SUPPORTING INFORMATION:

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

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

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

![Chemical structure of Compound 8](image)

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

$^1$H-NMR (250 MHz, DMSO-d$_6$): $\delta$(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$_6$): $\delta$(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:

![Chemical structure of Compound 9](image)

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

$^1$H-NMR (250 MHz, DMSO-d$_6$): $\delta$(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$_6$): $\delta$(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$_6$): $\delta$(ppm) 11.09 (s, 1 H), 8.51 (s, 3H), 7.49 (d, $J = 7.5$ Hz, 1H), 7.37 (d, $J = 7.8$Hz, 1H), 7.23 (d, $J = 2.3$Hz, 1H), 7.09 (t, $J = 7.1$ Hz, 1H), 7.00 (t, $J = 7.1$Hz, 1H), 4.24 (br, 1H), 3.65 (s, 3H), 3.28 (dd, $J = 3.6$Hz, 6.3Hz, 2H). $^{13}$C-NMR (63 MHz, DMSO-d$_6$): $\delta$(ppm) 169.81, 136.25, 125.02, 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$_6$): $\delta$(ppm) 8.96 (s, 1 H), 7.46 (s, 1H), 4.41 (t, $J = 7.5$ Hz, 1H), 3.72 (s, 3H), 5.12 (br, 2H), 3.35 (d, $J = 2.5$Hz, 2H). $^{13}$C-NMR (63 MHz, DMSO-d$_6$): $\delta$(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-\(d_6\)): \(\delta\) (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). \(^1\)\(^3\)C-NMR (63 MHz, DMSO-\(d_6\)): \(\delta\) (ppm) 170.5, 63.7, 58.4, 55.3.

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

![Chemical structure of Compound 13](image)

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

\(^1\)H-NMR (250 MHz, DMSO-\(d_6\)): \(\delta\) (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). \(^1\)\(^3\)C-NMR (63 MHz, DMSO-\(d_6\)): \(\delta\) (ppm) 169.59, 65.94, 58.81, 53.73, 20.93.

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

![Chemical structure of Compound 14](image)

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

\(^1\)H-NMR (250 MHz, DMSO-\(d_6\)): \(\delta\) (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).

\(^1\)\(^3\)C-NMR (100 MHz, DMSO-\(d_6\)): \(\delta\) (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:

![Chemical structure of Compound 15a]


**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:

![Chemical structure of Compound 15b]

**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:

![Chemical structure of Compound 16a]

**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:

![Chemical structure of Compound 16b]

**Mass:** ESI-MS m/z: calcd: 623.37 [M+H]^+; found: 624.3 [M+H]^+. 
HATU 1.5 equivalents:

![Fig. S5](image1) Mass spectrum of compounds 16 with 1.5 eq of HATU.

HATU 1.0 equivalent:

![Fig. S6](image2) 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:

![Chemical structure of Compound 17a]


**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):

![Chemical structure of Compound 17b]

**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:

![Chemical Structure of Compound 18a]

**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:

![Chemical Structure of Compound 18b]

**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.

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:

![Chemical Structure of Compound 19a](image)


**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:

![Chemical Structure of Compound 19b](image)

**Mass:** ESI-MS $m/z$: calcd: 484.32 [M+H]$^+$; found: -
HATU 1.5 equivalents:

![Mass spectrum of compounds 19 with 1.5 eq of HATU.](image)

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

HATU 1.0 equivalent:

![Mass spectrum of compounds 19 with 1 eq of HATU.](image)

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:

![Chemical structure of Compound 20a](image)


**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:

![Chemical structure of Compound 20b](image)

**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:

![Chemical structure of Compound 21a]

**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:

![Chemical structure of Compound 21b]

**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:

![Mass spectrum of compounds 21 with 1.5 eq of HBTU.](image)

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

HBTU 1.0 equivalents:

![Mass spectrum of compounds 21 with 1 eq of HBTU.](image)

**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 H₂O+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 ¹H-NMR of compound 6 in DMSO-d₆ 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 ¹H-NMR of compound 6 in D₂O 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$_2$O.

![Figure S31](image)

**Fig. S31** Overlay of 2D NMR TOCSY spectra of [D-Lys]$^5$-GnRH (red color) and compound 6 (black color) in D$_2$O at 298K

Analytical RP-HPLC of the purified compound 6:

![Figure S32](image)

**Fig. S32** Analytical RP-HPLC chromatogram of compound 6 in its pure state
(Gradient system: from 90/10% until 10/90% of H$_2$O+0.1%TFA/MeCN+0.1%TFA, in 30 mins at 214 nm).

HRMS characterization of compound 6:

![HRMS spectrum of compound 6](image)

**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$_2$O+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:**

![Structure of compound 22](image)

**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$_2$O+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]$^{3+}$)
1H-NMR characterization of compound 22:

![1H-NMR spectrum of compound 22 in DMSO-d\textsubscript{6} at 298K](image)

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:
**Fmoc-HER2-BP1**

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

**Purification of peptide Fmoc-HER2-BP1:**

![RP-HPLC chromatogram](image)

**Fig. S43** RP-HPLC chromatogram of the purification of peptide Fmoc-HER2-BP1. (Gradient system: from 80/20% until 20/80% of H$_2$O+0.1%TFA/MeCN+0.1%TFA, in 30 mins at 214 nm).

**Mass characterization of peptide Fmoc-HER2-BP1:**

![ESI-MS spectrum](image)

**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:

![Compound 23](image)

Fig. S45 Structure of compound 23.

Purification of compound 23:

![RP-HPLC chromatogram](image)

Fig. S46 RP-HPLC chromatogram of the purification of compound 23 (Gradient system: from 80/20% until 20/80% of H$_2$O+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+)](image)

1H-NMR characterization of compound 23:

![Fig. S48 1H-NMR spectrum of compound 23 in DMSO-d_6 at 298K](image)

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-NH₂ and Fmoc-Ser-Tyr-NH₂:

![Structures of dipeptides Fmoc-Cys-Tyr-NH₂ and Fmoc-Ser-Tyr-NH₂](image)

**Fig. S49** Structures of dipeptides Fmoc-Cys-Tyr-NH₂ and Fmoc-Ser-Tyr-NH₂

Purification of dipeptides Fmoc-Cys-Tyr-NH₂ and Fmoc-Ser-Tyr-NH₂:

**Fig. S50** RP-HPLC chromatogram of the purification of dipeptide Fmoc-Cys-Tyr-NH₂ (Gradient system: from 90/10% until 30/70% of H₂O+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-NH₂ (Gradient system: from 80/20% until 40/60% of H₂O+0.1%TFA/MeCN+0.1%TFA, in 30 mins at 214 nm).
Mass characterization of dipeptides Fmoc-Cys-Tyr-NH₂ and Fmoc-Ser-Tyr-NH₂:

Fig. S52 ESI-MS spectrum of dipeptide Fmoc-Cys-Tyr-NH₂ (528.2 [M+2H+Na]^{3+}; 381.3 [M+3H+Na]^{4+})

Fig. S53 ESI-MS spectrum of dipeptide Fmoc-Ser-Tyr-NH₂ (512.9 [M+2H+Na]^{3+}; 349.8 [M+3H+Na]^{4+})

The structures, RP-HPLC chromatogram, mass and ¹H-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:
Purification of compounds 24 and 25:

**Fig. S54** Structures 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$_2$O+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$_2$O+0.1%TFA/MeCN+0.1%TFA, in 30 mins at 214 nm).
Mass characterization of compounds 24 and 25:

![ESI-MS spectrum of compound 24 (353.1 \([\text{M+2H}]^{2+}\))](image1)

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

![ESI-MS spectrum of compound 25 (589.5 [M+H]+)](image2)

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

\(^1\text{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_{141-151} (Fig. S61) which was synthesized with SPPS, purified via RP-HPLC (Fig. S62) and characterized with ESI-MS (Fig. S63).

Structure of peptide C1B5_{141-151}:

![Structure of peptide C1B5_{141-151}](image)

**Fig. S61** Structure of the peptide C1B5_{141-151}

Purification of peptide C1B5_{141-151}:

![RP-HPLC chromatogram](image)

**Fig. S62** RP-HPLC chromatogram of the purification of peptide C1B5_{141-151} (Gradient system: from 90/10% until 30/70% of H₂O+0.1%TFA/MeCN+0.1%TFA, in 30 mins at 214 nm).
Mass characterization of peptide C1B5_{141-151}:

![ESI-MS spectrum of the peptide C1B5_{141-151} (700.7 [M+2H]^2+)](image)

**Fig. S63** ESI-MS spectrum of the peptide C1B5_{141-151} (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:**

![Structure of compound 26](image)

**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 H₂O+0.1%TFA/MeCN+0.1%TFA, in 30 mins at 214 nm).

Mass characterization of compound 26:

![Mass spectrum of compound 26](image)

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 ¹H-NMR spectra of compound 27 are illustrated in Fig. S67, Fig. S68, Fig. S69 and Fig. S70 respectively:
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 H₂O+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]⁺)
9. Mass characterization of expected compounds 28 (uronium) and 29 (guanidinium).

**Compound 28**

![Structure of Compound 28]

Molecular Weight: 573.67

**Compound 29**

![Structure of 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]^+)