Visible-Light-Mediated Selective Arylation of Cysteine in Batch and Flow

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General information

All components as well as reagents and solvents were used as received without further purification, unless stated otherwise. Reagents and solvents were bought from Sigma Aldrich and TCI and if applicable, kept under argon atmosphere. Technical solvents were bought from VWR International and used as received. Product isolation was performed using silica (60, F254, MerckTM), and TLC analysis was performed using Silica on aluminum foils TLC plates (F254, Supelco Sigma-AldrichTM) with visualization under ultraviolet light (254 nm and 365 nm) or appropriate TLC staining. $^1$H (400MHz or 500 MHz), $^{13}$C (100MHz) and $^{19}$F (376 MHz) NMR spectra were recorded on ambient temperature. $^1$H NMR spectra are reported in parts per million (ppm) downfield relative to Chloroform-d (7.26 ppm) and all $^{13}$C NMR spectra are reported in ppm relative to Chloroform-d (77.2 ppm). NMR spectra are described using the following abbreviations for assigning the multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, h = hextet, hept = heptet, m = multiplet, dd = double doublet, td = triple doublet. Known products were characterized by comparing to the corresponding $^1$H NMR and $^{13}$C NMR from literature.

For peptide synthesis: DMF and NMP (peptide synthesis grade) were purchased from Biosolve. Acetonitrile, methanol, diethyl ether, DIPEA (supplied as extra dry, redistilled, 99.5 % pure) and trisopropylsilane (TIPS) were purchased from Sigma Aldrich. Milli-Q grade water was obtained in-house either from a Millipore ROs 5 purification system or a Sartorius Arium 611 DI. H-Rink amide ChemMatrix (35 – 100 mesh, manufacturer’s loading: 0.4-0.6 mmol/g) was obtained from Sigma Aldrich. HBTU, TFA (peptide synthesis grade) and Nα-Fmoc protected amino acids used for peptide synthesis were obtained from Iris Biotech GmbH. All chiral α-amino acids used in this paper possessed the L configuration. Residues with standard acid-sensitive side-chain PGs were used: Cys(Trt) [C], Lys(Boc) [K], Ser(tBu) [S]. The peptide was N-terminally capped with acetamidobenzoic acid (ABA, Sigma Aldrich).
Reaction Set-ups

Batch Set-up

A 5 mL vial with septum was loaded with the reagents and placed in the proximity of a 24 W white CFL (around 2 cm distance). A needle was inserted in the septum for ensuring venting of N₂ from the reaction mixture. Cooling of the reaction mixture was insured via an air stream directed towards the vial.

Flow Set-up

All microfluidic fittings were purchased from IDEX Health and Science. The syringes were connected to the capillary using ¼-28 flat-bottom flangeless fittings. A syringe pump (Fusion 200 Classic) equipped with 20 mL syringes was used to feed liquid reagents through a high purity perfluoroalkoxyalkane (PFA) capillary tubing (ID = 500 µm) to a Tefzel® tee mixer (ID = 500 µm). The microcapillary was coiled around the inner cylinder of a 3D-printed reactor holder and irradiated with a white LED strip (3.12W, 78 lumen, 39 LEDs). Cooling of the reaction mixture was granted by a stream of compressed air inserted at the bottom of the reactor holder. A photo of the flow set-up used is represented in Figure S 1, while a schematic representation is shown in Scheme S 1. This set-up was employed both for the arylation of cysteine and of cysteine containing dipeptides. For a detailed procedure on the manufacturing of photomicroreactors, we refer to¹.

B) Arylation of cysteine in flow

| N-Ac-Cys-OMe, aniline | Eosin Y, PTSA H₂O, ACN |
|-----------------------|------------------------|
| 450 µL·min⁻¹           |                        |

| tBuONO, ACN            |
|------------------------|
| 450 µL·min⁻¹           |

Scheme S 1: Schematic representation of the flow set-up used for the arylation of cysteine derivatives and dipeptide arylated derivatives.

Figure S 1: Details of the photomicroreactor employed for the arylation of cysteine. a) microcapillary coiled around the inner cylinder of the reactor holder. b) White LED strip placed in the beaker of the reactor holder. c) View of the photochemical reactor during operation.
Light sources and their emission spectra
The emission of the used light sources was recorded using an integrating sphere equipped with a Labsphere LPS 100-0260 light detector array.

Calex daylight
Calex high quality CFL, daylight E-27 240V, 1460 Lumen, 24 Watt

Figure S 2: Emission spectrum for the 24 W white Calex CFL.
**White LED strip**

Paulmann YourLED, stripe white, 78 Lumen, 3.12 Watt, 39 LEDs

*Figure S 3 Emission spectrum for the Paulmann YourLED White strip.*
Synthesis of peptides 4, 5, 6 and 7 via native chemical ligation

The dipeptides used as starting materials for the synthesis of derivatives 8 to 11 were prepared through a two-step procedure adapted from literature.²

**Step 1: Formation of the thioester derivative:** L-Boc-Ala-OH or L-Boc-Leu-OH or L-Boc-Trp-OH or L-Boc-Phe-OH (1.0 equiv.) and DCM or ethyl acetate (0.5 mmol/mL) were added to an oven-dried flask and placed in an ice bath (0°C). Then, DCC (1 equiv.) and HOBt•H₂O (1 equiv.) were added together with thiophenol (1 equiv.). The flask was closed with a PTFE septum and the reaction mixture was placed under argon atmosphere. The reaction was checked for completion by TLC (4-24 hours). The reaction mixture was washed with HCl (1M), NaHCO₃ (sat) and brine. The organic layer was dried with MgSO₄. The crude was absorbed on silica gel and purified with PE:EtOAC 7:1. Purification by column chromatography (DCM:MeOH 9:1 + 1% acetic acid) afforded the desired thioester intermediates. (Figure S 4).

**Step 2: Native chemical ligation:** L-Cysteine methyl ester HCl (1.0 equiv.) and the thioester derivative obtained from the previous step (1.0 equiv.) were added to MeOH (0.25 mmol/mL) in an oven-dried flask kept under argon atmosphere and closed with a PTFE septum. Next tributylphosphine (0.6 equiv.) was added and the reaction mixture was stirred at r.t. until completion (24 hours). The crude was evaporated under vacuo, and re-dissolved in EtOAc. The organic layer was extracted with H₂O (3 times) and with brine (3 times). The organic layer was then dried with MgSO₄ and concentrated on silica gel under vacuo. Purification by column chromatography (DCM:MeOH 9:1 + 1% acetic acid) afforded the desired dipeptides Boc-Ala-Cys-OMe 4 (overall yield 37%), Boc-Leu-Cys-OMe 5 (overall yield 39%), Boc-Trp-Cys-OMe 6 (overall yield 77%) and Boc-Phe-Cys-OMe 7 (overall yield, 20%) (Figure S 4).

![Figure S 4: Schematic representation of the synthesis of dipeptides 4 to 7 via native chemical ligation. Yields reported are the overall yields of the two steps.](image-url)
Synthesis of peptide 12

Automated peptide synthesis were performed on a fully-automated SYRO Multiple Peptide Synthesizer robot, equipped with a vortexing unit for the 24-reactor block (MultiSynTech GmbH). Reactions were open to the atmosphere and executed at ambient temperature. Peptide 12 was synthesized using the Fmoc/tBu strategy with HBTU/DIPEA-mediated couplings.

Peptide sequence synthesized ABA-CGSSK-CONH₂

After the final Fmoc deprotection step, the peptide was manually cleaved from the resin. Cleavage cocktail (500 μL – 1 mL of 95% TFA, 2.5% TIPS and 2.5% H₂O) was added to peptide resin, and the reaction was shaken at r.t. for 2 h. Cleavage cocktail containing peptide was precipitated into cold ether and centrifuged (10 mins, 10 krpm). Ether was poured off, and the pellet was re-suspended in fresh cold ether and centrifuged (10 mins, 10 krpm). The resulting pellet peptide was dried on an oil pump. Peptide 12 was then analyzed by LC/MS and employed for the reaction without further purification.

Peptide analysis :LC-TIC-MS data (reversed phase) were recorded on an Agilent 1100 Series instrument with diode array detector (set to 214, 254, 280, 310, 360 nm), equipped with a Phenomenex Kinetex C18 100 Å (150 x 4.6 mm, 5 μm, at 35 °C), hyphenated to an Agilent ESI-single quadrupole MS detector type VL. Mass detection operated in the positive mode. Linear gradient elution was performed by flushing 0.5 min with A followed by 0 to 100% buffer B in 6 minutes and finally by a 2 min flushing with B using a binary solvent system composed of buffer A: 0.1% HCOOH in H₂O and B: MeCN with a flow of 1.0 mL/min at 35 °C.

LC-MS of crude peptide 12 (CH₃CN/H₂O/0.1% formic acid):

| theoretical | found  |
|-------------|--------|
| [M+H]⁺      | 641.27 | 641.2 |
| [M+Na]⁺     | 663.25 | 663.2 |

Starting material, peptide 12
Mass of peak at 3.46 min = starting material, peptide 12.

Deconvolution of peak at 3.46 min = starting material, peptide 12.
Photocatalytic synthesis of arylated peptide 13 and 14

Synthesis of arylated peptide 13
1 mg of para-F benzene diazonium salt (10 eq) was weighed in a 2.5 mL Eppendorf vial. To this vial, 150 μL (0.3 mg, 0.468 μmol, 1 eq) of a 3.15 μM solution of peptide 12 in PBS (2 mg of peptide in 1 ml PBS pH = 8) was added together with 30.3 μL (0.1 eq) of a solution Eosin Y in PBS (1 mg in 1 ml PBS). More PBS was added to reach a final volume of 1 mL. The Eppendorf vial then closed and positioned in the proximity of a 24W white CFL lamp. Prior to the reaction the lid of the vial was perforated with a needle to allow the evolution of gas. The reaction was magnetically stirred. After 30 minutes the reaction was analyzed in LC-MS.

LC-MS of formation of arylated peptide 13 after 30 minutes reaction time (CH₃CN/H₂O/0.1% formic acid):

| theoretical | found |
|-------------|-------|
| [M+H]^+    | 735.29 | 735.2 |

arylated peptide 13.

Mass of peak at 3.96 min = arylated peptide 13.
Synthesis of arylated peptide 14

1.3 mg of para-OCF₃ benzene diazonium salt (10 eq) was weighed in a 2.5 mL Eppendorf vial. To this vial, 150 μL (0.3 mg, 0.468 μmol, 1 eq) of a 3.15 μM solution of peptide 12 in PBS (2 mg of peptide in 1 mL PBS pH = 8) was added together with 30.3 μL (0.1 eq) of a solution Eosin Y in PBS (1 mg in 1 mL PBS). More PBS was added to reach a final volume of 1 mL. The Eppendorf vial then closed and positioned in the proximity of a 24W white CFL lamp. Prior to the reaction the lid of the vial was perforated with a needle to allow the evolution of gas. The reaction was magnetically stirred. After 30 minutes the reaction was analyzed in LC-MS.

LC-MS of formation of arylated peptide 14 after 30 minutes reaction time (CH₃CN/H₂O/0.1% formic acid):

The peak found at 3.35 min ([M]+ = 189) represents excess of unreacted diazonium salt.

|         | theoretical | found  |
|---------|-------------|--------|
| [M+H]+  | 801.28      | 801.2  |
| [M+Na]+ | 823.26      | 823.2  |

Peak at 3.3= excess diazonium salt.

Peak at 5 min= arylated peptide 14.

Mass of peak at 3.35 min = excess diazonium salt.
General Procedures

Synthesis of cysteine derivatives in batch (GP1)

In an oven-dried glass vial equipped with a magnetic stirrer and a PTFE septum, photoredox catalyst Eosin Y (1 mol%, 0.010 mmol) was added to a mixture of N-Ac-L-cysteine-OMe (1a) / cysteine containing peptide (1.0 mmol), aniline (1.3 eq, 1.3 mmol) and p-toluenesulfonic acid monohydrate (1.5 mol%, 0.015 mmol) in MeCN (10 mL). The vial was purged with argon and the PTFE septum was punched with a disposable syringe needle (for venting N₂). The vial was irradiated with a 24W CFL and tert-butylnitrite (2.0 mmol) was added dropwise to the mixture. The reaction was monitored by TLC, and stopped after 2 hours. After completion, water was added to the reaction mixture, and then extracted with EtOAc (3x). The organic layer was washed with brine, dried with MgSO₄ and evaporated under reduced pressure. The resulting crude compound was absorbed on silica gel and purified via column chromatography (EtOAc/Hexane, varying ratios). The isolated compound was analysed by GC-MS and NMR.

Synthesis of cysteine derivatives in continuous flow (GP2)

In an oven-dried glass vial equipped with a magnetic stirrer, photoredox catalyst Eosin Y (1 mol%, 0.02 mmol) was added to a mixture of N-Ac-L-cysteine-OMe (1a) / cysteine containing peptide (2.0 mmol), aniline (1.3 eq, 2.6 mmol) and p-toluenesulfonic acid monohydrate (4 mol%, 0.08 mmol) in MeCN (20 mL). The vial was fitted with a PTFE septum and purged with argon. A second oven-dried glass vial was fitted with a septum, purged with argon and tert-butylnitrite (459 μL, 4 mmol) was added through a syringe and diluted with 20 mL of MeCN. The two solutions were transferred into two 20 mL BD Discardit plastic syringes and introduced into the photo-microreactor through a syringe pump. The two liquid streams were merged with a T-Mixer (ID = 500 μm) before entering the reactor. The flow rate was set to 900 μL/min (450 μL/min per syringe), thus resulting in 30 seconds residence time (volume of reactor = 450 μL). After reaching steady state (approximately 4 residence times), a reaction sample was collected in a vial kept in the dark under argon atmosphere. The volume collected was measured and the sample was then diluted with water and extracted with EtOAc (3x). The organic layer was washed with brine, dried with MgSO₄ and evaporated under reduced pressure. The resulting crude compound was absorbed on silica gel and purified via column chromatography (EtOAc/Hexane, varying ratios). The isolated compound was analysed by GC-MS and NMR.
Compounds

3a: methyl N-acetyl-S-phenyl-L-cysteinate

\[
\begin{align*}
\text{HN} & \quad \text{C} \\
\text{S} & \quad \text{MeO} \\
\text{Me} & \quad \text{Me}
\end{align*}
\]

methyl N-acetyl-S-phenyl-L-cysteinate (3a) was made according to **GP1** on a 1.0 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 1:1) yielding 126.4 mg (0.50 mmol, 50 %) of 3a as a yellow oil. The reaction according to **GP2** on a 1.825 mmol scale afforded 328.3 mg (1.30 mmol, 72%) of 3a within 30 seconds residence time.

\(^1\)H NMR (400 MHz, Chloroform-d) \(\delta 7.41 \ (d, J = 8.1 \text{ Hz}, 2\text{H}), 7.32 – 7.27 \ (m, 2\text{H}), 7.23 \ (d, J = 7.6 \text{ Hz}, 1\text{H}), 6.20 \ (d, J = 6.6 \text{ Hz}, 1\text{H}), 4.92 – 4.79 \ (m, 1\text{H}), 3.56 \ (s, 3\text{H}), 3.52 – 3.33 \ (m, 2\text{H}), 1.87 \ (s, 3\text{H})\). \(^13\)C NMR (101 MHz, CDCl3) \(\delta 170.8, 169.9, 134.8, 131.2, 129.2, 127.3, 52.6, 52.5, 36.6, 23.0\). HRMS (ESI) m/z [M+H]^+ calculated for C\(_{12}\)H\(_{16}\)NO\(_3\)S: 254.0846, found 254.0849.

3b: methyl N-acetyl-S-(4-tert-butylphenyl)-L-cysteinate

\[
\begin{align*}
\text{HN} & \quad \text{C} \\
\text{S} & \quad \text{MeO} \\
\text{Me} & \quad \text{Me} \\
\text{tBu} & \quad \text{Me}
\end{align*}
\]

methyl N-acetyl-S-(4-tert-butylphenyl)-L-cysteinate (3b) was made according to **GP1** on a 1.0 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 1:1) yielding 101.4 (0.33 mmol, 33 %) of 3b as a yellow oil. The reaction according to **GP2** on a 1.75 mmol scale afforded 335.6 mg (1.09 mmol, 62%) of 3b within 150 seconds residence time.

\(^1\)H NMR (400 MHz, Chloroform-d) \(\delta 7.38 – 7.29 \ (m, 4\text{H}), 6.18 \ (d, J = 7.0 \text{ Hz}, 1\text{H}), 4.85 \ (dt, J = 7.6, 4.4 \text{ Hz}, 1\text{H}), 3.54 \ (s, 3\text{H}), 3.47 – 3.29 \ (m, 2\text{H}), 1.84 \ (s, 3\text{H}), 1.29 \ (s, 9\text{H})\). \(^13\)C NMR (101 MHz, CDCl3) \(\delta 170.8, 169.9, 150.7, 131.5, 131.1, 126.2, 52.6, 52.5, 36.9, 34.6, 31.3, 23.0\). HRMS (ESI) m/z [M + H]^+ calculated for C\(_{16}\)H\(_{24}\)NO\(_3\)S: 310.1472, found 310.1479.

3c: methyl N-acetyl-S-mesityl-L-cysteinate

\[
\begin{align*}
\text{HN} & \quad \text{C} \\
\text{S} & \quad \text{MeO} \\
\text{Me} & \quad \text{Me} \\
\text{Me} & \quad \text{Me}
\end{align*}
\]

methyl N-acetyl-S-mesityl-L-cysteinate (3c) was made according to **GP1** on a 1.0 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 1:1) yielding 159.9 mg (0.59 mmol, 59 %) of 3c as a yellow oil.
1H NMR (400 MHz, Chloroform-d) δ 6.91 (s, 2H), 6.17 (d, J = 6.72 Hz, 1H), 4.82 – 4.73 (m, 1H), 3.59 (s, 3H), 3.29 – 3.07 (m, 2H), 2.47 (s, 6H), 2.24 (s, 3H), 1.91 (s, 3H). 13C NMR (101 MHz, Chloroform-d) δ 171.1, 169.8, 142.5, 138.5, 129.4, 129.0, 52.8, 52.7, 36.6, 23.1, 21.9, 21.1. HRMS (ESI) m/z [M + H]+ calculated for C15H22NO3S: 296.1315, found 296.1317

3d: methyl N-acetyl-S-(4-ethynylphenyl)-L-cysteinate

methyl N-acetyl-S-(4-ethynylphenyl)-L-cysteinate (3d) was made according to GP1 on a 1.0 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 1:1) yielding 136.4 (0.49 mmol, 49 %) of 3d as a yellow oil. The reaction according to GP2 on a 1.75 mmol scale afforded 296.3 mg (1.07 mmol, 61%) of 3d within 60 seconds residence time.

1H NMR (399 MHz, Chloroform-d) δ 7.42 – 7.30 (m, 4H), 6.24 (d, J = 7.4 Hz, 1H), 4.87 (dt, J = 7.4, 4.5 Hz, 1H), 3.59 (s, 3H), 3.44 (ddd, J = 57.0, 14.3, 4.5 Hz, 2H), 3.10 (s, 1H), 1.90 (s, 3H). 13C NMR (100 MHz, Chloroform-d) δ 170.7, 169.9, 136.4, 132.7, 129.9, 120.6, 83.0, 78.2, 77.5, 77.2, 76.8, 52.8, 52.4, 35.9, 23.1. HRMS (ESI) m/z [M + H]+ calculated for C14H16NO3S: 278.0846, found 278.0853

3e: methyl N-acetyl-S-(4-methoxy-2-methylphenyl)-L-cysteinate

methyl N-acetyl-S-(4-methoxy-2-methylphenyl)-L-cysteinate (3e) was made according to GP1 on a 1.0 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 1:1) yielding 83.4 mg (0.28 mmol, 28 %) of 3e as a yellow oil.

1H NMR (399 MHz, Chloroform-d) δ 7.37 (d, J = 8.54 Hz, 1H), 6.76 (d, J = 2.77 Hz, 1H), 6.69 (dd, J = 8.53, 2.84 Hz, 1H), 6.18 (d, J = 6.91 Hz, 1H), 4.79 (dt, J = 7.54, 4.58 Hz, 1H), 3.77 (s, 3H), 3.57 (s, 3H), 3.34 – 3.16 (m, 2H), 2.43 (s, 3H), 1.91 (s, 3H). 13C NMR (100 MHz, Chloroform-d) δ 171.0, 169.8, 159.7, 142.4, 135.4, 124.0, 116.2, 112.2, 55.4, 52.6, 52.5, 37.2, 23.1, 21.2. HRMS (ESI) m/z [M + H]+ calculated for C14H20NO4S: 298.1108, found 298.1113

3f: methyl N-acetyl-S-(2-methyl-4-nitrophenyl)-L-cysteinate

methyl N-acetyl-S-(2-methyl-4-nitrophenyl)-L-cysteinate (3f) was made according to GP1 on a 1.0 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 1:1) yielding 206.2 (0.66 mmol,
66 %) of 3f as a yellow oil. The reaction according to GP2 on a 1.85 mmol scale afforded 421.4 mg (1.35 mmol, 73%) of 3f within 30 seconds residence time.

\[ \text{3f: methyl N-acetyl-S-(4-fluorophenyl)-L-cysteinate} \]

\[
\begin{array}{c}
\text{MeO} \\
\text{S} \\
\text{Me} \\
\text{H} \\
\text{F}
\end{array}
\]

methyl N-acetyl-S-(4-fluorophenyl)-L-cysteinate (3g) was made according to GP1 on a 1.0 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 1:1) yielding 159.9 mg (0.59 mmol, 59%) of 3g as a yellow oil. The reaction according to GP2 on a 1.85 mmol scale afforded 396.1 mg (1.46 mmol, 79%) of 3g within 30 seconds residence time.

\[ \text{1H NMR (400 MHz, Chloroform-}d\text{) } \delta 7.51 – 7.36 (m, 2H), 7.10 – 6.94 (m, 2H), 6.20 (s, 1H), 4.83 (dt, J = 7.50, 4.55 Hz, 1H), 3.58 (s, 3H), 3.43 (dd, J = 14.28, 4.56 Hz, 1H), 3.30 (dd, J = 14.28, 4.56 Hz, 1H), 1.92 (s, 3H). \]

\[ \text{13C NMR (101 MHz, Chloroform-}d\text{) } \delta 170.8, 169.8, 162.3 (d, J = 246.57 Hz), 134.5, 129.9 (d, J = 8.13 Hz), 124.7 (d, J = 3.81 Hz), 121.4 (d, J = 17.47 Hz), 116.1 (d, J = 22.76 Hz), 52.6, 52.3, 36.0, 23.1. \]

\[ \text{19F NMR (376 MHz, Chloroform-}d\text{) } \delta -105.69 – -111.27 (m). \]

\[ \text{HRMS (ESI) m/z } [\text{M+H}]^+ \text{ calculated for } C_{12}H_{15}FNO_3S: 272.0751, \text{ found 272.0750.} \]

\[ \text{3g: methyl N-acetyl-S-(4-fluorophenyl)-L-cysteinate} \]

\[ \text{methyl N-acetyl-S-(2-fluorophenyl)-L-cysteinate (3h) was made according to GP1 on a 1.0 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 1:1) yielding 189.9 mg (0.70 mmol, 70%) of 3h as a yellow oil.} \]

\[ \text{1H NMR (400 MHz, Chloroform-}d\text{) } \delta 7.45 (td, J = 7.84, 1.76 Hz, 1H), 7.31 – 7.19 (m, 1H), 7.13 – 7.01 (m, 2H), 6.28 (d, J = 6.26 Hz, 1H), 5.14 – 4.59 (m, 1H), 3.54 (s, 3H), 3.40 (dd, J = 4.45, 3.27 Hz, 2H), 1.92 (s, 3H). \]

\[ \text{13C NMR (101 MHz, Chloroform-}d\text{) } \delta 170.7, 169.8, 162.3 (d, J = 246.57 Hz), 134.5, 129.9 (d, J = 8.02 Hz), 124.7 (d, J = 3.81 Hz), 121.4 (d, J = 17.47 Hz), 116.1 (d, J = 22.76 Hz), 52.6, 52.3, 36.0, 23.1. \]

\[ \text{19F NMR (376 MHz, Chloroform-}d\text{) } \delta -105.69 – -111.27 (m). \]

\[ \text{HRMS (ESI) m/z } [\text{M+H}]^+ \text{ calculated for } C_{12}H_{15}FNO_3S: 272.0751, \text{ found 272.0750.} \]

\[ \text{3h: methyl N-acetyl-S-(2-fluorophenyl)-L-cysteinate} \]

\[ \text{methyl N-acetyl-S-(perfluorophenyl)-L-cysteinate (3i) was made according to GP1 on a 1.0 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 1:1} \]

\[ \text{1H NMR (400 MHz, Chloroform-}d\text{) } \delta 7.45 (td, J = 7.84, 1.76 Hz, 1H), 7.31 – 7.19 (m, 1H), 7.13 – 7.01 (m, 2H), 6.28 (d, J = 6.26 Hz, 1H), 5.14 – 4.59 (m, 1H), 3.54 (s, 3H), 3.40 (dd, J = 4.45, 3.27 Hz, 2H), 1.92 (s, 3H). \]

\[ \text{13C NMR (101 MHz, Chloroform-}d\text{) } \delta 170.7, 169.8, 162.3 (d, J = 246.57 Hz), 134.5, 129.9 (d, J = 8.02 Hz), 124.7 (d, J = 3.81 Hz), 121.4 (d, J = 17.47 Hz), 116.1 (d, J = 22.76 Hz), 52.6, 52.3, 36.0, 23.1. \]

\[ \text{19F NMR (376 MHz, Chloroform-}d\text{) } \delta -105.69 \] -111.27 (m). \]

\[ \text{HRMS (ESI) m/z } [\text{M+H}]^+ \text{ calculated for } C_{12}H_{15}FNO_3S: 272.0751, \text{ found 272.0750.} \]

\[ \text{3i: methyl N-acetyl-S-(perfluorophenyl)-L-cysteinate} \]
methyl N-acetyl-S-(perfluorophenyl)-L-cysteinate (3i) was made according to GP2 on a 1.725 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 1:1) yielding 235.6 mg (0.67 mmol, 40%) of 3i as a yellow oil within 150 seconds residence time.

\[
\text{H NMR (400 MHz, Chloroform-}d\text{) }\delta 6.35 (d, J = 6.21 Hz, 1H), 4.79 (dt, J = 7.11, 4.62 Hz, 1H), 3.67 (s, 3H), 3.51 – 3.24 (m, 2H), 2.00 (s, 3H). 13C NMR (101 MHz, Chloroform-}d\text{) }\delta 170.2, 169.7, 148.7, 146.4, 138.9, 132.8, 129.2, 111.1, 52.8, 52.0, 36.5, 22.9. 19F NMR (376 MHz, Chloroform-}d\text{) }\delta -131.69 (dd, J = 22.97, 8.69 Hz), -151.41 (t, J = 20.90 Hz), -160.52 (dt, J = 22.39, 6.40 Hz). HRMS (ESI) m/z [M + H]+ calculated for C12H11F5NO3S: 344.0380, found 344.0377.
\]

3j: methyl N-acetyl-S-(perfluoro-[1,1'-biphenyl]-4-yl)-L-cysteinate

methyl N-acetyl-S-(perfluoro-[1,1'-biphenyl]-4-yl)-L-cysteinate (3j) was made according to GP1 on a 1.0 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 1:1) yielding 206.4 mg (0.42 mmol, 42%) of 3j as a yellow oil. The reaction according to GP2 on a 1.875 mmol scale afforded 403.1 mg (0.84 mmol, 45%) of 3j within 30 seconds residence time.

\[
\text{H NMR (399 MHz, Chloroform-}d\text{) }\delta 6.42 (d, J = 7.09 Hz, 1H), 4.87 (dt, J = 7.04, 4.36 Hz, 1H), 3.65 (s, 3H), 3.61 – 3.50 (m, 2H), 2.00 (s, 3H). 13C NMR (100 MHz, Chloroform-}d\text{) }\delta 170.2, 170.1, 169.7, 148.4, 145.9, 143.3, 143.0, 142.8, 139.3, 136.9, 116.5, 52.9, 52.4, 36.1, 23.0. 19F NMR (376 MHz, Chloroform-}d\text{) }\delta -131.55 – -131.79 (m), -137.34 – -137.57 (m), -149.55 – -149.74 (m), -160.13 – -160.33 (m). HRMS (ESI) m/z [M + H]+ calculated for C18H11F9NO3S: 429.0310, found 429.0353.
\]

3k: methyl N-acetyl-S-(4-trifluoromethylphenyl)-L-cysteinate

methyl N-acetyl-S-(4-trifluoromethylphenyl)-L-cysteinate (3k) was made according to GP1 on a 1.0 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 1:1) yielding 192.6 mg (0.60 mmol, 60%) of 3k as a yellow oil. The reaction according to GP2 on a 1.700 mmol scale afforded 511.2 mg (1.59 mmol, 89%) of 3k within 30 seconds residence time.
$^1$H NMR (400 MHz, Chloroform-d) δ 7.51 (d, $J = 8.27$ Hz, 2H), 7.44 (d, $J = 8.21$ Hz, 2H), 6.38 (s, 1H), 4.87 (dt, $J = 7.31$, 4.70 Hz, 1H), 3.60 (s, 3H), 3.55 (dd, $J = 14.19$, 4.78 Hz, 1H), 3.39 (dd, $J = 14.18$, 4.65 Hz, 1H), 1.88 (s, 3H).

$^{13}$C NMR (101 MHz, Chloroform-d) δ 170.5, 169.9, 152.8, 140.3, 129.2, 125.7 (q, $J = 3.81$ Hz), 125.2, 52.6, 52.2, 35.2, 22.8.  $^{19}$F NMR (376 MHz, Chloroform-d) δ -62.67. HRMS (ESI) m/z [M+H]$^+$ calculated for C$_{13}$H$_{15}$F$_3$NO$_3$S: 322.0718, found 322.0724.

3l: methyl N-acetyl-S-(3-trifluoromethylphenyl)-L-cysteinate

methyl N-acetyl-S-(3-trifluoromethylphenyl)-L-cysteinate (3l) was made according to GP2 on a 1.775 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 1:1) yielding 518.9 mg (1.62 mmol, 81%) of 3l as a yellow oil within 30 seconds residence time.

$^1$H NMR (400 MHz, Chloroform-d) δ 7.63 (s, 1H), 7.56 (d, $J = 7.58$ Hz, 1H), 7.43 (dt, $J = 15.53$, 7.84 Hz, 2H), 6.32 (s, 1H), 4.66 (dt, $J = 7.36$, 4.51 Hz, 1H), 3.95 (s, 1H), 3.54 (dd, $J = 14.32$, 4.74 Hz, 1H), 3.41 (dd, $J = 14.25$, 4.39 Hz, 1H), 1.91 (s, 3H).  $^{13}$C NMR (101 MHz, Chloroform-d) δ 170.7, 169.9, 136.7, 133.6, 131.6 (q, $J = 32.51$ Hz), 129.6, 127.0 (q, $J = 3.86$ Hz), 123.8 (q, $J = 272.59$ Hz), 123.7 (q, $J = 3.76$ Hz), 52.8, 52.4, 36.3, 23.1.  $^{19}$F NMR (188 MHz, Chloroform-d) δ -62.86. HRMS (ESI) m/z [M+H]$^+$ calculated for C$_{13}$H$_{15}$F$_3$NO$_3$S: 322.0718, found 322.0730.

3m: methyl N-acetyl-S-(4-trifluoromethoxyphenyl)-L-cysteinate

methyl N-acetyl-S-(4-trifluoromethoxyphenyl)-L-cysteinate (3m) was made according to GP2 on a 1.85 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 1:1) yielding 389.0 mg (1.15 mmol, 62%) of 3m as a yellow oil within 30 seconds residence time.

$^1$H NMR (400 MHz, Chloroform-d) δ 7.49 – 7.39 (m, 2H), 7.20 – 7.08 (m, 2H), 6.22 (d, $J = 6.2$ Hz, 1H), 4.94 – 4.78 (m, 1H), 3.57 (s, 3H), 3.56 – 3.28 (m, 2H), 1.89 (s, 3H).  $^{13}$C NMR (101 MHz, Chloroform-d) δ 170.7, 169.9, 148.4, 133.7, 132.6, 121.8, 119.2, 52.6, 52.5, 36.9, 23.0.  $^{19}$F NMR (188 MHz, Chloroform-d) δ -58.01. HRMS (ESI) m/z [M+H]$^+$ calculated for C$_{13}$H$_{15}$F$_3$NO$_4$S: 338.0669, found 338.0673. HRMS (ESI) m/z [M+H]$^+$ calculated for C$_{13}$H$_{15}$F$_3$NO$_4$S: 338.0674, found 338.0673.

3n: methyl N-acetyl-S-(2-bromophenyl)-L-cysteinate

methyl N-acetyl-S-(2-bromophenyl)-L-cysteinate (3n) was made according to GP1 on a 1.0 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 1:1) yielding 254.7 mg (0.79 mmol, 79%) of 3n as a yellow oil.
$^1$H NMR (400 MHz, Chloroform-d) δ 7.53 – 7.46 (m, 1H), 7.37 (dd, J = 7.85, 1.18 Hz, 1H), 7.24 – 7.17 (m, 1H), 7.01 (td, J = 7.87, 1.30 Hz, 1H), 6.32 (d, J = 6.17 Hz, 1H), 4.81 (dt, J = 7.47, 4.74 Hz, 1H), 3.57 (s, 3H), 3.39 (qd, J = 14.01, 4.75 Hz, 2H), 1.86 (s, 3H). $^{13}$C NMR (101 MHz, Chloroform-d) δ 170.7, 169.9, 135.9, 133.4, 131.5, 128.3, 128.0, 125.9, 52.8, 52.1, 35.9, 23.1. HRMS (ESI) m/z [M+H]$^+$ calculated for $C_{12}H_{15}BrNO_3S$: 331.9951, found 331.9951.

$3o$: methyl N-acetyl-S-(2-chlorophenyl)-L-cysteinate

methyl N-acetyl-S-(2-chlorophenyl)-L-cysteinate ($3o$) was made according to GP1 on a 1.0 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 1:1) yielding 224.7 mg (0.78 mmol, 78%) of $3o$ as a yellow oil.

$^1$H NMR (400 MHz, Chloroform-d) δ 7.41 (ddd, J = 24.53, 7.74, 1.61 Hz, 2H), 7.18 (dtd, J = 20.96, 7.47, 1.57 Hz, 2H), 6.36 (d, J = 6.68 Hz, 1H), 4.86 (dt, J = 7.46, 4.71 Hz, 1H), 3.61 (s, 3H), 3.51 – 3.37 (m, 2H), 1.92 (s, 3H). $^{13}$C NMR (101 MHz, Chloroform-d) δ 170.7, 169.9, 135.6, 133.8, 131.9, 130.1, 127.4, 52.7, 52.1, 35.5, 23.1. HRMS (ESI) m/z [M+H]$^+$ calculated for $C_{12}H_{15}ClNO_3S$: 288.0456, found 288.0463.

$3p$: methyl N-acetyl-S-(4-chlorophenyl)-L-cysteinate

methyl N-acetyl-S-(4-chlorophenyl)-L-cysteinate ($3p$) was made according to GP1 on a 1.0 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 1:1) yielding 117.6 mg (0.62 mmol, 62%) of $3p$ as a brown oil.

$^1$H NMR (399 MHz, Chloroform-d) δ 7.31 (d, J = 8.69 Hz, 2H), 7.24 (d, J = 8.69 Hz, 2H), 6.34 (d, J = 6.91 Hz, 1H), 4.82 (dt, J = 7.48, 4.71 Hz, 1H), 3.57 (s, 3H), 3.37 (ddd, J = 55.08, 14.21, 4.72 Hz, 2H), 1.89 (s, 3H). $^{13}$C NMR (100 MHz, Chloroform-d) δ 170.7, 169.9, 133.4, 133.2, 132.3, 129.2, 52.6, 52.3, 36.7, 23.0. HRMS (ESI) m/z [M+H]$^+$ calculated for $C_{12}H_{15}ClNO_3S$: 288.0456, found 288.0461.

$3q$: methyl N-acetyl-S-(4-iodophenyl)-L-cysteinate

methyl N-acetyl-S-(4-iodophenyl)-L-cysteinate ($3q$) was made according to GP1 on a 1.0 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 1:1) yielding 133.2 mg (0.35 mmol, 35%) of $3q$ as a yellow oil.
$^1$H NMR (399 MHz, Chloroform-d) δ 7.60 (d, $J = 8.52$ Hz, 2H), 7.12 (d, $J = 8.52$ Hz, 2H), 6.24 (d, $J = 6.69$ Hz, 1H), 4.84 (dt, $J = 7.38$, 4.59 Hz, 1H), 3.59 (s, 3H), 3.52 – 3.26 (m, 2H), 1.90 (s, 3H). $^{13}$C NMR (100 MHz, Chloroform-d) δ 170.7, 169.8, 138.1, 135.0, 132.5, 131.2, 129.2, 92.2, 52.7, 52.4, 36.4, 23.1. HRMS (ESI) m/z [M+H]$^+$ calculated for C$_{12}$H$_{15}$INO$_3$S: 379.9812, found 379.9817.

3r: methyl N-acetyl-S-(3-iodophenyl)-L-cysteinate

methyl N-acetyl-S-(3-iodophenyl)-L-cysteinate (3r) was made according to GP1 on a 1.0 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 1:1) yielding 155.6 mg (0.41 mmol, 41%) of 3r as a yellow oil.

$^1$H NMR (399 MHz, Chloroform-d) δ 7.73 (t, $J = 1.65$ Hz, 1H), 7.53 (dt, $J = 7.85$, 1.17 Hz, 1H), 7.34 (dt, $J = 7.83$, 1.26 Hz, 1H), 7.00 (t, $J = 7.87$ Hz, 1H), 6.29 (d, $J = 5.83$ Hz, 1H), 4.87 (dt, $J = 7.32$, 4.43 Hz, 1H), 3.60 (s, 3H), 3.41 (ddd, $J = 51.73$, 14.25, 4.44 Hz, 2H), 1.92 (s, 3H). $^{13}$C NMR (100 MHz, Chloroform-d) δ 170.7, 169.9, 138.8, 137.4, 136.0, 130.6, 129.7, 94.6, 52.8, 52.4, 36.3, 23.1. HRMS (ESI) m/z [M+H]$^+$ calculated for C$_{12}$H$_{15}$INO$_3$S: 379.9812, found 379.9824.

3s: methyl N-acetyl-S-(4-acetylphenyl)-L-cysteinate

methyl N-acetyl-S-(4-acetylphenyl)-L-cysteinate (3s) was made according to GP1 on a 1.0 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 1:1) yielding 230.6 mg (0.78 mmol, 78%) of 3s as a yellow oil.

$^1$H NMR (400 MHz, Chloroform-d) δ 7.87 (d, $J = 8.52$ Hz, 2H), 7.40 (d, $J = 8.52$ Hz, 2H), 6.24 (d, $J = 6.76$ Hz, 1H), 4.91 (dt, $J = 7.24$, 4.67 Hz, 1H), 3.65 (s, 3H), 3.52 (ddd, $J = 62.48$, 14.15, 4.68 Hz, 2H), 2.57 (s, 3H), 1.91 (s, 3H). $^{13}$C NMR (101 MHz, Chloroform-d) δ 197.2, 170.7, 169.9, 142.3, 135.0, 129.0, 128.4, 52.9, 52.3, 34.9, 26.7, 23.1. HRMS (ESI) m/z [M+H]$^+$ calculated for C$_{14}$H$_{18}$NO$_4$S: 296.0951, found 296.0946.

3t: methyl (R)-2-((2-acetamido-3-methoxy-3-oxopropyl)thio)benzoate

methyl (R)-2-((2-acetamido-3-methoxy-3-oxopropyl)thio)benzoate (3t) was made according to GP1 on a 1.0 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 1:1) yielding 233.6 mg (0.75 mmol, 75%) of 3t as a yellow oil.
$^1$H NMR (399 MHz, Chloroform-$d$) $\delta$ 7.85 (d, $J = 7.55$ Hz, 1H), 7.46 (s, 2H), 7.25 – 7.19 (m, 1H), 6.55 (s, 1H), 5.02 – 4.72 (m, 1H), 3.92 (s, 3H), 3.68 (s, 3H), 3.57 – 3.36 (m, 2H), 1.91 (s, 3H). $^{13}$C NMR (101 MHz, Chloroform-$d$) $\delta$ 170.8, 170.1, 167.3, 138.3, 132.3, 130.9, 130.2, 128.6, 125.5, 52.8, 52.4, 51.9, 35.2, 23.0. HRMS (ESI) m/z [M+H]$^+$ calculated for C$_{14}$H$_{18}$NO$_5$S: 312.0900, found 312.0885.

3u: methyl N-acetyl-S-(4-acetylphenyl)-L-cysteinate

$m$ethyl N-acetyl-S-(6-chloropyridin-3-yl)-L-cysteinate (3u) was made according to GP2 on a 1.875 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 1:1) yielding 371.1 mg (1.29 mmol, 69%) of 3u as a yellow oil within 30 seconds residence time.

$^1$H NMR (400 MHz, Chloroform-$d$) $\delta$ 8.39 (dd, $J = 2.6$, 0.7 Hz, 1H), 7.71 (dd, $J = 8.3$, 2.6 Hz, 1H), 7.29 – 7.26 (m, 1H), 6.24 (d, $J = 7.0$ Hz, 1H), 4.84 (dt, $J = 7.89$, 4.18 Hz, 1H), 3.64 (s, 3H), 3.53 – 3.30 (m, 2H), 1.96 (s, 3H). $^{13}$C NMR (101 MHz, Chloroform-$d$) $\delta$ 170.5, 169.9, 151.4, 150.4, 141.3, 131.1, 124.6, 52.9, 52.3, 36.8, 23.1. HRMS (ESI) m/z [M+H]$^+$ calculated for C$_{11}$H$_{13}$ClN$_2$O$_3$S: 289.0408, found 289.0422.

4: methyl (tert-butoxycarbonyl)-L-alanyl-L-cysteinate

4 was made according to the 2-step native chemical ligation procedure (see Page 8). The crude product was purified by flash chromatography (DCM 100%) yielding 4 as a yellow solid.

$^1$H NMR (200 MHz, Chloroform-$d$) $\delta$ 7.02 (d, $J = 5.04$ Hz, 1H), 5.05 (s, 1H), 4.84 (dt, $J = 7.89$, 4.18 Hz, 1H), 4.30 – 4.06 (m, 1H), 3.78 (s, 3H), 2.99 (ddd, $J = 9.04$, 4.20, 1.82 Hz, 2H), 1.44 (s, 9H), 1.41 – 1.33 (m, 4H). $^{13}$C NMR (101 MHz, Chloroform-$d$) $\delta$ 172.7, 170.4, 155.6, 53.8, 53.5, 52.9, 28.4, 26.8, 13.7. HRMS (ESI) m/z [M-BOC+2H]$^+$ calculated for C$_7$H$_{15}$N$_2$O$_3$S: 207.0803, found 207.0798.

8a: Methyl-N-((tert-butoxycarbonyl)-L-alanyl)-S-(4-(trifluoromethyl)phenyl)-L-cysteinate

8a was made according to GP1 on a 0.26 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 2:1) yielding 70.2 mg (0.156 mmol, 60%) of 8a as a yellow solid.
\[ ^1\text{H NMR (200 MHz, Chloroform-}d\text{)} \delta 7.50 (q, J = 9.10\text{ Hz}, 4H), 6.94 \text{ (s, 1H), 4.90} \text{ – 4.75 (m, 1H), 4.71 (d, J = 8.42 Hz, 1H), 4.22} \text{ – 4.00 (m, 1H), 3.62 \text{ (s, 3H), 3.54} \text{ – 3.43 (m, 2H), 1.46 \text{ (s, 9H), 1.29 \text{ (d, J = 7.11 Hz, 3H),}}}
\]

\[ ^{19}\text{F NMR (376 MHz, Chloroform-}d\text{)} \delta -62.67. \text{HRMS (ESI) m/z [M+H]}^+ \text{ calculated C}_{19}\text{H}_{26}\text{F}_{3}\text{N}_{2}\text{O}_{5}\text{S: 451.1509, found 451.0895.}
\]

**8b:** Methyl-N-((tert-butoxycarbonyl)-L-alanyl)-S-(4-fluorophenyl)-L-cysteinate

![Structure of 8b](image)

8b was made according to GP1 on a 0.125 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 2:1) yielding 28.2 mg (0.07 mmol, 56%) of 8b as a yellow solid.

\[ ^1\text{H NMR (400 MHz, Chloroform-}d\text{)} \delta 7.42 \text{ (dd, J = 8.89, 5.23 Hz, 2H), 7.00 \text{ (t, J = 8.63 Hz, 2H), 6.88 \text{ (s, 1H), 4.78 (s, 1H), 4.13 \text{ (s, 1H), 3.65 \text{ (s, 1H), 3.57 \text{ (s, 3H), 3.42} \text{ – 3.25 (m, 2H), 1.47 (s, 9H), 1.31 \text{ (d, J = 7.01 Hz, 3H).}}}}}
\]

\[ ^{19}\text{F NMR (376 MHz, Chloroform-}d\text{)} \delta -113.89 \text{. HRMS (ESI) m/z [M-BOC+2H]}^+ \text{ calculated for C}_{13}\text{H}_{18}\text{FN}_{2}\text{O}_{3}\text{S: 301.1022, found 301.1033.}}
\]

**8c:** Methyl-N-((tert-butoxycarbonyl)-L-alanyl)-S-(4-tert-butyl-phenyl)-L-cysteinate

![Structure of 8c](image)

8c was made according to GP1 on a 0.25 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 2:1) yielding 46.6 mg (0.105 mmol, 42%) of 8c as a yellow solid.

\[ ^{1}\text{H NMR (400 MHz, Chloroform-}d\text{)} \delta 7.34 \text{ (q, J = 8.64 Hz, 4H), 6.87 \text{ (d, J = 7.00 Hz, 1H), 4.81 (dt, J = 7.61, 4.51 Hz, 1H), 4.59} \text{ – 4.44 (m, 1H), 4.12 \text{ (s, 1H), 3.56 \text{ (s, 3H), 3.38 \text{ (ddd, J = 47.26, 14.35, 4.45 Hz, 2H), 1.47 \text{ (s, 9H), 1.29 (s, 9H), 1.26} \text{ – 1.20 (m, 3H).}}}}
\]

\[ ^{13}\text{C NMR (101 MHz, Chloroform-}d\text{)} \delta 172.2, 170.5, 150.7, 131.5, 131.4, 126.4, 126.3, 52.7, 52.6, 50.0, 35.8 \text{ (d, J = 223.40 Hz), 31.4, 28.5, 18.1. HRMS (ESI) m/z [M-BOC+2H]}^+ \text{ calculated for C}_{17}\text{H}_{27}\text{N}_{2}\text{O}_{3}\text{S: 339.1742, found 339.1760.}}
\]

**5:** methyl (tert-butoxycarbonyl)-L-leucyl-L-cysteinate

![Structure of 5](image)
5 was made according to the 2-step native chemical ligation procedure (see Page 8). The crude product was purified by flash chromatography (DCM 100%) yielding 5 as a yellow solid.

\[^{1}\text{H NMR (400 MHz, Chloroform-}d\text{)} \delta 6.96 (d, J = 6.92 Hz, 1H), 4.91 (d, J = 7.59 Hz, 1H), 4.87 – 4.77 (m, 1H), 4.19 – 4.05 (m, 1H), 3.78 (s, 3H), 3.11 – 2.89 (m, 2H), 1.75 – 1.60 (m, 2H), 1.48 – 1.46 (m, 1H), 1.44 (s, 9H), 1.39 – 1.34 (m, 1H), 0.94 (t, J = 6.11 Hz, 6H). \[^{13}\text{C NMR (101 MHz, Chloroform-}d\text{)} \delta 172.6, 170.4, 155.8, 80.3, 53.8, 53.3, 52.8, 41.0, 28.4, 26.7, 24.8, 23.0. \] HRMS (ESI) m/z [M-BOC+2H\(^+\)] calculated for \(\text{C}_{10}\text{H}_{21}\text{N}_{2}\text{O}_{3}\text{S}: 249.1273\), found 249.1269.

\[^{9}\text{a: Methyl-N-((tert-butoxycarbonyl)-L-leucyl)-S-(4-(trifluoromethylphenyl)-L-cysteinate}}\]

\[^{9}\text{a} \text{was made according to GP1 on a 0.25 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 2:1) yielding 75.2 mg (0.15 mmol, 61\%) of \[^{9}\text{a} \text{as a yellow solid. The reaction according to GP2 on a 0.9 mmol scale afforded 366.2 mg (0.75 mmol, 86\%) of \[^{9}\text{a} \text{within 30 seconds residence time.}}\]

\[^{1}\text{H NMR (399 MHz, Chloroform-}d\text{)} \delta 7.58 – 7.43 (m, 1H), 6.92 (d, J = 6.26 Hz, 4H), 4.83 (dt, J = 7.18, 4.97 Hz, 1H), 4.62 (d, J = 4.71 Hz, 1H), 4.14 – 4.04 (m, 1H), 3.62 (s, 3H), 3.55 – 3.38 (m, 2H), 1.71 – 1.59 (m, 2H), 1.45 (s, 9H), 1.41 – 1.31 (m, 1H), 0.92 (t, J = 5.82 Hz, 6H). \[^{13}\text{C NMR (100 MHz, Chloroform-}d\text{)} \delta 206.9, 172.8, 170.3, 155.6, 140.5, 129.1, 128.2 (q, J = 34.47 Hz), 125.7 (q, J = 3.68 Hz), 122.6 (q, J = 272.27 Hz), 114.0, 53.0, 52.5, 51.9, 40.9, 35.1, 30.8, 28.2, 24.6, 22.9, 21.7. \[^{19}\text{F NMR (188 MHz, Chloroform-}d\text{)} \delta -62.60. \] HRMS (ESI) m/z [M-BOC+2H\(^+\)] calculated for \(\text{C}_{17}\text{H}_{24}\text{F}_{3}\text{N}_{2}\text{O}_{3}\text{S}: 393.1460\), found 393.1498.

\[^{9}\text{b: Methyl-N-((tert-butoxycarbonyl)-L-leucyl)-S-(4-fluorophenyl)-L-cysteinate}}\]

\[^{9}\text{b} \text{was made according to GP1 on a 0.25 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 4:1) yielding 51.1 mg (0.12 mmol, 46\%) of \[^{9}\text{b} \text{as a yellow solid. The reaction according to GP2 on a 0.75 mmol scale afforded 260.2 mg (0.59 mmol, 78\%) of \[^{9}\text{b} \text{within 30 seconds residence time.}}\]

\[^{1}\text{H NMR (399 MHz, Chloroform-}d\text{)} \delta 7.52 (d, J = 8.49 Hz, 2H), 7.45 (d, J = 8.24 Hz, 2H), 6.96 (d, J = 5.60 Hz, 1H), 4.82 (dt, J = 7.31, 4.98 Hz, 1H), 4.68 (d, J = 6.88 Hz, 1H), 4.10 (s, 1H), 3.61 (s, 3H), 3.55 – 3.33 (m, 2H), 1.62 (s, 3H), 1.44 (s, 9H), 0.91 (t, J = 5.92 Hz, 6H). \[^{13}\text{C NMR (100 MHz, Chloroform-}d\text{)} \delta 172.7, 172.5, 170.4, 163.5, 161.0, 155.5 (d, J = 10.35 Hz), 134.0 (d, J = 8.09 Hz), 129.6 (d, J = 3.46 Hz), 116.2 (d, J = 21.85 Hz), 59.5, 53.5, 52.4, 51.9, 37.6, 29.8, 28.3, 24.7, 22.9, 21.8. \[^{19}\text{F NMR (376 MHz, Chloroform-}d\text{)} \delta -126.91 (tt, J = 8.66, 4.46 Hz). \] HRMS (ESI) m/z [M-BOC+2H\(^+\)] calculated for \(\text{C}_{16}\text{H}_{24}\text{F}_{2}\text{N}_{2}\text{O}_{3}\text{S}: 343.1492\), found 343.1503.

\[^{9}\text{c: Methyl-N-((tert-butoxycarbonyl)-L-leucyl)-S-(perfluorophenyl)-L-cysteinate}}\]
9c was made according to GP2 on a 0.9 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 7:1) yielding 185 mg (0.36 mmol, 40%) of 9c as a yellow solid within 150 seconds of residence time.

$^1$H NMR (399 MHz, Chloroform-d) $\delta$ 7.01 (d, $J = 7.23$ Hz, 1H), 4.86 (d, $J = 7.56$ Hz, 1H), 4.73 (dt, $J = 7.44, 4.93$ Hz, 1H), 4.16 – 4.00 (m, 1H), 3.65 (s, 3H), 3.36 (d, $J = 4.94$ Hz, 2H), 1.76 – 1.58 (m, 3H), 1.44 (s, 9H), 0.93 (t, $J = 6.59$ Hz, 6H). $^{19}$F NMR (188 MHz, Chloroform-d) $\delta$ -131.77 (dd, $J = 23.92, 7.71$ Hz), -151.44 (t, $J = 20.87$ Hz), -160.50 (dt, $J = 21.09, 11.73$ Hz). $^{13}$C NMR (100 MHz, Chloroform-d) $\delta$ 172.6, 169.9, 155.6, 148.9, 139.0, 136.4, 107.9, 100.0, 80.4, 53.2, 52.7, 51.9, 41.0, 36.4, 28.2, 24.7, 22.9, 21.8. HRMS (ESI) m/z [M-BOC+2H]$^+$ calculated for C$_{16}$H$_{20}$F$_5$N$_2$O$_3$S: 415.1115, found 415.1113.

9d: Methyl-N-((tert-butoxycarbonyl)-L-leucyl)-S-(4-tert-butylphenyl)-L-cysteinate

9d was made according to GP1 on a 0.25 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 4:1) yielding 38.6 mg (0.08 mmol, 46%) of 9d as a yellow solid. The reaction according to GP2 on a 0.73 mmol scale afforded 215 mg (0.45 mmol, 61%) of 9d within 30 seconds residence time.

$^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.32 (q, $J = 8.60$ Hz, 1H), 6.97 (d, $J = 7.16$ Hz, 4H), 4.78 (dt, $J = 7.59, 4.77$ Hz, 1H), 4.60 (d, $J = 7.59$ Hz, 1H), 4.11 (s, 1H), 3.52 (s, 3H), 3.33 (m, 2H), 1.72 – 1.51 (m, 2H), 1.45 (s, 9H), 1.27 (s, 9H), 1.22 – 1.16 (m, 1H), 0.95 – 0.86 (m, 6H). $^{13}$C NMR (100 MHz, Chloroform-d) $\delta$ 172.4, 170.5, 155.6, 150.5, 131.5, 131.2, 126.2, 80.2, 64.3, 52.4, 41.0, 36.9, 34.6, 31.3, 28.4, 25.3, 24.7, 23.1, 21.8. HRMS (ESI) m/z [M-BOC+2H]$^+$ calculated for C$_{20}$H$_{33}$N$_2$O$_3$S: 381.2212, found 381.2244.

6: methyl (tert-butoxycarbonyl)-L-tryptophyl-L-cysteinate

6 was made according to the 2-step native chemical ligation procedure (see Page 8). The crude product was purified by flash chromatography (DCM 100%) yielding 6 as a yellow solid.
**1H NMR (399 MHz, Chloroform-d) δ 8.22 (s, 1H), 7.64 (d, J = 7.88 Hz, 1H), 7.36 (d, J = 8.10 Hz, 1H), 7.20 (t, J = 7.53 Hz, 1H), 7.17 – 7.07 (m, 2H), 6.70 (d, J = 6.95 Hz, 1H), 5.15 (d, J = 6.77 Hz, 1H), 4.75 – 4.64 (m, 1H), 4.48 (d, J = 7.69 Hz, 1H), 3.68 (s, 3H), 3.40 (dd, J = 14.62, 5.31 Hz, 1H), 3.17 (dd, J = 14.60, 6.75 Hz, 1H), 2.98 – 2.75 (m, 2H), 1.44 (s, 9H), 0.94 (t, J = 7.15, 1.62 Hz, 1H).**

**13C NMR (100 MHz, Chloroform-d) δ 171.8, 170.1, 155.5, 136.4, 127.6, 123.3, 122.5, 120.0, 118.9, 111.4, 110.5, 55.3, 53.9, 52.8, 28.4, 28.0, 26.7.**

**HRMS (ESI) m/z [M-BOC+2H]+ calculated for C15H20N3O3S: 322.1225, found 322.1217.**

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**1H NMR (400 MHz, Chloroform-d) δ 8.34 (s, 1H), 7.66 (d, J = 9.42 Hz, 1H), 7.37 (d, J = 8.06 Hz, 1H), 7.31 – 7.18 (m, 5H), 7.14 (t, J = 7.45 Hz, 1H), 7.09 (s, 1H), 6.71 (d, J = 7.41 Hz, 1H), 5.11 (s, 1H), 4.80 – 4.67 (m, 1H), 4.46 (s, 1H), 3.44 (s, 3H), 3.35 – 3.13 (m, 4H), 1.47 (s, 9H), 1.29 (s, 9H).**

**13C NMR (101 MHz, Chloroform-d) δ 171.5, 170.3, 161.7, 150.6, 143.0, 132.6, 131.4, 131.0, 129.5, 127.8, 126.2, 123.3, 122.4, 119.9, 119.0, 111.3, 52.4, 52.2, 37.1, 34.7, 31.3, 28.5, 22.1.**

**HRMS (ESI) m/z [M-BOC+2H]+ calculated for C25H32N3O3S: 454.2164, found 454.2161.**

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**7: methyl (tert-butoxycarbonyl)-L-phenylalanyl-L-cysteinate**

7 was made according to the 2-step native chemical ligation procedure (see Page 8). The crude product was purified by flash chromatography (DCM 100%) yielding 7 as a white solid.

**1H NMR (400 MHz, Chloroform-d) δ 7.25 – 7.10 (m, 5H), 6.75 (d, J = 6.84 Hz, 1H), 4.93 (s, 1H), 4.72 (dt, J = 7.42, 3.95 Hz, 1H), 4.41 – 4.21 (m, 1H), 3.68 (s, 3H), 3.11 – 2.98 (m, 2H), 2.96 – 2.81 (m, 2H), 1.36 (s, 9H), 1.35 – 1.28 (m, 1H).**

**13C NMR (100 MHz, Chloroform-d) δ 171.4, 170.1, 155.4, 136.5, 129.3, 128.6, 126.9, 80.1, 55.6, 53.9, 52.7, 38.0, 28.2, 26.6.**

**HRMS (ESI) m/z [M-BOC+2H]+ calculated for C13H19N2O3S: 283.1110, found 283.1159.**

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**11a: Methyl-N-((tert-butoxycarbonyl)-L-tryptophyl)-S-(4-tert-butylphenyl)-L-cysteinate**
**11a** was made according to GP1 on a 0.5 mmol scale. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate 4:1) yielding 144.7 mg (0.28 mmol, 55%) of **11a** as a yellow solid.

$^1$H NMR (400 MHz, Chloroform-$d$) $\delta$ 7.53 (d, $J$ = 8.14 Hz, 2H), 7.44 (d, $J$ = 8.20 Hz, 2H), 7.31 (q, $J$ = 8.24, 7.55 Hz, 3H), 7.22 (d, $J$ = 6.23 Hz, 2H), 6.80 (d, $J$ = 6.68 Hz, 1H), 4.91 – 4.78 (m, 2H), 4.46 – 4.27 (m, 1H), 3.60 (s, 3H), 3.44 (qd, $J$ = 14.07, 4.99 Hz, 2H), 3.18 – 2.92 (m, 2H), 1.43 (d, $J$ = 2334.78 Hz, 9H). $^{19}$F NMR (376 MHz, Chloroform-$d$) $\delta$ -62.54. $^{13}$C NMR (101 MHz, Chloroform-$d$) $\delta$ 171.3, 170.1, 140.3, 136.5, 129.4, 129.3, 128.8, 127.1, 125.9 (q, $J$ = 3.73 Hz), 125.4, 122.7, 53.6, 52.7, 52.2, 38.0, 35.5, 29.8, 28.3. HRMS (ESI) m/z [M -BOC+2H]$^+$ calculated for C$_{20}$H$_{22}$F$_3$N$_2$O$_3$S: 427.1298, found 427.1382
[Chemical structure image]

NMR spectrum with chemical shifts in ppm:
- 21.07, 21.92, 23.13, 36.61, 52.66, 52.80, 76.88 (CDCl₃), 77.20 (CDCl₃), 77.52 (CDCl₃), 128.95, 129.36, 138.50, 142.54, 169.85, 171.09
Eob-Ab-Cyc-DMe
**E6c-Ala-Cys\textsubscript{OMe}**

![Chemical Structure](image-url)
Eoc-Leu-Cys OMe

[Chemical Structure Image]

[Graph Image]
Eos Lau-Cys OMe
References:

1. Straathof, N. J. W.; Su, Y.; Hessel, V.; Noël, T., Nat. Protoc. 2015, 11, 10-21.
2. Markey, L.; Giordani, S.; Scanlan, E. M., J. Org. Chem. 2013, 78, 4270-4277.