Supplementary Information

Design and Synthesis of Non-Peptide Mimetics Mapping the Immunodominant Myelin Basic Protein (MBP_{83-96}) Epitope to Function as T-cell Receptor Antagonists

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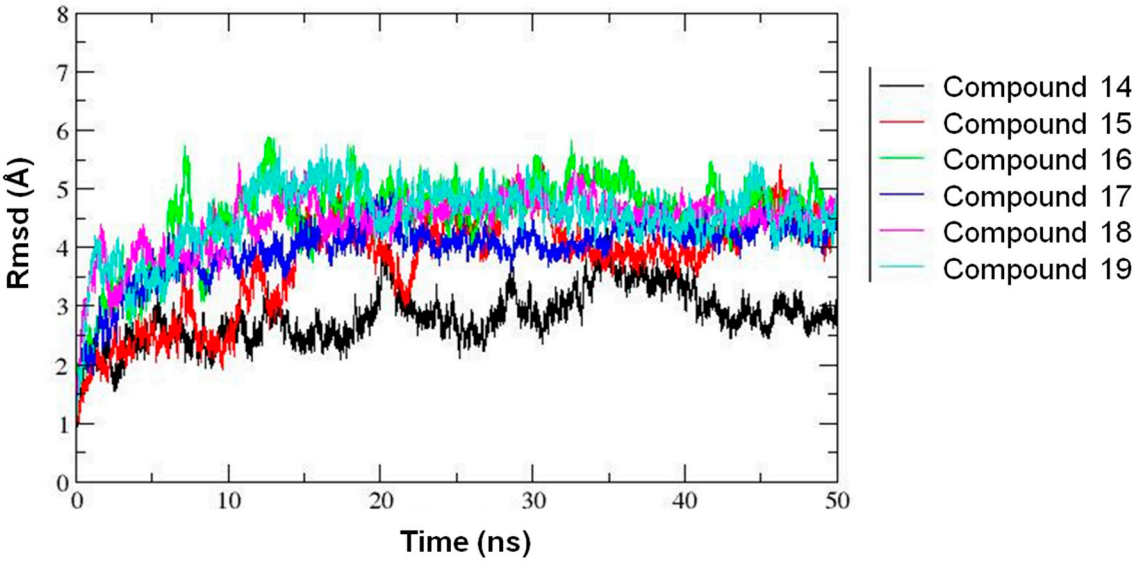
(a)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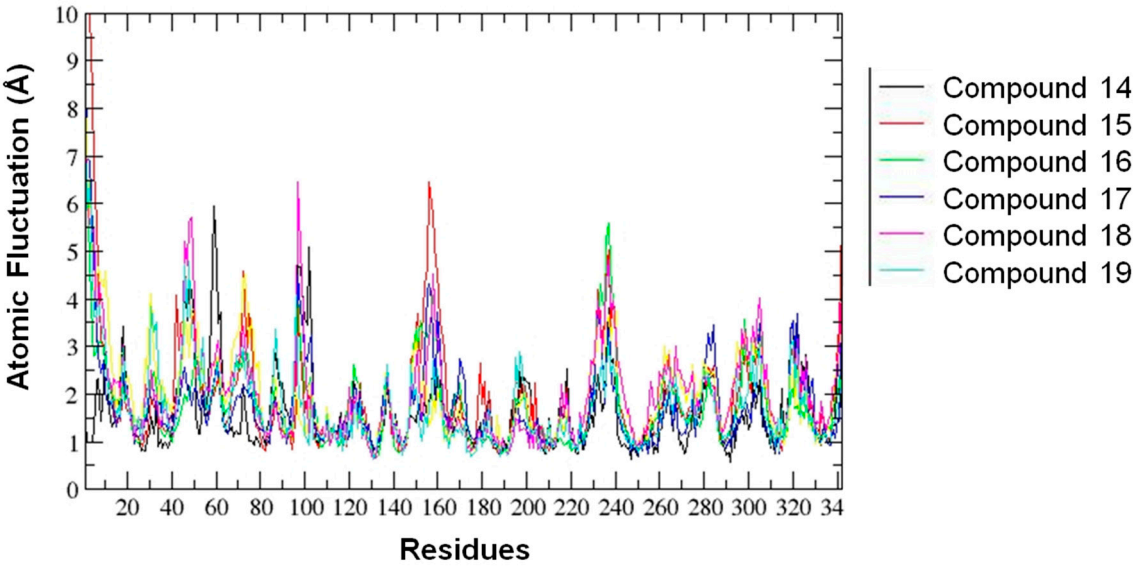
Compound 15
Compound 16
Compound 17
Compound 18
Compound 19

(b)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Compound 18
Compound 19

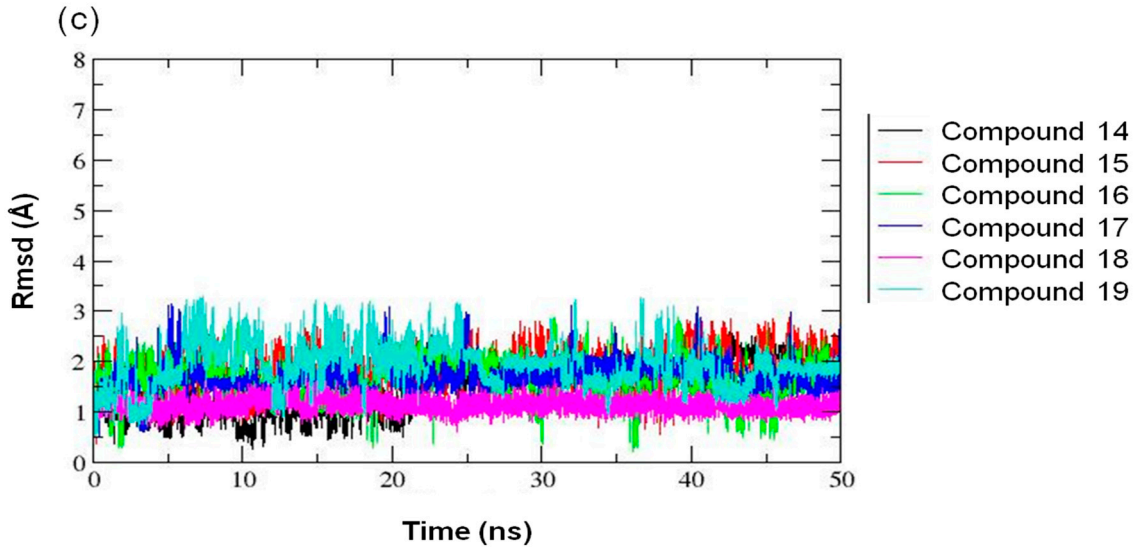
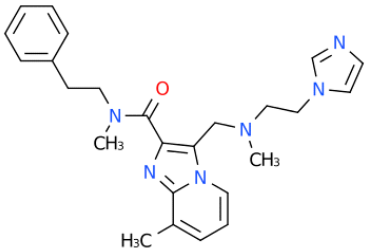
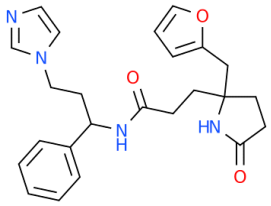
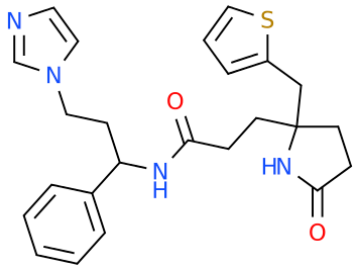
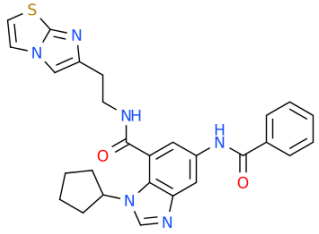
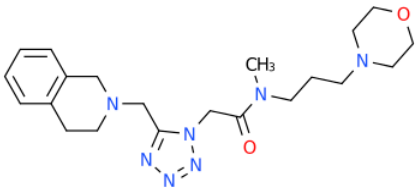
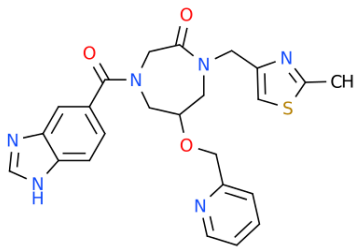
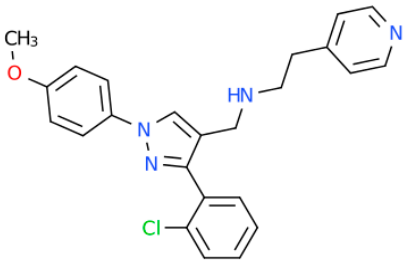
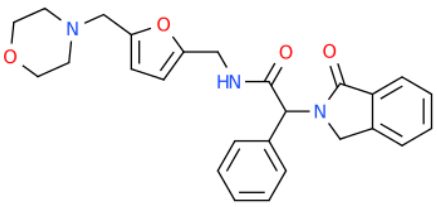
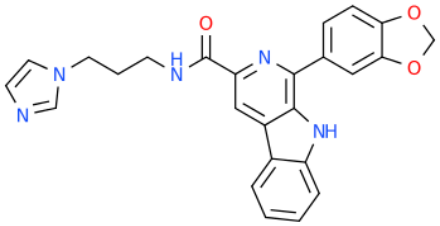
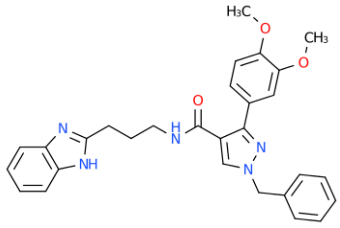
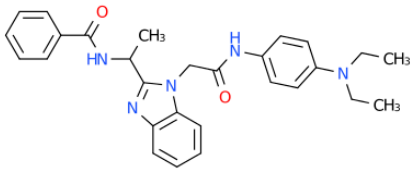
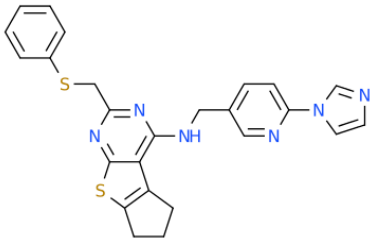
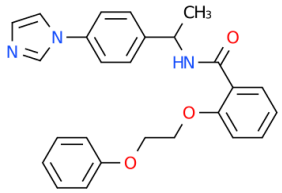


Figure S1. (**a**) Plot of rms values over time for the Cα atoms of T cell receptor (TCR) residues in complex with compounds **14-19**, (**b**) atomic positional fluctuations for the different residues in the TCR for the different molecular dynamics (MD) simulation runs, and (**c**) rms values for compounds **14-19**, during the MD simulations.

Table S1. The chemical structures and properties of compounds **1-13**, purchased from Ambinter Chemicals.

Ambinter Code	**Structure**	**Molecular Weight**	**logP[a]**	**PSA[b]**
Amb11063559 $C_{25}H_{30}N_6O$		430.545	3.2859	58.67
Amb10213450 $C_{24}H_{28}N_4O_3$		420.504	4.1151	89.16

Amb11124336 $C_{24}H_{28}N_4O_2S$		436.57	4.5836	104.26
Amb11124920 $C_{27}H_{26}N_6O_2S$		498.599	5.5493	121.56
Amb11049469 $C_{21}H_{31}N_7O_2$		413.517	0.2879	79.62
Amb11020966 $C_{24}H_{24}N_6O_3S$		476.551	2.6686	132.55
Amb11084608 $C_{24}H_{23}ClN_4O$		418.919	5.3195	51.97
Amb20310491 $C_{26}H_{27}N_3O_4$		445.51	3.392	75.02

Amb562959 $C_{25}H_{21}N_5O_3$		439.466	4.5193	94.06
Amb499010 $C_{29}H_{29}N_5O_3$ **(LEAD MOLECULE)**		495.572	5.2454	94.06
Amb409596 $C_{28}H_{31}N_5O_2$		469.578	5.4761	79.26
Amb509000 $C_{25}H_{22}N_6S_2$		470.612	5.7381	122.06
Amb58395 $C_{26}H_{25}N_3O_3$		427.495	5.212	65.38

[a,b] logP and PSA values are reported as shown on Ambinter Chemicals catalogue: (http://www.ambinter.com/)

Table S2. Semi-empirical (SE) binding energy in solvent, for molecule **15** in complex with the whole TCR and with selected binding site residues (kcal/mol).

Method	Whole Receptor	Selected Site Residues
PM7	-34.39	-24.09
PM6-D	6.00	0.26
PM6-DH2	-0.57	-6.16
AM1	19.30	16.41
PM6	27.89	18.178
RM1	28.60	23.21
PM3	25.65	18.71
PM6-D3	-1.02	-6.21
PM6-DH+	-1.56	-5.87

Table S3. Interaction energies for 20 MD snapshots of analogue **15**, using PM7 in solvent.

MD Snapshot	**Analogue 15** **Interaction Energy (Kcal/mol)**
1	-53.6571
2	-68.5798
3	-51.6185
4	-52.5194
5	-43.5009
6	-47.5047
7	-46.076
8	-43.8191
9	-39.9552
10	-55.3105
11	-48.8477
12	-50.9680
13	-28.8528
14	-44.3055
15	-45.3845
16	-42.7999
17	-52.5803
18	-38.2581
19	-44.1766
20	-48.007
Mean	-47.2926
Standard Deviation	7.9095
Standard Error (SE)	1.77

Table S4. Interaction energy for molecule **15** in solvent as calculated by different density functional theory (DFT) methodologies, employing different basis sets.

DFT Method in Solvent	**Basis Set**					
	cc-pVTZ	**cc-pVDZ**	**6-311G**	**6-31G**	**6-31G****	**6-31+G****
B3lyp	-10.88	-24.40	-18.46	-23.84	-23.78	-6.66
Cam-b3lyp	-18.58	-32.12	-26.74	-31.53		
M06	-33.25	-40.09		-38.58		
M06-2X	-31.63	-40.75				
B97D	-42.41			-54.58		
MPW1PW91	-11.33			-22.23		
BHandH	-41.77			-51.86		
B3lyp-D	-42.85					

Table S5. Interaction energies for analogues **17-19**, using PM7.

Compounds	**Interaction Energy (Kcal/mol)**
17	-35.39
18	-37.28
19	-35.40

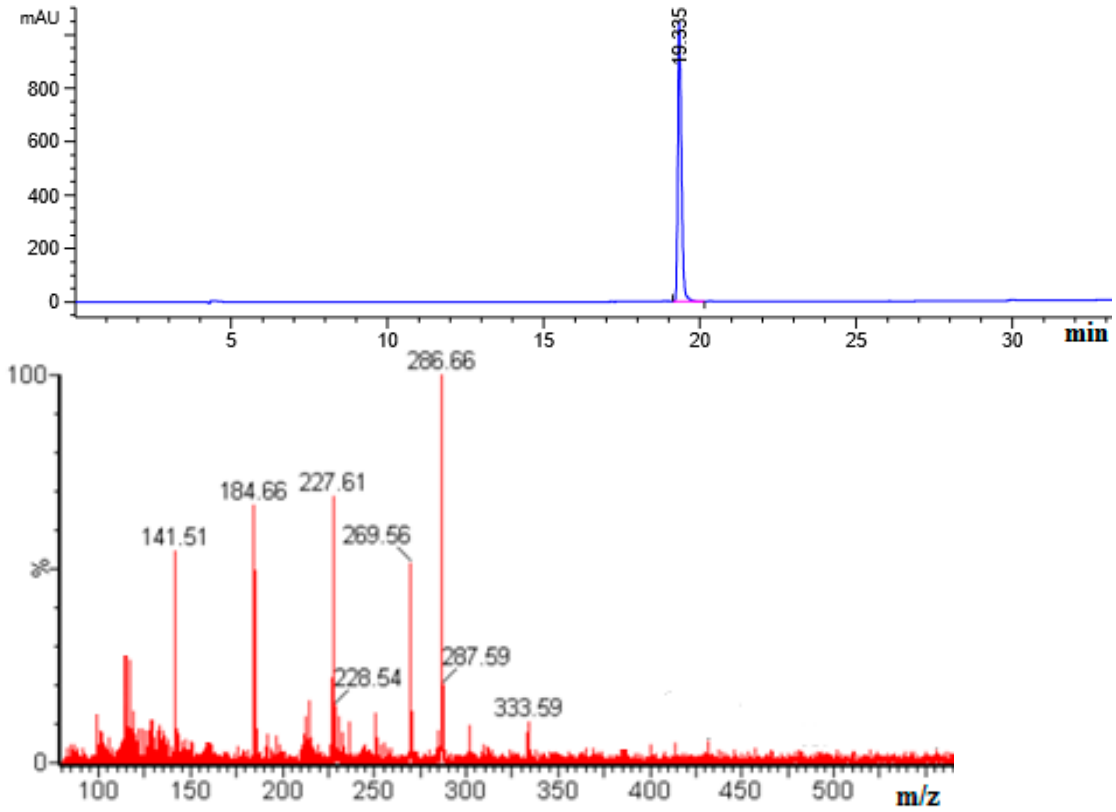


Figure S2. RP-HPLC chromatogram (top) and ESI-MS (bottom) of final analogue **15** ($MW_{theoretical}$: 285.34).

RP-HPLC Conditions:

i) Column: Agilent ZORBAX Eclipse Plus C18 (3.5μm, 100x4.6mm),

ii) Solvents: H_2O (0.08% TFA), AcN (0.08%TFA),

iii) Gradient elution: from 10% AcN to 100% AcN over 30min.

iv) t_R: 19.3 min, Purity: 99%

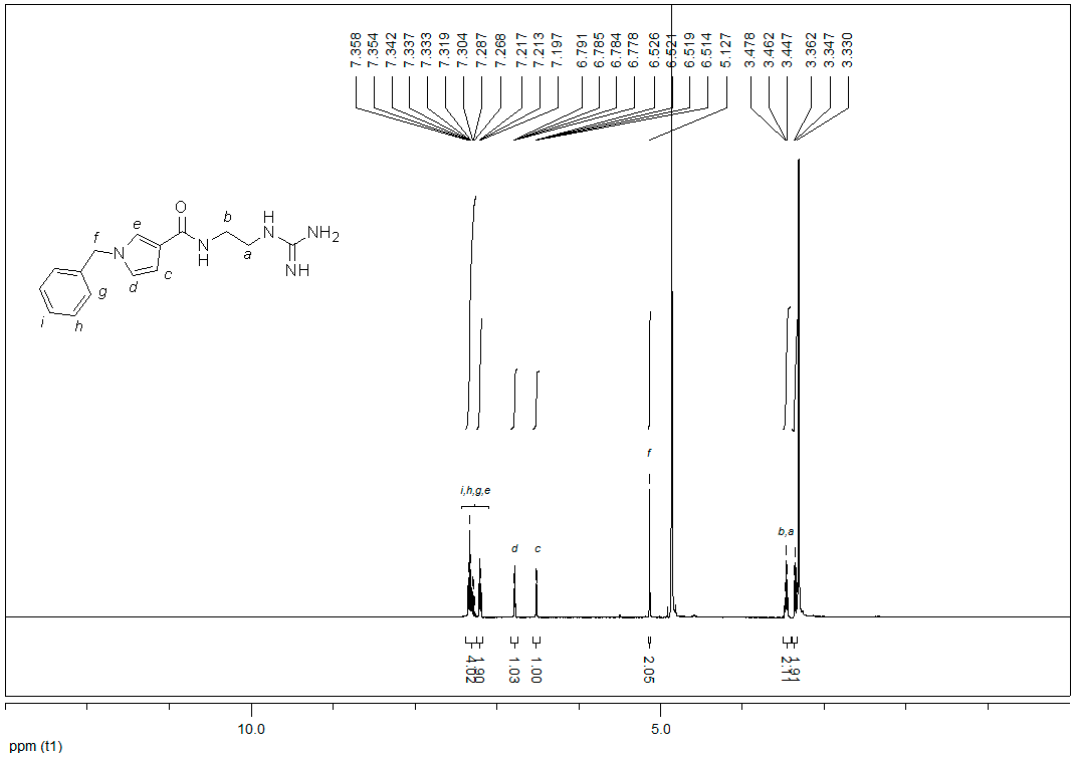


Figure S3. [1]H NMR spectra of analogue **15** (400 MHz, CD_3OD) δ 7.27-7.36 (m, 4 H, Ph, Ar), 7.20-7.22 (m, 2 H, Ph), 6.78 (dd, 1 H, *J* = 2.8, 2.4 Hz, Ar), 6.52 (dd, 1 H, *J* = 2.8, 2.0 Hz, Ar), 5.13 (s, 2 H, CH_2Ph), 3.46 (t, 2 H, *J* = 6.3 Hz, CH_2), 3.35 (t, 2 H, *J* = 6.3 Hz, CH_2).

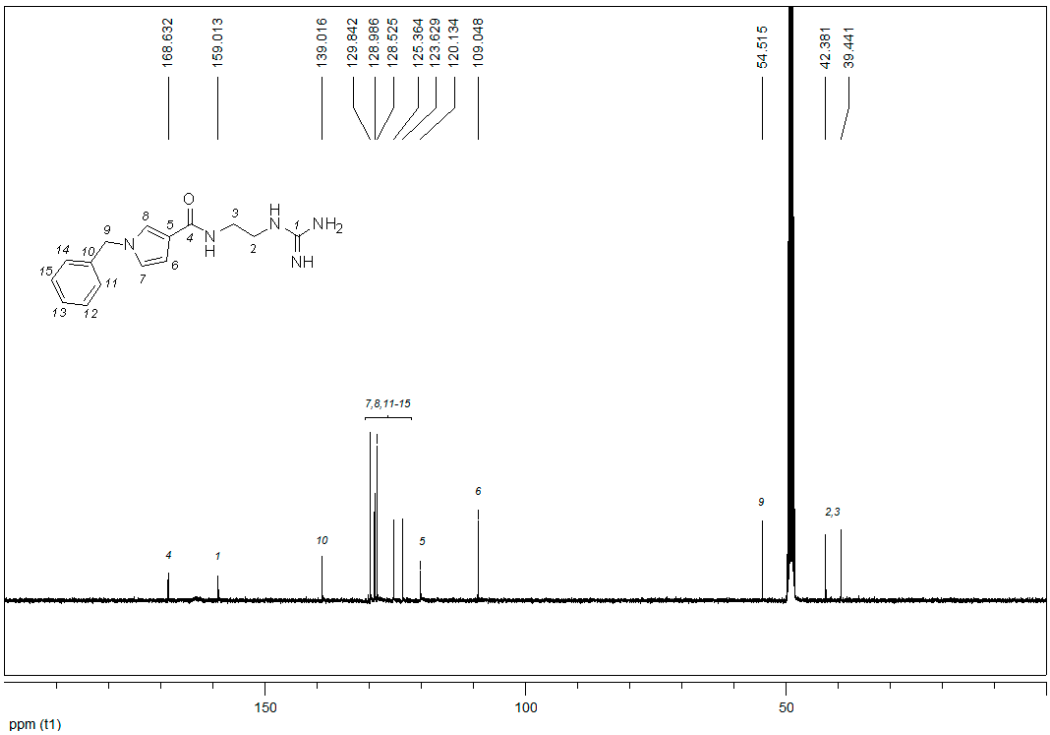


Figure S4. [13]C NMR spectra of analogue **15** (100 MHz, CD_3OD) δ 168.6 (C=O), 159.0 (C=NH), 139.0 (C Ph), 129.8 (2 × CH Ph), 129.0 (CH), 128.5 (2 × CH Ph), 125.4 (CH), 123.6 (CH), 120.1 (C Ar), 109.0 (CH Ar), 54.5 (CH_2Ph), 42.4 (CH_2), 39.4 (CH_2).

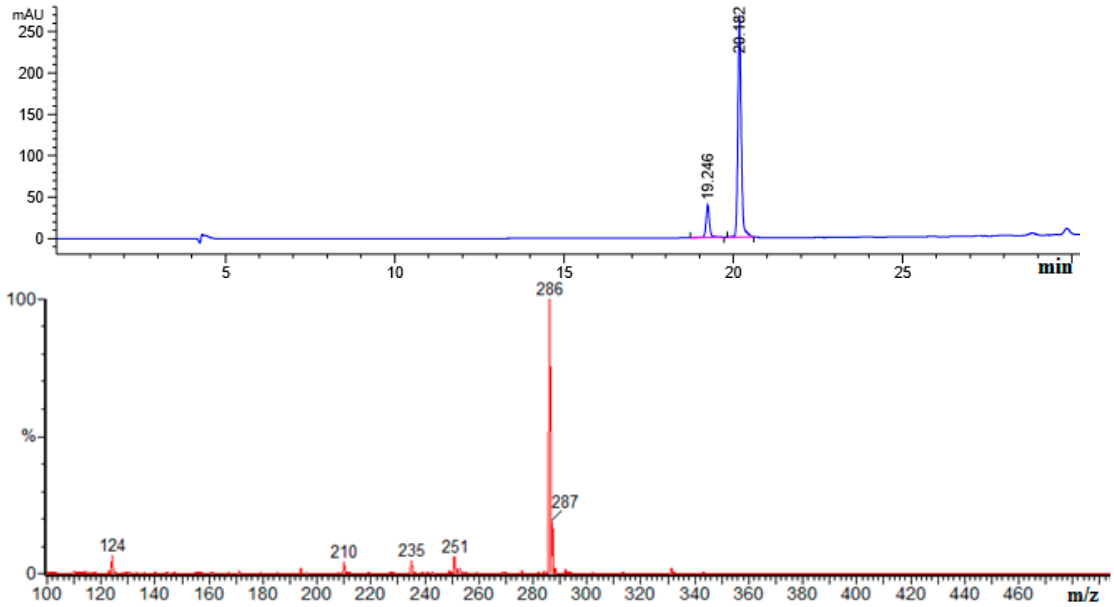


Figure S5. RP-HPLC chromatogram (top) and ESI-MS (bottom) of final analogue **16** ($MW_{theoretical}$: 285.34).

RP-HPLC Conditions:

i) Column: Agilent ZORBAX Eclipse Plus C18 (3.5µm, 100x4.6mm),

ii) Solvents: H_2O (0.08% TFA), AcN (0.08%TFA),

iii) Gradient elution: from 5% AcN to 100% AcN over 30min.

iv) t_R: 20.2 min.

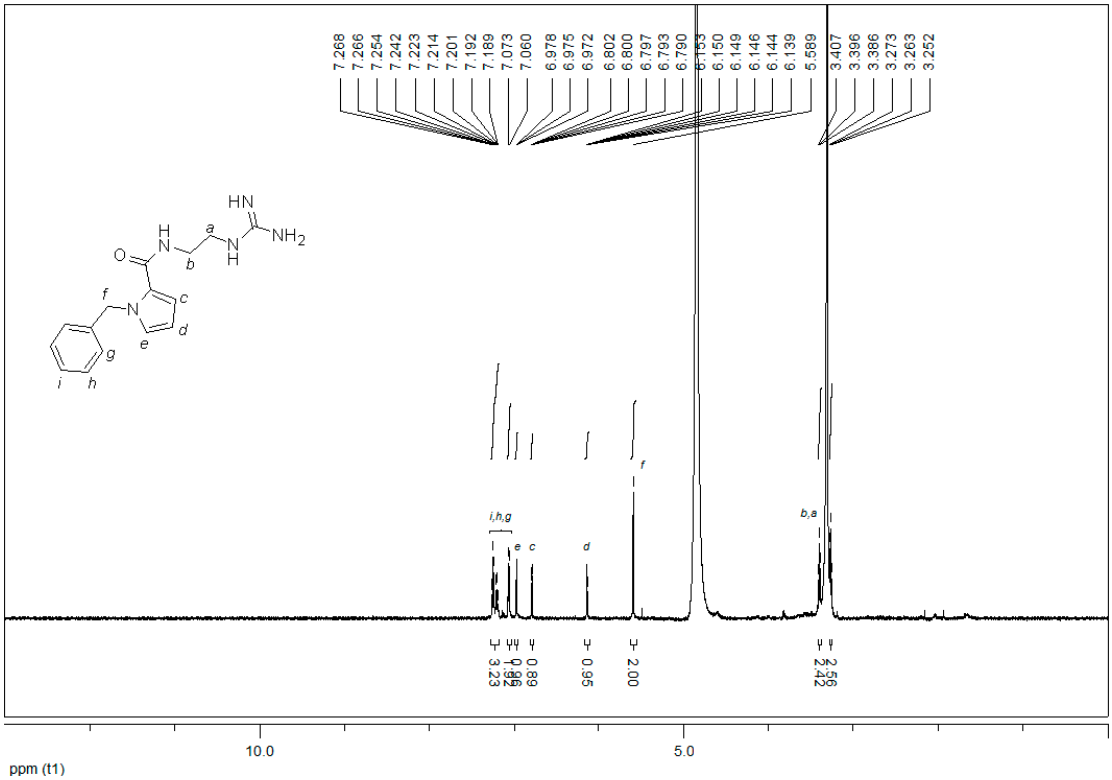


Figure S6. ^{1}H NMR spectra of analogue **16** (600 MHz, CD_3OD) δ 7.19-7.27 (m, 3 H, Ph), 7.07 (d, 2 H, = 7.8 Hz, Ph), 6.97-6.98 (m, 1 H, Ar), 6.79-6.80 (m, 1 H, Ar), 6.14-6.15 (m, 1 H, Ar), 5.59 (s, 2 H, 3.40 (t, 2 H, *J* = 6.3 Hz, CH_2), 3.26 (t, 2 H, *J* = 6.3 Hz, CH_2).

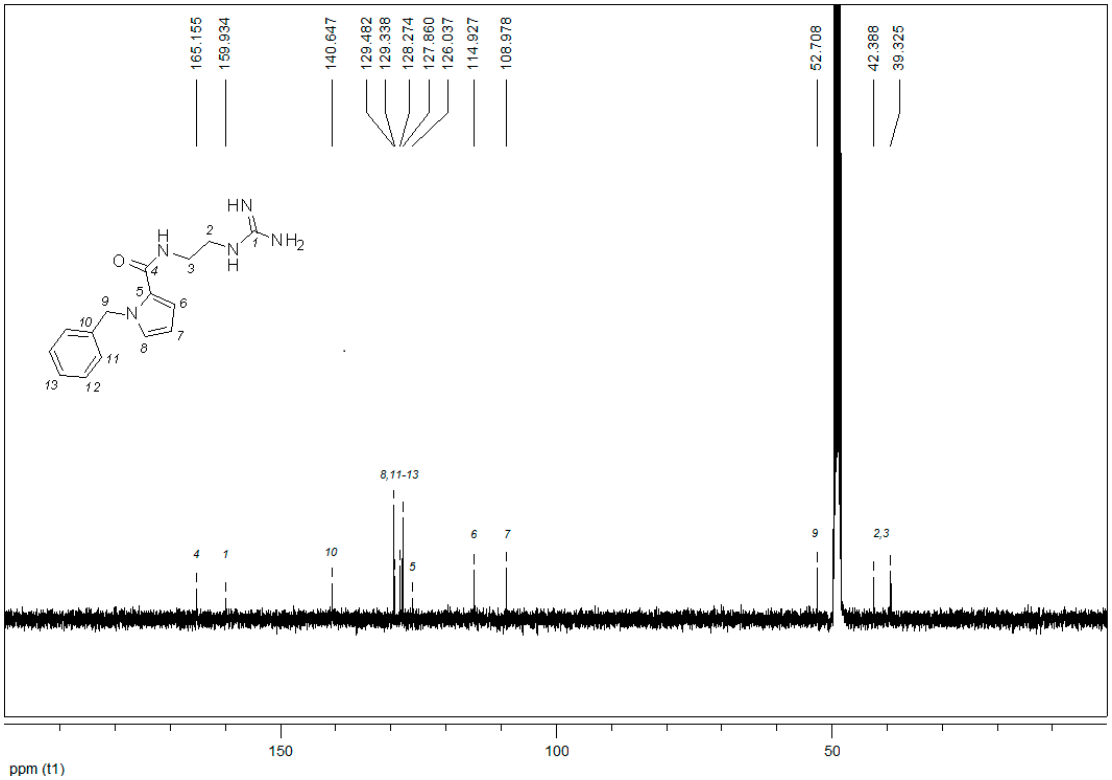


Figure S7. ^{13}C NMR spectra of analogue **16** (100 MHz, CD_3OD) δ 165.2 (C=O), 159.9 (C=NH), 140.6 Ph), 129.5 (2 × CH Ph), 129.3 (CH), 128.3 (CH), 127.9 (2 × CH Ph), 126.0 (C Ar), 114.9 (CH Ar), 109.0 (CH Ar), 52.7 (CH_2Ph), 42.4 (CH_2), 39.3 (CH_2).

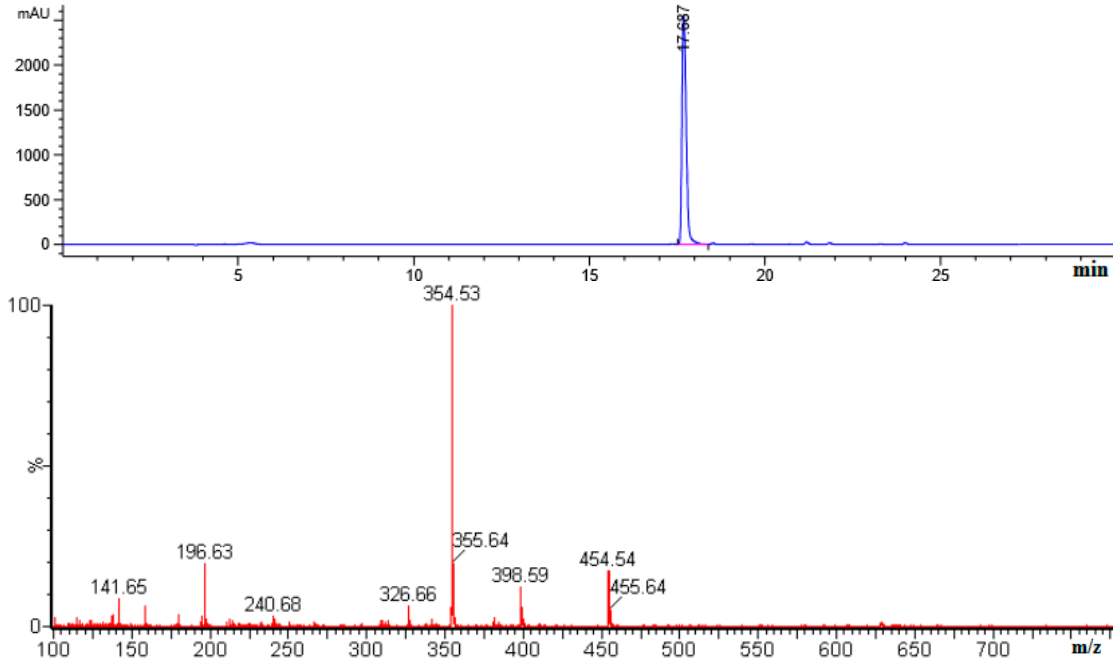


Figure S8. RP-HPLC chromatogram (top) and ESI-MS (bottom) of final analogue **17** ($MW_{theoretical}$: 353.48).

RP-HPLC Conditions:

i) Column: Agilent ZORBAX Eclipse Plus C18 (3.5μm, 100x4.6mm),

ii) Solvents: H_2O (0.08% TFA), AcN (0.08%TFA),

iii) Gradient elution: from 10% AcN to 100% AcN over 30min.

iv) t_R: 17.7 min, Purity: 99%

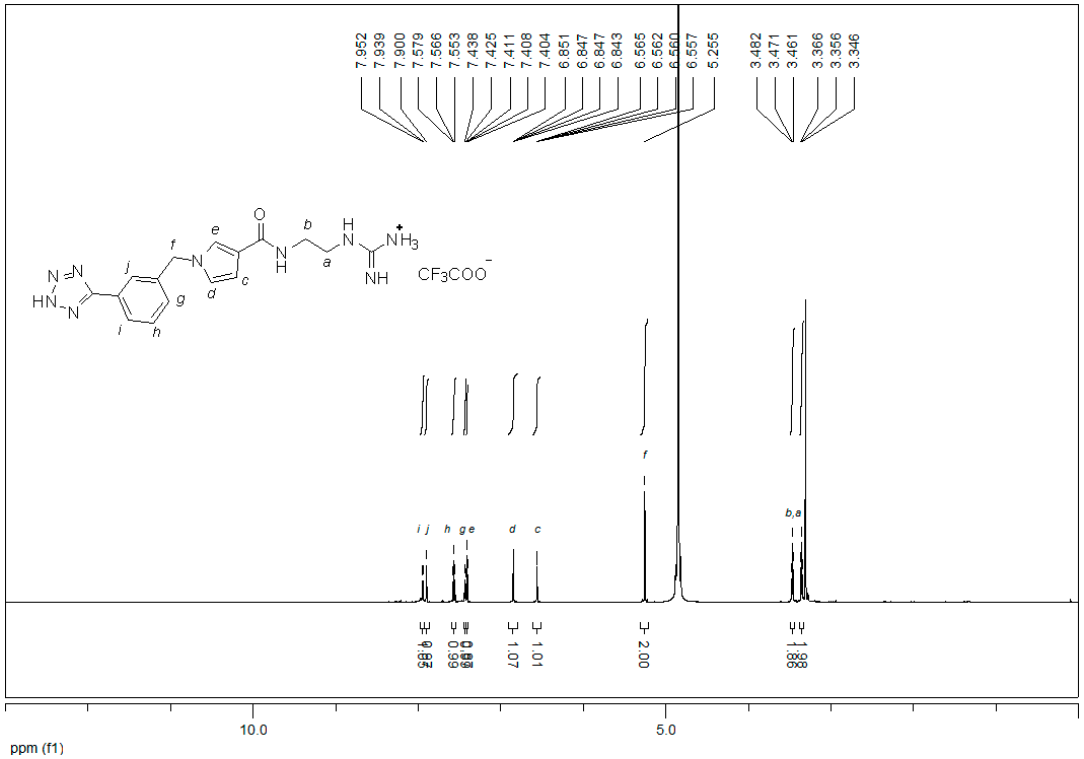


Figure S9. ^{1}H NMR spectra of analogue **17** (600 MHz, CD_3OD) δ 7.95 (d, 1 H, *J* = 7.8 Hz, Ar'), 7.90 (s, 1 H, Ar'), 7.57 (t, 1 H, *J* = 7.8 Hz, Ar'), 7.43 (d, 1 H, *J* = 7.8 Hz, Ar'), 7.41 (app dd, 1 H, *J* = 2.4, 1.8 Hz, Ar), 6.85 (dd, 1 H, *J* = 3.0, 2.4 Hz, Ar), 6.56 (dd, 1 H, *J* = 3.0, 1.8 Hz, Ar), 5.26 (s, 2 H, CH_2Ar'), 3.47 (t, 2 H, *J* = 6.0 Hz, CH_2), 3.36 (t, 2 H, *J* = 6.0 Hz, CH_2).

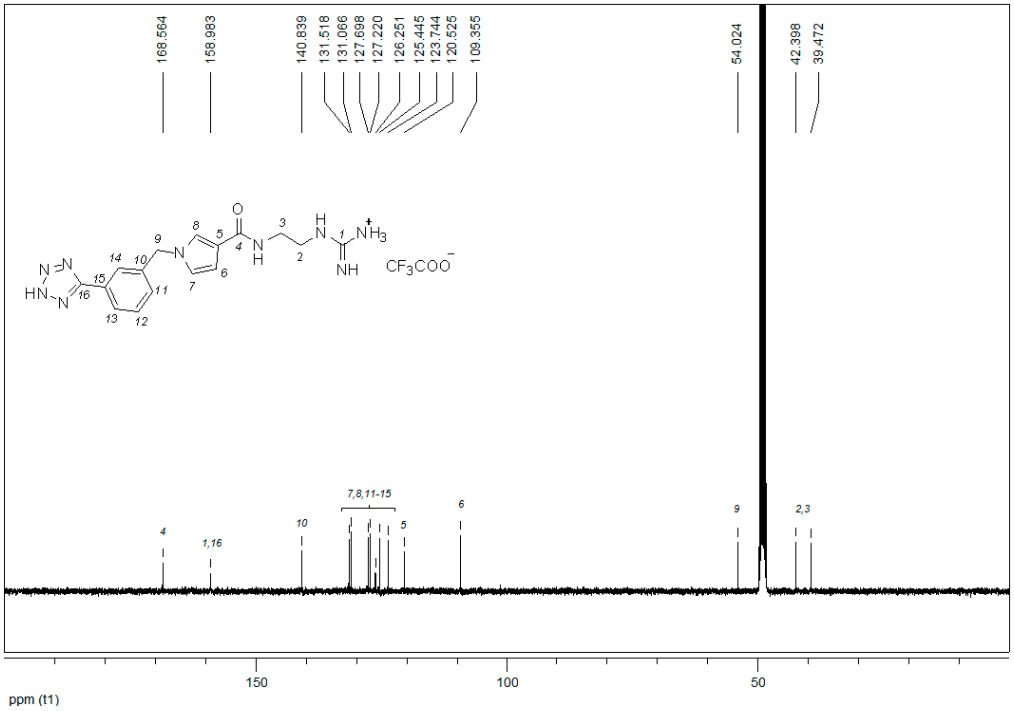


Figure S10. ^{13}C NMR spectra of analogue **17** (100 MHz, CD_3OD) δ 168.6 (C=O), 159.0 (2 × C=NH), 140.8 (C Ar'), 131.5 (CH), 131.1 (CH), 127.7 (CH), 127.2 (CH), 126.3 (C Ar'), 125.4 (CH), 123.7 (CH), 120.5 (C Ar), 109.4 (CH Ar), 54.0 (CH_2Ar'), 42.4 (CH_2), 39.5 (CH_2).

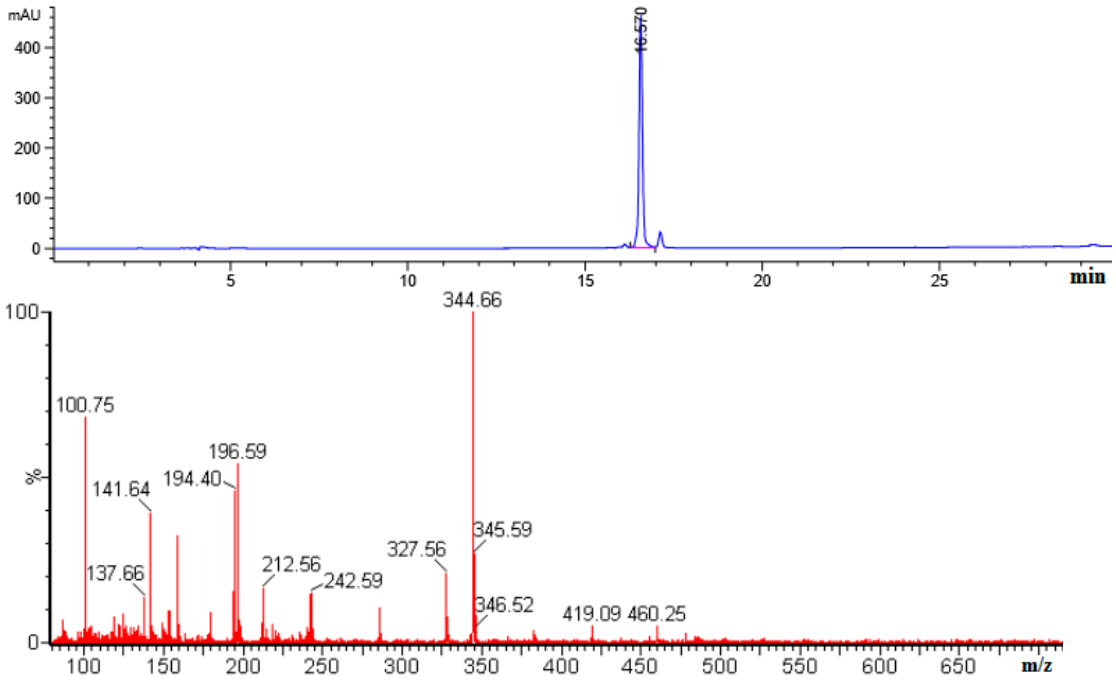


Figure S11. RP-HPLC chromatogram (top) and ESI-MS (bottom) of final analogue **18** ($MW_{theoretical}$: 343.39).

RP-HPLC Conditions:

i) Column: Agilent ZORBAX Eclipse Plus C18 (3.5µm, 100x4.6mm),

ii) Solvents: H_2O (0.08% TFA), AcN (0.08%TFA),

iii) Gradient elution: from 10% AcN to 100% AcN over 30min.

iv) t_R: 16.6 min, Purity: 98%.

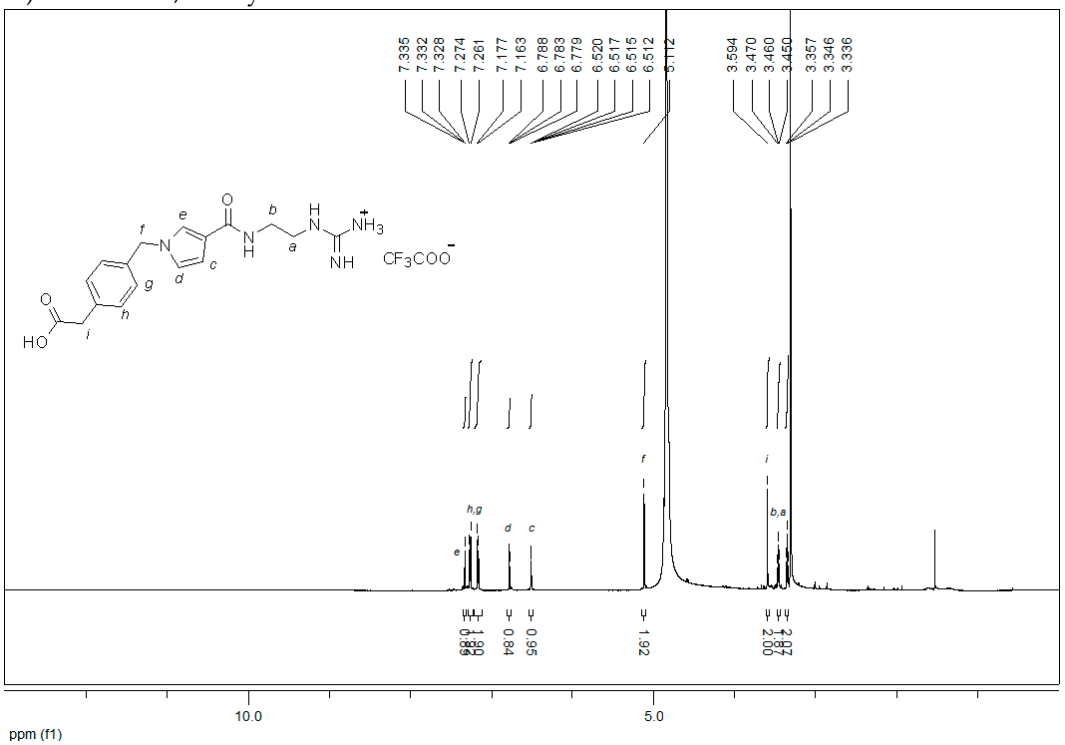


Figure S12. ^{1}H NMR spectra of analogue **18** (600 MHz, CD_3OD) δ 7.33 (app dd, 1 H, *J* = 2.4, 1.8 Hz, Ar), 7.27 (d, 2 H, *J* = 8.1 Hz, Ar'), 7.17 (d, 2 H, *J* = 8.1 Hz, Ar'), 6.78 (dd, 1 H, *J* = 3.0, 2.4 Hz, Ar), 6.52 (dd, 1 H, *J* = 3.0, 1.8 Hz, Ar), 5.11 (s, 2 H, CH_2Ar'), 3.59 (s, 2 H, CH_2CO_2H), 3.46 (t, 2 H, *J* = 6.0 Hz, CH_2), 3.35 (t, 2 H, *J* = 6.0 Hz, CH_2).

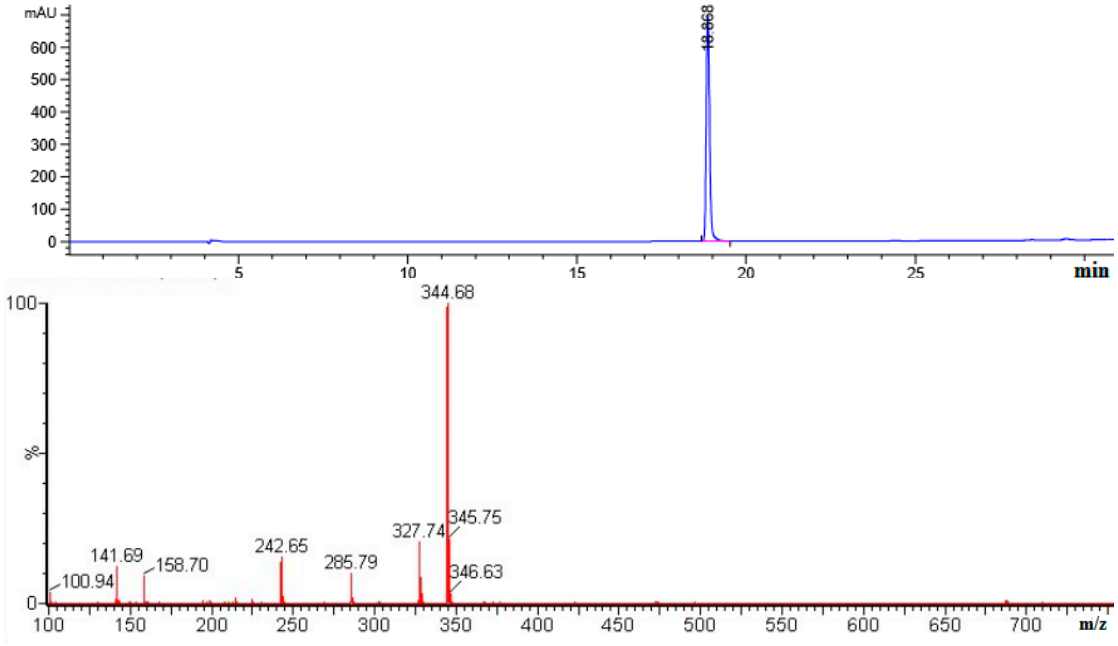


Figure S13. RP-HPLC chromatogram (top) and ESI-MS (bottom) of final analogue **19** ($MW_{theoretical}$: 343.38).

RP-HPLC Conditions:

i) Column: Agilent ZORBAX Eclipse Plus C18 (3.5µm, 100x4.6mm),

ii) Solvents: H_2O (0.08% TFA), AcN (0.08%TFA),

iii) Gradient elution: from 5% AcN to 100% AcN over 30min.

iv) t_R: 18.9 min, Purity: 99%.

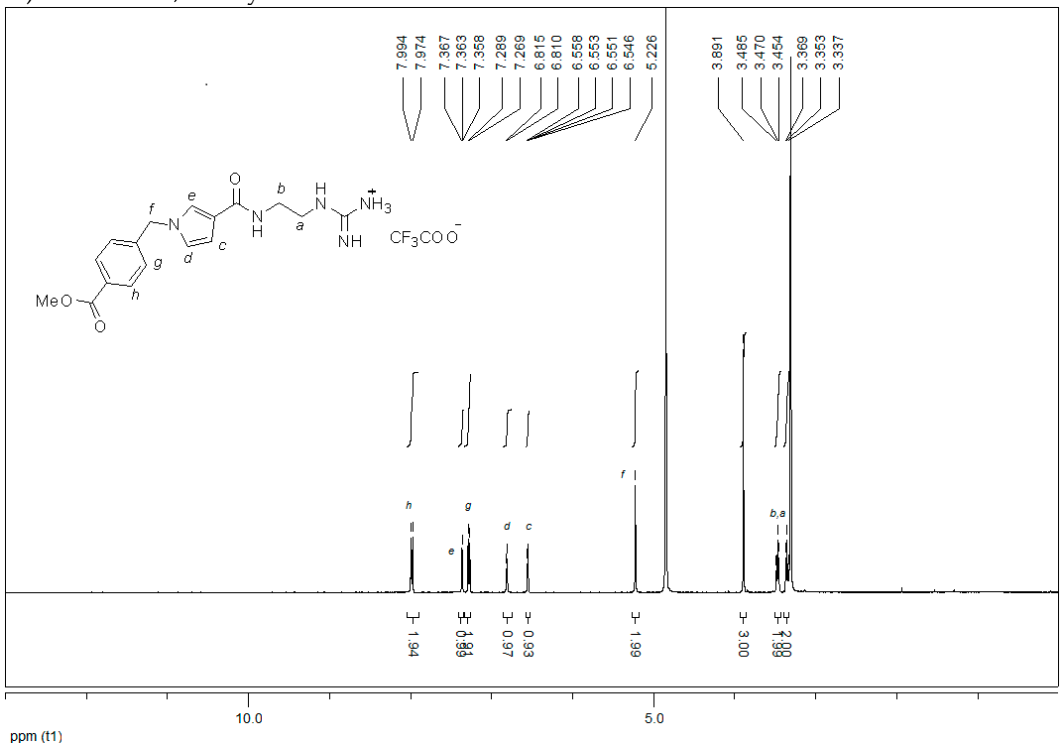


Figure S14. ^1H NMR spectra of analogue **19** (400 MHz, CD_3OD) δ 7.98 (d, 2 H, *J* = 8.0 Hz, Ar'), 7.36 (app t, 1 H, *J* = 2.0 Hz, Ar), 7.28 (d, 2 H, *J* = 8.0 Hz, Ar'), 6.81-6.82 (m, 1 H, Ar), 6.55 (dd, 1 H, *J* = 2.8, 2.0 Hz, Ar), 5.23 (s, 2 H, CH_2Ar'), 3.89 (s, 3 H, OCH_3), 3.47 (t, 2 H, *J* = 6.4 Hz, CH_2), 3.35 (t, 2 H, *J* = 6.4 Hz, CH_2).