 mL of 25 mM phosphate buffer (pH 5.5): DMSO (4:1) at room temperature. % Conversion to the Petasis products were determined by injecting the sample in HPLC after regular intervals of time and analysis by MS.

Method for analysis of diastereoselectivity of SASP reaction and determination of the absolute configuration of SASP product. Product 4b obtained by reaction of peptide PAF with SAL, and PMB. The relative stereochemistry of SASP product **4b** was determined by through-space correlations (i.e., ROEs) from the ROESY spectrum and then verified by computational chemistry predictions of the NMR chemical shifts. DFT calculations (PCM-mPW1PW91/6-31+G**//B3LYP/6-31G*) of ^{1}H and ^{13}C chemical shifts for the Petasis product **4b** was performed. The calculated chemical shifts were Boltzmann averaged based on the conformer distribution. There were 13 conformers less than 5 kcal/mol in energy for the *S*-configuration and 14 conformers less than 5 kcal/mol for the *R*-configuration. In both cases, there was a single dominant conformer accounting for greater than 40% of the Boltzmann population. Using the DP4+ method, the *R*-configuration was predicted to be the correct stereoisomer with >99% probability.[2] See below for details.



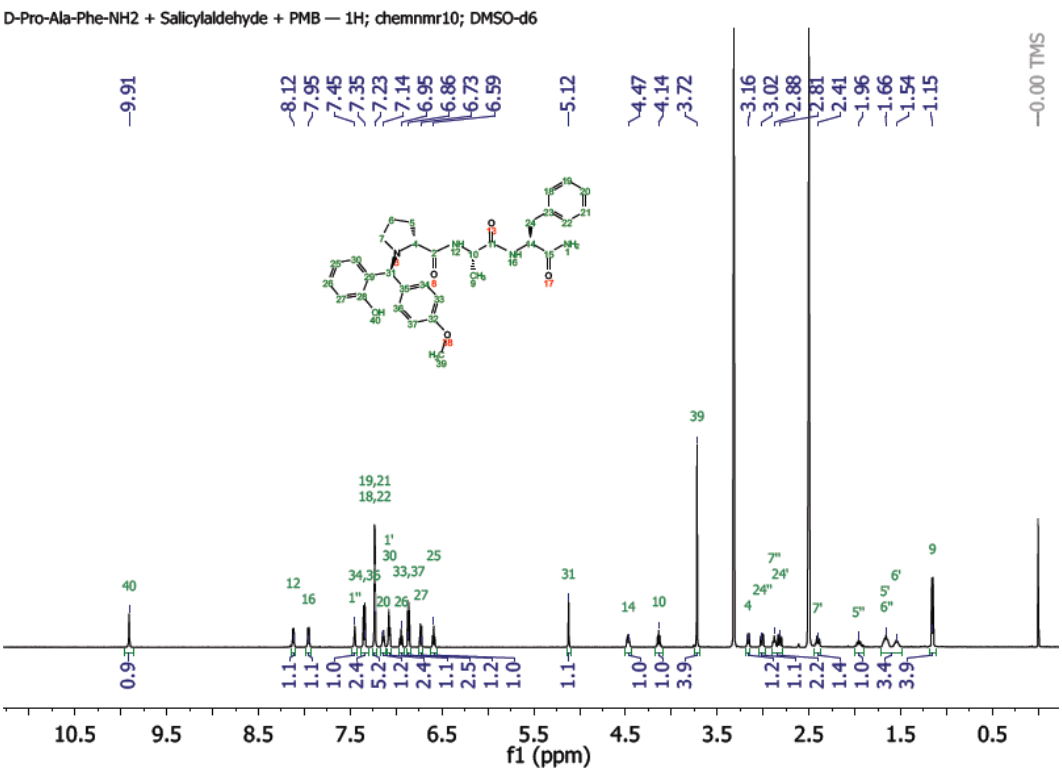
Functional	Solvent?	Basis Set	
mPW1PW91	PCM	6-31+G(d,p)	
	Isomer 1	Isomer 2	Isomer 3	Isomer 4
sDP4+ (H data)	0.15%	99.85%	-	-
sDP4+ (C data)	90.62%	9.38%	-	-
sDP4+ (all data)	1.47%	98.53%	-	-
uDP4+ (H data)	7.84%	92.16%	-	-
uDP4+ (C data)	0.01%	99.99%	-	-
uDP4+ (all data)	0.00%	100.00%	-	-
DP4+ (H data)	0.01%	99.99%	-	-
DP4+ (C data)	0.05%	99.95%	-	-
DP4+ (all data)	0.00%	100.00%	-	-

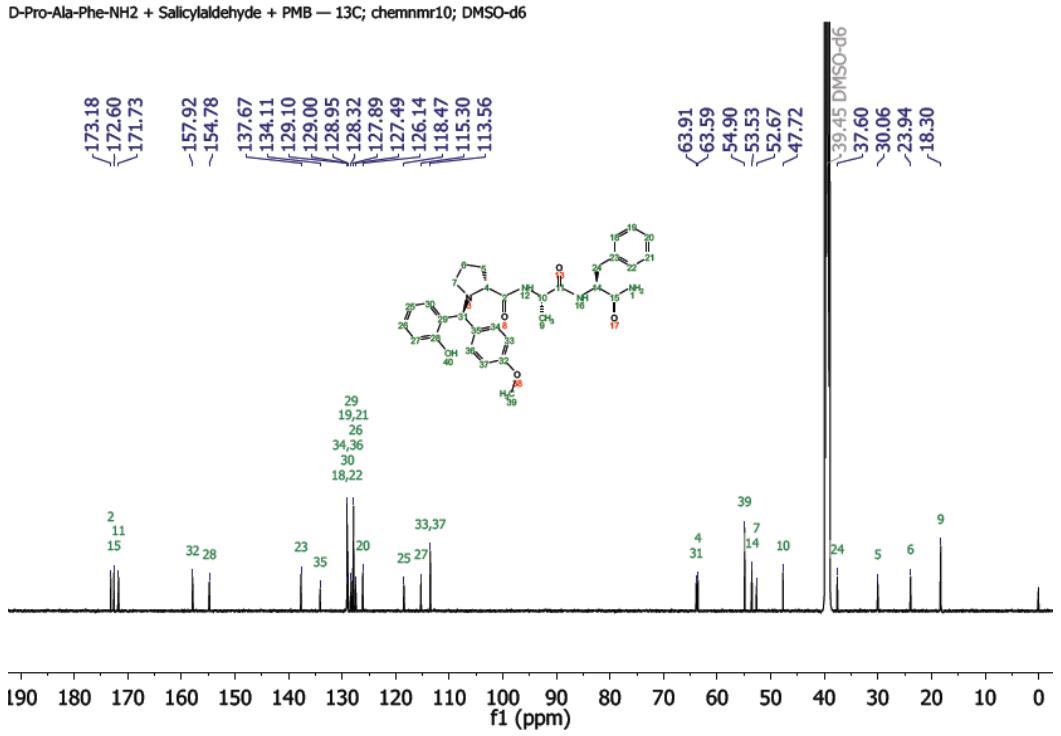
Fig. S12. Determination of the absolute configuration of the product 4b and stereoselective nature of SASP reaction. Comparison of ROEs from ROESY spectrum with conformers from a MMFF94s molecular mechanics Monte Carlo search in Spartan '14 are consistent with the *R* stereo configuration. Key ROEs are shown above. DP4+ calculations based on experimental ^{13}C and ^{1}H NMR chemical shifts are consistent with the formation of only one diastereoisomer (de >99%).

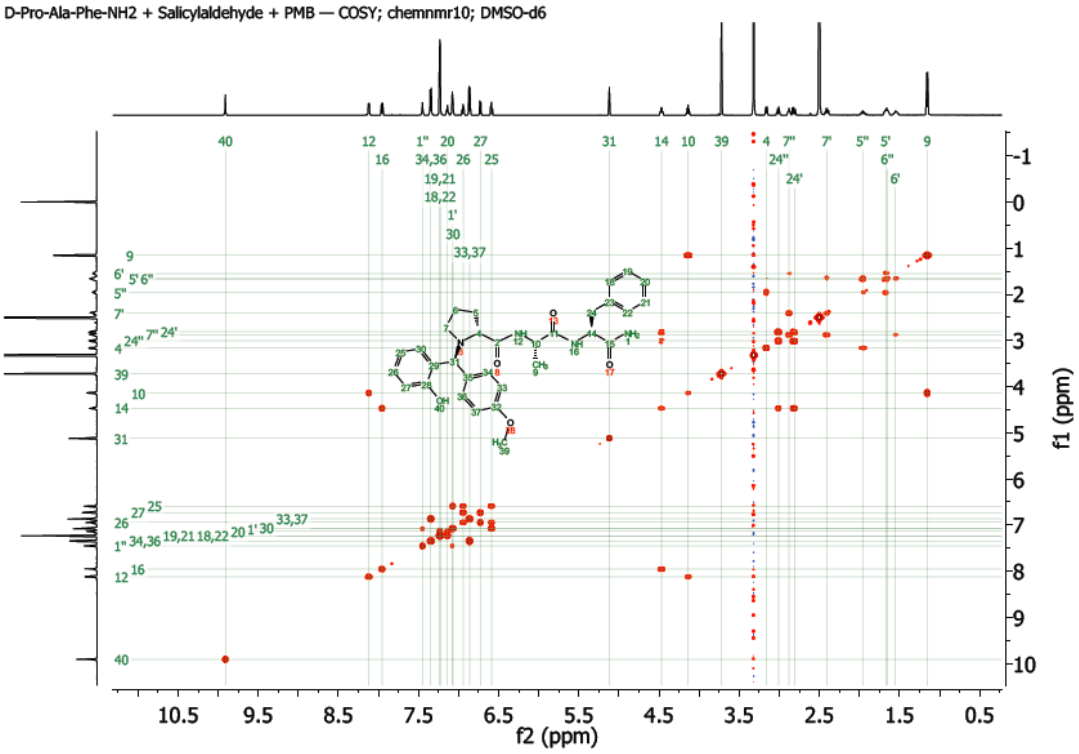
Synthesis of small molecules. D-Pro-L-Ala-L-Phe SASP product 4B with SAL and PMB. To a solution of D-pro-L-Ala-L-Phe **pAF 1B** (8 mg, 0.024 mmol) in 25 mM phosphate buffer pH 7.3: DMSO (4:1) (1 mL) was added SAL **2a** (4.36 mg, 0.036 mmol) and PMB **3b** (29.0 mg, 0.192 mmol). The resulting solution was stirred at room temperature. After 16 h, the reaction was concentrated by lyophilization. The resulting material was purified by HPLC to afford the product **4B** as a single diastereomer. HPLC: 0.1% FA (v/v) in water (solvent A): 0.1% FA (v/v) acetonitrile (solvent B); gradient 0-60 %, 0.1% FA (v/v) acetonitrile in 25 min, flow rate = 1.0 mL/min, detection wavelength 220 nm.

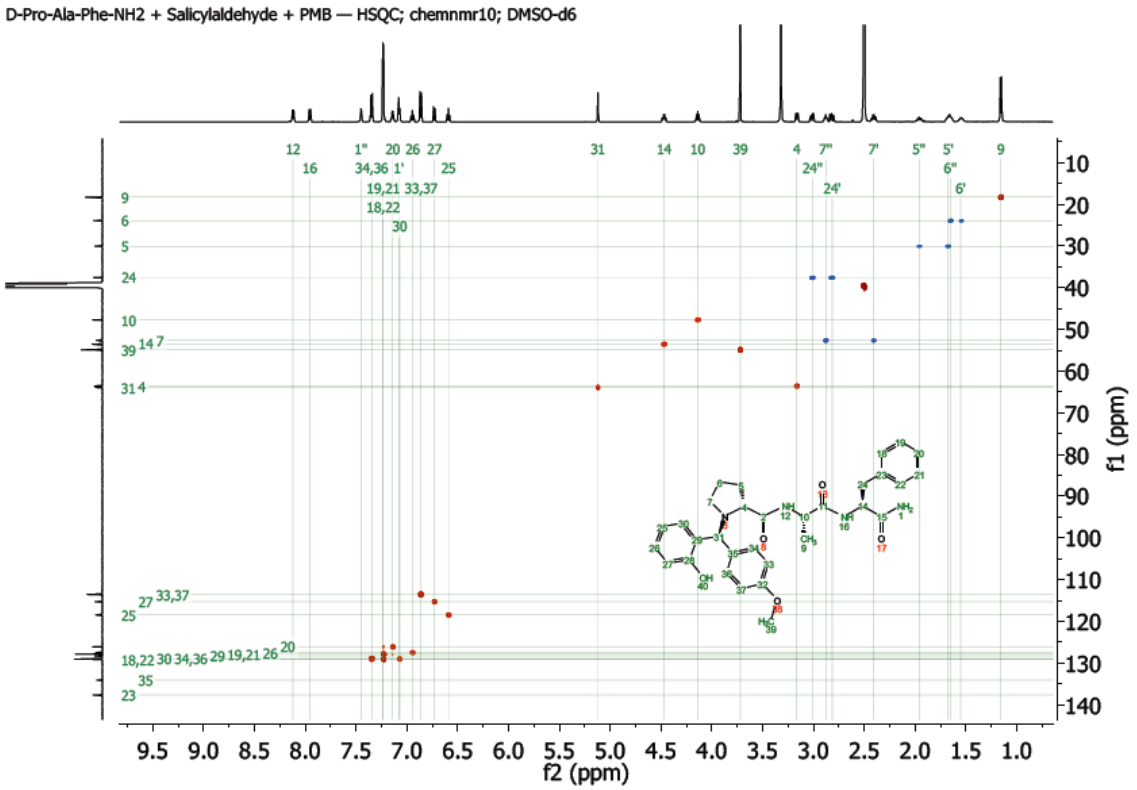
Single diastereomer: ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.91 (s, 1H), 8.12 (d, *J* = 7.7 Hz, 1H), 7.95 (d, *J* = 8.2 Hz, 1H), 7.45 (s, 1H), 7.35 (d, *J* = 8.2 Hz, 2H), 7.29-7.27 (m, 2H), 7.27-7.24 (m, 2H), 7.19 (t, *J* = 6.9 Hz, 1H), 7.08 (s, 1H), 7.02 (d, *J* = 7.4 Hz, 1H), 6.87 (d, *J* = 8.2 Hz, 1H), 6.75 (d, *J* = 7.7 Hz, 2H), 6.66 (t, *J* = 7.7 Hz, 1H), 6.55 (m,1H), 5.10 (s,1H), 4.55-4.49 (m,1H), 4.10 (p, *J* = 7.0 Hz,1H), 3.71 (s, 3H), 3.14 (m,1H), 3.02 (dd, *J* = 13.8, 4.8 Hz,1H), 2.91 (m,1H), 2.82 (dd, *J* = 13.8, 9.6 Hz,1H), 2.44 (m,1H), 1.98 (m,1H), 1.74-1.60 (m, 2H), 1.72-1.65 (m,1H), 1.13 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (151 MHz, DMSO-d_6): 173.2, 172.6, 171.7, 157.9, 154.8, 137.7, 134.1, 129.1, 129.0, 128.9, 128.3, 127.9, 127.5, 126.1, 118.5, 115.3, 113.6, 63.9, 63.6, 54.9, 53.5, 52.7, 47.7, 37.6, 30.1, 23.9, 18.3. See below for ^{1}H, ^{13}C, COSY, HSQC, HMBC, TOCSY and ROESY NMR spectra.

Method for analysis of diastereoselectivity of SASP reaction and determination of the absolute configuration of SASP product. Product 4B obtained by the reaction of peptide pAF with SAL, and PMB. The relative stereochemistry of SASP product **4B** was determined by through-space correlations (i.e., ROEs) from the ROESY spectrum and then verified by computational chemistry predictions of the NMR chemical shifts as mentioned above. Using the DP4+ method, the *S*-configuration was predicted to be the correct stereoisomer with >99% probability. See below for details.

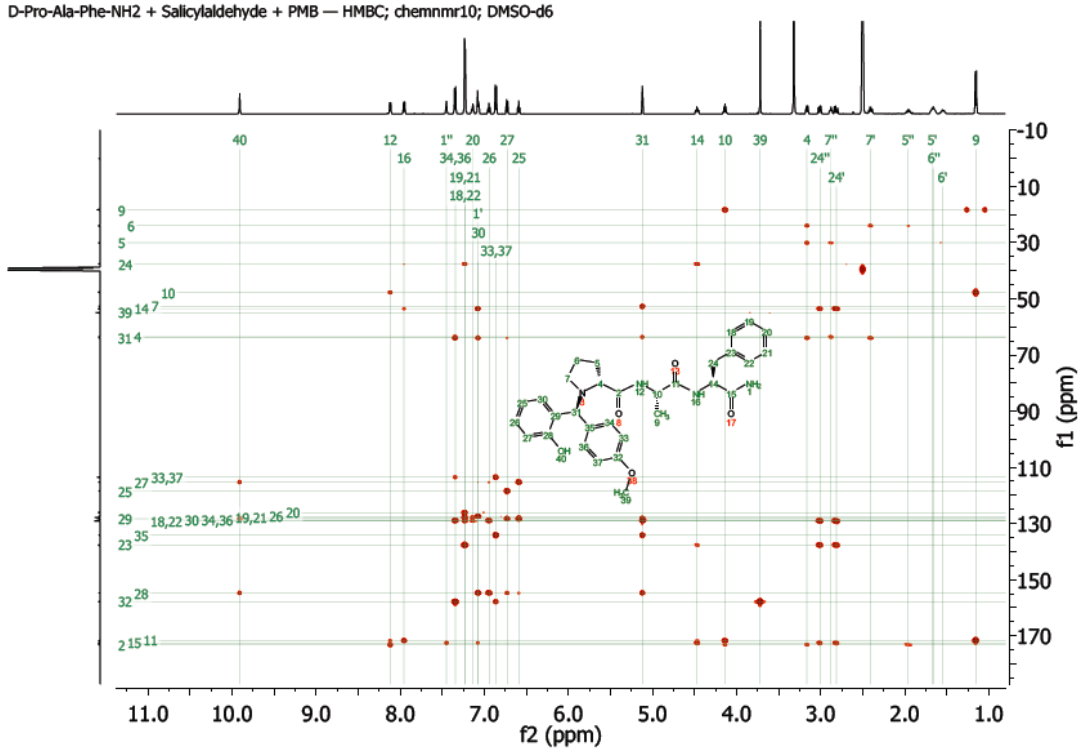

D-Pro-Ala-Phe-NH2 + Salicylaldehyde + PMB — 1H; chemnmr10; DMSO-d6
9.91
8.12
7.95
7.45
7.35
7.23
7.14
6.95
6.86
6.73
6.59
5.12
4.47
4.14
3.72
3.16
3.02
2.88
2.81
2.41
1.96
1.66
1.54
1.15
0.00 TMS
f1 (ppm)


D-Pro-Ala-Phe-NH2 + Salicylaldehyde + PMB — 13C; chemnmr10; DMSO-d6
173.18
172.60
171.73
157.92
154.78
137.67
134.11
129.10
129.00
128.95
128.32
127.89
127.49
126.14
118.47
115.30
113.56
63.91
63.59
54.90
53.53
52.67
47.72
39.45 DMSO-d6
37.60
30.06
23.94
18.30
f1 (ppm)

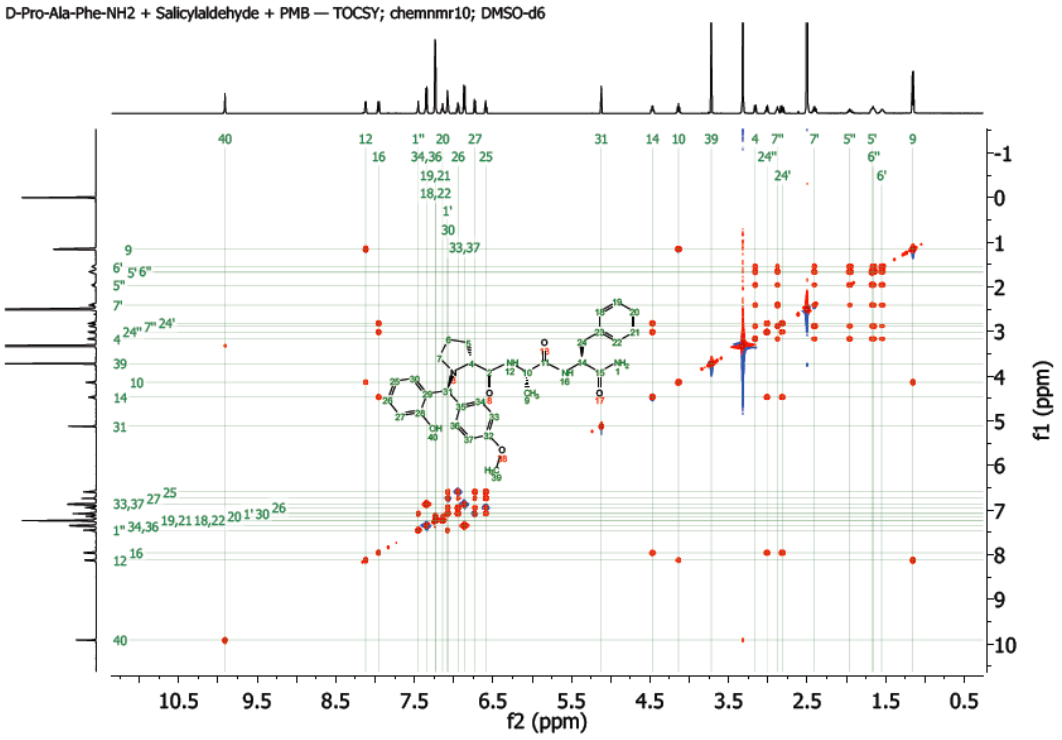

D-Pro-Ala-Phe-NH2 + Salicylaldehyde + PMB — COSY; chemnmr10; DMSO-d6
f2 (ppm)
f1 (ppm)


D-Pro-Ala-Phe-NH2 + Salicylaldehyde + PMB — HSQC; chemnmr10; DMSO-d6
f2 (ppm)
f1 (ppm)

D-Pro-Ala-Phe-NH2 + Salicylaldehyde + PMB — HMBC; chemnmr10; DMSO-d6



D-Pro-Ala-Phe-NH2 + Salicylaldehyde + PMB — TOCSY; chemnmr10; DMSO-d6



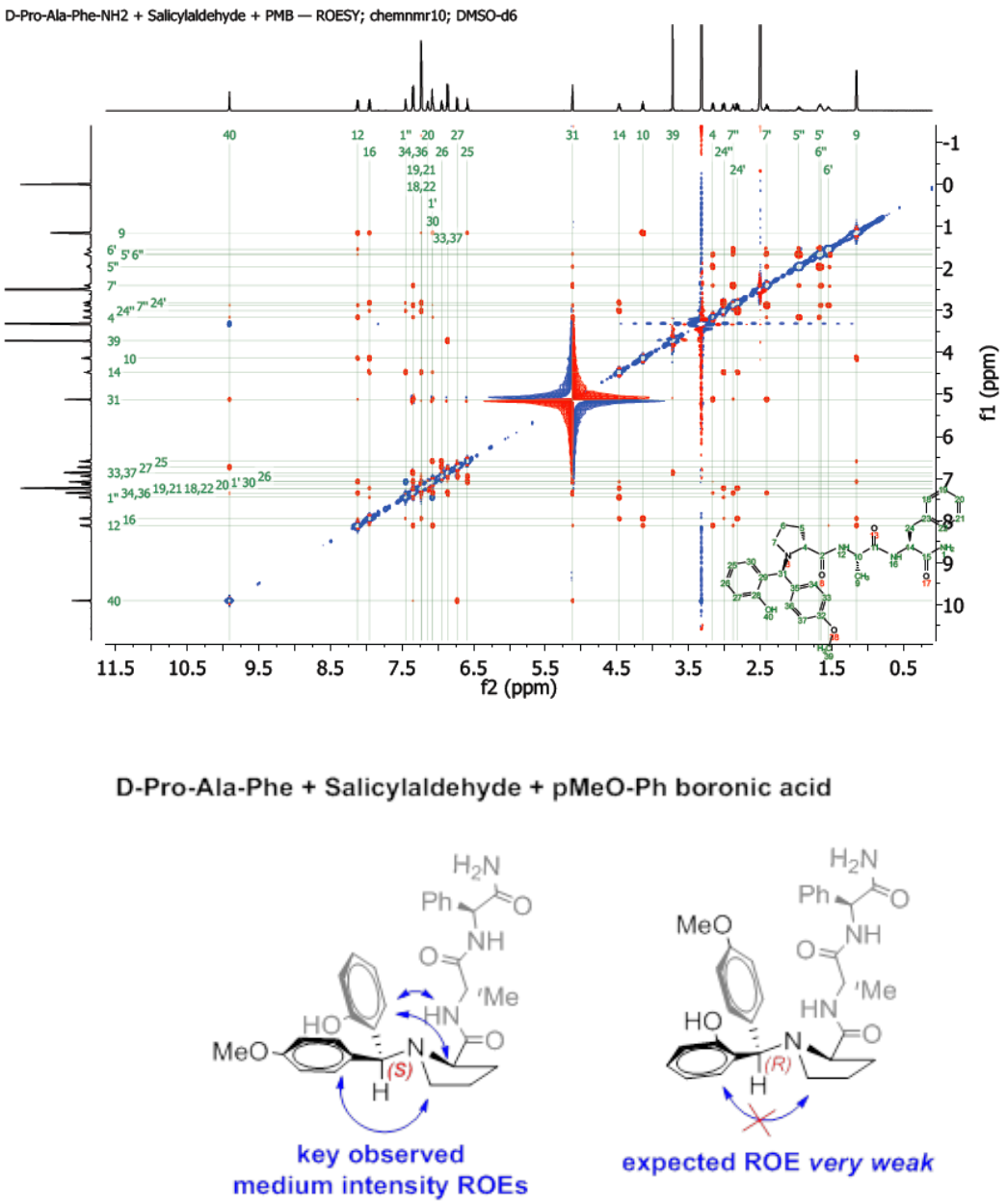
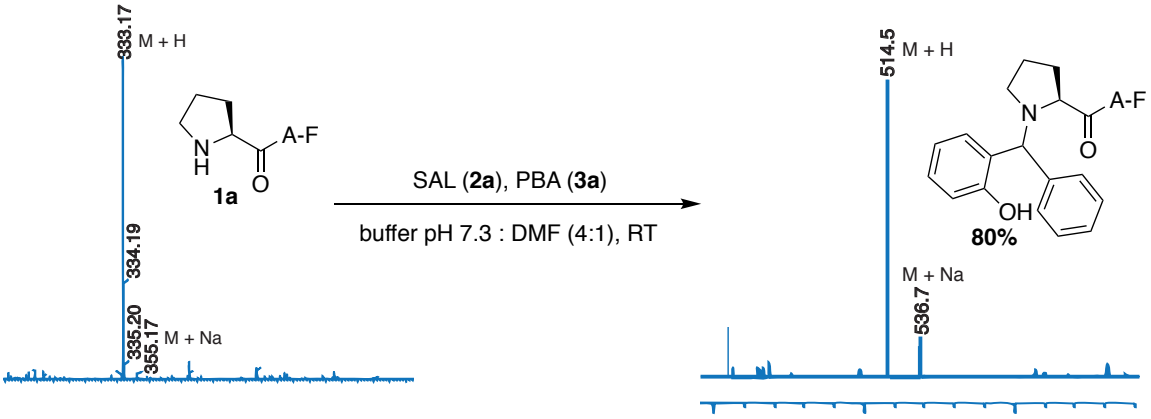
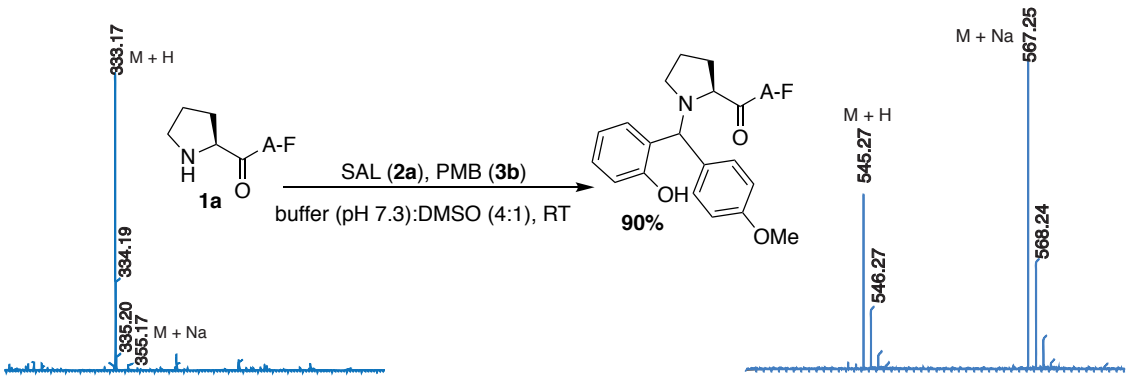


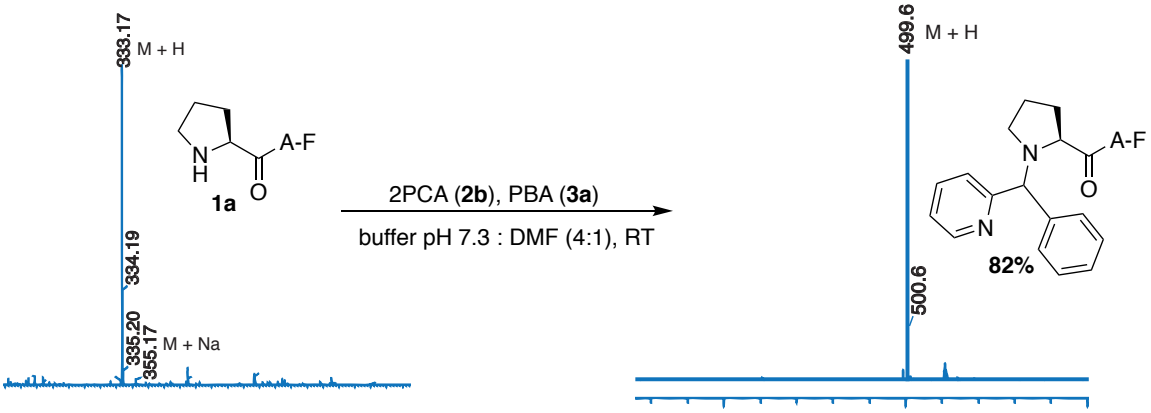
Fig. S13. SASP product 4B characterization. ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, TOCSY and ROESY NMR of the product **4B**. The reaction afforded a single diastereoisomer with (de > 99%). D-Pro gives opposite diastereoisomer as compared to L-proline. Comparison of ROEs from ROESY spectrum with conformers from a MMFF94s molecular mechanics Monte Carlo search in Spartan '14 are consistent with the *S* stereo configuration. Key ROEs are shown above. DP4+ calculations based on experimental ^{13}C and ^{1}H NMR chemical shifts are consistent with the formation of only one diastereoisomer (de >99%).

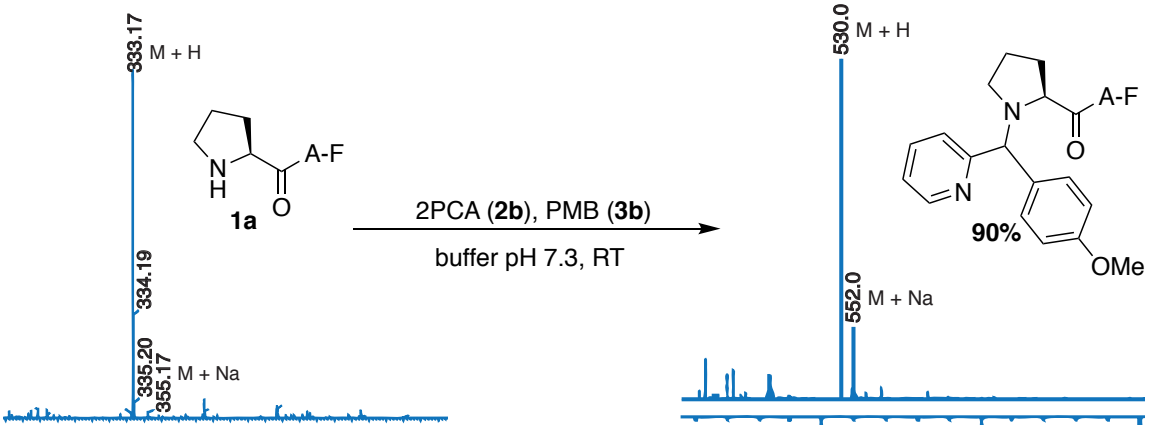
General method for screening of aldehydes with peptide PAF 1a. To a peptide PAF **1a** (2 mg, 12 mM) in 0.5 mL solution of 25 mM phosphate buffer (pH 7.3): DMSO (4:1) was added a variety of aldehydes (1.5 equiv., 18 mM) and PBA **3a**/PMB **3b** (8 equiv., 96 mM). The reactions were stirred at room temperature for 4-24 h. The reactions were analyzed by LC/MS. LC: 0.1% FA (v/v) in water (solvent A): 0.1% FA (v/v) acetonitrile (solvent B); gradient 0-60 % or 0-80 %, depending on nature of peptides, 0.1% FA (v/v) acetonitrile in 25 min, flow rate = 1.0 mL/min, detection wavelength 220 nm.

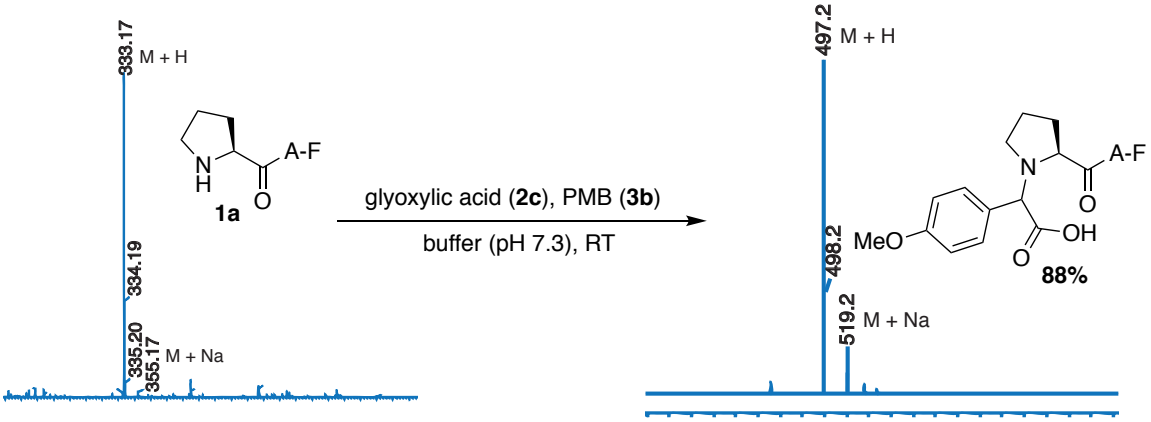
General method for screening of boronic acids with peptide PAF. To a peptide PAF **1a** (2 mg, 12 mM), in 0.5 mL solution of 25 mM phosphate buffer (pH 7.3): DMSO (4:1) was added SAL **2a** or 2PCA **2b** (1.5 equiv., 18 mM) and a variety of boronic acids (8 equiv., 96 mM). The reactions were stirred at room temperature for 2-24 h. The reactions were analyzed by LC/MS. LC: 0.1% FA (v/v) in water (solvent A): 0.1% FA (v/v) acetonitrile (solvent B); gradient 0-60 % or 0-80 %, depending on nature of peptides, 0.1% FA (v/v) acetonitrile in 25 min, flow rate = 1.0 mL/min, detection wavelength 220 nm.

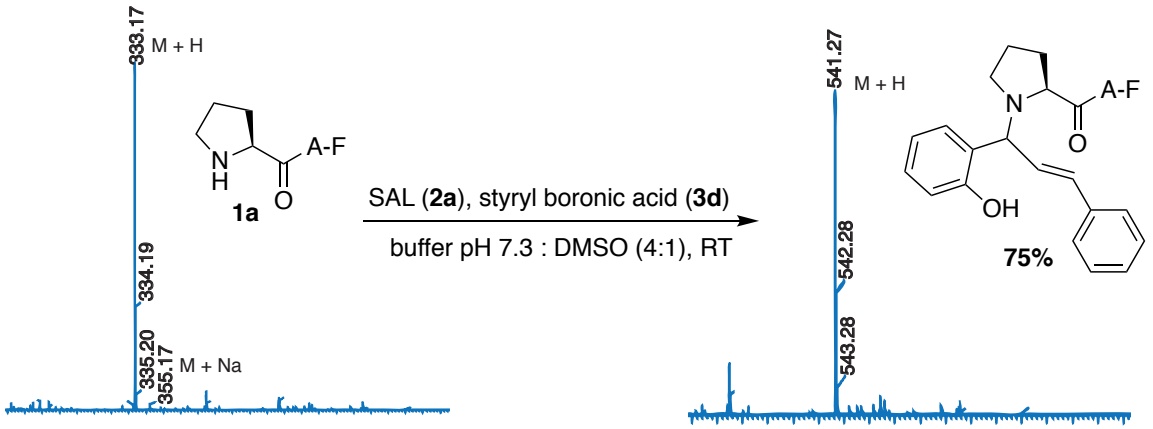


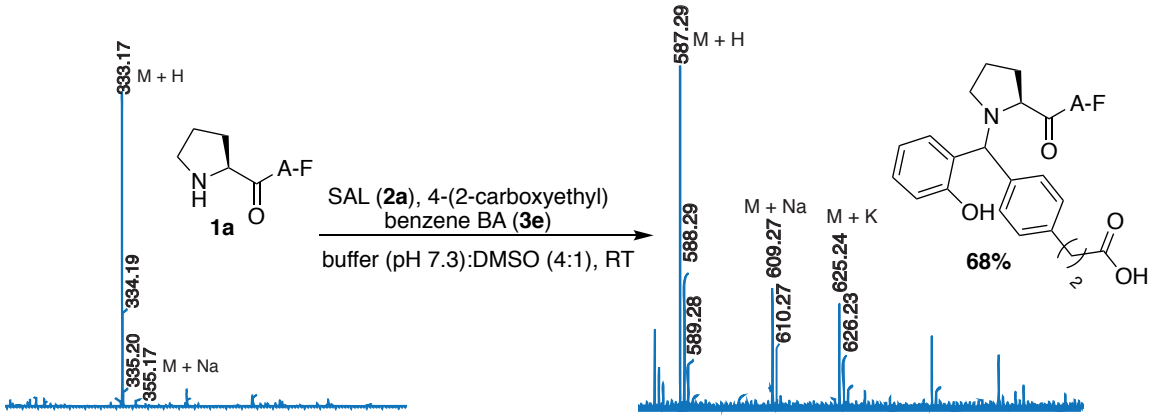


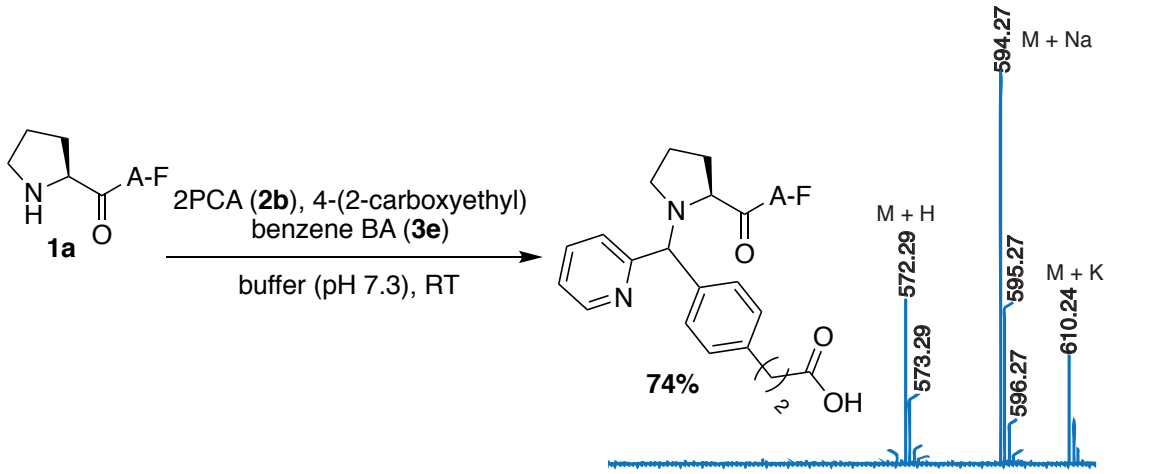

333.17
M + H
334.19
335.20
355.17
M + Na
A-F
1a
2PCA (2b), PBA (3a)
buffer pH 7.3 : DMF (4:1), RT
499.6
M + H
500.6
82%

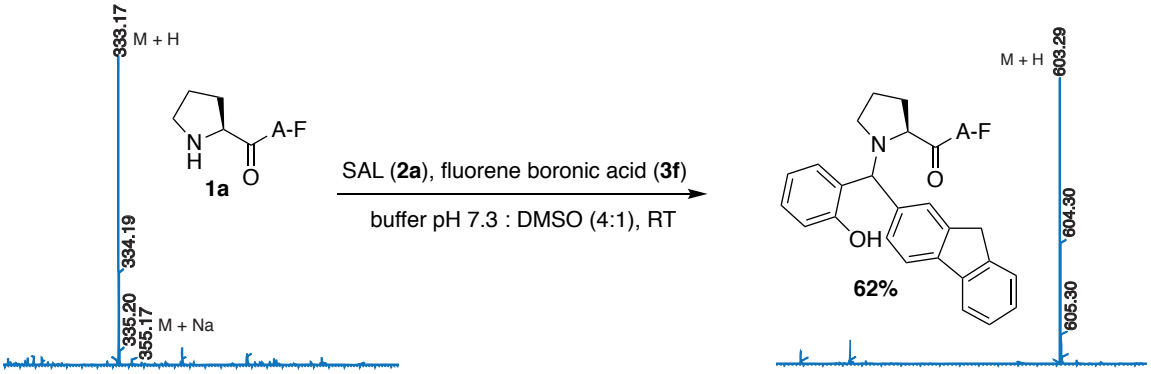

333.17
M + H
334.19
335.20
355.17
M + Na
A-F
1a
2PCA (2b), PMB (3b)
buffer pH 7.3, RT
530.0
M + H
552.0
M + Na
90%
OMe

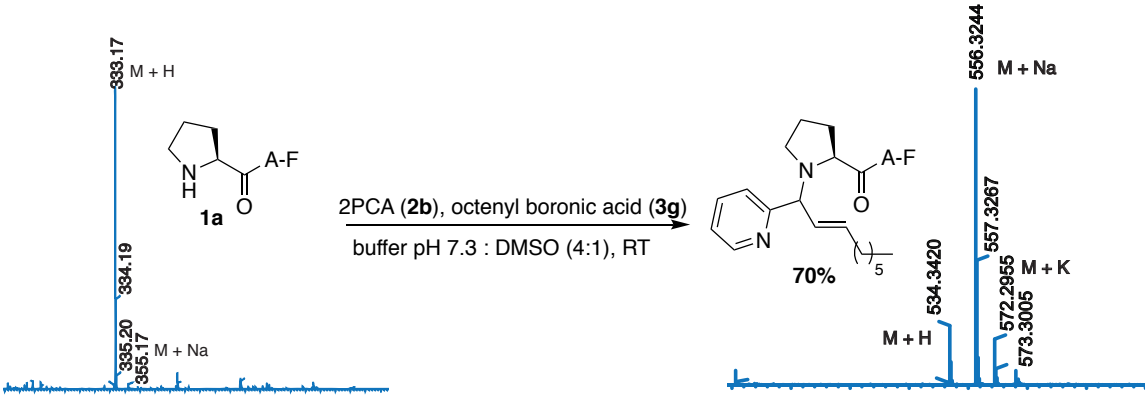

333.17
M + H
334.19
335.20
355.17
M + Na
A-F
1a
glyoxylic acid (2c), PMB (3b)
buffer (pH 7.3), RT
497.2
M + H
498.2
519.2
M + Na
MeO
OH
88%

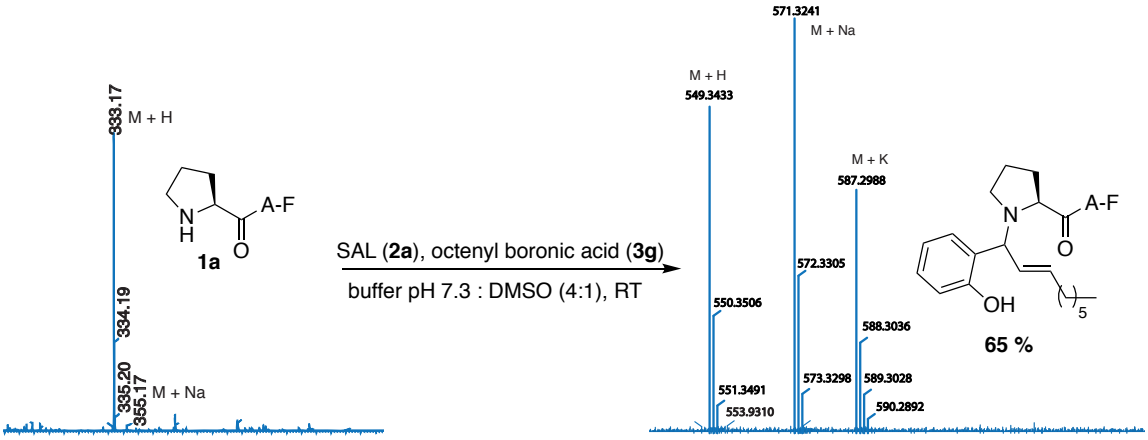

333.17
M + H
334.19
335.20
355.17
M + Na
A-F
1a
SAL (2a), styryl boronic acid (3d)
buffer pH 7.3 : DMSO (4:1), RT
541.27
M + H
542.28
543.28
OH
75%

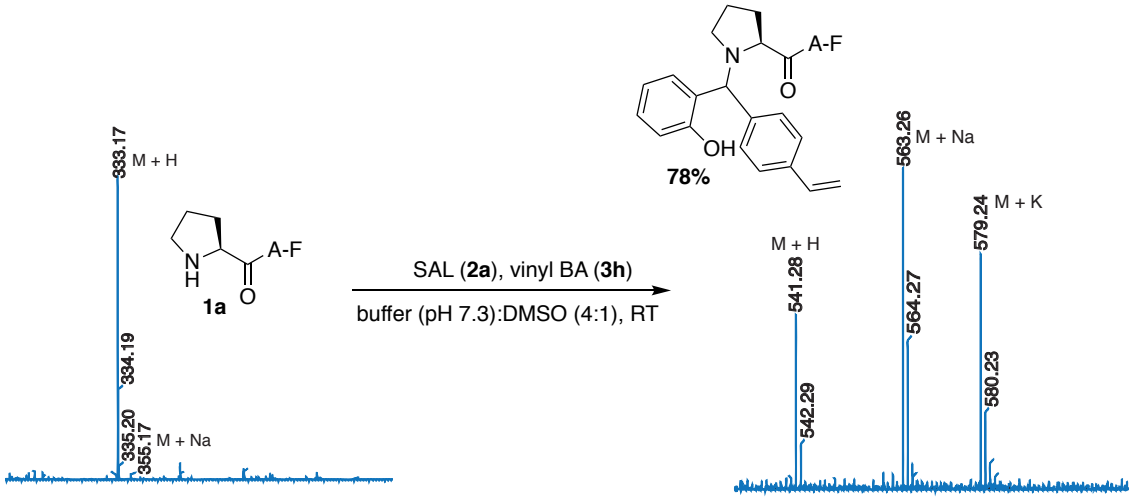

333.17
M + H
334.19
335.20
355.17
M + Na
A-F
1a
SAL (2a), 4-(2-carboxyethyl)
benzene BA (3e)
buffer (pH 7.3):DMSO (4:1), RT
587.29
M + H
588.29
589.28
M + Na
609.27
610.27
M + K
625.24
626.23
OH
68%

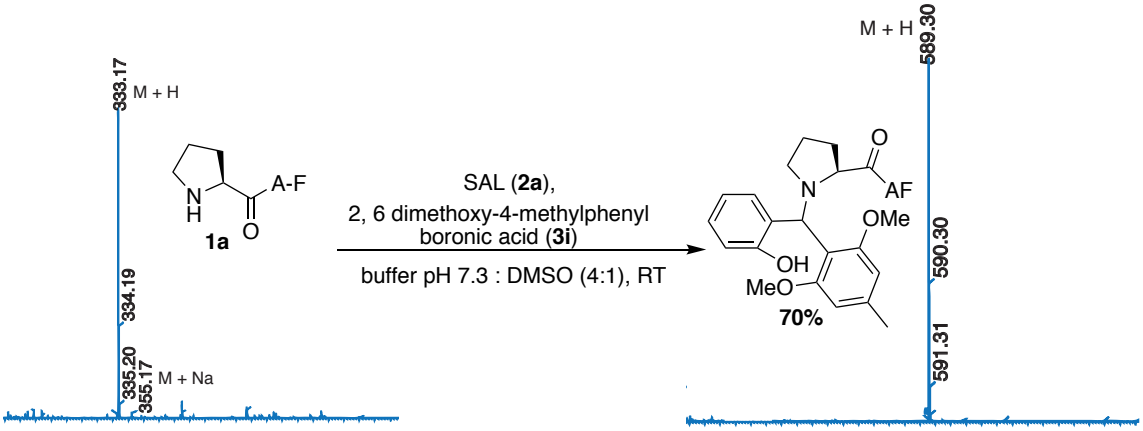

A-F
1a
2PCA (2b), 4-(2-carboxyethyl)
benzene BA (3e)
buffer (pH 7.3), RT
594.27
M + Na
M + H
572.29
573.29
595.27
596.27
M + K
610.24
74%
OH

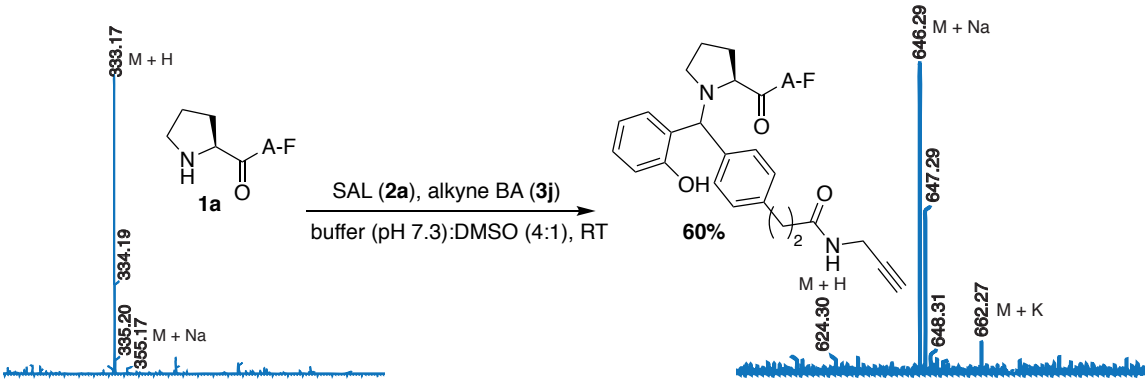

333.17
M + H
334.19
335.20
355.17
M + Na
1a
A-F
SAL (2a), fluorene boronic acid (3f)
buffer pH 7.3 : DMSO (4:1), RT
62%
603.29
M + H
604.30
605.30


333.17
M + H
334.19
335.20
355.17
M + Na
1a
A-F
2PCA (2b), octenyl boronic acid (3g)
buffer pH 7.3 : DMSO (4:1), RT
70%
556.3244
M + Na
557.3267
M + K
534.3420
M + H
572.2955
573.3005


333.17
M + H
334.19
335.20
355.17
M + Na
1a
A-F
SAL (2a), octenyl boronic acid (3g)
buffer pH 7.3 : DMSO (4:1), RT
65 %
571.3241
M + Na
M + H
549.3433
M + K
587.2988
572.3305
550.3506
588.3036
551.3491
573.3298
589.3028
553.9310
590.2892


333.17
M + H
334.19
335.20
355.17
M + Na
1a
A-F
SAL (2a), vinyl BA (3h)
buffer (pH 7.3):DMSO (4:1), RT
78%
OH
563.26
M + Na
579.24
M + K
M + H
541.28
564.27
542.29
580.23


333.17
M + H
334.19
335.20
355.17
M + Na
1a
A-F
SAL (2a),
2, 6 dimethoxy-4-methylphenyl
boronic acid (3i)
buffer pH 7.3 : DMSO (4:1), RT
70%
OMe
OH
MeO
M + H
589.30
590.30
591.31


333.17
M + H
334.19
335.20
355.17
M + Na
1a
A-F
SAL (2a), alkyne BA (3j)
buffer (pH 7.3):DMSO (4:1), RT
60%
OH
646.29
M + Na
647.29
M + H
624.30
648.31
662.27
M + K

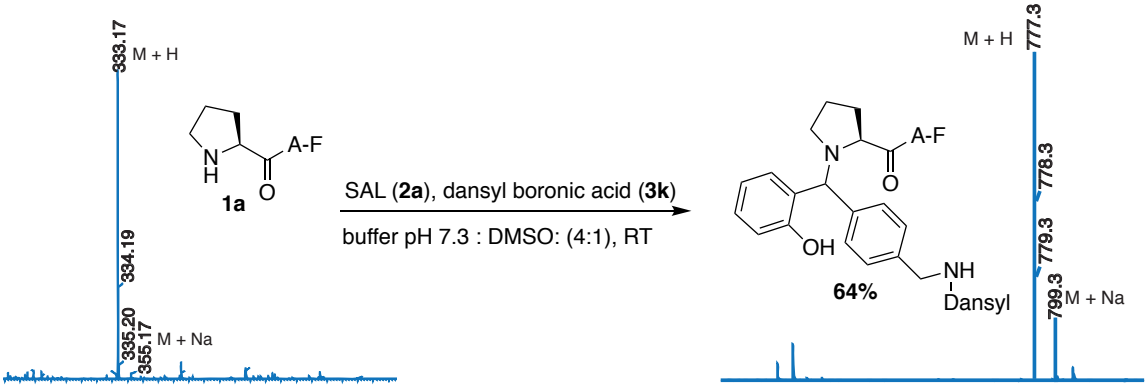
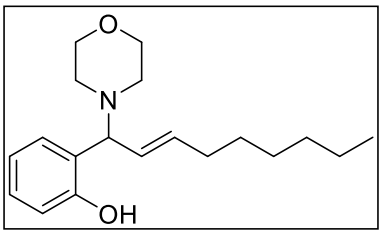
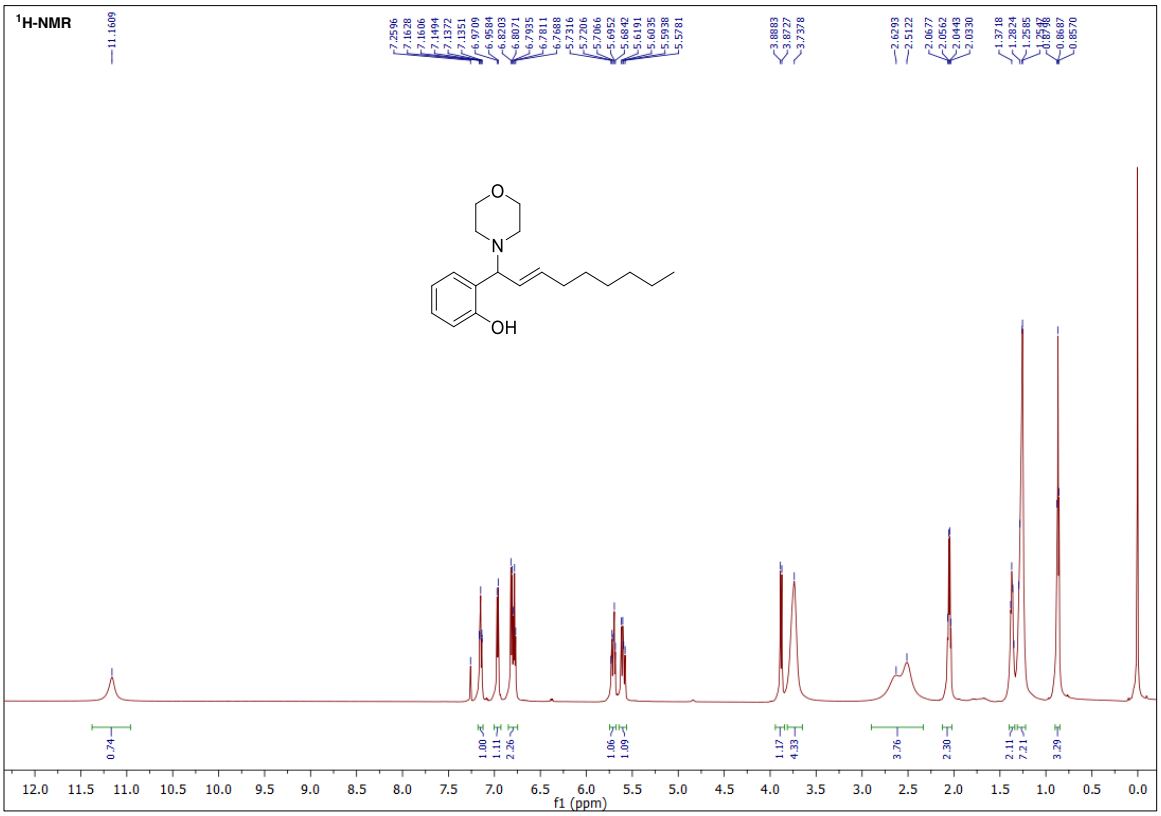
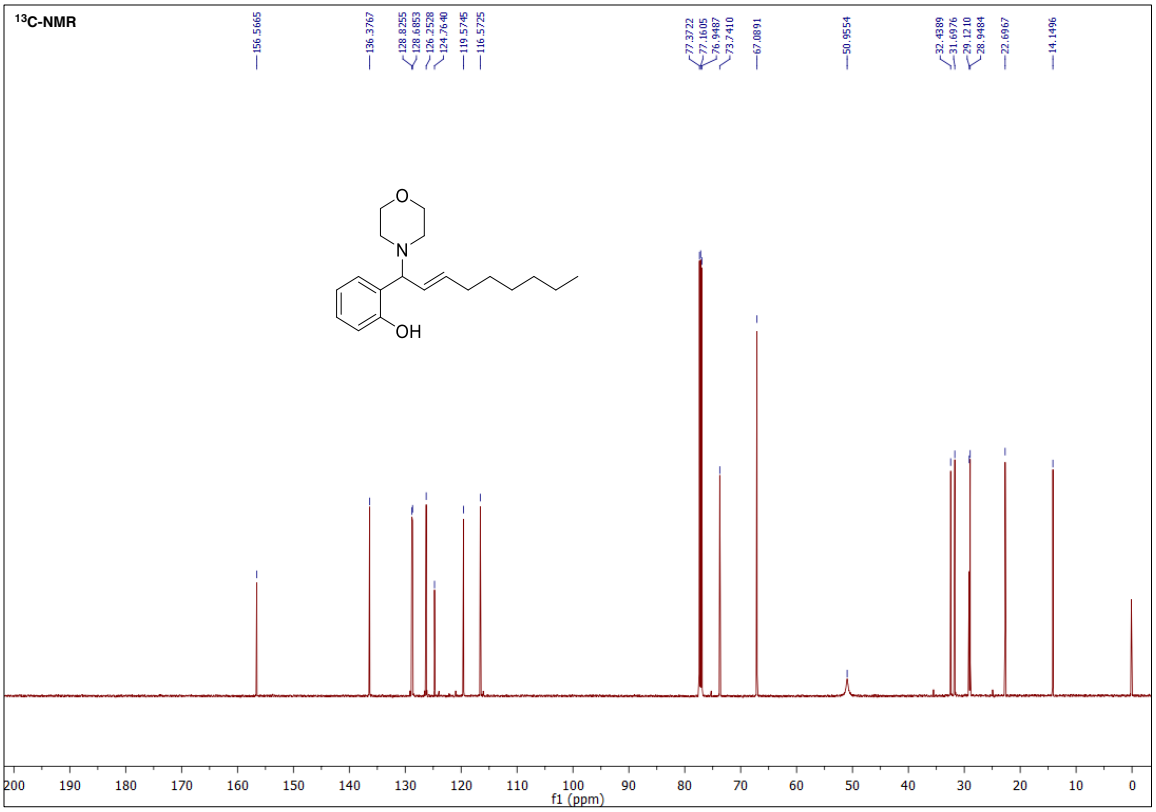


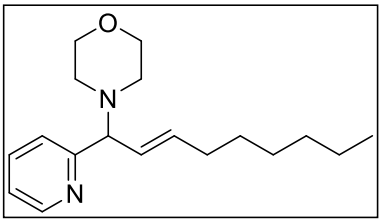
Fig. S14. HRMS of SASP product obtained from Petasis reaction on peptide PAF **1a** with different combination of aldehydes and boronic acid derivatives. Shown are representative mass spectra of modified peptides.



^{1}H NMR (600 MHz, $CDCl_3$) δ 11.16 (s, 1H), 7.16 – 7.14 (m, 1H), 6.96 (d, *J* = 7.2 Hz, 1H), 6.82 – 6.77 (m, 2H), 5.73 – 5.68 (m, 1H), 5.60 (dd, *J* = 15.2, 9.4 Hz, 1H), 3.88 (d, *J* = 9.4 Hz, 1H), 3.74 (s, 4H), 2.63-2.51 (bs, 4H), 2.07-2.03 (m, 2H), 1.38-1.35 (m, 2H), 1.29-1.25 (m, 6H), 0.87 (t, *J* = 6.7 Hz, 3H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 156.6, 136.4, 128.8, 128.7, 126.3, 124.8, 119.6, 116.6, 73.7, 67.1, 51.0, 32.4, 31.7, 29.1, 28.9, 22.7, 14.1.







^{1}H NMR (600 MHz, $CDCl_3$) δ 8.56 (d, *J* = 4.7 Hz, 1H), 7.64 (td, *J* = 7.7, 1.7 Hz, 1H), 7.37 (d, *J* = 7.8 Hz, 1H), 7.15 – 7.12 (m, 1H), 5.73 (dt, *J* = 15.1, 6.7 Hz, 1H), 5.55 (dd, *J* = 15.2, 9.1 Hz, 1H), 3.76 (d, *J* = 9.0 Hz, 1H), 3.71-3.69 (m, 4H), 2.57 (bs, 2H), 2.29-2.26 (m, 2H), 2.00 (dd, *J* = 13.7, 6.7 Hz, 2H), 1.34 – 1.32 (m, 2H), 1.25-1.22 (m, 6H), 0.84 (t, *J* = 6.7 Hz, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 161.8, 149.6, 136.8, 135.0, 130.0, 122.7, 122.2, 76.5, 67.2, 52.2, 32.5, 31.7, 29.1, 28.9, 22.7, 14.2.

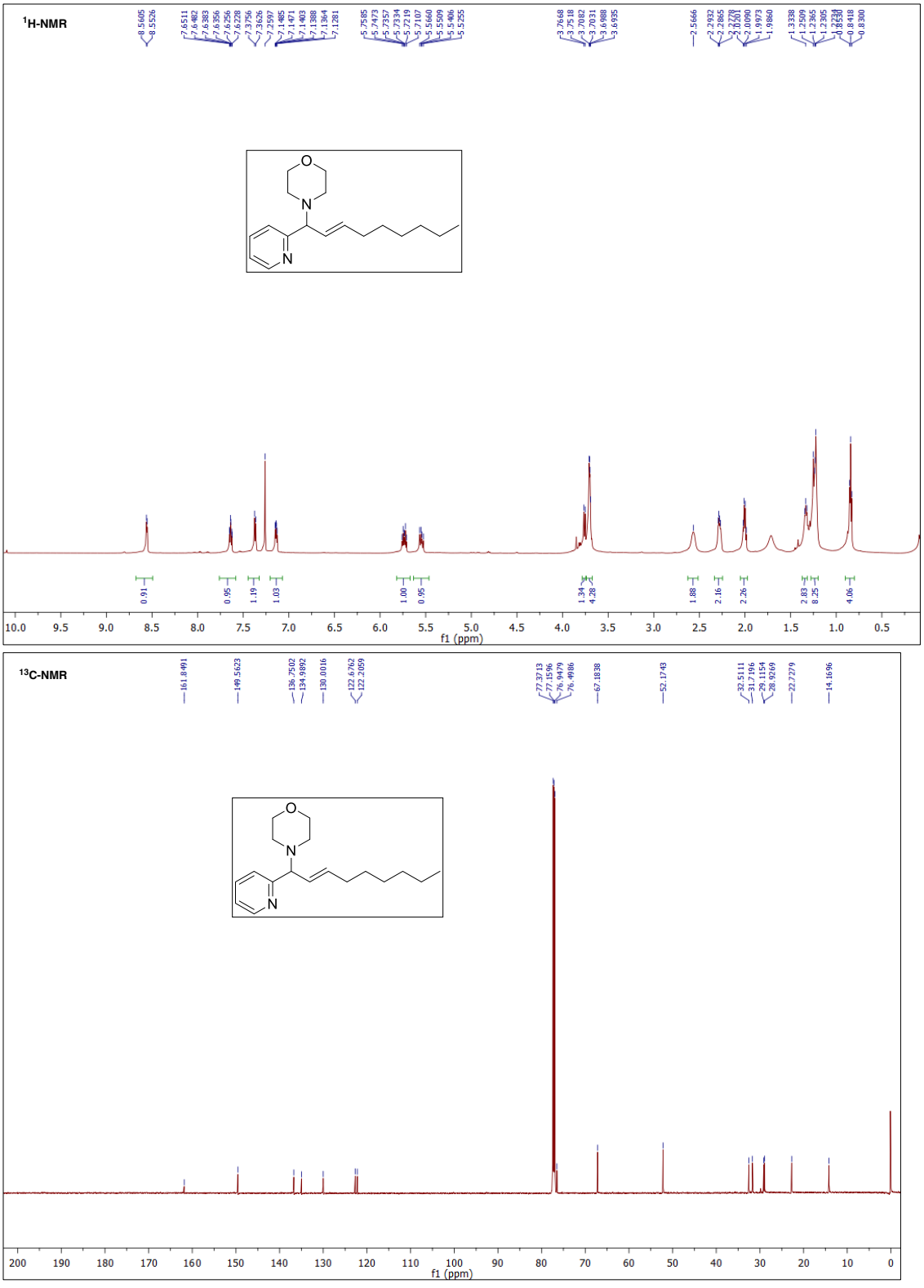
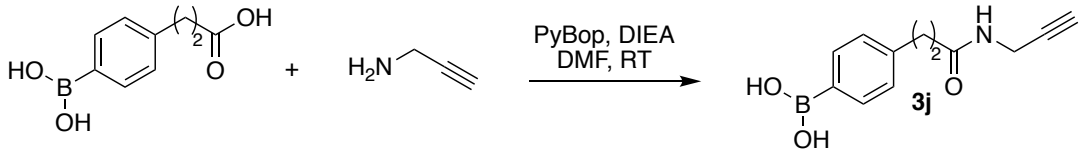
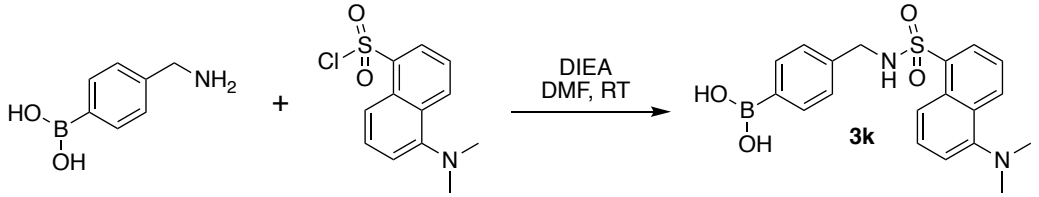


Fig. S15 Characterization of SASP product obtained by the reaction of morpholine with octenylboronic acid and SAL **2a**/2PCA **2b** by ^{1}H NMR, ^{13}C NMR of products.

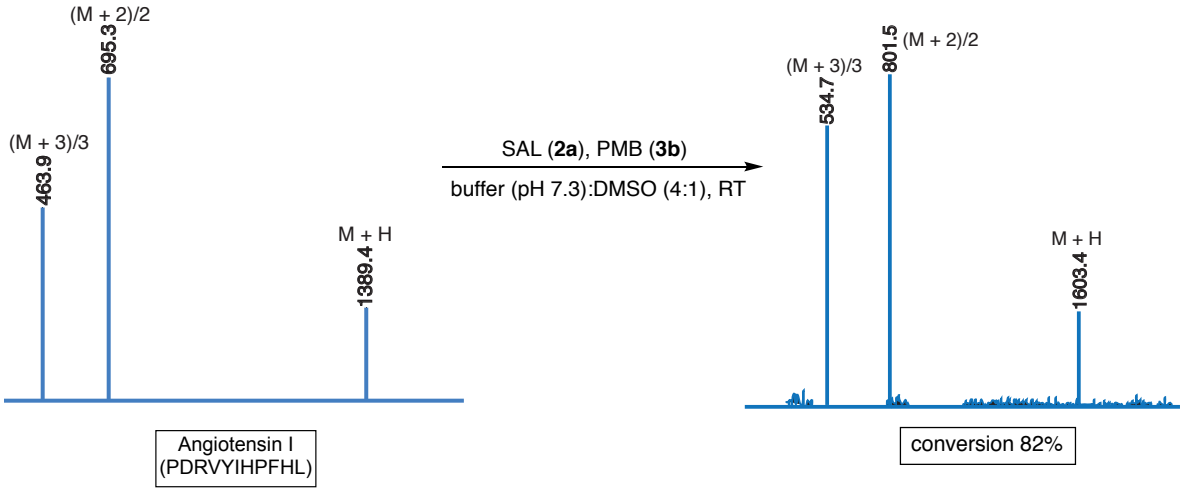


Synthesis of alkyneboronic acid 3j. To a solution of 4-(2-Carboxyethyl)benzeneboronic acid (100 mg) and PyBOP (1 equiv.) in DMF, propargylamine (2 equiv.) was added followed by addition of DIEA (3.2 equiv.). The reaction mixture was stirred overnight at room temperature. The reaction was analyzed by HRMS and purified by HPLC to obtain white solid. HPLC: 0.1% FA (v/v) in water (solvent A): 0.1% FA (v/v) acetonitrile (solvent B); gradient 0-80 % with flow rate = 1.0 mL/min, detection wavelength 220 nm.



Synthesis of dansylboronic acid 3k. To 1.0 mL solution of dansyl chloride (1.5 equiv.), diisopropyl amine (1.5 equiv.) in DMF, 4-(aminomethyl)phenylboronic acid (20 mg) was added. The solution was stirred at room temperature for 12 h. The reaction was analyzed by MS and purified by HPLC to obtain yellow solid. HPLC: 0.1% FA (v/v) in water (solvent A): 0.1% FA (v/v) acetonitrile (solvent B); gradient 0-80 %, depending on nature of peptides, 0.1% FA (v/v) acetonitrile in 25 min, flow rate = 1.0 mL/min, detection wavelength 220 nm.

Fig. S16. Synthesis of derivatives of boronic acids (3j and 3k). Procedure for the synthesis of alkyneboronic acid **3j** and dansylboronic acid **3k**.



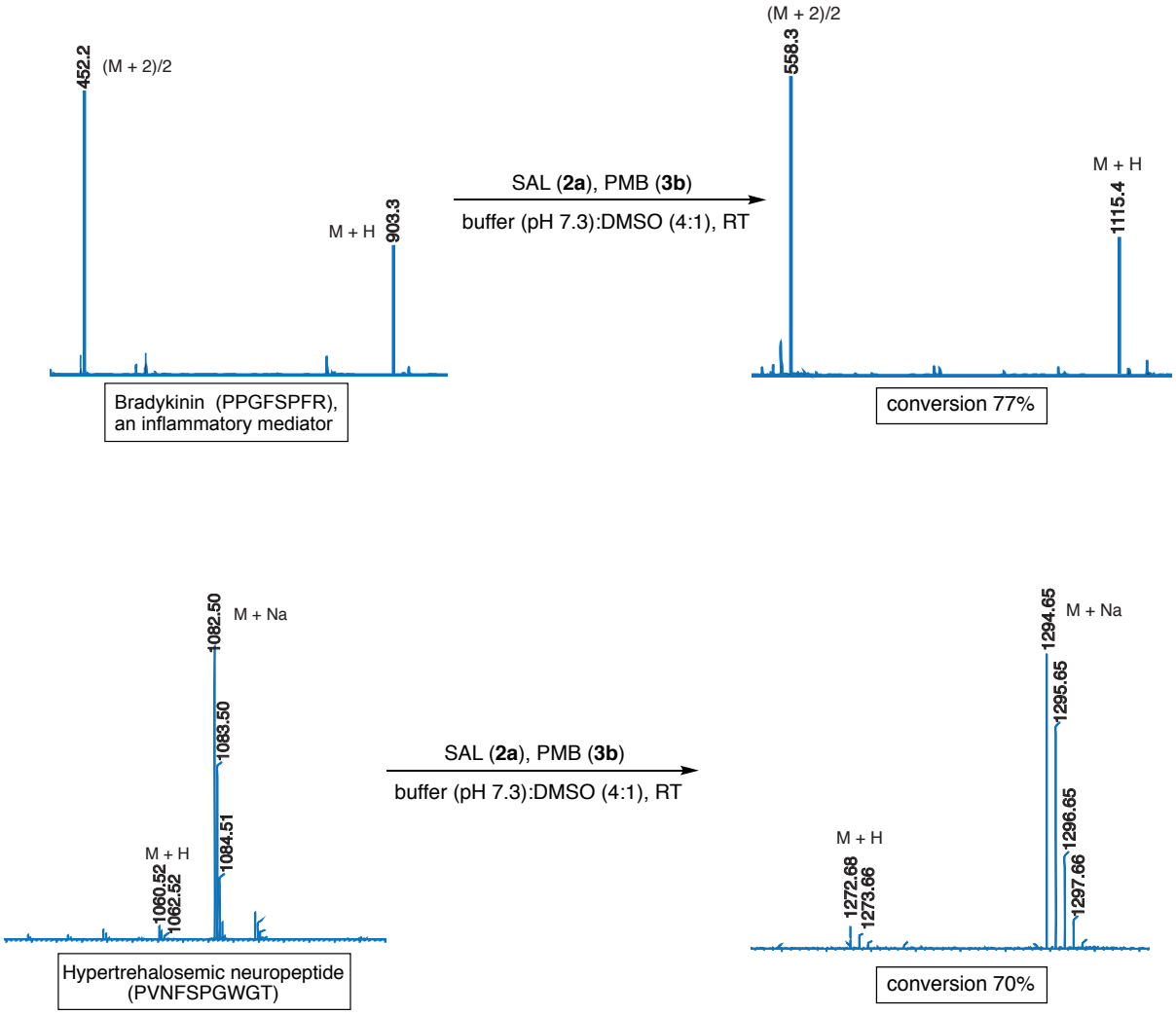


Fig. S17. **HRMS of bioactive peptides after modification with SAL 2a and PMB 3b.** Shown are representative mass spectra of modified bioactive peptides after a 2 mM solution of the peptide was reacted with SAL **2a** (1.5 equiv., 3 mM) and PMB **3b** (8 equiv., 16 mM) in 0.5 mL 25 mM phosphate buffer pH 7.3: DMSO (4:1) and reactions were stirred at room temperature for 15 h. The reactions were analyzed by LC-MS.

Protein modification. **Method for testing the reactivity of SASP reaction on proteins**. A general description of the reaction follows. The reaction was prepared in a 1.0 mL microcentrifuge tube. A protein creatine kinase (ck) (2 mg, 100 μM) was dissolved in 400 μL of 25 mM phosphate buffer pH 7.3. 2PCA (0.5 mM) and PMB (5 mM) was added in the solution. The reaction was agitated and incubated at room temperature. After various time points, the reaction was purified by repeated (five times) centrifugal filtration against a 0.5-mL Amicon Ultra-4 Centrifugal Filter spin concentrator with an appropriate molecular weight cutoff (EMD Millipore, USA). Modification was monitored by SDS-PAGE or LC-MS.

Stability studies of SASP product of protein creatine kinase (ck). To a modified creatine kinase (1 μM), 0.5 mL of 25 mM phosphate buffer (pH ranging from 3.5 to 9.5): ACN (9:1) was added and the resulting solution was incubated at room temperature. The stability of the modified creatine kinase **ck** was monitored by injecting samples in the LC-MS after regular intervals of time 12 h and 24 h.

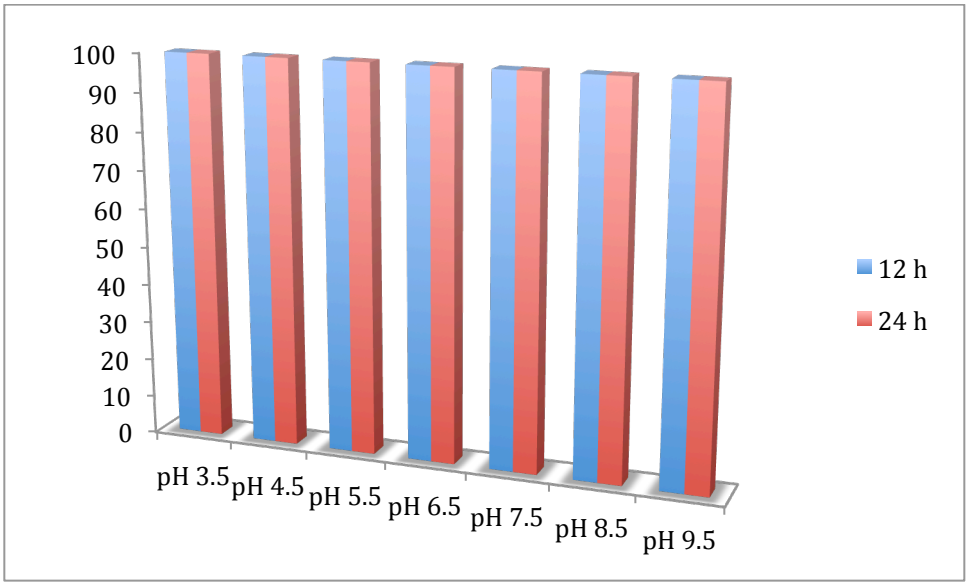


Fig. S18. Stability studies of SASP product of protein creatine kinase (ck). The modified ck obtained from SASP reaction with 2-PCA **2a** and PMB **3b** was incubated under different pH conditions ranging from 3.5-9.5. Reaction conditions: ck-modified product (1 μM) was incubated in 25 mM phosphate buffer at different pH ranging from 3.5 to 9.5 at room temperature. The reaction was monitored by injecting the sample in ESI-MS after 12 h and 24 h. No degradation of the ck-modified Petasis product was observed after 24 h at different pH conditions.

References.

1. W. C. Chan and P. D. White, Fmoc solid phase peptide synthesis: a practical approach, Oxford university press: New York, (2000).
2. N. Grimblat, M. M. Zanardi and A. M. Sarotti, *J. Org. Chem.,* 2015, **80**, 12526-12534.