SUPPORTING INFORMATION:

Unveiling and Tackling Guanidinium Peptide Coupling Reagent Side Reactions towards the Development of Peptide-Drug Conjugates

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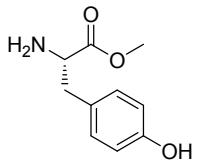
Contents

1. NMR characterization of the methyl esters of amino acids 8-14

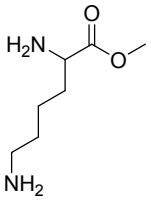
Compound 8 (Tyr-OMe): (S)-methyl 2-amino-3-(4-hydroxyphenyl)propanoate:



The amino acid analogue of tyrosine was obtained as a white solid in 98% yield.

^{1}H-NMR (250 MHz, DMSO-d_6): δ(ppm) 9.46 (s, 1H), 8.52 (s, 3H), 7.04 (d, J = 8.4 Hz, 2H), 6.75 (d, J= 8.4Hz, 2H), 4.22 (t, J = 6.4Hz, 1H), 3.71 (s, 3H), 3.04 (t, J= 6.1Hz, 1H). ^{13}C-NMR (63 MHz, DMSO-d_6): δ(ppm) 170.44, 157.03, 131.34, 125.21, 116.38, 54.36, 53.54, 36.09.

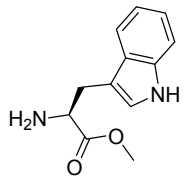
Compound 9 (Lys-OMe): Methyl 2, 6-diaminohexanoate hydrochloride:



The amino acid analogue of lysine was obtained as a white solid in 96% yield.

^{1}H-NMR (250 MHz, DMSO-d_6): δ(ppm) 8.62 (br, 2H), 8.09 (br, 2H), 3.97 (t, J = 5 Hz, 1H), 3.73 (s, 3H), 3.34 (d, J = 2.5Hz, 2H), 2.72 (t, J = 7.5 Hz, 2H), 1.84-1.75 (m, 2H), 1.61-1.48 (m, 2H). ^{13}C-NMR (63 MHz, DMSO-d_6): δ(ppm) 169.87, 52.8, 51.61, 38.14, 29.27, 26.20, 21.18

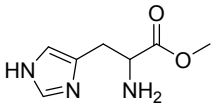
Compound 10 (Trp-OMe): (S)-methyl 2-amino-3-(1H-indol-3-yl)propanoate:



The amino acid analogue of tryptophan was obtained as a brown solid in 96% yield.

^{1}H-NMR (250 MHz, DMSO-d_6): δ(ppm) 11.09 (s, 1 H), 8.51 (s, 3H), 7.49 (d, J = 7.5 Hz, 1H), 7.37 (d, J= 7.8Hz, 1H), 7.23 (d, J = 2.3Hz, 1H), 7.09 (t, J = 7.1 Hz, 1H), 7.00 (t, J= 7.1Hz, 1H), 4.24 (br, 1H), 3.65 (s, 3H), 3.28 (dd, J= 3.6Hz, 6.3Hz, 2H).^{13}C-NMR (63 MHz, DMSO-d_6): δ(ppm) 169.81, 136.25, 126.90, 125.02, 121.25, 118.7, 117.99, 111.63, 106.29, 52.73, 52.69, 26.15.

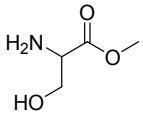
Compound 11 (His-OMe): Methyl 2-amino-3-(1H-imidazol-4-yl)propanoate:



The amino acid analogue of histidine was obtained as a grey solid in 94% yield.

^{1}H-NMR (250 MHz, DMSO-d_6): δ (ppm) 8.96 (s, 1H), 7.46 (s, 1H), 4.41 (t, J = 7.5 Hz, 1H), 3.72 (s, 3H), 5.12 (br, 2H), 3.35 (d, J = 2.5Hz, 2H). ^{13}C-NMR (63 MHz, DMSO-d_6): δ(ppm) 168.75, 134.26, 126.99, 118.01, 52.9, 51.12, 25.25.

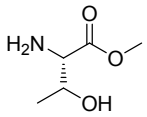
Compound 12 (Ser-OMe): Methyl 2-amino-3-hydroxypropanoate:



The amino acid analogue of serine was obtained as a white solid in 89% yield.

^{1}H-NMR (500 MHz, DMSO-d_6): δ(ppm) 5.54 (br, 2H), 4.06 (d, J = 2.5Hz, 2H), 3.59 (s, 3H), 3.40 (br, 1H), 3.19 (t, J = 5Hz, 2H). ^{13}C-NMR (63 MHz, DMSO-d_6): δ(ppm) 170.5, 63.7, 58.4, 55.3.

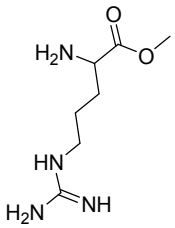
Compound 13 (Thr-OMe): (2S,3S)-methyl 2-amino-3-hydroxybutanoate:



The amino acid analogue of threonine was obtained as a sticky white solid in 91.5% yield.

^{1}H-NMR (250 MHz, DMSO-d_6): δ(ppm) 8.42 (s, 3H), 5.68 (d, J= 4.4Hz, 1H), 4.16 (m, 1H), 3.96 (d, J= 3.8Hz, 1H), 3.78 (s, 3H), 1.24 (d, J = 6.6 Hz, 3H). ^{13}C-NMR (63 MHz, DMSO-d_6): δ(ppm) 169.59, 65.94, 58.81, 53.73, 20.93.

Compound 14 (Arg-OMe): Methyl 2-amino-5-guanidinopentanoate:



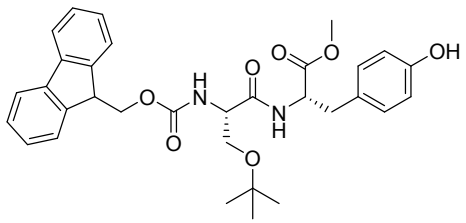
The amino acid analogue of arginine was obtained as a whitesolid in 97% yield.

^{1}H-NMR (250 MHz, DMSO-d_6): δ(ppm) 2.29 (dt, *J* = 1.4, 5.4 Hz, 2H), 3.09 (m, 5H), 3.61 (m, 3H), 3.67 (s, 3H), 7.41 (m, 10H), 7.91 (m, 2H), 8.43 (br, 1H).
^{13}C-NMR (100 MHz, DMSO-d_6): δ(ppm) 24.8, 30.0, 44.3, 46.4, 52.7, 53.3, 54.0, 120.6, 121.9, 127.9, 129.5, 157.6, 159.4, 161.3, 172.6, 173.9.

2. Mass characterization of the amino dipeptide coupling products

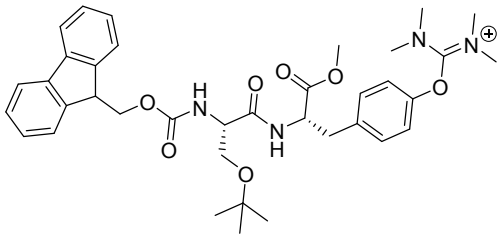
Compounds 15 (Fmoc-Ser(tBu)-Tyr-OMe)

Compound 15a: (S)-methyl 2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tert-butoxy)propanamido)-3-(4-hydroxyphenyl)propanoate:



Mass: ESI-MS *m/z*: calcd: 560.25 $[M+H]^+$; found: 599.97 $[M+K]^+$, 583.99 $[M+Na]^+$.

Compound 15b: 2-(4-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tert-butoxy)propanamido)-3-methoxy-3-oxopropyl)phenyl)-1,1,3,3-tetramethylisouronium:



Mass: ESI-MS *m/z*: calcd: 659.34 $[M+H]^+$; found: 660.14 $[M+H]^+$.

HATU 1.5 equivalents:

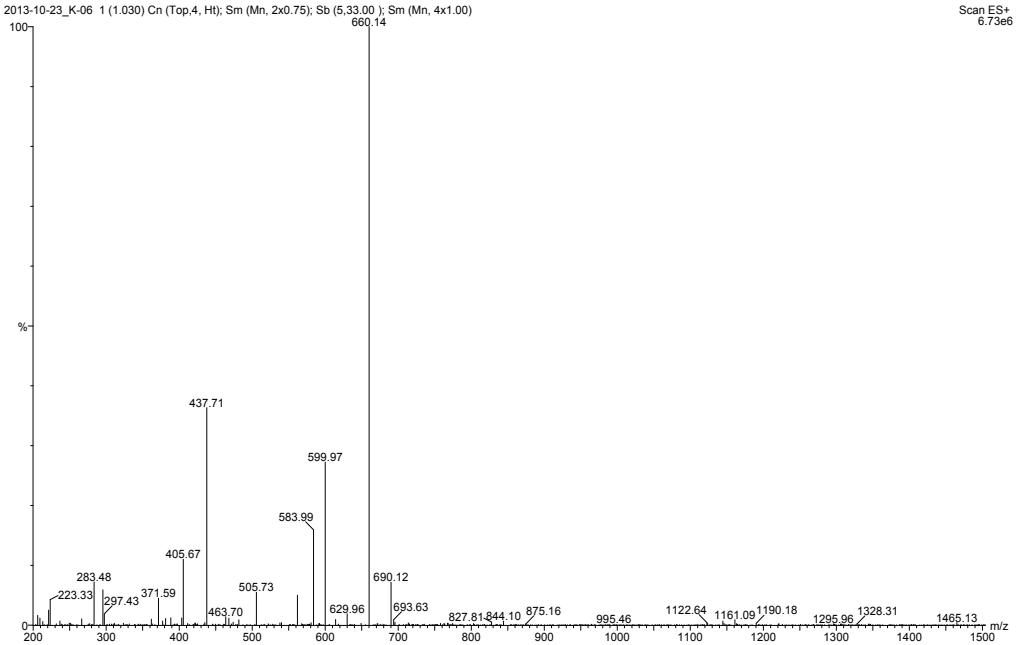


Fig. S1 Mass spectrum of compounds 15 with 1.5 eq of HATU.

HATU 1.0 equivalent:

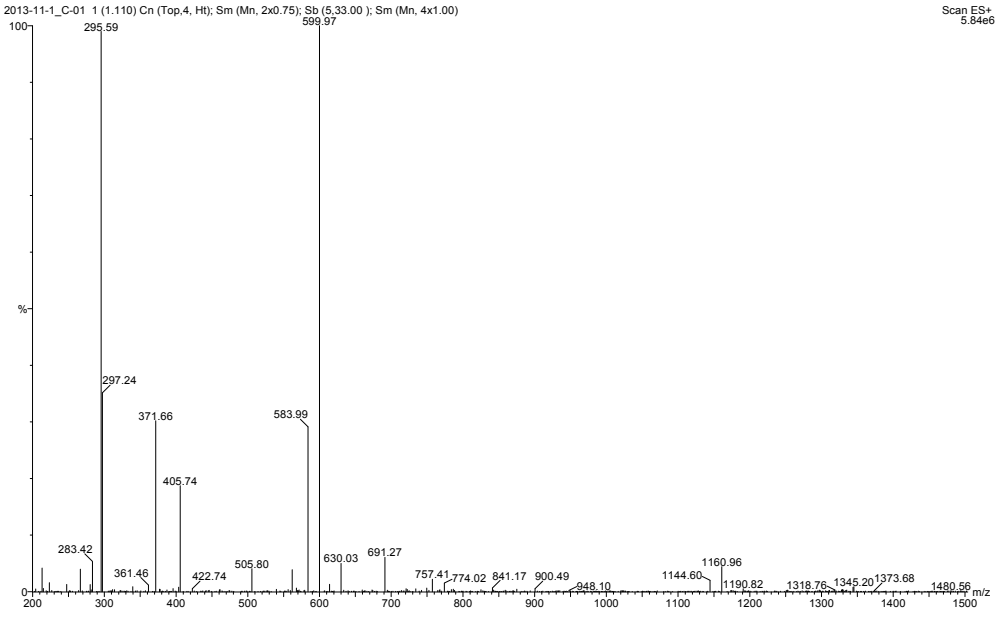


Fig. S2 Mass spectrum of compounds 15 with 1 eq of HATU.

HBTU 1.5 equivalents:

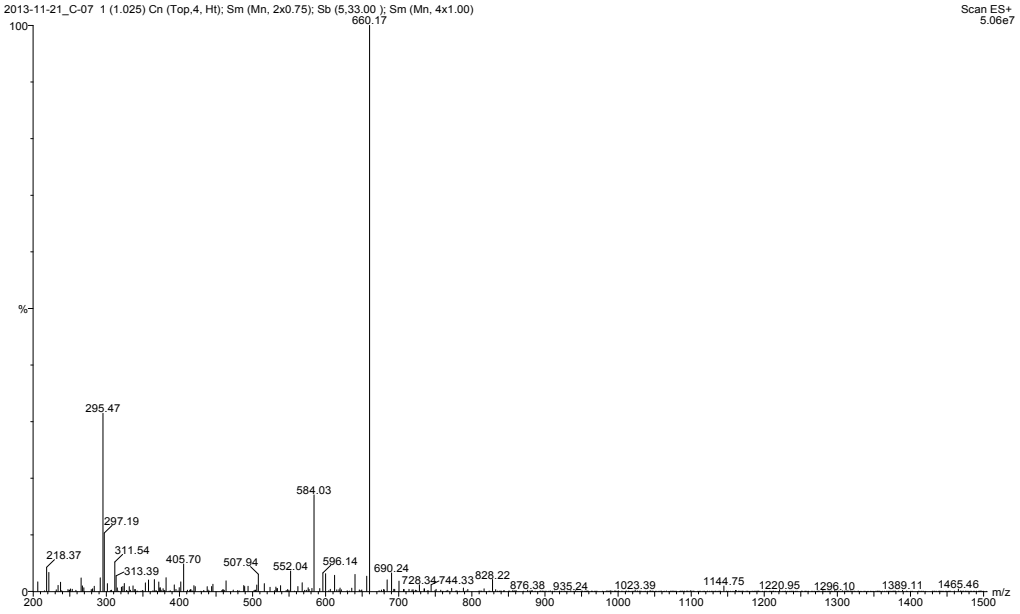


Fig. S3 Mass spectrum of compounds 15 with 1.5 eq of HBTU.

HBTU 1.0 equivalent:

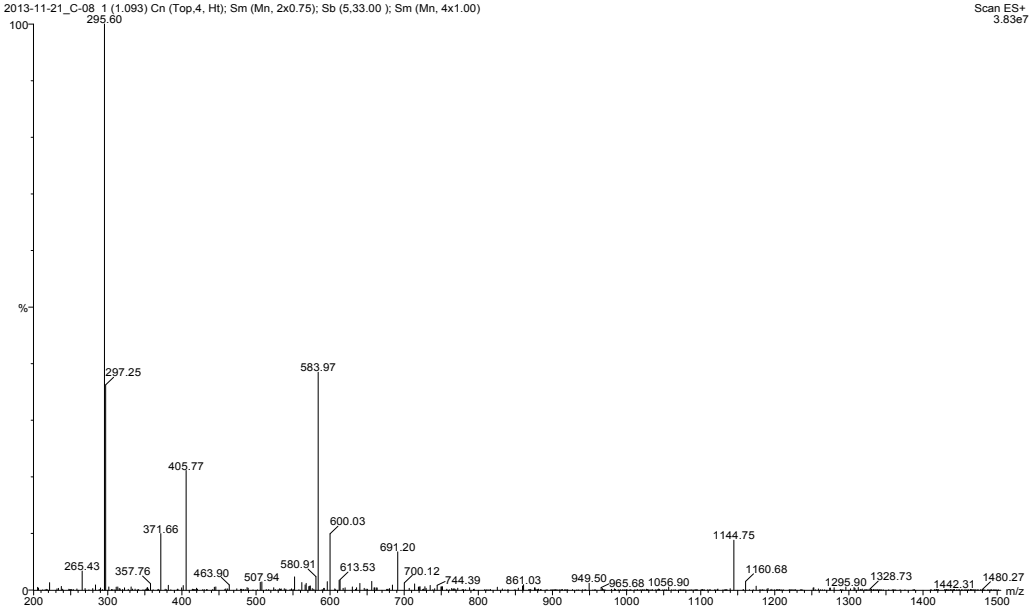
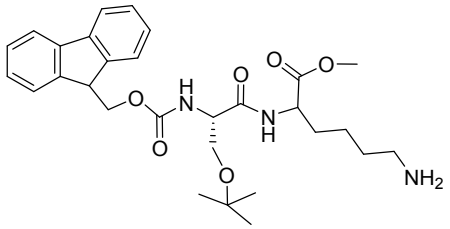


Fig. S4 Mass spectrum of compounds 15 with 1 eq of HBTU.

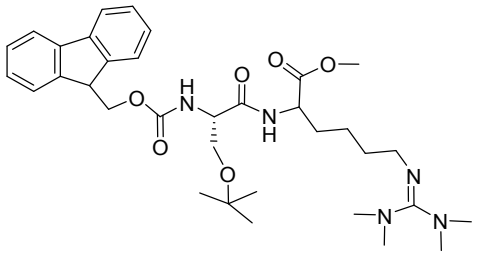
Compounds 16 (Fmoc-Ser(tBu)-Lys-OMe)

Compound 16a: Methyl 2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tert-butoxy)propanamido)-6-aminohexanoate:



Mass: ESI-MS *m/z*: calcd: 525.28 $[M+H]^+$; found: 526.2 $[M+K]^+$.

Compound 16b: (S)-methyl 2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tert-butoxy)propanamido)-3-(4-hydroxyphenyl)propanoate**:**



Mass: ESI-MS *m/z*: calcd: 623.37 $[M+H]^+$; found: 624.3 $[M+H]^+$.

HATU 1.5 equivalents:

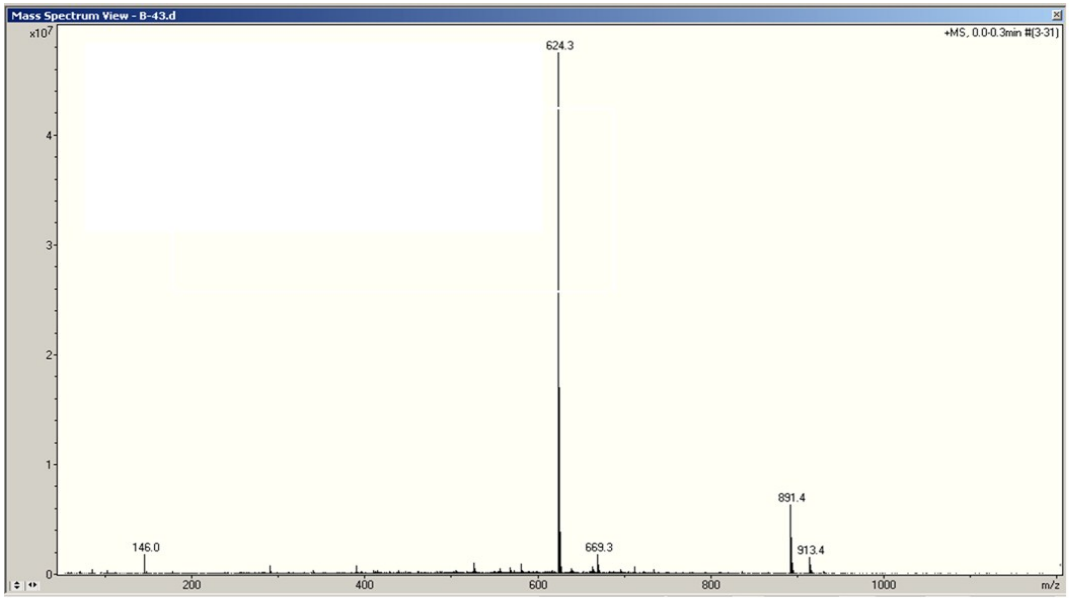


Fig. S5 Mass spectrum of compounds 16 with 1.5 eq of HATU.

HATU 1.0 equivalent:

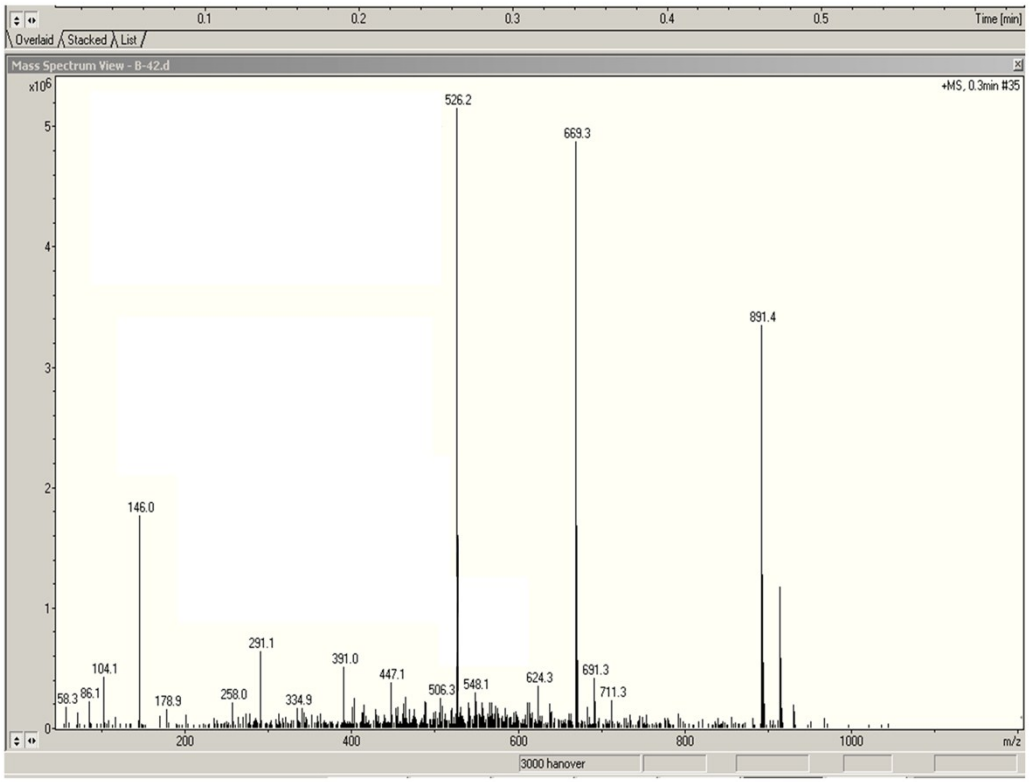


Fig. S6 Mass spectrum of compounds 16 with 1 eq of HATU.

HBTU 1.5 equivalents:

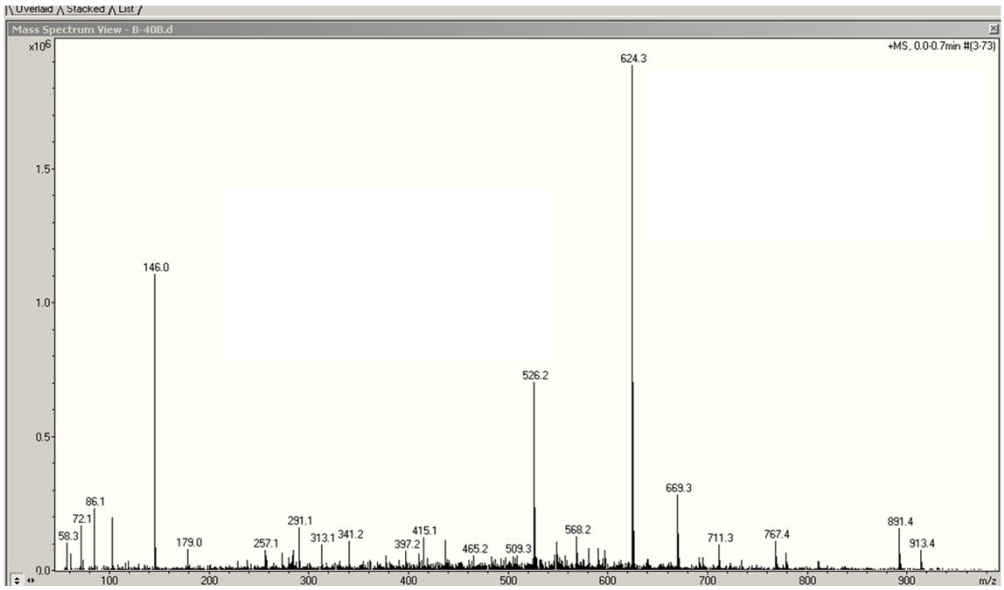


Fig. S7 Mass spectrum of compounds 16 with 1.5 eq of HBTU.

HBTU 1.0 equivalent:

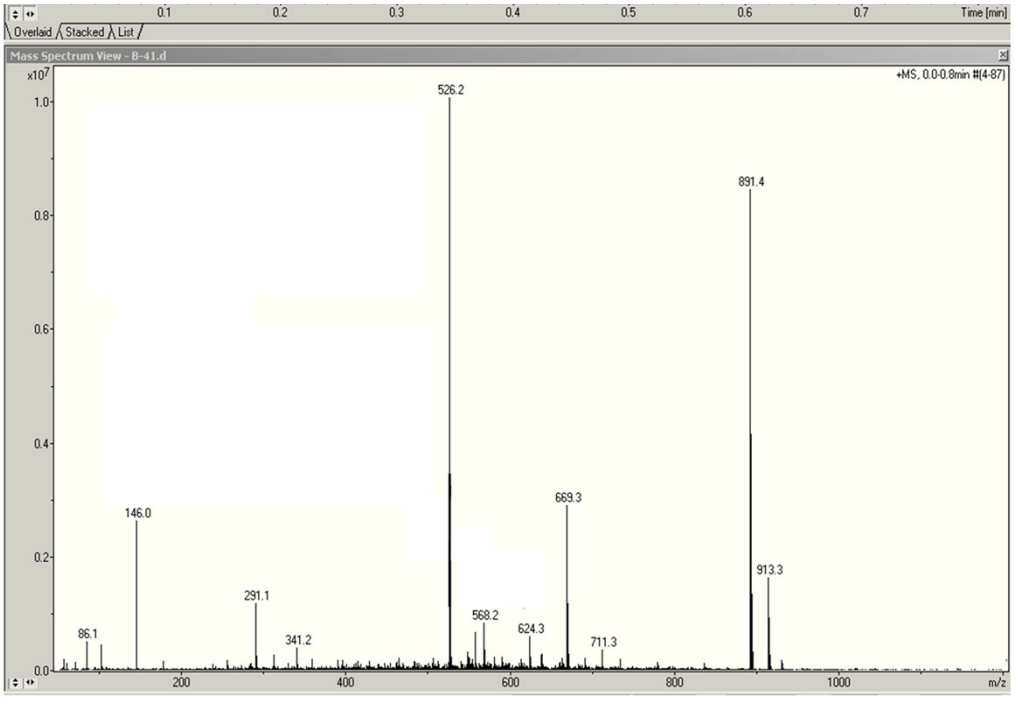
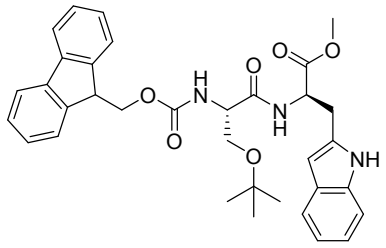


Fig. S8 Mass spectrum of compounds 16 with 1 eq of HBTU.

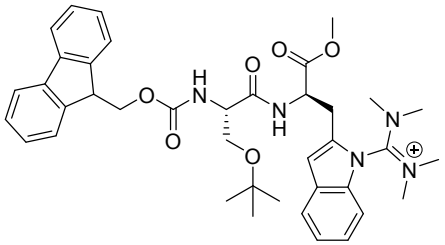
Compounds 17 (Fmoc-Ser(tBu)-Trp-OMe)

Compound 17a: (R)-methyl 2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tert-butoxy)propanamido)-3-(1H-indol-2-yl)propanoate:



Mass: ESI-MS *m/z*: calcd: 583.27 $[M+H]^+$; found: 584.2754 $[M+H]^+$, 623.02 $[M+K]^+$, 607.04 $[M+Na]^+$.

Compound 17b: N-((2-((R)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tert-butoxy)propanamido)-3-methoxy-3-oxopropyl)-1H-indol-1-yl)(dimethylamino)methylene)-N-methylmethanaminium):



Mass: ESI-MS *m/z*: calcd: 682.36 $[M+H]^+$; found: -

HATU 1.5 equivalents:

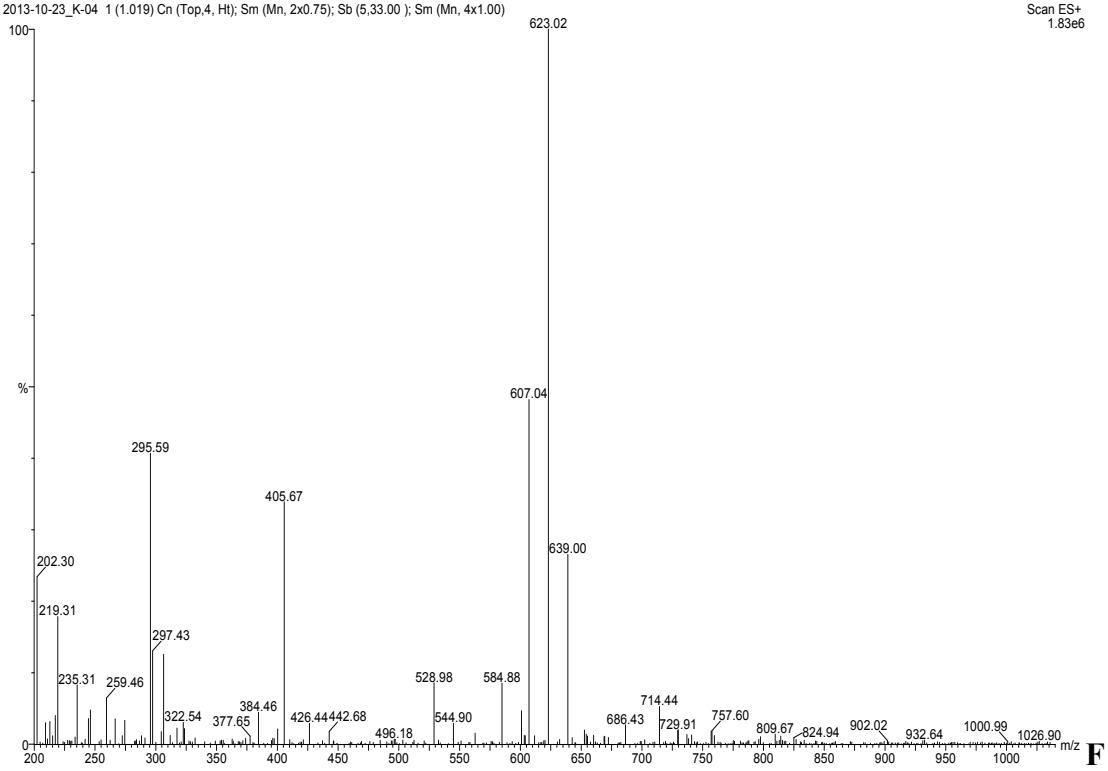


Fig. S9 Mass spectrum of compounds 17 with 1.5 eq of HATU.

HATU 1.0 equivalent:

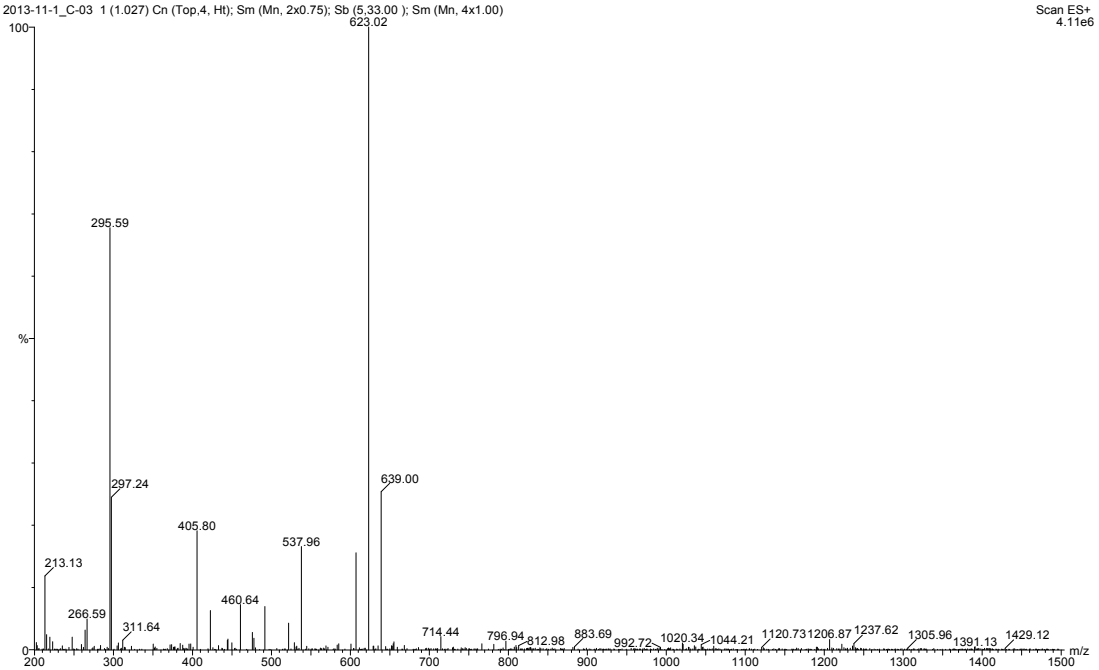


Fig. S10 Mass spectrum of compounds 17 with 1 eq of HATU.

HBTU 1.5 equivalents:

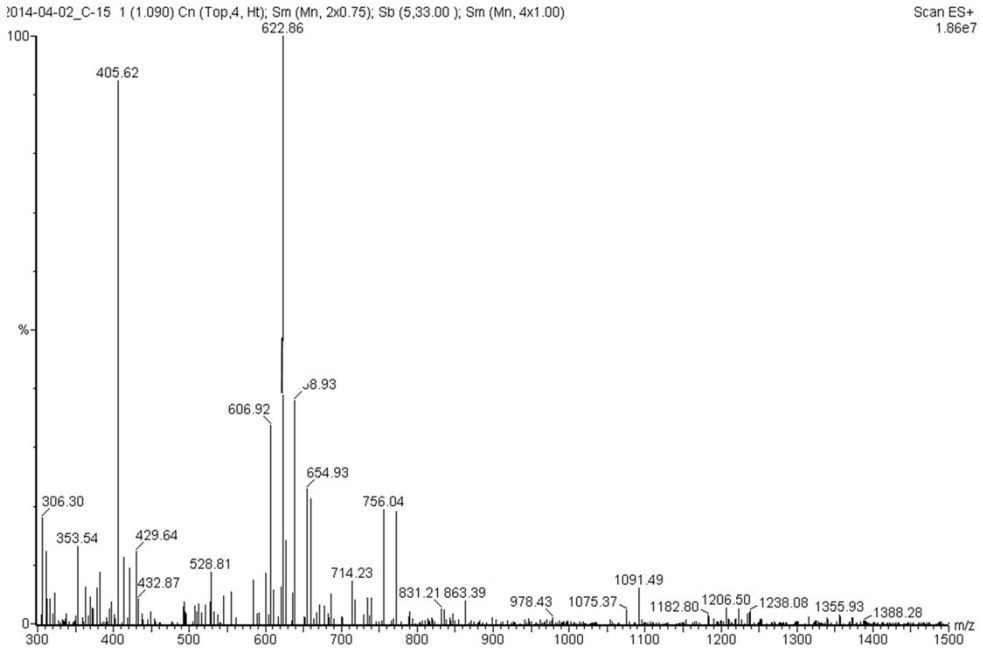


Fig. S11 Mass spectrum of compounds 17 with 1.5 eq of HBTU.

HBTU 1.0 equivalent:

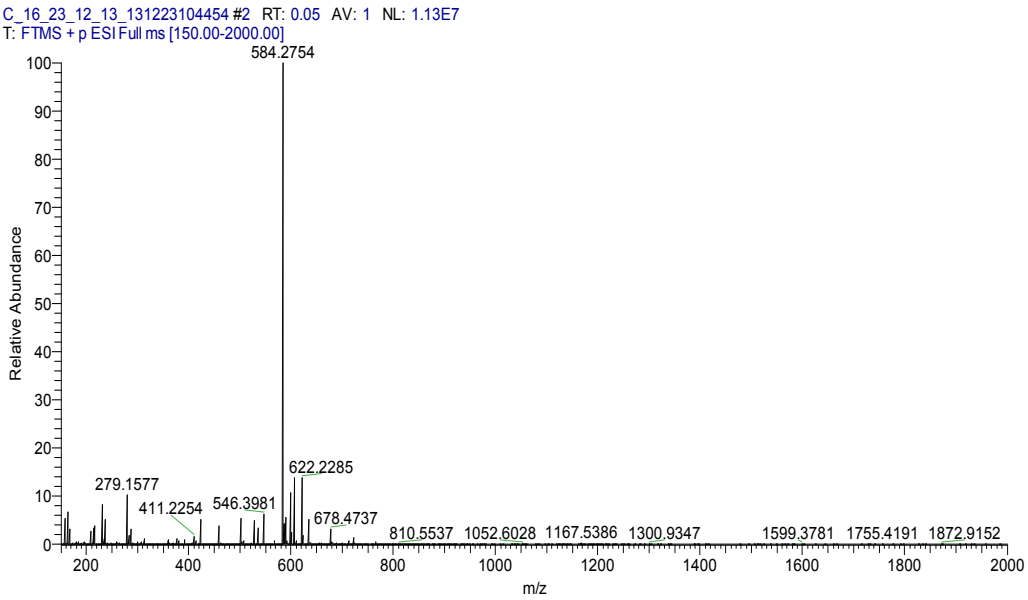
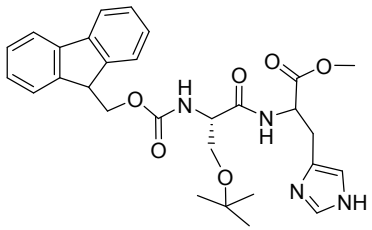


Fig. S12 Mass spectrum of compounds 17 with 1 eq of HBTU.

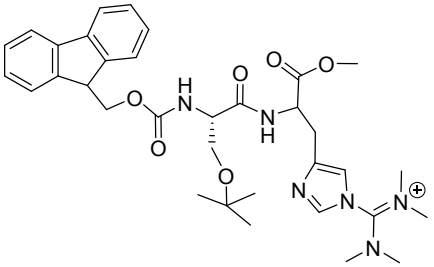
Compounds 18 (Fmoc-Ser(tBu)-His-OMe)

Compound 18a: Methyl 2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tert-butoxy)propanamido)-3-(1H-imidazol-4-yl)propanoate**:**



Mass: ESI-MS *m/z*: calcd: 534.25 $[M+H]^+$; found: 535.2548 $[M+H]^+$.

Compound 18b: N-((4-(2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tert-butoxy)propanamido)-3-methoxy-3-oxopropyl)-1H-imidazol-1-yl)(dimethylamino)methylene)-N-methylmethanaminium:



Mass: ESI-MS *m/z*: calcd: 633.34 $[M+H]^+$; found: -

HATU 1.5 equivalents:

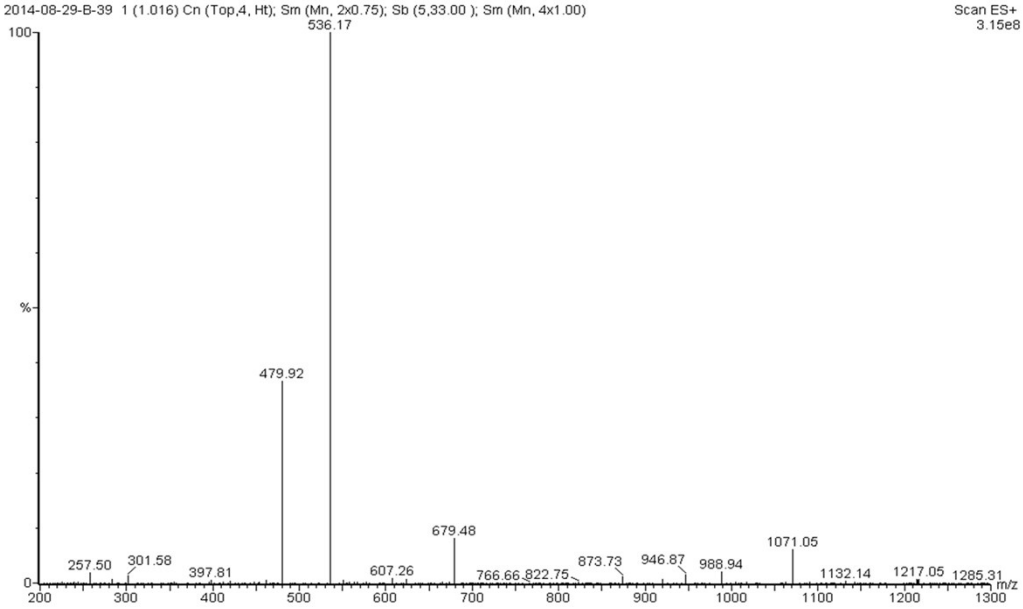


Fig. S13 Mass spectrum of compounds 18 with 1.5 eq of HATU.

HATU 1.0 equivalent:

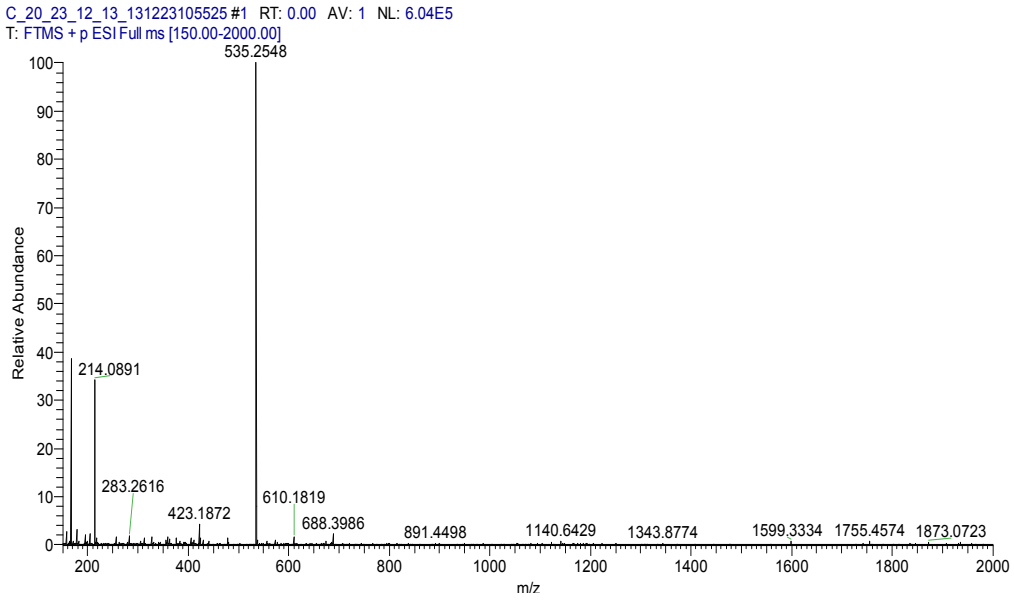


Fig. S14 Mass spectrum of compounds 18 with 1 eq of HATU.

HBTU 1.5 equivalents:

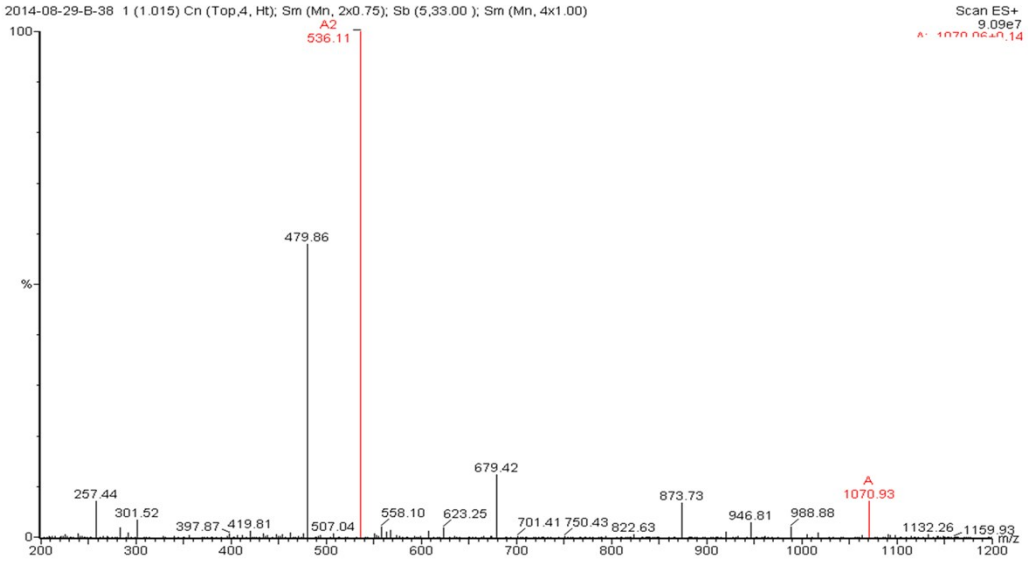


Fig. S15 Mass spectrum of compounds 18 with 1.5 eq of HBTU.

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HBTU 1.0 equivalent:

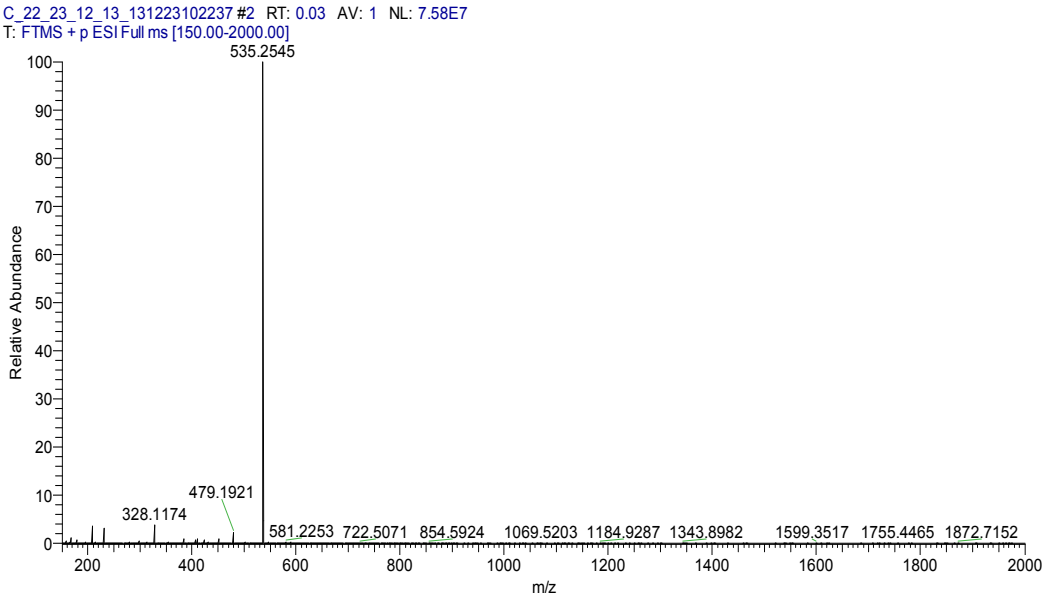
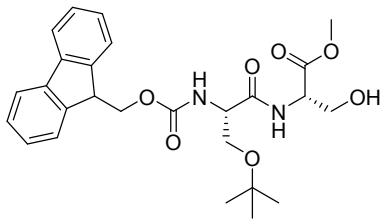


Fig. S16 Mass spectrum of compounds 18 with 1 eq of HBTU.

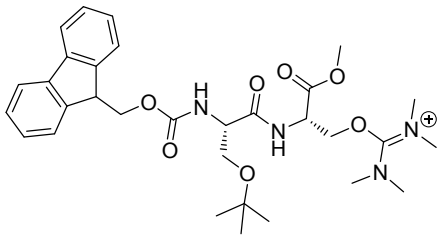
Compounds 19 (Fmoc-Ser(tBu)-Ser-OMe)

Compound 19a: (S)-methyl 2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tert-butoxy)propanamido)-3-hydroxypropanoate:



Mass: ESI-MS *m/z*: calcd: 383.17 $[M+H]^+$; found: 421.81 $[M+K]^+$, 405.75 $[M+Na]^+$.

Compound 19b: 2-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tert-butoxy)propanamido)-3-methoxy-3-oxopropyl)-1,1,3,3-tetramethylisouronium:



Mass: ESI-MS *m/z*: calcd: 484.32 $[M+H]^+$; found: -

HATU 1.5 equivalents:

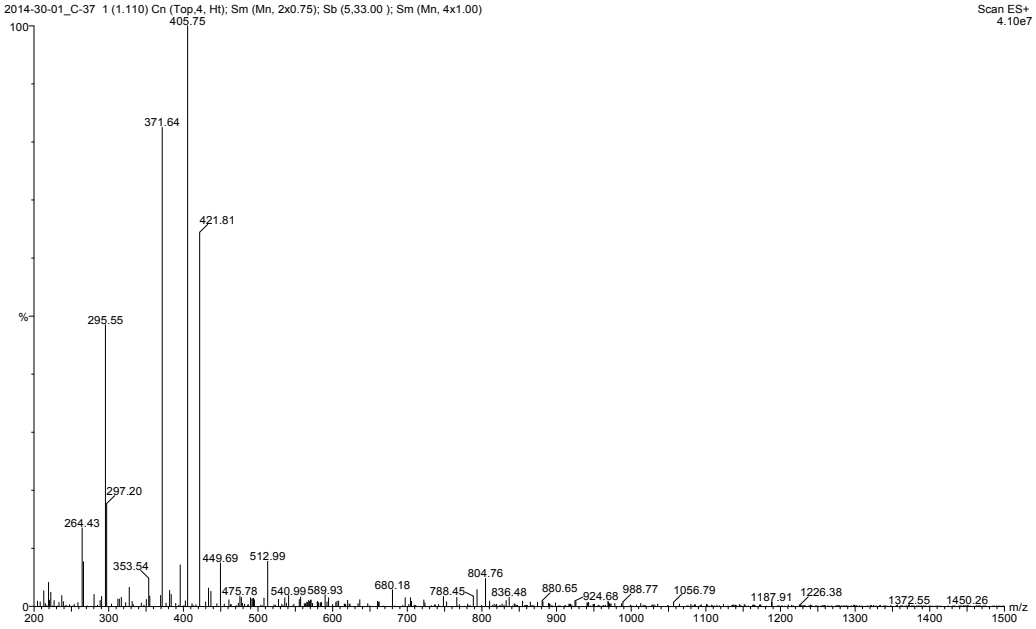


Fig. S17 Mass spectrum of compounds 19 with 1.5 eq of HATU.

HATU 1.0 equivalent:

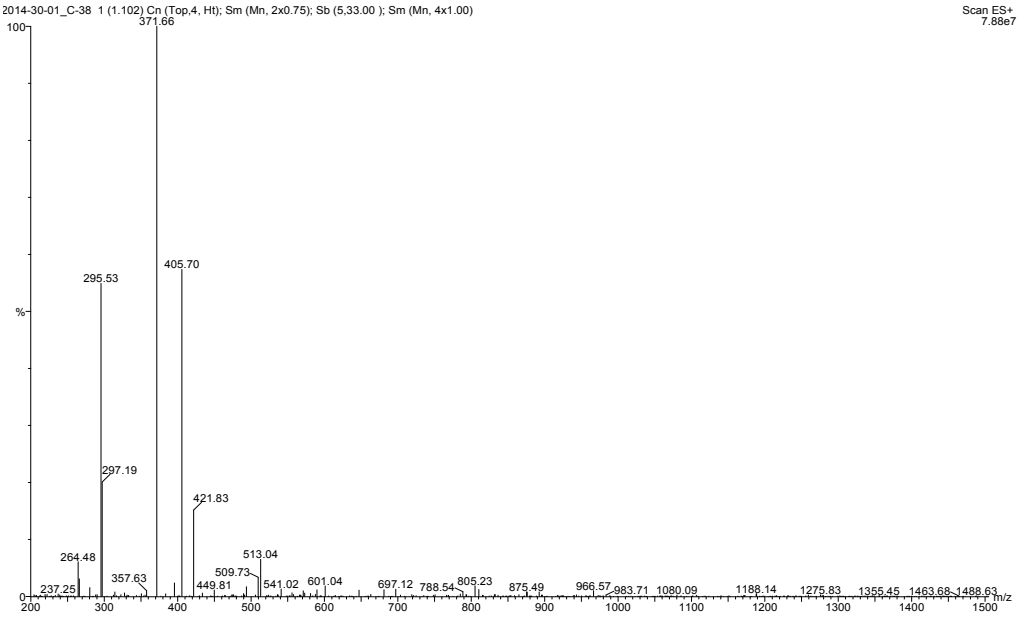


Fig. S18 Mass spectrum of compounds 19 with 1 eq of HATU.

HBTU 1.5 equivalents:

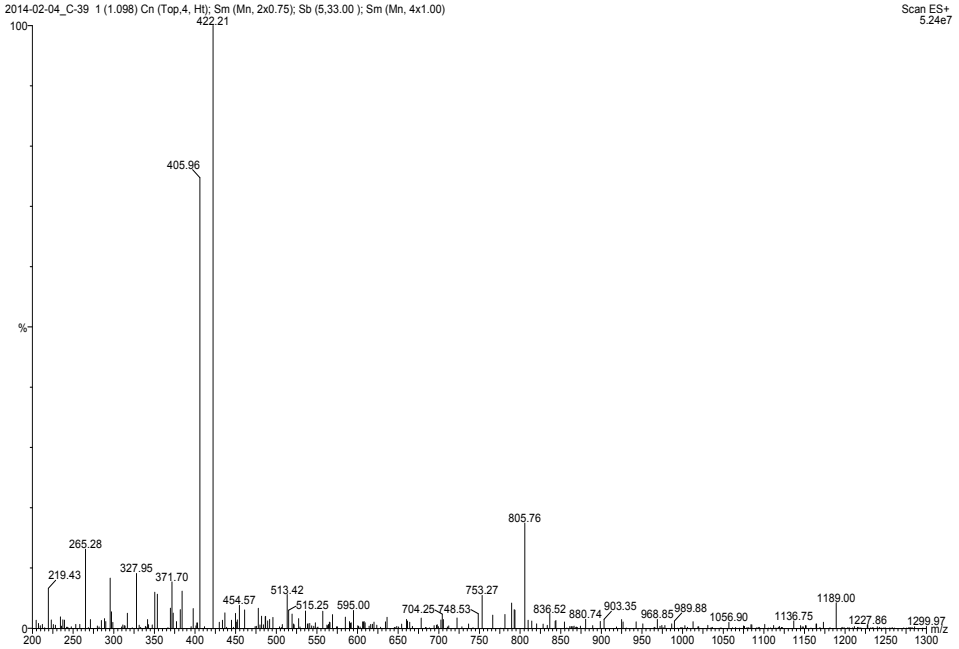


Fig. S19 Mass spectrum of compounds 19 with 1.5 eq of HBTU.

HBTU 1.0 equivalent:

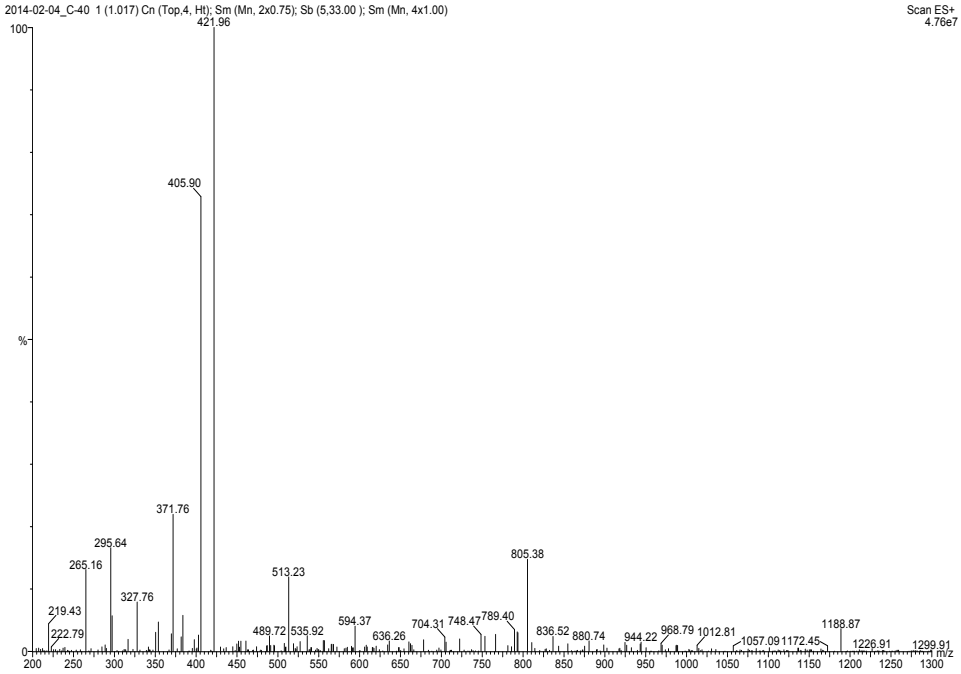
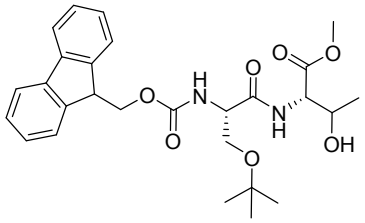


Fig. S20 Mass spectrum of compounds 19 with 1 eq of HBTU.

Compounds 20 (Fmoc-Ser(tBu)-Thr-OMe)

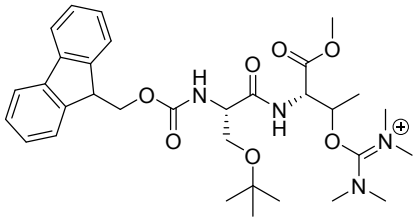
Compound 20a: (2S)-methyl 2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tert-butoxy)propanamido)-3-hydroxybutanoate:



Exact Mass: 498.24

Mass: ESI-MS *m/z*: calcd: 498.24 $[M+H]^+$; found: 499.2431 $[M+H]^+$, 537.83 $[M+K]^+$, 521.84 $[M+Na]^+$.

Compound 20b: 2-((3S)-3-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tert-butoxy)propanamido)-4-methoxy-4-oxobutan-2-yl)-1,1,3,3-tetramethylisouronium:



Mass: ESI-MS *m/z*: calcd: 597.33 $[M+H]^+$; found: -

HATU 1.5 equivalents:

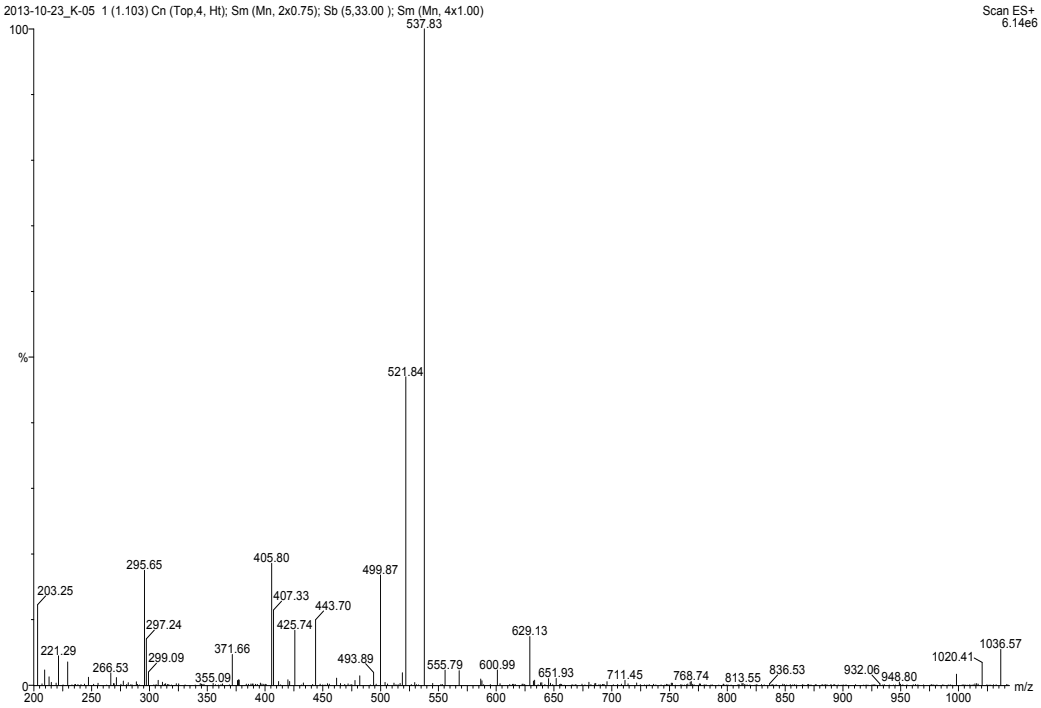


Fig. S21 Mass spectrum of compounds 20 with 1.5 eq of HATU.

HATU 1.0 equivalent:

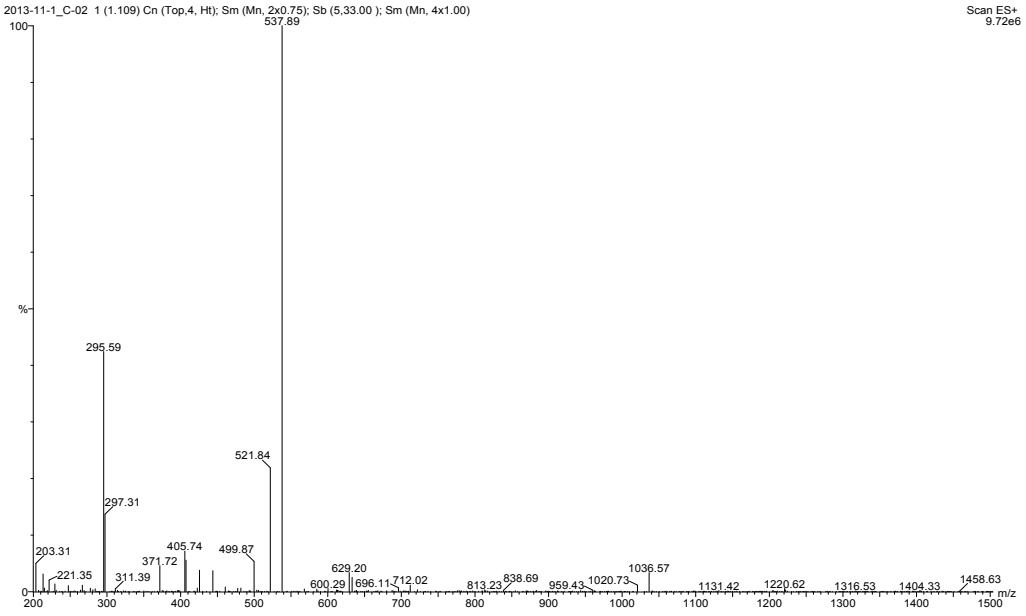


Fig. S22 Mass spectrum of compounds 20 with 1 eq of HATU.

HBTU 1.5 equivalents:

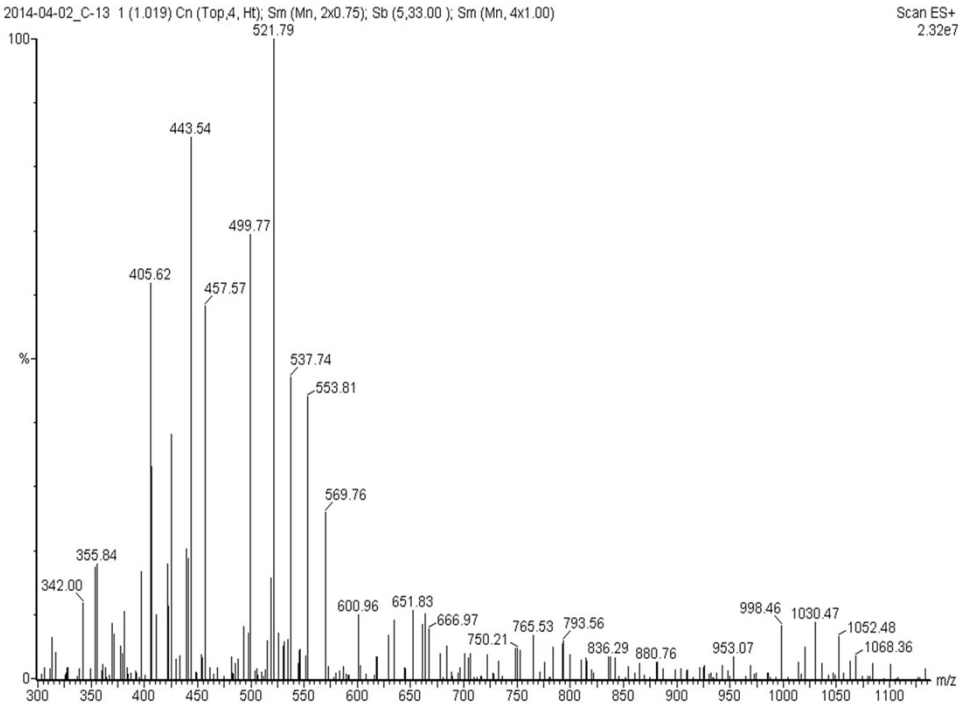


Fig. S23 Mass spectrum of compounds 20 with 1.5 eq of HBTU.

HBTU 1.0 equivalent:

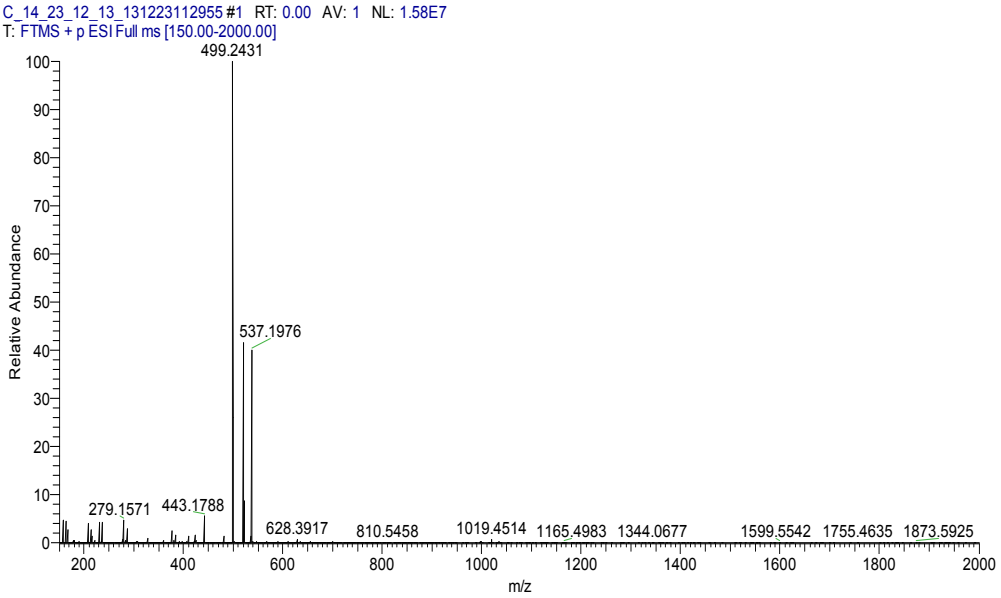
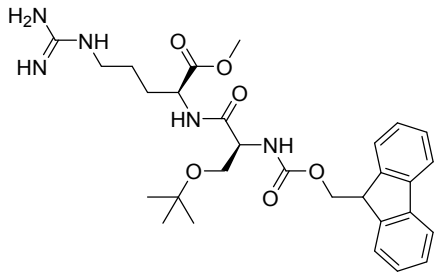


Fig. S24 Mass spectrum of compounds 20 with 1 eq of HBTU.

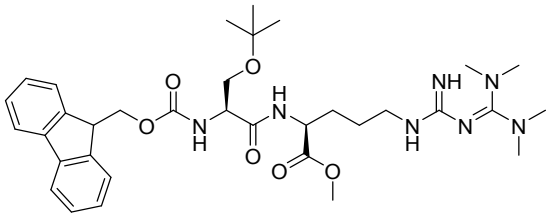
Compounds 21 (Fmoc-Ser(tBu)-Arg-OMe)

Compound 21a: (S)-methyl 2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tert-butoxy)propanamido)-3-hydroxypropanoate:



Mass: ESI-MS *m/z*: calcd: 555.29 $[M+H]^+$; found: 555.13 $[M+H]^+$.

Compound 21b: 2-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tert-butoxy)propanamido)-3-methoxy-3-oxopropyl)-1,1,3,3-tetramethylisouronium:



Mass: ESI-MS *m/z*: calcd: 651.37 $[M+H]^+$; found: -

HATU 1.5 equivalents:

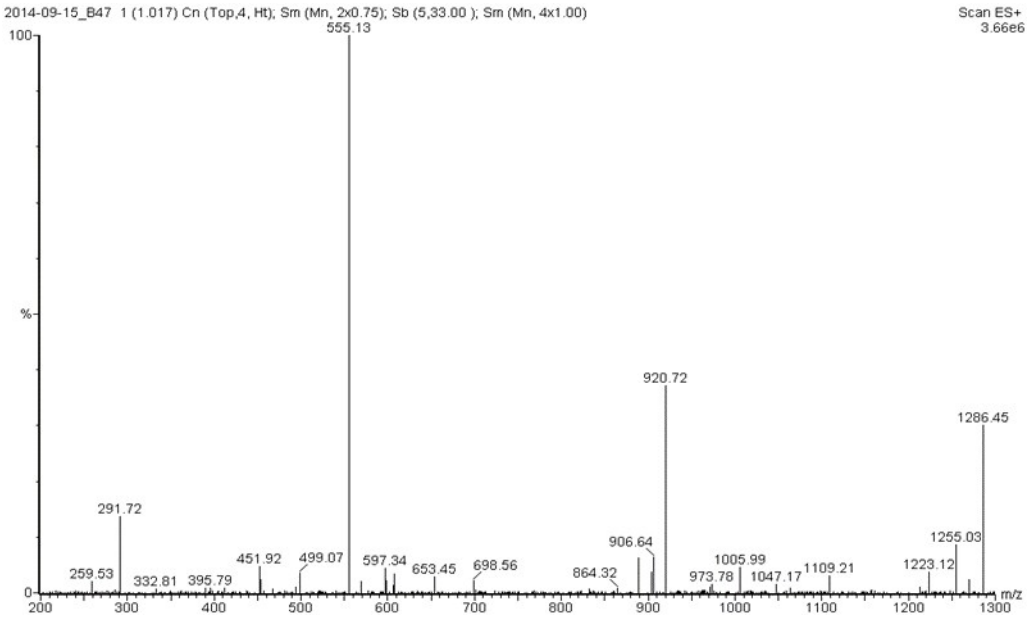


Fig. S25 Mass spectrum of compounds 21 with 1.5 eq of HATU.

HATU 1.0 equivalent:

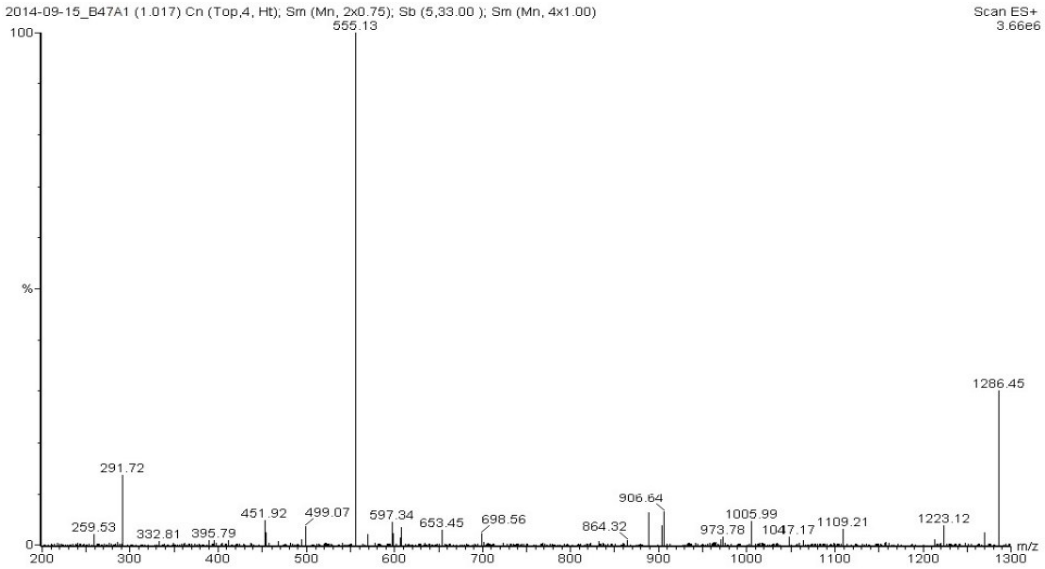


Fig. S26 Mass spectrum of compounds 21 with 1 eq of HATU.

HBTU 1.5 equivalents:

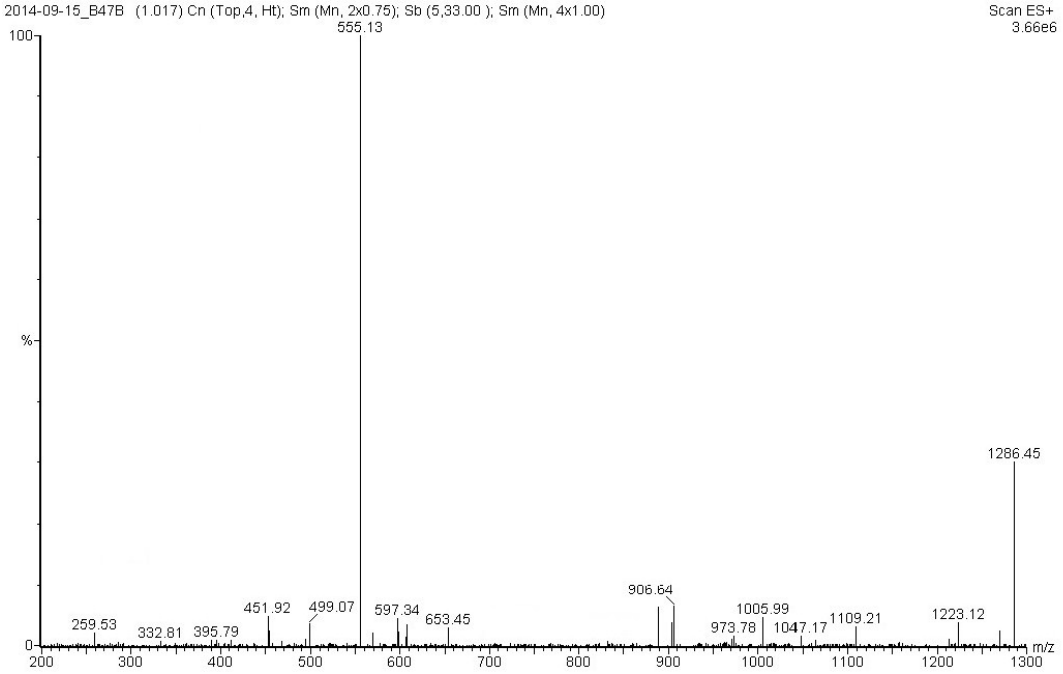


Fig. S27 Mass spectrum of compounds 21 with 1.5 eq of HBTU.

HBTU 1.0 equivalents:

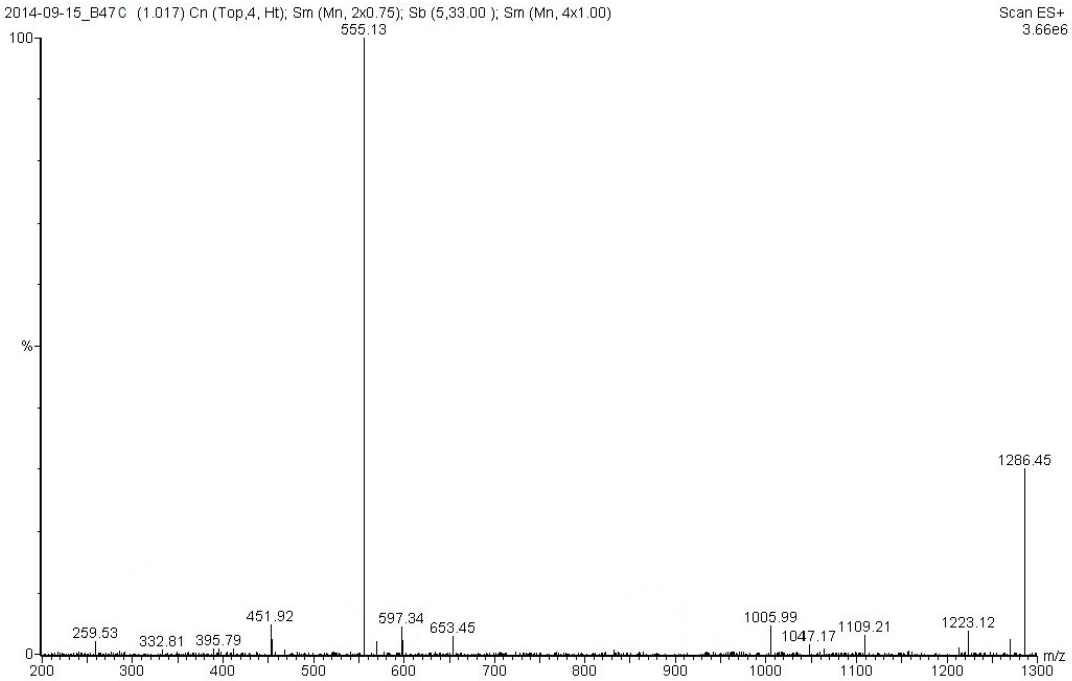


Fig. S28 Mass spectrum of compounds 21 with 1 eq of HBTU.

3. Purification and characterization of compound 6

Purification of compound **6**:

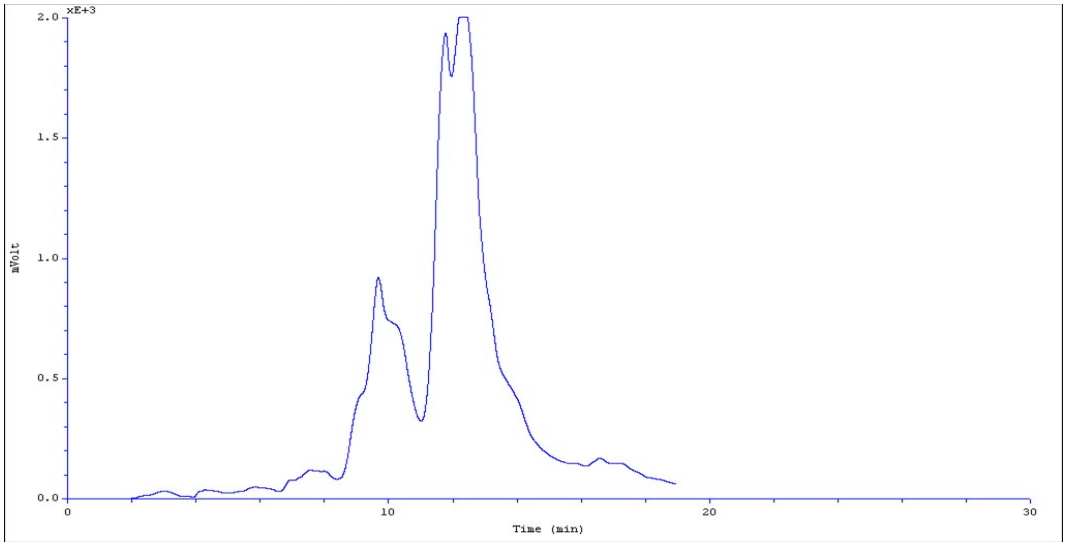


Fig. S29 Semi-prep RP-HPLC chromatogram during the purification of compound **5** and compound **6** (Gradient system: from 90/10% until 60/40% of H_2O+0.1%TFA/MeCN+0.1%TFA, in 20mins at 214nm).

1D/2D NMR characterization of compound **6:**

The formation of compound **6** is verified with 1D/2D NMR spectroscopy, as shown below:

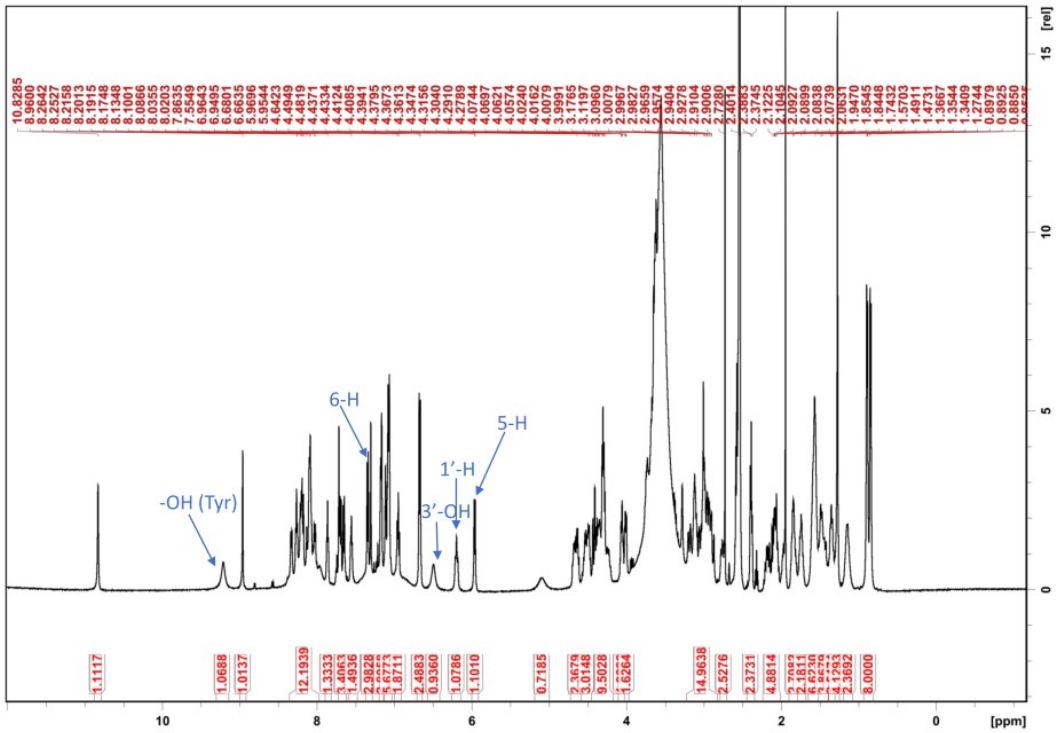


Fig. S30 ^{1}H-NMR of compound **6** in DMSO-d_6 at 298K. The peaks of gemcitabine and of the phenol of tyrosine are highlighted.

In **Fig. S30** the peaks of both gemcitabine and GnRH can be seen. Moreover, the peak of -OH group of tyrosine is clearly proving the formation of compound **6** and not of compound **5**.

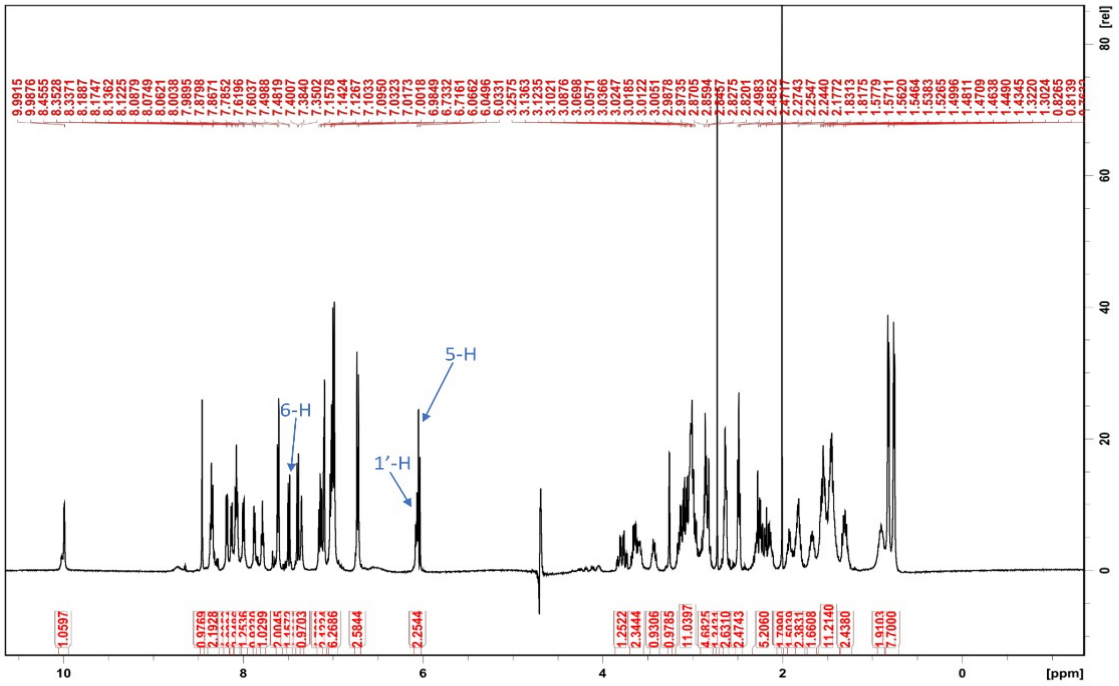


Fig. S31 ^{1}H-NMR of compound **6** in D_2O at 298K. The peaks of gemcitabine are highlighted.

In **Fig. S31** the peaks of both gemcitabine and GnRH can be seen. Moreover, the peak regarding 3'-OH of gemcitabine and the peak of -OH group of tyrosine are absent because of proton exchange due to the presence of D_2O.

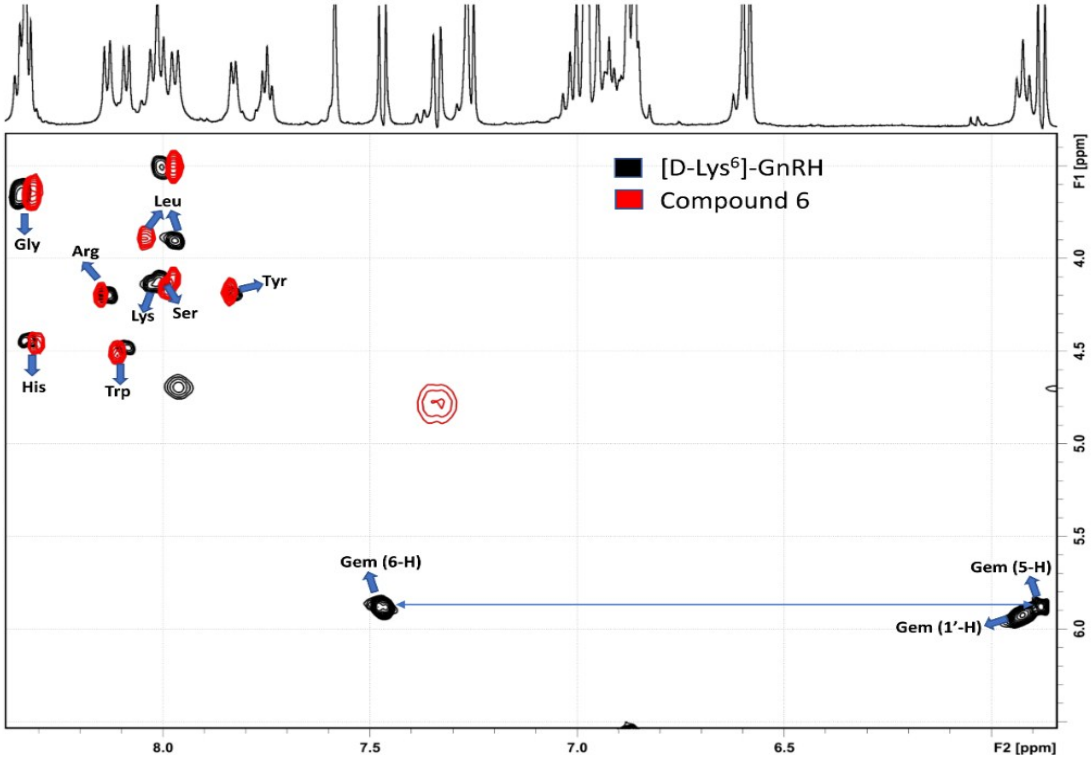


Fig. S32 Overlay of 2D NMR TOCSY spectra of [D-Lys]^6-GnRH (red color) and compound **6** (black color) in D_2O at 298K

Analytical RP-HPLC of the purified compound **6**:

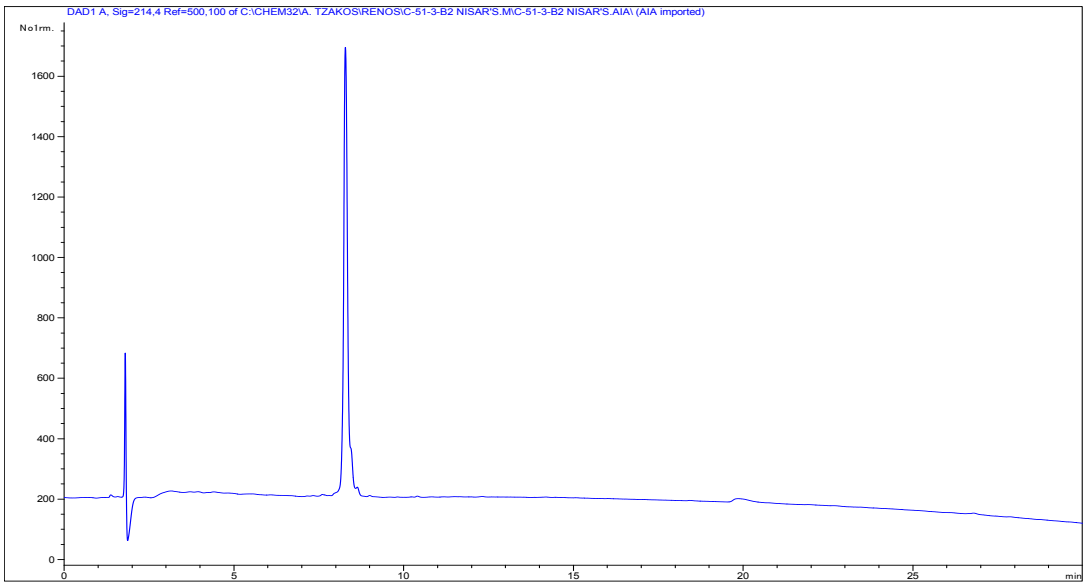


Fig. S33 Analytical RP-HPLC chromatogram of compound **6** in its pure state

(Gradient system: from 90/10% until 10/90% of H_2O+0.1%TFA/MeCN+0.1%TFA, in 30 mins at 214 nm).

HRMS characterization of compound **6**:

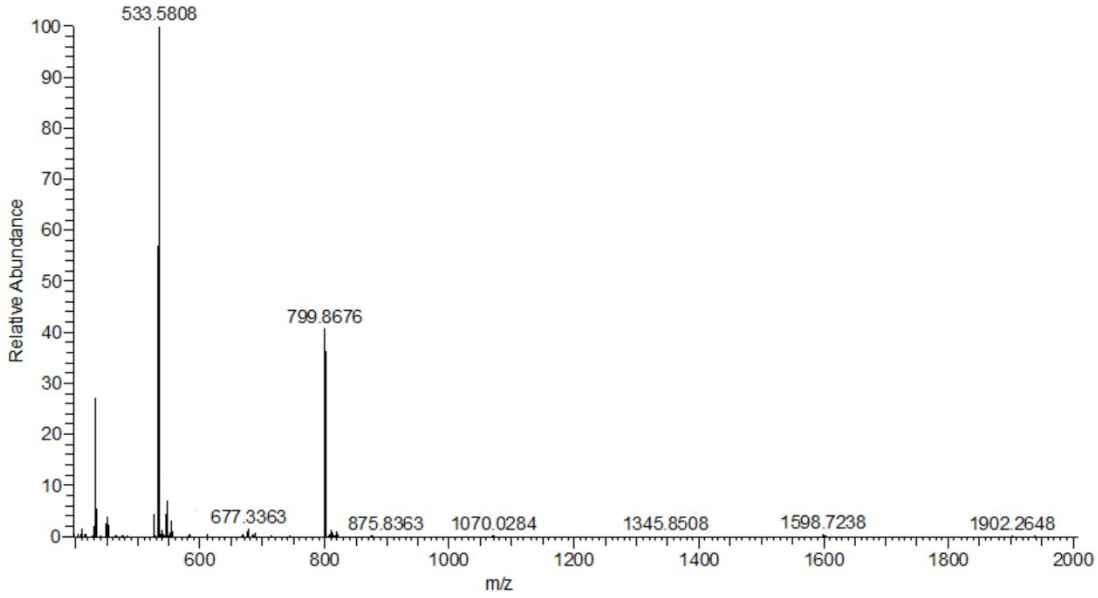


Fig. S34 HRMS of compound **6** (799.8676 $[M+2H]^{2+}$; 535.5806 $[M+3H]^{3+}$)

4. Purification and characterization of compound 22

The synthesis of compound **22** was based on the peptide [D-Lys]^6-GnRH (**Fig. S35**) which was synthesized with SPPS, purified via RP-HPLC (**Fig. S36**) and characterized with ESI-MS (**Fig. S37).**

Structure of peptide [D-Lys]^6-GnRH:

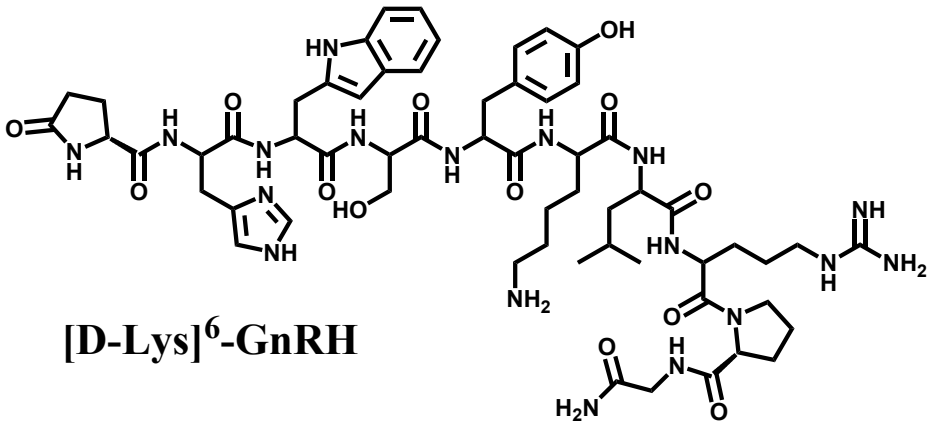


Fig. S35 Structure of peptide [D-Lys]^6-GnRH

Purification of peptide [D-Lys]^6-GnRH:

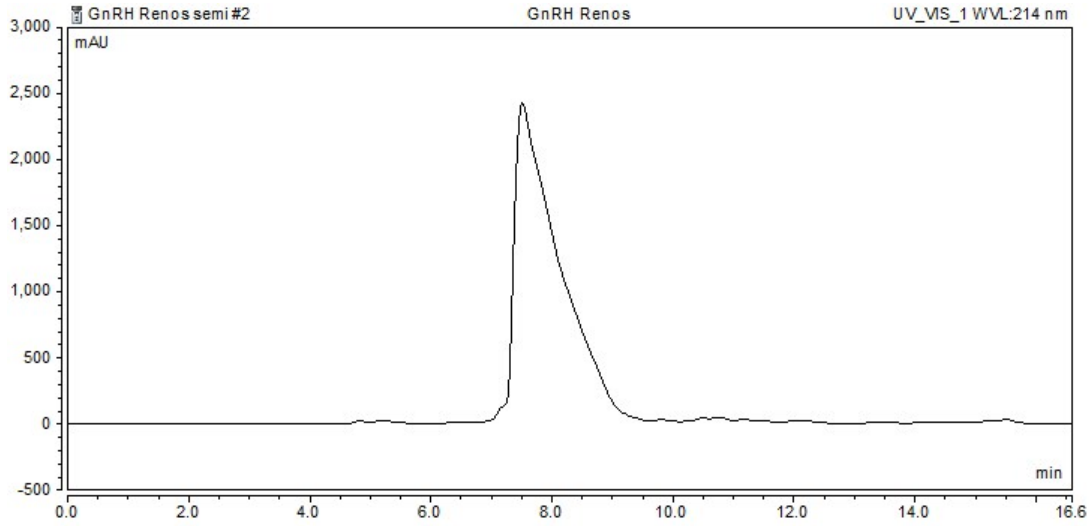


Fig. S36 RP-HPLC chromatogram of peptide [D-Lys]^6-GnRH (Gradient system: from 85/15% until 55/45% of H_2O+0.1%TFA/MeCN+0.1%TFA, in 20 mins at 214 nm).

Mass characterization of peptide [D-Lys]^6-GnRH:

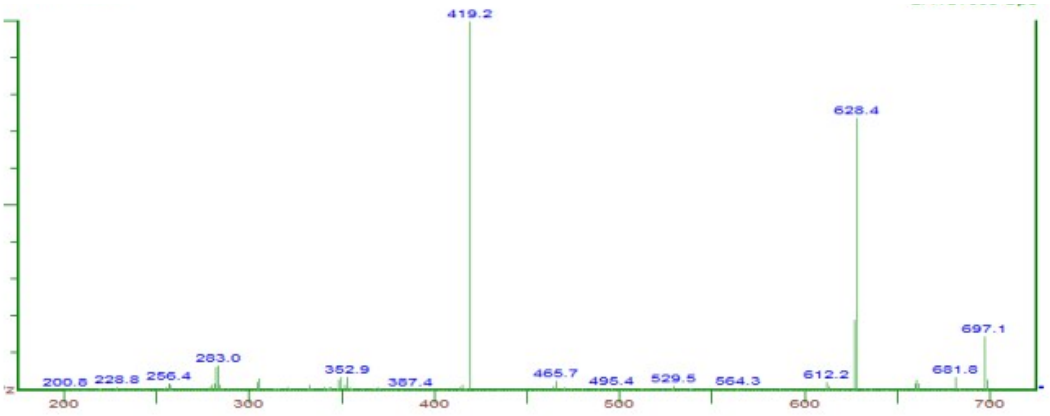


Fig. S37 ESI-MS spectrum of peptide [D-Lys]^{6}-GnRH (628.4 $[M+2H]^{2+}$; 419.2 $[M+3H]^{3+}$)

The structure, RP-HPLC chromatogram, mass and ^{1}H-NMR spectra of compound **22** are illustrated in **Fig. S38**, **Fig. S39**, **Fig. S40** and **Fig. S41** respectively:

Structure of compound **22**:

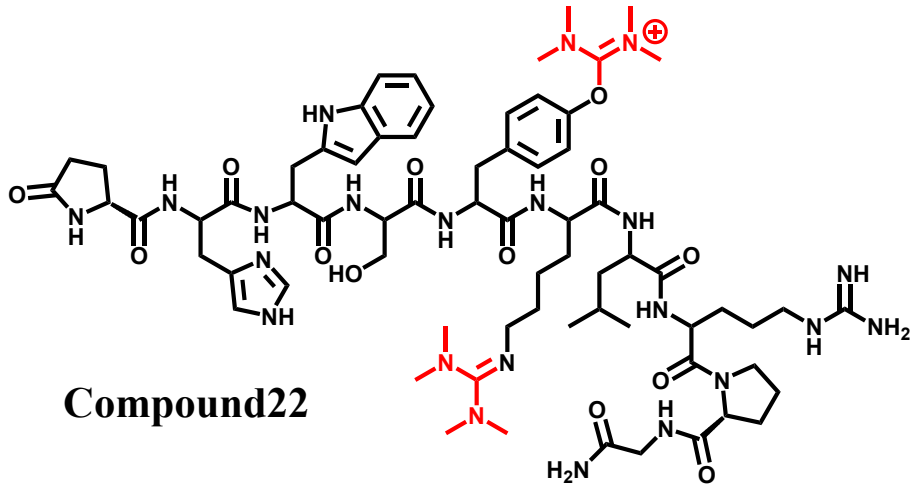


Fig. S38 Structure of compound **22**.

Purification of compound **22**:

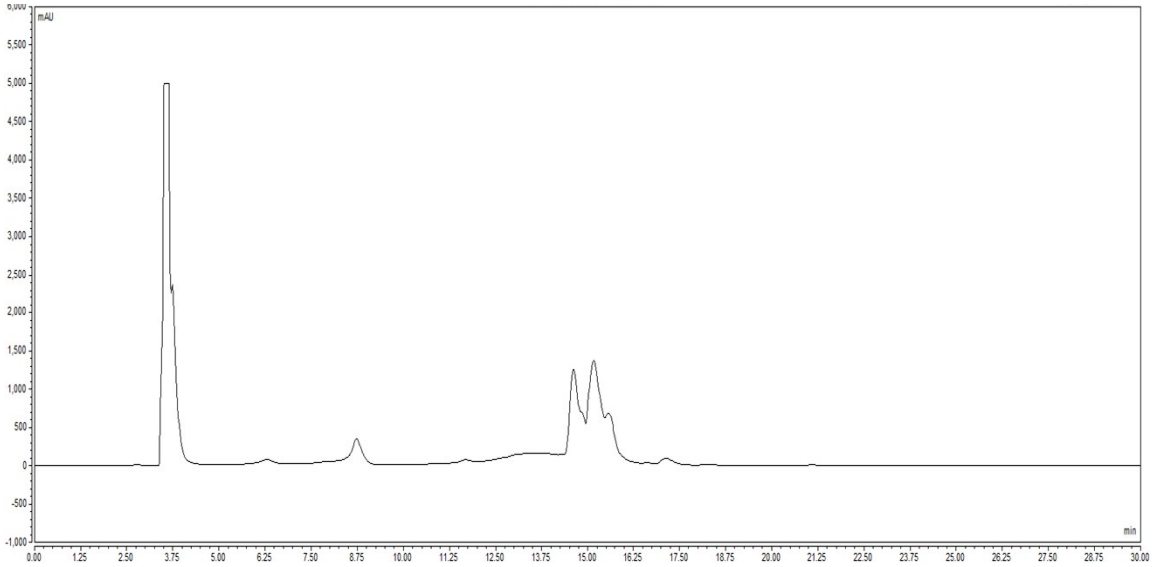


Fig. S39 RP-HPLC chromatogram of the purification of compound **22** (Gradient system: from 85/15% until 55/45% of H_2O+0.1%TFA/MeCN+0.1%TFA, in 30 mins at 214 nm).

Mass characterization of compound 22:

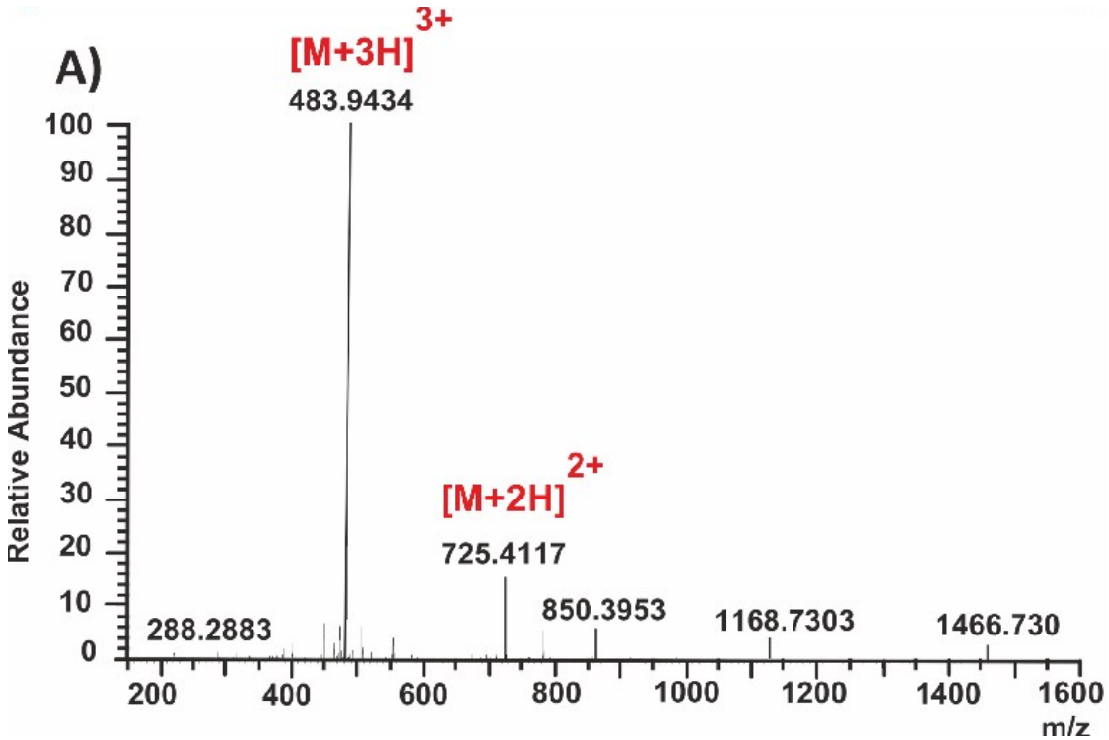


Fig. S40 HRMS spectrum of compound **22** (725.4117 $[M+2H]^{2+}$; 483.9434 $[M+3H]^{3+}$)

^{1}H-NMR characterization of compound **22**:

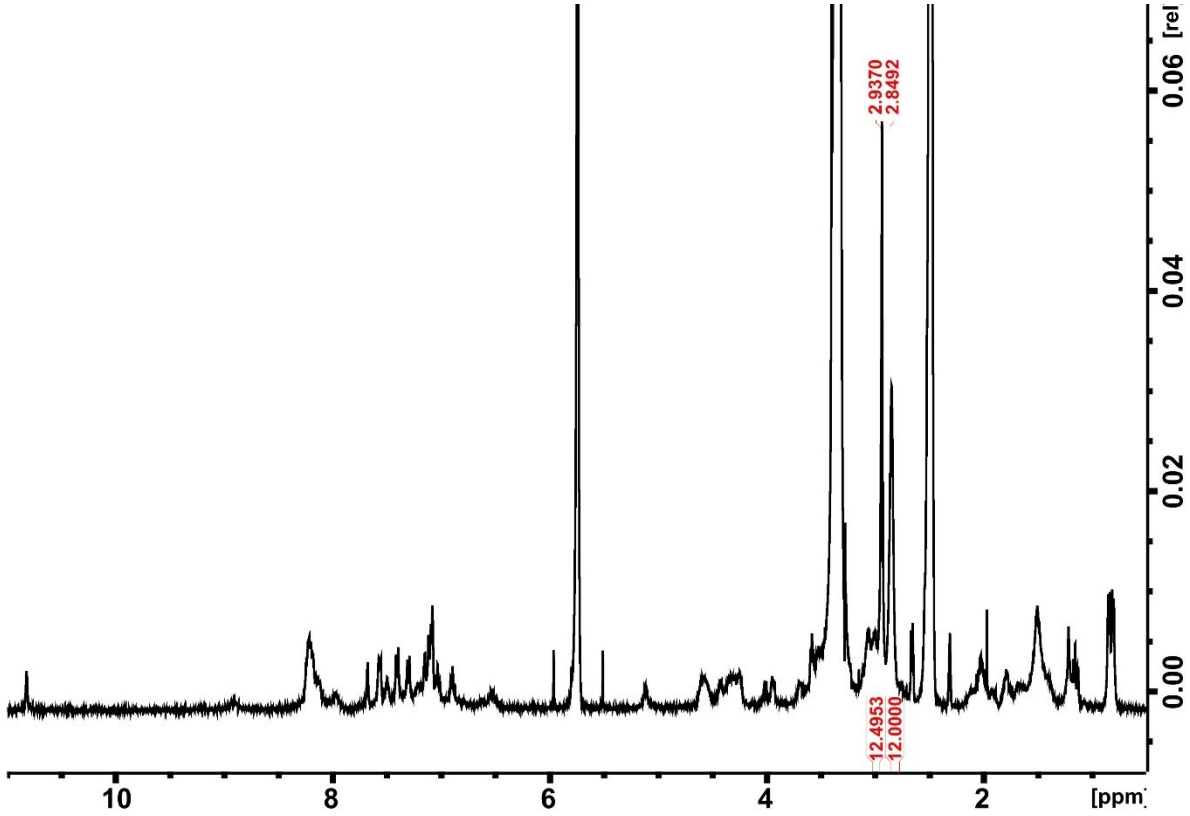


Fig. S41 ^{1}H-NMR spectrum of compound **22** in DMSO-d_6 at 298K

5. Purification and characterization of compound 23

The synthesis of compound **23** was based on the peptide Fmoc-HER2-BP1 (**Fig. S42**) which was synthesized with SPPS, purified via RP-HPLC (**Fig. S43**) and characterized with ESI-MS (**Fig. S44).**

Structure of peptide Fmoc-HER2-BP1:

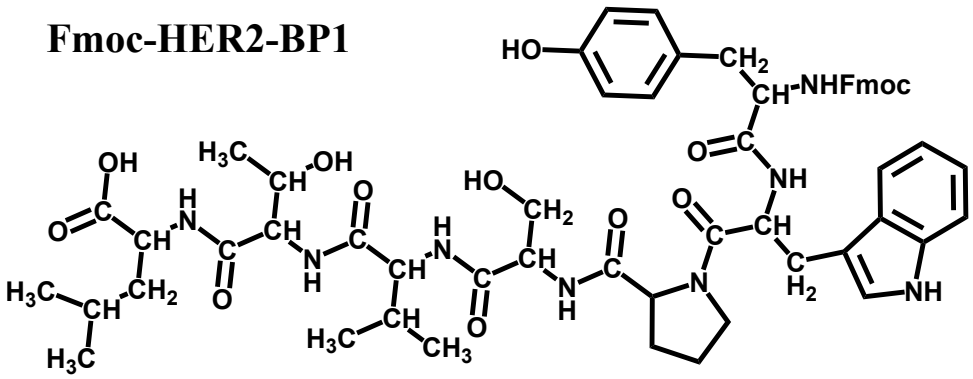


Fig. S42 Structure of peptide Fmoc-HER2-BP1.

Purification of peptide Fmoc-HER2-BP1:

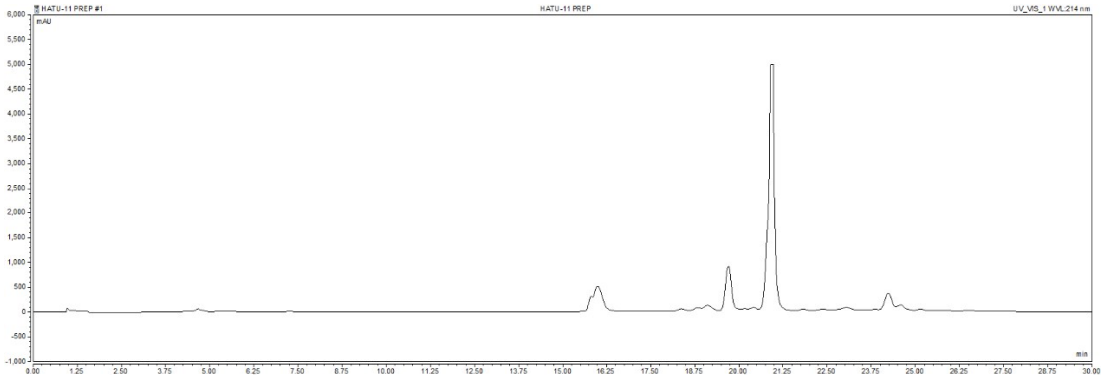


Fig. S43 RP-HPLC chromatogram of the purification of peptide Fmoc-HER2-BP1. (Gradient system: from 80/20% until 20/80% of H_2O+0.1%TFA/MeCN+0.1%TFA, in 30 mins at 214 nm).

Mass characterization of peptide Fmoc-HER2-BP1:

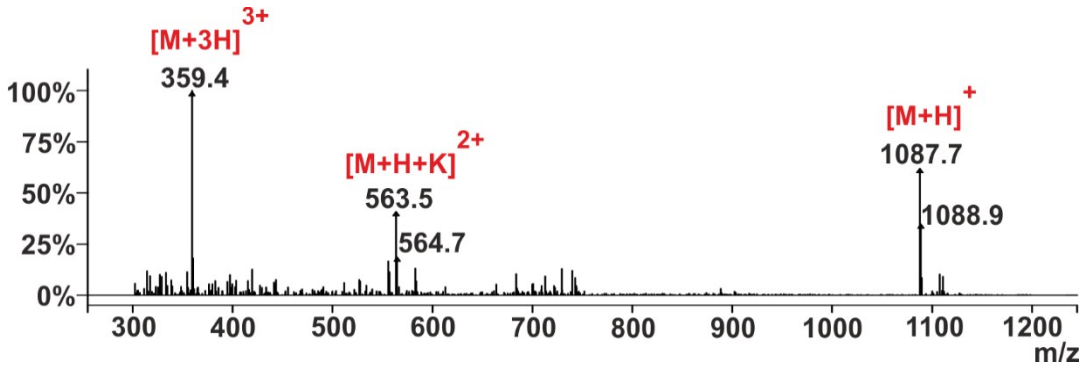


Fig. S44 ESI-MS spectrum of peptide Fmoc-HER2-BP1 (1087.7 $[M+H]^+$; 563.5 $[M+H+K]^{2+}$; 359.4 $[M+3H]^{3+}$)

The structure, RP-HPLC chromatogram, mass and ^{1}H-NMR spectra of compound **23** are illustrated in **Fig. S45**, **Fig. S46**, **Fig. S47** and **Fig. S48** respectively:

Structure of compound **23**:

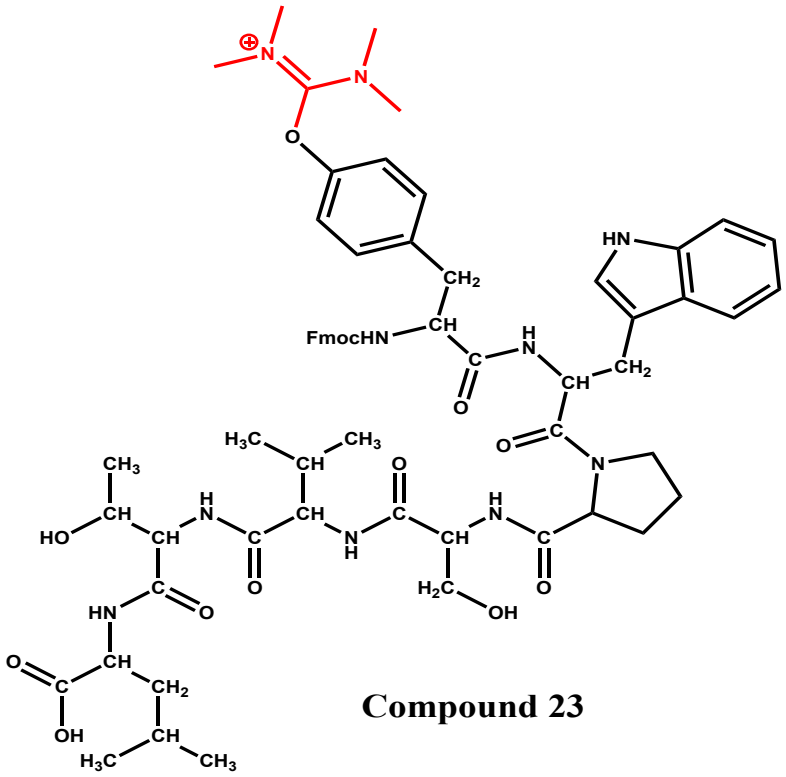


Fig. S45 Structure of compound **23**.

Purification of compound **23**:

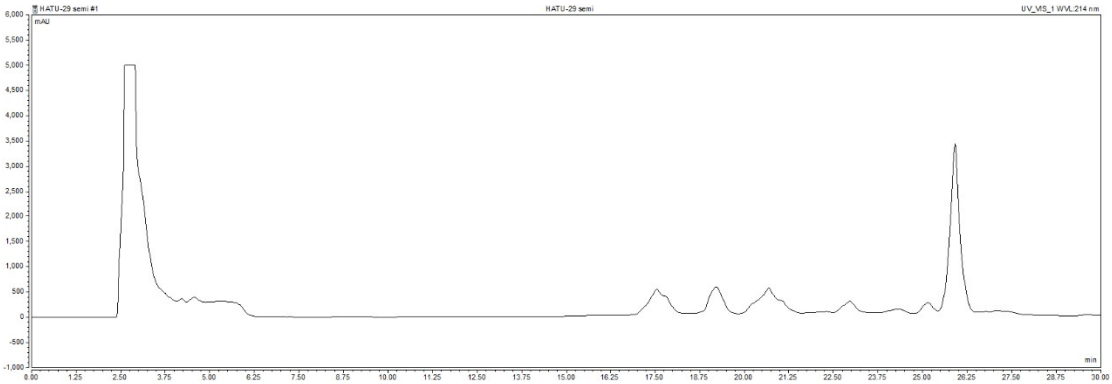


Fig. S46 RP-HPLC chromatogram of the purification of compound **23** (Gradient system: from 80/20% until 20/80% of H_2O+0.1%TFA/MeCN+0.1%TFA, in 30 mins at 214 nm).

Mass characterization of compound **23**:

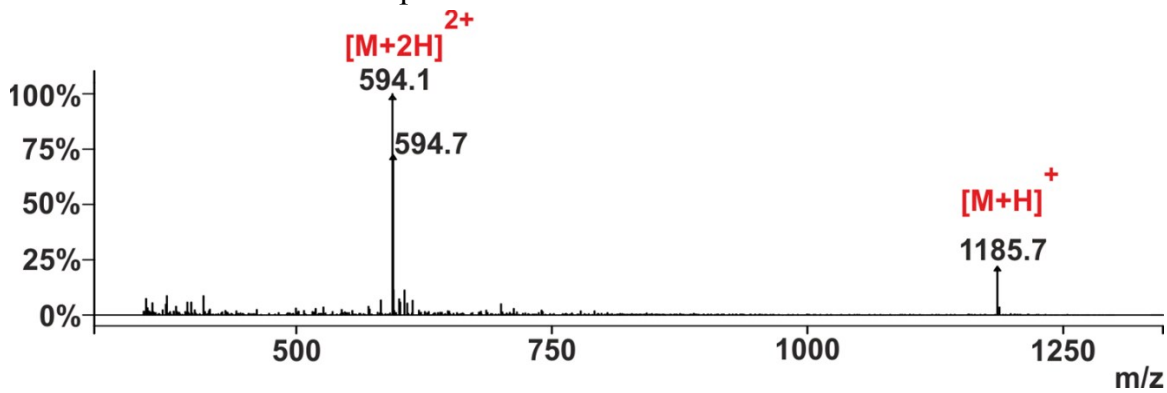


Fig. S47 ESI-MS spectrum of compound **23** (1185.7 $[M+H]^+$; 594.1 $[M+2H]^{2+}$)

^{1}H-NMR characterization of compound **23**:

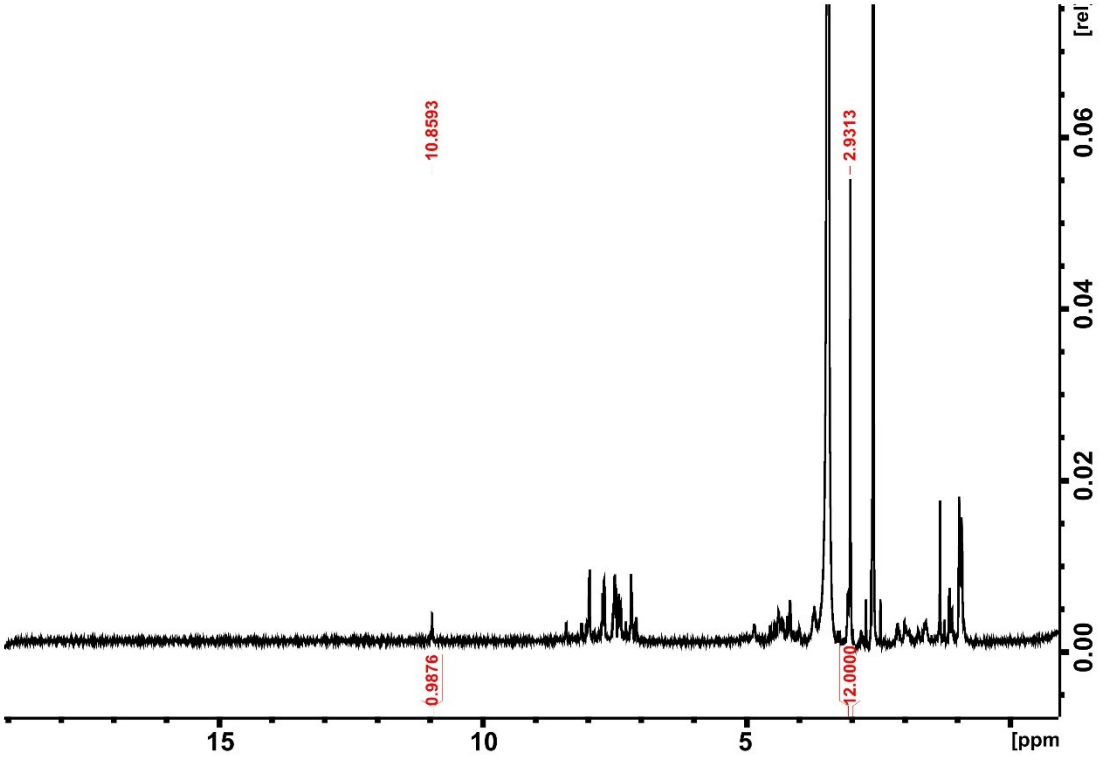


Fig. S48 ^{1}H-NMR spectrum of compound **23** in DMSO-d_6 at 298K

6. Purification and characterization of compounds 24 and 25

The synthesis of compounds **24** and **25** were based on the dipeptides Fmoc-Cys-Tyr-NH_2 and Fmoc-Ser-Tyr-NH_2 respectively (**Fig. S49**) which were synthesized with SPPS, purified via RP-HPLC (**Fig. S50/S51**) and characterized with ESI-MS (**Fig. S52/S53).**

Structures of dipeptides Fmoc-Cys-Tyr-NH_2 and Fmoc-Ser-Tyr-NH_2:

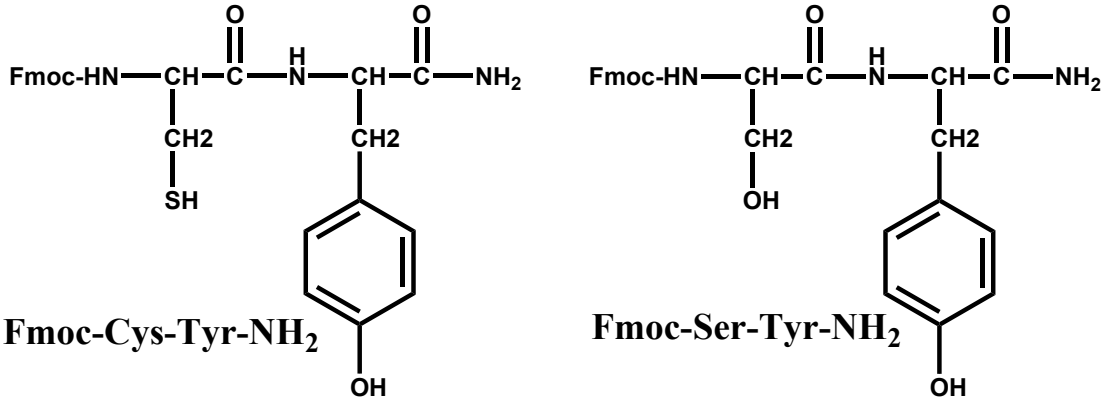


Fig. S49 Structures of dipeptides Fmoc-Cys-Tyr-NH_2 and Fmoc-Ser-Tyr-NH_2

Purification of dipeptides Fmoc-Cys-Tyr-NH_2 and Fmoc-Ser-Tyr-NH_2:

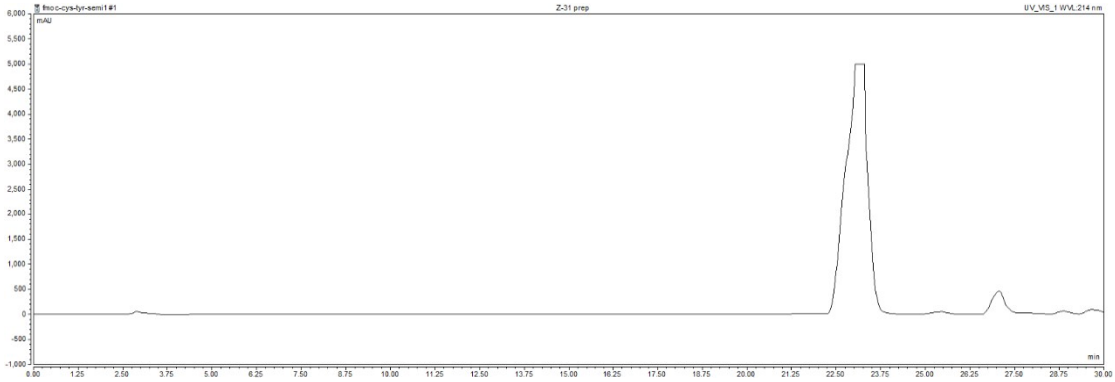


Fig. S50 RP-HPLC chromatogram of the purification of dipeptide Fmoc-Cys-Tyr-NH_2 (Gradient system: from 90/10% until 30/70% of H_2O+0.1%TFA/MeCN+0.1%TFA, in 30 mins at 214 nm).

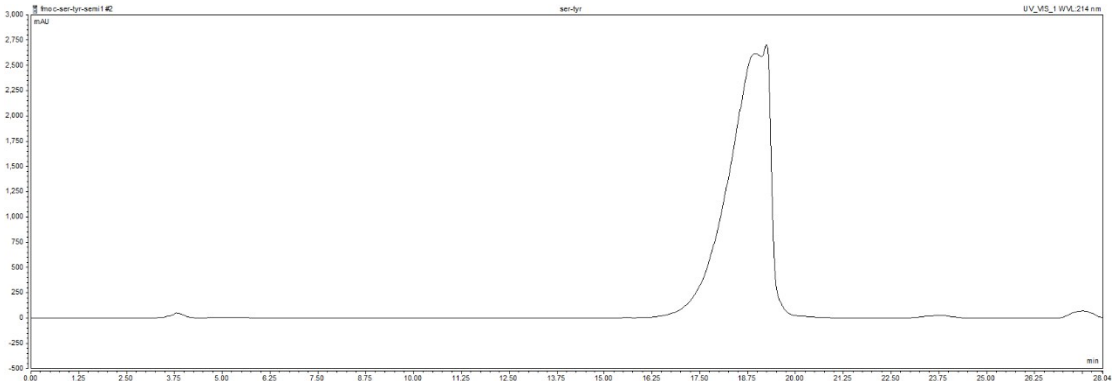


Fig. S51 RP-HPLC chromatogram of the purification of dipeptide Fmoc-Ser-Tyr-NH_2 (Gradient system: from 80/20% until 40/60% of H_2O+0.1%TFA/MeCN+0.1%TFA, in 30 mins at 214 nm).

Mass characterization of dipeptides Fmoc-Cys-Tyr-NH_2 and Fmoc-Ser-Tyr-NH_2:

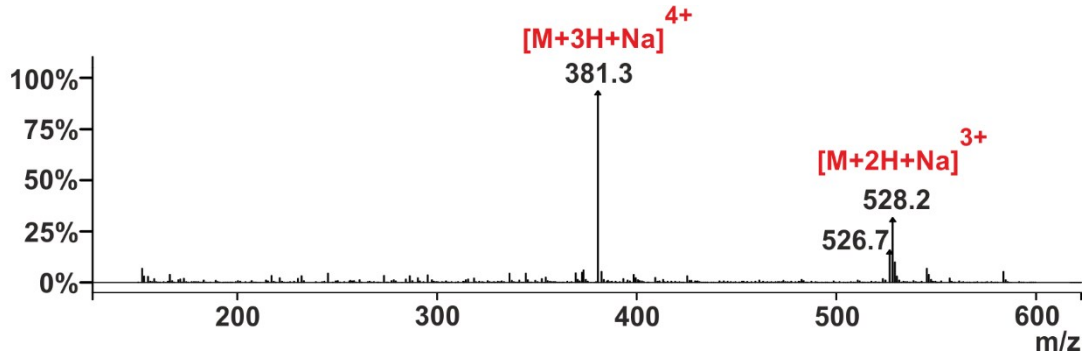


Fig. S52 ESI-MS spectrum of dipeptide Fmoc-Cys-Tyr-NH_2 (528.2 $[M+2H+Na]^{3+}$; 381.3 $[M+3H+Na]^{4+}$)

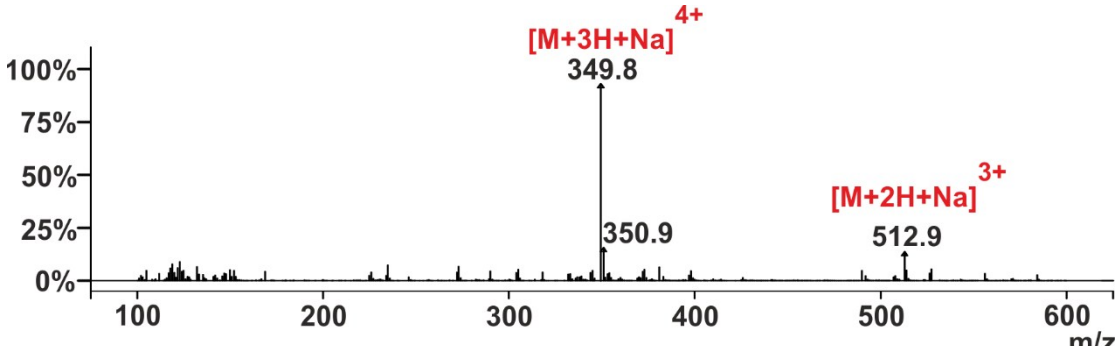


Fig. S53 ESI-MS spectrum of dipeptide Fmoc-Ser-Tyr-NH_2 (512.9 $[M+2H+Na]^{3+}$; 349.8 $[M+3H+Na]^{4+}$)

The structures, RP-HPLC chromatogram, mass and ^{1}H-NMR spectra of compounds **24** and **25** are illustrated in **Fig. S54**, **Fig. S55/S56**, **Fig. S57/S58** and **Fig. S59/S60** respectively:

Structures of compounds **24** and **25**:

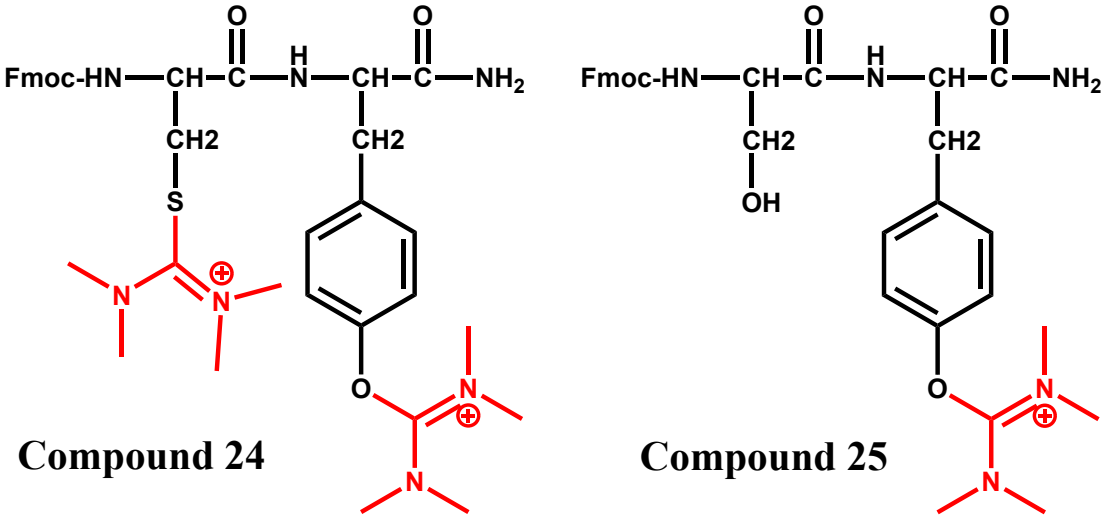


Fig. S54 Structures of compounds **24** and **25**

Purification of compounds **24** and **25**:

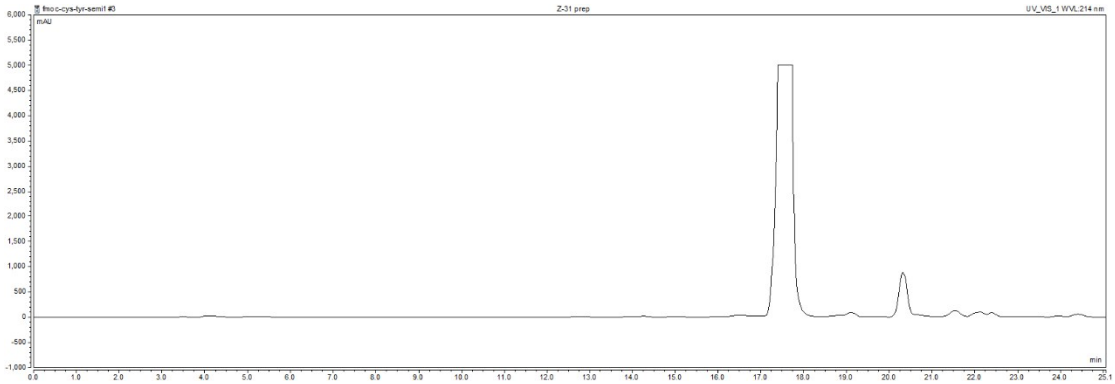


Fig. S55 RP-HPLC chromatogram of the purification of compound **24** (Gradient system: from 90/10% until 30/70% of H_2O+0.1%TFA/MeCN+0.1%TFA, in 30 mins at 214 nm).

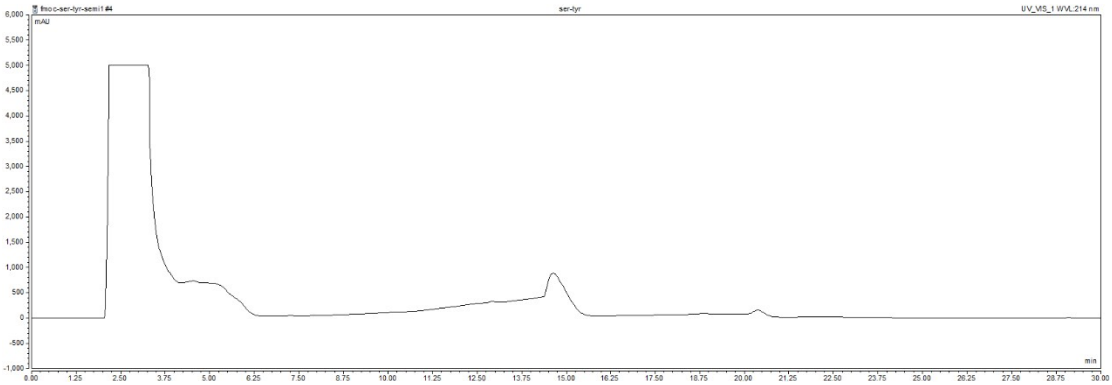
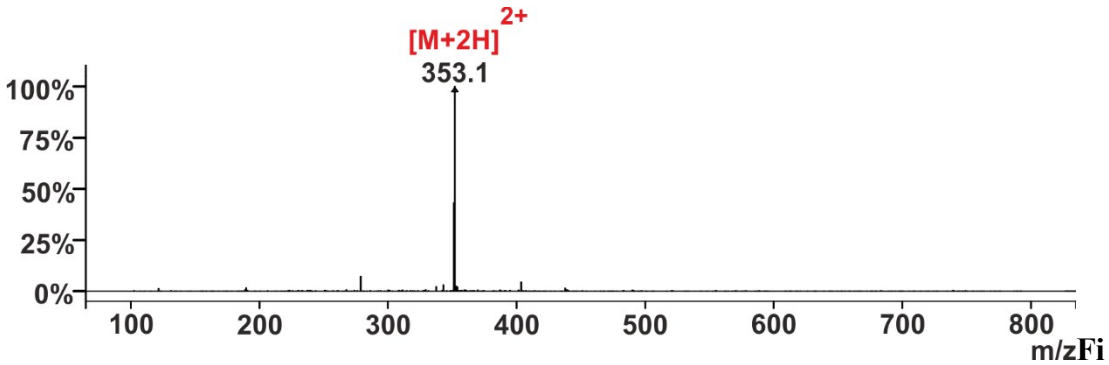
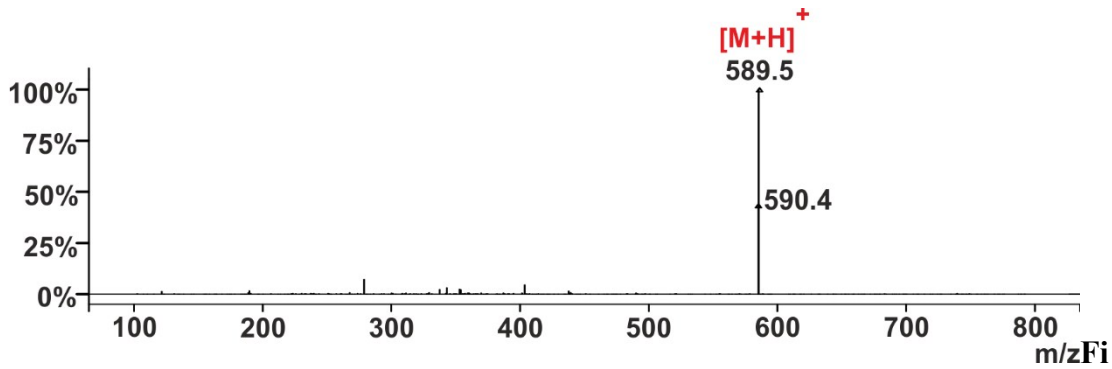


Fig. S56 RP-HPLC chromatogram of the purification of compound **25** (Gradient system: from 80/20% until 40/60% of H_2O+0.1%TFA/MeCN+0.1%TFA, in 30 mins at 214 nm).

Mass characterization of compounds **24** and **25**:



g. S57 ESI-MS spectrum of compound **24** (353.1 $[M+2H]^{2+}$)



g. S58 ESI-MS spectrum of compound **25** (589.5 $[M+H]^{+}$)

^{1}H-NMR characterization of compounds **24** and **25**:

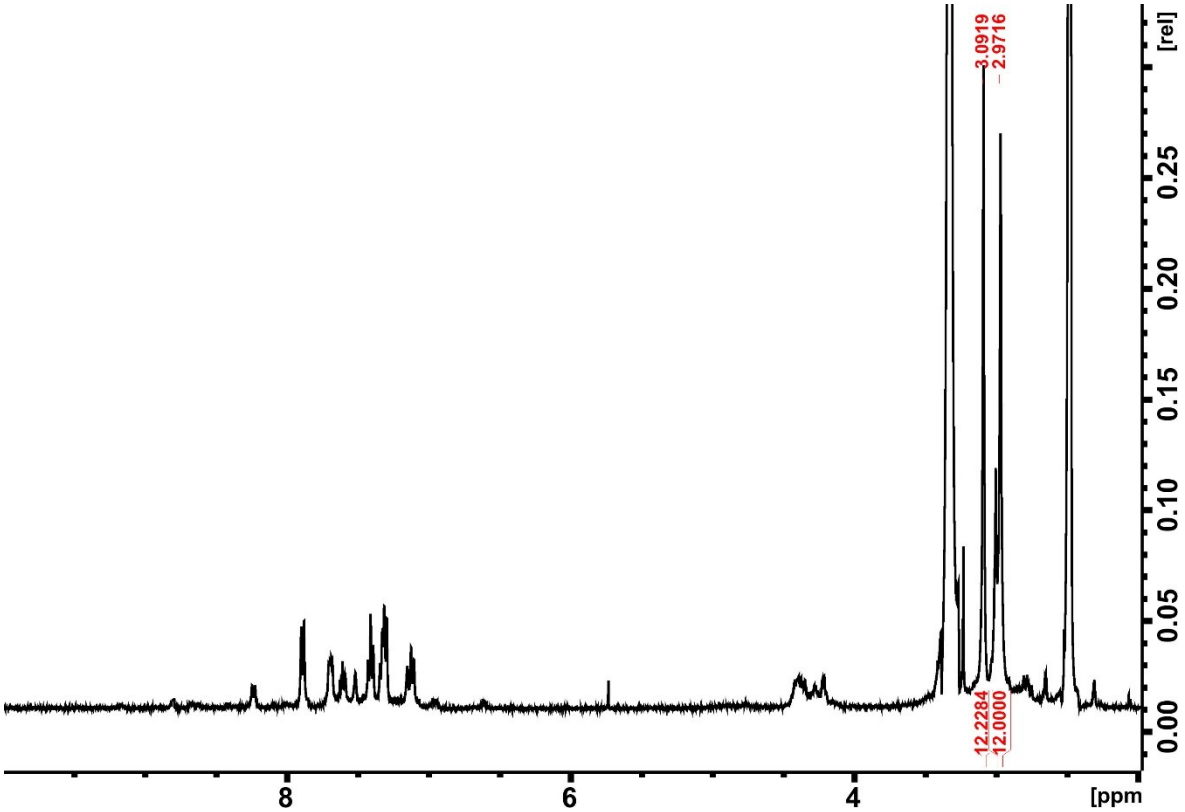


Fig. S59 ^{1}H-NMR spectrum of compound **24** in DMSO-d_6 at 298K

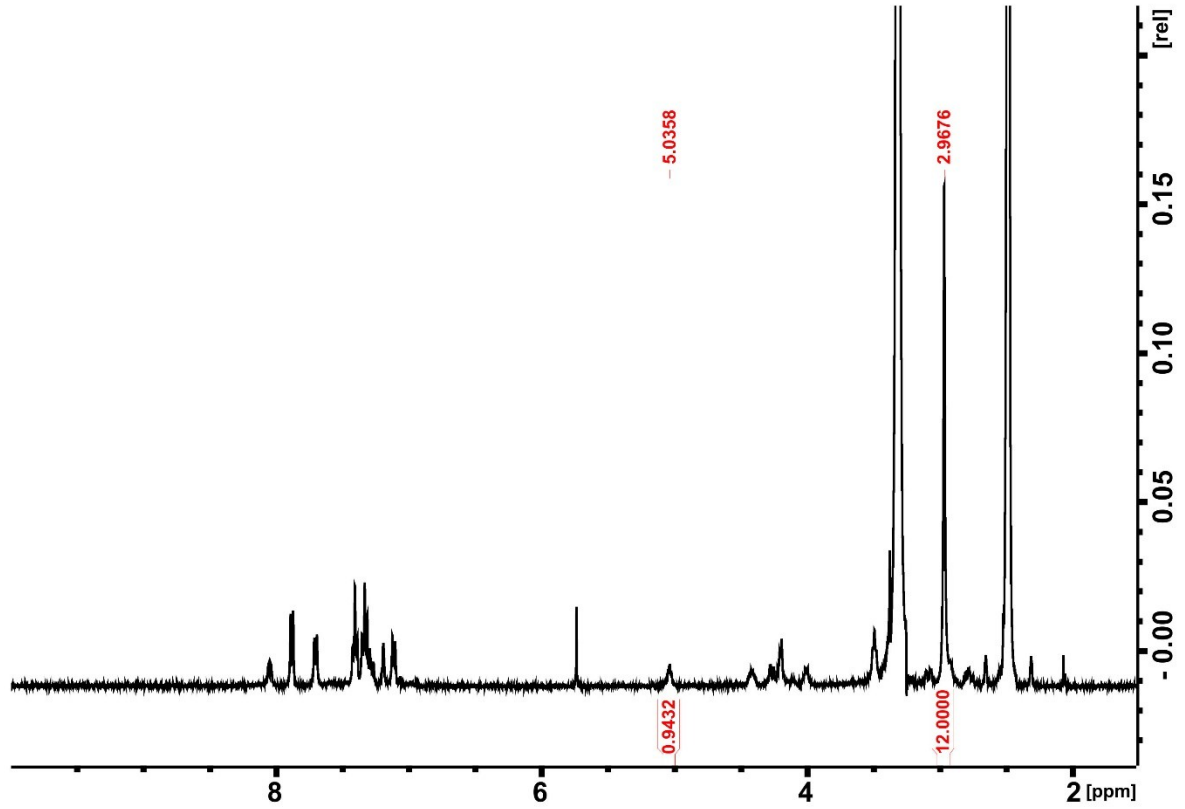


Fig. S60 ^{1}H-NMR spectrum of compound **25** in DMSO-d_6 at 298K

7. Purification and characterization of compound 26

The synthesis of compound **26** was based on the peptide C1B5$_{141\text{-}151}$ (**Fig. S61**) which was synthesized with SPPS, purified via RP-HPLC (**Fig. S62**) and characterized with ESI-MS (**Fig. S63).**

Structure of peptide C1B5$_{141\text{-}151}$:

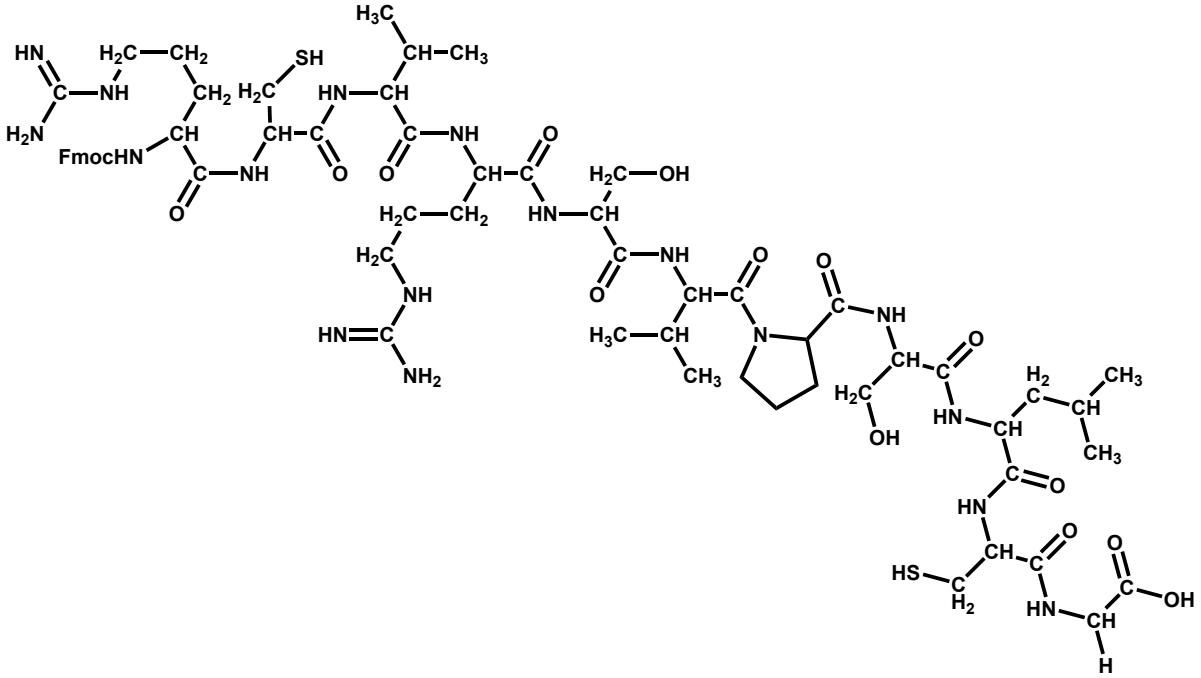


Fig. S61 Structure of the peptide C1B5$_{141\text{-}151}$

Purification of peptide C1B5$_{141\text{-}151}$:

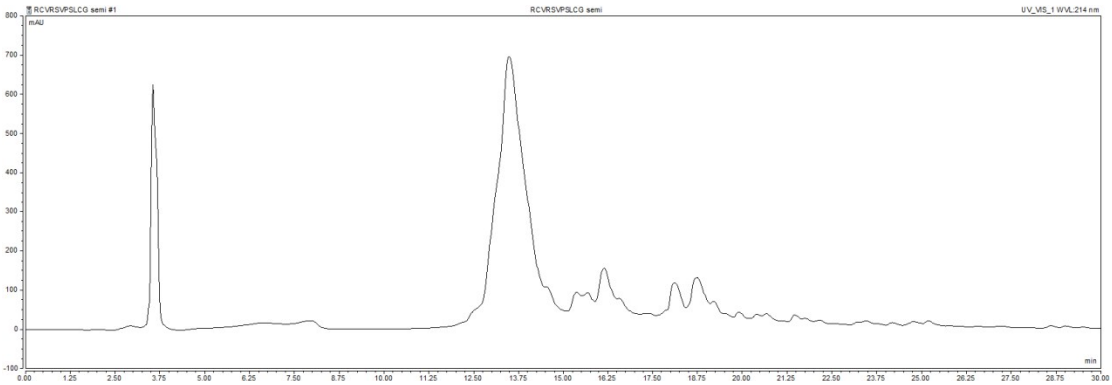


Fig. S62 RP-HPLC chromatogram of the purification of peptide C1B5$_{141\text{-}151}$ (Gradient system: from 90/10% until 30/70% of H_2O+0.1%TFA/MeCN+0.1%TFA, in 30 mins at 214 nm).

Mass characterization of peptide C1B5$_{141\text{-}151}$:

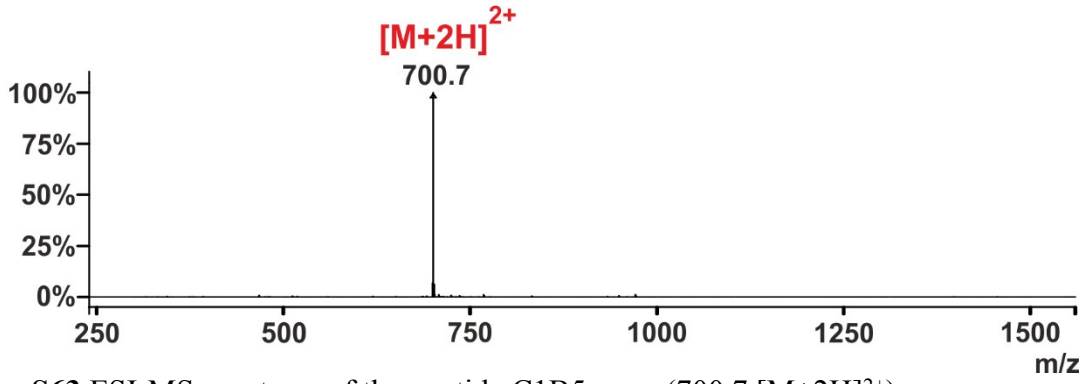


Fig. S63 ESI-MS spectrum of the peptide C1B5$_{141\text{-}151}$ (700.7 $[M+2H]^{2+}$)

The structure, RP-HPLC chromatogram and mass spectrum of compound **26** are illustrated in **Fig. S64**, **Fig. S65** and **Fig. S66** respectively:

Structure of compound **26**:

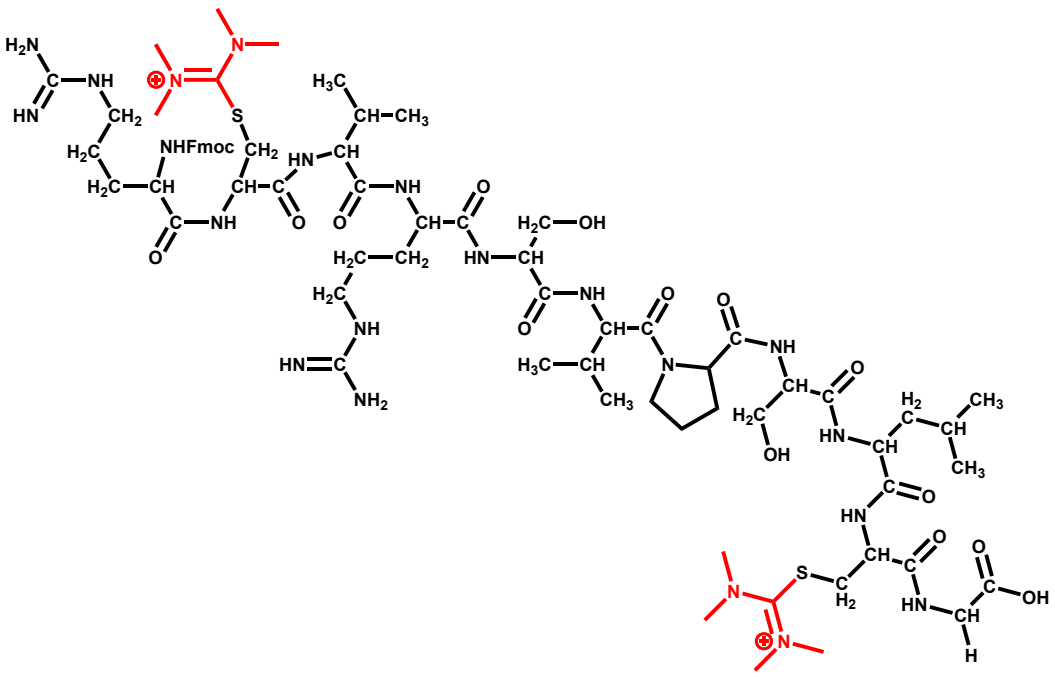


Fig. S64 Structure of compound **26**

Purification of compound **26**:

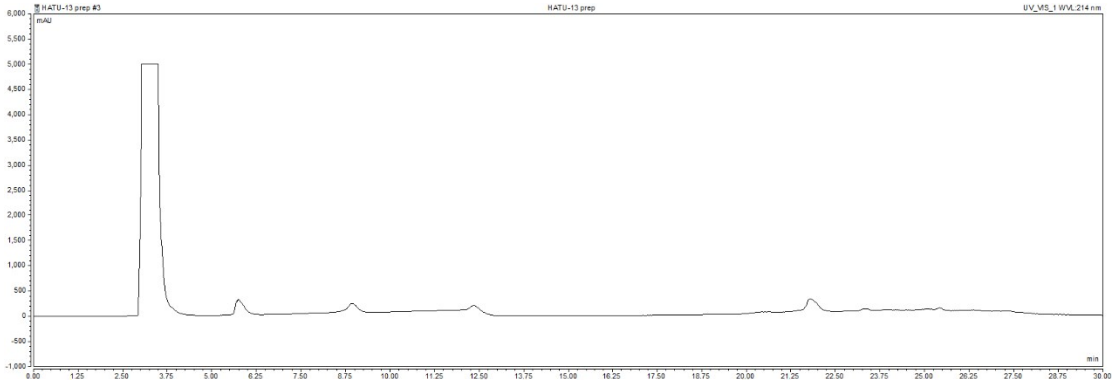


Fig. S65 RP-HPLC chromatogram of the purification of compound **26** (Gradient system: from 98/2% until 50/50% of H_2O+0.1%TFA/MeCN+0.1%TFA, in 30 mins at 214 nm).

Mass characterization of compound **26**:

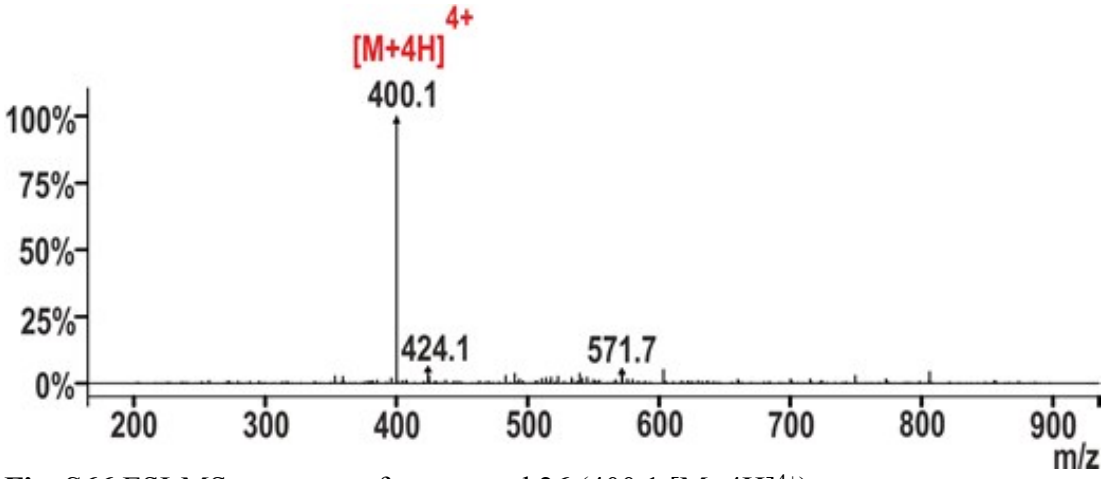
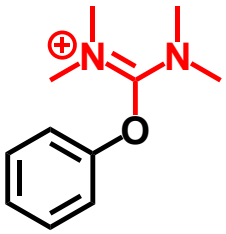


Fig. S66 ESI-MS spectrum of compound **26** (400.1 $[M+4H]^{4+}$)

8. Purification and characterization of compound 27

The structure, RP-HPLC chromatogram, mass and ^{1}H-NMR spectra of compound **27** are illustrated in **Fig. S67**, **Fig. S68**, **Fig. S69** and **Fig. S70** respectively:



Compound 27

Fig. S67 Structure of compound **27**

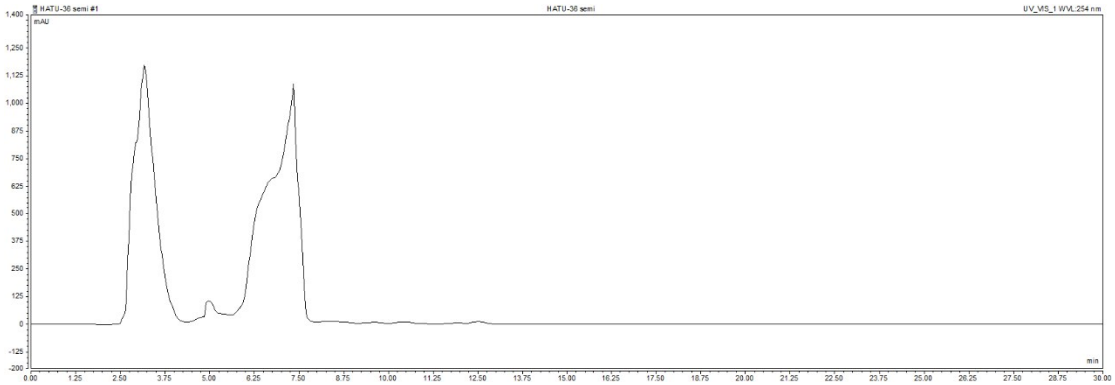


Fig. S68 RP-HPLC chromatogram of the purification of compound **27** (Gradient system: from 70/30% until 0/100% of H_2O+0.1%TFA/MeCN+0.1%TFA, in 30 mins at 214 nm).

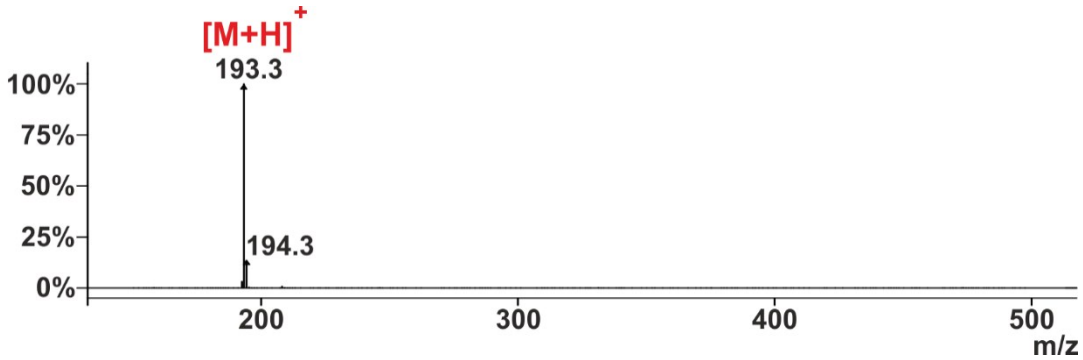


Fig. S69 ESI-MS spectrum of compound **27** (193.3 $[M+H]^+$)

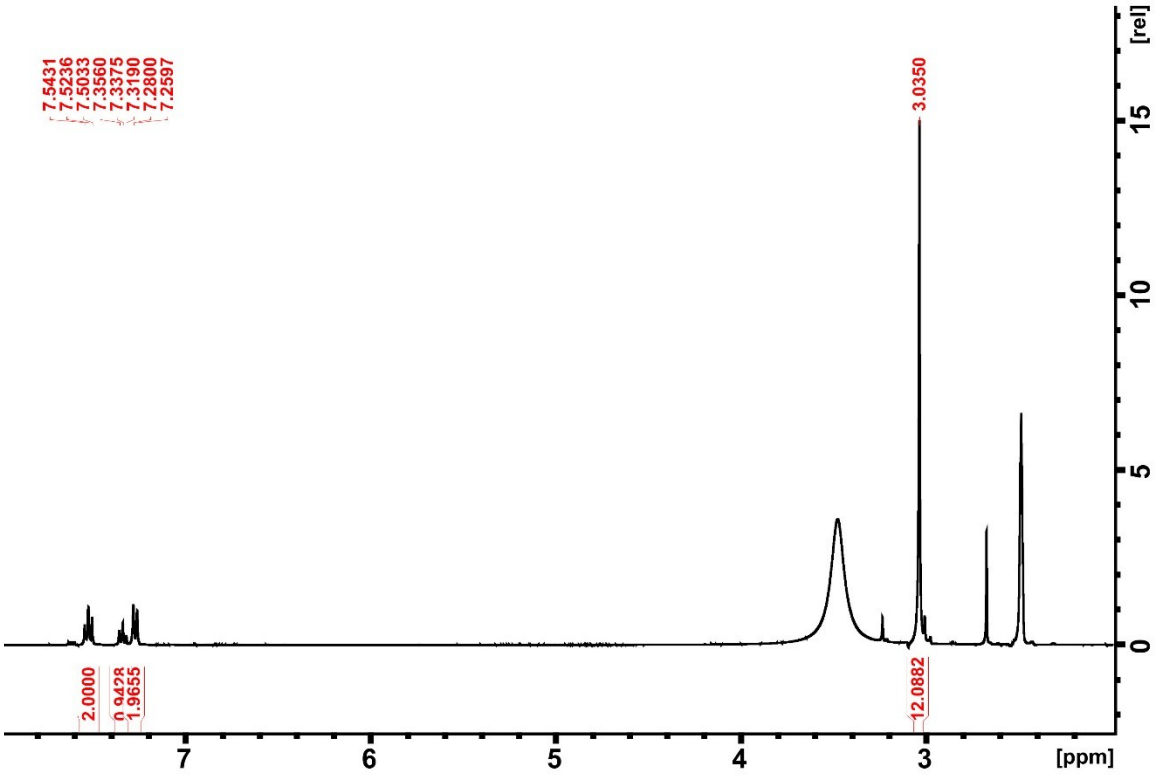
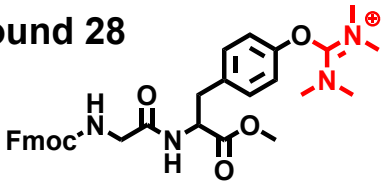


Fig. S70 ^{1}H-NMR spectrum of compound **27** in DMSO-d_6 at 298K

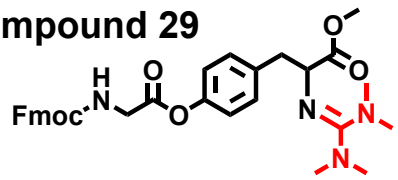
9. Mass characterization of expected compounds 28 (uronium) and 29 (guanidinium).

Compound 28



Molecular Weight: 573.67

Compound 29



Molecular Weight: 572.66

Fig. S71 Structures of expected compounds **28** (uronium) and **29** (guanidinium).

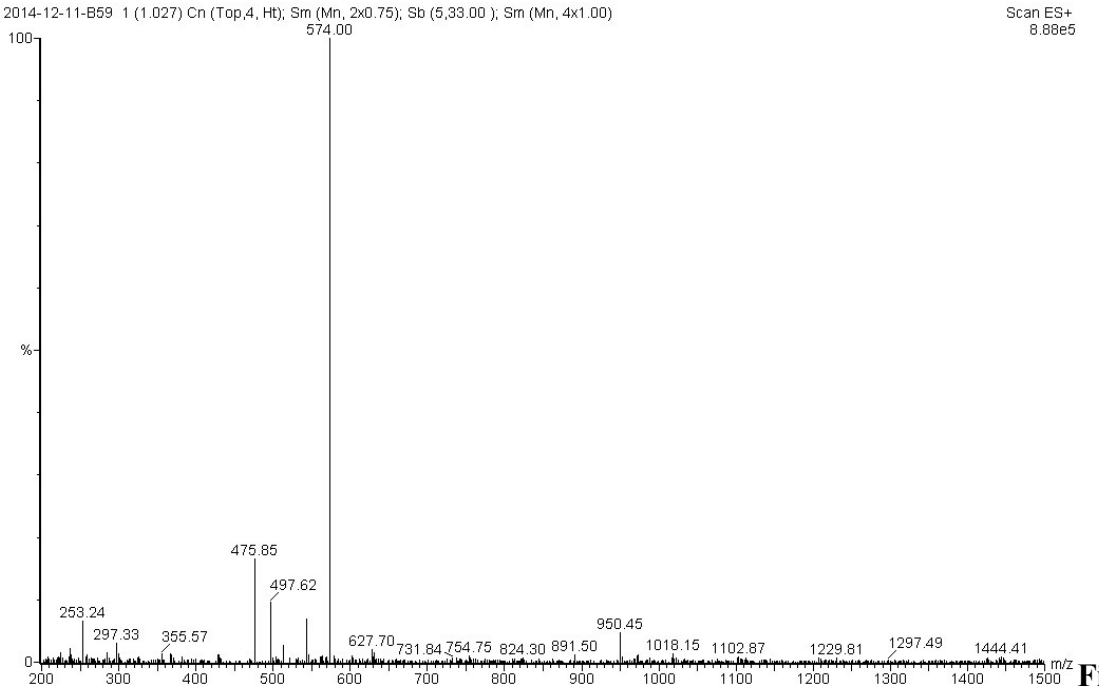


Fig. S72 Mass spectrum of compound **28** (574.00 $[28+H]^+$)