Supporting Information

Solid Phase Peptide Synthesis using side-chain unprotected arginine and histidine, with Oxyma Pure/TBEC in green solvents.

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General methods

Unless otherwise specified, all solvents and reagents were obtained from commercial suppliers, of the best grade, and used without further purification. Specifically, Fmoc amino acids and resins were supplied by Iris Biotech, Alfa Aesar, Merck or Fluorochem. Coupling reagents were purchased from Merck or Novabiochem. Piperidine (PIP) and 3-(Diethylamino)propylamine (DEAPA) were supplied by Merck or TCI (purity >99%). N,N-dimetilformammide (DMF), N-octylpyrrolidone (NOP), N-butyl-pyrrolidone (NBP), Dimethyl carbonate (DMC), Ethyl acetate (AcOEt), Dimethyl sulfoxide (DMSO) and HPLC-quality acetonitrile (CH_3CN) were purchased from Merck. Milli-Q water was used for RP-HPLC analyses. Automated solid-phase peptide syntheses were carried out manually. SPPS at 35 °C was performed using a Minichiller 300 from Huber. The ^{1}H NMR and ^{13}C NMR spectra were recorded by using an INOVA 600 MHz instrument with a 5 mm probe. All chemical shifts were quoted relative to deuterated solvent signals.

Analytical methods

HPLC-MS analyses were performed on Agilent 1260 Infinity II system coupled to ESI mass spectrometer (positive-ion mode, m/z = 100–3000 amu, fragmentor 30 V), with the following parameters:

- column Phenomenex Luna C18 5 µm, 250 x 4.6 mm or InfinityLab Poroshell C18 2.7 µm, 150 x 3.0 mm
- temperature: 35°C
- injection volume: 10 µL
- UV: 210 nm
- mobile phases: H_2O+0.08%TFA (mobile phase A) and ACN+0.08%TFA (mobile phase B)
- flow: 1.0 mL/min

The gradient of analytical methods reported across the paper are defined as follow:

Method 1		
Column: Phenomenex Luna C18 5 µm, 250 x 4.6 mm		
Flow: 1 ml/min		
Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	95	5
22	5	95
25	5	95
28	95	5
30	95	5

Method 2		
Column: Phenomenex Luna C18 5 µm, 250 x 4.6 mm		
Flow: 1 ml/min		
Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	90	10
13	70	30
15	50	50
18	10	90
20	10	90
22	90	10
25	90	10

Method 3		
Column: Phenomenex Luna C18 5 µm, 250 x 4.6 mm		
Flow: 1 ml/min		
Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	95	5
1	95	5
30	85	15
32	5	95
32	5	95
34	95	5
36	95	5

Method 4		
Column: InfinityLab Poroshell C18 2.7 µm, 150 x 3.0 mm		
Flow: 1 ml/min		
Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	80	20
7	20	80
10	20	80
12	80	20
15	80	20

Method 5		
Column: Phenomenex Luna C18 5 µm, 250 x 4.6 mm		
Flow: 1 ml/min		
Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	95	5
4	80	20
15	70	30
19	50	50
22	5	95
24	5	95
27	95	5
30	95	5

ChemStation software was used for data processing. Percentage areas of integrated peaks are reported in mAu.

1. NMR studies

^{1}H NMR of 1a and 1b

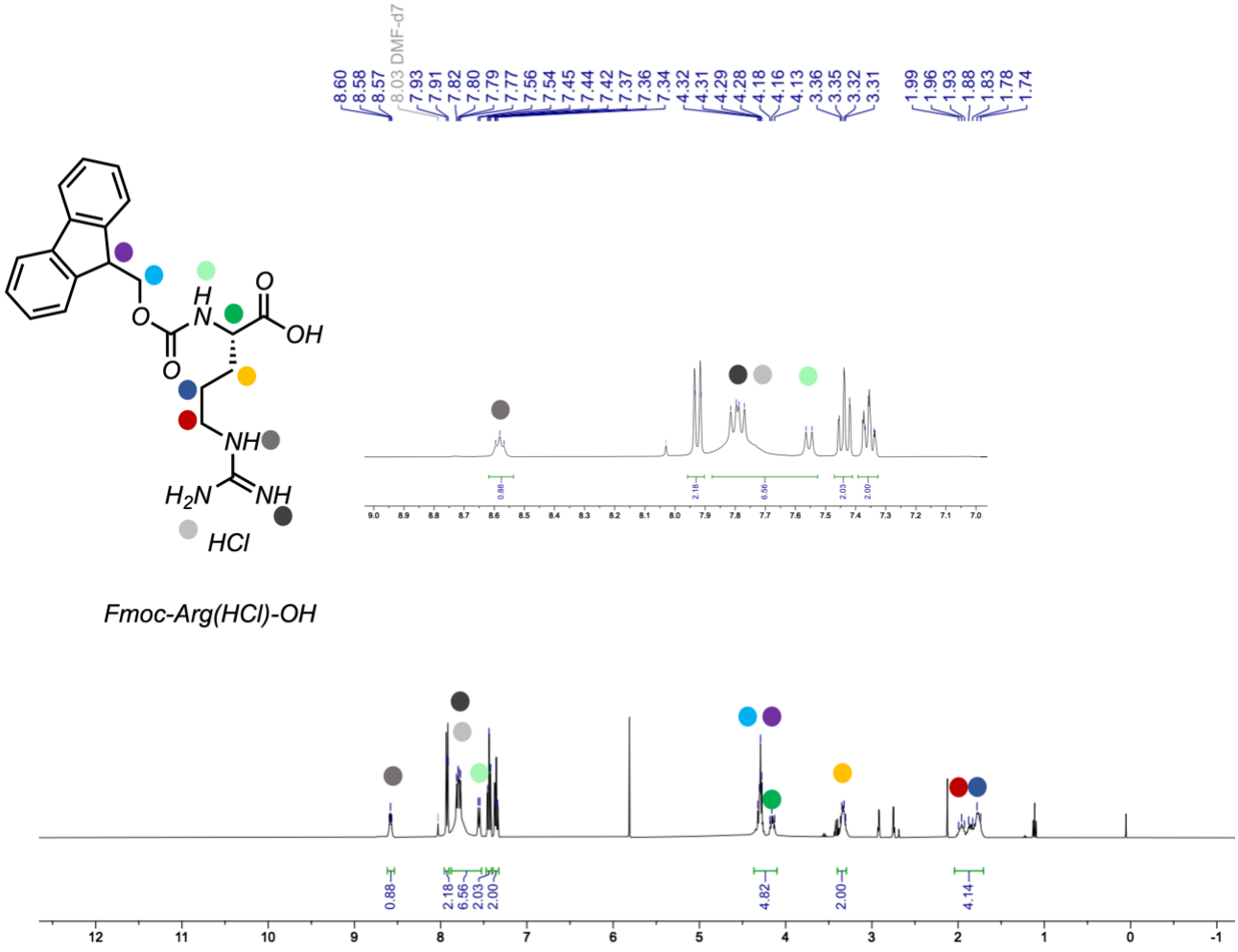


Figure S1. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH

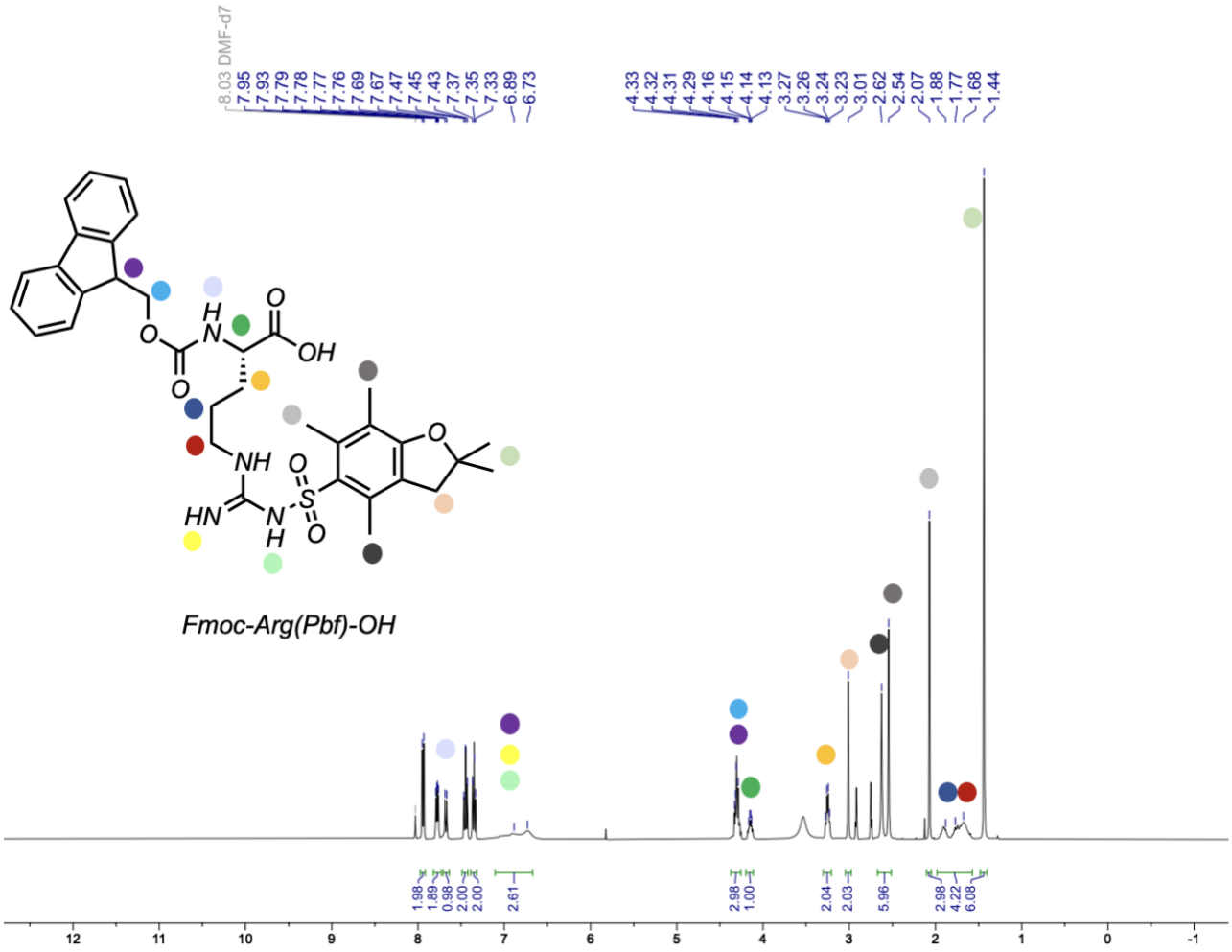
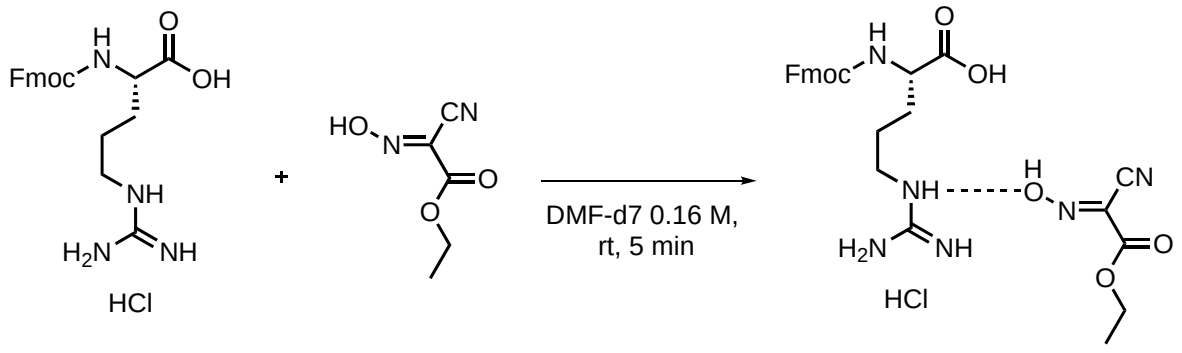


Figure S2. ^{1}H NMR spectrum of Fmoc-Arg(Pbf)-OH

Fmoc-Arg(HCl)-OH

Fmoc-Arg(HCl)-OH with 1.0 eq of Oxyma Pure



Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma Pure (0.1 mmol, 14.3 mg, 1.0 eq) were dissolved in a glass-vial with 0.6 mL of DMF-d_7. After 5 minutes the ^{1}H NMR spectrum was acquired.

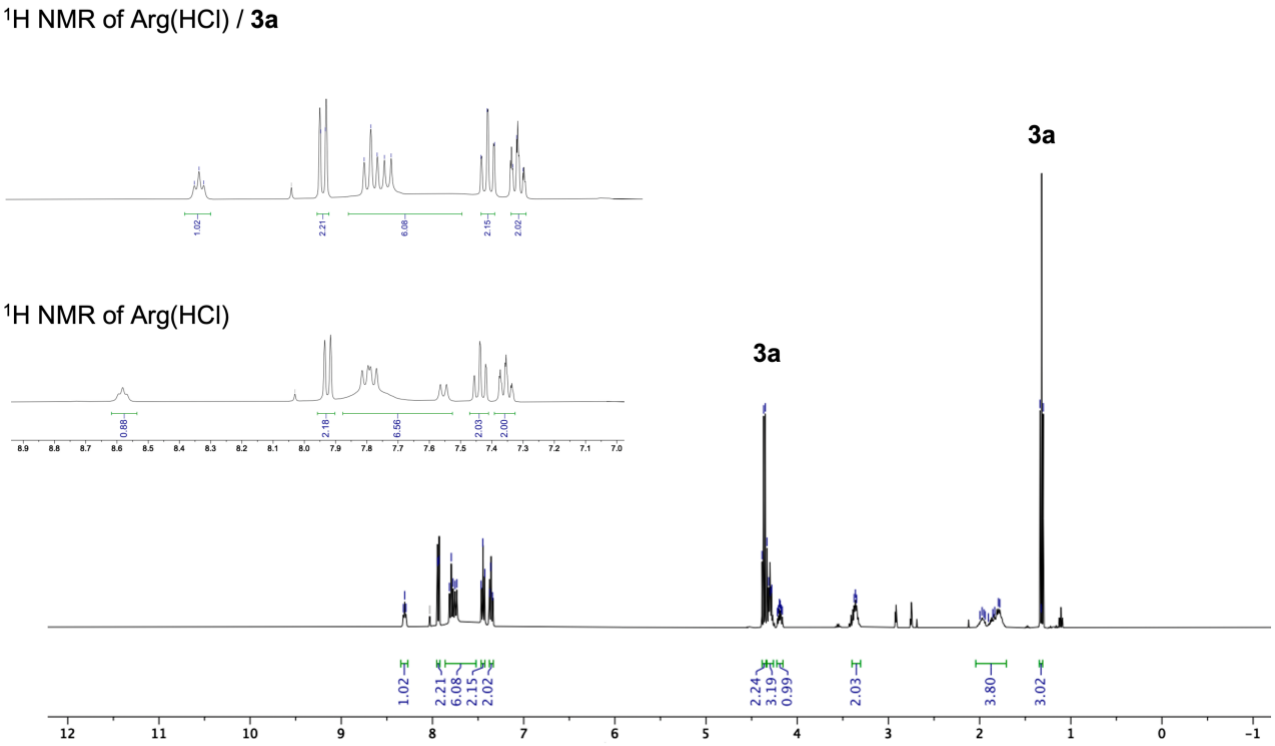
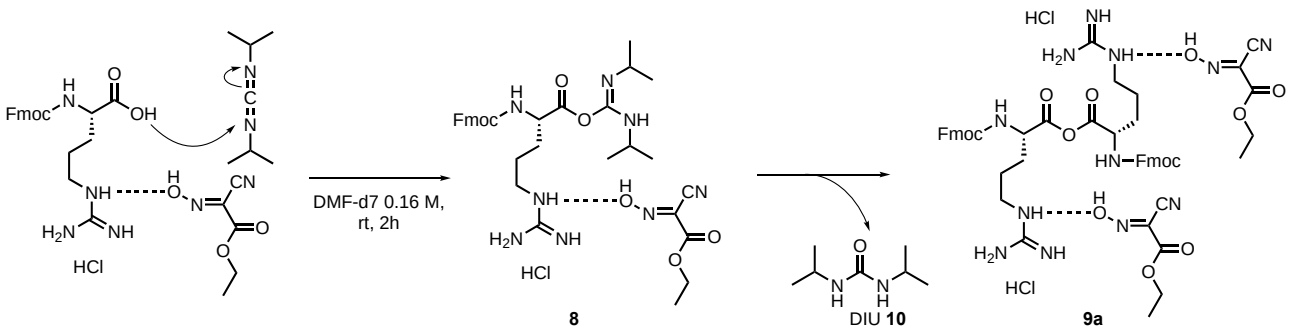


Figure S3. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH and 1.0 eq of Oxyma pure

Fmoc-Arg(HCl)-OH with 1.0 eq of Oxyma Pure and 1.0 eq of DIC



Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma Pure (0.1 mmol, 14.3 mg, 1.0 eq) were dissolved in a glass-vial with 0.6 mL of DMF-d_7. After 5 minutes N,N'-diisopropyl-carbodiimide (DIC) (0.1 mmol, 15.5 µL, 1.0 eq) was added and the reaction stirred at room temperature. After 2h, the ^{1}H NMR spectrum was acquired.

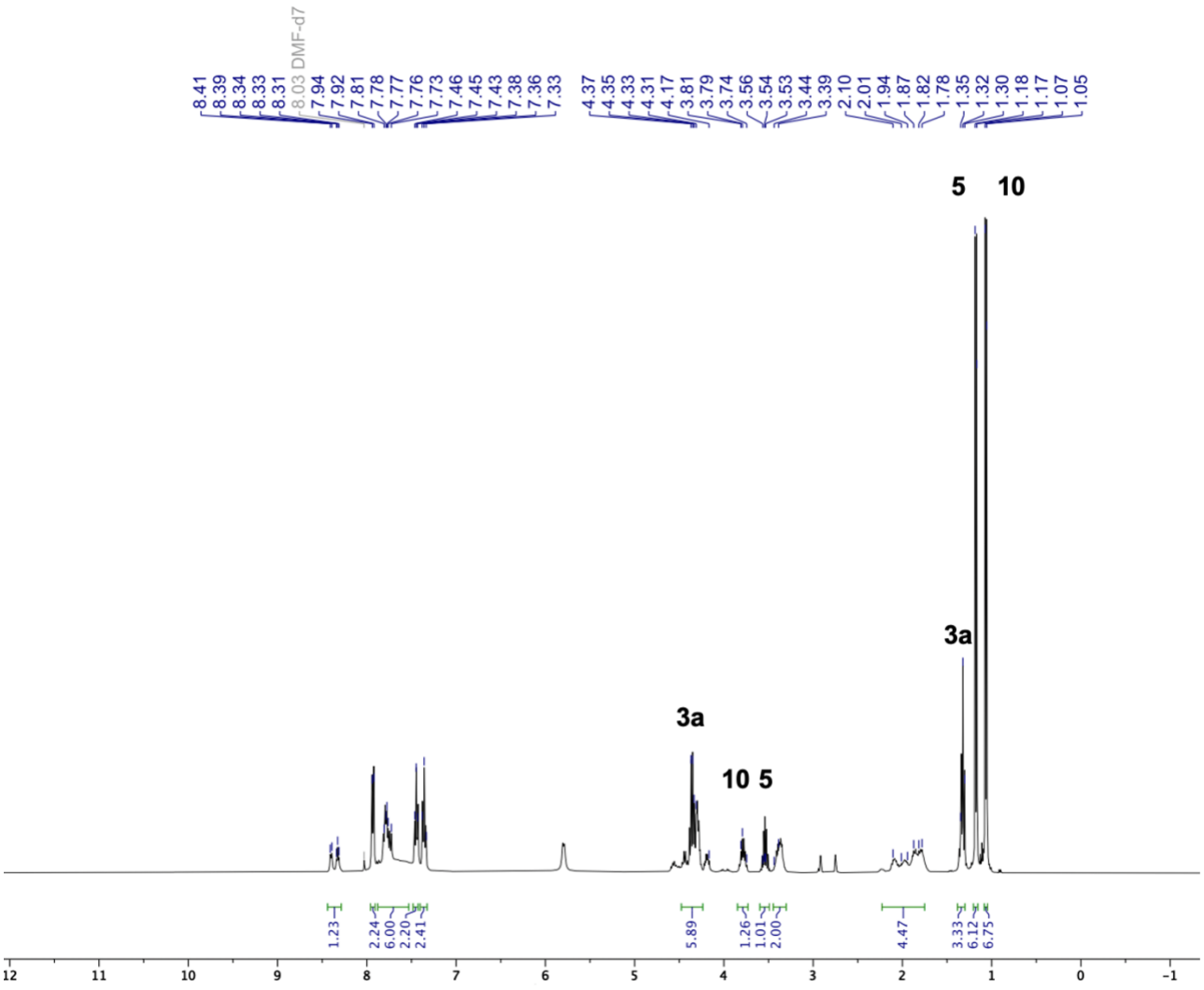
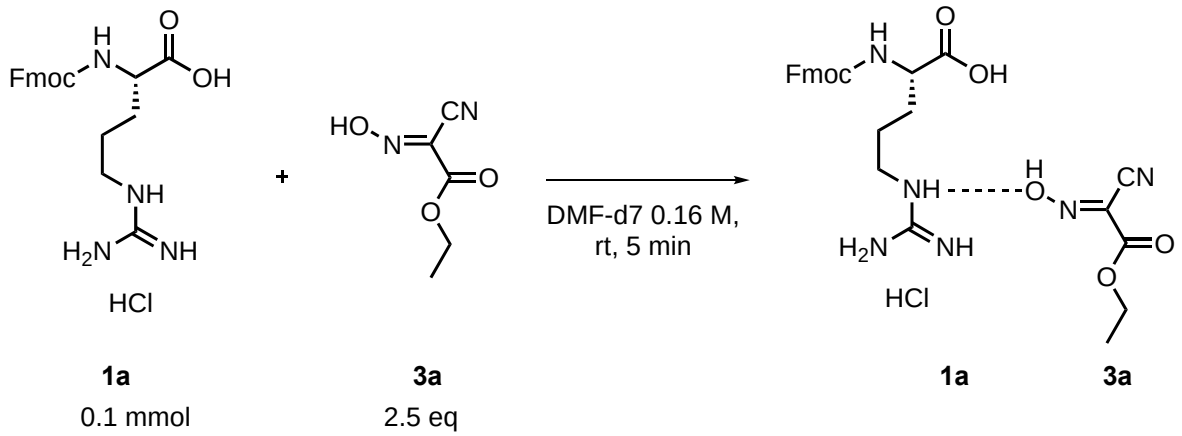


Figure S4. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH, 1.0 eq of Oxyma pure and 1.0 eq of DIC

Fmoc-Arg(HCl)-OH with 2.5 eq of Oxyma Pure



Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma Pure (0.25 mmol, 35.6 mg, 2.5 eq) were dissolved in a glass-vial with 0.6 mL of DMF-d_7. After 5 minutes the ^{1}H NMR spectrum was acquired

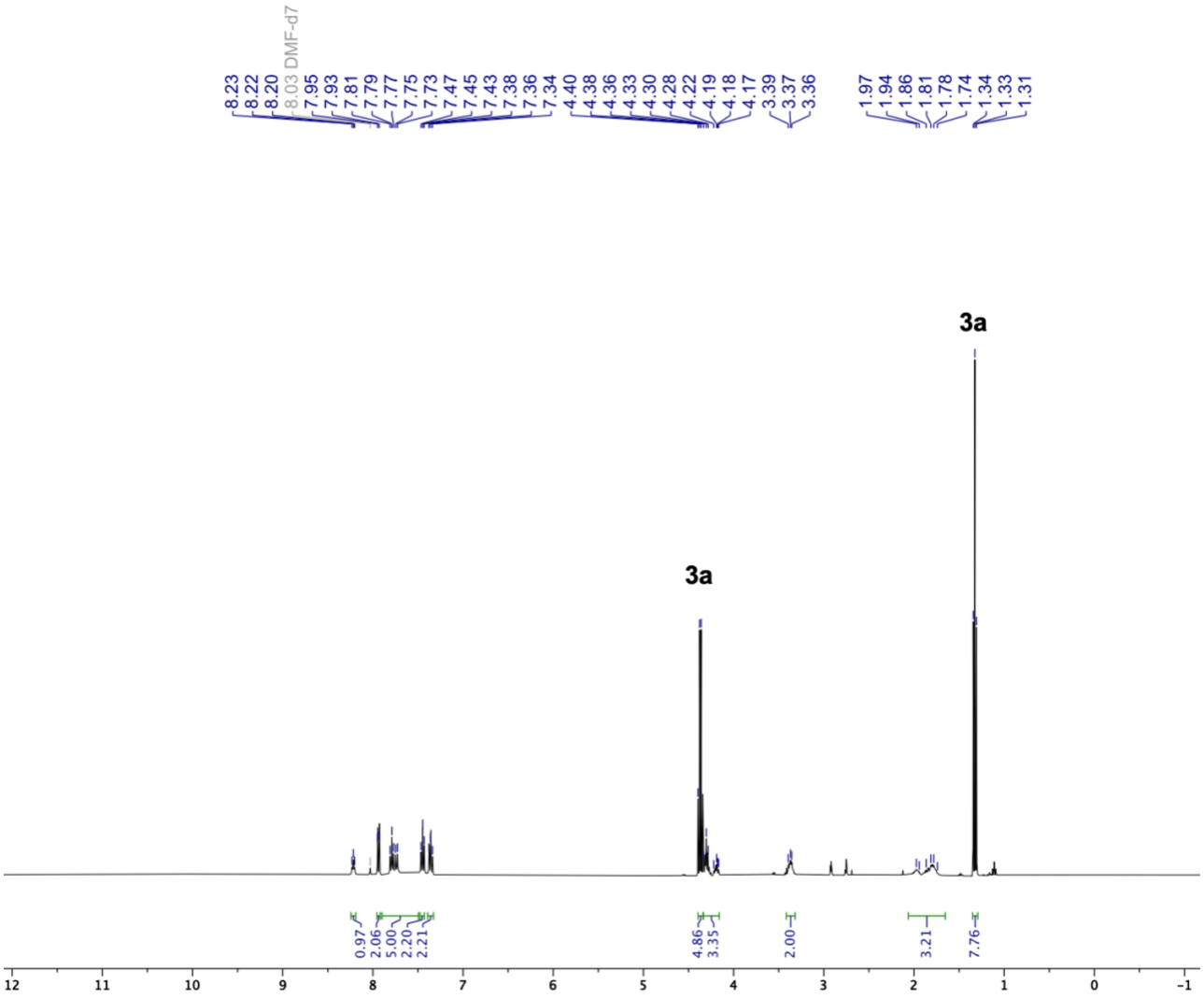
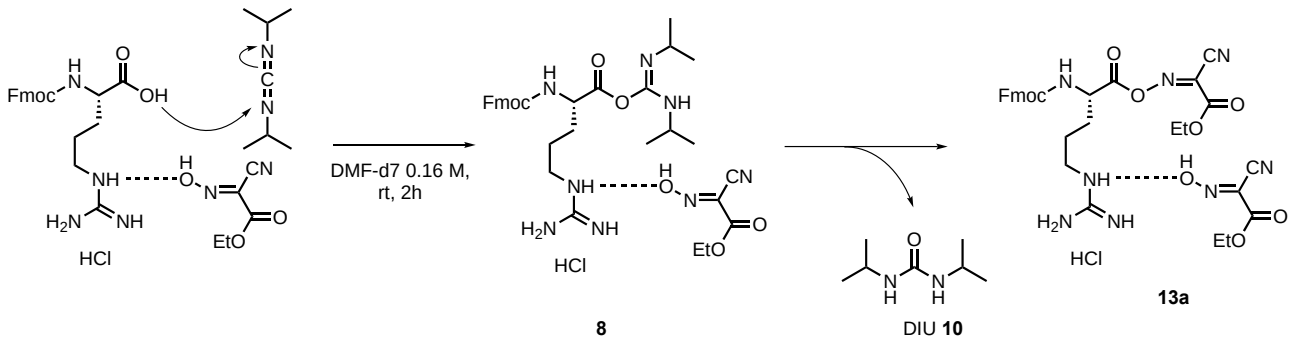


Figure S5. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH and 2.5 eq of Oxyma Pure

Fmoc-Arg(HCl)-OH with 2.5 eq of Oxyma Pure and 1.0 eq of DIC



Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma Pure (0.25 mmol, 35.6 mg, 2.5 eq) were dissolved in a glass-vial with 0.6 mL of DMF-d_7. After 5 minutes DIC (0.1 mmol, 15.5 µL, 1.0 eq) was added and the reaction stirred at room temperature. After 2h, the ^{1}H NMR spectrum was acquired.

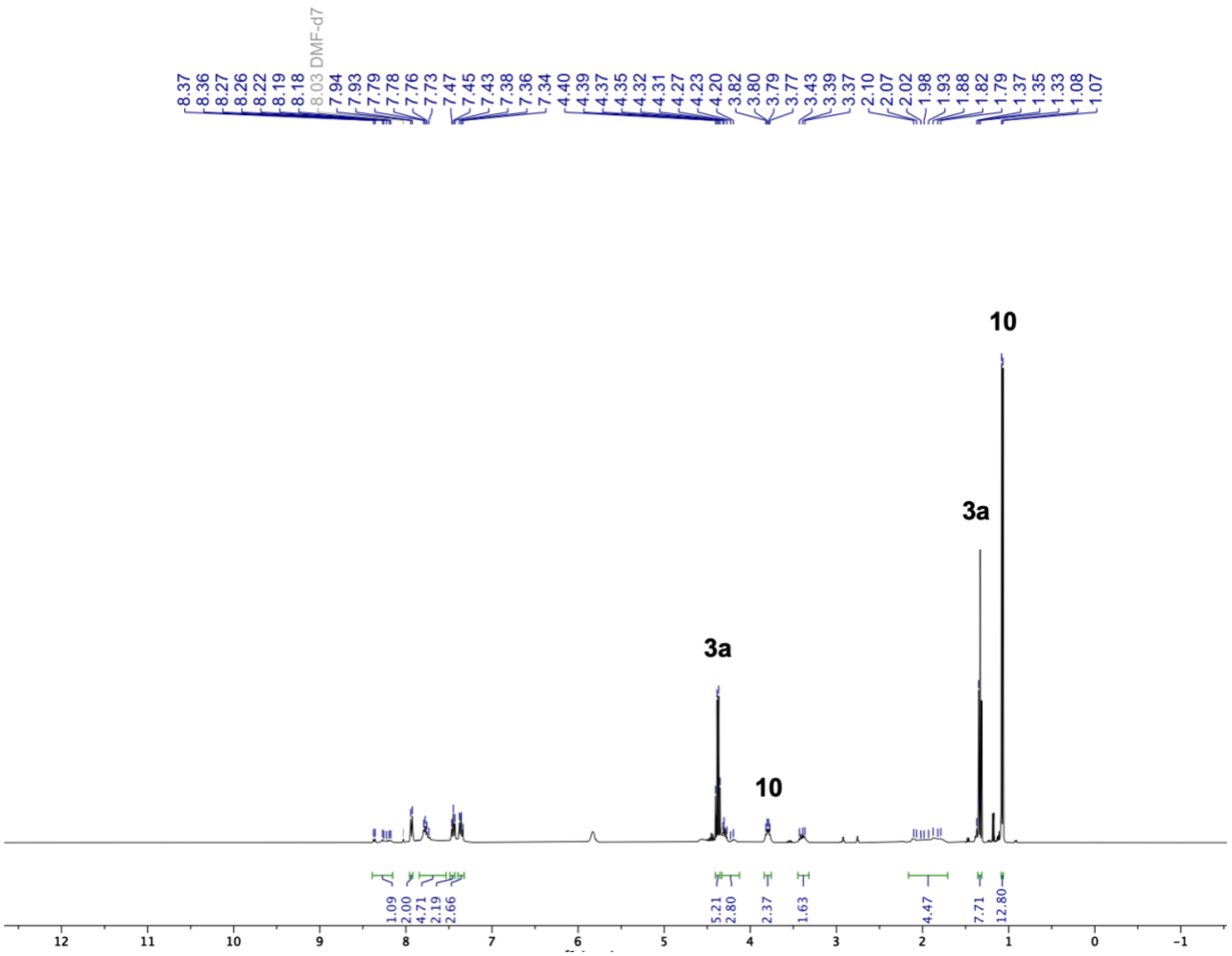
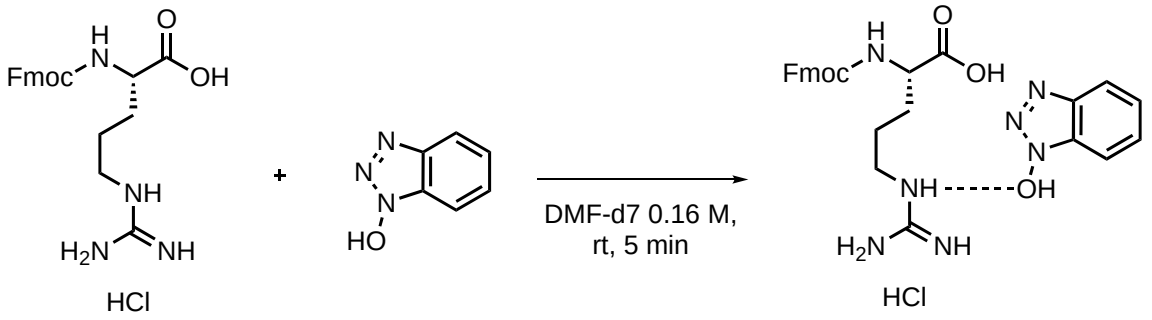


Figure S6. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH, 2.5 eq of Oxyma Pure and 1.0 eq of DIC

Fmoc-Arg(HCl)-OH with 1.0 eq of HOBt



1a 0.1 mmol **3b** 1.0 eq **1a** **3b**

Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and HOBt (0.1 mmol, 13.5 mg, 1.0 eq) were dissolved in a glass-vial with 0.6 mL of DMF-d_7. After 5 minutes the ^{1}H NMR spectrum was acquired.

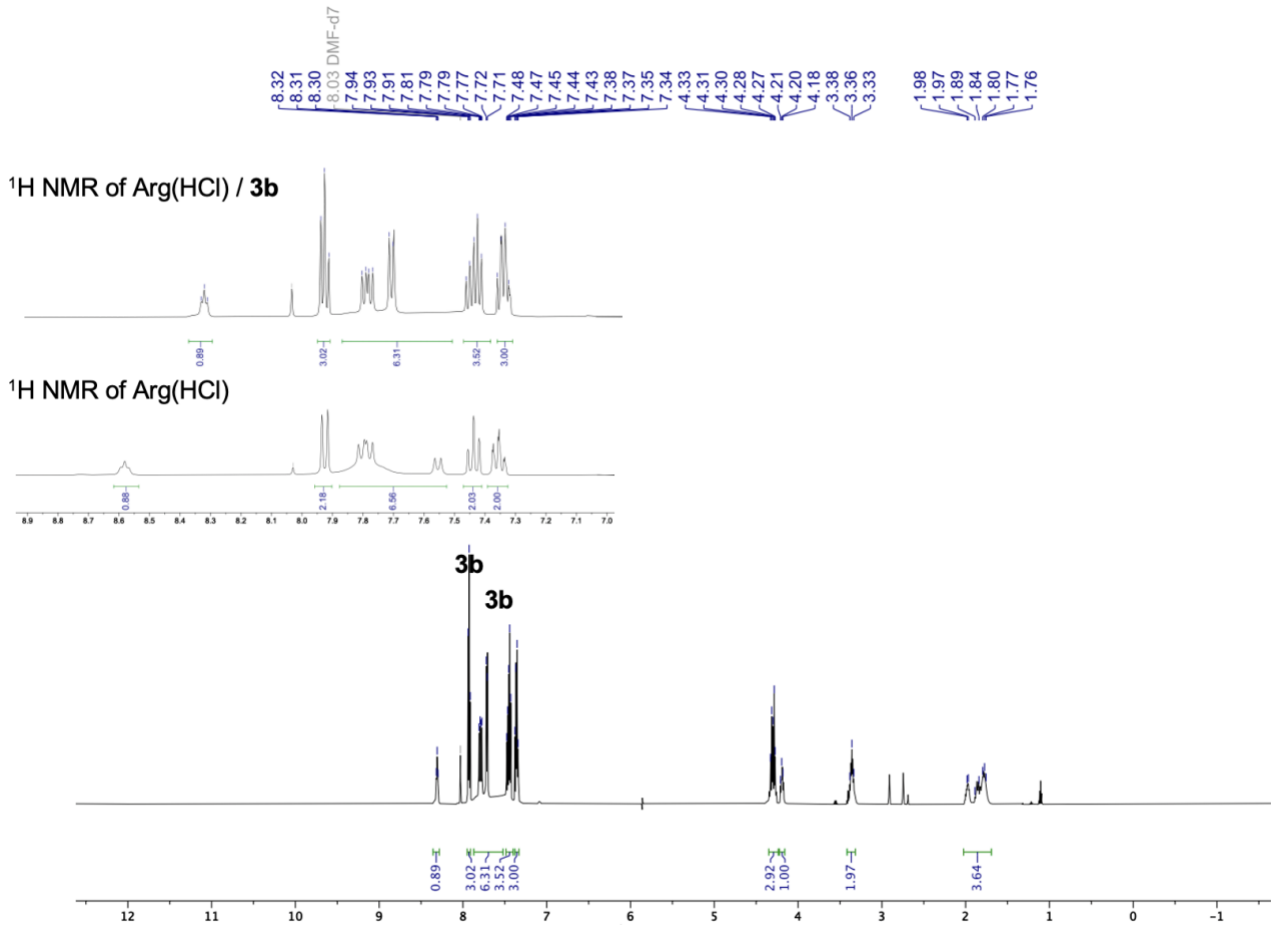
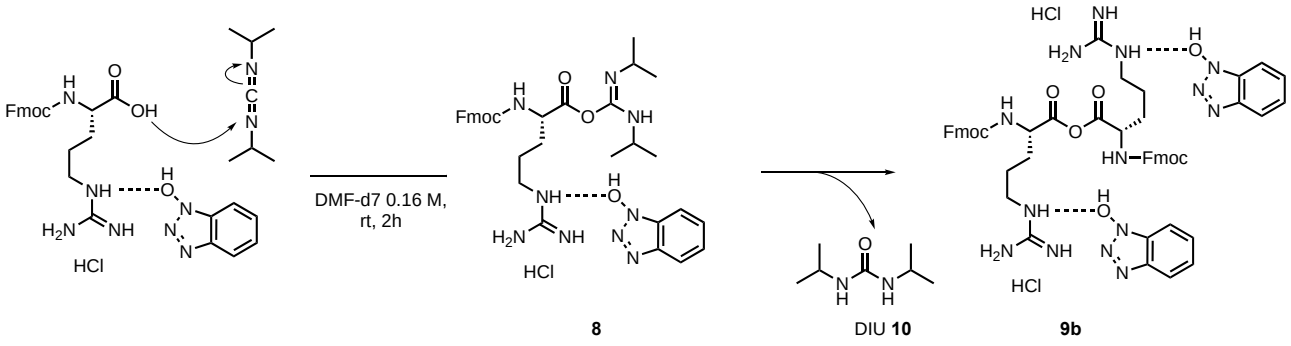


Figure S7. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH and 1.0 eq of HOBt

Fmoc-Arg(HCl)-OH with 1.0 eq of HOBt and 1.0 eq of DIC



Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and HOBt (0.1 mmol, 13.5 mg, 1.0 eq) were dissolved in a glass-vial with 0.6 mL of DMF-d_7. After 5 minutes DIC (0.1 mmol, 15.5 µL, 1.0 eq) was added and the reaction stirred at room temperature. After 2h, the ^{1}H NMR spectrum was acquired.

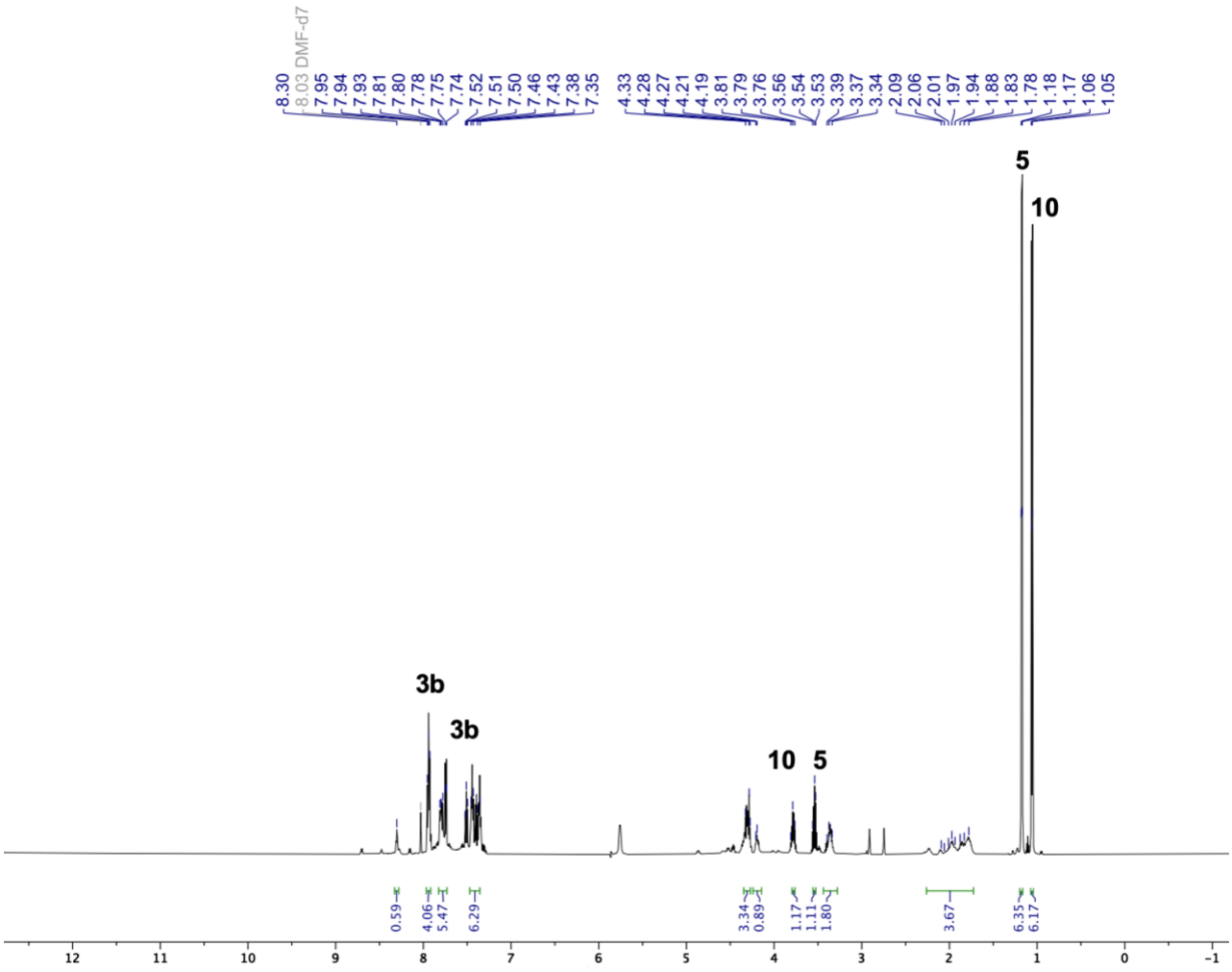
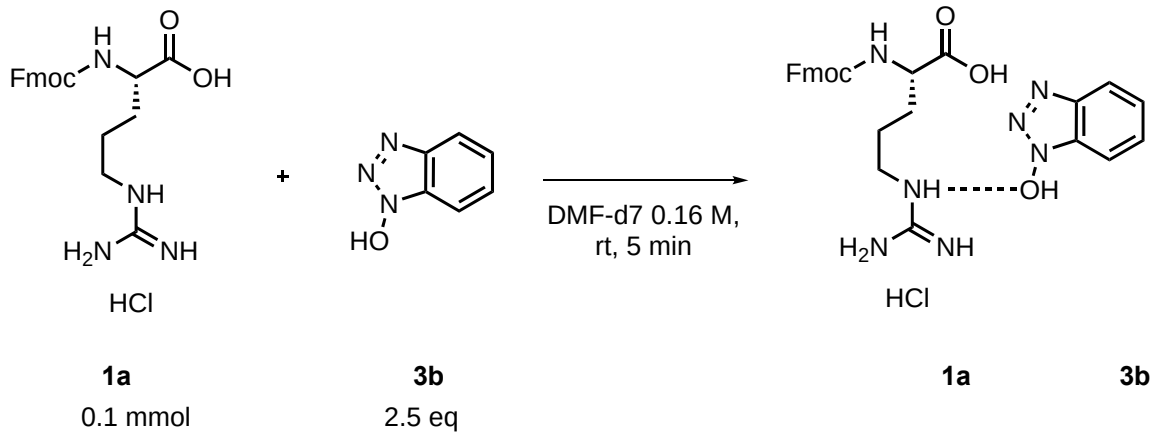


Figure S8. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH, 1.0 eq of HOBt and 1.0 eq of DIC

Fmoc-Arg(HCl)-OH with 2.5 eq of HOBt



1a 0.1 mmol **3b** 2.5 eq **1a** **3b**

Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and HOBt (0.25 mmol, 33.7 mg, 2.5 eq) were dissolved in a glass-vial with 0.6 mL of DMF-d7. After 5 minutes the ^{1}H NMR spectrum was acquired.

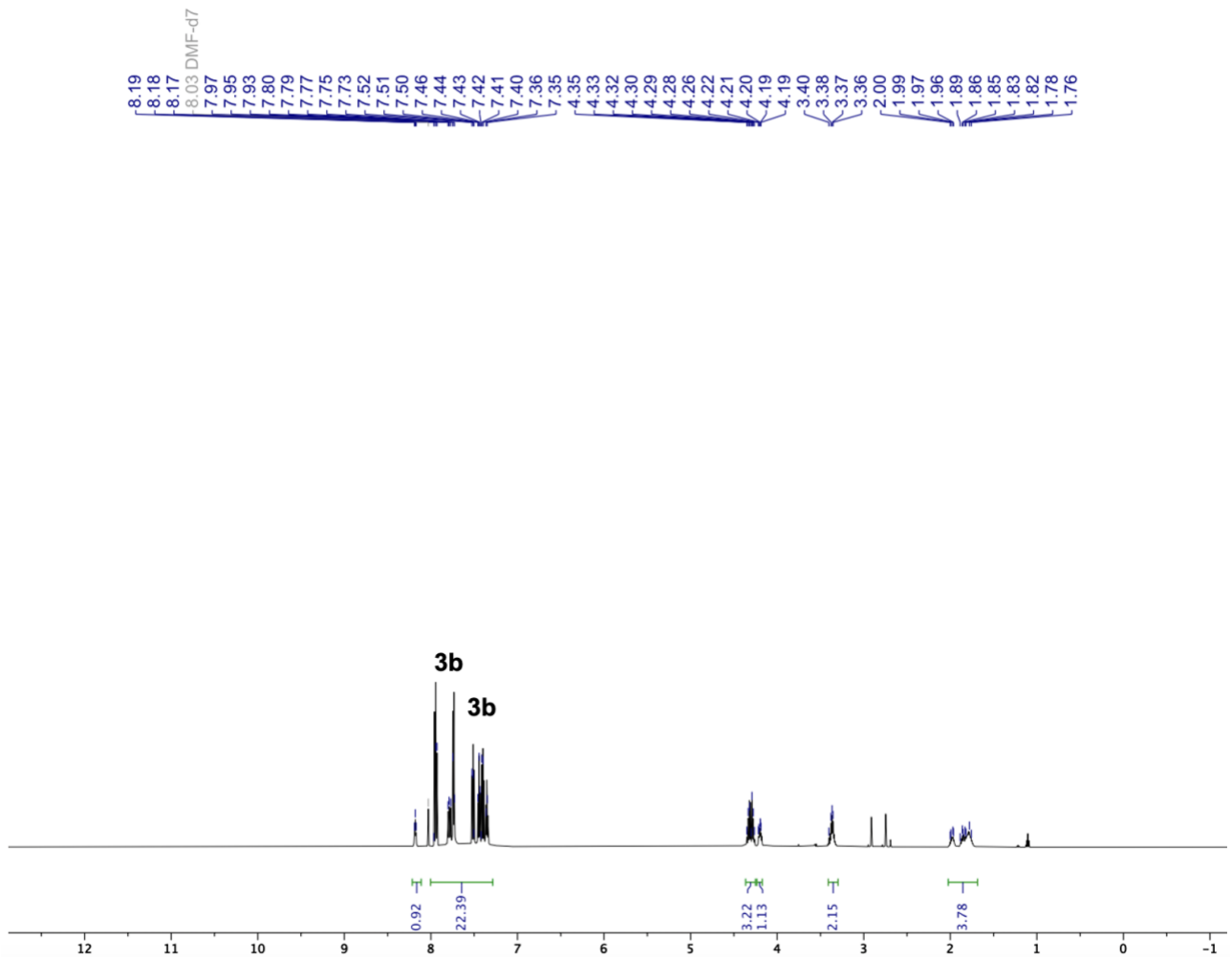
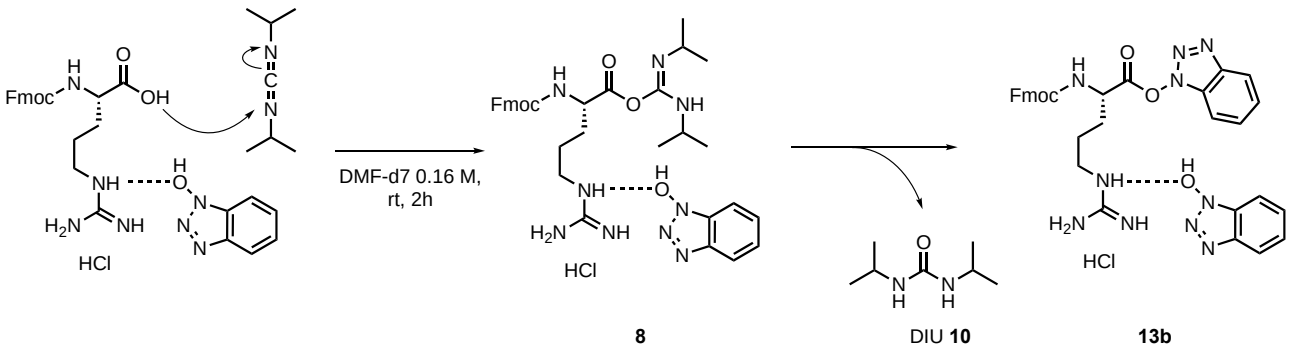


Figure S9. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH and 2.5 eq of HOBt

Fmoc-Arg(HCl)-OH with 2.5 eq of HOBt and 1.0 eq of DIC



Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and HOBt (0.25 mmol, 33.7 mg, 2.5 eq) were dissolved in a glass-vial with 0.6 mL of DMF-d7. After 5 minutes DIC (0.1 mmol, 15.5 µL, 1.0 eq) was added and the reaction stirred at room temperature. After 2h, the ^{1}H NMR spectrum was acquired.

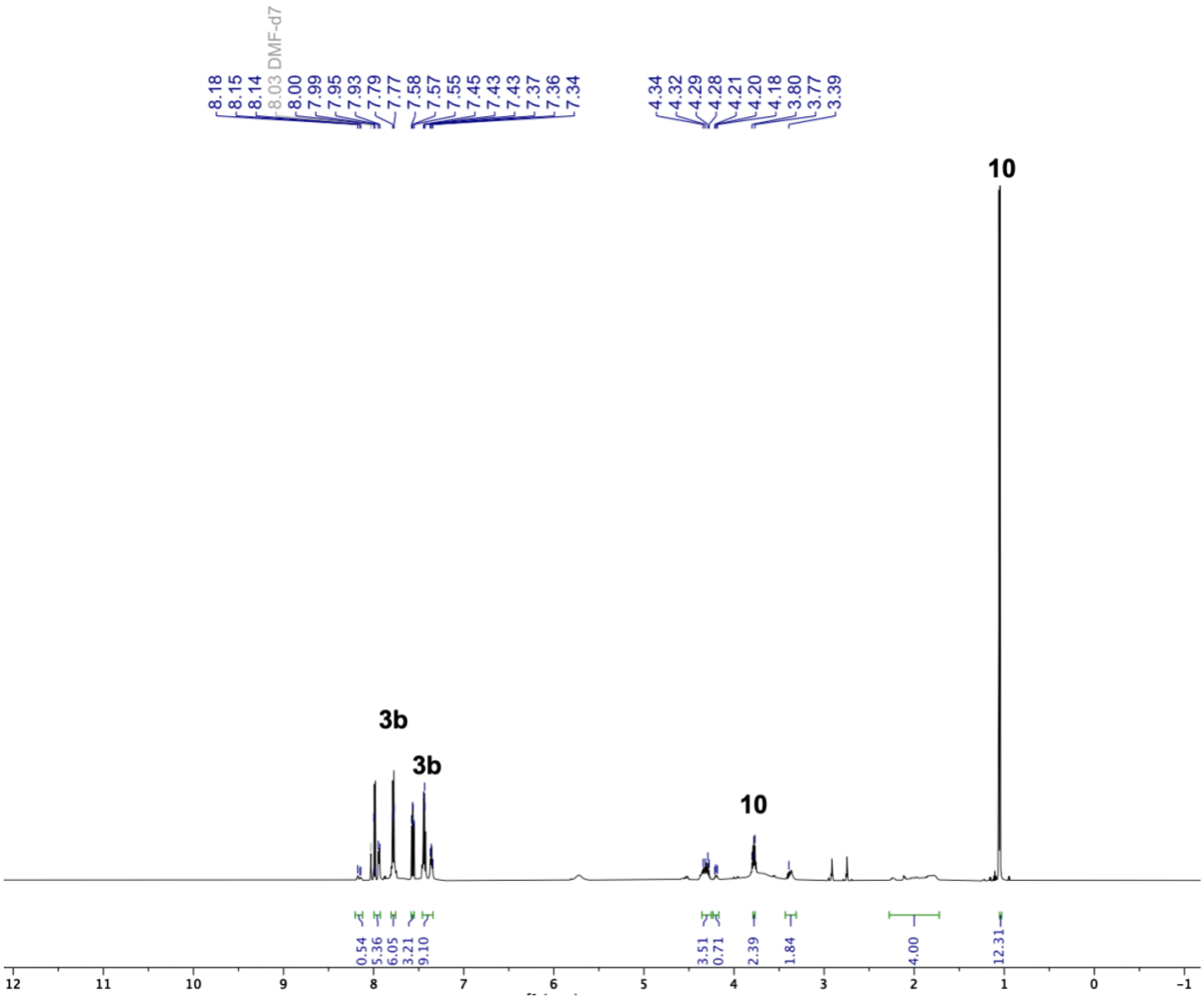
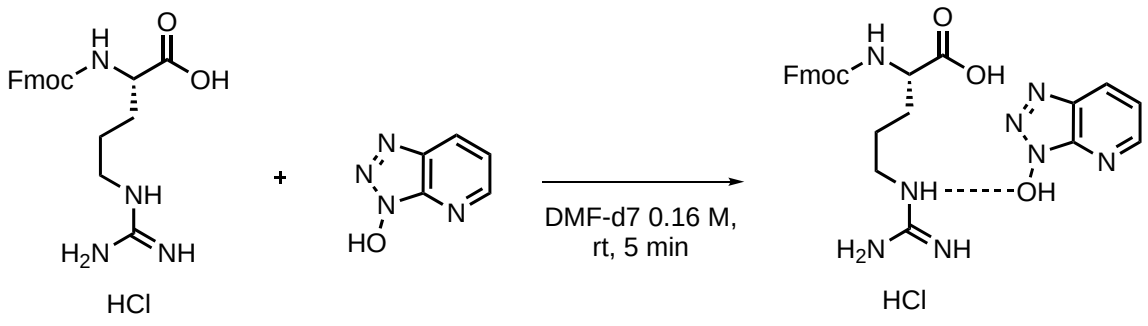


Figure S10. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH, 2.5 eq of HOBt and 1.0 eq of DIC

Fmoc-Arg(HCl)-OH with 1.0 eq of HOAt



1a 0.1 mmol

3c 1.0 eq

1a **3c**

Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and HOAt (0.1 mmol, 13.6 mg, 1.0 eq) were dissolved in a glass-vial with 0.6 mL of DMF-d_7. After 5 minutes the ^{1}H NMR spectrum was acquired.

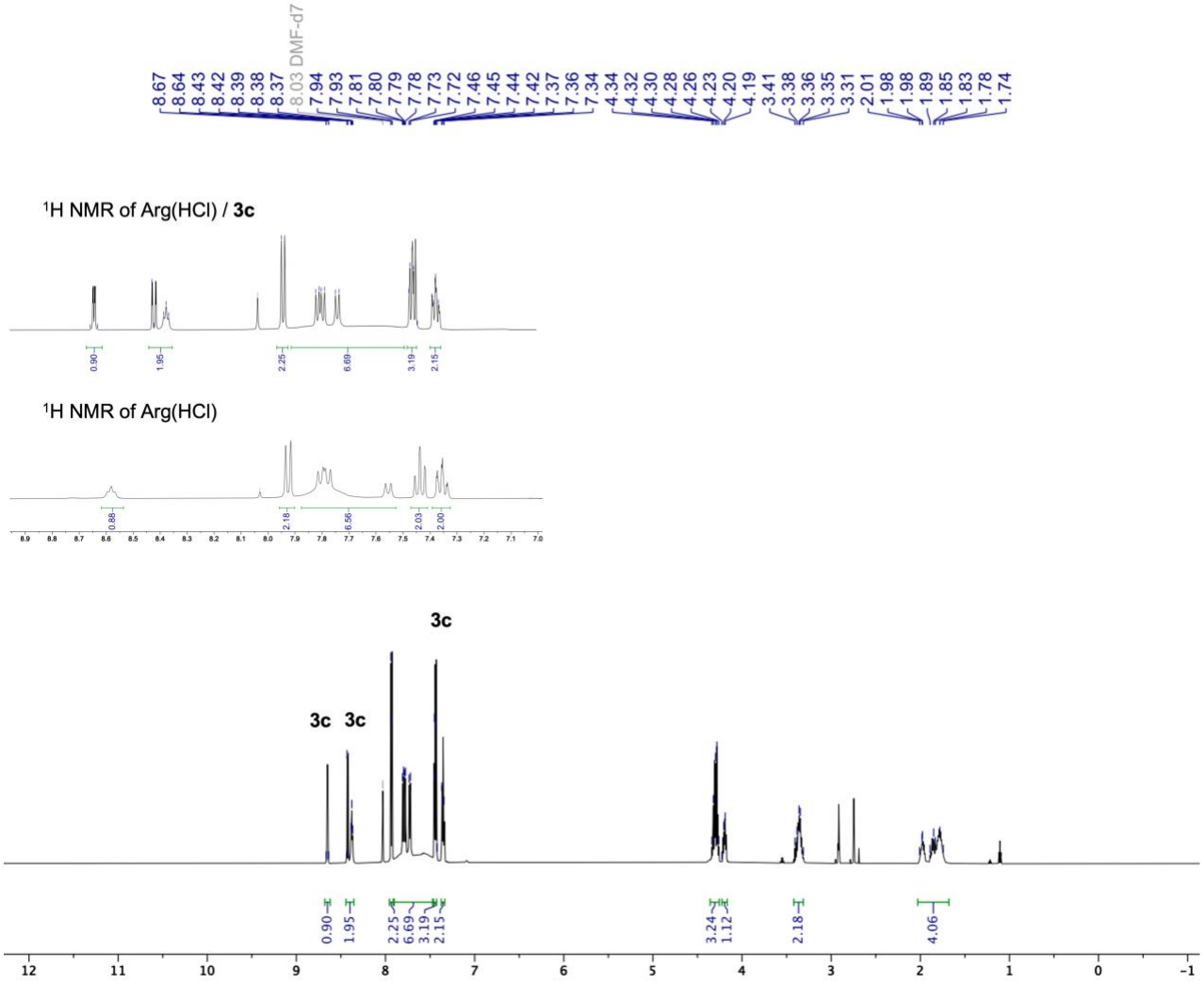
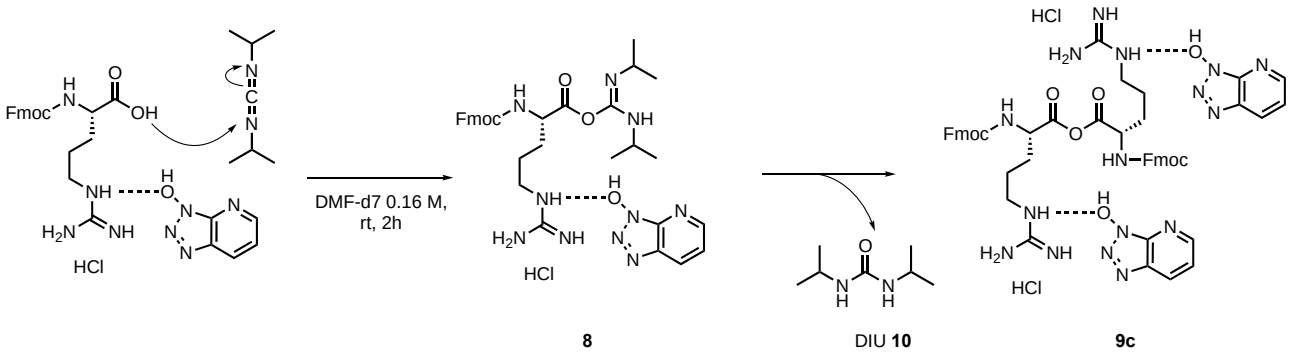


Figure S11. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH and 1.0 eq of HOAt

Fmoc-Arg(HCl)-OH with 1.0 eq of HOAt and 1.0 eq of DIC



Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and HOAt (0.1 mmol, 13.6 mg, 0.1 eq) were dissolved in a glass-vial with 0.6 mL of DMF-d_7. After 5 minutes DIC (0.1 mmol, 15.5 µL, 1.0 eq) was added and the reaction stirred at room temperature. After 2h, the ^{1}H NMR spectrum was acquired.

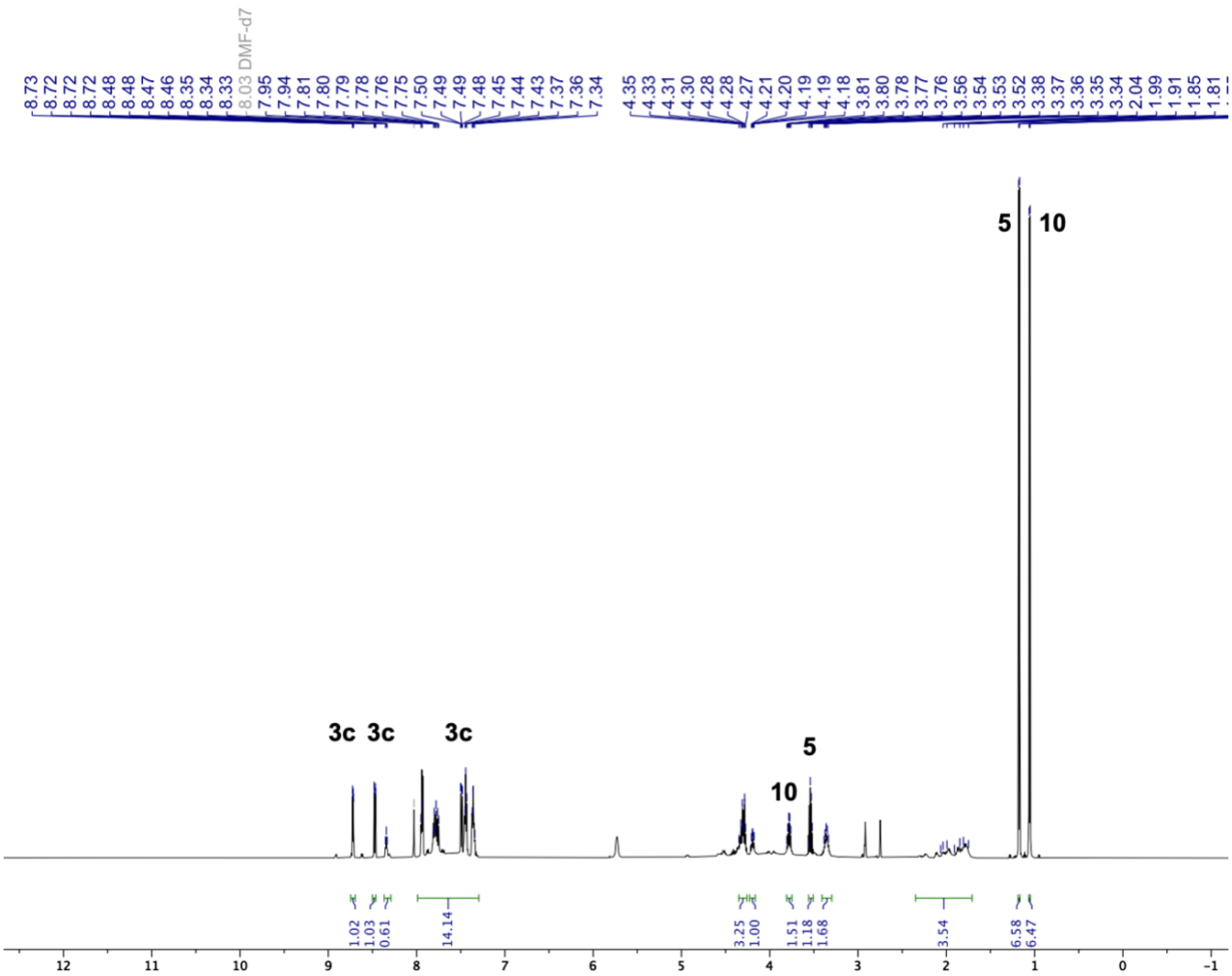
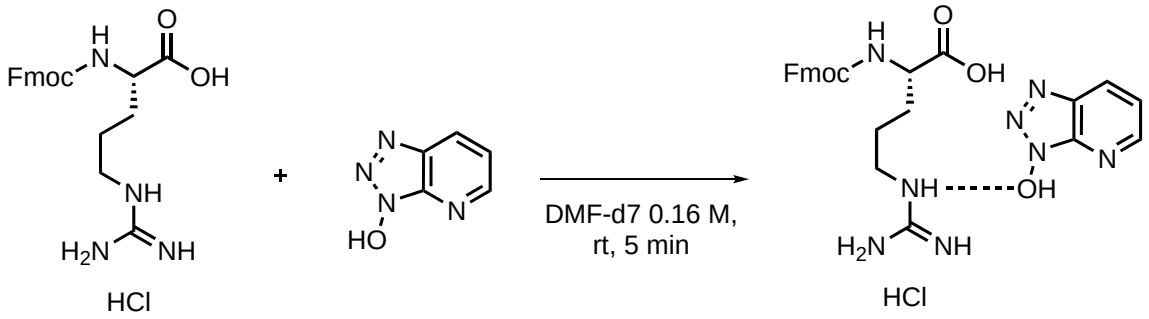


Figure S12. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH, 1.0 eq of HOAt and 1.0 eq of DIC

Fmoc-Arg(HCl)-OH with 2.5 eq of HOAt



1a 0.1 mmol **3c** 2.5 eq **1a** **3c**

Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and HOAt (0.25 mmol, 34.0 mg, 2.5 eq) were dissolved in a glass-vial with 0.6 mL of DMF-d_7. After 5 minutes the ^{1}H NMR spectrum was acquired.

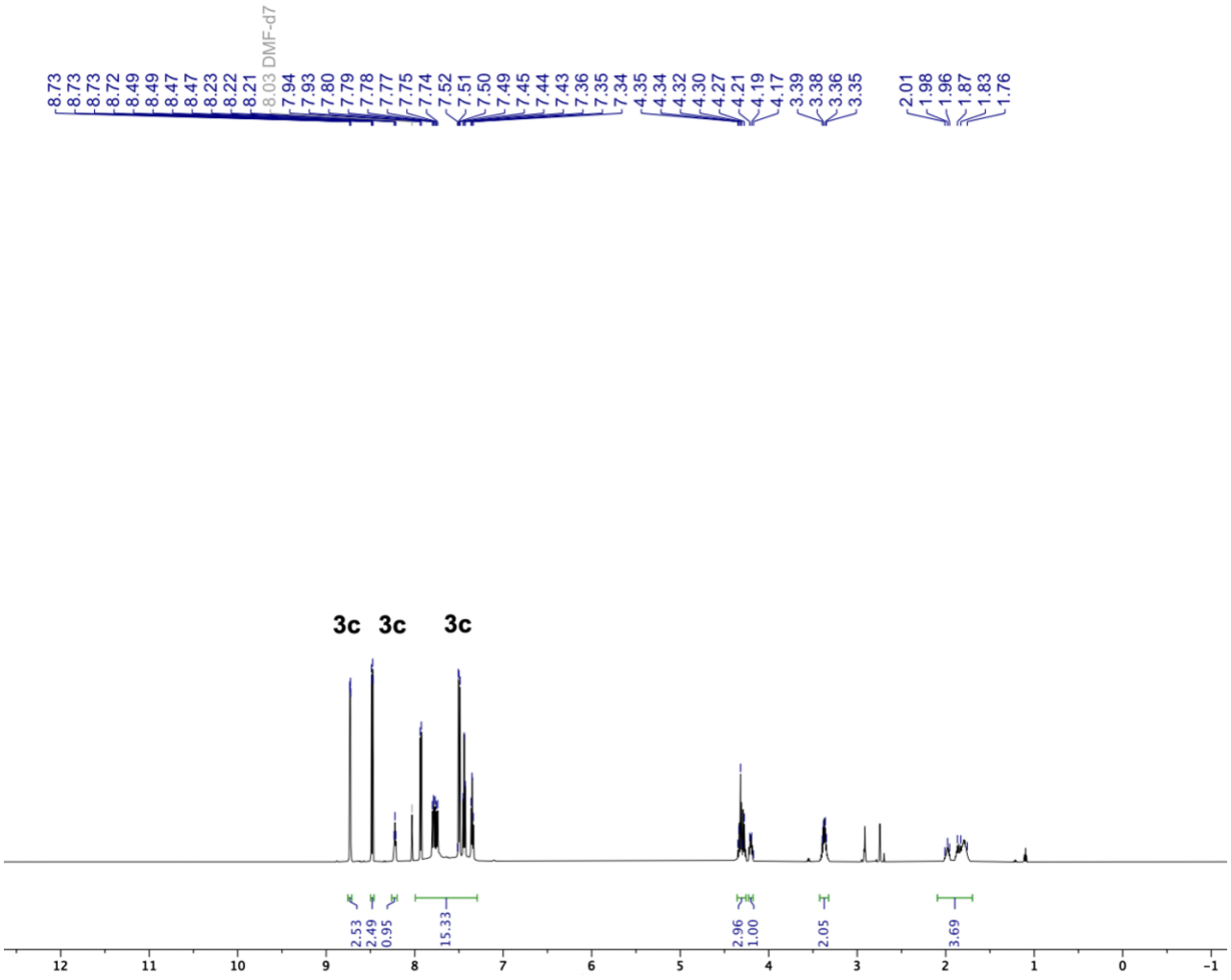
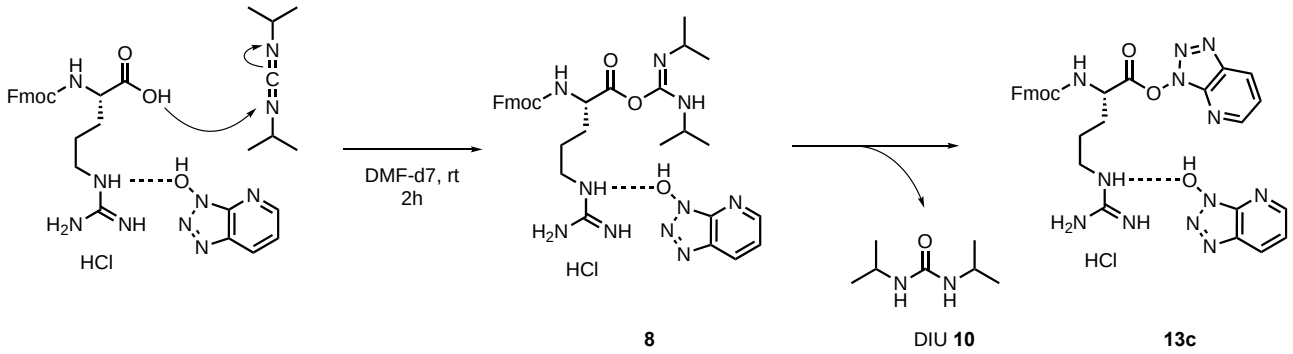


Figure S13. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH and 2.5 eq of HOAt

Fmoc-Arg(HCl)-OH with 2.5 eq of HOAt and 1.0 eq of DIC



Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and HOAt (0.25 mmol, 34.0 mg, 0.25 eq) were dissolved in a glass-vial with 0.6 mL of DMF-d_7. After 5 minutes, DIC (0.1 mmol, 15.5 µL, 1.0 eq) was added and the reaction stirred at room temperature. After 2h, the ^{1}H NMR spectrum was acquired.

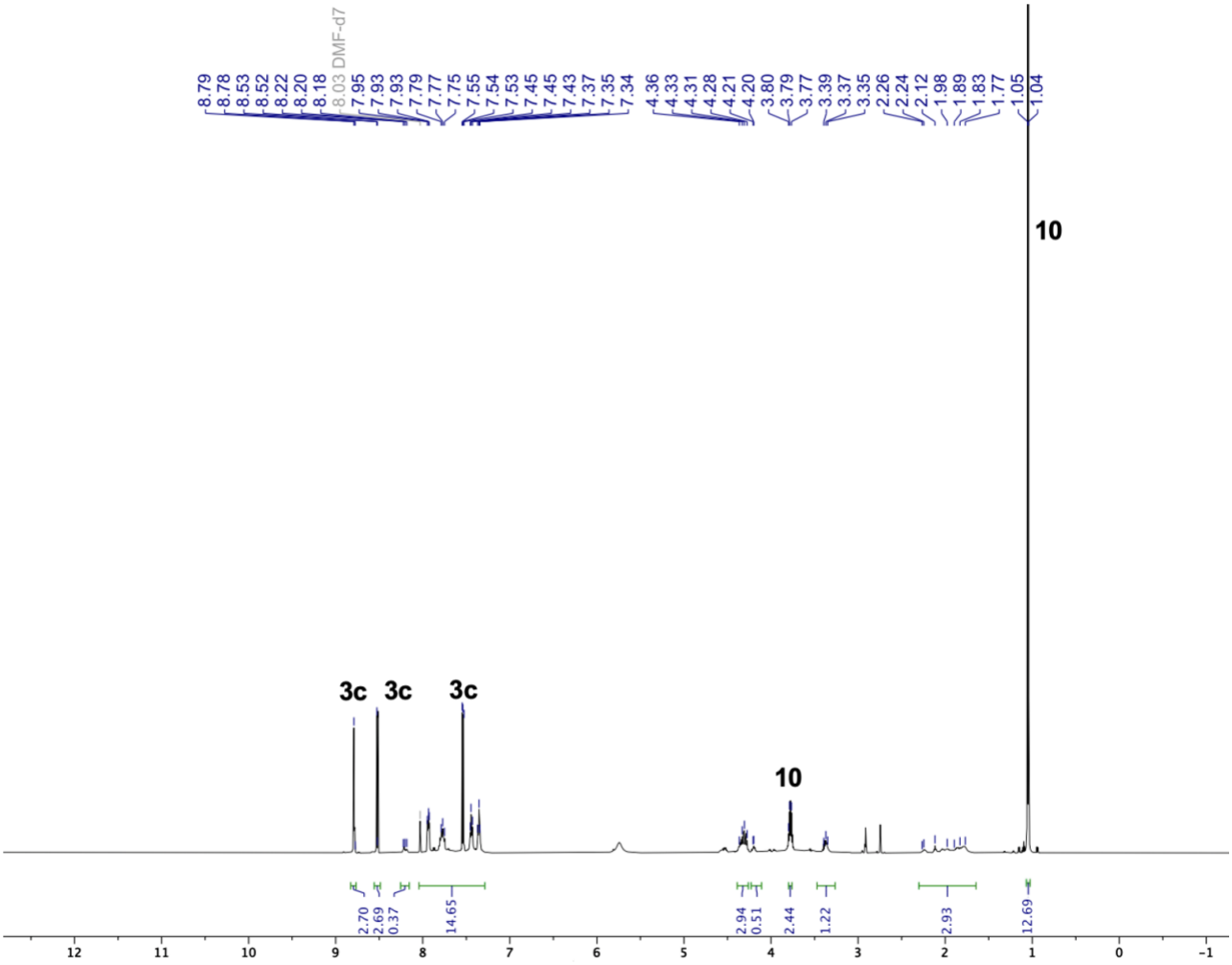
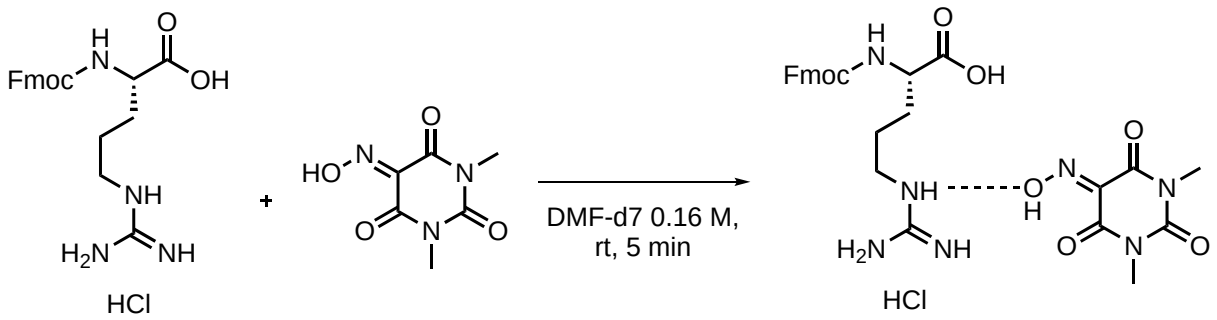


Figure S14. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH, 2.5 eq of HOAt and 1.0 eq of DIC

Fmoc-Arg(HCl)-OH with 1.0 eq of Oxyma B



1a 0.1 mmol

3d 1.0 eq

1a **3d**

Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma B (0.1 mmol, 18.5 mg, 1.0 eq) were dissolved in a glass-vial with 0.6 mL of DMF-d_7. After 5 minutes the ^{1}H NMR spectrum was acquired.

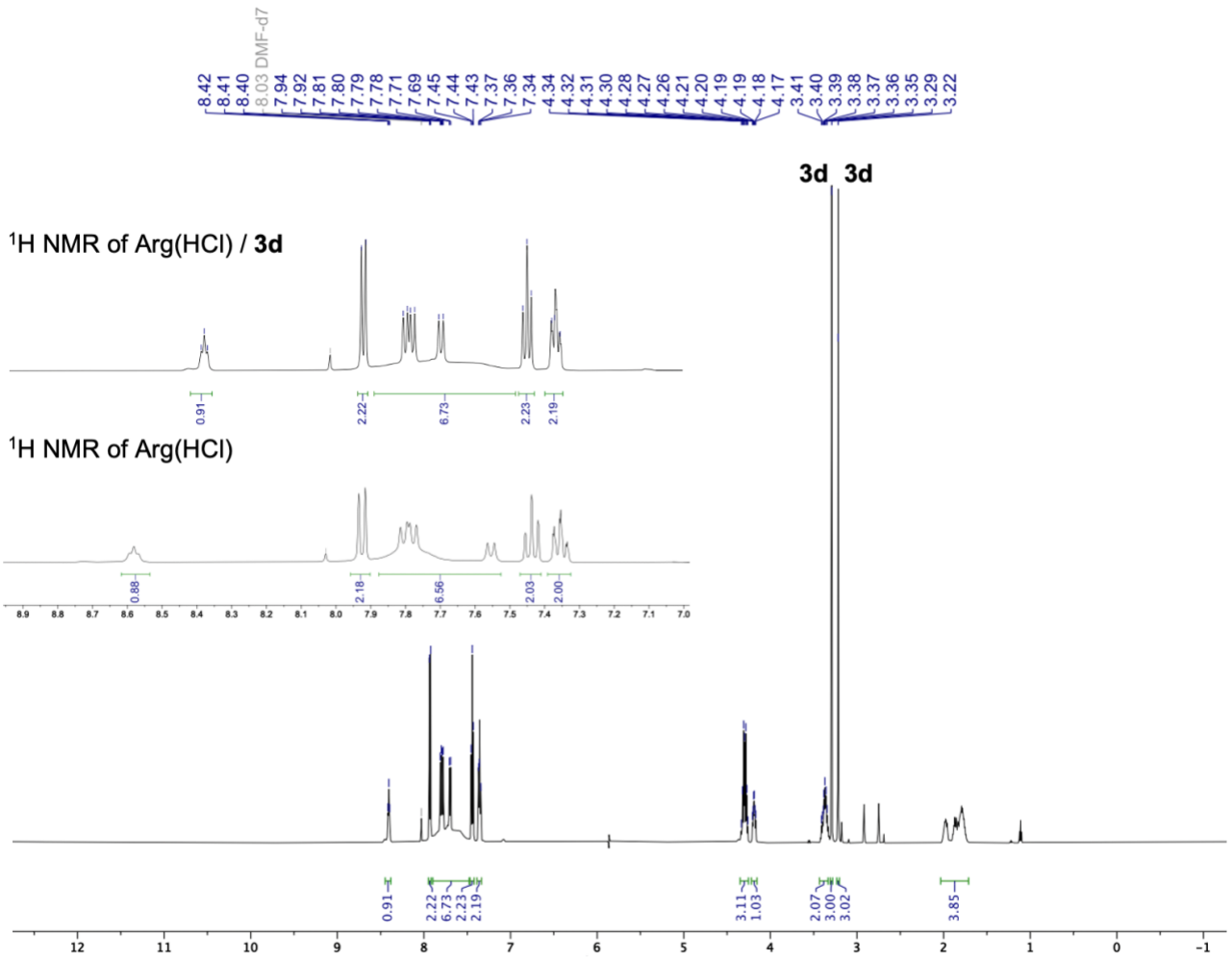
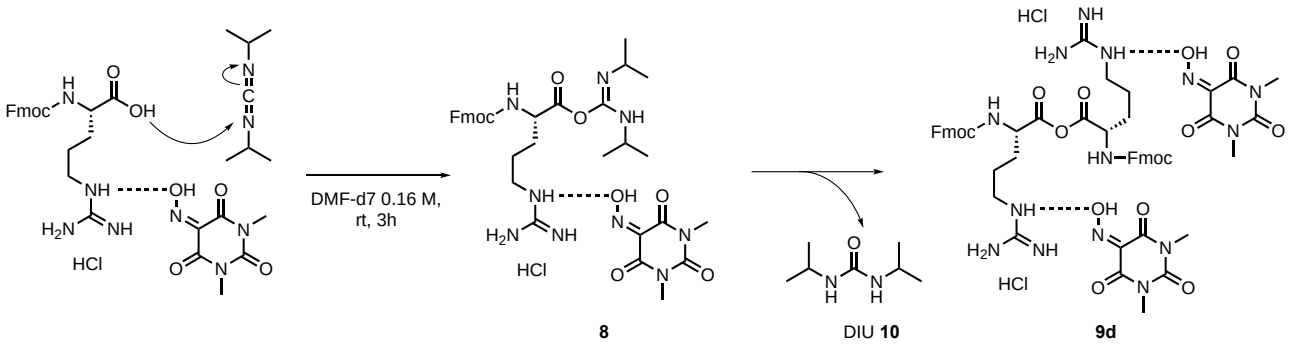


Figure S15. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH and 1.0 eq of Oxyma B

Fmoc-Arg(HCl)-OH with 1.0 eq of Oxyma B and 1.0 eq of DIC



Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma B (0.1 mmol, 18.5 mg, 0.1 eq) were dissolved in a glass-vial with 0.6 mL of DMF-d_7. After 5 minutes DIC (0.1 mmol, 15.5 µL, 1.0 eq) was added and the reaction stirred at room temperature. After 3h, the ^{1}H NMR spectrum was acquired.

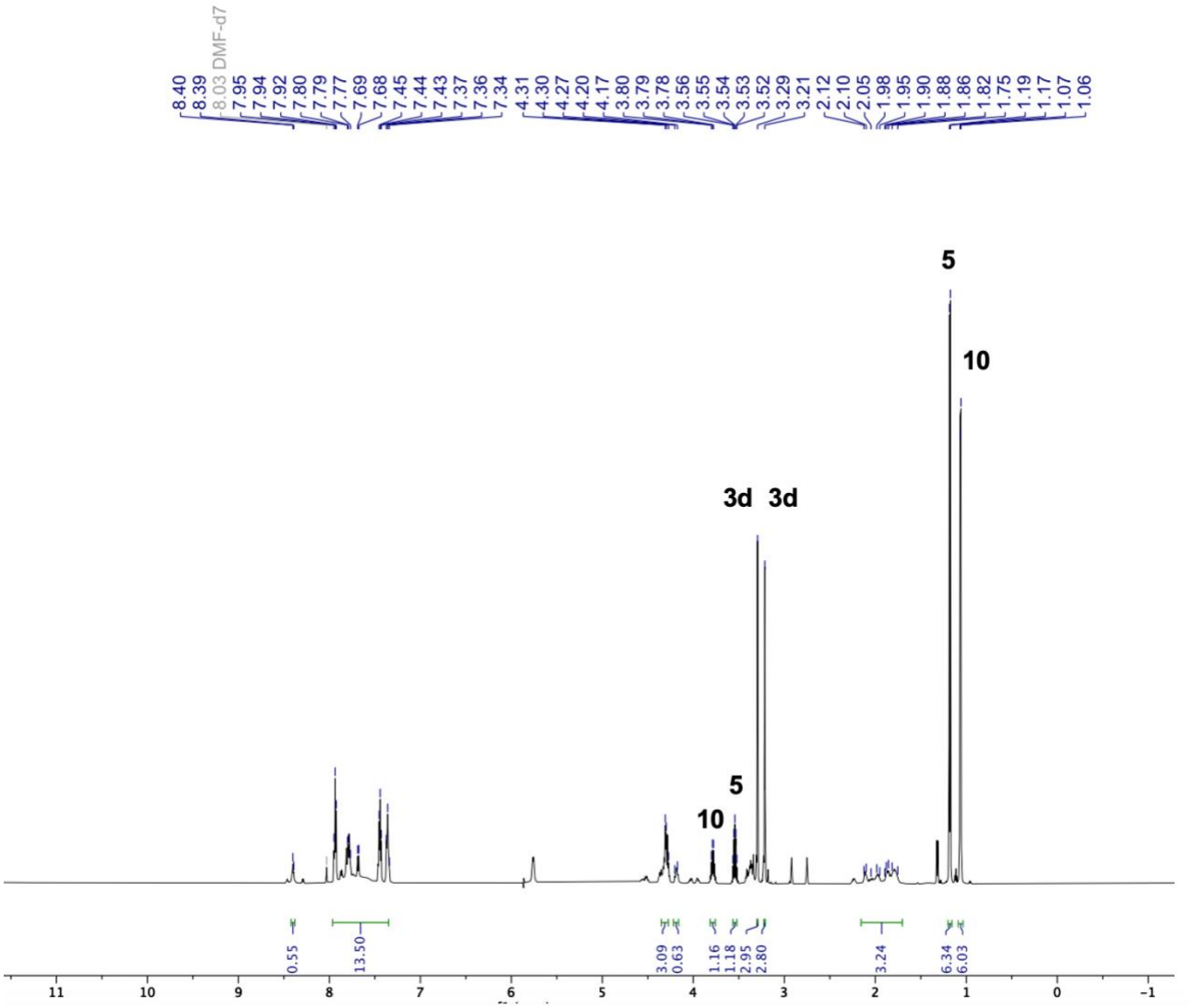
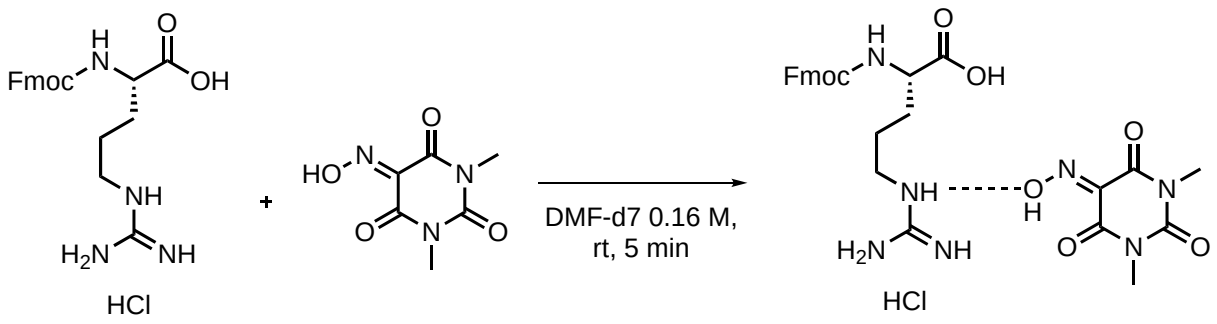


Figure S16. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH, 1.0 eq of Oxyma B and 1.0 eq of DIC

Fmoc-Arg(HCl)-OH with 2.5 eq of Oxyma B



1a 0.1 mmol

3d 2.5 eq

1a **3d**

Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma B (0.25 mmol, 40.2 mg, 0.25 eq) were dissolved in a glass-vial with 0.6 mL of DMF-d_7. After 5 minutes the ^{1}H NMR spectrum was acquired.

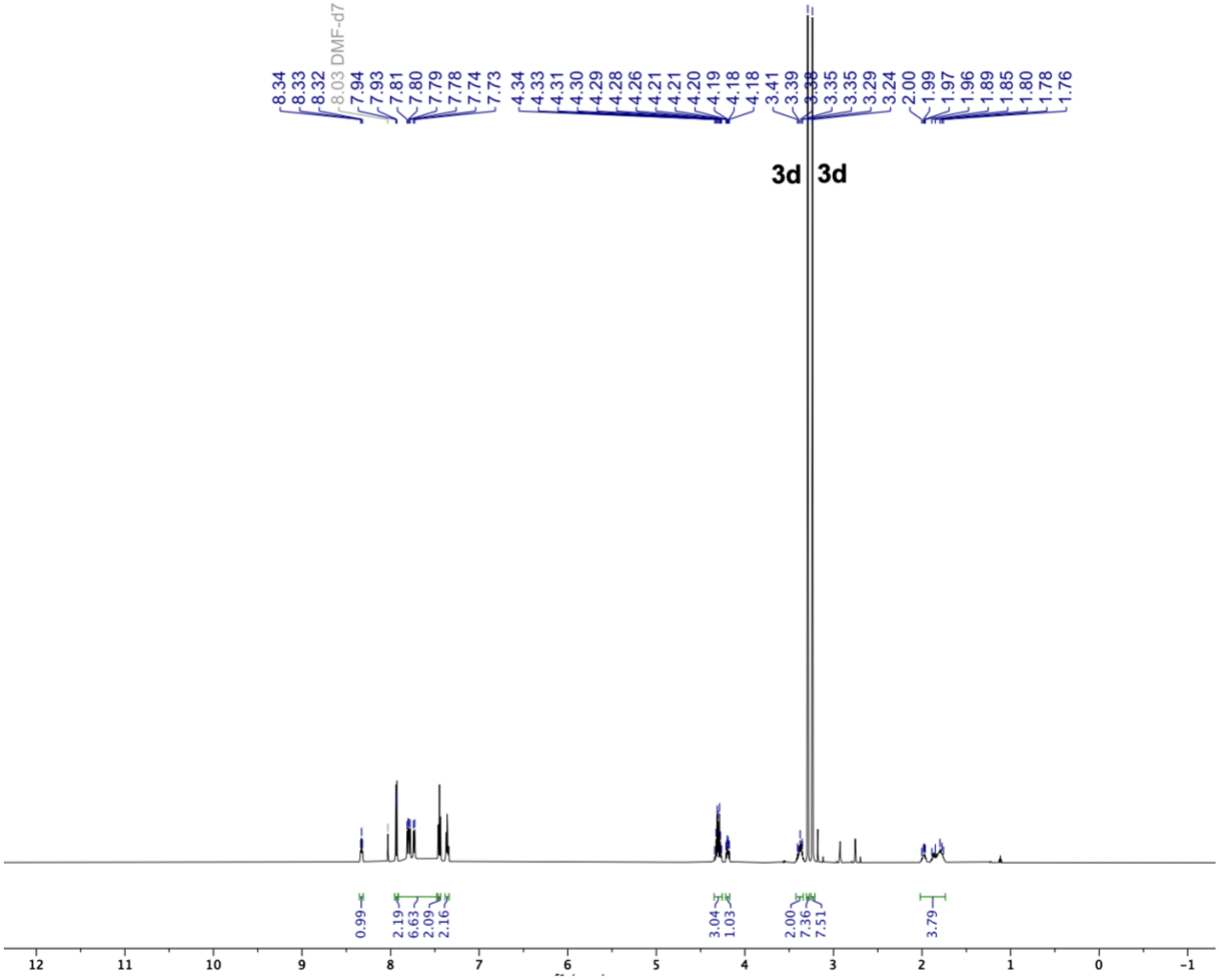
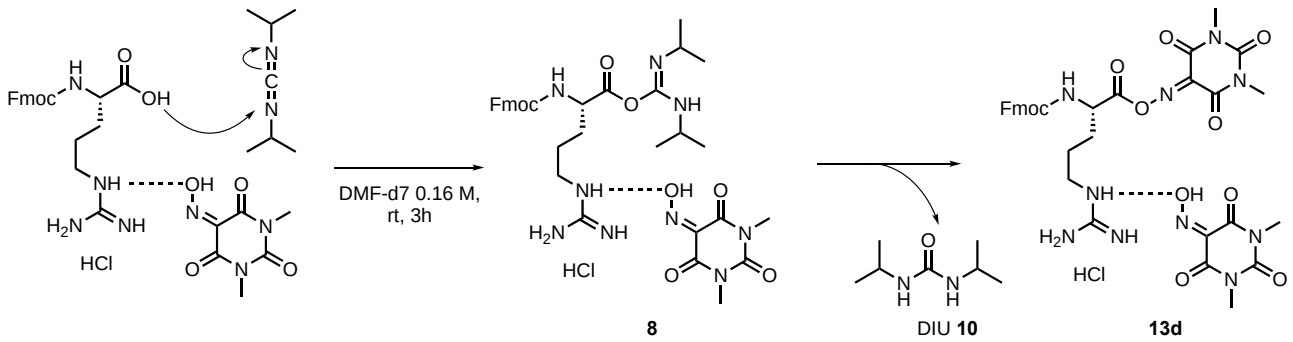


Figure S17. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH and 2.5 eq of Oxyma B

Fmoc-Arg(HCl)-OH with 2.5 eq of Oxyma B and 1.0 eq of DIC



Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma B (0.25 mmol, 40.2 mg, 0.25 eq) were dissolved in a glass-vial with 0.6 mL of DMF-d_7. After 5 minutes DIC (0.1 mmol, 15.5 µL, 1.0 eq) was added and the reaction stirred at room temperature. After 3h, the ^{1}H NMR spectrum was acquired.

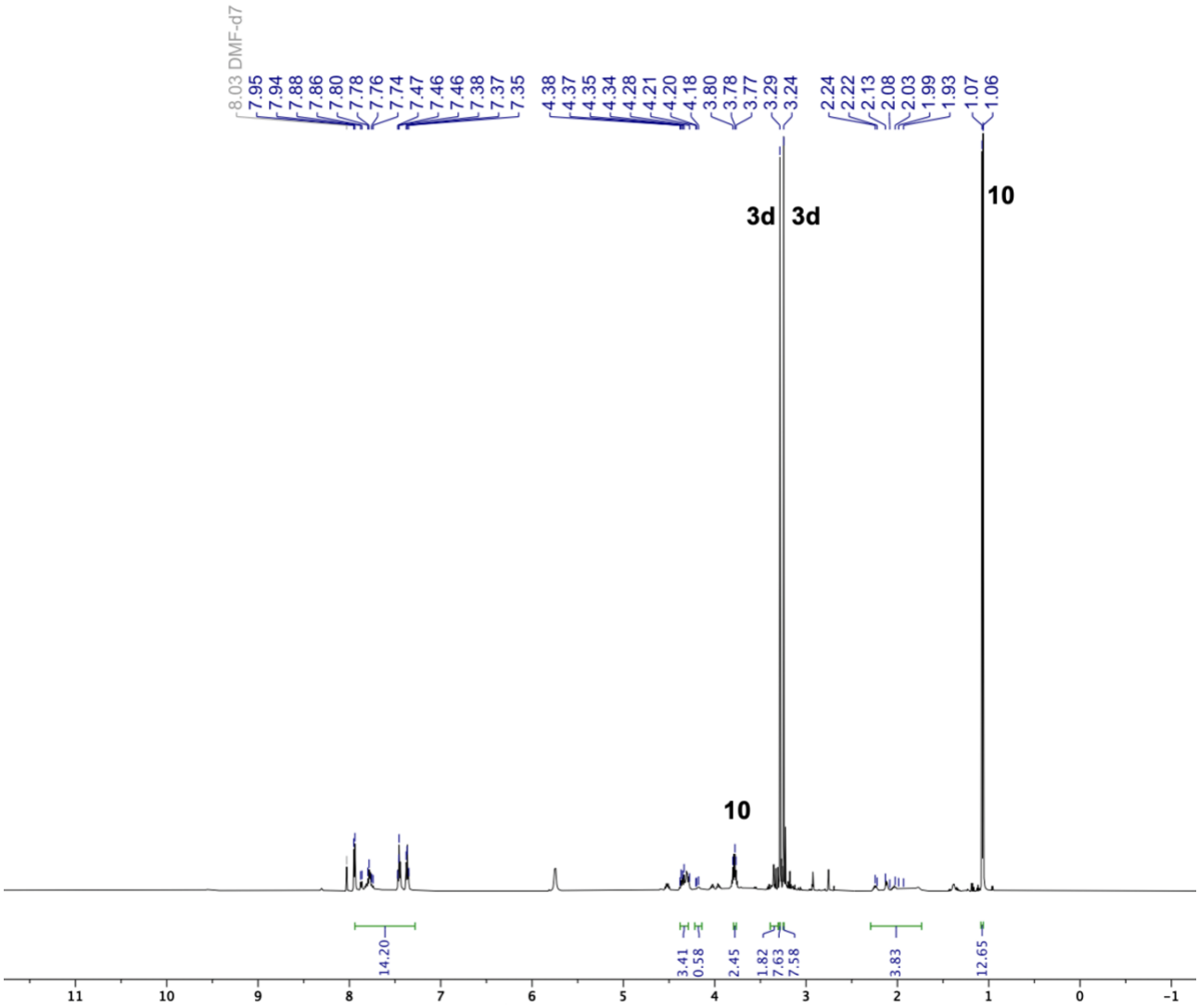
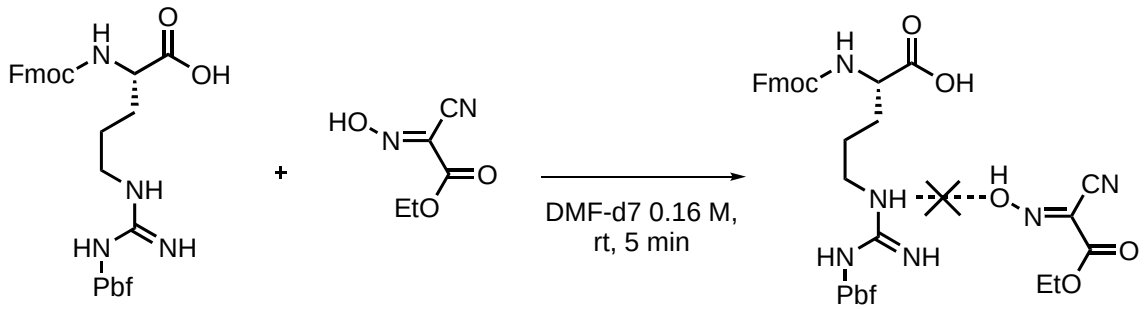


Figure S18. [1]H NMR spectrum of Fmoc-Arg(HCl)-OH, 2.5 eq of Oxyma B and 1.0 eq of DIC

Fmoc-Arg(Pbf)-OH

Fmoc-Arg(Pbf)-OH and 1.0 eq of Oxyma Pure



1b 0.1 mmol

3a 1.0 eq

1b **3a**

Fmoc-Arg(Pbf)-OH (0.1 mmol, 64.9 mg, 1.0 eq) and Oxyma Pure (0.1 mmol, 14.3 mg, 1.0 eq) were dissolved in a glass-vial with 0.6 mL of DMF-d_7. After 5 minutes the ^{1}H NMR spectrum was acquired

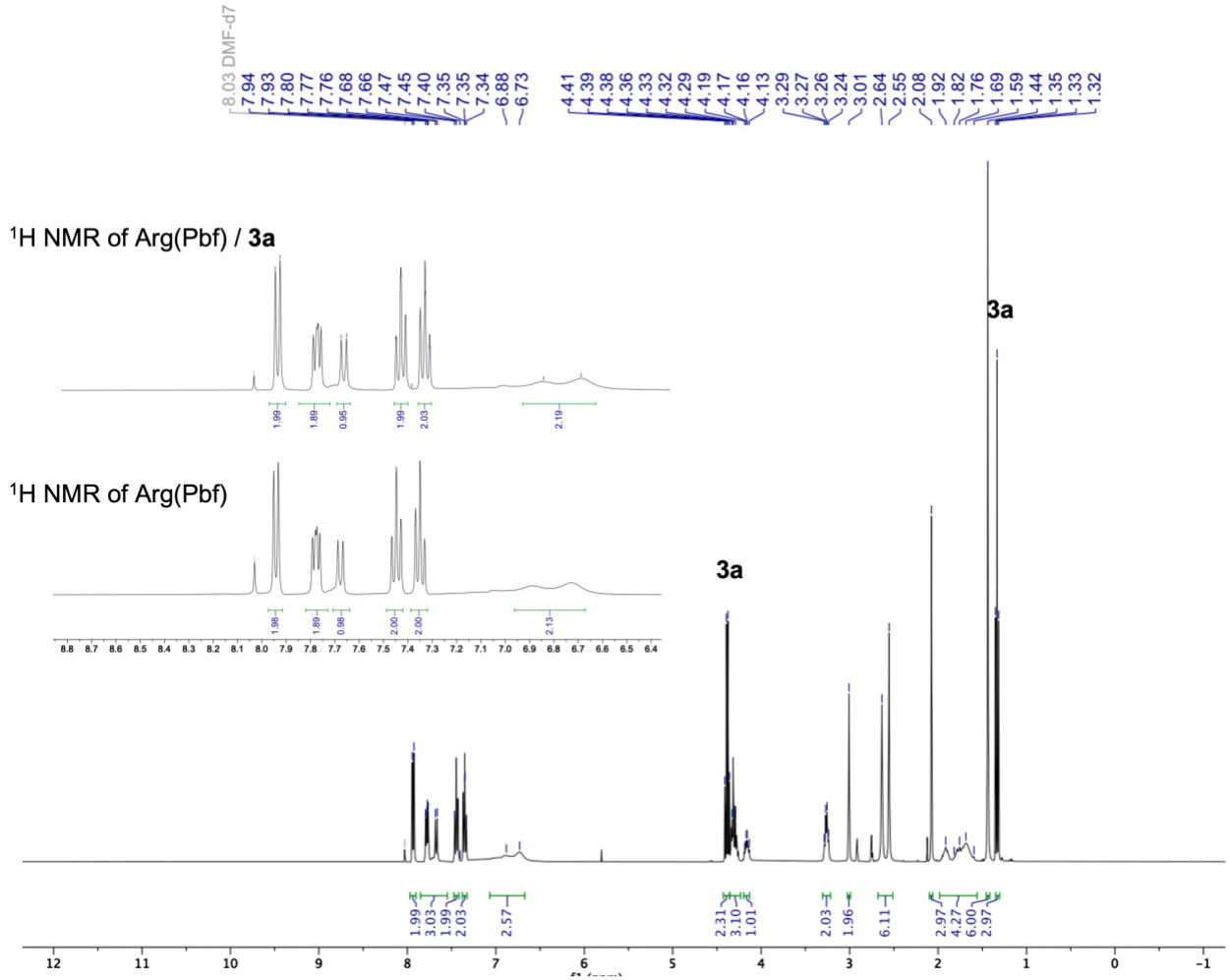
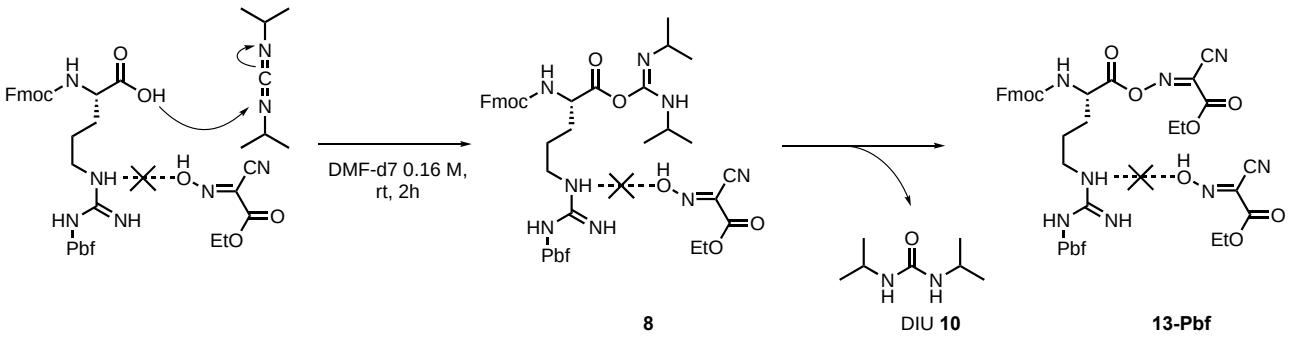


Figure S19. ^{1}H NMR spectrum of Fmoc-Arg(Pbf)-OH and 1.0 eq of Oxyma pure

Fmoc-Arg(Pbf)-OH with 1.0 eq of Oxyma Pure and 1.0 eq of DIC



Fmoc-Arg(Pbf)-OH (0.1 mmol, 64.9 mg, 1.0 eq) and Oxyma Pure (0.1 mmol, 14.3 mg, 1.0 eq) were dissolved in a glass-vial with 0.6 mL of DMF-d_7. After 5 minutes DIC (0.1 mmol, 15.5 µL, 1.0 eq) was added and the reaction stirred at room temperature. After 2h, the ^{1}H NMR spectrum was acquired.

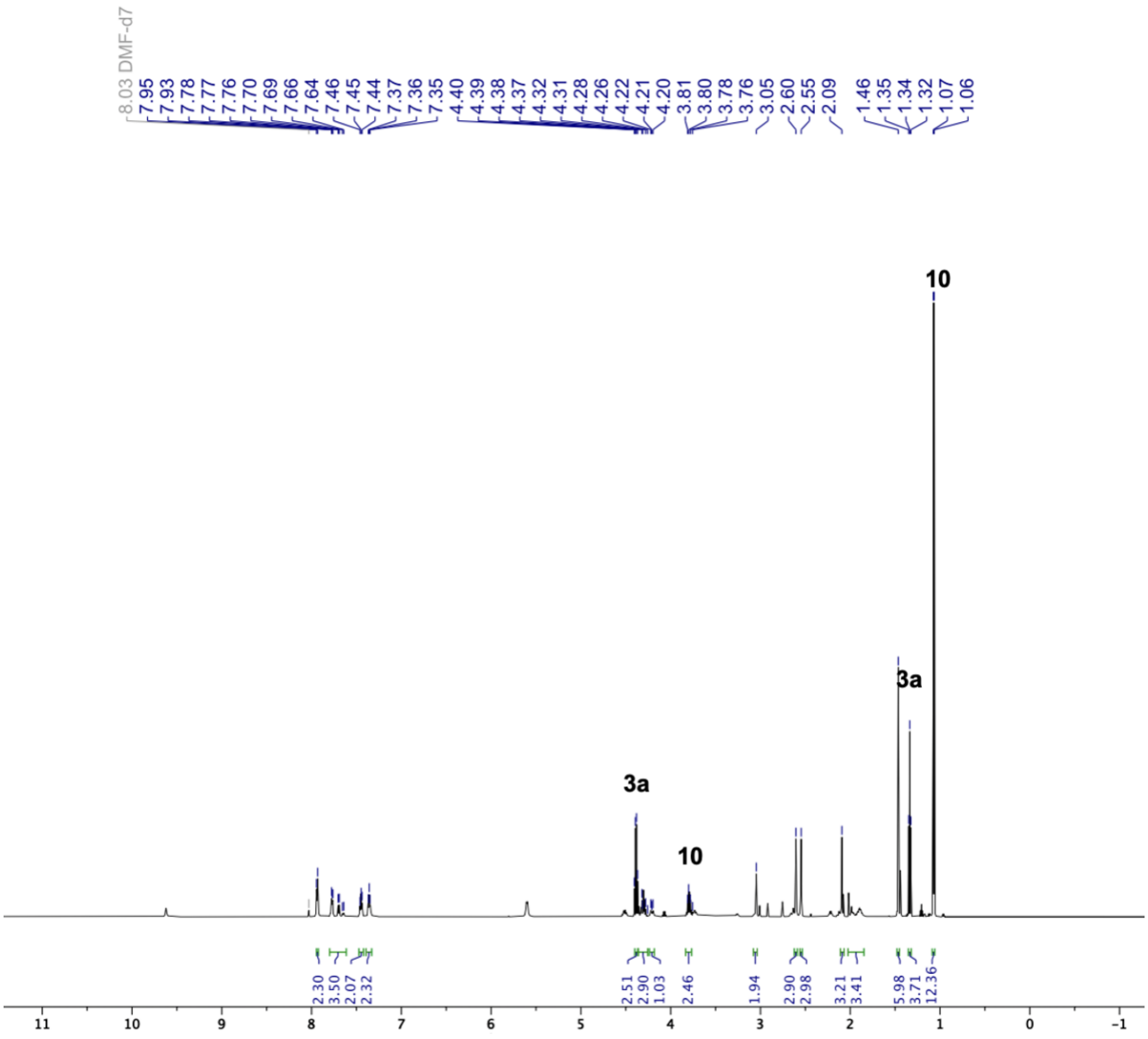
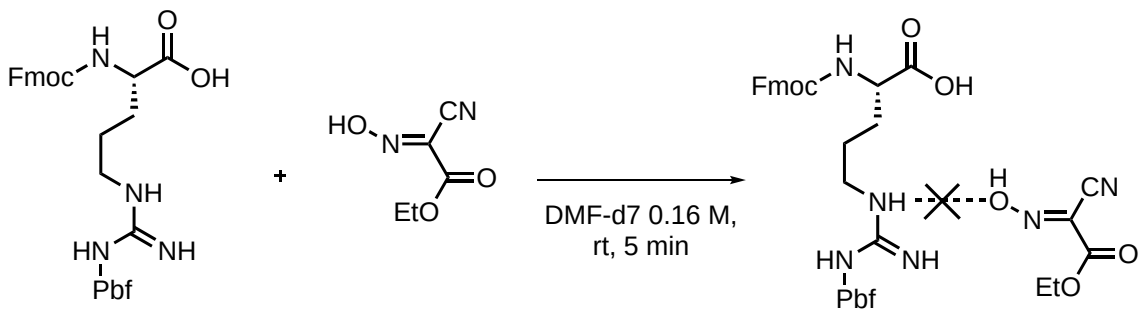


Figure S20. ^{1}H NMR spectrum of Fmoc-Arg(Pbf)-OH, 1.0 eq of Oxyma pure and 1.0 eq of DIC

Fmoc-Arg(Pbf)-OH and 2.5 eq of Oxyma Pure



1b 0.1 mmol + **3a** 2.5 eq → **1b** **3a**

Fmoc-Arg(Pbf)-OH (0.1 mmol, 64.9 mg, 1.0 eq) and Oxyma Pure (0.25 mmol, 35.7 mg, 2.5 eq) were dissolved in a glass-vial with 0.6 mL of DMF-d_7. After 5 minutes the ^{1}H NMR spectrum was acquired

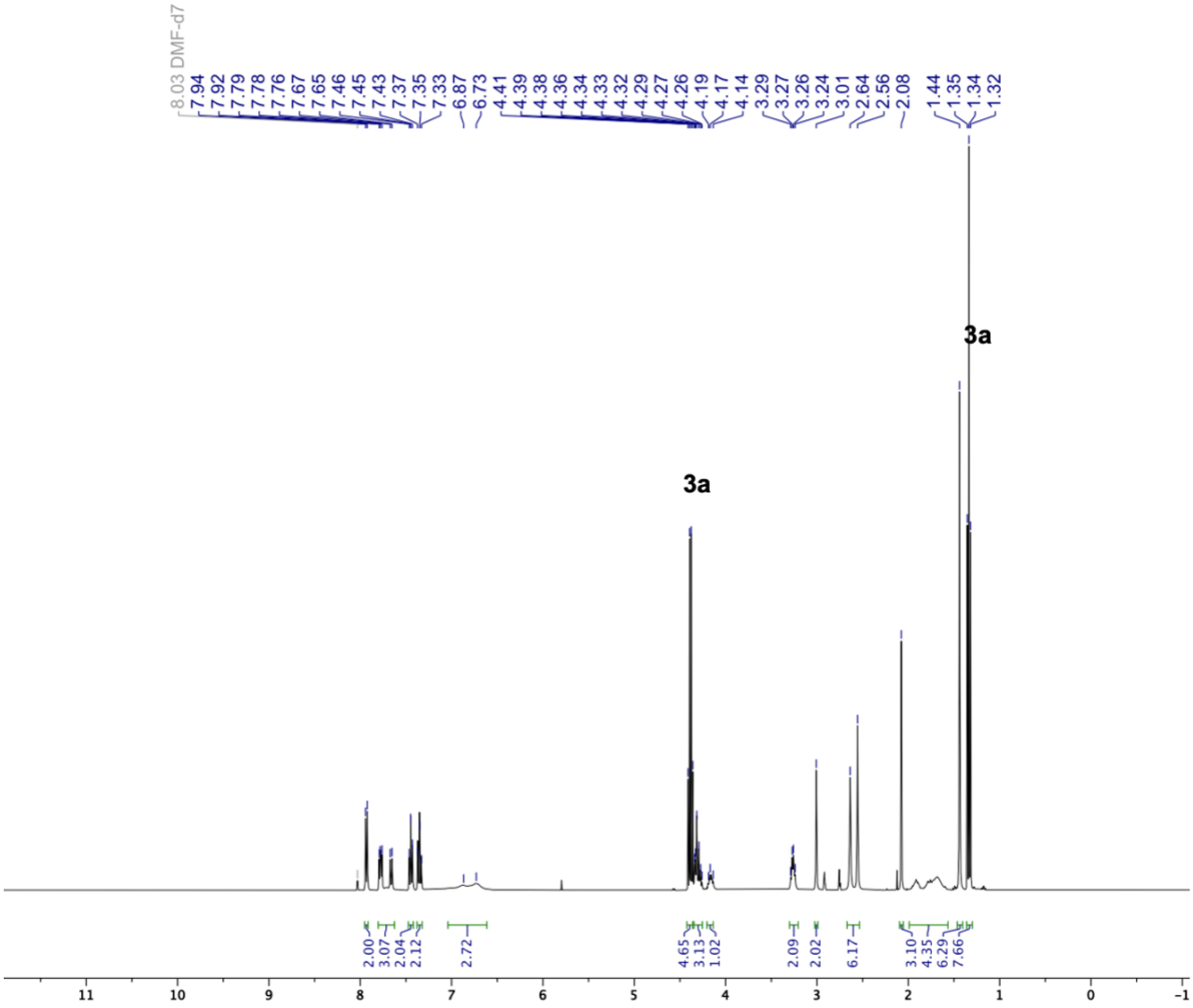
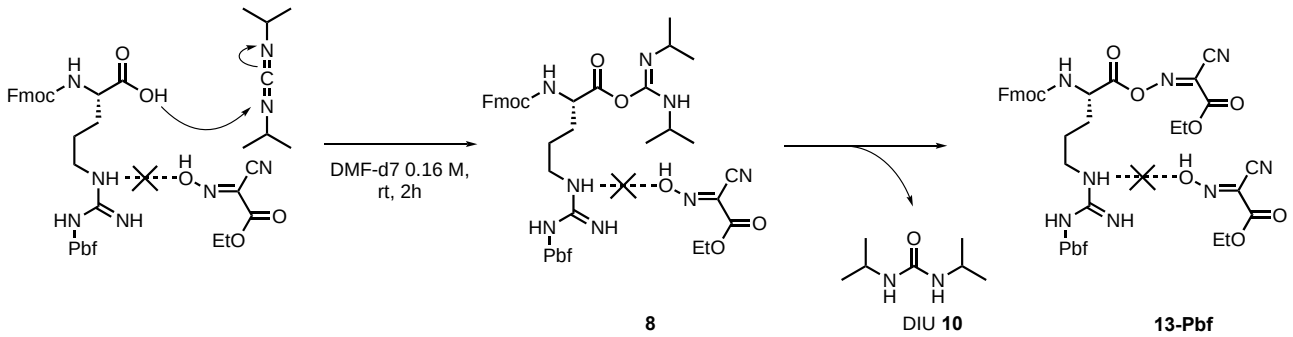


Figure S21. ^{1}H NMR spectrum of Fmoc-Arg(Pbf)-OH and 2.5 eq of Oxyma pure

Fmoc-Arg(Pbf)-OH with 2.5 eq of Oxyma Pure and 1.0 eq of DIC



Fmoc-Arg(Pbf)-OH (0.1 mmol, 64.9 mg, 1.0 eq) and Oxyma Pure (0.25 mmol, 35.7 mg, 2.5 eq) were dissolved in a glass-vial with 0.6 mL of DMF-d7. After 5 minutes DIC (0.1 mmol, 15.5 µL, 1.0 eq) was added and the reaction stirred at room temperature. After 2h, the ^{1}H NMR spectrum was acquired.

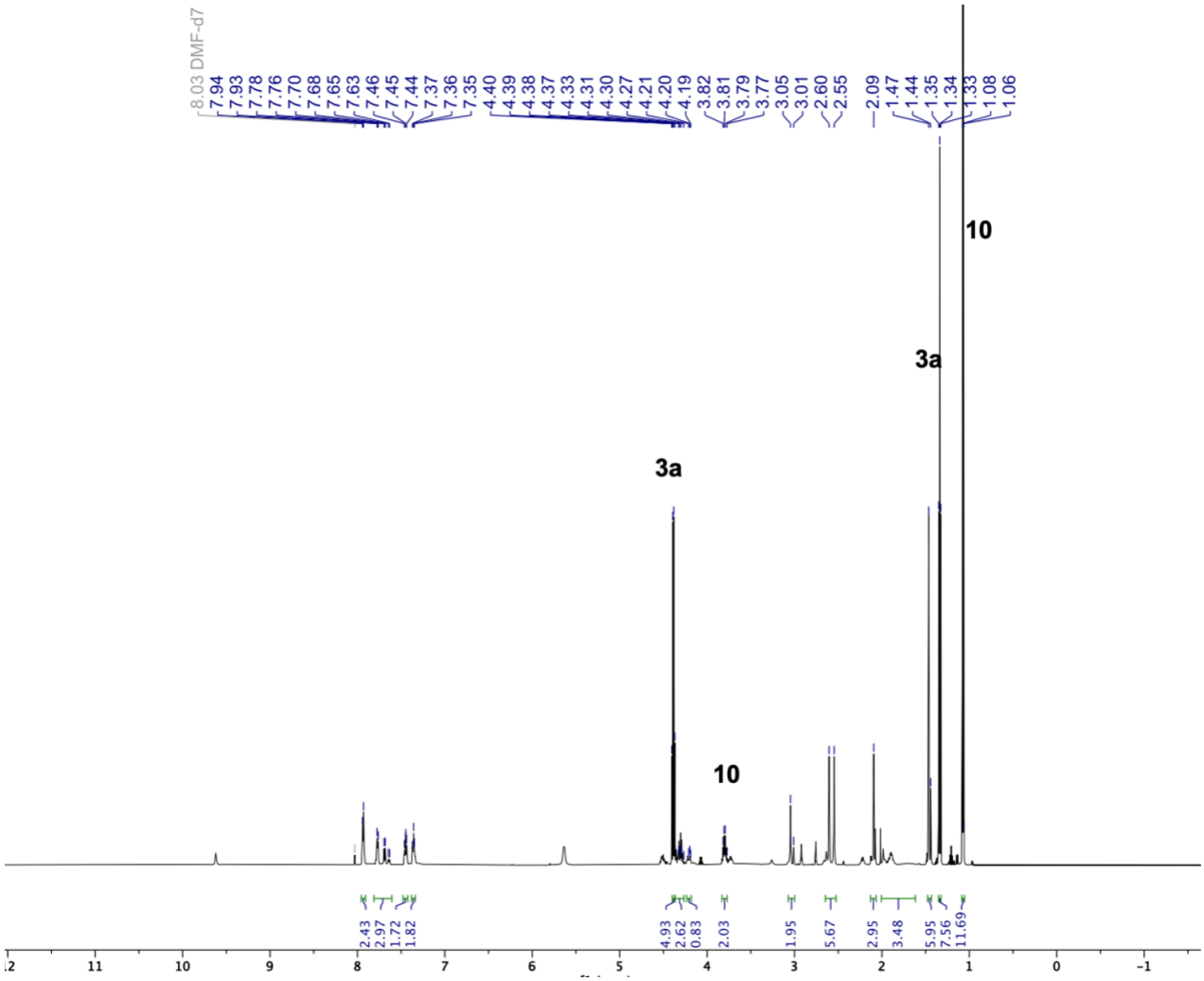
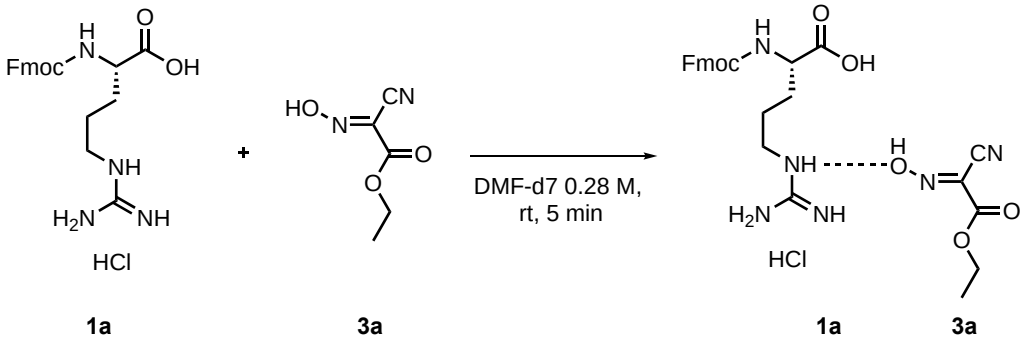


Figure S22. ^{1}H NMR spectrum of Fmoc-Arg(Pbf)-OH, 2.5 eq of Oxyma pure and 1.0 eq of DIC

NMR of Fmoc-Arg(HCl)-OH with different solvents and concentrations

^{1}H NMR in concentrated systems

Fmoc-Arg(HCl)-OH with 1.0 eq of Oxyma Pure



1a 0.1 mmol

3a 1.0 eq

1a **3a**

Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma Pure (0.1 mmol, 14.3 mg, 1.0 eq) were dissolved in a glass-vial with 0.35 mL of DMF-d_7. After 5 minutes the ^{1}H NMR spectrum was acquired.

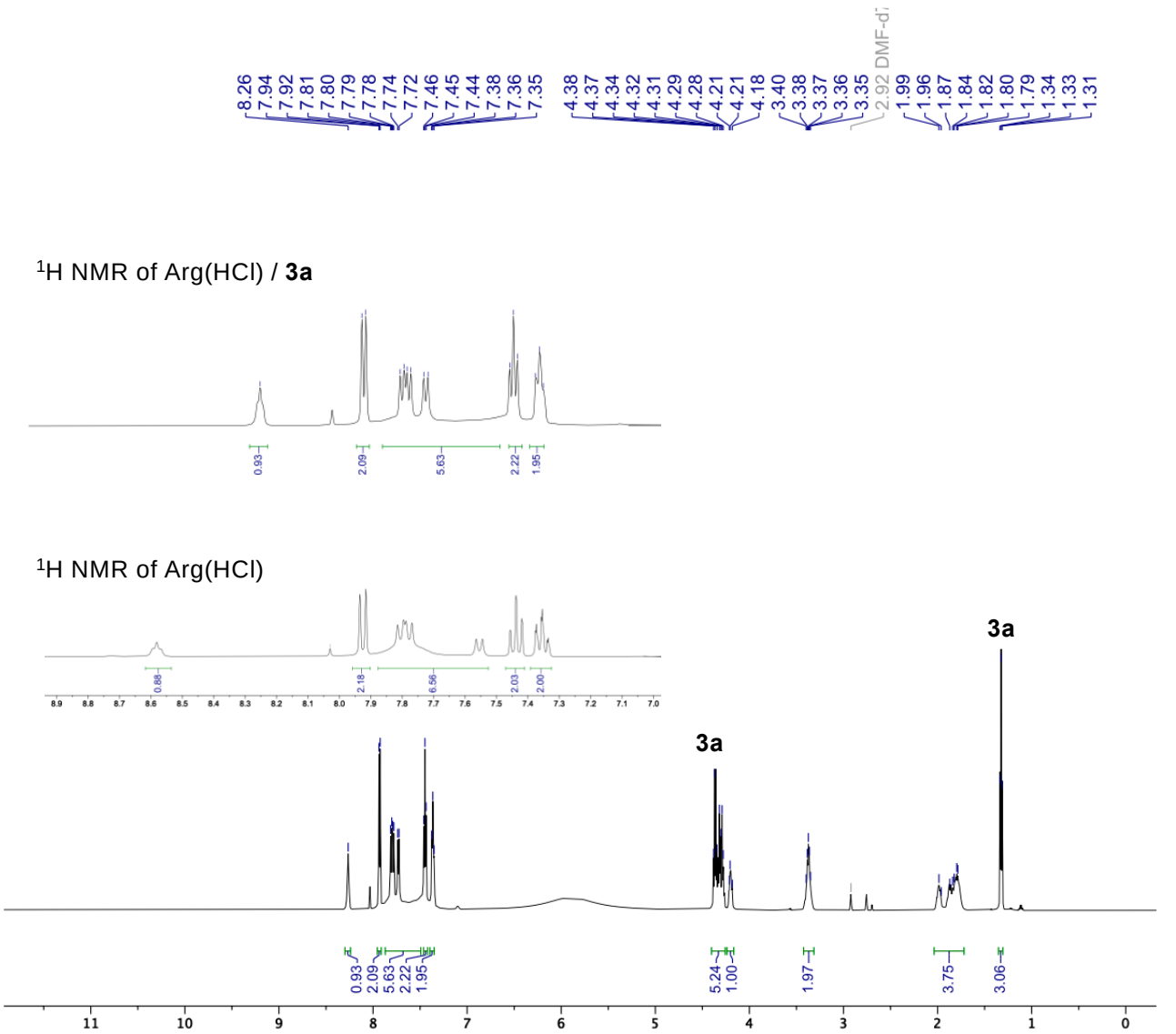
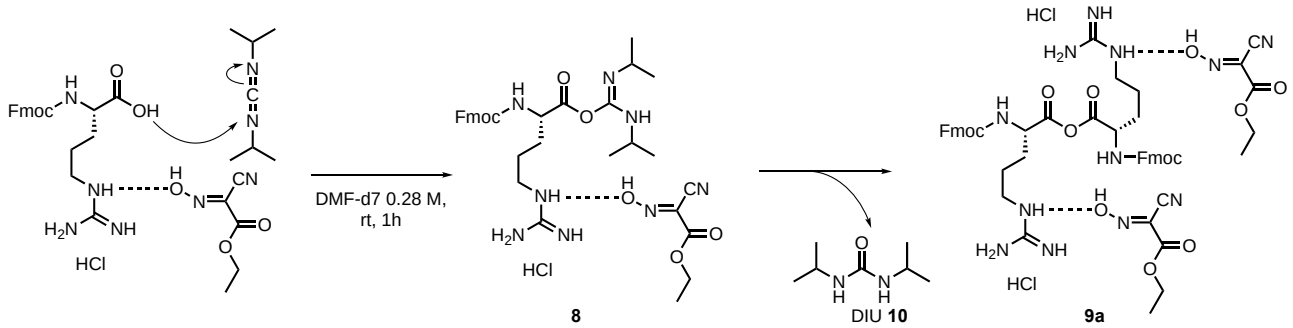


Figure S23. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH and 1.0 eq of Oxyma pure

Fmoc-Arg(HCl)-OH with 1.0 eq of Oxyma Pure and 1.0 eq of DIC



Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma Pure (0.1 mmol, 14.3 mg, 1.0 eq) were dissolved in a glass-vial with 0.35 mL of DMF-d_7. After 5 minutes DIC (0.1 mmol, 15.5 µL, 1.0 eq) was added and the reaction stirred at room temperature. After 1h, the ^{1}H NMR spectrum was acquired.

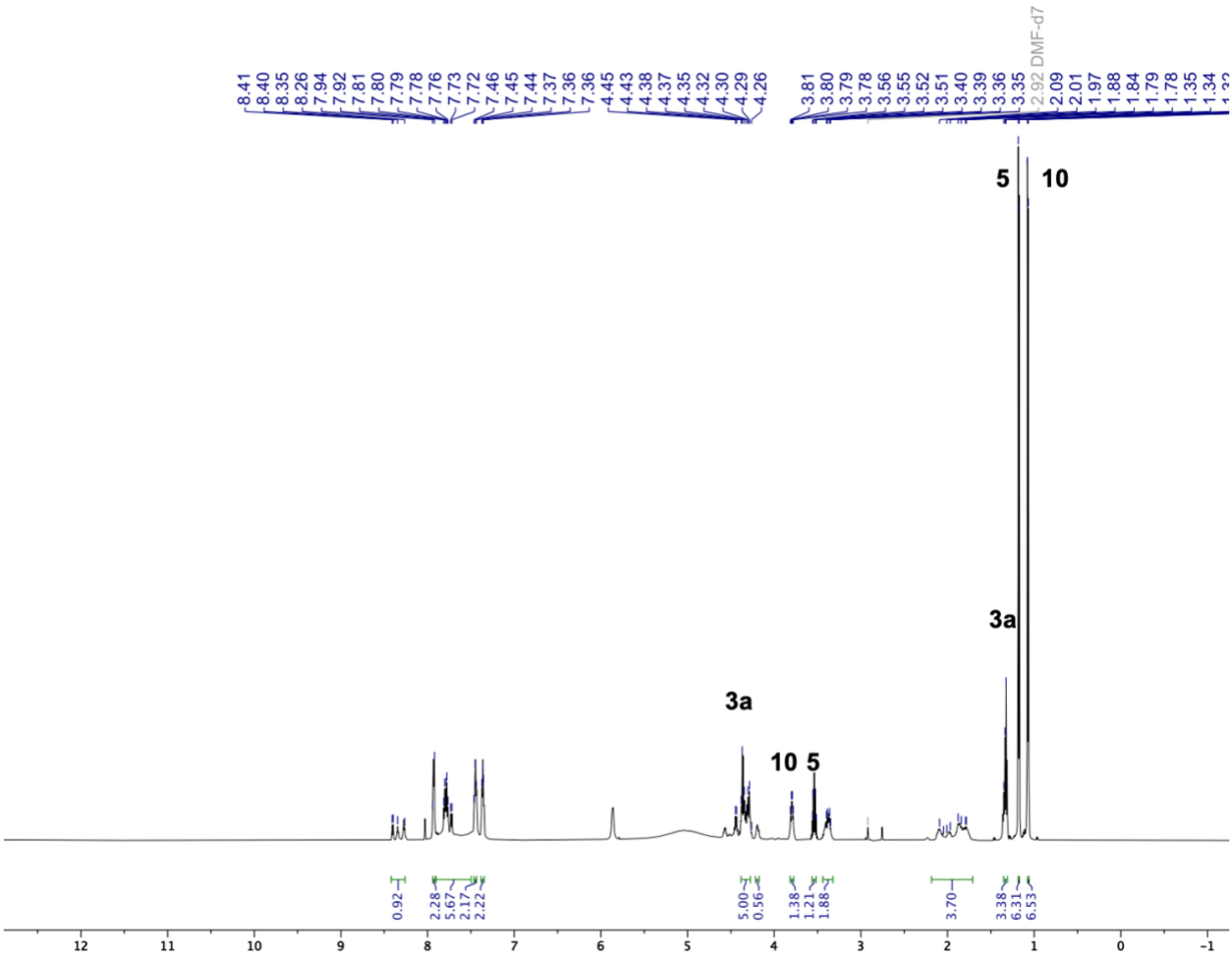
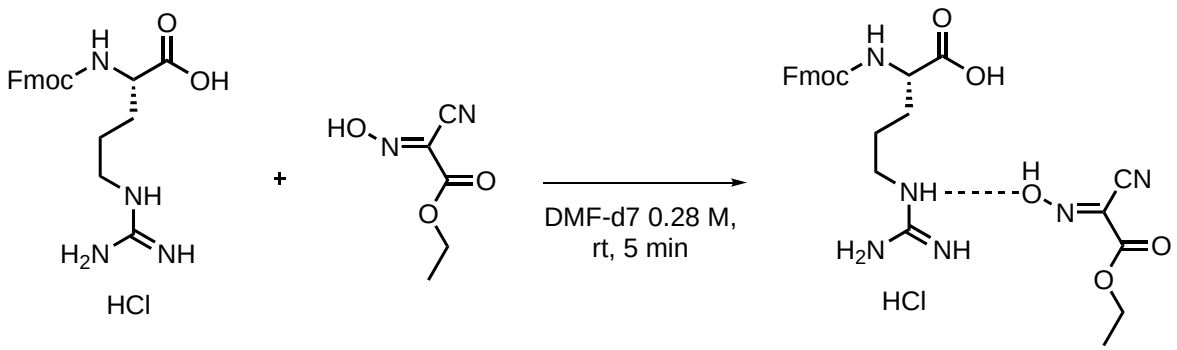


Figure S24. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH, 1.0 eq of Oxyma pure and 1.0 eq of DIC

Fmoc-Arg(HCl)-OH with 2.5 eq of Oxyma Pure



1a
0.1 mmol

3a
2.5 eq

Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma Pure (0.25 mmol, 35.6 mg, 2.5 eq) were dissolved in a glass-vial with 0.35 mL of DMF-d_7. After 5 minutes the ^{1}H NMR spectrum was acquired

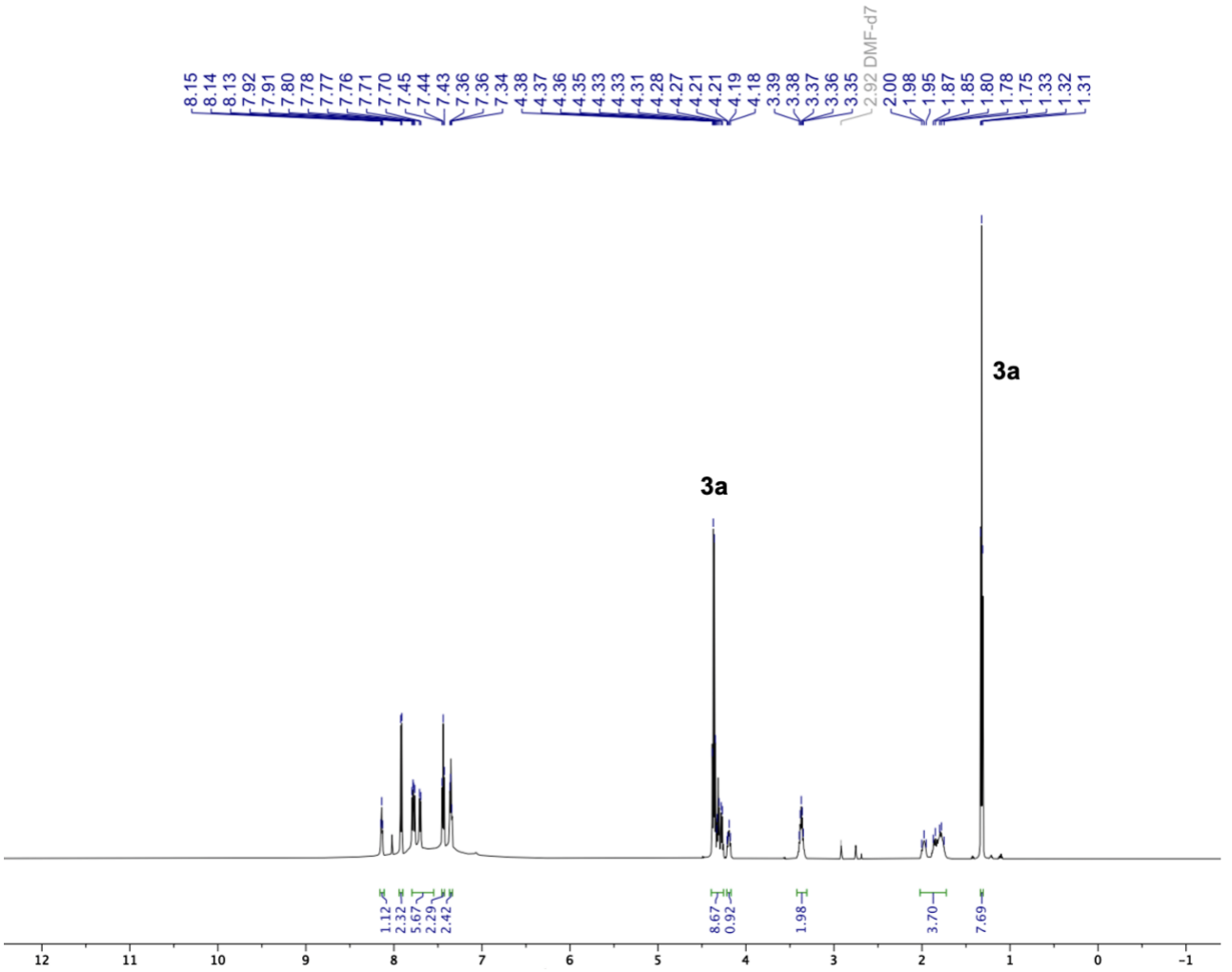
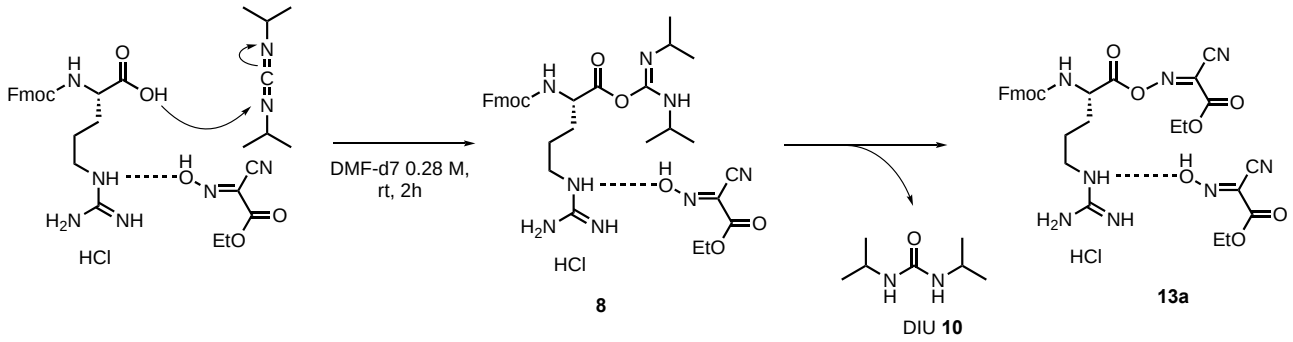


Figure S25. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH and 2.5 eq of Oxyma pure

Fmoc-Arg(HCl)-OH with 2.5 eq of Oxyma Pure and 1.0 eq of DIC



Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma Pure (0.25 mmol, 35.6 mg, 2.5 eq) were dissolved in a glass-vial with 0.35 mL of DMF-d_7. After 5 minutes DIC (0.1 mmol, 15.5 µL, 1.0 eq) was added and the reaction stirred ad room temperature. After 1h, the ^{1}H NMR spectrum was acquired.

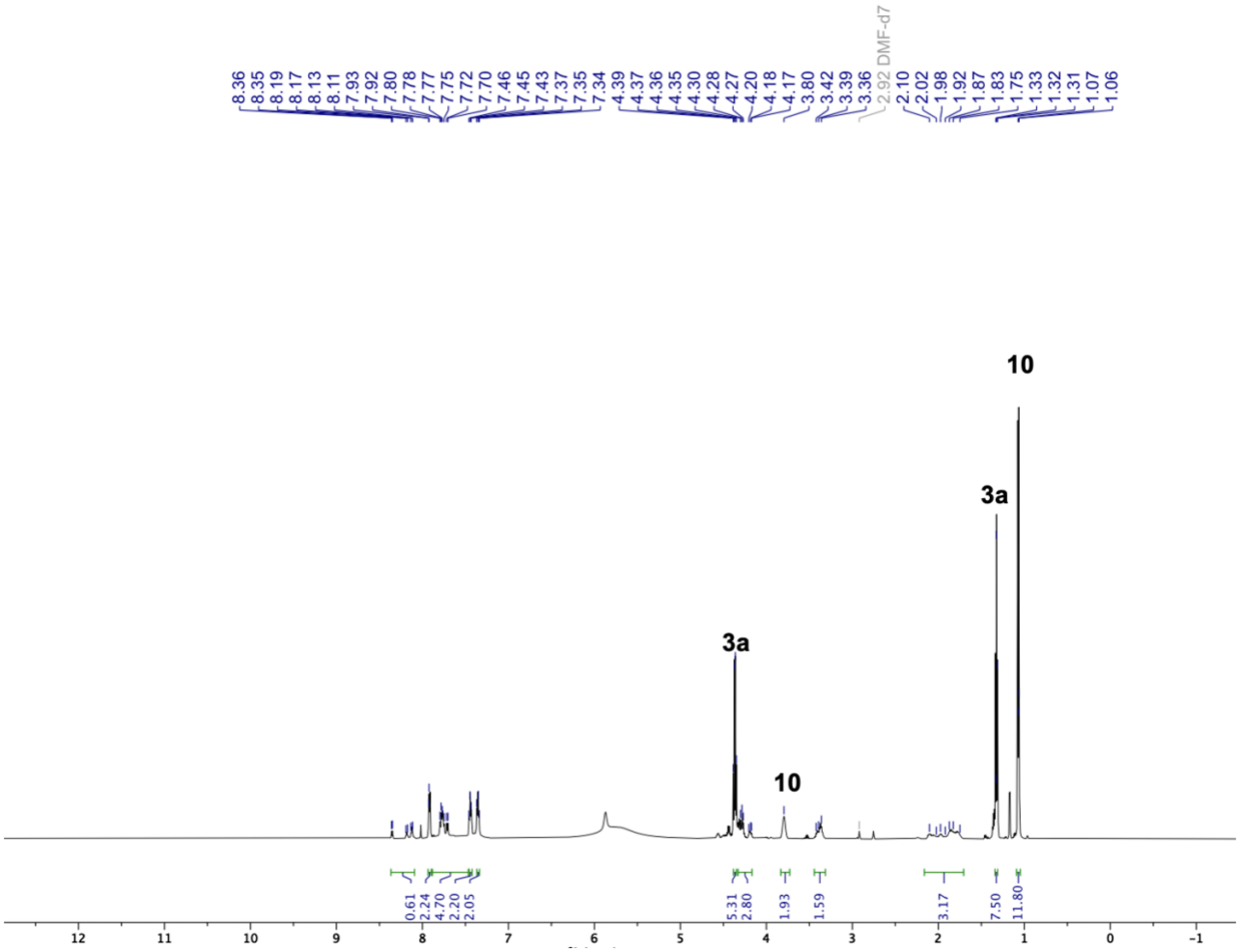
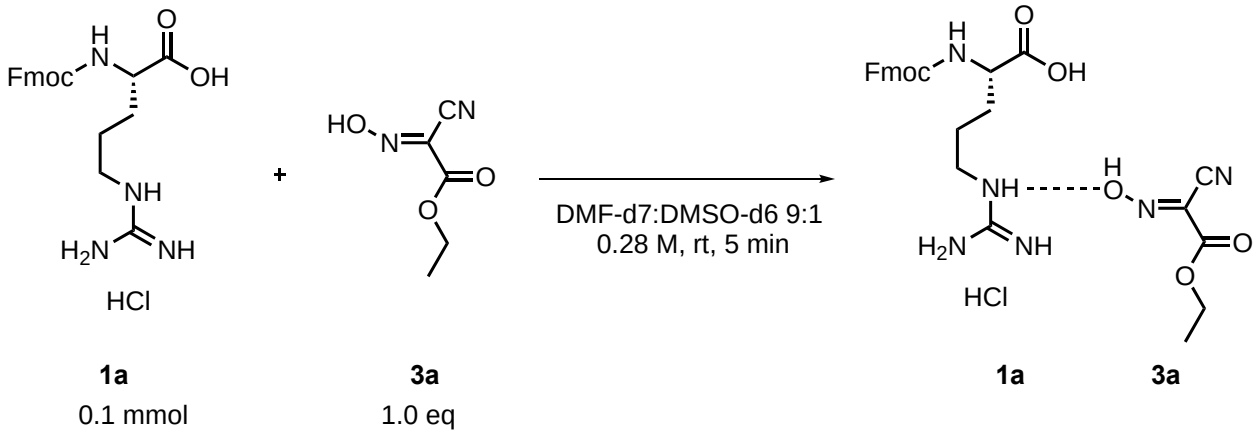


Figure S26. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH, 2.5 eq of Oxyma pure and 1.0 eq of DIC

Fmoc-Arg(HCl)-OH with 1.0 eq of Oxyma Pure in DMF:DMSO 9:1



Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma Pure (0.1 mmol, 14.3 mg, 1.0 eq) were dissolved in a glass-vial with 0.35 mL of DMF-d_7:DMSO-d_6 9:1. After 5 minutes the ^{1}H NMR spectrum was acquired.

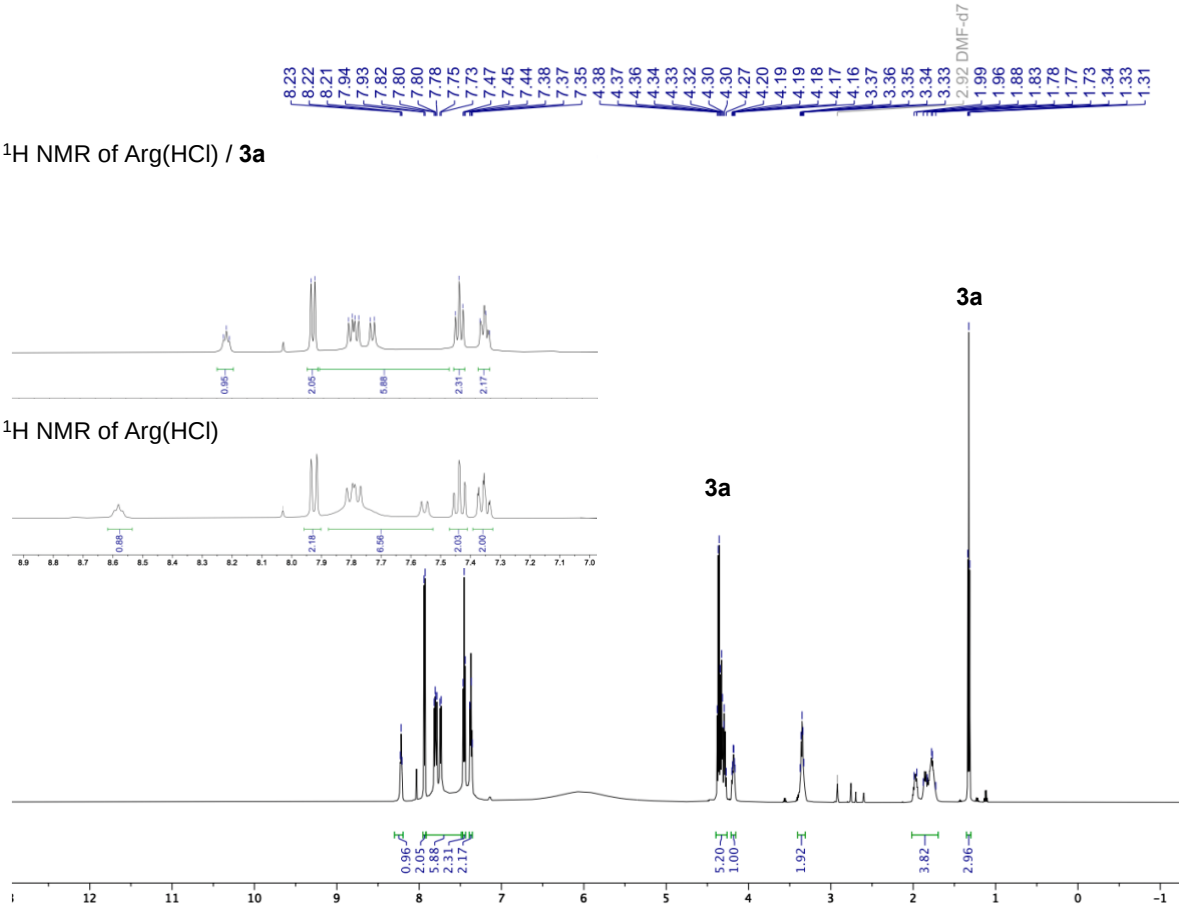
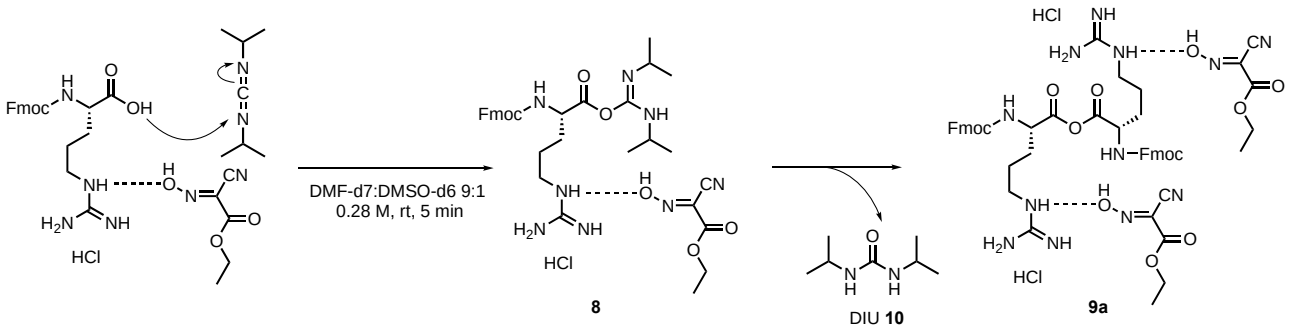


Figure S27. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH and 1.0 eq of Oxyma pure

Fmoc-Arg(HCl)-OH with 1.0 eq of Oxyma Pure and 1.0 eq of DIC in DMF:DMSO 9:1



Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma Pure (0.25 mmol, 35.6 mg, 2.5 eq) were dissolved in a glass-vial with 0.35 mL of DMF-d_7:DMSO-d_6 9:1. After 5 minutes DIC (0.1 mmol, 15.5 µL, 1.0 eq) was added and the reaction stirred at room temperature. After 1h, the ^{1}H NMR spectrum was acquired.

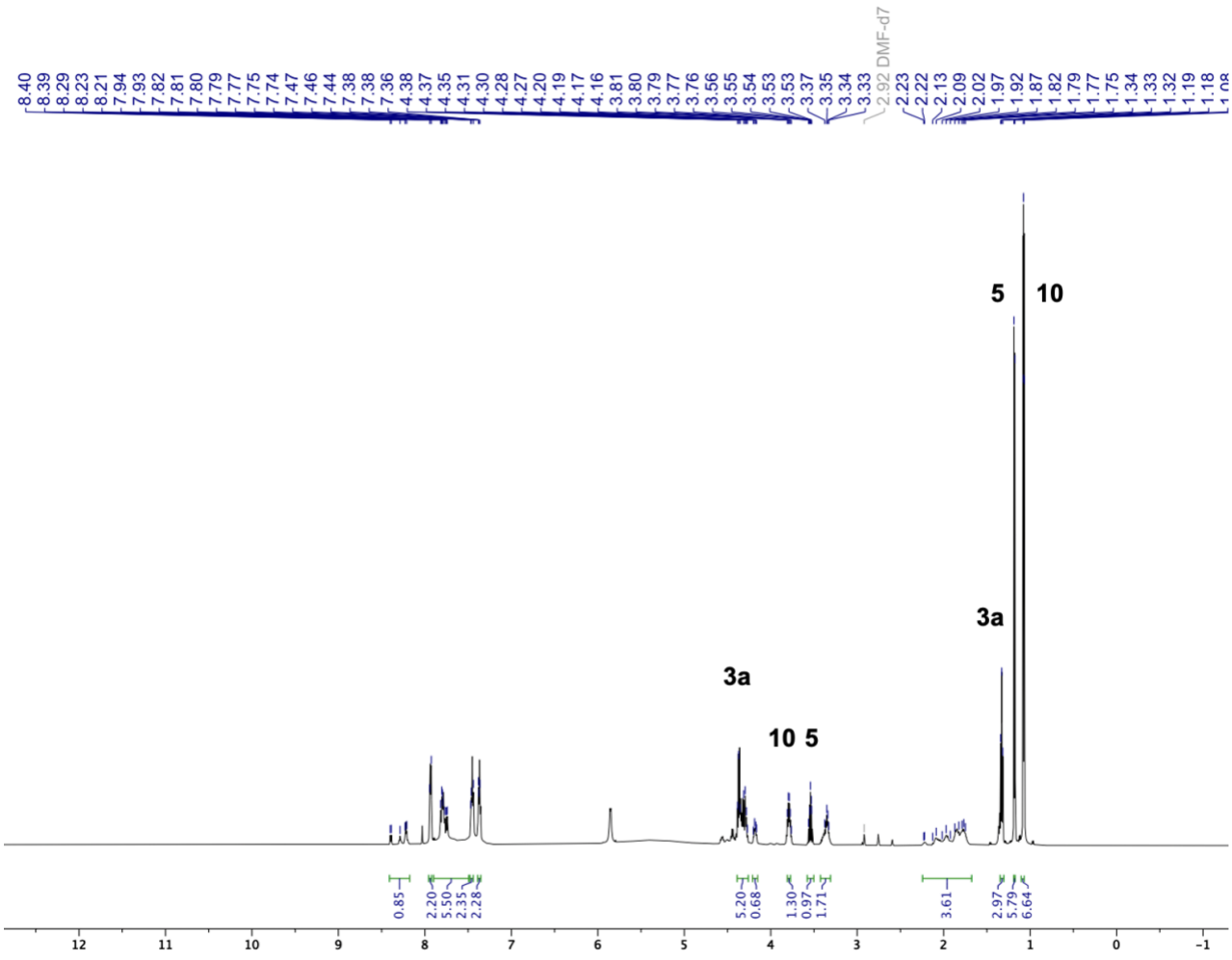
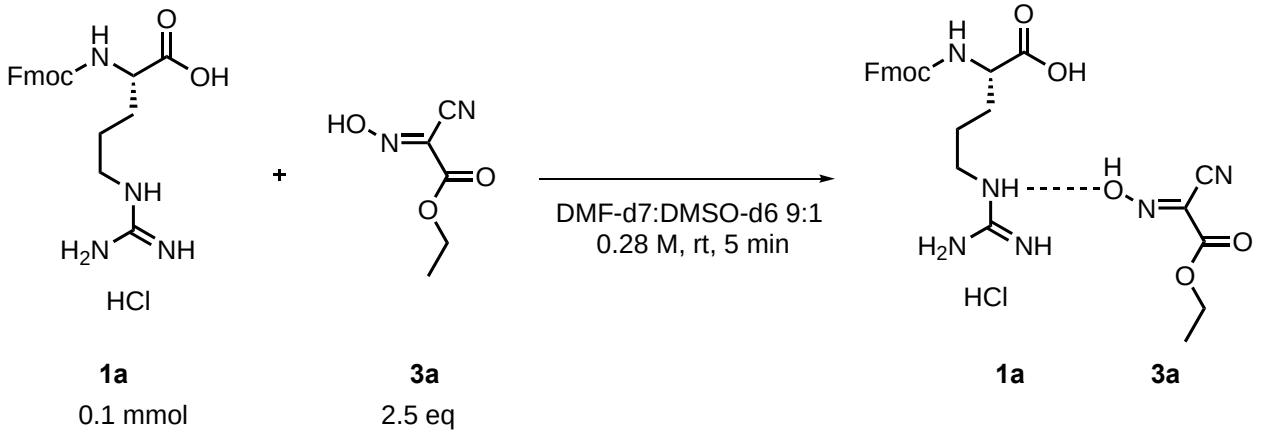


Figure S28. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH, 1.0 eq of Oxyma pure and 1.0 eq of DIC

Fmoc-Arg(HCl)-OH with 2.5 eq of Oxyma Pure in DMF:DMSO 9:1



1a 0.1 mmol **3a** 2.5 eq **1a** **3a**

Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma Pure (0.25 mmol, 35.6 mg, 2.5 eq) were dissolved in a glass-vial with 0.35 mL of DMF-d_7:DMSO-d_6 9:1. After 5 minutes the ^{1}H NMR spectrum was acquired

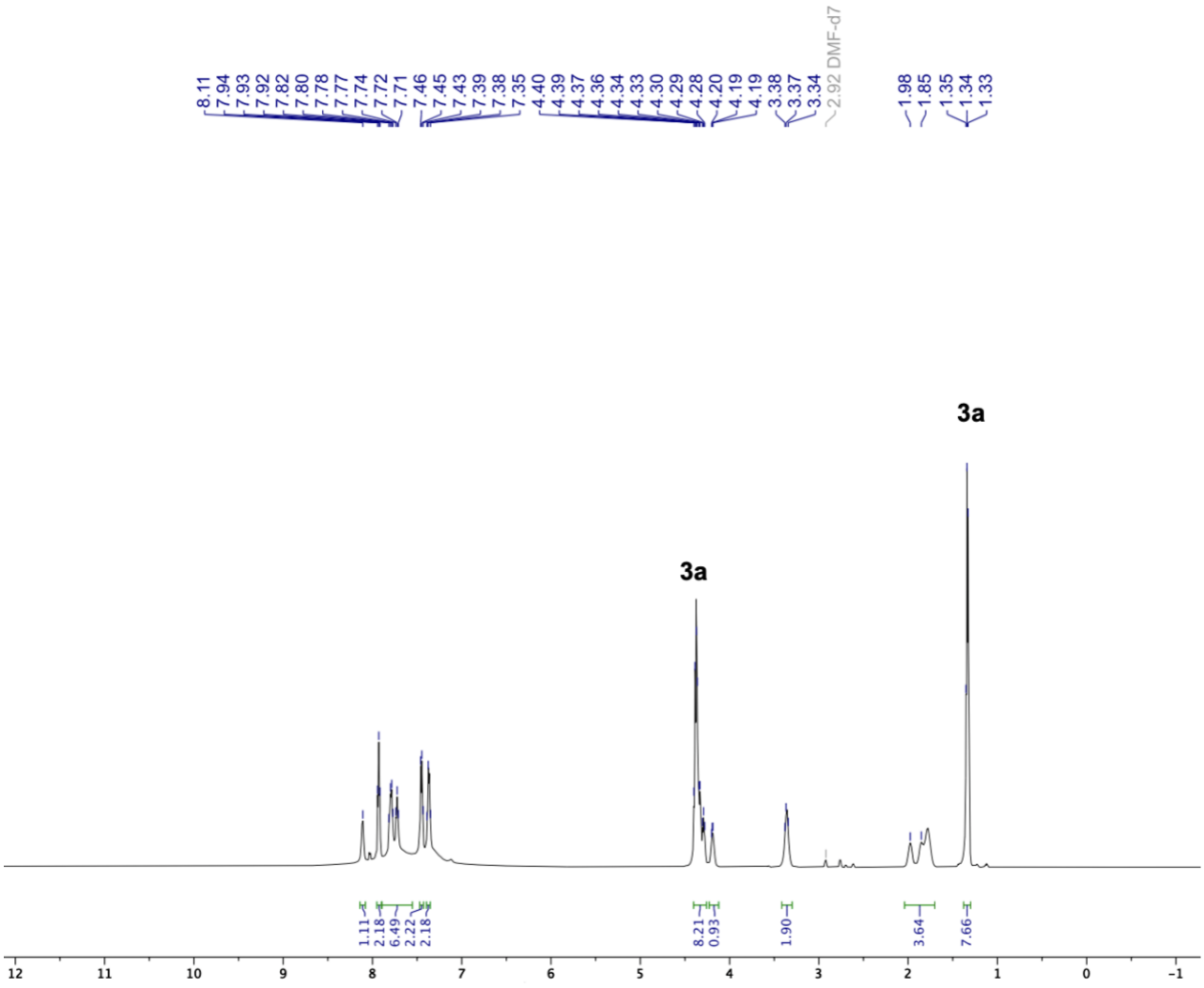
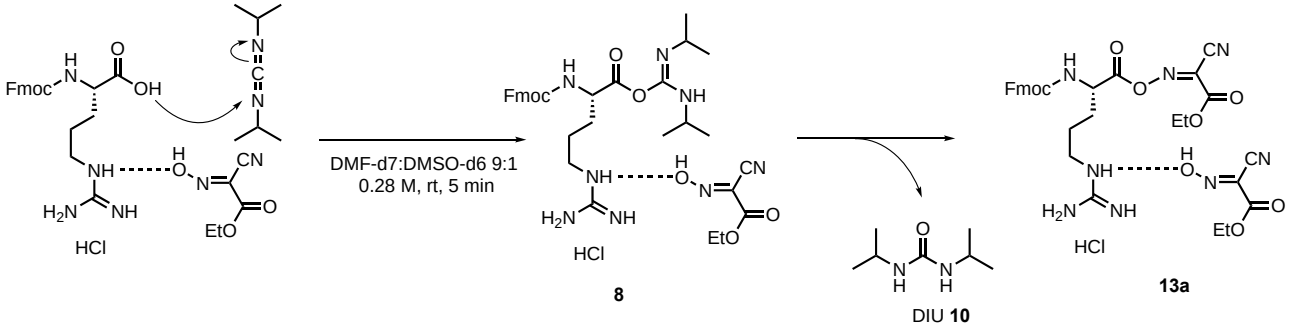


Figure S29. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH and 2.5 eq of Oxyma pure

Fmoc-Arg(HCl)-OH with 2.5 eq of Oxyma Pure and 1.0 eq of DIC in DMF:DMSO 9:1



Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma Pure (0.25 mmol, 35.6 mg, 2.5 eq) were dissolved in a glass-vial with 0.35 mL of DMF-d_7:DMSO-d_6 9:1. After 5 minutes DIC (0.1 mmol, 15.5 µL, 1.0 eq) was added and the reaction stirred at room temperature. After 1h, the ^{1}H NMR spectrum was acquired.

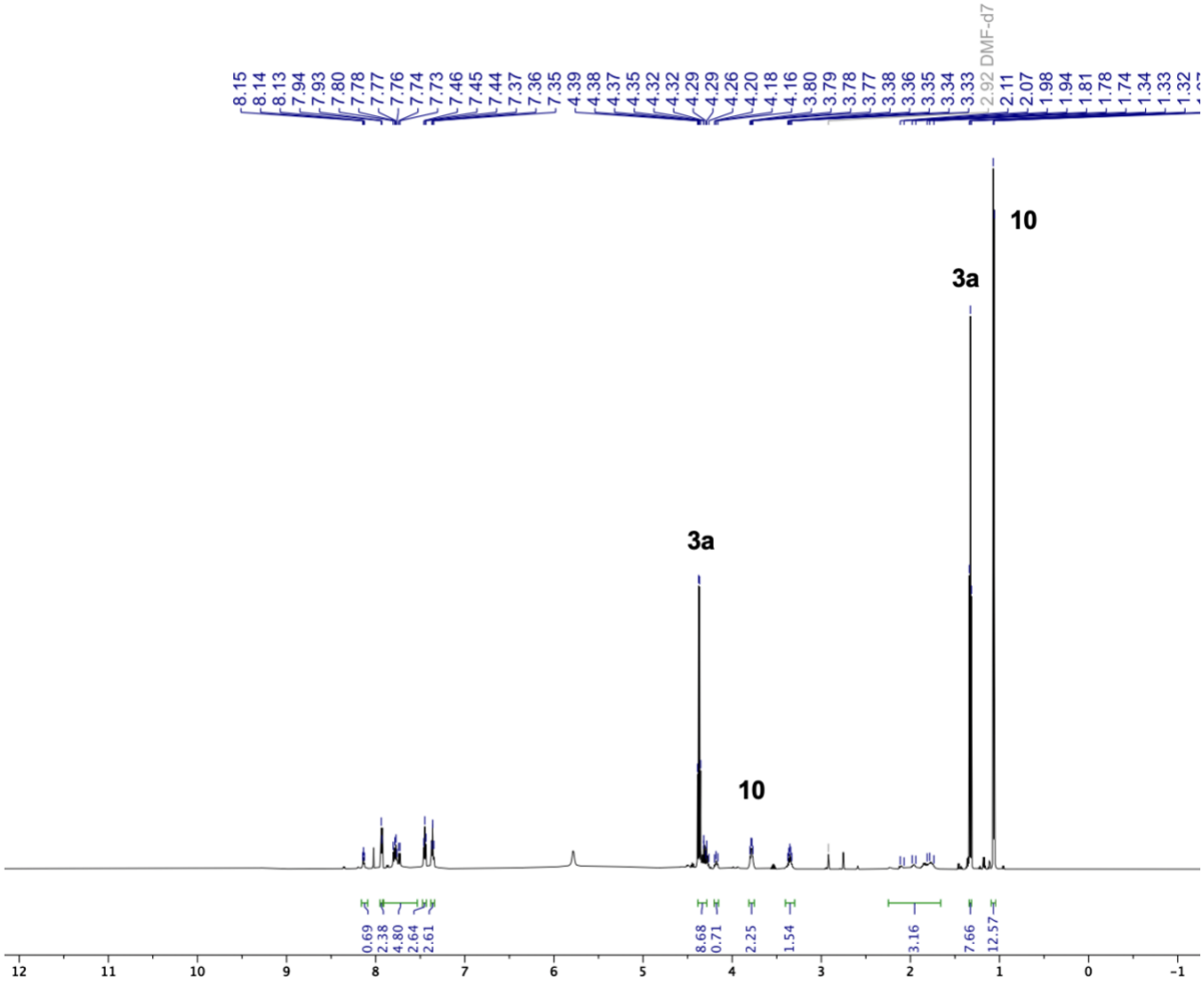
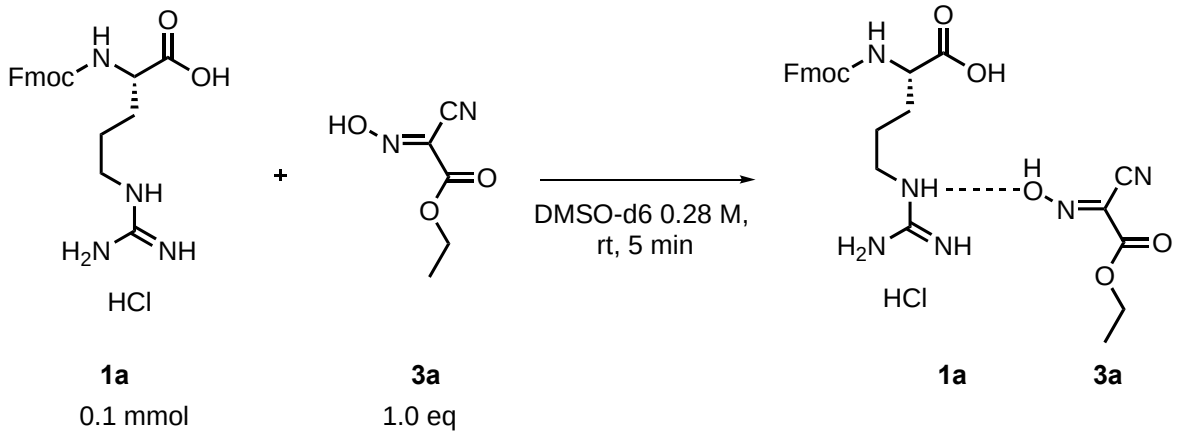


Figure S30. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH, 1.0 eq of Oxyma pure and 1.0 eq of DIC

Fmoc-Arg(HCl)-OH with 1.0 eq of Oxyma Pure in DMSO



1a 0.1 mmol **3a** 1.0 eq **1a** **3a**

Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma Pure (0.1 mmol, 14.3 mg, 1.0 eq) were dissolved in a glass-vial with 0.35 mL of DMSO-d_6. After 5 minutes the ^{1}H NMR spectrum was acquired.

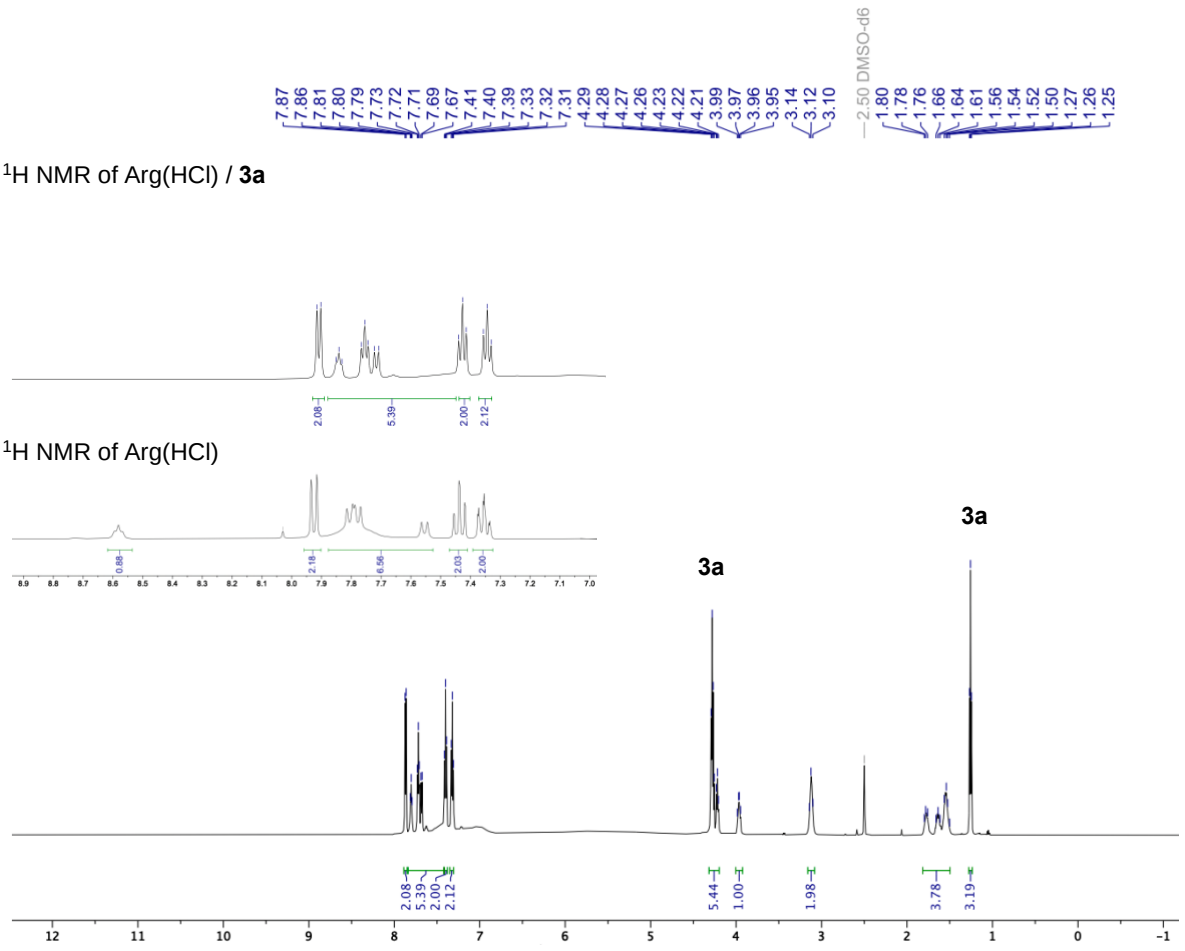
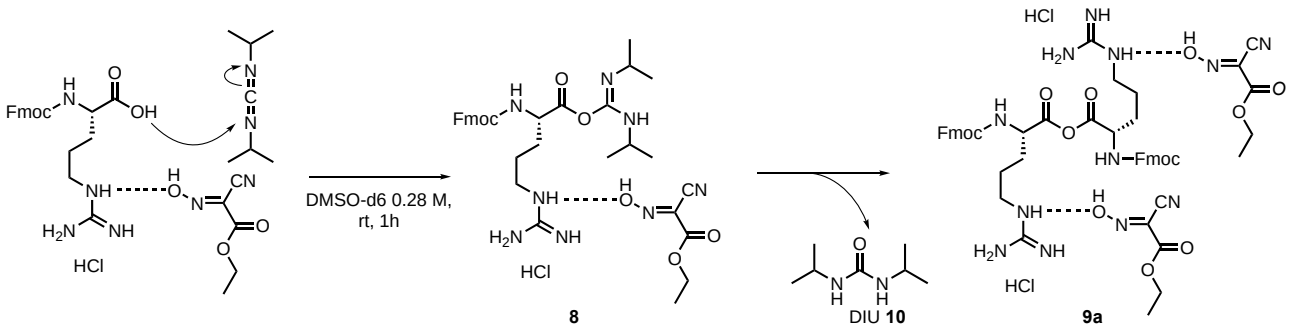


Figure S31. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH and 1.0 eq of Oxyma pure

Fmoc-Arg(HCl)-OH with 1.0 eq of Oxyma Pure in DMSO and 1.0 eq of DIC



Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma Pure (0.25 mmol, 35.6 mg, 2.5 eq) were dissolved in a glass-vial with 0.35 mL of DMSO-d_6. After 5 minutes DIC (0.1 mmol, 15.5 µL, 1.0 eq) was added and the reaction stirred ad room temperature. After 1h, the ^{1}H NMR spectrum was acquired.

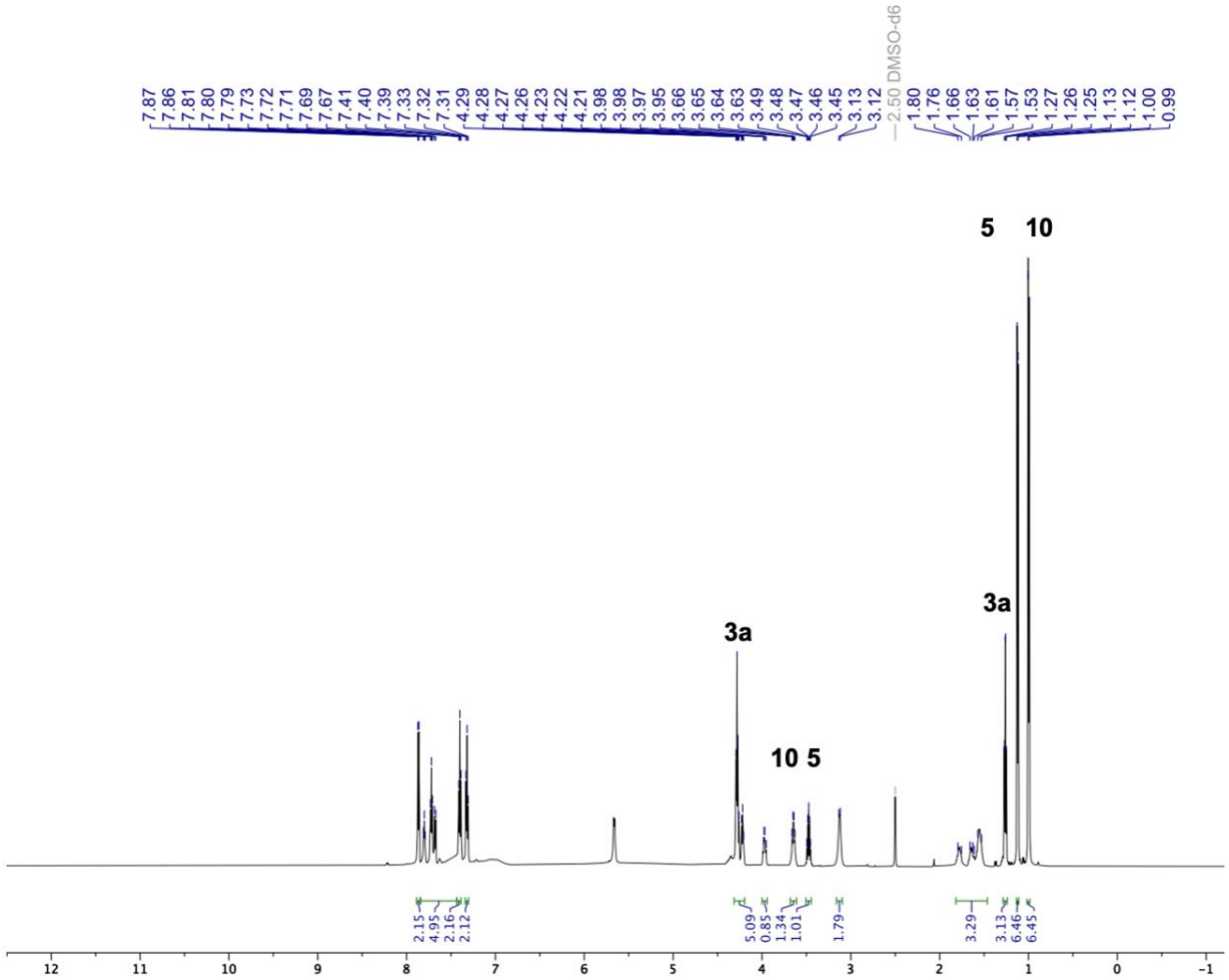
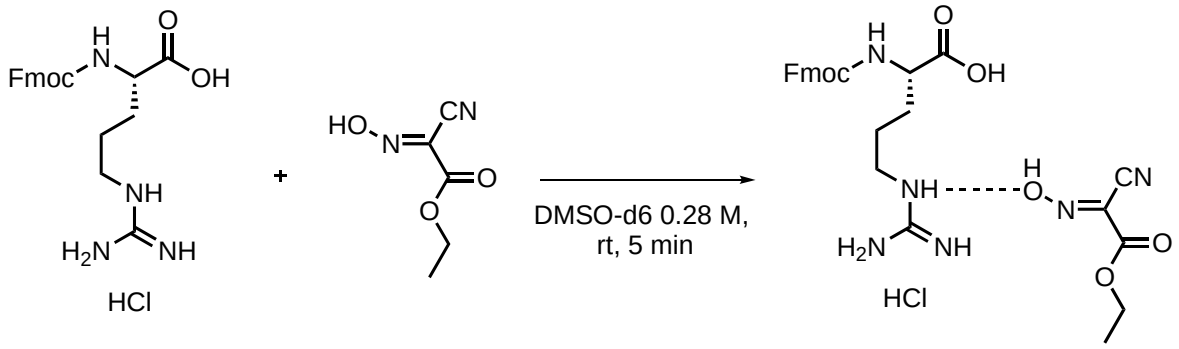


Figure S32. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH and 1.0 eq of Oxyma pure and 1.0 eq of DIC

Fmoc-Arg(HCl)-OH with 2.5 eq of Oxyma Pure in DMSO



1a 0.1 mmol

3a 2.5 eq

1a **3a**

Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma Pure (0.25 mmol, 35.6 mg, 2.5 eq) were dissolved in a glass-vial with 0.35 mL of DMSO-d_6. After 5 minutes the ^{1}H NMR spectrum was acquired

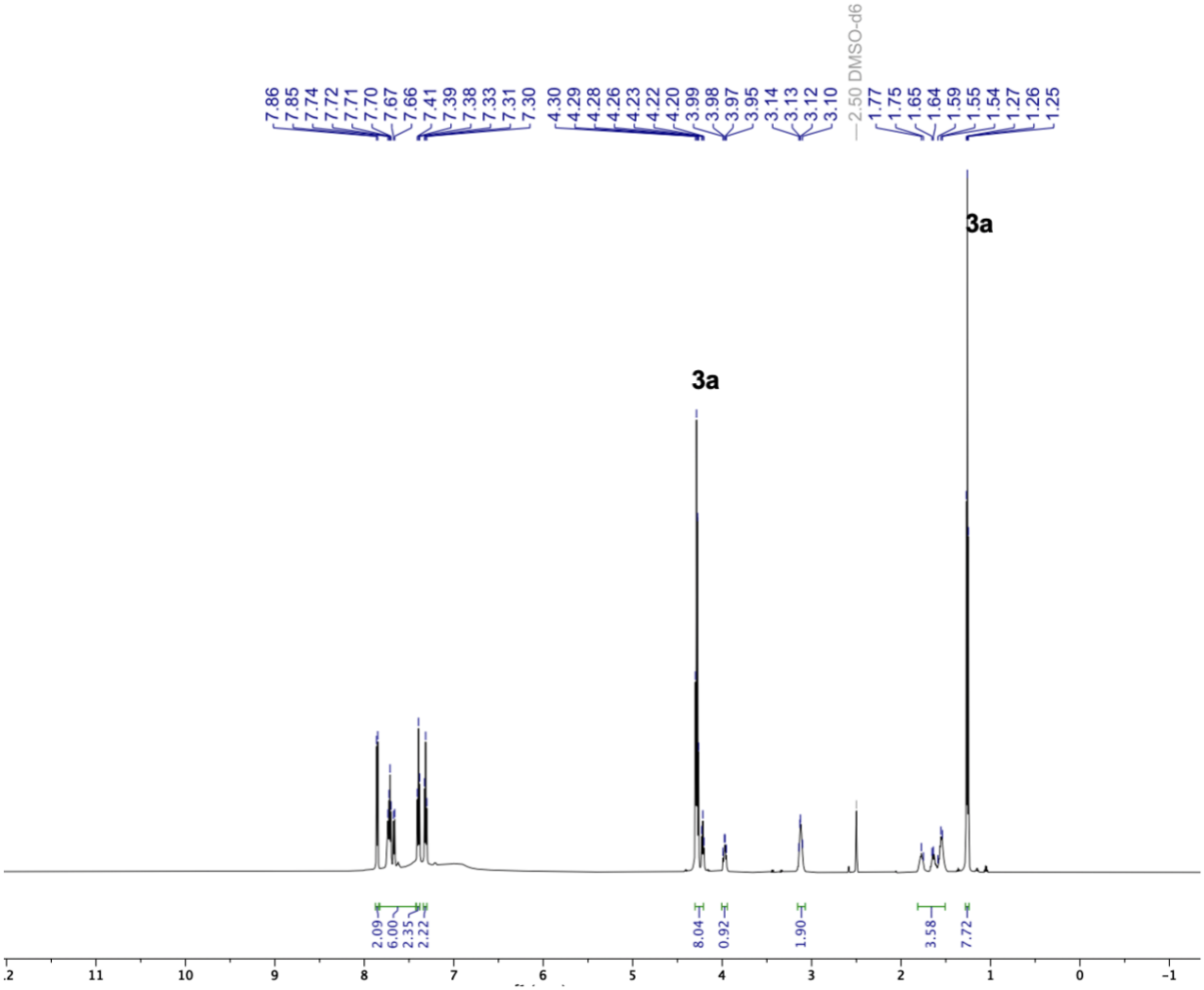
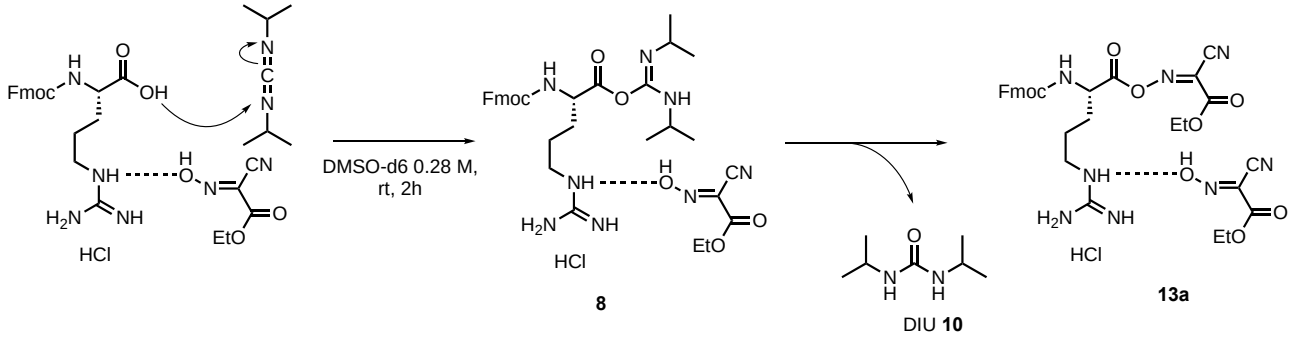


Figure S33. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH and 2.5 eq of Oxyma pure

Fmoc-Arg(HCl)-OH with 2.5 eq of Oxyma Pure in DMSO and 1.0 eq of DIC



Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma Pure (0.25 mmol, 35.6 mg, 2.5 eq) were dissolved in a glass-vial with 0.35 mL of DMSO-d_6. After 5 minutes DIC (0.1 mmol, 15.5 µL, 1.0 eq) was added and the reaction stirred at room temperature. After 1h, the ^{1}H NMR spectrum was acquired.

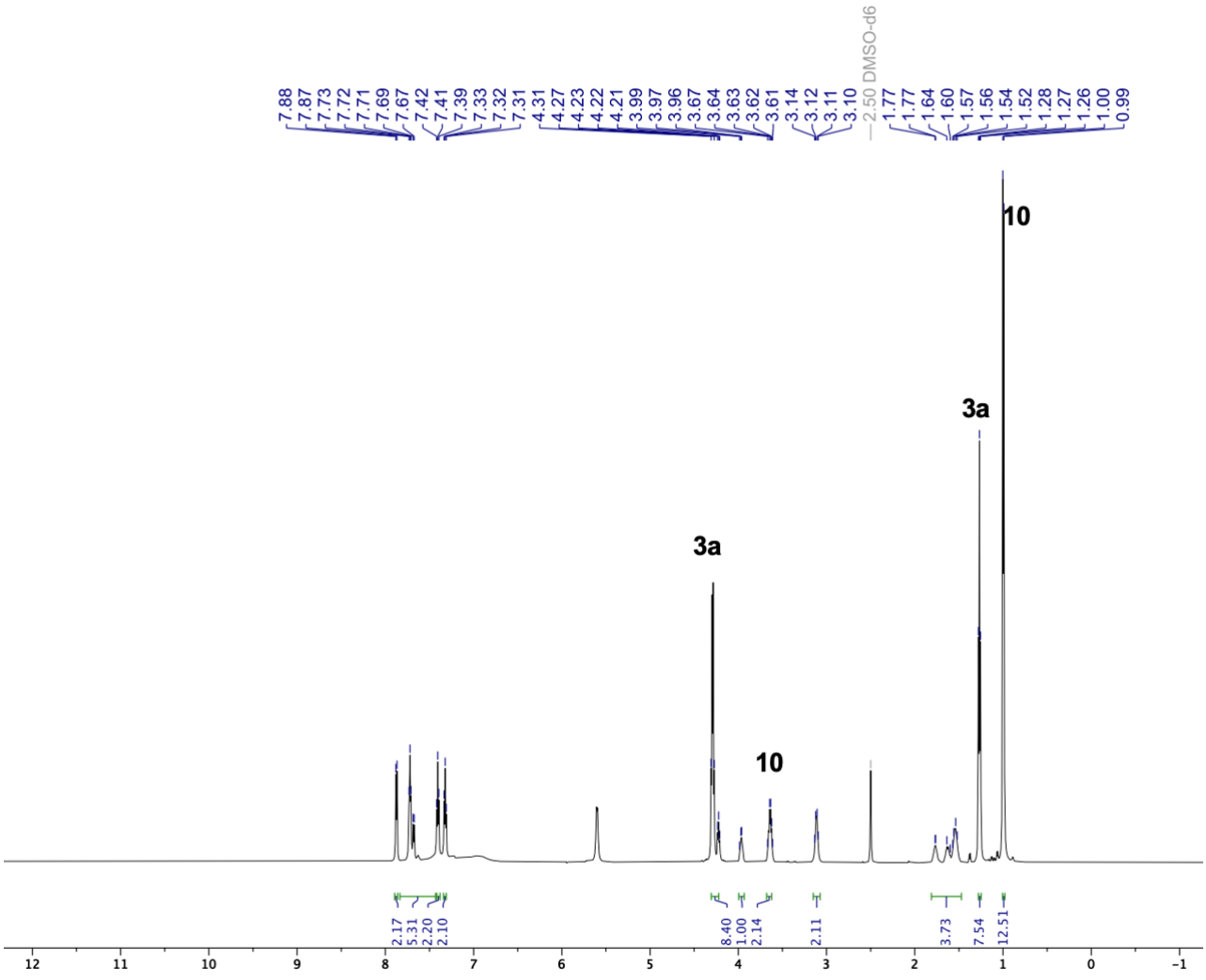
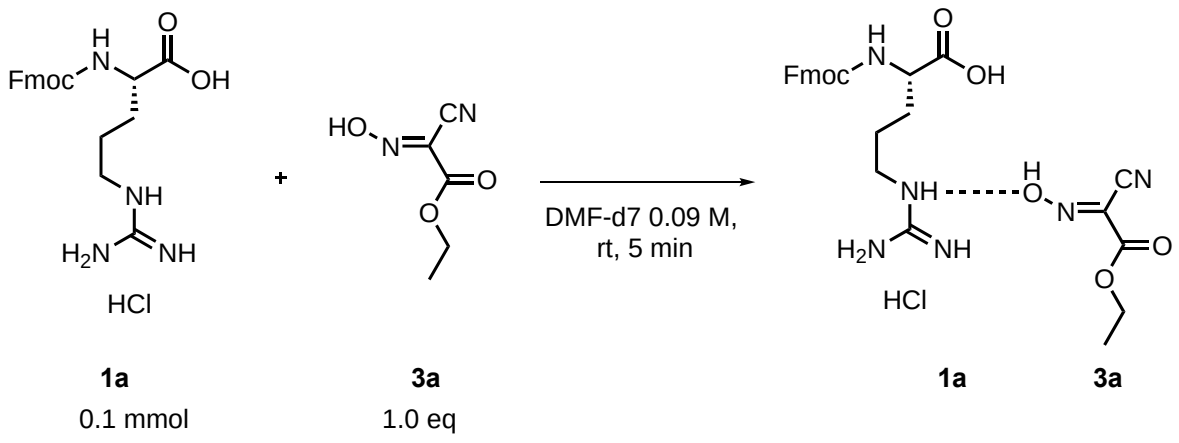


Figure S34. [1]H NMR spectrum of Fmoc-Arg(HCl)-OH and 2.5 eq of Oxyma pure and 1.0 eq of DIC

[1]H NMR in diluted systems

Fmoc-Arg(HCl)-OH with 1.0 eq of Oxyma Pure



1a 0.1 mmol

3a 1.0 eq

1a **3a**

Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma Pure (0.1 mmol, 14.3 mg, 1.0 eq) were dissolved in a glass-vial with 1.1 mL of DMF-d_7. After 5 minutes the ^{1}H NMR spectrum was acquired.

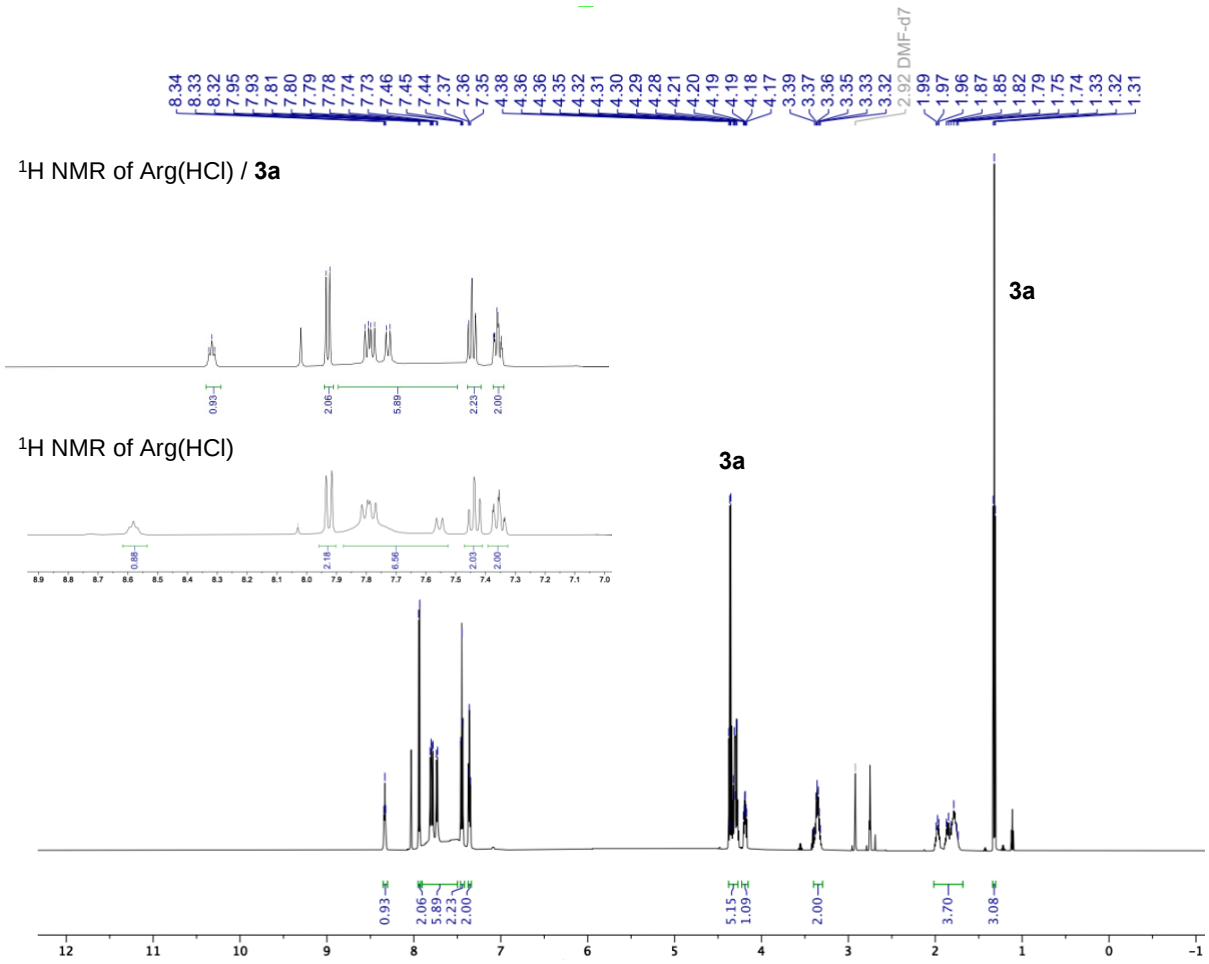
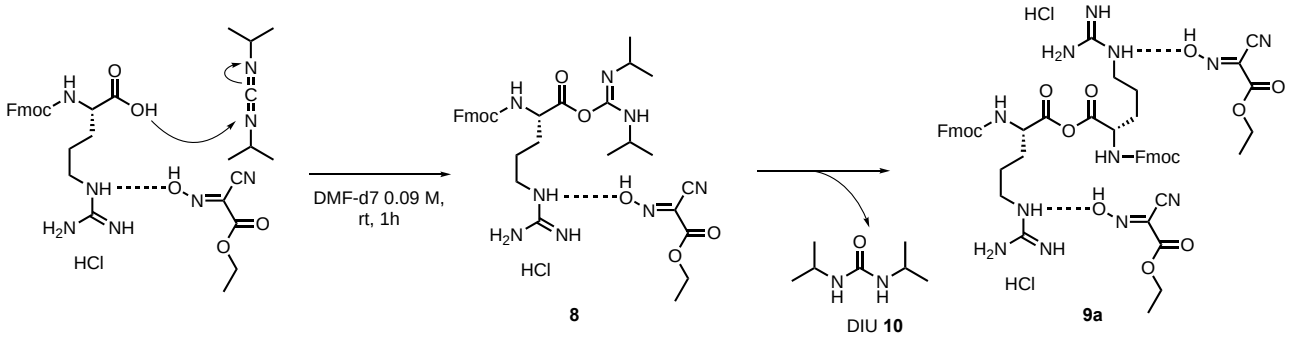


Figure S35. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH and 1.0 eq of Oxyma pure

Fmoc-Arg(HCl)-OH with 1.0 eq of Oxyma Pure and 1.0 eq of DIC



Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma Pure (0.25 mmol, 35.6 mg, 2.5 eq) were dissolved in a glass-vial with 1.1 mL of DMF-d_7. After 5 minutes DIC (0.1 mmol, 15.5 µL, 1.0 eq) was added and the reaction stirred at room temperature. After 3h, the ^{1}H NMR spectrum was acquired.

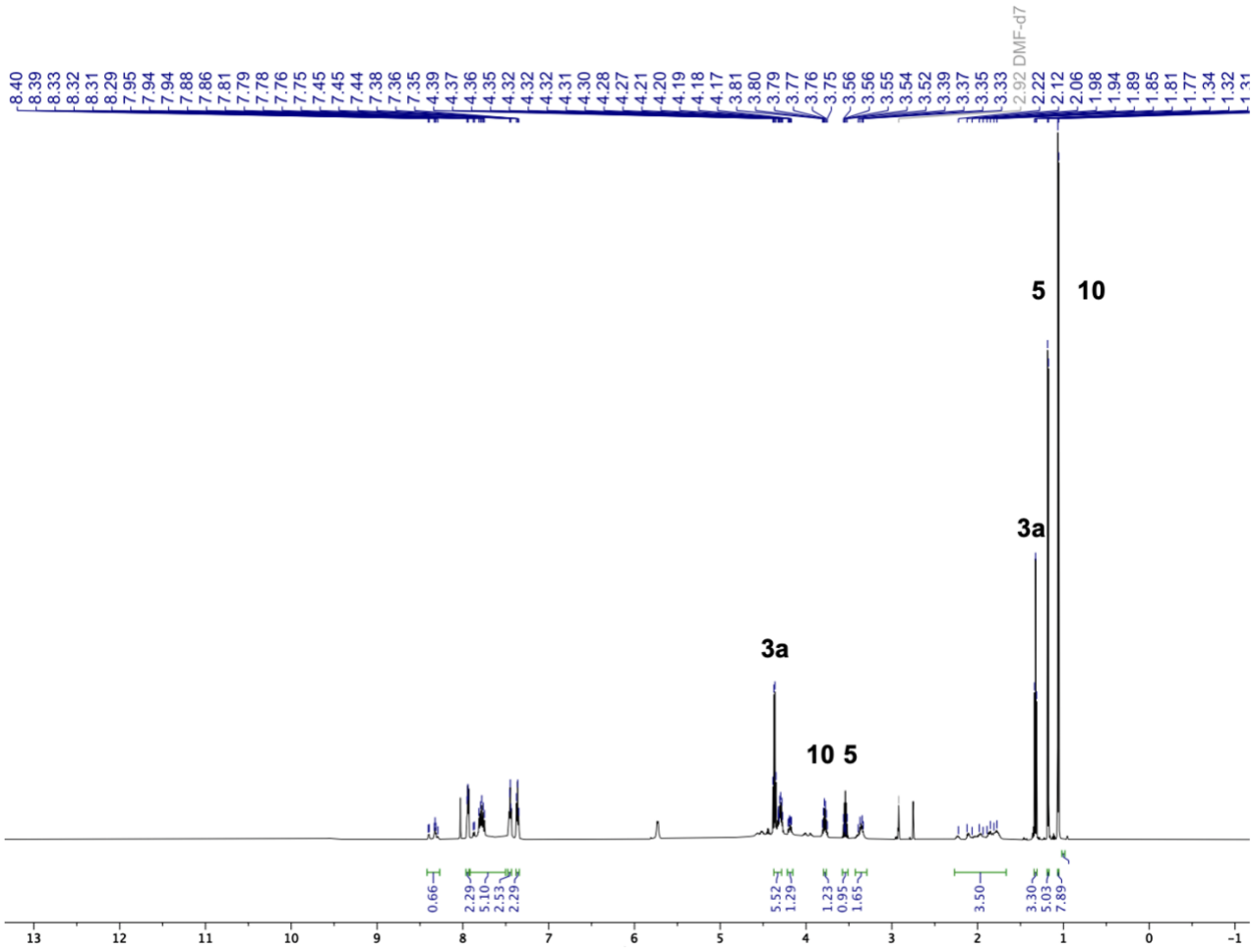
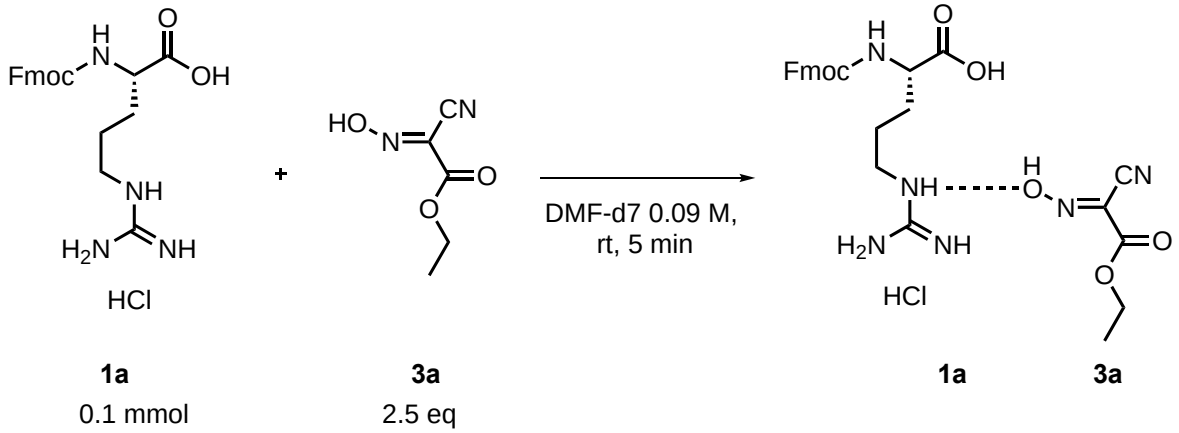


Figure S36. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH, 1.0 eq of Oxyma pure and 1.0 eq of DIC

Fmoc-Arg(HCl)-OH with 2.5 eq of Oxyma Pure



1a 0.1 mmol + **3a** 2.5 eq → **1a** **3a**

Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma Pure (0.25 mmol, 35.6 mg, 2.5 eq) were dissolved in a glass-vial with 1.1 mL of DMF-d_7. After 5 minutes the ^{1}H NMR spectrum was acquired

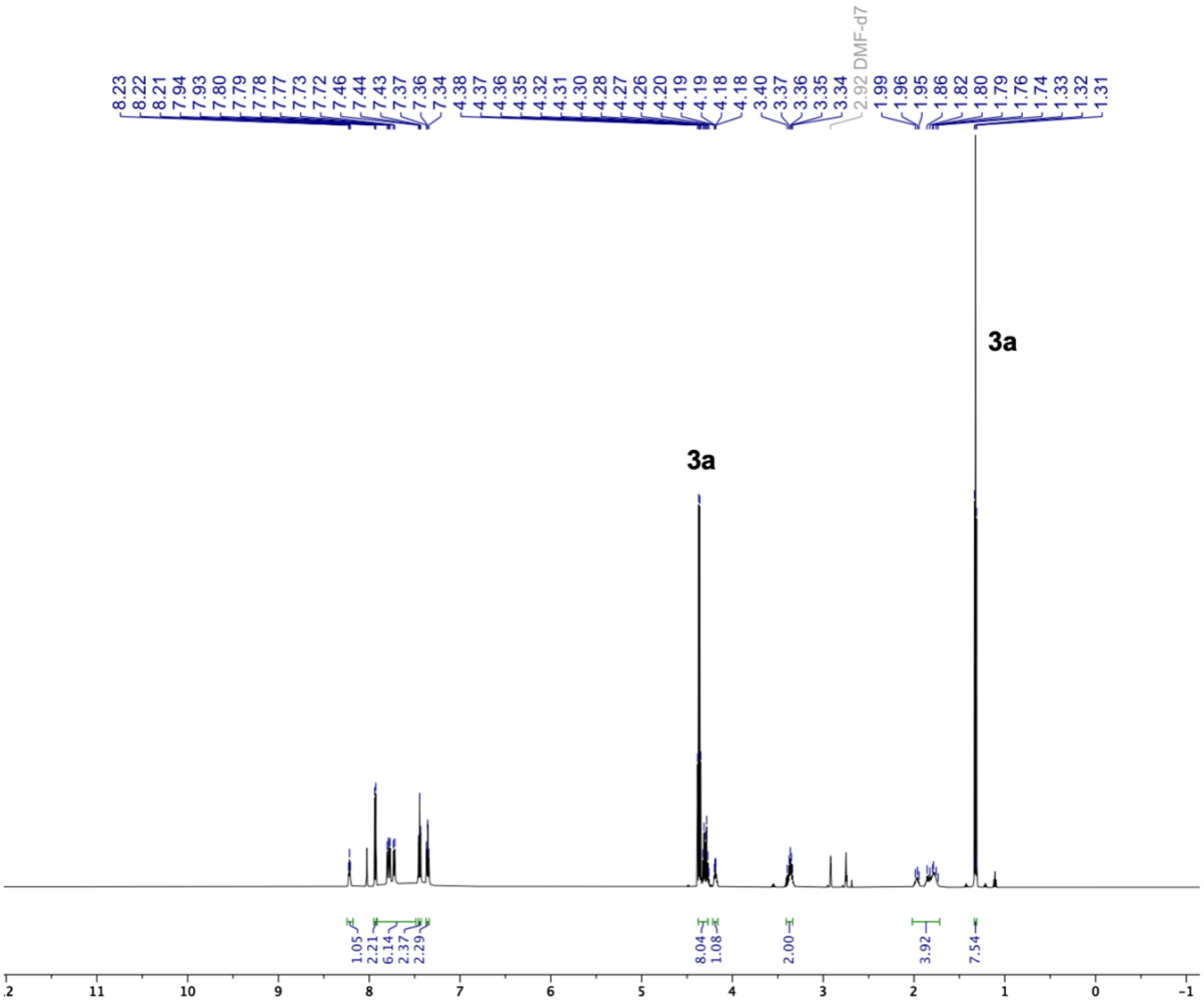
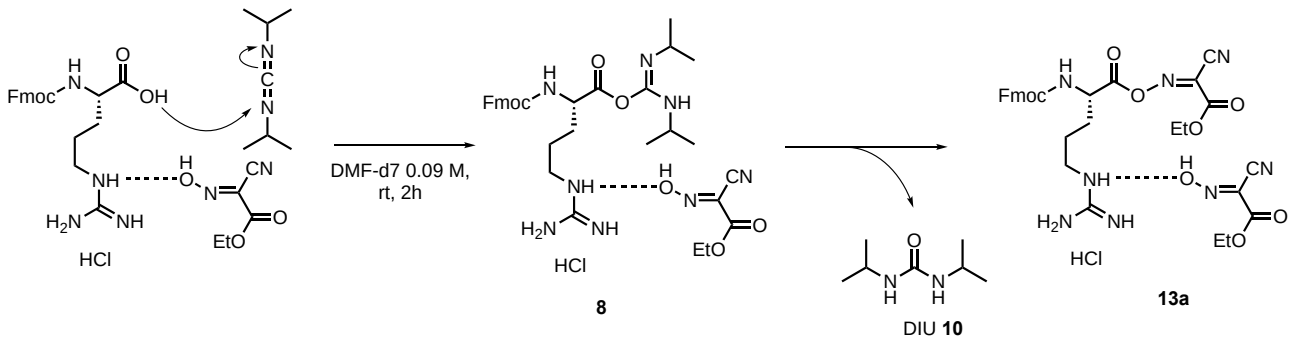


Figure S37. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH and 2.5 eq of Oxyma pure

Fmoc-Arg(HCl)-OH with 2.5 eq of Oxyma Pure and 1.0 eq of DIC



Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and Oxyma Pure (0.25 mmol, 35.6 mg, 2.5 eq) were dissolved in a glass-vial with 1.1 mL of DMF-d_7. After 5 minutes DIC (0.1 mmol, 15.5 µL, 1.0 eq) was added and the reaction stirred ad room temperature. After 3h, the ^{1}H NMR spectrum was acquired.

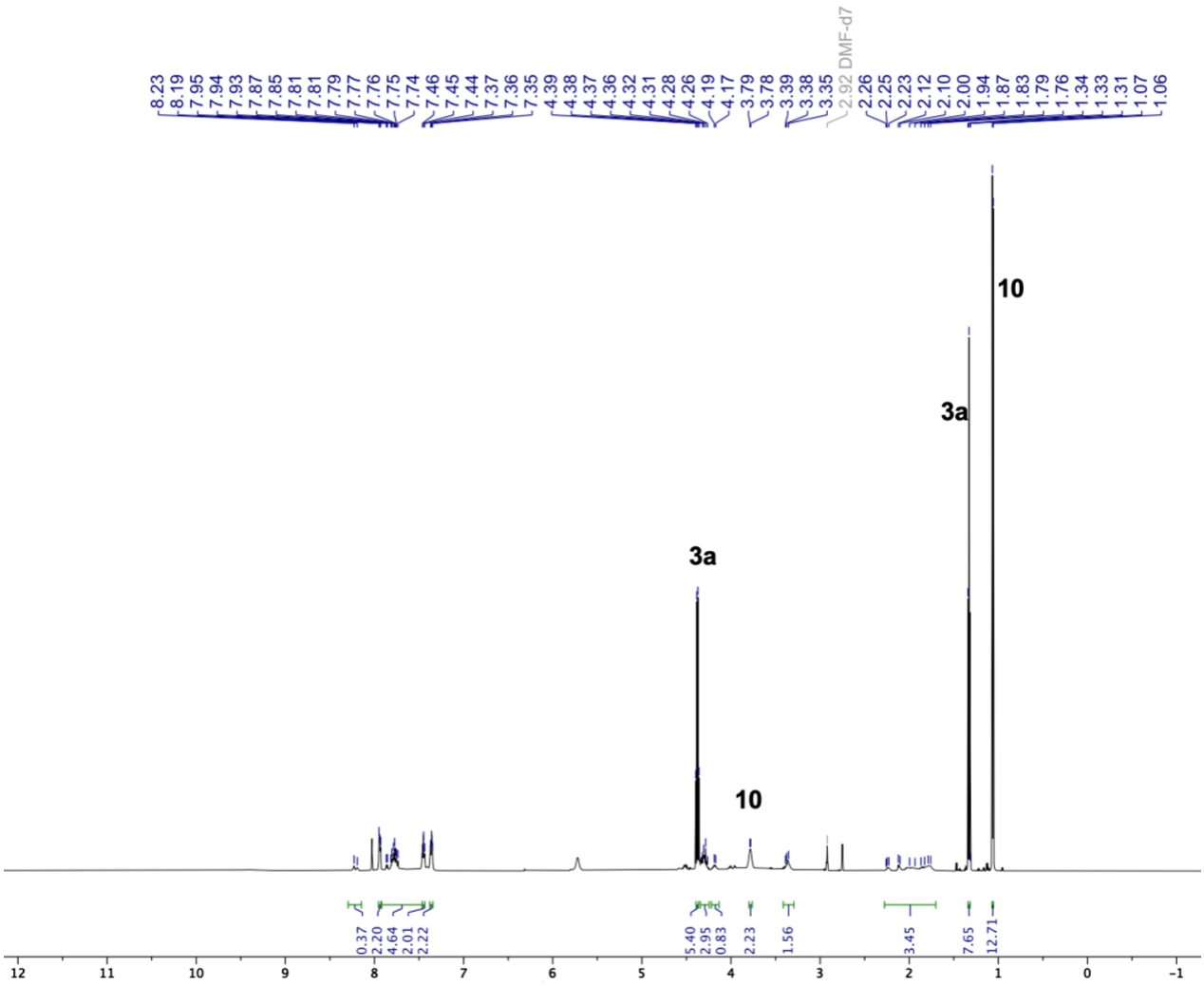
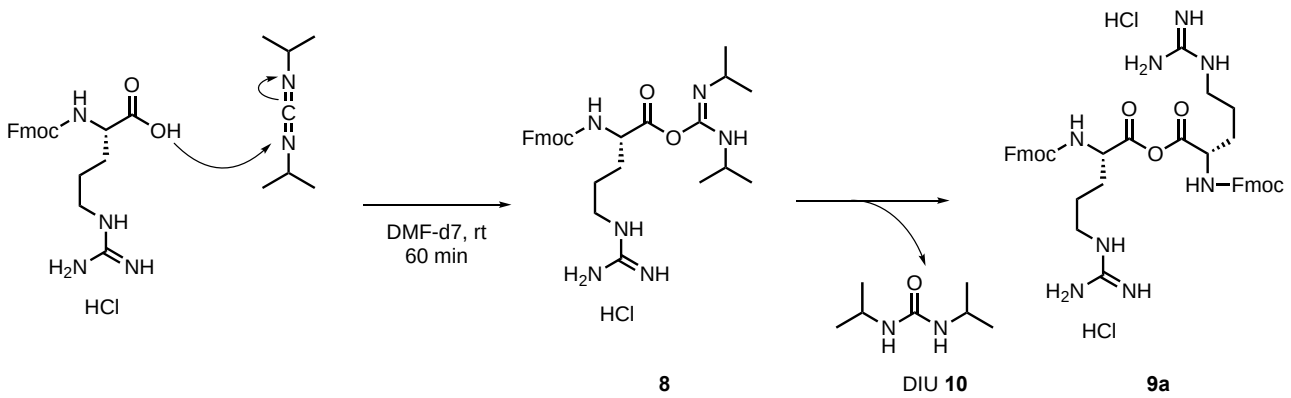


Figure S38. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH, 2.5 eq of Oxyma pure and 1.0 eq of DIC

Anhydride formation



Fmoc-Arg(HCl)-OH (0.1 mmol, 42.9 mg, 1.0 eq) and DIC (0.1 mmol, 15.5 µL, 1.0 eq) were dissolved in a glass-vial with 0.6 mL of DMF-d_7. After 60 minutes the ^{1}H NMR spectrum was acquired

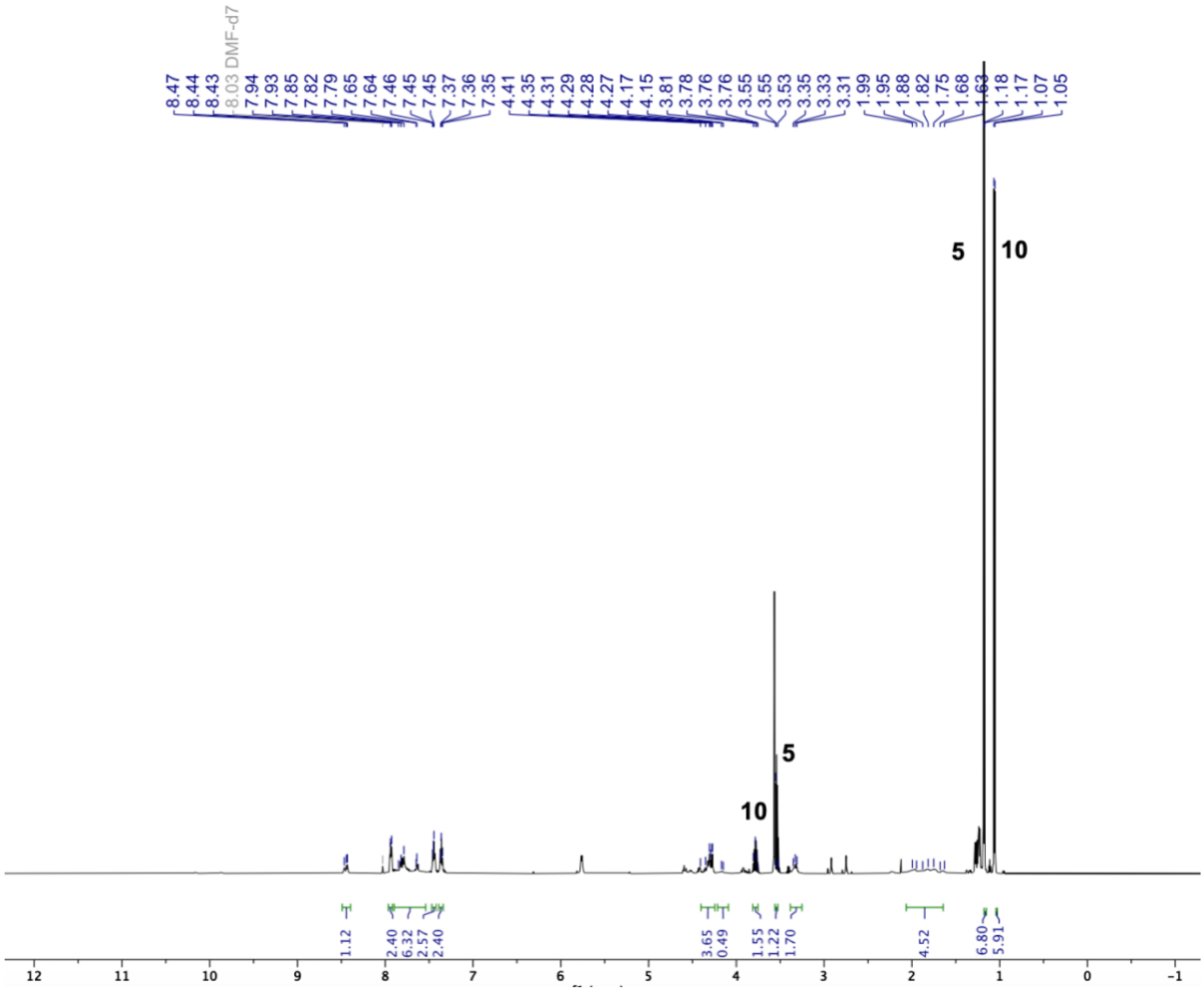


Figure S39. ^{1}H NMR spectrum of Fmoc-Arg(HCl)-OH and 1.0 eq of DIC to form the anhydride

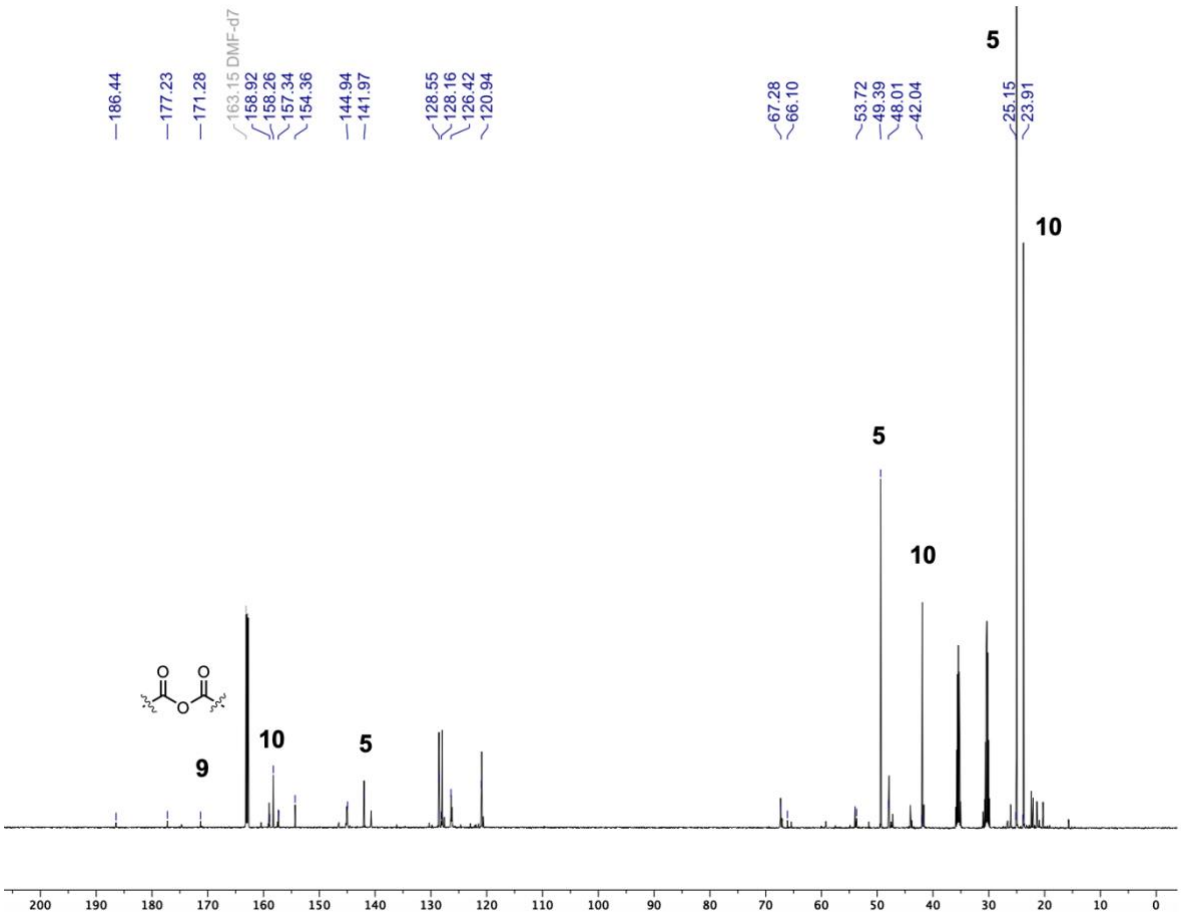


Figure S40. ^{13}C NMR spectrum of Fmoc-Arg(HCl)-OH and 1.0 eq of DIC to form the anhydride

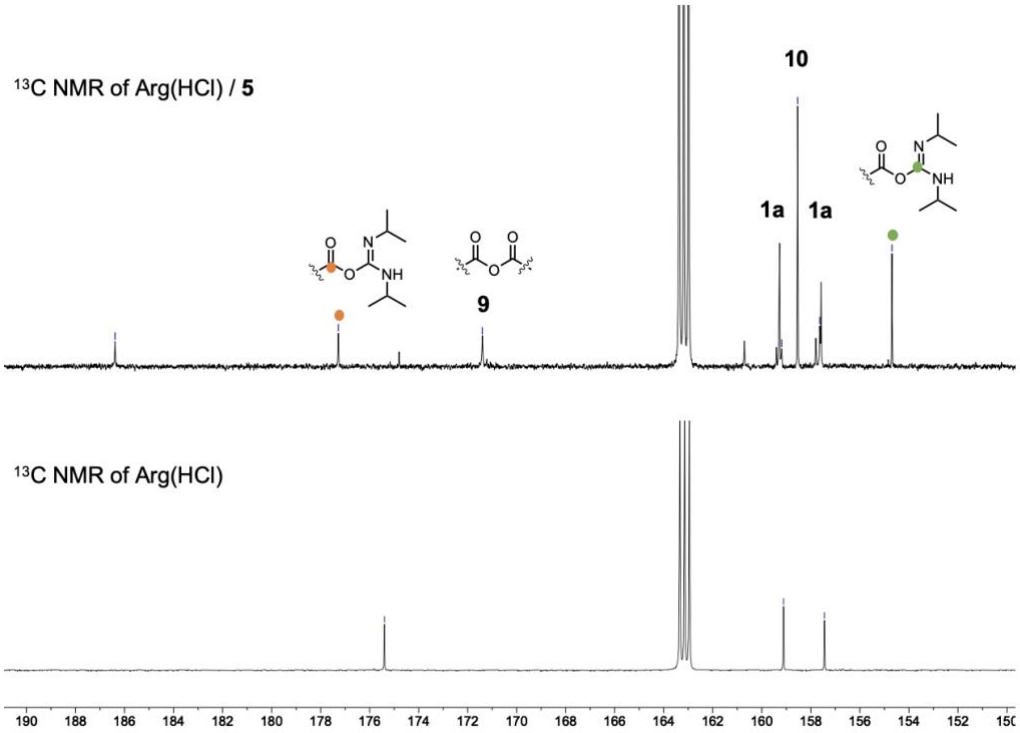


Figure S41. ^{13}C NMR spectrum of Fmoc-Arg(HCl)-OH and 1.0 eq of DIC to form the anhydride

2. Terminal arginine coupling agent efficiency

General procedure

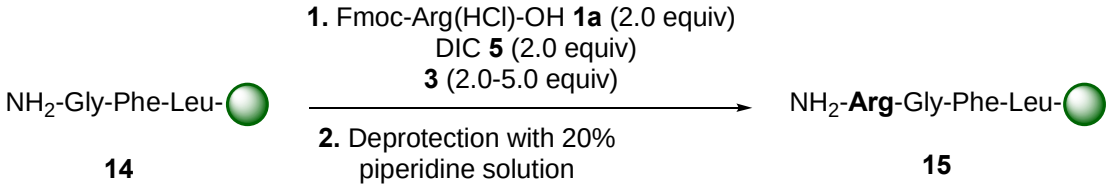


Table S1. Evaluation of the coupling agent efficiency with terminal arginine

Entry[a]	Auxiliary (equiv)	Solvent	pKa	15/14[b]
1	**3a** (2)	DMF	4.6	>99/1
2	**3a** (5)	DMF	4.6	>99/1
3	**3b** (5)	DMF	4.6	>99/1
4	**3c** (5)	DMF	3.3	>99/1
5	**3d** (5)	DMF	8.2	87/13
6	**3a** (5)	NMP	8.2	>99/1
7	**3a** (5)	DMSO/AcOEt 1/9	4.6	83/17
8	**3a** (5)	NOP/DMC 8/2	4.6	97/3
9	**3a** (5)	NBP/DMC 8/2	4.6	>99/1
10	**3a** (5)	NBP/AcOEt 8/2	4.6	>99/1

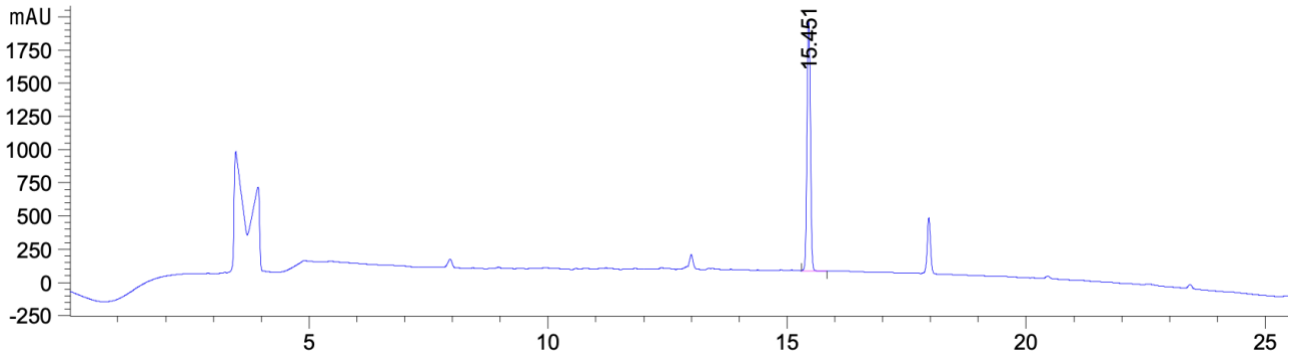
[a]**1a**/**3**/**5** in a 2/X/2 respect to **14** were dissolved in the solvent and after 5 min activation added to the resin at room temperature. [b]Determined by HPLC-MS after cleavage and precipitation.

The synthesis was carried out by using Gly-Phe-Leu-CTC resin (200 mg, loading 0.7 mmol g^{-1}). After swelling of the resin in 2 mL of the selected solvent, Fmoc-L-Arg(HCl)-OH (2.0 eq, 120.1 mg, 0.28 mmol) and the coupling additive (2.0-5.0 eq, 0.28-0.70 mmol) were diluted in 1 mL of the selected solvent and preactivated by DIC (2.0 eq, 43.5 µL , 0.28 mmol) for 5 min, added to the resin and stirred at room temperature. After 90 min, 5 mg of the resin was taken, washed with DCM to perform the mini cleavage to evaluate the conversion into Fmoc-Arg-Gly-Phe-Leu-OH. The dry peptide resin was suspended in 0.25 mL of the mixture TFA/H_2O (95.0/5.0 v/v), stirred for 2 h, filtered and the conversion was monitored by HPLC-MS analysis (Analytical Method 1).

At this point, the Fmoc protective group was removed by treating the peptide resin with a 20% piperidine solution in the solvent (2 × 1 mL, 15 min each) and the resin was washed with the solvent (3 × 1 mL).

After the Fmoc-cleavage of the N-terminal alpha-amino group, the peptide resin was washed with the selected solvent (3 × 1 mL) and DCM (3 × 1 mL). The dry peptide resin was suspended in 2 mL of the mixture TFA/H_2O (95.0/5.0 v/v) and stirred for 2 h. The resin was filtered off and diisopropylether (10 mL) cooled to 4 °C was added to the solution. The peptide was filtered and dried in vacuo to obtain crude H-Arg-Gly-Phe-Leu-OH and the ratio **14/15** monitored by HPLC-MS (Analytical Method 1).

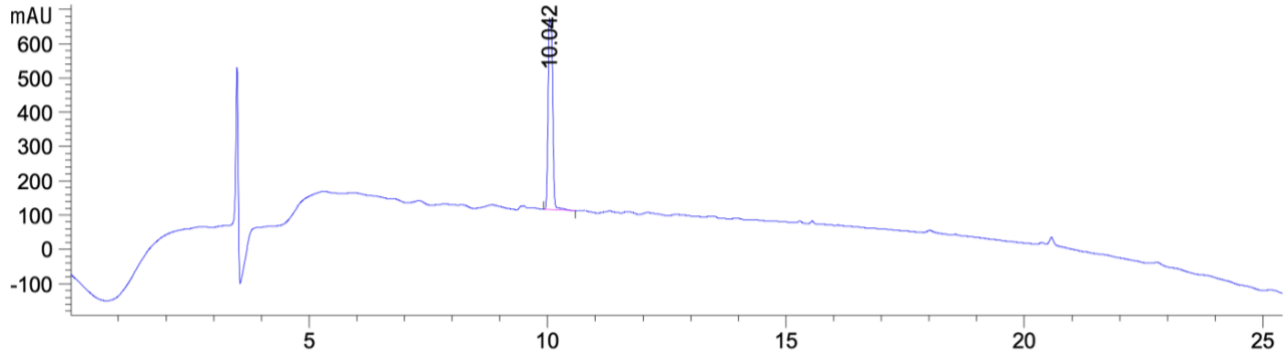
Entry 1, Table S1



Product	m/z observed	Rt (min)	Area (%)
Fmoc-R-G-F-L	714.5 $[M+H]^+$	15.451	>99
G-F-L	336.3 $[M+H]^+$	10.739	n.d

Figure S42. Chromatogram after the mini cleavage of Fmoc-Arg-Gly-Phe-Leu-OH synthesis in DMF with Oxyma pure/DIC 2/2 ratio (Entry 1, Table S1).

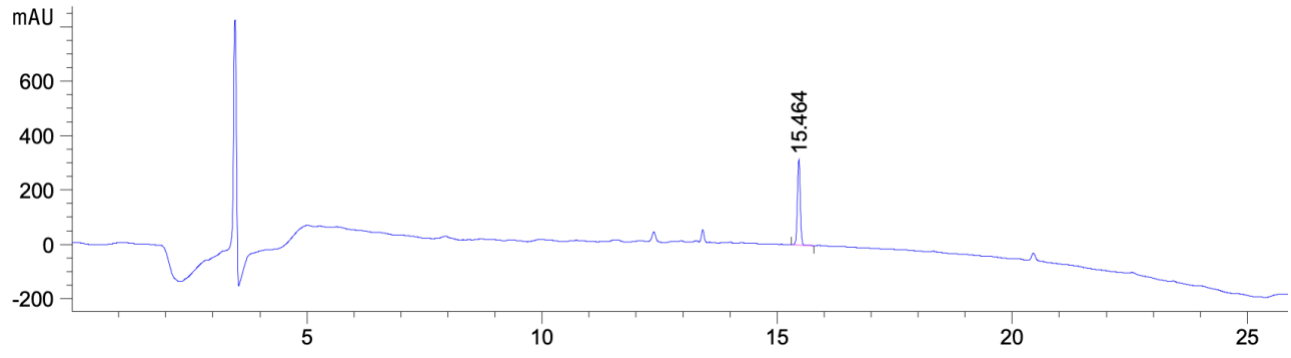
Entry 1, Table S1



Product	m/z observed	Rt (min)	Area (%)
R-G-F-L	492.2 $[M+H]^+$	10.042	>99
G-F-L	336.3 $[M+H]^+$	10.730	n.d

Figure S43. Chromatogram of H-Arg-Gly-Phe-Leu-OH synthesis in DMF with Oxyma pure/DIC 2/2 ratio (Entry 1, Table S1).

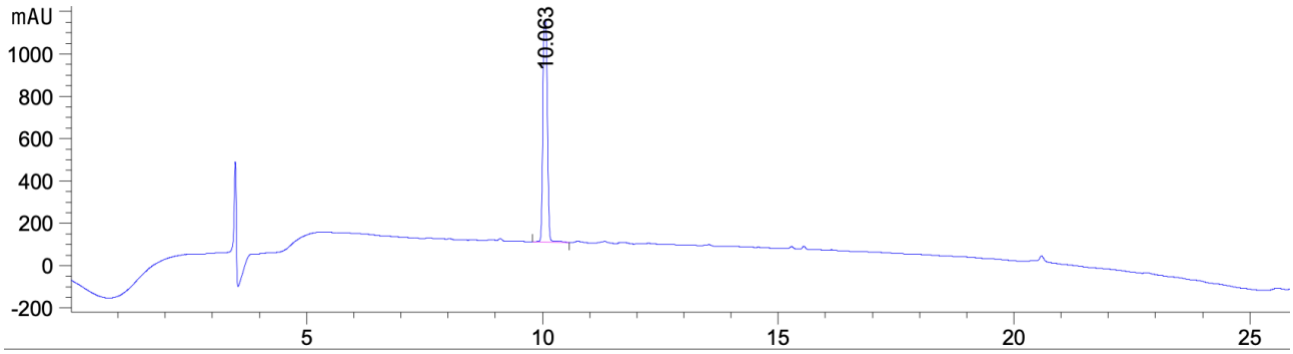
Entry 2, Table S1



Product	m/z observed	Rt (min)	Area (%)
Fmoc-R-G-F-L	714.5 $[M+H]^+$	15.464	>99
G-F-L	336.3 $[M+H]^+$	10.730	n.d

Figure S44. Chromatogram after the mini cleavage of Fmoc-Arg-Gly-Phe-Leu-OH synthesis in DMF with Oxyma pure/DIC 5/2 ratio (Entry 2, Table S1).

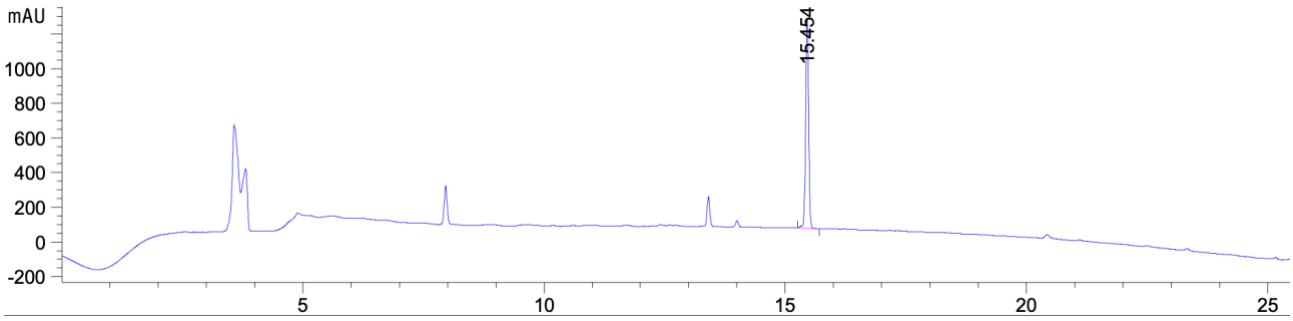
Entry 2, Table S1



Product	m/z observed	Rt (min)	Area (%)
R-G-F-L	492.2 $[M+H]^+$	10.063	>99
G-F-L	336.3 $[M+H]^+$	10.738	n.d

Figure S45. Chromatogram of H-Arg-Gly-Phe-Leu-OH synthesis in DMF with Oxyma pure/DIC 5/2 ratio (Entry 2, Table S1).

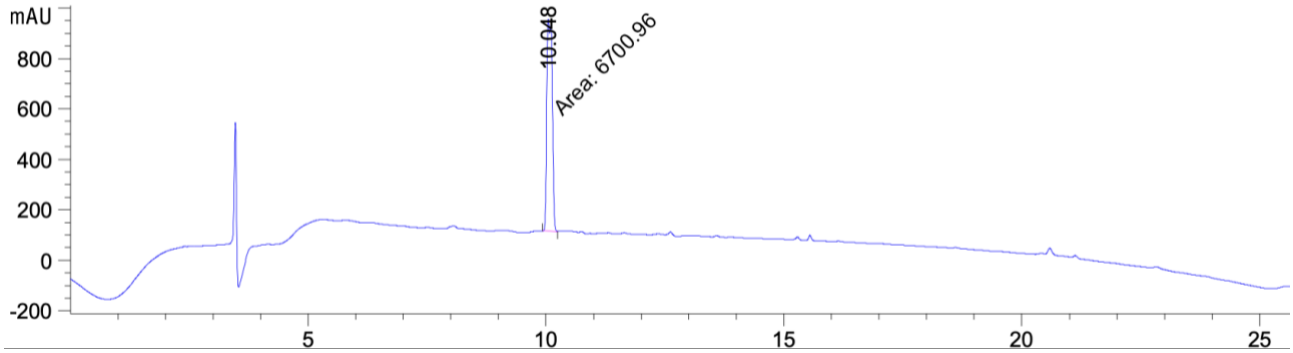
Entry 3, Table S1



Product	m/z observed	Rt (min)	Area (%)
Fmoc-R-G-F-L	714.5 $[M+H]^+$	15.454	>99
G-F-L	336.3 $[M+H]^+$	10.733	n.d

Figure S46. Chromatogram after the mini cleavage of Fmoc-Arg-Gly-Phe-Leu-OH synthesis in DMF with HOBt/DIC 5/2 ratio (Entry 3, Table S1).

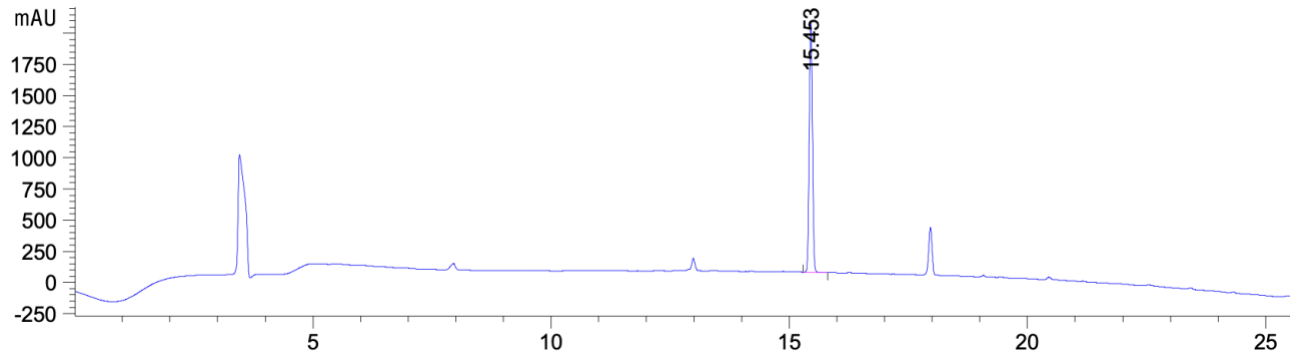
Entry 3, Table S1



Product	m/z observed	Rt (min)	Area (%)
R-G-F-L	492.2 $[M+H]^+$	10.048	>99
G-F-L	336.3 $[M+H]^+$	10.694	n.d

Figure S47. Chromatogram of H-Arg-Gly-Phe-Leu-OH synthesis in DMF with HOBt/DIC 5/2 ratio (Entry 3, Table S1).

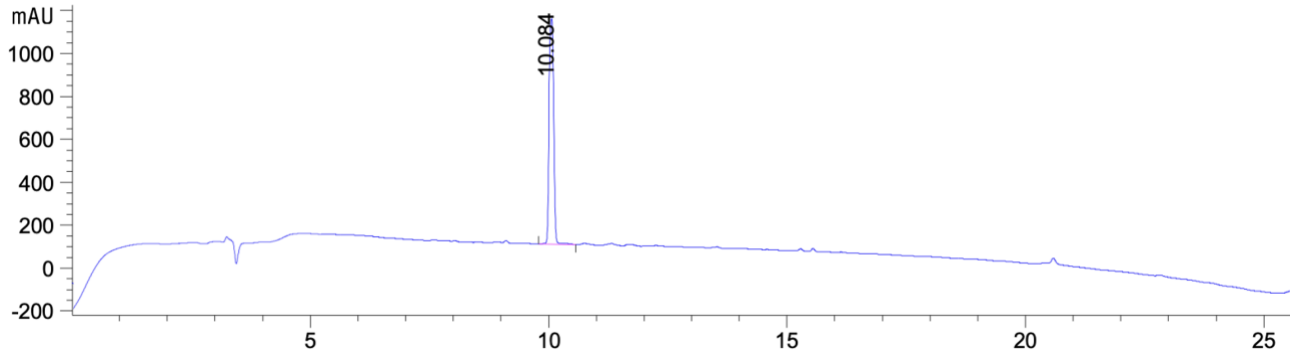
Entry 4, Table S1



Product	m/z observed	Rt (min)	Area (%)
Fmoc-R-G-F-L	714.5 $[M+H]^+$	15.453	>99
G-F-L	336.3 $[M+H]^+$	10.731	n.d

Figure S48. Chromatogram after the mini cleavage of Fmoc-Arg-Gly-Phe-Leu-OH synthesis in DMF with HOAt/DIC 5/2 ratio (Entry 4, Table S1).

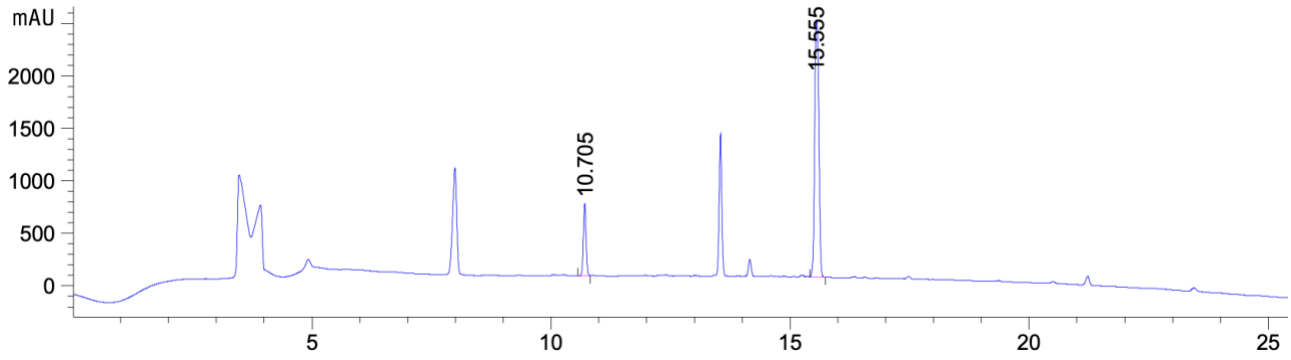
Entry 4, Table S1



Product	m/z observed	Rt (min)	Area (%)
R-G-F-L	492.2 $[M+H]^+$	10.084	>99
G-F-L	336.3 $[M+H]^+$	10.699	n.d

Figure S49. Chromatogram of H-Arg-Gly-Phe-Leu-OH synthesis in DMF with HOAt/DIC 5/2 ratio (Entry 4, Table S1).

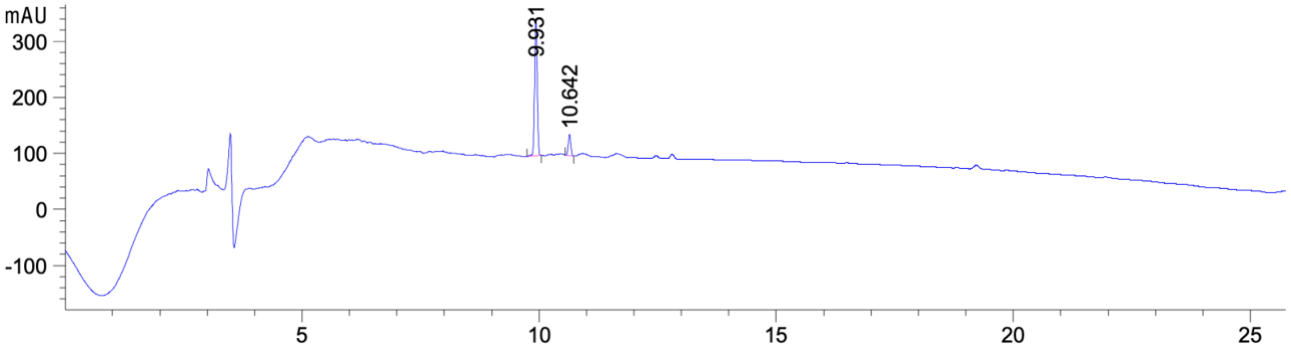
Entry 5, Table S1



Product	m/z observed	Rt (min)	Area (%)
Fmoc-R-G-F-L	714.5 $[M+H]^+$	15.555	87.2
G-F-L	336.3 $[M+H]^+$	10.705	12.8

Figure S50. Chromatogram after the mini cleavage of Fmoc-Arg-Gly-Phe-Leu-OH synthesis in DMF with Oxyma B/DIC 5/2 ratio (Entry 5, Table S1).

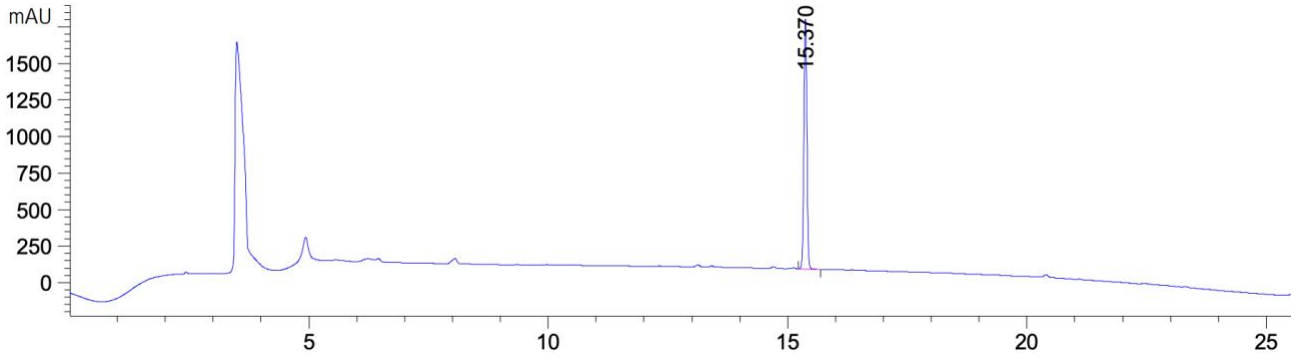
Entry 5, Table S1



Product	m/z observed	Rt (min)	Area (%)
R-G-F-L	492.2 $[M+H]^+$	9.931	86.4
G-F-L	336.3 $[M+H]^+$	10.642	13.4

Figure S51. Chromatogram of H-Arg-Gly-Phe-Leu-OH synthesis in DMF with Oxyma B/DIC 5/2 ratio (Entry 5, Table S1).

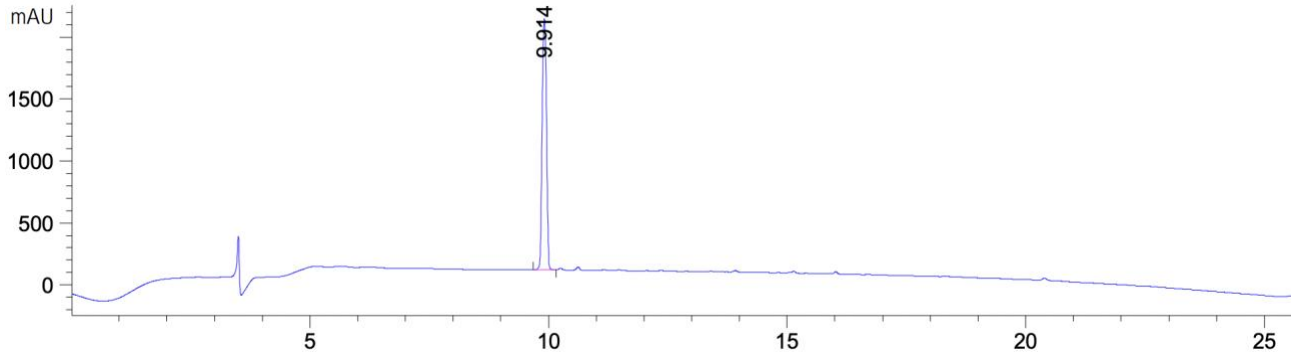
Entry 6, Table S1



Product	m/z observed	Rt (min)	Area (%)
Fmoc-R-G-F-L	714.5 $[M+H]^+$	15.370	>99
G-F-L	336.3 $[M+H]^+$	10.701	n.d

Figure S52. Chromatogram after the mini cleavage of Fmoc-Arg-Gly-Phe-Leu-OH synthesis in NMP with Oxyma Pure/DIC 5/2 ratio (Entry 6, Table S1).

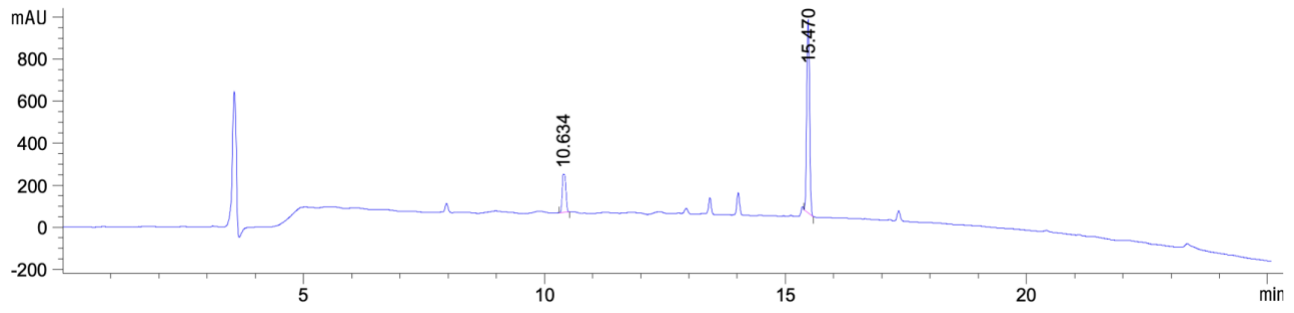
Entry 6, Table S1



Product	m/z observed	Rt (min)	Area (%)
R-G-F-L	492.2 $[M+H]^+$	9.914	>99
G-F-L	336.3 $[M+H]^+$	10.665	n.d

Figure S53. Chromatogram of H-Arg-Gly-Phe-Leu-OH synthesis in NMP with Oxyma Pure/DIC 5/2 ratio (Entry 6, Table S1).

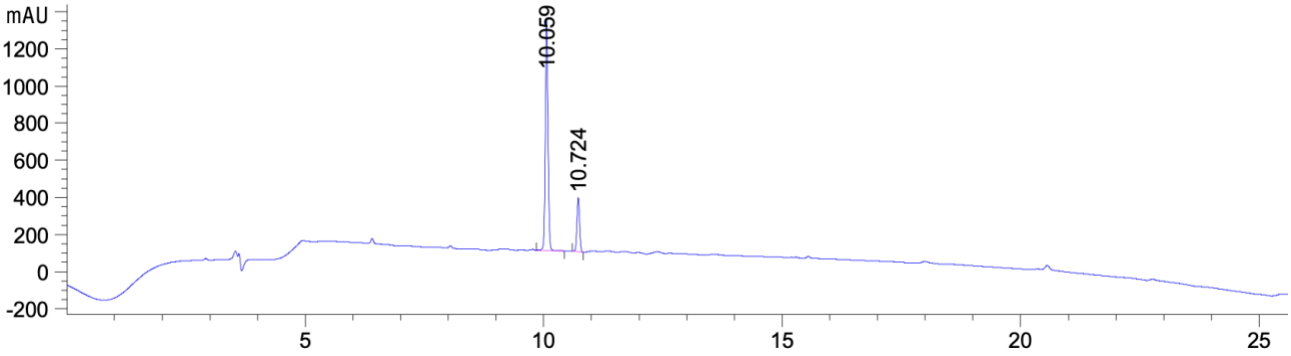
Entry 7, Table S1



Product	m/z observed	Rt (min)	Area (%)
Fmoc-R-G-F-L	714.5 $[M+H]^+$	15.470	84.2
G-F-L	336.3 $[M+H]^+$	10.634	13.8

Figure S54. Chromatogram after the mini cleavage of Fmoc-Arg-Gly-Phe-Leu-OH synthesis in DMSO/AcOEt 1:9 with Oxyma pure/DIC 5/2 ratio (Entry 7, Table S1).

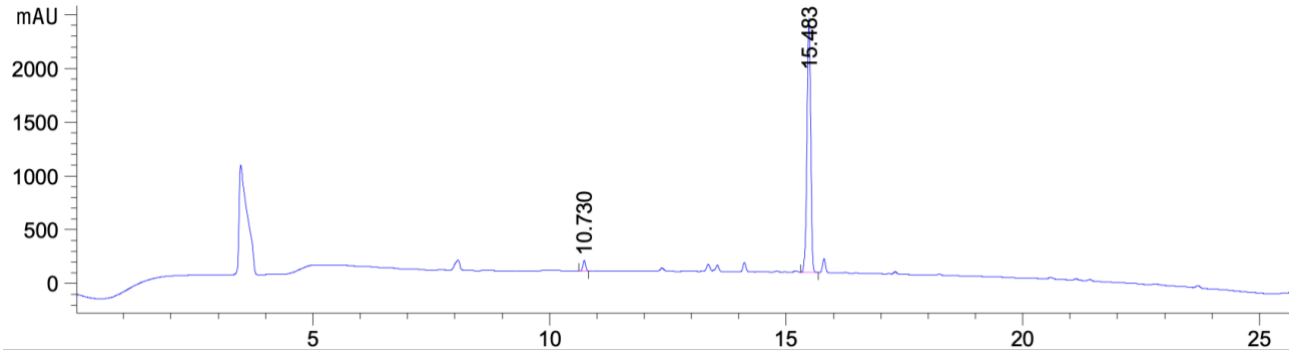
Entry 7, Table S1



Product	m/z observed	Rt (min)	Area (%)
R-G-F-L	492.2 $[M+H]^+$	10.059	83.1
G-F-L	336.3 $[M+H]^+$	10.724	16.9

Figure S55. Chromatogram of H-Arg-Gly-Phe-Leu-OH synthesis in DMSO/AcOEt 1:9 with Oxyma B/DIC 5/2 ratio (Entry 7, Table S1).

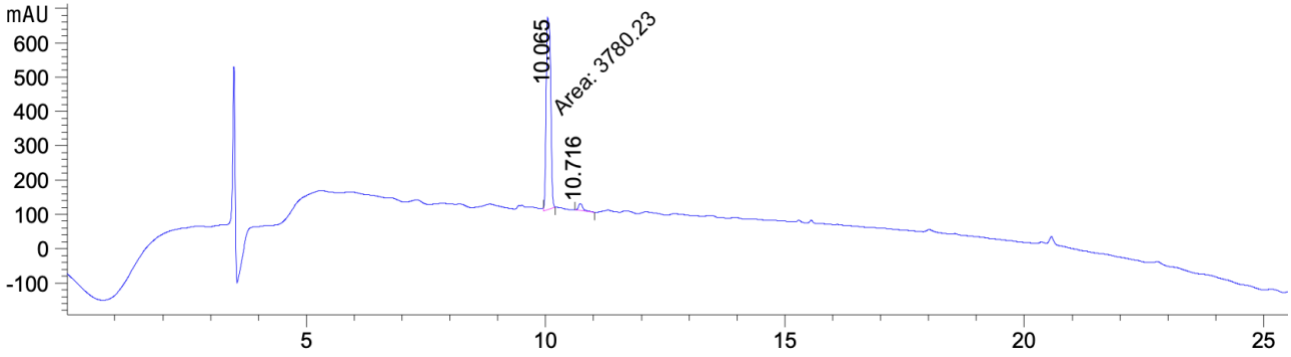
Entry 8, Table S1



Product	m/z observed	Rt (min)	Area (%)
Fmoc-R-G-F-L	714.5 $[M+H]^+$	15.483	97.2
G-F-L	336.3 $[M+H]^+$	10.730	2.8

Figure S56. Chromatogram after the mini cleavage of Fmoc-Arg-Gly-Phe-Leu-OH synthesis in NOP/DMC 8:2 with Oxyma pure/DIC 5/2 ratio (Entry 8, Table S1).

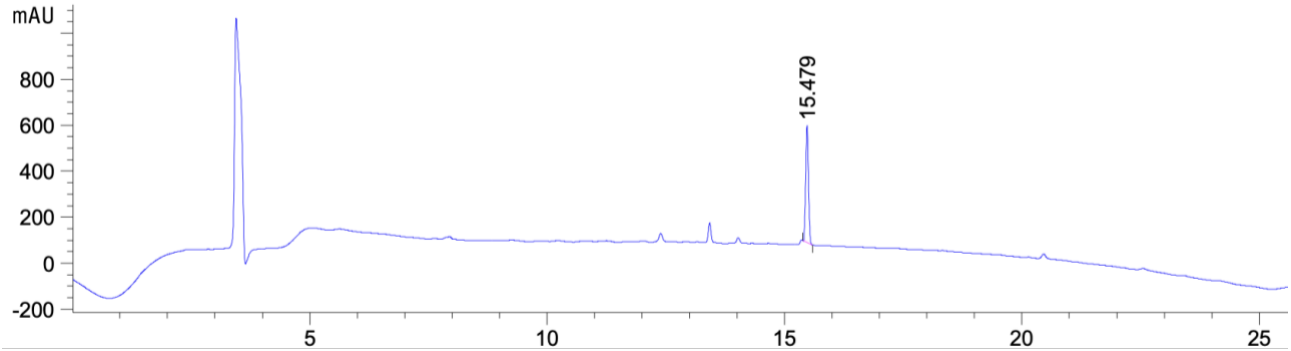
Entry 8, Table S1



Product	m/z observed	Rt (min)	Area (%)
R-G-F-L	492.2 $[M+H]^+$	10.065	96.8
G-F-L	336.3 $[M+H]^+$	10.716	3.2

Figure S57. Chromatogram of H-Arg-Gly-Phe-Leu-OH synthesis NOP/DMC 8:2 with Oxyma pure/DIC 5/2 ratio (Entry 8, Table S1).

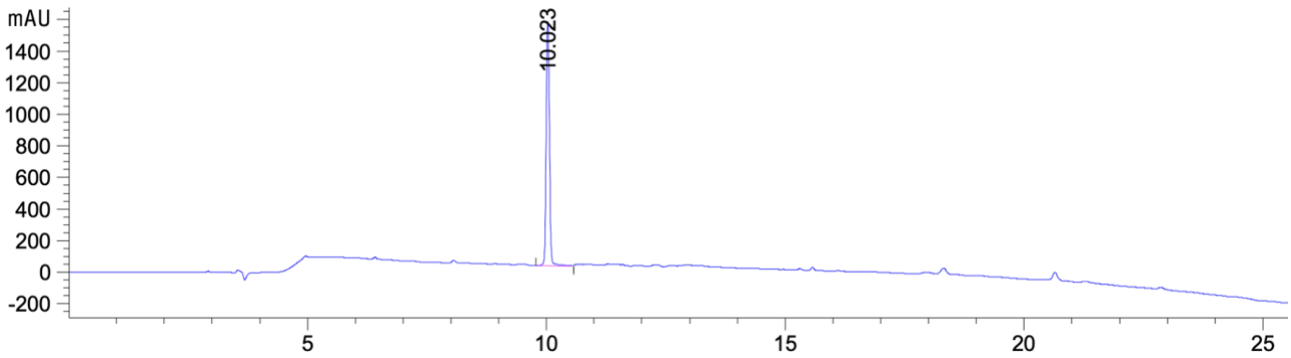
Entry 9, Table S1



Product	m/z observed	Rt (min)	Area (%)
Fmoc-R-G-F-L	714.5 $[M+H]^+$	15.479	>99
G-F-L	336.3 $[M+H]^+$	10.737	n.d

Figure S58. Chromatogram after the mini cleavage of Fmoc-Arg-Gly-Phe-Leu-OH synthesis in NBP/DMC 8:2 with Oxyma pure/DIC 5/2 ratio (Entry 9, Table S1).

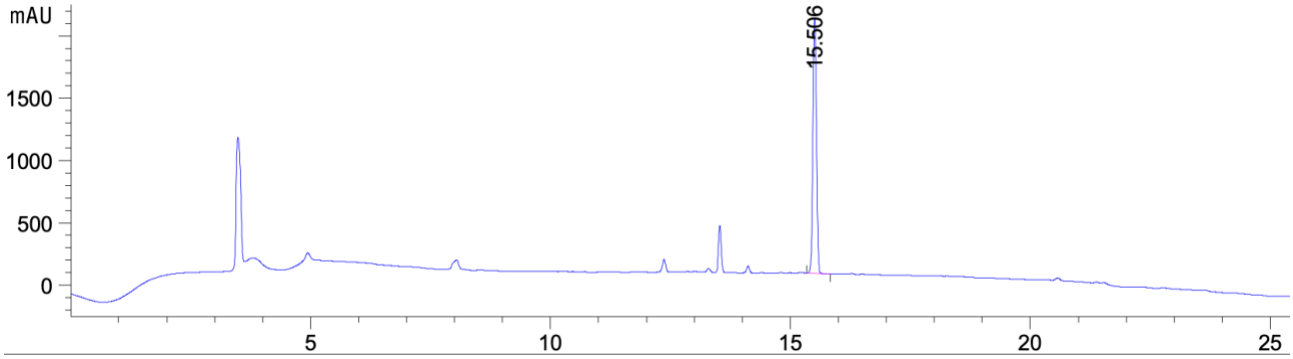
Entry 9, Table S1



Product	m/z observed	Rt (min)	Area (%)
R-G-F-L	492.2 $[M+H]^+$	10.023	>99
G-F-L	336.3 $[M+H]^+$	10.692	n.d

Figure S59. Chromatogram of H-Arg-Gly-Phe-Leu-OH synthesis NBP/DMC 8:2 with Oxyma pure/DIC 5/2 ratio (Entry 9, Table S1).

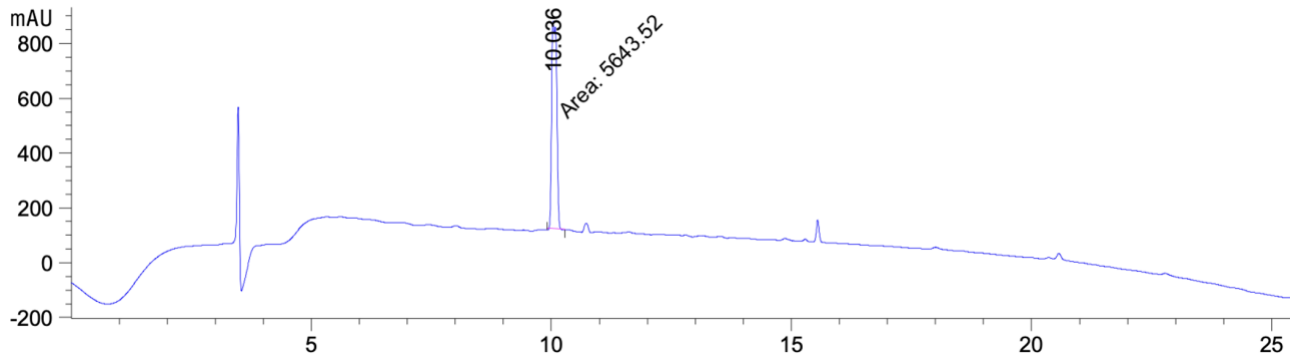
Entry 10, Table S1



Product	m/z observed	Rt (min)	Area (%)
Fmoc-R-G-F-L	714.5 $[M+H]^+$	15.506	>99
G-F-L	336.3 $[M+H]^+$	10.735	n.d

Figure S60. Chromatogram after the mini cleavage of Fmoc-Arg-Gly-Phe-Leu-OH synthesis in NBP/AcOEt 8:2 with Oxyma pure/DIC 5/2 ratio (Entry 10, Table S1).

Entry 10, Table S1



Product	m/z observed	Rt (min)	Area (%)
R-G-F-L	492.2 $[M+H]^+$	10.036	99.1
G-F-L	336.3 $[M+H]^+$	10.721	0.9

Figure S61. Chromatogram of H-Arg-Gly-Phe-Leu-OH synthesis NBP/AcOEt 8:2 with Oxyma pure/DIC 5/2 ratio (Entry 10, Table S1).

3. Coupling efficiency with arginine as penultimate amino acid

General procedure

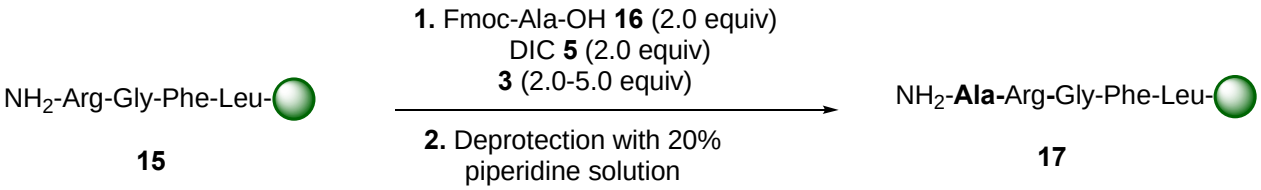


Table S2. Evaluation of the coupling efficiency with arginine as penultimate amino acid

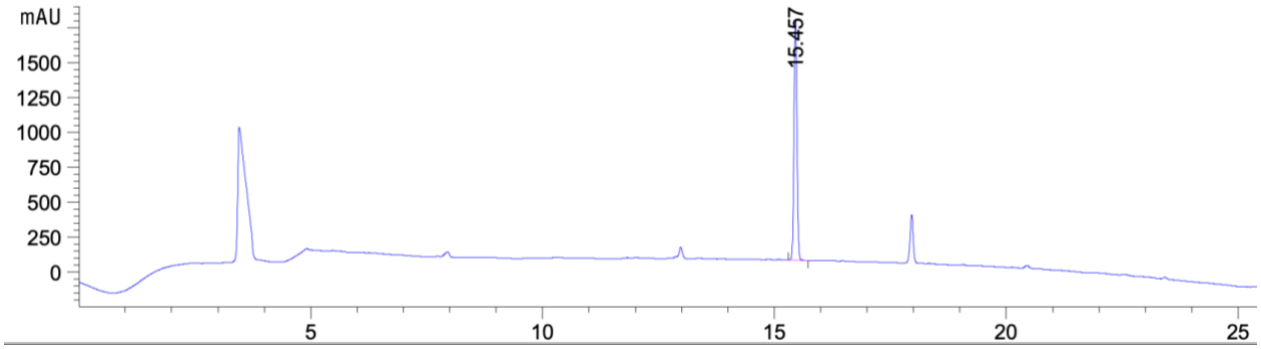
Entry[a]	Auxiliary (equiv)	Solvent	15/14[b]
1	**3** (2)	DMF	>99/1
2	**3** (5)	DMF	>99/1
3	**3** (2)	NBP/DMC 8:2	>99/1
4	**3** (2)	NBP/AcOEt 8:2	>99/1

[a]**16**/**3**/**5** in a 2/X/2 respect to **15** were dissolved in the solvent and after 5 min activation added to the resin at room temperature. [b]Determined by HPLC after cleavage and precipitation.

The synthesis of **15** was carried out according to procedure in Table S1 by using Gly-Phe-Leu-CTC (200 mg, loading 0.7 mmol g^{-1}). After the Fmoc removal by treating the peptide resin with a 20% piperidine solution in the selected solvent (2 × 1 mL, 15 min each), the resin was washed with the solvent (3 × 1 mL) and Fmoc-L-Ala-OH (2.0 eq, 87.0 mg, 0.28 mmol) and Oxyma pure (2.0-5.0 eq, 0.28-0.70 mmol) were diluted in 1 mL of the selected solvent, preactivated by DIC (2.0 eq, 43.5 µL , 0.28 mmol) for 5 min and added to the resin and stirred at room temperature. After 90 min, 5 mg of the resin was taken, washed with DCM to perform the mini cleavage to evaluate the conversion into Fmoc-Ala-Arg-Gly-Phe-Leu-OH. The dry peptide resin was suspended in 0.25 mL of the mixture TFA/H_2O (95.0/5.0 v/v), stirred for 2 h, filtered and the conversion was monitored by HPLC-MS analysis (Analytical Method 1).

At this point, the Fmoc protective group was removed by treating the peptide resin with a 20% piperidine solution in the solvent (2 × 2 mL, 15 min each) and the resin was washed with the selected solvent (3 × 1 mL). After the Fmoc-cleavage of the N-terminal alpha-amino group, the peptide resin was washed with the selected solvent (3 × 2 mL) and DCM (3 × 2 mL). The dry peptide resin was suspended in 2 mL of the mixture TFA/H_2O (95.0/5.0 v/v) and stirred for 2 h. The resin was filtered off and diisopropylether (10 mL) cooled to 4 °C was added to the solution. The peptide was filtered and dried in vacuo to obtain crude H-Ala Arg-Gly-Phe-Leu-OH and the ratio **15/17** monitored by HPLC (Analytical Method 1).

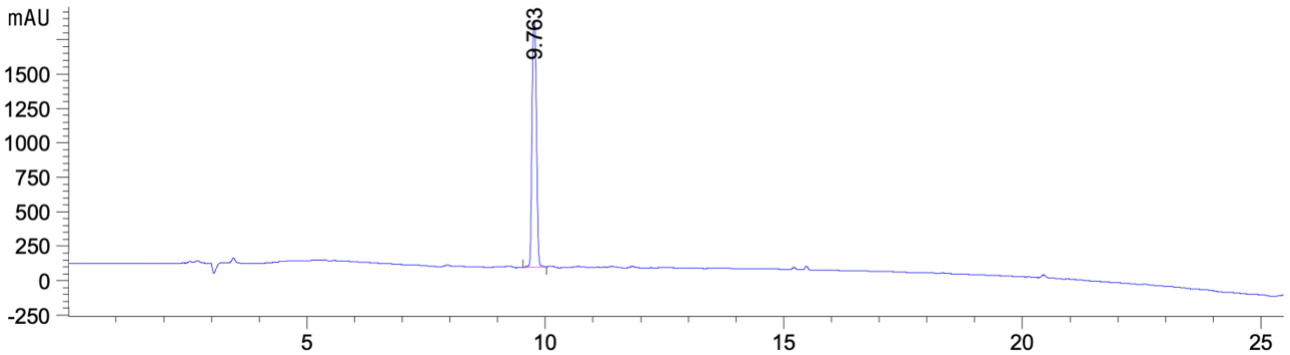
Entry 1, Table S2



Product	m/z observed	Rt (min)	Area (%)
Fmoc-A-R-G-F-L	785.2 $[M+H]^+$	15.457	>99
R-G-F-L	492.2 $[M+H]^+$	10.021	n.d

Figure S62. Chromatogram after the mini cleavage of Fmoc-Ala-Arg-Gly-Phe-Leu-OH synthesis in DMF with Oxyma pure/DIC 5/2 ratio (Entry 1, Table S2).

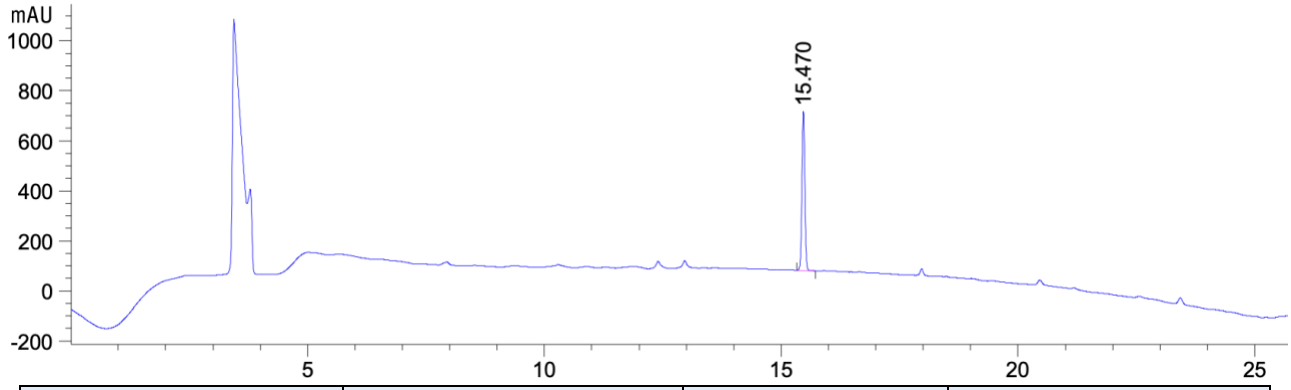
Entry 1, Table S2



Product	m/z observed	Rt (min)	Area (%)
A-R-G-F-L	563.6 $[M+H]^+$	9.763	>99
R-G-F-L	492.2 $[M+H]^+$	10.032	n.d

Figure S63. Chromatogram of H-Ala-Arg-Gly-Phe-Leu-OH synthesis in DMF with Oxyma pure/DIC 5/2 ratio (Entry 1, Table S2).

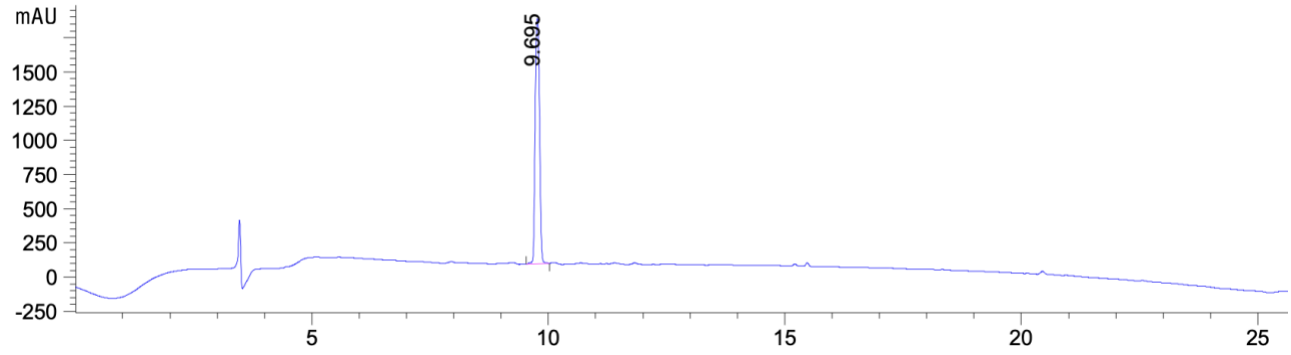
Entry 2, Table S2



Product	m/z observed	Rt (min)	Area (%)
Fmoc-A-R-G-F-L	785.2 $[M+H]^+$	15.470	>99
R-G-F-L	492.2 $[M+H]^+$	10.024	n.d

Figure S64. Chromatogram after the mini cleavage of Fmoc-Ala-Arg-Gly-Phe-Leu-OH synthesis in DMF with Oxyma pure/DIC 2/2 ratio (Entry 2, Table S2).

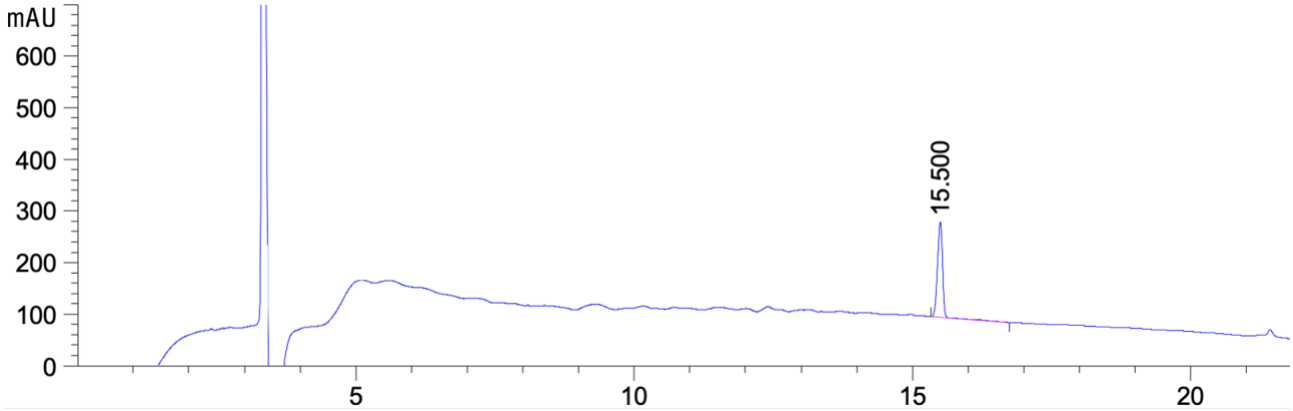
Entry 2, Table S2



Product	m/z observed	Rt (min)	Area (%)
A-R-G-F-L	563.6 $[M+H]^+$	9.695	>99
R-G-F-L	492.2 $[M+H]^+$	10.019	n.d

Figure S65. Chromatogram of H-Ala-Arg-Gly-Phe-Leu-OH synthesis in DMF with Oxyma pure/DIC 2/2 ratio (Entry 2, Table S2).

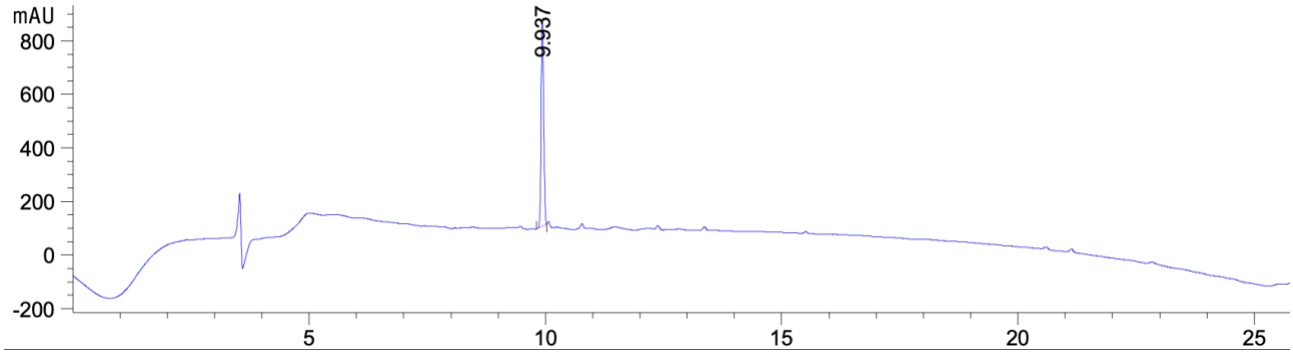
Entry 3, Table S2



Product	m/z observed	Rt (min)	Area (%)
Fmoc-A-R-G-F-L	785.2 $[M+H]^+$	15.500	>99
R-G-F-L	492.2 $[M+H]^+$	10.026	n.d

Figure S66. Chromatogram after the mini cleavage of Fmoc-Ala-Arg-Gly-Phe-Leu-OH synthesis in NBP/DMC 8:2 with Oxyma pure/DIC 2/2 ratio (Entry 3, Table S2).

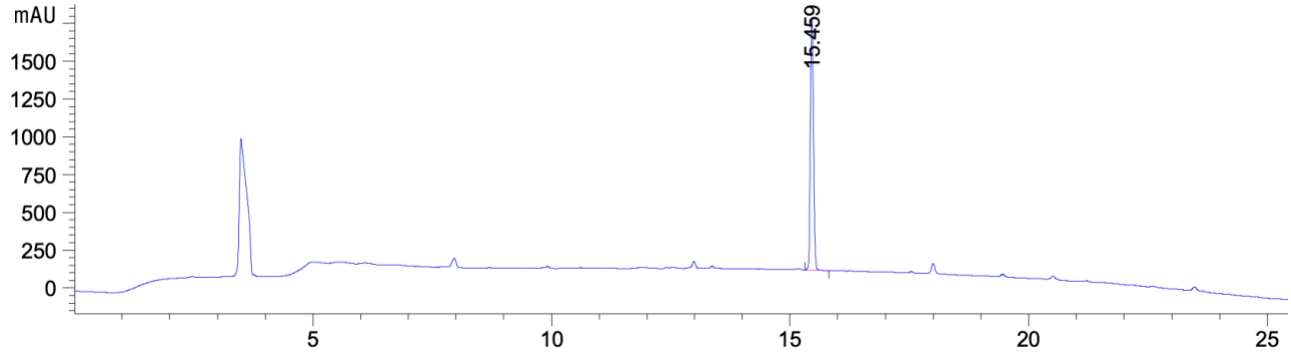
Entry 3, Table S2



Product	m/z observed	Rt (min)	Area (%)
A-R-G-F-L	563.6 $[M+H]^+$	9.937	99.1
R-G-F-L	492.2 $[M+H]^+$	10.029	0.9

Figure S67. Chromatogram of H-Ala-Arg-Gly-Phe-Leu-OH synthesis in NBP/DMC 8:2 with Oxyma pure/DIC 2/2 ratio (Entry 3, Table S2)

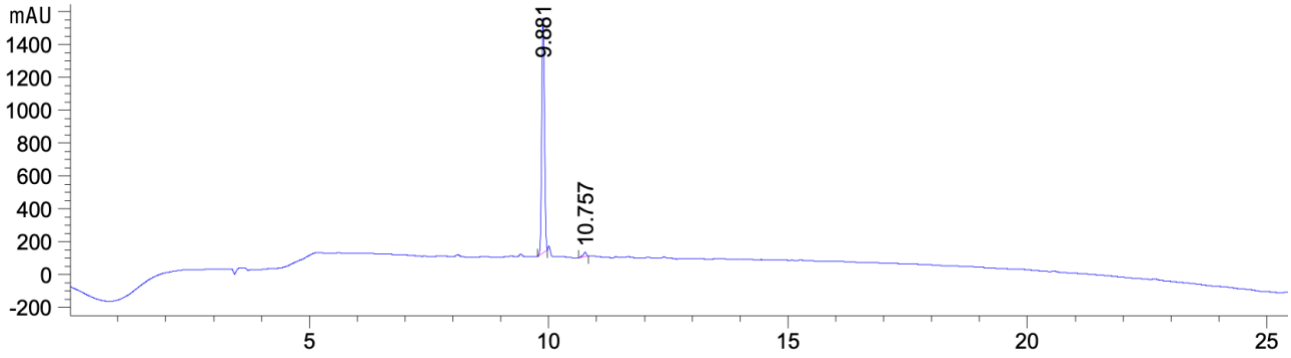
Entry 4, Table S2



Product	m/z observed	Rt (min)	Area (%)
Fmoc-A-R-G-F-L	785.2 $[M+H]^+$	15.459	>99
R-G-F-L	492.2 $[M+H]^+$	10.031	n.d

Figure S68. Chromatogram after the mini cleavage of Fmoc-Ala-Arg-Gly-Phe-Leu-OH synthesis in NBP/AcOEt 8:2 with Oxyma pure/DIC 2/2 ratio (Entry 4, Table S2).

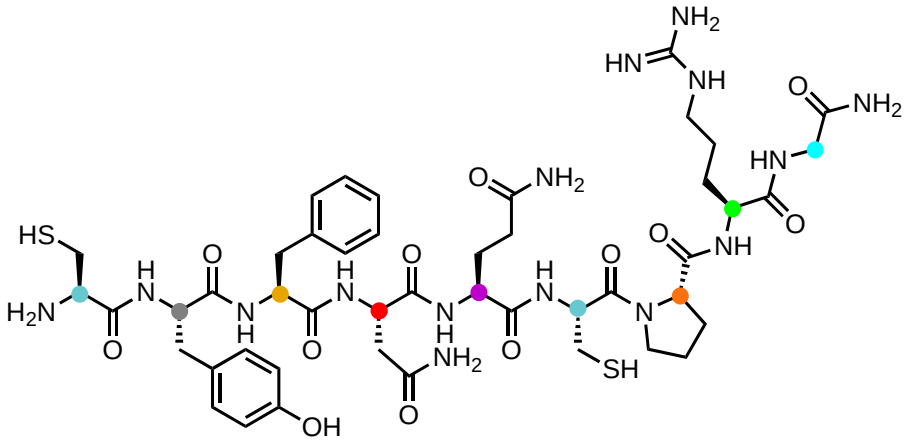
Entry 4, Table S2



Product	m/z observed	Rt (min)	Area (%)
A-R-G-F-L	563.6 $[M+H]^+$	9.881	99.0
R-G-F-L	492.2 $[M+H]^+$	10.028	1.0

Figure S69. Chromatogram of H-Ala-Arg-Gly-Phe-Leu-OH synthesis in NBP/AcOEt 8:2 with Oxyma pure/DIC 2/2 ratio (Entry 4, Table S2)

4. Synthesis of linear vasopressin.



Cys Tyr Phe Asn Gln Cys Pro Arg Gly

Solubility test

Table S3. Solubility test of the amino acids in different solvents.

AA	DMF	NOP/DMC 8:2	NBP/DMC 8:2	NBP/AcOEt 8:2	Anisole/DMC 8:2
Arg	YES	NO	YES	YES	NO
Pro	YES	YES	YES	YES	YES
Cys	YES	YES	YES	YES	YES
Asn	YES	NO	YES	YES	NO
Gln[a]	YES	NO	NO	NO	NO
Phe	YES	YES	YES	YES	YES
Tyr	YES	YES	YES	YES	YES
Cys	YES	YES	YES	YES	YES

[a]Soluble at 70°C after addition of **3a** and **5**

1 mL of the desired solvent was added to 0.2 mmol of the amino acid/coupling reagent and stirred at room temperature until a clear solution was observed.

General procedure

Table S4. Effect of oxyma pure **3a** excess in SPPS of linear vasopressin **18**

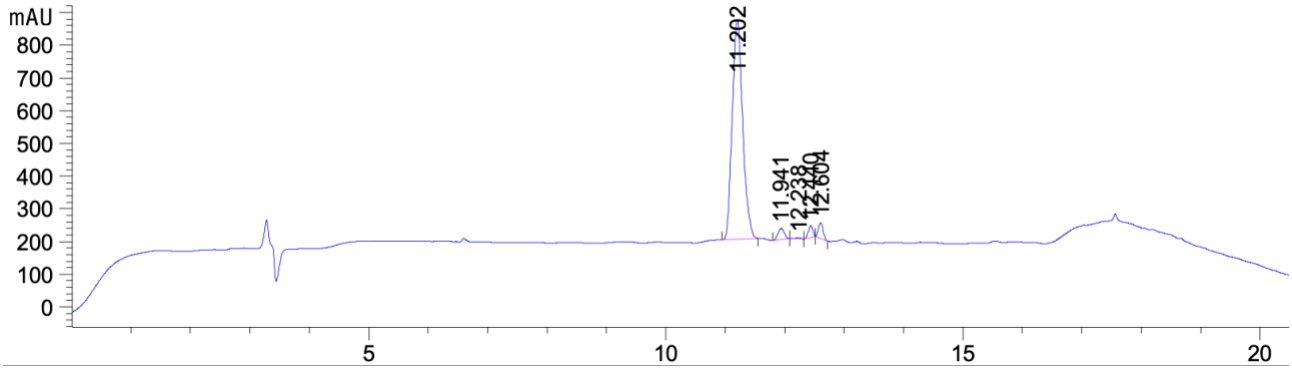
Entry	Solvent	Base	Purity %	Des %	23 %
1[a]	DMF	PIP	86.4	10.3	4.3
2	DMF	PIP	94.8	1.1	4.1
3	NBP/DMC 8:2	PIP	77.2	17.3	5.5
4[b]	NBP/DMC 8:2	PIP	92.5	2.6	4.9
5[b]	NBP/DMC 8:2	DEAPA	93.2	2.7	4.1

[a]Synthesis carried out using **1a**/**3a**/**5** in 2/2/2 ratio for all the couplings. [b]Syntheses carried out performing double coupling on Gln and Asn residues.

Linear vasopressin syntheses were carried out at room temperature in glass syringes fitted with a polyethylene porous disc and connected to a vacuum source to remove excess reagents and solvents, by using 0.3 g of preloaded Fmoc-Gly-Rink Amide resin (loading 0.5 mmol g^{-1}). After the swelling of the resin in 3.0 mL of the selected solvent, the Fmoc protective group was removed by 20% of base (PIP or DEAPA) in the selected solvent (2 times × 1.5 mL, 15 min each) and then the resin was washed with the solvent (3 times × 1.5 mL, 2 min each). Fmoc-Arg(HCl)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asn-OH, Fmoc-Gln-OH, Fmoc-Phe-OH and Fmoc-Tyr-OH (2.0 eq, 0.30 mmol) were pre-activated by Oxyma pure (2.0-5.0 eq, 0.30-0.75 mmol) and DIC (2.0 eq, 46.4 µL, 0.30 mmol) for 5 minutes and coupled to the resin. After each coupling step, the Fmoc protective group was removed by treating the peptide resin with 20% of base (PIP or DEAPA) in the selected solvent (2 times × 1.5 mL, 15 min each) and the resin was washed with the selected solvent (3 times × 1.5 mL, 2 min each). After the Fmoc cleavage of the N-terminal amino group, the peptide resin was further washed with DCM (3 times × 1.5 mL, 2 min each) and dried under vacuum for 12 hours. The dry peptide resin was suspended in 3 mL of the TFA/TIS/H_2O (95/2.5/2.5 v/v/v) mixture and stirred for 2 h. The resin was filtered off, washed with TFA (1 time × 1 mL, 1 min) and diisopropylether (10 mL) cooled to 4 °C was added to the solution dropwise. The peptide was filtered and dried in vacuo to obtain crude linear vasopressin **18** that was directly analyzed by HPLC-MS (Analytical Method 2).

Entry 1, Table S4

AA	Eq	Ac	Ad	Ratio AA/Ac/Ad	Reaction time h	Fmoc deprotection
Arg	2	DIC	Oxyma	1/1/1	2.5	20% PIP in DMF
Pro	2	DIC	Oxyma	1/1/1	1.5	20% PIP in DMF
Cys	2	DIC	Oxyma	1/1/1	1.5	20% PIP in DMF
Asn	2	DIC	Oxyma	1/1/1	1.5	20% PIP in DMF
Gln	2	DIC	Oxyma	1/1/1	1.5	20% PIP in DMF
Phe	2	DIC	Oxyma	1/1/1	1.5	20% PIP in DMF
Tyr	2	DIC	Oxyma	1/1/1	1.5	20% PIP in DMF
Cys	2	DIC	Oxyma	1/1/1	1.5	20% PIP in DMF

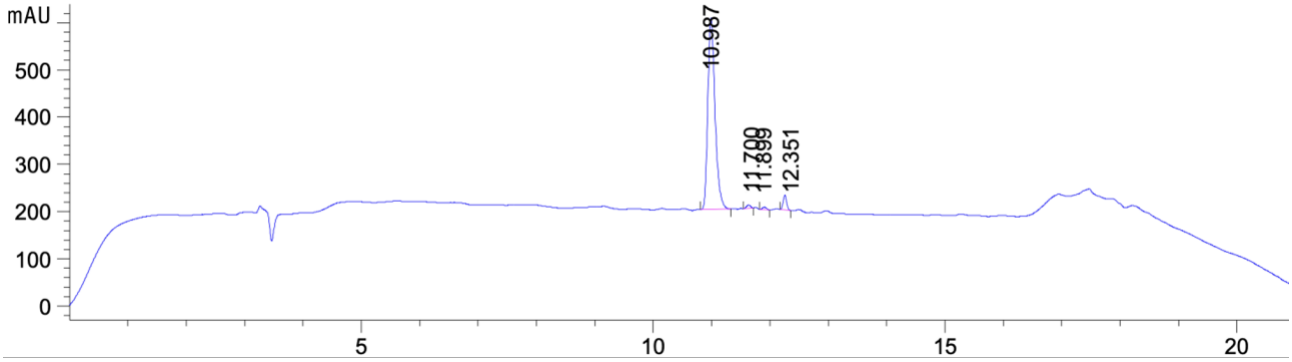


Product	m/z observed	Rt (min)	Area (%)
Target peptide	1087.4 $[M+H]^+$	11.202	86.4
Des-Gln	960.1 $[M+H]^+$	11.941	4.3
Des-Gln-Asn	845.9 $[M+H]^+$	12.238	0.9
Des-Asn	974.1 $[M+H]^+$	12.440	4.1
Aspartimide	1069.4 $[M+H]^+$	12.604	4.3

Figure S70. Chromatogram of linear vasopressin synthesis in DMF (Entry 1, Table S4)

Entry 2, Table S4

AA	Eq	Ac	Ad	Ratio AA/Ac/Ad	Reaction time h	Fmoc deprotection
Arg	2	DIC	Oxyma	1/1/2.5	2.5	20% PIP in DMF
Pro	2	DIC	Oxyma	1/1/2.5	1.5	20% PIP in DMF
Cys	2	DIC	Oxyma	1/1/2.5	1.5	20% PIP in DMF
Asn	2	DIC	Oxyma	1/1/2.5	1.5	20% PIP in DMF
Gln	2	DIC	Oxyma	1/1/2.5	1.5	20% PIP in DMF
Phe	2	DIC	Oxyma	1/1/2.5	1.5	20% PIP in DMF
Tyr	2	DIC	Oxyma	1/1/2.5	1.5	20% PIP in DMF
Cys	2	DIC	Oxyma	1/1/2.5	1.5	20% PIP in DMF

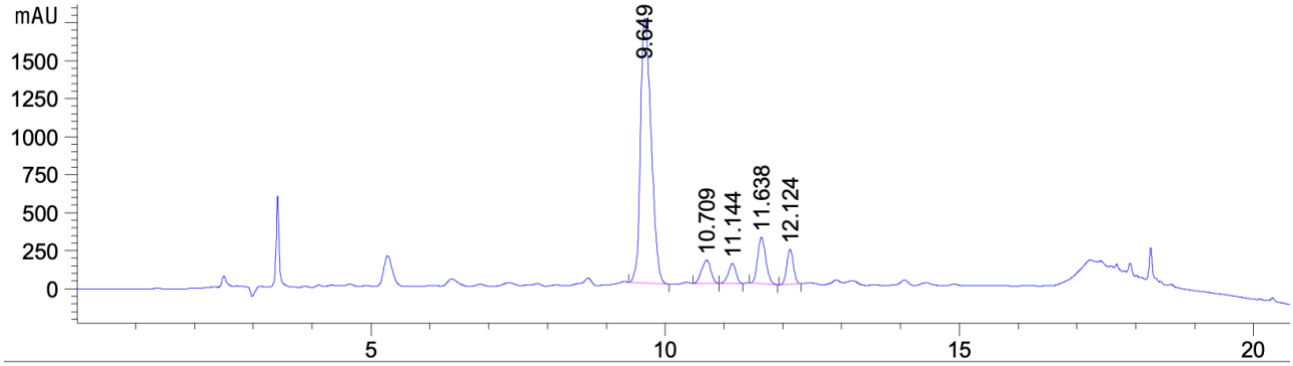


Product	m/z observed	Rt (min)	Area (%)
Target peptide	1087.4 $[M+H]^+$	10.987	94.8
Des-Gln	960.1 $[M+H]^+$	11.700	0.6
Des-Asn	974.1 $[M+H]^+$	11.899	0.5
Aspartimide	1069.4 $[M+H]^+$	12.351	4.1

Figure S71. Chromatogram of linear vasopressin synthesis in DMF (Entry 2, Table S4)

Entry 3, Table S4

AA	Eq	Ac	Ad	Ratio AA/Ac/Ad	Reaction time h	Fmoc deprotection
Arg	2	DIC	Oxyma	1/1/2.5	2.5	20% PIP in NBP/DMC
Pro	2	DIC	Oxyma	1/1/2.5	1.5	20% PIP in NBP/DMC
Cys	2	DIC	Oxyma	1/1/2.5	1.5	20% PIP in NBP/DMC
Asn	2	DIC	Oxyma	1/1/2.5	1.5	20% PIP in NBP/DMC
Gln	2	DIC	Oxyma	1/1/2.5	1.5	20% PIP in NBP/DMC
Phe	2	DIC	Oxyma	1/1/2.5	1.5	20% PIP in NBP/DMC
Tyr	2	DIC	Oxyma	1/1/2.5	1.5	20% PIP in NBP/DMC
Cys	2	DIC	Oxyma	1/1/2.5	1.5	20% PIP in NBP/DMC



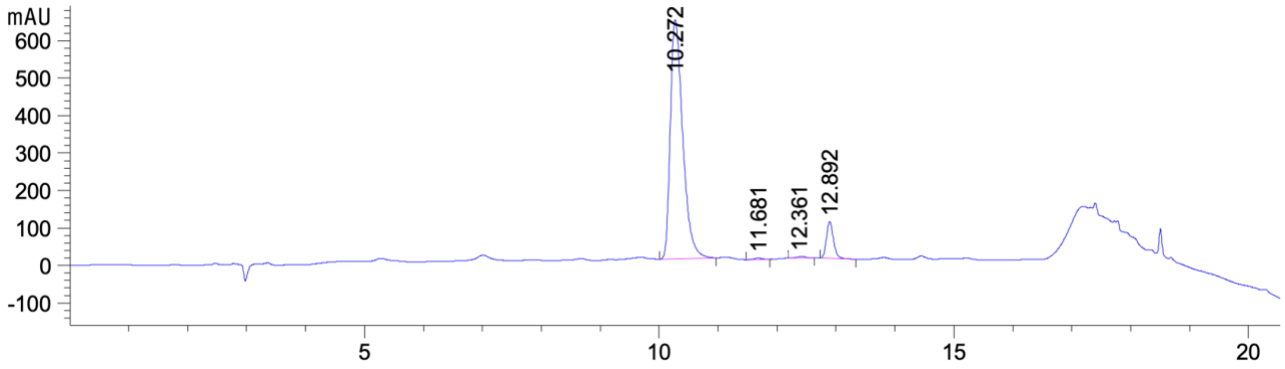
Product	m/z observed	Rt (min)	Area (%)
Target peptide	1087.4 $[M+H]^+$	9.649	72.2
Des-Gln	960.1 $[M+H]^+$	10.709	5.1
Des-Gln-Asn	845.9 $[M+H]^+$	11.144	4.7
Des-Asn	974.1 $[M+H]^+$	11.638	7.5
Aspartimide	1069.4 $[M+H]^+$	12.124	5.5

Figure S72. Chromatogram of linear vasopressin synthesis in NBP/DMC 8:2 (Entry 3, Table S4)

Entry 4, Table S4

AA	Eq	Ac	Ad	Ratio AA/Ac/Ad	Reaction time h	Fmoc deprotection
Arg	2	DIC	Oxyma	1/1/2.5	2.5	20% PIP in NBP/DMC
Pro	2	DIC	Oxyma	1/1/2.5	1.5	20% PIP in NBP/DMC
Cys	2	DIC	Oxyma	1/1/2.5	1.5	20% PIP in NBP/DMC
Asn	2	DIC	Oxyma	1/1/2.5 x 2	1.5	20% PIP in NBP/DMC
Gln[a]	2	DIC	Oxyma	1/1/2.5 x 2	1.5	20% PIP in NBP/DMC
Phe	2	DIC	Oxyma	1/1/2.5	1.5	20% PIP in NBP/DMC
Tyr	2	DIC	Oxyma	1/1/2.5	1.5	20% PIP in NBP/DMC
Cys	2	DIC	Oxyma	1/1/2.5	1.5	20% PIP in NBP/DMC

[a]Preactivation performed at 70°C for 5 minutes because of solubility problems.



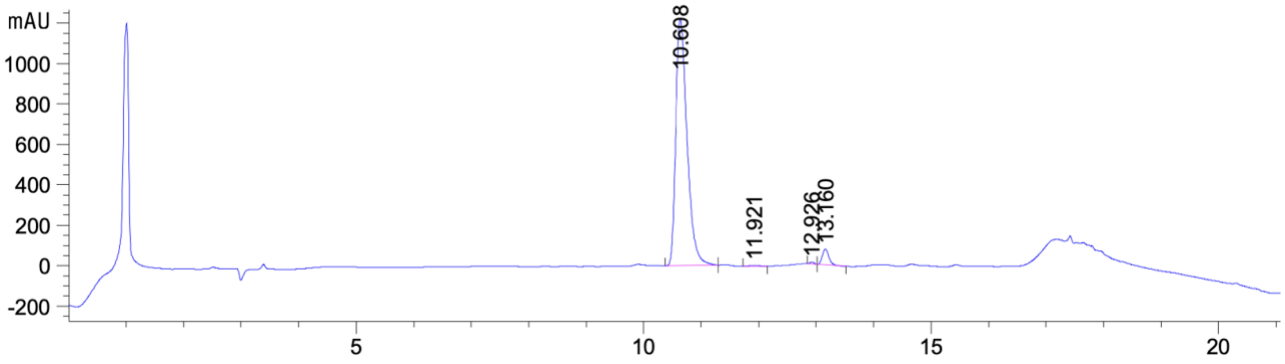
Product	m/z observed	Rt (min)	Area (%)
Target peptide	1087.4 $[M+H]^+$	10.272	92.5
Des-Gln	960.1 $[M+H]^+$	11.681	1.2
Des-Asn	974.1 $[M+H]^+$	12.361	1.4
Aspartimide	1069.4 $[M+H]^+$	12.892	4.9

Figure S73. Chromatogram of linear vasopressin synthesis in NBP/DMC 8:2 (Entry 4, Table S4)

Entry 5, Table S4

AA	Eq	Ac	Ad	Ratio AA/Ac/Ad	Reaction time h	Fmoc deprotection
Arg	2	DIC	Oxyma	1/1/2.5	1.5	20% DEAPA in NBP/DMC
Pro	2	DIC	Oxyma	1/1/2.5	1.5	20% DEAPA in NBP/DMC
Cys	2	DIC	Oxyma	1/1/2.5	1.5	20% DEAPA in NBP/DMC
Asn	2	DIC	Oxyma	1/1/2.5 x 2	1.5	20% DEAPA in NBP/DMC
Gln[a]	2	DIC	Oxyma	1/1/2.5 x 2	1.5	20% DEAPA in NBP/DMC
Phe	2	DIC	Oxyma	1/1/2.5	1.5	20% DEAPA in NBP/DMC
Tyr	2	DIC	Oxyma	1/1/2.5	1.5	20% DEAPA in NBP/DMC
Cys	2	DIC	Oxyma	1/1/2.5	1.5	20% DEAPA in NBP/DMC

[a]Preactivation performed at 70°C for 5 minutes because of solubility problems.

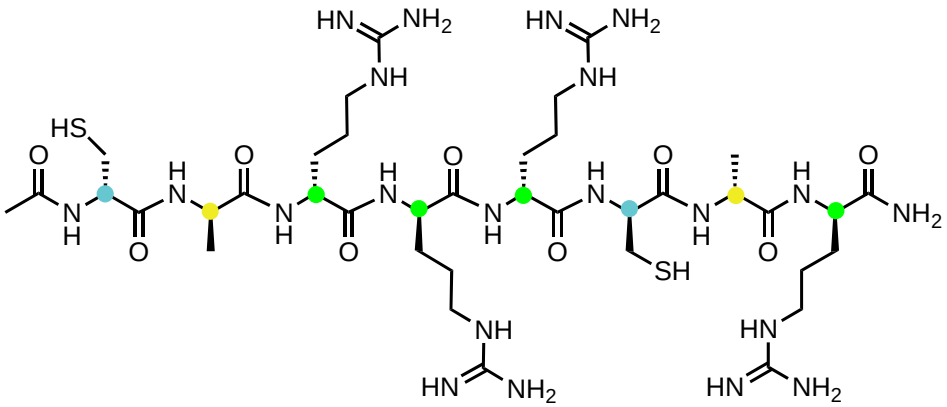


Product	m/z observed	Rt (min)	Area (%)
Target peptide	1087.4 $[M+H]^+$	10.608	93.2
Des-Gln	960.1 $[M+H]^+$	11.921	1.5
Des-Asn	974.1 $[M+H]^+$	12.926	1.2
Aspartimide	1069.4 $[M+H]^+$	13.160	4.1

Figure S74. Chromatogram of linear Vasopressin synthesis in NBP/DMC 8:2 (Entry 5, Table S4)

5. Synthesis of etelcalcetide-1.

General procedure



Ac-D-Cys D-Ala D-Arg D-Arg D-Arg D-Cys D-Ala D-Arg

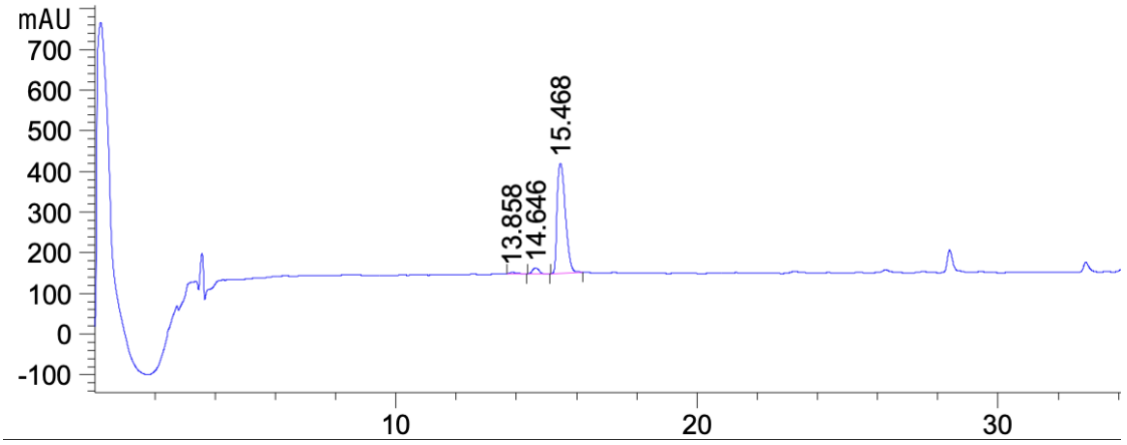
Table S5. Effect of oxyma pure **3a** excess in SPPS of Etecalcetide-1 **20**

Entry	Solvent	Base	Purity %	Des-Arg %
1	DMF	PIP	94.8	5.2
2	NBP/DMC 8:2	PIP	93.0	7.0

Etelcalcetide-1 syntheses were carried out at room temperature in glass syringes fitted with a polyethylene porous disc and connected to a vacuum source to remove excess reagents and solvents, by using 0.3 g of rink amide resin (loading 0.7 mmol g^{-1}). After the swelling of the resin in 3.0 mL of the selected solvent, the Fmoc protective group was removed by 20% Piperidine solution in the selected solvent (2 times × 1.5 mL, 15 min each) and then the resin was washed with the selected solvent (3 times × 1.5 mL, 2 min each). After the loading of the first Fmoc-Arg(HCl)-OH (2.0 eq, 180.1 mg, 0.42 mmol) the resin was washed with the selected solvent (3 times × 1.5 mL, 2 min each) and the capping of the resin was performed with a 1/1/1 solution of AcOH/DIC/Oxyma pure (5.0 eq, 1.05 mmol) for 1 h. The resin was then washed again with the selected solvent (3 times × 1.5 mL, 2 min each). Fmoc-D-Ala-OH, Fmoc-D-Arg(HCl)-OH and Fmoc-D-Cys(Trt)-OH (2.0 eq, 0.42 mmol) were pre-activated by Oxyma pure (2.0-5.0 eq, 0.42-1.05 mmol) and DIC (2.0 eq, 65.0 µL, 0.42 mmol) for 5 minutes and coupled to the resin. After each coupling step, the Fmoc protective group was removed by treating the peptide resin with 20% Piperidine solution in the selected solvent (2 times × 1.5 mL, 15 min each) and the resin was washed with the solvent (2 times × 1.5 mL, 2 min each) and with a 0.5 M solution of Oxyma pure (2 times × 1.5 mL, 2 min each). After the Fmoc cleavage of the N-terminal amino group, the last amino acid was acetylated after treating the resin with a solution of AcOH/DIC/Oxyma pure 1/1/1 (5.0 eq ,1.05 mmol) for 1 h. The peptide resin was then washed with the selected solvent (3 times × 1.5 mL,2 min each), with DCM (3 times × 1.5 mL, 2 min each) and dried under vacuum for 12 hours. The dry peptide resin was suspended in 3 mL of the TFA/TIS/H_2O (95/2.5/2.5 v/v/v) mixture and stirred for 2 h. The resin was filtered off, washed with TFA (1 time × 1 mL, 1 min) and diisopropylether (10 mL) cooled to 4 °C was added to the solution dropwise. The peptide was filtered and dried in vacuo to obtain crude etelcalcetide-1 **20** that was directly analyzed by HPLC-MS (Analytical Method 3).

Entry 1, Table S5

AA	Eq	Ac	Ad	Ratio AA/Ac/Ad	Reaction time h	Fmoc deprotection
Arg	2	DIC	Oxyma	1/1/2.5	2.5	20% PIP in DMF
Ala	2	DIC	Oxyma	1/1/1	1.5	20% PIP in DMF
Arg	2	DIC	Oxyma	1/1/2.5	2.5	20% PIP in DMF
Arg	2	DIC	Oxyma	1/1/2.5	2.5	20% PIP in DMF
Arg	2	DIC	Oxyma	1/1/2.5	2.5	20% PIP in DMF
Ala	2	DIC	Oxyma	1/1/1	1.5	20% PIP in DMF
Cys	2	DIC	Oxyma	1/1/1	1.5	20% PIP in DMF

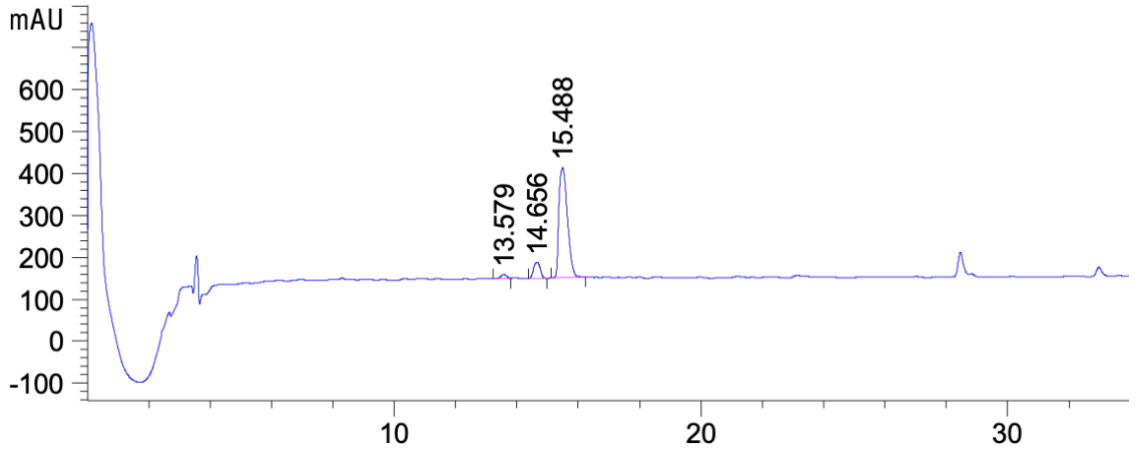


Product	m/z observed	Rt (min)	Area (%)
Target peptide	930.5 $[M+H]^+$	15.468	94.8
Des-Arg	774.4 $[M+H]^+$	14.646	4.6
Des-2Arg	618.3 $[M+H]^+$	13.858	0.6

Figure S75. Chromatogram of Etelcalcetide-1 synthesis in DMF (Entry 1, Table S5)

Entry 2, Table S5

AA	Eq	Ac	Ad	Ratio AA/Ac/Ad	Reaction time h	Fmoc deprotection
Arg	2	DIC	Oxyma	1/1/2.5	2.5	20% PIP in NBP/DMC
Ala	2	DIC	Oxyma	1/1/1	1.5	20% PIP in NBP/DMC
Arg	2	DIC	Oxyma	1/1/2.5	2.5	20% PIP in NBP/DMC
Arg	2	DIC	Oxyma	1/1/2.5 x 2	2.5	20% PIP in NBP/DMC
Arg	2	DIC	Oxyma	1/1/2.5 x 2	2.5	20% PIP in NBP/DMC
Ala	2	DIC	Oxyma	1/1/1	1.5	20% PIP in NBP/DMC
Cys	2	DIC	Oxyma	1/1/1	1.5	20% PIP in NBP/DMC



Product	m/z observed	Rt (min)	Area (%)
Target peptide	930.5 $[M+H]^+$	15.488	93.0
Des-Arg	774.4 $[M+H]^+$	14.656	5.9
Des-2Arg	618.3 $[M+H]^+$	13.579	1.1

Figure S76. Chromatogram of Etelcalcetide-1 synthesis in NBP/DMC 8:2 (Entry 2, Table S5)

6. DIC versus TBEC as coupling reagent with unprotected Histidine

General procedure

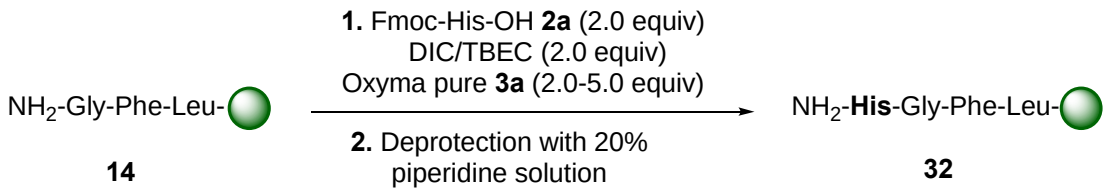


Table S6. Evaluation of the coupling efficiency with unprotected Histidine

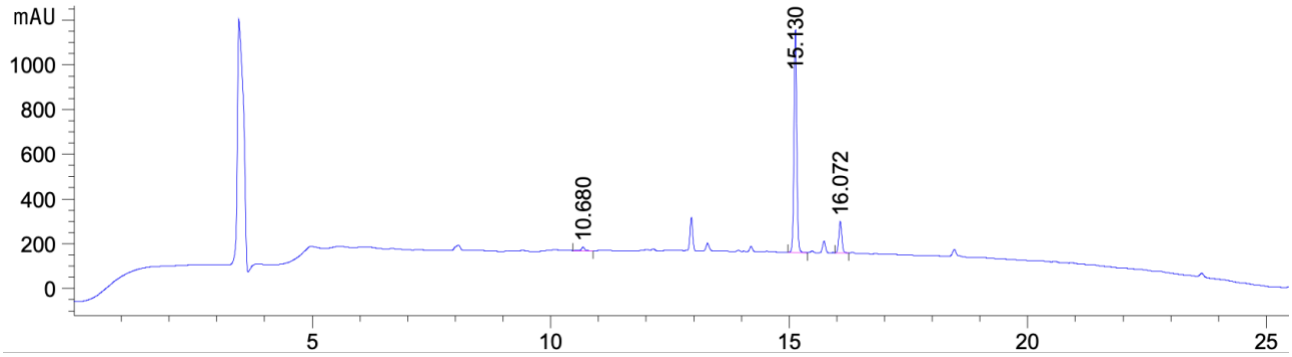
Entry[a]	Solvent	3a equiv.	RN=C=NR	Equiv.	Adduct %	14/32 %[b]	L/D-His %[c]
1	DMF	2	DIC	2	13	>99	99.4/0.6
2	DMF	5	DIC	2	16	>99	99.1/0.9
3	DMF	2	TBEC	2	n.d	>99	99.4/0.6
4	DMF	2	EDC	2	25	>99	98.9/1.1
5	DMF	2	DCC	2	n.d	>99	99.2/0.8
6	NBP/DMC 8:2	2	TBEC	2	n.d	>99	99.4/0.6
7	NBP/DMC 8:2	2	TBEC	20	1.3	>99	99.7/0.3

[a]**2a**/**3**/**Ac** in a 2/X/2 respect to **14** were dissolved in the solvent and after 15 min activation at 0°C added to the resin at room temperature. [b]Determined by HPLC after cleavage and precipitation. [c]Evaluated after the synthesis of the D analogue of **32**

The synthesis was carried out by using Gly-Phe-Leu-CTC resin (200 mg, loading 0.7 mmol g^{-1}). After swelling of the resin in 2 mL of the selected solvent, Fmoc-L-His-OH (2.0 eq, 105.6 mg, 0.28 mmol) and the Oxyma pure (2.0-5.0 eq, 0.28-0.70 mmol) were diluted in 1 mL of the selected solvent and preactivated by the carbodiimide (2.0 eq, 0.28 mmol) for 15 min at 0°C, added to the resin and stirred at room temperature. After 90 min, 5 mg of the resin was taken, washed with DCM to perform the mini cleavage to evaluate the conversion into Fmoc-His-Gly-Phe-Leu-OH. The dry peptide resin was suspended in 0.25 mL of the mixture TFA/H_2O (95.0/5.0 v/v), stirred for 2 h, filtered and the conversion was monitored by HPLC-MS analysis (Analytical Method 1).

At this point, the Fmoc protective group was removed by treating the peptide resin with a 20% piperidine solution in the solvent (2 × 1 mL, 15 min each) and the resin was washed with the selected solvent (3 × 1 mL). After the Fmoc-cleavage of the N-terminal alpha-amino group, the peptide resin was washed with the selected solvent (3 × 1 mL) and DCM (3 × 1 mL). The dry peptide resin was suspended in 2 mL of the mixture TFA/H_2O (95.0/5.0 v/v) and stirred for 2 h. The resin was filtered off and diisopropylether (10 mL) cooled to 4 °C was added to the solution. The peptide was filtered and dried in vacuo to obtain crude H-His-Gly-Phe-Leu-OH and the ratio **14/32** monitored by HPLC (Analytical Method 5).

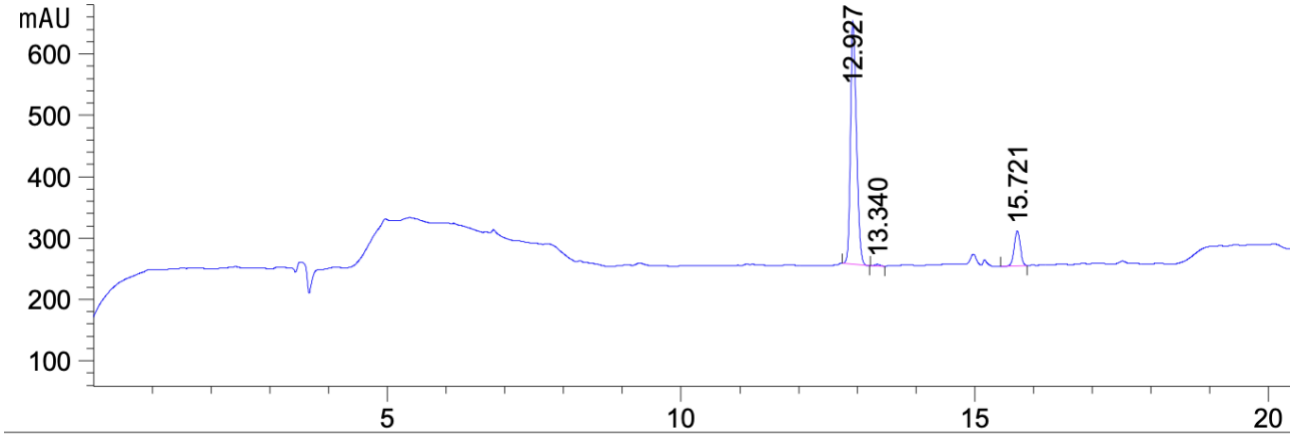
Entry 1, Table S6



Product	m/z observed	Rt (min)	Area (%)
Fmoc-H-G-F-L	695.2 $[M+H]^+$	15.130	86.5
DIC adduct	821.3 $[M+H]^+$	16.072	13.1
G-F-L	336.3 $[M+H]^+$	10.680	0.4

Figure S77. Chromatogram after the mini cleavage of Fmoc-HIs-Gly-Phe-Leu-OH synthesis in DMF with Oxyma pure/DIC 2/2 ratio (Entry 1, Table S6).

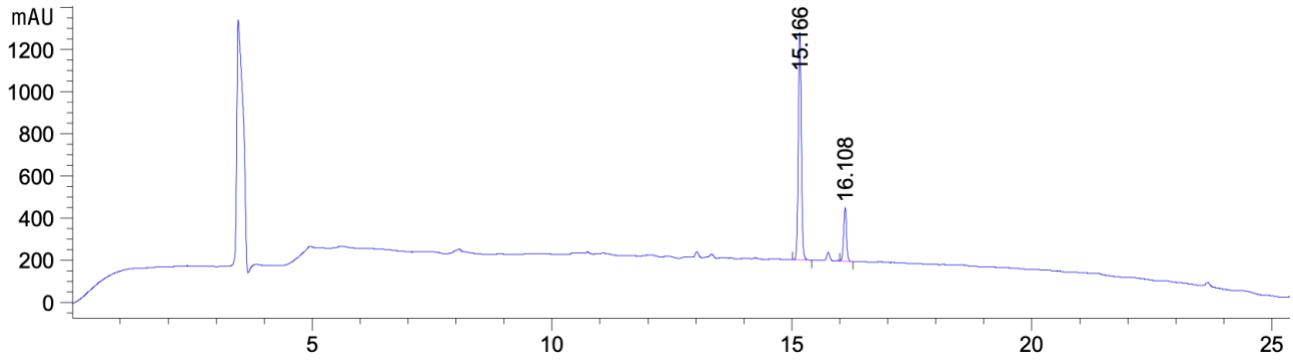
Entry 1, Table S6



Product	m/z observed	Rt (min)	Area (%)
H-G-F-L	473.2 $[M+H]^+$	12.927	86.9
D-H-G-F-L	473.2 $[M+H]^+$	13.340	0.3
DIC-Adduct	599.3 $[M+H]^+$	15.721	13.1
G-F-L	336.3 $[M+H]^+$	10.715	n.d

Figure S78. Chromatogram of H-His-Gly-Phe-Leu-OH synthesis in DMF with Oxyma pure/DIC 2/2 ratio (Entry 1, Table S6).

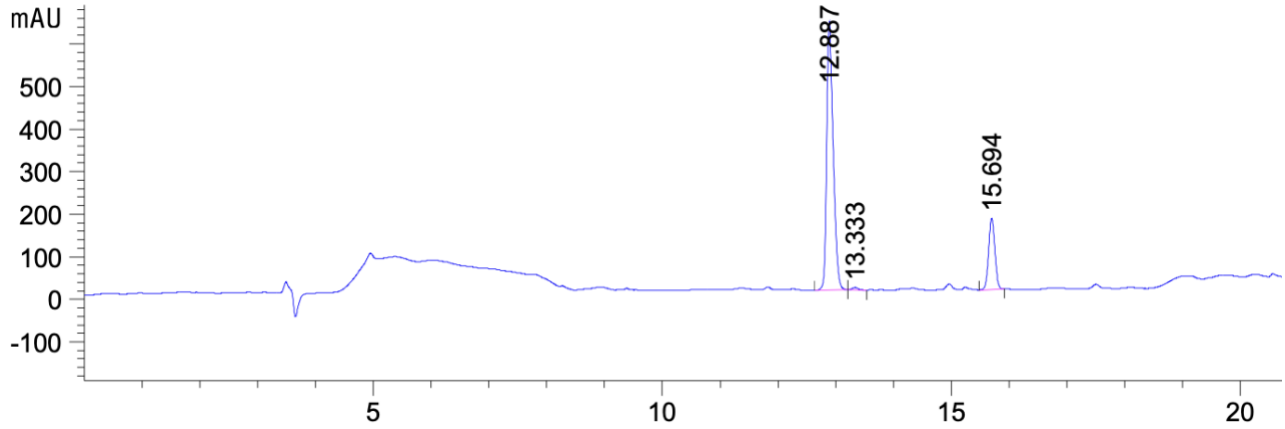
Entry 2, Table S6



Product	m/z observed	Rt (min)	Area (%)
Fmoc-H-G-F-L	695.2 $[M+H]^+$	15.166	83.6
DIC adduct	821.3 $[M+H]^+$	16.108	16.4
G-F-L	336.3 $[M+H]^+$	10.682	n.d

Figure S79. Chromatogram after the mini cleavage of Fmoc-His-Gly-Phe-Leu-OH synthesis in DMF with Oxyma pure/DIC 5/2 ratio (Entry 2, Table S6).

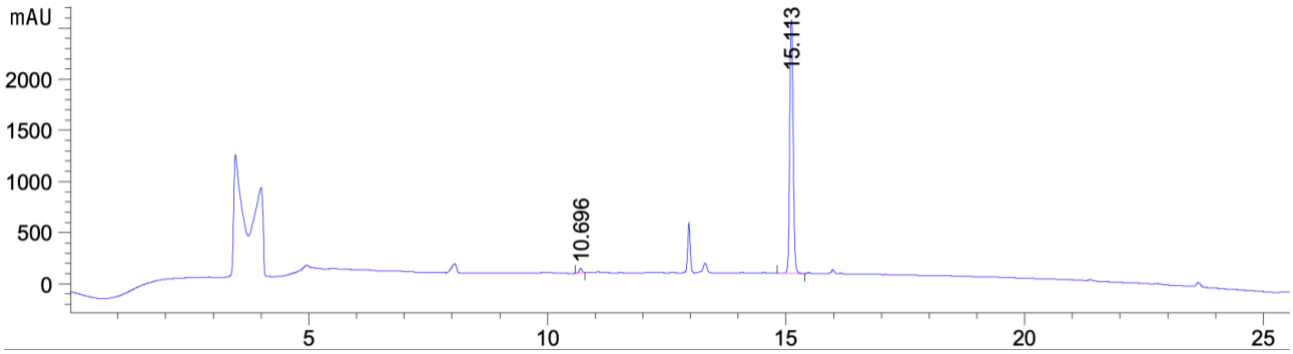
Entry 2, Table S6



Product	m/z observed	Rt (min)	Area (%)
H-G-F-L	473.2 $[M+H]^+$	12.927	82.8
D-H-G-F-L	473.2 $[M+H]^+$	13.333	0.8
DIC-Adduct	599.3 $[M+H]^+$	15.721	17.2
G-F-L	336.3 $[M+H]^+$	10.715	n.d

Figure S80. Chromatogram of H-His-Gly-Phe-Leu-OH synthesis in DMF with Oxyma pure/DIC 5/2 ratio (Entry 2, Table S6).

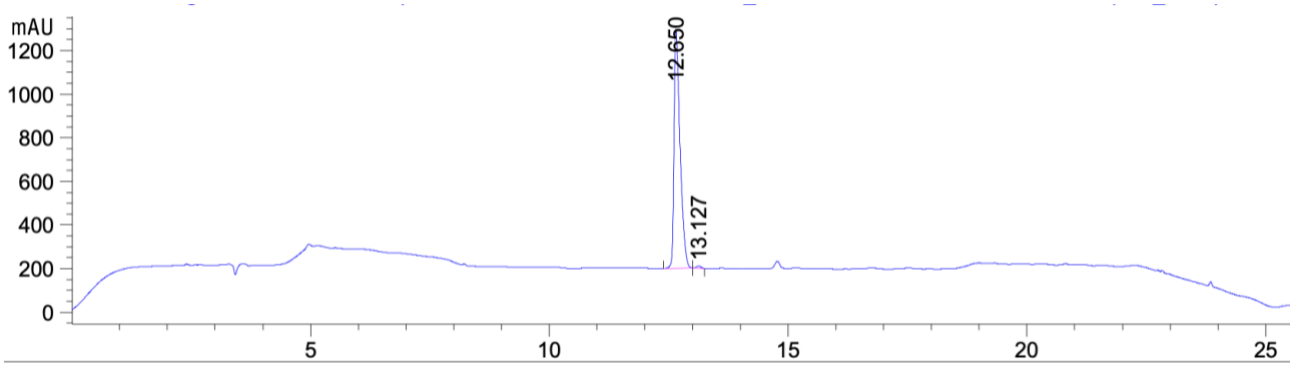
Entry 3, Table S6



Product	m/z observed	Rt (min)	Area (%)
Fmoc-H-G-F-L	695.2 $[M+H]^+$	15.113	99.5
TBEC-Adduct	821.3 $[M+H]^+$	16.019	n.d
G-F-L	336.3 $[M+H]^+$	10.696	0.5

Figure S81. Chromatogram after the mini cleavage of Fmoc-His-Gly-Phe-Leu-OH synthesis in DMF with Oxyma pure/TBEC 2/2 ratio (Entry 3, Table S6).

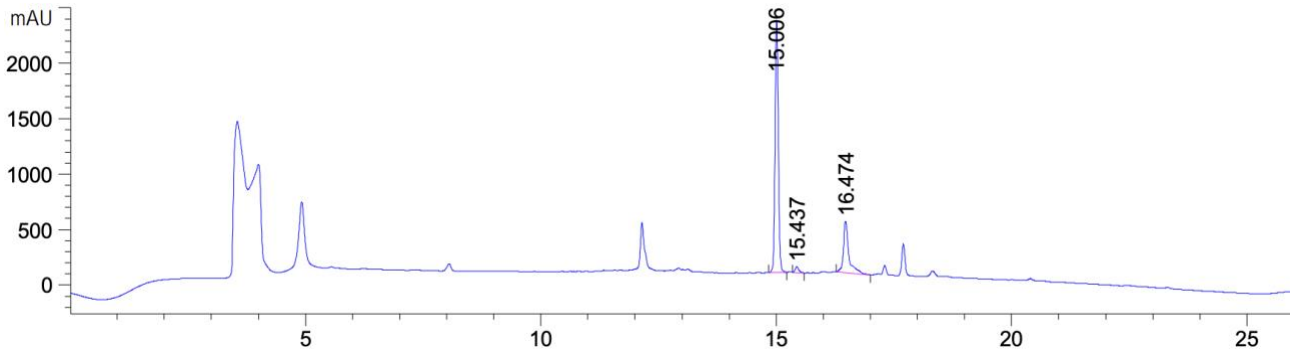
Entry 3, Table S6



Product	m/z observed	Rt (min)	Area (%)
H-G-F-L	473.2 $[M+H]^+$	12.650	99.4
D-H-G-F-L	473.2 $[M+H]^+$	13.127	0.6
TBEC-Adduct	599.3 $[M+H]^+$	15.749	n.d
G-F-L	336.3 $[M+H]^+$	10.714	n.d

Figure S82. Chromatogram of H-His-Gly-Phe-Leu-OH synthesis in DMF with Oxyma pure/TBEC 2/2 ratio (Entry 3, Table S6)

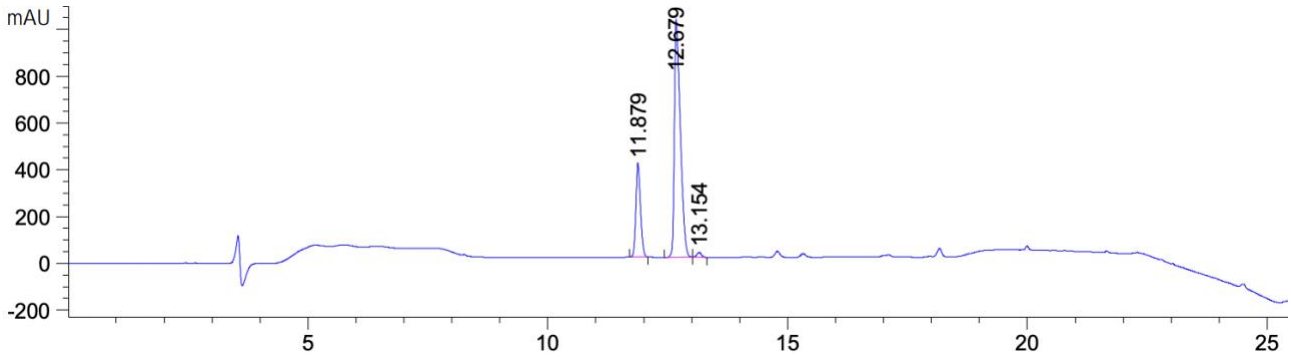
Entry 4, Table S6



Product	m/z observed	Rt (min)	Area (%)
Fmoc-H-G-F-L	695.2 $[M+H]^+$	15.006	73.8
Fmoc-*D*-H-G-F-L	695.2 $[M+H]^+$	15.437	1.1
EDC-Adduct	850.3 $[M+H]^+$	16.019	25.1
G-F-L	336.3 $[M+H]^+$	10.699	n.d

Figure S83. Chromatogram after the mini cleavage of Fmoc-His-Gly-Phe-Leu-OH synthesis in DMF with Oxyma pure/EDC 2/2 ratio (Entry 4, Table S6).

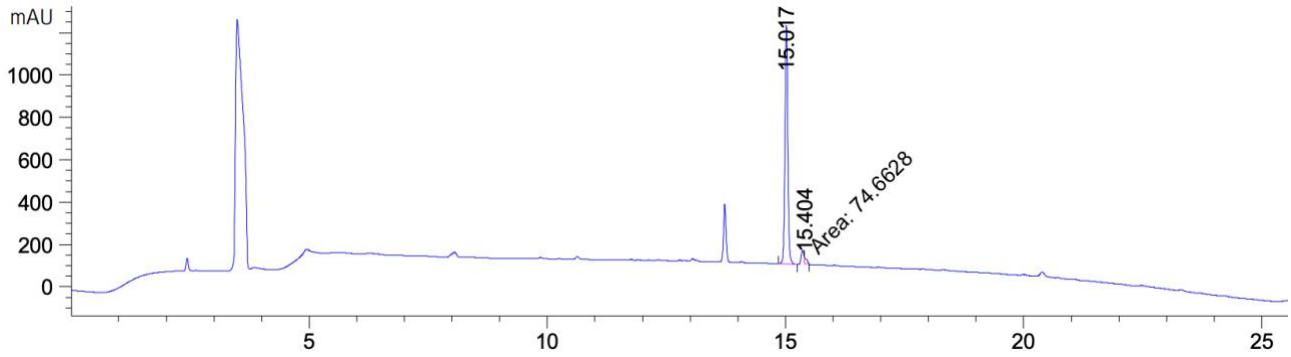
Entry 4, Table S6



Product	m/z observed	Rt (min)	Area (%)
H-G-F-L	473.2 $[M+H]^+$	12.679	75.6
D-H-G-F-L	473.2 $[M+H]^+$	13.154	1.1
EDC-Adduct	599.3 $[M+H]^+$	11.879	23.2
G-F-L	336.3 $[M+H]^+$	10.718	n.d

Figure S84. Chromatogram of H-His-Gly-Phe-Leu-OH synthesis in DMF with Oxyma pure/EDC 2/2 ratio (Entry 4, Table S6)

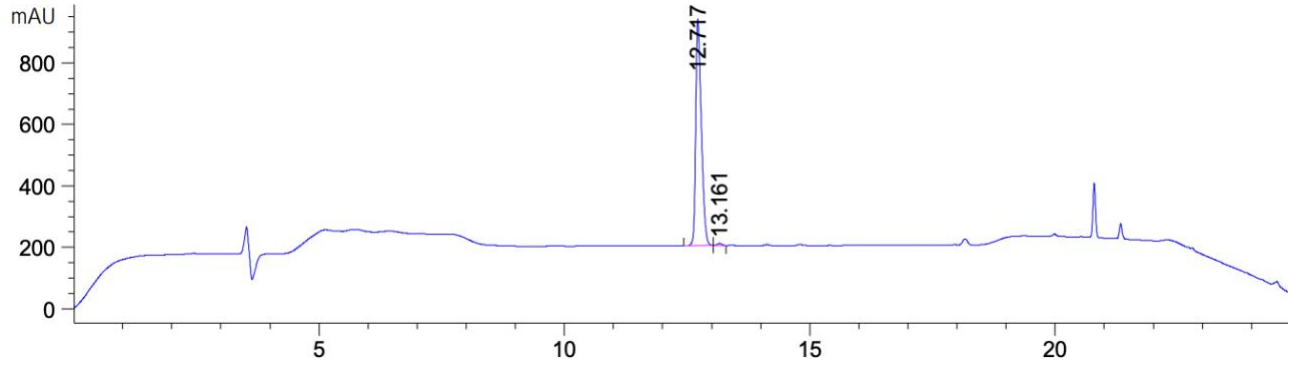
Entry 5, Table S6



Product	m/z observed	Rt (min)	Area (%)
Fmoc-H-G-F-L	695.2 $[M+H]^+$	15.017	99.1
Fmoc-*D*-H-G-F-L	695.2 $[M+H]^+$	15.404	0.9
DCC-Adduct	901.3 $[M+H]^+$	n.d	n.d
G-F-L	336.3 $[M+H]^+$	n.d	n.d

Figure S85. Chromatogram after the mini cleavage of Fmoc-His-Gly-Phe-Leu-OH synthesis in DMF with Oxyma pure/DCC 2/2 ratio (Entry 5, Table S6).

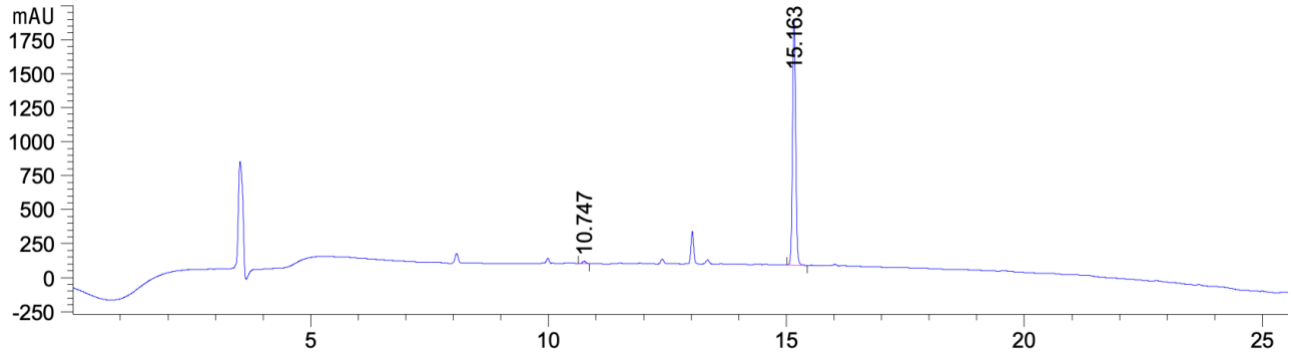
Entry 5, Table S6



Product	m/z observed	Rt (min)	Area (%)
H-G-F-L	473.2 $[M+H]^+$	12.717	99.2
D-H-G-F-L	473.2 $[M+H]^+$	13.161	0.8
DCC-Adduct	599.3 $[M+H]^+$	-	n.d
G-F-L	336.3 $[M+H]^+$	10.787	n.d

Figure S86. Chromatogram of H-His-Gly-Phe-Leu-OH synthesis in DMF with Oxyma pure/DCC 2/2 ratio (Entry 5, Table S6)

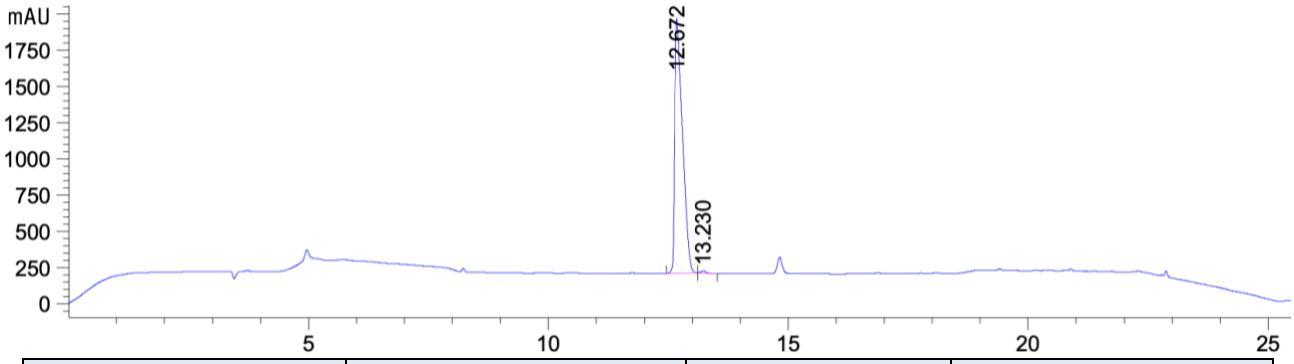
Entry 6, Table S6



Product	m/z observed	Rt (min)	Area (%)
Fmoc-H-G-F-L	695.2 $[M+H]^+$	15.163	99.6
TBEC-Adduct	821.3 $[M+H]^+$	16.023	n.d
G-F-L	336.3 $[M+H]^+$	10.747	0.4

Figure S87. Chromatogram after the mini cleavage of Fmoc-His-Gly-Phe-Leu-OH synthesis in NBP/DMC 8:2 with Oxyma pure/TBEC 2/2 ratio (Entry 6, Table S6).

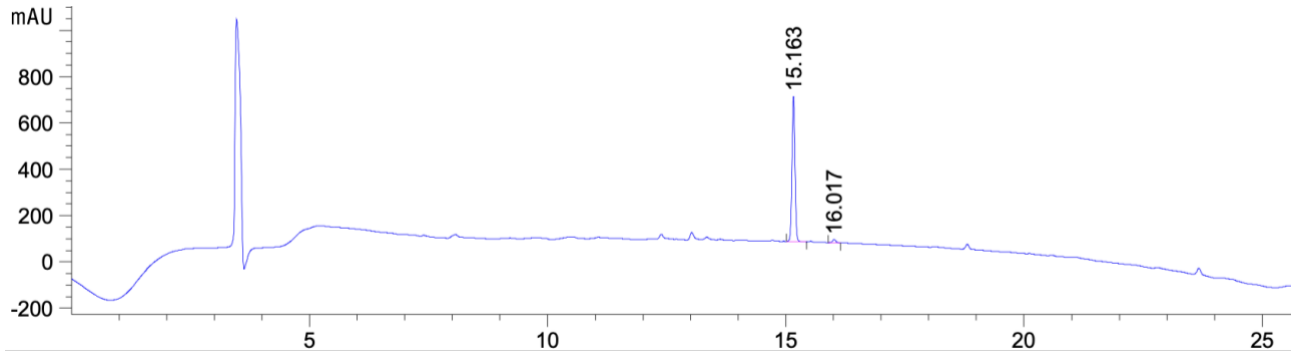
Entry 6, Table S6



Product	m/z observed	Rt (min)	Area (%)
H-G-F-L	473.2 $[M+H]^+$	12.672	99.4
D-H-G-F-L	473.2 $[M+H]^+$	13.230	0.6
TBEC-Adduct	599.3 $[M+H]^+$	15.845	n.d
G-F-L	336.3 $[M+H]^+$	10.729	n.d

Figure S88. Chromatogram of H-His-Gly-Phe-Leu-OH synthesis in NBP/DMC 8:2 with Oxyma pure/TBEC 2/2 ratio (Entry 6, Table S6)

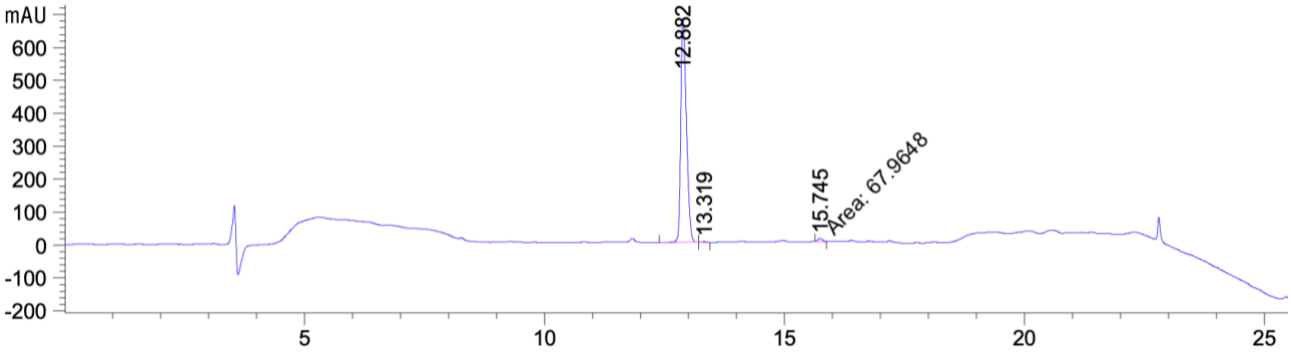
Entry 7, Table S6



Product	m/z observed	Rt (min)	Area (%)
Fmoc-H-G-F-L	695.2 $[M+H]^+$	15.163	98.7
TBEC-Adduct	821.3 $[M+H]^+$	16.017	1.3
G-F-L	336.3 $[M+H]^+$	10.725	n.d

Figure S89. Chromatogram after the mini cleavage of Fmoc-His-Gly-Phe-Leu-OH synthesis in NBP/DMC 8:2 with Oxyma pure/TBEC 2/20 ratio (Entry 7, Table S6).

Entry 7, Table S6



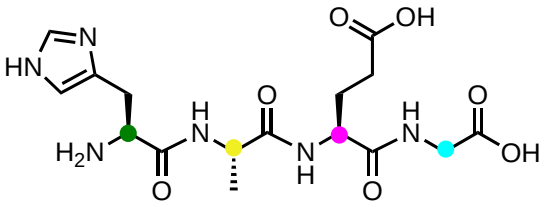
Product	m/z observed	Rt (min)	Area (%)
H-G-F-L	473.2 $[M+H]^+$	12.882	98.5
D-H-G-F-L	473.2 $[M+H]^+$	13.319	0.3
TBEC-Adduct	599.3 $[M+H]^+$	15.745	1.2
G-F-L	336.3 $[M+H]^+$	10.724	n.d

Figure S90. Chromatogram of H-His-Gly-Phe-Leu-OH synthesis in NBP/DMC 8:2 with Oxyma pure/TBEC 2/20 ratio (Entry 7, Table S6)

7. Synthesis of Liraglutide and Semaglutide residues

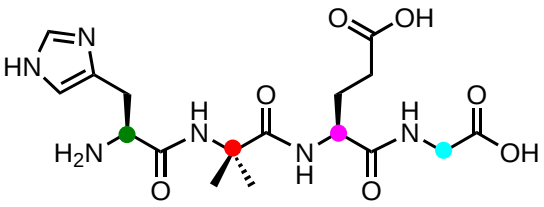
General procedure

Liraglutide residue



His Ala Glu Gly

Semaglutide residue



His Aib Glu Gly

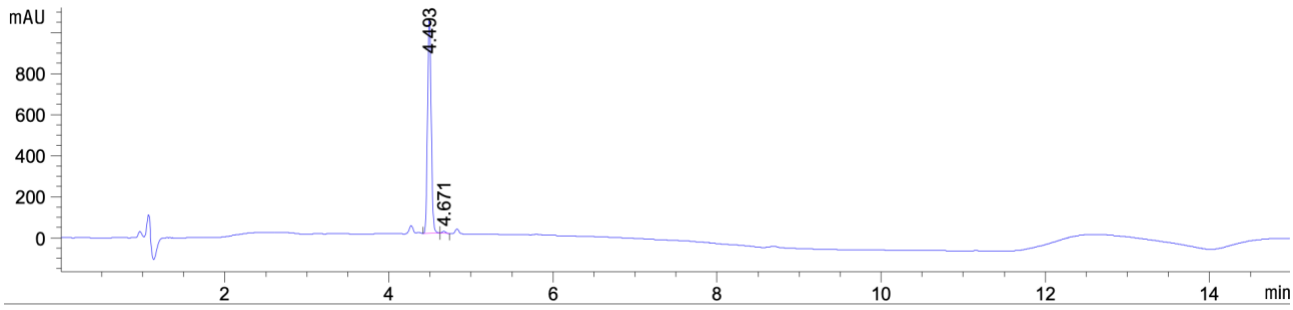
Table S7. Synthesis of Liraglutide and Semaglutide residues with TBEC in NBP/DMC 8:2

Entry	Product	Purity
1	Liraglutide	>99
2	Semaglutide	>97

Liraglutide and semaglutide syntheses were carried out at room temperature in glass syringes fitted with a polyethylene porous disc and connected to a vacuum source to remove excess reagents and solvents, by using 0.3 g of preloaded Fmoc-Gly-MBH resin (loading 0.5 mmol g^{-1}). After the swelling of the resin in 3.0 mL of NBP/DMC 8:2, the Fmoc protective group was removed by 20% Piperidine solution in NBP/DMC 8:2 (2 times × 1.5 mL, 15 min each) and then the resin was washed with NBP/DMC 8:2 (3 times × 1.5 mL, 2 min each). Fmoc-Glu(OtBu)-OH, Fmoc-Ala-OH, Fmoc-Aib-OH and Fmoc-His-OH (2.0 eq, 0.30 mmol) were pre-activated by Oxyma pure (2.0 eq, 42.8 mg, 0.30 mmol) and TBEC (2.0 eq, 46.4 µL, 0.30 mmol) for 5 minutes and coupled to the resin for 2 h. After each coupling step, the Fmoc protective group was removed by 20% Piperidine solution in NBP/DMC 8:2 (2 times × 1.5 mL, 15 min each) and then the resin was washed with NBP/DMC 8:2 (3 times × 1.5 mL, 2 min each). After the coupling of the last amino acid the peptide resin was washed with NBP/DMC 8:2 (3 times × 1.5 mL, 2 min each), DCM (3 times × 1.5 mL, 2 min each) and dried under a vacuum for 12 hours. The dry peptide resin was suspended in 3 mL of the TFA/TIS/H_2O (95/2.5/2.5 v/v/v) mixture and stirred for 2 h. The resin was filtered off, washed with TFA (1 time × 1 mL, 1 min) and diisopropylether (10 mL) cooled to 4 °C was added to the solution dropwise. The peptide was filtered and dried in vacuo to obtain crude product that was directly analyzed by HPLC-MS (Analytical Method 4)

Entry 1, Table S7

AA	Eq	Ac	Ad	Ratio AA/Ac/Ad	Reaction time h	Fmoc deprotection
Glu	2	TBEC	Oxyma	1/1/1	2.0	20% PIP in NBP/DMC
Ala	2	TBEC	Oxyma	1/1/1	2.0	20% PIP in NBP/DMC
His	2	TBEC	Oxyma	1/1/1	2.0	-



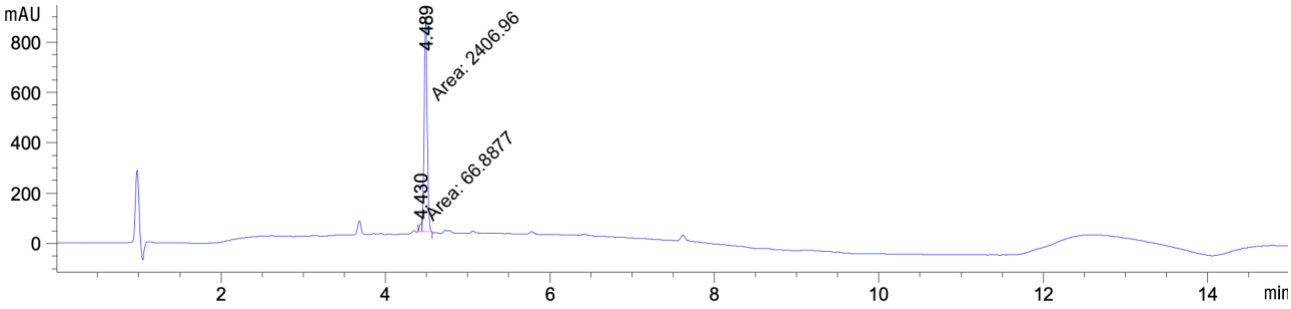
Product	m/z observed	Rt (min)	Area (%)
Target peptide	635.1 $[M+H]^+$	4.493	99.2
D-analogue	635.1 $[M+H]^+$	4.671	0.8

Figure S91. Chromatogram of liraglutide residue synthesis in NBP/DMC 8:2 (Entry 1, Table S7)

Entry 2, Table S7

AA	Eq	Ac	Ad	Ratio AA/Ac/Ad	Reaction time h	Fmoc deprotection
Glu	2	TBEC	Oxyma	1/1/1	2.0	20% PIP in NBP/DMC
Aib[a]	2	TBEC	Oxyma	1/1/1 x 2	2.0	20% PIP in NBP/DMC
His	2	TBEC	Oxyma	1/1/1	2.0	-

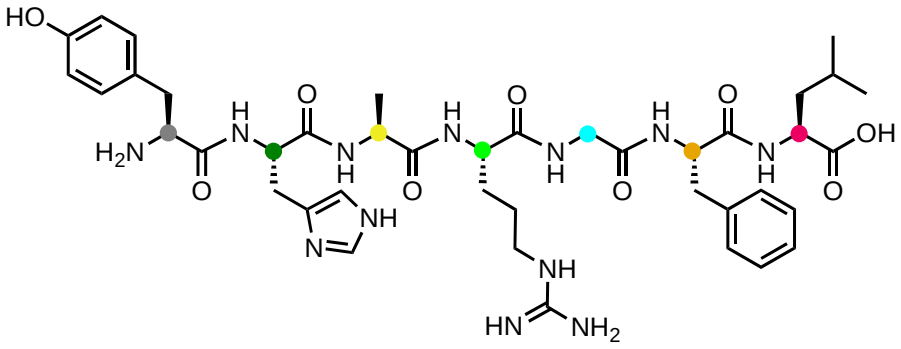
[a]Coupling performed at 35°C



Product	m/z observed	Rt (min)	Area (%)
Target peptide	649.1 $[M+H]^+$	4.472	97.2
Des-Aib	564.1 $[M+H]^+$	4.403	3.8

Figure S92. Chromatogram of semaglutide residue synthesis in NBP/DMC 8:2 (Entry 2, Table S7)

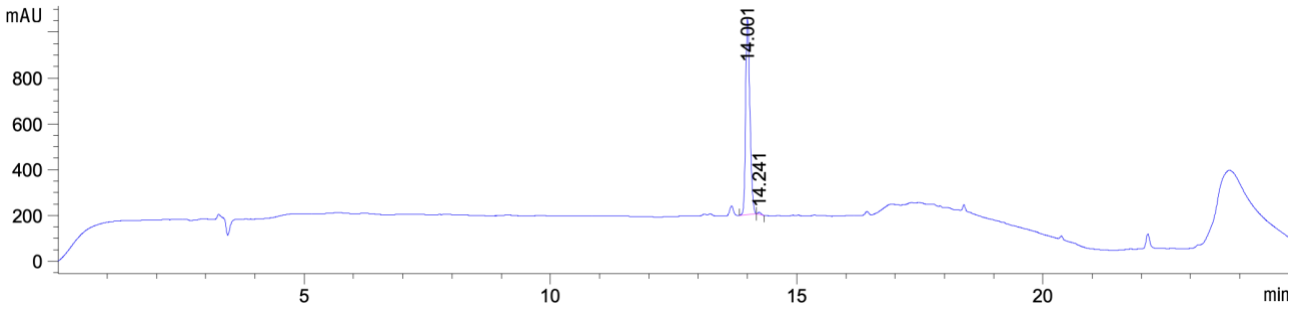
8. Synthesis of Y-H-A-R-G-F-L



Tyr His Ala Arg Gly Phe Leu

The synthesis of **33** was carried out at room temperature in glass syringes fitted with a polyethylene porous disc and connected to a vacuum source to remove excess reagents and solvents, by using 0.3 g of preloaded Fmoc-Gly-Phe-Leu-CTC resin (loading 0.7 mmol g^{-1}). After the swelling of the resin in 3.0 mL of NBP/DMC 8:2, Fmoc-Arg(HCl)-OH, Fmoc-Ala-OH, Fmoc-His-OH and Fmoc-Tyr-OH (2.0 eq, 0.42 mmol) were pre-activated by Oxyma pure (5.0 eq, 1.05 mmol) and TBEC (2.0 eq, 65.0 µL, 0.42 mmol) for 5 minutes and coupled to the resin for 2 h (Fmoc-His-OH was pre-activated for 15 min at 0°C). After each coupling step, the Fmoc protective group was removed by 20% Piperidine solution in NBP/DMC 8:2 (2 times × 1.5 mL, 15 min each) and then the resin was washed with NBP/DMC 8:2 (3 times × 1.5 mL, 2 min each). After the Fmoc cleavage of the N-terminal amino group, the resin was washed with NBP/DMC 8:2 (3 times × 1.5 mL, 2 min each), DCM (3 times × 1.5 mL, 2 min each) and dried under a vacuum for 12 hours. The dry peptide resin was suspended in 3 mL of the TFA/H_2O (95/5.0 v/v) mixture and stirred for 2 h. The resin was filtered off, washed with TFA (1 time × 1 mL, 1 min) and diisopropylether (10 mL) cooled to 4 °C was added to the solution dropwise. The peptide was filtered and dried in vacuo to obtain crude **33** that was directly analyzed by HPLC-MS (Analytical Method 1).

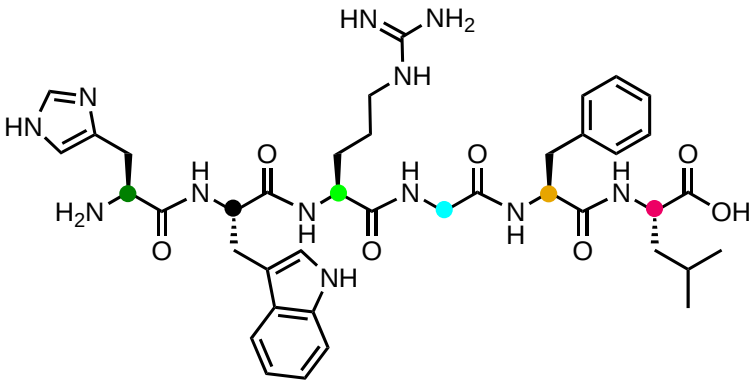
AA	Eq	Ac	Ad	Ratio AA/Ac/Ad	Reaction time h	Fmoc deprotection
Arg	2	TBEC	Oxyma	1/1/2.5	2.0	20% PIP in NBP/DMC
Ala	2	TBEC	Oxyma	1/1/2.5	2.0	20% PIP in NBP/DMC
His	2	TBEC	Oxyma	1/1/2.5 x 2	2.0	20% PIP in NBP/DMC
Tyr	2	TBEC	Oxyma	1/1/2.5	2.0	20% PIP in NBP/DMC



Product	m/z observed	Rt (min)	Area (%)
Target peptide	863.4 $[M+H]^+$	14.001	97.1
D-analogue	863.4 $[M+H]^+$	14.241	0.4

Figure S93. Chromatogram of **33** synthesis with TBEC in NBP/DMC 8:2

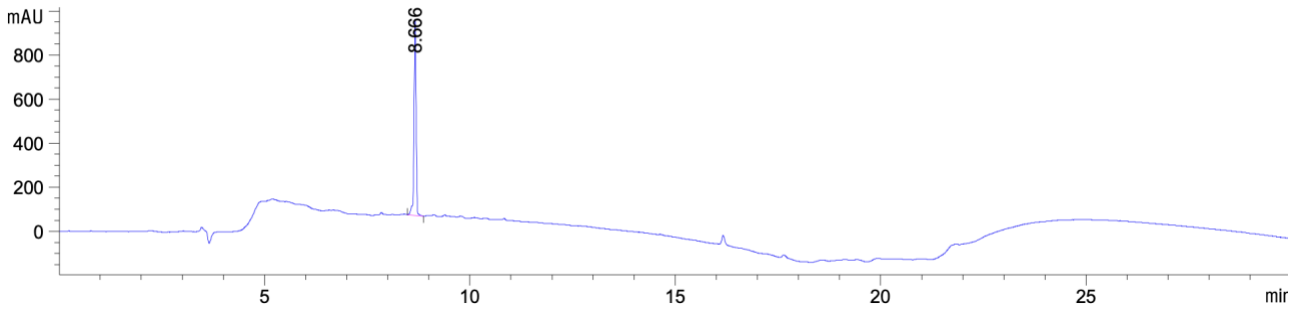
9. Synthesis of H-W-R-G-F-L



His Trp Arg Gly Phe Leu

The synthesis of **34** was carried out at room temperature in glass syringes fitted with a polyethylene porous disc and connected to a vacuum source to remove excess reagents and solvents, by using 0.3 g of preloaded Fmoc-Gly-Phe-Leu-CTC resin (loading 0.7 mmol g^{-1}). After the swelling of the resin in 3.0 mL of NBP/DMC 8:2, Fmoc-Arg(HCl)-OH, Fmoc-Trp-OH and Fmoc-His-OH (2.0 eq, 0.42 mmol) were pre-activated by Oxyma pure (5.0 eq, 1.05 mmol) and TBEC (2.0 eq, 65.0 µL, 0.42 mmol) for 5 minutes and coupled to the resin for 2 h (Fmoc-His-OH was pre-activated for 15 min at 0°C). After each coupling step, the Fmoc protective group was removed by 20% Piperidine solution in NBP/DMC 8:2 (2 times × 1.5 mL, 15 min each) and then the resin was washed with NBP/DMC 8:2 (3 times × 1.5 mL, 2 min each). After the Fmoc cleavage of the N-terminal amino group, the resin was washed with NBP/DMC 8:2 (3 times × 1.5 mL, 2 min each), DCM (3 times × 1.5 mL, 2 min each) and dried under a vacuum for 12 hours. The dry peptide resin was suspended in 3 mL of the TFA/H_2O (95/5.0 v/v) mixture and stirred for 2 h. The resin was filtered off, washed with TFA (1 time × 1 mL, 1 min) and diisopropylether (10 mL) cooled to 4 °C was added to the solution dropwise. The peptide was filtered and dried in vacuo to obtain crude **34** that was directly analyzed by HPLC-MS (Analytical Method 1).

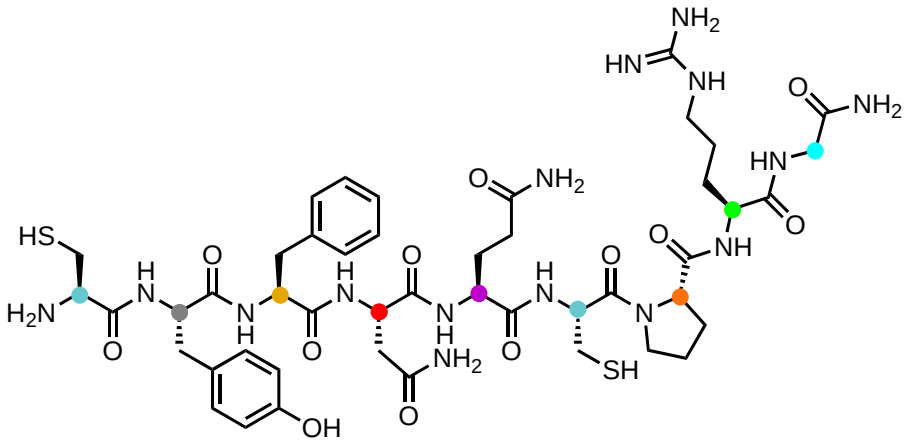
AA	Eq	Ac	Ad	Ratio AA/Ac/Ad	Reaction time h	Fmoc deprotection
Arg	2	TBEC	Oxyma	1/1/2.5	2.0	20% PIP in NBP/DMC
Trp	2	TBEC	Oxyma	1/1/2.5	2.0	20% PIP in NBP/DMC
His	2	TBEC	Oxyma	1/1/2.5 x 2	2.0	20% PIP in NBP/DMC



Product	m/z observed	Rt (min)	Area (%)
Target peptide	815.4 $[M+H]^+$	8.666	>99

Figure S94. Chromatogram of **34** synthesis with TBEC in NBP/DMC 8:2

10. Synthesis of linear Vasopressin with TBEC

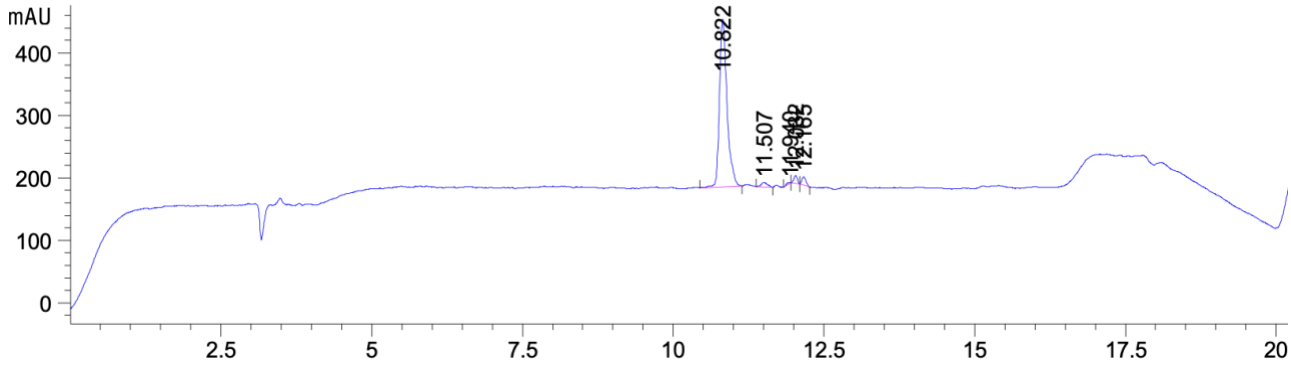


Cys Tyr Phe Asn Gln Cys Pro Arg Gly

Linear vasopressin **18** synthesis were carried out at room temperature in glass syringes fitted with a polyethylene porous disc and connected to a vacuum source to remove excess reagents and solvents, by using 0.3 g of preloaded Fmoc-Gly-Rink Amide resin (loading 0.5 mmol g^{-1}). After the swelling of the resin in 3.0 mL of NBP/DMC 8:2, the Fmoc protective group was removed by 20% DEAPA solution in NBP/DMC 8:2 (2 times × 1.5 mL, 15 min each) and then the resin was washed with NBP/DMC 8:2 (3 times × 1.5 mL, 2 min each). Fmoc-Arg(HCl)-OH, Fmoc-Pro-OH, Fmoc-Cys(Trt)-OH, Fmoc-Asn-OH, Fmoc-Gln-OH, Fmoc-Phe-OH and Fmoc-Tyr-OH (2.0 eq, 0.30 mmol) were pre-activated by Oxyma pure (5.0 eq, 107.1 mg, 0.75 mmol) and TBEC (2.0 eq, 46.4 µL, 0.30 mmol) for 5 minutes and coupled to the resin for 2 h. After each coupling step, the Fmoc protective group was removed by treating the peptide resin with 20% DEAPA solution in NBP/DMC 8:2 (2 times × 1.5 mL, 15 min each) and then the resin was washed with NBP/DMC 8:2 (3 times × 1.5 mL, 2 min each). After the Fmoc cleavage of the N-terminal amino group, the peptide resin was further washed with DCM (3 times × 1.5 mL,21 min each) and dried under vacuum for 12 hours. The dry peptide resin was suspended in 3 mL of the TFA/TIS/H_2O (95/2.5/2.5 v/v/v) mixture and stirred for 2 h. The resin was filtered off, washed with TFA (1 time × 1 mL, 1 min) and diisopropylether (10 mL) cooled to 4 °C was added to the solution dropwise. The peptide was filtered and dried in vacuo to obtain crude linear vasopressin **18** that was directly analyzed by HPLC-MS (Analytical Method 2).

AA	Eq	Ac	Ad	Ratio AA/Ac/Ad	Reaction time h	Fmoc deprotection
Arg	2	TBEC	Oxyma	1/1/2.5	3.0	20% DEAPA in NBP/DMC
Pro	2	TBEC	Oxyma	1/1/2.5	2.0	20% DEAPA in NBP/DMC
Cys	2	TBEC	Oxyma	1/1/2.5	2.0	20% DEAPA in NBP/DMC
Asn	2	TBEC	Oxyma	1/1/2.5 x 2	2.0	20% DEAPA in NBP/DMC
Gln[a]	2	TBEC	Oxyma	1/1/2.5 x 2	2.0	20% DEAPA in NBP/DMC
Phe	2	TBEC	Oxyma	1/1/2.5	2.0	20% DEAPA in NBP/DMC
Tyr	2	TBEC	Oxyma	1/1/2.5	2.0	20% DEAPA in NBP/DMC
Cys	2	TBEC	Oxyma	1/1/2.5	2.0	20% DEAPA in NBP/DMC

[a]Preactivation performed at 70°C for 5 minutes because of solubility problems.



Product	m/z observed	Rt (min)	Area (%)
Target peptide	1087.4 $[M+H]^+$	10.822	93.0
Des-Gln	960.1 $[M+H]^+$	11.507	1.1
Des-Asn	974.1 $[M+H]^+$	12.032	1.6
Aspartimide	1069.4 $[M+H]^+$	12.165	4.3

Figure S95. Chromatogram of linear vasopressin synthesis with TBEC in NBP/DMC 8:2