Electrochemical Chemoselective Hydroxyl Group Transformation: Anthranilic Acyl modification of Tyrosine Bioconjugations

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1. General Information

Unless otherwise stated, analytical grade solvents and commercially available reagents were used without further purification. All solvents were analytical reagent or better and were degassed prior to use. The instrument for electrolysis was dual display potentiostat (DJS-292B) (made in China). The anode electrode is platinum plate electrodes (Φ 6 mm) and the cathode electrode is lead plate electrodes (15 mm×15 mm×0.3 mm). Thin layer chromatography (TLC) employed glass 0.25 mm silica gel plates. Flash chromatography columns were packed with 200-300 mesh silica gel in petroleum (boiling point is between 60-90°C). Gradient flash chromatography was conducted eluting with a continuous gradient from DCM to the indicated solvent, and they are listed as volume/volume ratios. High resolution mass spectra (HRMS) for polypeptides were measured with an Agilent 6224 instrument and accurate masses were reported for the molecular ion + Hydrogen (M+H) or molecular ion + Sodium (M+Na). The $^1$H, $^{13}$C and $^{19}$F NMR spectra were recorded on a Bruker Advance III (400 MHz) spectrometers with tetramethylsilane as an internal standard. All chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. For $^1$H NMR, chemical shifts (δ) are given in ppm relatives to internal standard (TMS at 0 ppm, CDCl$_3$ at 7.26 ppm, MeOH-$d_4$ at 3.31 ppm, DMSO-$d_6$ at 2.50 ppm). For $^{13}$C-NMR, chemical shifts (δ) were reported in ppm using solvent as internal standard (CDCl$_3$ at 77.00 ppm, MeOH-$d_4$ at 49.00 ppm, DMSO-d6 at 39.50 ppm).
2. Synthesis of Starting Materials

2.1 Synthesis of starting materials dipeptides\(^1\)[\(^2\)]

To a solution of Boc-L-tyrosine \(\text{A} (410 \text{ mg}, 2.0 \text{ mmol}, 1.0 \text{ equiv.})\) in 40 mL \(\text{CH}_2\text{Cl}_2\) was added HOBT (1-hydroxybenzotriazole) (2.4 mmol), EDCI (1-ethyl-3(3-dimethylpropylamine) carbodiimide) (2.4 mmol) and peptide \(\text{B} (2.0 \text{ mmol})\). The mixture was stirred for 10 min at room temperature, and then triethylamine (3.0 mmol) was added to the solution. The reaction was stirred overnight. After regular workup, the reaction mixture washed 2M hydrochloric acid solution (40 mL x 3) and \(\text{H}_2\text{O}\) (40 mL x 3). The organic layers were combined, dried over \(\text{Na}_2\text{SO}_4\), and concentrated. The resulting crude product was purified by flash chromatography (DCM/MeOH) to afford corresponding dipeptides \(2\text{aa-2ag, 2aj, 2ak}\).

Dipeptide \(2\text{aa Boc-Tyr-Gly-OMe}\), white solid. \(^1\text{H} \text{NMR (400 MHz, Chloroform-d)} \delta 7.65 \text{ (s, 1H)}, 7.54 \text{ (s, 1H)}, 6.99 \text{ (dt, } J = 6.1, 0.8 \text{ Hz, 2H)}, 6.68 - 6.63 \text{ (m, 2H)}, 6.44 \text{ (d, } J = 8.2 \text{ Hz, 1H), } 4.40 \text{ (dt, } J = 8.2, 5.6 \text{ Hz, 1H)}, 3.99 - 3.87 \text{ (m, 2H)}, 3.69 \text{ (s, 2H), } 3.15 - 3.04 \text{ (m, 2H), } 1.41 \text{ (s, 6H).}
$^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 172.25, 170.46, 156.17, 155.61, 130.62, 129.22, 115.74, 115.73, 79.37, 55.03, 52.28, 41.53, 37.72, 28.30.

![2ab](image)

Dipeptide 2ab Boc-Tyr-Val-OMe, white solid. $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.56 (s, 1H), 7.52 (d, $J = 9.5$ Hz, 1H), 6.99 (dt, $J = 6.0$, 0.9 Hz, 2H), 6.65 (d, $J = 6.0$ Hz, 2H), 6.38 (d, $J = 8.3$ Hz, 1H), 4.49 (d, $J = 8.4$ Hz, 1H), 4.11 (dd, $J = 9.4$, 5.6 Hz, 1H), 3.65 (s, 3H), 3.17 – 2.95 (m, 2H), 2.21 – 2.03 (m, 1H), 1.41 (s, 9H), 1.01 (d, $J = 5.4$ Hz, 3H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 173.43, 172.16, 156.42, 154.83, 130.64, 130.49, 115.78, 79.34, 57.76, 55.37, 52.40, 37.75, 30.35, 28.29, 19.06, 19.03.

![2ac](image)

Dipeptide 2ac Boc-Tyr-Leu-OMe, white solid. $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.60 (d, $J = 10.4$ Hz, 1H), 7.56 (s, 1H), 6.99 (dt, $J = 6.0$, 0.9 Hz, 2H), 6.68 – 6.62 (m, 2H), 6.38 (d, $J = 8.3$ Hz, 1H), 4.45 – 4.50 (m, 1H), 4.40 – 4.32 (m, 1H), 3.68 (s, 3H), 3.03 (qdt, $J = 9.9$, 5.7, 0.8 Hz, 2H), 1.69 – 1.55 (m, 3H), 1.41 (s, 9H), 0.90 (d, $J = 5.1$ Hz, 3H), 0.85 (d, $J = 5.2$ Hz, 3H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 173.30, 172.00, 156.09, 154.82, 130.64, 130.49, 115.69, 115.67, 79.35, 55.57, 52.40, 51.33, 40.76, 37.75, 28.29, 24.44, 22.52, 22.46.
Dipeptide 2ad **Boc-Tyr-Phe-OMe**, white solid. $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.69 (d, $J$ = 11.3 Hz, 1H), 7.57 (s, 1H), 7.26 (dd, $J$ = 1.7, 0.8 Hz, 2H), 7.25 – 7.22 (m, 3H), 7.02 (dt, $J$ = 6.1, 0.8 Hz, 2H), 6.66 (d, $J$ = 6.1 Hz, 2H), 6.34 (d, $J$ = 8.2 Hz, 1H), 4.59 (dt, $J$ = 9.0, 5.6 Hz, 1H), 4.49 (dt, $J$ = 9.0, 5.6 Hz, 1H), 4.09 (s, 3H), 3.12 – 2.92 (m, 4H), 1.41 (s, 9H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 171.74, 171.32, 156.09, 154.80, 136.71, 130.80, 130.55, 129.27, 129.18, 128.63, 127.01, 115.63, 79.39, 55.59, 53.79, 52.39, 37.76, 37.74, 28.29.

Dipeptide 2ae **Boc-Tyr-Ser-OMe**, white solid, $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.56 (s, 1H), 7.51 (d, $J$ = 9.6 Hz, 1H), 6.99 (dt, $J$ = 6.0, 0.9 Hz, 2H), 6.67 (d, $J$ = 6.0 Hz, 2H), 6.42 (d, $J$ = 8.2 Hz, 1H), 4.50 (dt, $J$ = 8.2, 5.6 Hz, 1H), 4.33 – 4.26 (m, 1H), 4.25 (s, 1H), 3.78 (td, $J$ = 5.6, 2.1 Hz, 2H), 3.69 (s, 3H), 3.12 – 3.00 (m, 2H), 1.41 (s, 9H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 172.28, 170.96, 156.22, 154.83, 130.58, 115.70, 79.34, 64.84, 55.57, 54.42, 52.61, 37.75, 28.28.
Dipeptide **2af Boc-Tyr-Cys-OMe**, white solid, $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.70 (d, $J = 8.5$ Hz, 1H), 7.56 (s, 1H), 6.99 (dd, $J = 6.4$, 2.2 Hz, 2H), 6.65 (d, $J = 6.0$ Hz, 2H), 6.42 (d, $J = 8.2$ Hz, 1H), 4.52 – 4.44 (m, 2H), 3.72 (s, 3H), 3.15 – 2.99 (m, 2H), 2.89 (s, 1H), 2.84 – 2.68 (m, 2H), 1.41 (s, 9H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 172.78, 171.95, 156.22, 154.83, 130.86, 130.49, 115.73, 79.32, 55.56, 53.97, 52.37, 37.75, 29.69, 28.29.

Dipeptide **2ag Boc-Tyr-Met-OMe**, light yellow solid, $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.62 (d, $J = 8.5$ Hz, 1H), 7.54 (s, 1H), 6.98 (dd, $J = 6.0$, 2.0 Hz, 2H), 6.66 (d, $J = 6.0$ Hz, 2H), 6.41 (d, $J = 8.2$ Hz, 1H), 4.49 (dt, $J = 8.2$, 5.6 Hz, 1H), 4.26 (dt, $J = 8.8$, 5.6 Hz, 1H), 3.69 (s, 3H), 3.07 – 2.98 (m, 2H), 2.69 – 2.55 (m, 2H), 2.07 (s, 3H), 2.11 – 1.93 (m, 2H), 1.42 (s, 9H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 173.06, 171.99, 156.08, 154.82, 130.64, 129.95, 115.64, 79.39, 52.47, 51.59, 37.74, 31.04, 30.82, 28.29, 14.78.

Dipeptide **2aj Methyl (R)-2-(2-((tert-butoxycarbonyl)amino)-3-(4-hydroxyphenyl)propamido)acrylate**, white solid, $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 8.99 (s, 1H), 7.55 (s, 1H), 6.99 (dd, $J = 6.0$, 2.0 Hz, 2H), 6.69 (d, $J = 6.0$ Hz, 2H), 6.43 (s, 1H), 5.91 (d, $J = 2.0$ Hz, 1H), 5.32 (d, $J = 2.0$ Hz, 1H), 4.49 (dt, $J = 8.2$, 5.6 Hz, 1H), 3.78 (s, 3H), 3.13 – 3.01 (m, 2H), 1.41 (s, 9H=).
Dipeptide 2ak Ethyl (R)-4-(2-((tert-butoxycarbonyl)amino)-3-(4-hydroxyphenyl)propanamido)butanoate, white solid, $^1$H NMR (400 MHz, Chloroform-d) δ 7.56 (s, 1H), 7.42 (s, 1H), 6.99 (dt, $J = 6.0, 0.9$ Hz, 2H), 6.65 (d, $J = 6.1$ Hz, 2H), 6.26 (d, $J = 8.2$ Hz, 1H), 4.38 (dt, $J = 8.2, 5.6$ Hz, 1H), 4.18 – 4.04 (m, 2H), 3.32 – 3.17 (m, 2H), 3.01 – 2.85 (m, 2H), 2.37 (td, $J = 5.7, 2.0$ Hz, 2H), 1.82 – 1.66 (m, 2H), 1.41 (s, 9H), 1.22 (t, $J = 6.4$ Hz, 3H). $^{13}$C NMR (101 MHz, Chloroform-d) δ 173.13, 172.17, 156.42, 155.62, 130.64, 130.49, 115.77, 79.34, 60.23, 54.94, 39.98, 37.74, 32.23, 28.29, 25.17, 14.19.

In a round bottomed flask, equipped with a stir bar, peptide A (2.0 mmol), HOBT (1-hydroxybenzotriazole) (2.4 mmol), EDCI (1-ethyl-3(3-dimethylpropylamine) carbodiimide) (2.4 mmol) (2.4 mmol), dichloromethane (40 mL) and peptide B (2.0 mmol) were combined and added. The mixture was stirred for 10 min at room temperature, and then, triethylamine (3.0 mmol) was added to the solution. The reaction was stirred overnight. After regular workup, the reaction mixture washed by saturated 2 M hydrochloric acid solution (40 mL x 3) and H$_2$O (40 mL x 3).
The organic layers were combined, dried over Na$_2$SO$_4$, and concentrated. The resulting crude product was purified by flash chromatography (DCM/MeOH) to afford corresponding dipeptides 2ah-i.

Dipeptide 2ah Ac-Trp-Try-OMe, light yellow solid, $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 8.06 (d, $J=8.3$ Hz, 1H), 7.72 (d, $J=9.0$ Hz, 1H), 7.60 (dd, $J=5.8$, 1.4 Hz, 1H), 7.55 (s, 1H), 7.34 (dd, $J=6.0$, 1.3 Hz, 1H), 7.16 – 7.08 (m, 2H), 7.07 (td, $J=5.9$, 1.3 Hz, 1H), 7.01 (dt, $J=6.0$, 0.9 Hz, 2H), 6.68 – 6.62 (m, 2H), 4.62 – 4.50 (m, 2H), 3.68 (s, 3H), 3.01 (qdt, $J=6.0$, 0.9 Hz, 2H), 1.91 (s, 3H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 172.40, 171.97, 171.29, 156.09, 136.71, 131.54, 129.91, 127.73, 123.46, 121.78, 120.03, 118.64, 115.66, 112.31, 110.09, 54.23, 53.68, 52.36, 37.33, 28.33, 22.61.

Dipeptide 2ai Methyl ((S)-2-((tert-butoxycarbonyl)amino)pent-4-ynoyl)-L-tyrosinate, white solid, $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.80 (d, $J=9.0$ Hz, 1H), 7.55 (s, 1H), 7.01 (dt, $J=6.0$, 0.9 Hz, 2H), 6.64 (d, $J=6.0$ Hz, 2H), 6.32 (d, $J=7.4$ Hz, 1H), 4.56 – 4.50 (m, 1H), 4.47 – 4.39 (m, 1H), 3.67 (s, 3H), 3.02 – 2.99 (m, 2H), 2.74 – 2.61 (m, 3H), 1.41 (s, 9H). $^{13}$C NMR (101
MHz, Chloroform-d) δ 171.63, 171.42, 156.42, 155.09, 131.39, 129.98, 115.72, 80.61, 79.34, 73.04, 53.67, 53.04, 52.36, 37.3, 28.28, 24.03.

2.2 Synthesis of starting materials tripeptides [3]

In a round bottomed flask, equipped with a stir bar, peptide A (2 mmol), HOBT (1-hydroxybenzotriazole) (2.4 mmol), EDCI (1-ethyl-3(3-dimethylpropylamine) carbodiimide) (2.4 mmol) (2.4 mmol), dichloromethane (40 mL) and peptide B (2.0 mmol) were combined and added. The mixture was stirred for 10 min at room temperature, and then, triethylamine (3.0 mmol) was added to the solution. The reaction was stirred overnight. After regular workup, the reaction mixture washed by saturated 2 M hydrochloric acid solution (40 mL x 3) and H₂O (40 mL x 3). The organic layers were combined, dried over Na₂SO₄, and concentrated. The resulting crude product was purified by flash chromatography (DCM/MeOH) to afford corresponding dipeptide C. To a solution of dipeptide C (2.0 mmol) in dichloromethane (18 mL) at 0 °C was added trifluoroacetic acid (2 mL) to give a 10% solution. The reaction was stirred 6 h at room temperature. After removing the solvent in rotary evaporator, the product was obtained as white solid after freeze drying. Then, to a solution of the product in DCM (30 mL) was added HOBT (1-hydroxybenzotriazole) (2.4 mmol), EDCI (1-ethyl-3(3-dimethylpropylamine) carbodiimide) (2.4 mmol) (2.4 mmol) and peptide D (2.0 mmol). After 10 min, triethylamine (3.0 mmol) was added to the solution. The reaction was stirred overnight. After regular workup, the reaction mixture washed by saturated 2 M hydrochloric acid solution (40 mL x 3) and H₂O (40 mL x 3). The organic layers were combined, dried over Na₂SO₄, and concentrated. The resulting crude
product was purified by flash chromatography (DCM/MeOH) to afford corresponding tripeptide 2ba-f.

2.3 Synthesis of Isatin derivatives[^4]

Amino acid (2.0 mmol), HATU (3.0 mmol) and DIPEA (3.0 mmol) were dissolved in DCM (20 mL) and the solution was stirred at 0 °C under an argon atmosphere for 10 min. Then, 2,3-Dioxoindoline-5-carboxylic Acid (2.0 mmol) in DCM (10 mL) was added dropwise, and the reaction mixture was stirred overnight at 0 °C to room temperature. The solvent was removed by reduced pressure, and the crude product was purified by silica gel chromatography with CH₂Cl₂/MeOH to get the product 1h-j.

^1H NMR (400 MHz, Chloroform-d) δ 8.78 (s, 1H), 8.29 (d, J = 6.9 Hz, 1H), 8.13 (d, J = 1.3 Hz, 1H), 8.07 (dd, J = 6.0, 1.2 Hz, 1H), 7.65 (d, J = 6.0 Hz, 1H), 4.10 – 4.01 (m, 2H), 3.67 (s, 3H).

^13C NMR (101 MHz, Chloroform-d) δ 183.96, 170.65, 167.06, 160.54, 148.87, 130.31, 129.92, 125.37, 121.69, 112.46, 52.36, 41.97.
\(^1\)H NMR (400 MHz, Methanol-d4) \(\delta \) 8.10 (d, \(J = 1.2 \) Hz, 1H), 8.06 (dd, \(J = 6.0, 1.2 \) Hz, 1H), 7.90 (d, \(J = 6.2 \) Hz, 1H), 7.62 (d, \(J = 4.8 \) Hz, 1H), 4.31 (dq, \(J = 7.8, 5.4 \) Hz, 1H), 3.67 (s, 3H), 1.38 (d, \(J = 5.4 \) Hz, 3H). \(^{13}\)C NMR (101 MHz, Methanol-d4) \(\delta \) 183.60, 173.02, 166.92, 161.76, 148.92, 130.30, 129.80, 126.13, 121.27, 112.60, 52.58, 48.78, 17.77.

![Molecule](image1)

\(^1\)H NMR (400 MHz, Methanol-d4) \(\delta \) 8.09 – 8.02 (m, 1H), 7.92 – 7.79 (m, 1H), 6.97 (dd, \(J = 21.8, 8.2 \) Hz, 1H), 4.49 – 4.45 (m, 1H), 3.75 (s, 3H), 2.28 – 2.21 (m, 1H), 1.02 (dd, \(J = 11.5, 6.8 \) Hz, 6H). \(^{13}\)C NMR (101 MHz, Methanol-d4) \(\delta \) 183.37, 172.46, 167.37, 160.07, 153.03, 128.82, 123.90, 123.40, 117.69, 111.91, 58.88, 51.27, 30.34, 18.30, 17.88.

3. General Procedure

3.1 Reaction optimization

In an oven-dried undivided three-necked bottle (25 mL) equipped with a stir bar, isatin (0.3 mmol), Tyrosine residue (0.6 mmol) and \(\#\)Bu\(_4\)NF·3H\(_2\)O (0.3 mmol) were combined and added. Then, solvent (6 mL) were injected into the tubes via syringes. The bottle was equipped with platinum plate (15 mm×15 mm×0.3 mm) as the anode and plumbum plate (15 mm×15 mm×0.3 mm) as the cathode. The reaction mixture was stirred and electrolysis at constant current under room temperature. When the reaction was finished, the solvent was removed by reduced pressure and the crude product was purified by flash column chromatography on silica gel (eluent: petroleum ether/ethyl acetate= 5:1). A summary of optimization results is presented in Table S1 below.
Table S1. Investigation of the reaction conditions

<table>
<thead>
<tr>
<th>Entry</th>
<th>Variation from Standard Conditions[^{[a]}]</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>none</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>5 mL CH(_3)CN, 1 mL PBS was used</td>
<td>67</td>
</tr>
<tr>
<td>3</td>
<td>CH(_2)Cl(_2) as the solvent</td>
<td>Trace</td>
</tr>
<tr>
<td>4</td>
<td>Acetone as the solvent</td>
<td>45</td>
</tr>
<tr>
<td>5</td>
<td>DMF as the solvent</td>
<td>N.D.</td>
</tr>
<tr>
<td>6</td>
<td>(^{\text{t}})Bu(_4)NPF(_6) instead of (^{\text{t}})Bu(_4)NF-3H(_2)O</td>
<td>N.D.</td>
</tr>
<tr>
<td>7</td>
<td>(^{\text{t}})Bu(_4)NBr instead of (^{\text{t}})Bu(_4)NF-3H(_2)O</td>
<td>N.D.</td>
</tr>
<tr>
<td>8</td>
<td>3 mA, 360 min</td>
<td>60</td>
</tr>
<tr>
<td>9</td>
<td>10 mA, 120 min</td>
<td>55</td>
</tr>
<tr>
<td>10</td>
<td>20 mA, 60 min</td>
<td>47</td>
</tr>
</tbody>
</table>

\[^{[a]}\]Reaction conditions: platinum plate anode (15 mm×15 mm×0.3 mm), plumbum plate cathode, (15 mm×15 mm×0.3 mm), constant current = 5 mA, 1a (0.3 mmol), 2a (2.0 equiv.), \(^{\text{t}}\)Bu\(_4\)NF-3H\(_2\)O (1.0 equiv.), 5 mL MeCN, undivided cell, 4 h. Yields of isolated products are shown. N.D. = Not Detected.
3.2 General procedure for cyclic voltammetry (CV)

Cyclic voltammetry was performed in a three-electrode cell connected to a schlenk line at room temperature. The working electrode was a steady glassy carbon disk electrode, the counter electrode was a platinum wire. The reference was an Ag/AgCl electrode submerged in saturated aqueous KCl solution and separated from a reaction by a salt bridge. The cyclic voltammetry (CV) experiments on 0.015 M $\text{Bu}_4\text{NF}$ or $\text{Bu}_4\text{NBF}_4$ with 0.003 M 2a, 2af and 2ac were performed, respectively. The scan rate is 0.1 V/s. The positive scan range was from 0 V to -3.0 V.
Figure S1. As shown in this graphic, the cyclic voltammograms showed irreversible reduction waves.

3.3 Dipeptides scope and characterization

**General procedure for product (3aa-k):** In an oven-dried undivided three-necked bottle (25 mL) equipped with a stir bar, isatin (0.3 mmol), dipeptide (0.6 mmol) and Bu₄NF·3H₂O (0.3 mmol) were combined and added. Then, CH₃CN (6 mL) were injected into the tubes via syringes. The bottle was equipped with platinum plate (15 mm×15 mm×0.3 mm) as the anode and plumbum plate (15 mm×15 mm×0.3 mm) as the cathode. The reaction mixture was stirred and electrolysis at a constant current of 5 mA under room temperature for 4 h. After completion of the reaction, as indicated by TLC and LC-MS, the pure product was obtained by flash column chromatography on silica gel (eluent: petroleum ether/ethyl acetate).

**Detailed descriptions for products:**
(R)-4-(2-((tert-butoxycarbonyl)amino)-3-((2-methoxy-2-oxoethyl)amino)-3-oxopropyl)phenyl 2-aminobenzoate (3aa): light yellow oil (Yield: 60 %, 84.78 mg), $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.99 (dd, J = 8.3, 1.3 Hz, 1H), 7.30 – 7.24 (m, 1H), 7.20 (d, J = 8.9 Hz, 2H), 7.05 (d, J = 8.4 Hz, 2H), 6.64 (dd, J = 7.9, 5.6 Hz, 2H), 6.49 (s, 1H), 5.69 (s, 2H), 4.99 (s, 1H), 4.37 (s, 1H), 3.93 (qd, J = 18.3, 5.4 Hz, 2H), 3.67 (s, 3H), 3.05 (d, J = 6.4 Hz, 2H), 1.35 (s, 9H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 171.46, 169.89, 166.78, 155.47, 151.27, 149.79, 134.92, 134.08, 131.58, 130.41, 122.19, 116.80, 116.43, 109.57, 80.47, 55.54, 52.40, 41.22, 37.60, 28.29. HRMS (ESI) cald. for (M+H)$^+$ C$_{24}$H$_{30}$N$_3$O$_7$: 472.2078 found, 472.2075.

4-((R)-2-((tert-butoxycarbonyl)amino)-3-(((R)-1-methoxy-3-methyl-1-oxobutan-2-yl)amin-o)-3-oxopropyl)phenyl 2-aminobenzoate (3ab): light yellow oil (Yield: 63 %, 96.97 mg), $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.98 (d, J = 8.2 Hz, 1H), 7.26 (t, J = 7.7 Hz, 1H), 7.21 (d, J = 9.4 Hz, 2H), 7.05 (d, J = 8.4 Hz, 2H), 6.77 – 6.57 (m, 2H), 6.43 (d, J = 8.1 Hz, 2H), 5.68 (s, 2H), 4.98 (s, 1H), 4.41 (d, J = 13.6 Hz, 1H), 4.30 (d, J = 6.9 Hz, 1H), 3.64 (s, 3H), 3.04 (d, J = 6.7 Hz, 2H), 2.06 (dq, J = 13.5, 6.8 Hz, 1H), 1.37 (s, 9H), 0.81 (dd, J = 11.3, 6.9 Hz, 6H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 171.84, 171.08, 166.73, 155.48, 151.28, 149.77, 134.90, 134.13, 131.55, 130.42, 122.15, 116.80, 116.41, 109.58, 80.40, 57.30, 55.80, 52.16, 37.19, 31.29, 28.29, 18.87, 17.79. HRMS (ESI) cald. for (M+H)$^+$ C$_{27}$H$_{36}$N$_3$O$_7$: 514.2548 found, 514.2547.
4-((R)-2-((tert-butoxycarbonyl)amino)-3-(((R)-1-methoxy-4-methyl-1-oxopentan-2-yl)amino)-3-oxopropyl)phenyl 2-aminobenzoate (3ac): light yellow oil (Yield: 65 %, 102.68 mg), $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.97 (d, J = 8.2 Hz, 1H), 7.25 (t, J = 7.7 Hz, 1H), 7.20 (d, J = 8.2 Hz, 2H), 7.04 (d, J = 8.2 Hz, 2H), 6.62 (dd, J = 7.8, 4.5 Hz, 2H), 6.36 (d, J = 7.6 Hz, 1H), 5.67 (s, 2H), 5.02 (d, J = 6.1 Hz, 1H), 4.54 – 4.47 (m, 1H), 4.30 (d, J = 6.1 Hz, 1H), 3.63 (s, 3H), 3.02 (d, J = 6.6 Hz, 2H), 1.56 – 1.40 (m, 2H), 1.36 (s, 9H), 0.84 (t, J = 5.2 Hz, 6H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 172.89, 170.95, 166.72, 155.44, 151.31, 149.77, 134.88, 134.11, 131.54, 130.46, 128.81, 122.11, 116.80, 116.37, 109.54, 80.36, 55.57, 52.29, 50.80, 41.49, 37.32, 28.27, 24.68, 22.77, 21.87. HRMS (ESI) calcd. for (M+H)$^+$ $C_{28}H_{38}N_3O_7$: 528.2704 found, 528.2706.

4-((R)-2-((tert-butoxycarbonyl)amino)-3-(((R)-1-methoxy-1-oxo-3-phenylpropan-2-yl)amino)-3-oxopropyl)phenyl 2-aminobenzoate (3ad): white oil (Yield: 55 %, 92.67 mg), $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.98 (dd, J = 1.4, 8.3 Hz, 1H), 7.28 – 7.24 (m, 1H), 7.20 – 7.15 (m, 5H), 7.03 (d, J = 8.4 Hz, 2H), 6.96 (d, J = 6.3 Hz, 2H), 6.63 (dd, J = 7.8, 6.3 Hz, 2H), 6.31 (d, J = 7.6 Hz, 1H), 5.69 (s, 2H), 4.92 (s, 1H), 4.72 (q, J = 6.2 Hz, 1H), 4.27 (d, J = 5.8 Hz, 1H), 3.62 (s, 3H), 2.99 (tt, J = 13.7, 7.1 Hz, 4H), 1.34 (s, 9H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 171.44, 170.75, 166.72, 155.32, 151.28, 149.79, 135.66, 134.92, 134.01, 131.55, 130.44, 129.26, 128.63, 127.18, 122.17, 116.81, 116.41, 109.54, 80.35, 55.62, 53.34, 52.38, 37.95, 37.55, 28.28. HRMS
4-((R)-2-((tert-butoxycarbonyl)amino)-3-((R)-3-hydroxy-1-methoxy-1-oxopropan-2-yl)amino)-3-oxopropyl)phenyl 2-aminobenzoate (3ae): light yellow oil (Yield: 50 %, 75.34 mg), $^1$H NMR (400 MHz, Methanol-d$_4$) $\delta$ 8.00 – 7.95 (m, 1H), 7.36 – 7.27 (m, 3H), 7.11 (d, $J = 8.3$ Hz, 2H), 6.79 (d, $J = 8.4$ Hz, 1H), 6.66 – 6.60 (m, 1H), 4.54 (t, $J = 4.2$ Hz, 1H), 4.40 (dd, $J = 9.0$, 5.2 Hz, 1H), 3.91 (dd, $J = 11.3$, 4.4 Hz, 1H), 3.81 (dd, $J = 11.3$, 4.1 Hz, 1H), 3.74 (s, 3H), 3.17 (dd, $J = 13.9$, 5.1 Hz, 1H), 2.89 (dd, $J = 13.8$, 9.3 Hz, 1H), 1.39 (s, 9H). $^{13}$C NMR (101 MHz, Methanol-d$_4$) $\delta$ 172.94, 170.63, 166.74, 156.33, 152.20, 149.77, 134.70, 134.46, 130.95, 130.07, 121.59, 116.46, 115.26, 108.60, 79.44, 61.47, 55.84, 54.77, 51.50, 37.13, 27.28. HRMS (ESI) cald. for (M+H)$^+$ C$_{25}$H$_{32}$N$_3$O$_8$: 502.2184, found, 502.2188.

4-((R)-2-((tert-butoxycarbonyl)amino)-3-(((S)-3-mercapto-1-methoxy-1-oxopropan-2-yl)amino)-3-oxopropyl)phenyl 2-aminobenzoate (3af): yellow oil (Yield: 45 %, 69.78 mg), $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 8.06 (d, $J = 7.2$ Hz, 1H), 7.35 (t, $J = 7.7$ Hz, 1H), 7.24 (d, $J = 8.2$ Hz, 2H), 7.08 (d, $J = 8.2$ Hz, 2H), 6.72 (dd, $J = 8.0$, 5.9 Hz, 2H), 6.64 (d, $J = 7.1$ Hz, 1H), 5.75 (s, 2H), 5.31 (d, $J = 8.3$ Hz, 1H), 4.81 (q, $J = 6.8$, 6.4 Hz, 1H), 4.56 (s, 1H), 3.72 (s, 3H), 3.17 (dd, $J = 13.9$, 4.6 Hz, 1H), 3.07 (d, $J = 5.8$ Hz, 2H), 3.00 – 2.89 (m, 2H), 1.40 (s, 9H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 173.17, 170.45, 166.79, 155.38, 151.26, 150.20, 135.12, 134.37, 131.66,
4-((R)-2-((tert-butoxycarbonyl)amino)-3-((R)-1-methoxy-4-(methylthio)-1-oxobutan-2-yl)amino)-3-oxopropyl)phenyl 2-aminobenzoate (3ag): light yellow oil (Yield: 55%, 89.91 mg). $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.98 (dd, J = 8.3, 1.3 Hz, 1H), 7.29 – 7.24 (m, 1H), 7.20 (d, J = 6.9 Hz, 2H), 7.06 (d, J = 8.4 Hz, 2H), 6.67 – 6.58 (m, 3H), 5.70 (s, 2H), 4.98 (d, J = 6.7 Hz, 1H), 4.60 (q, J = 7.3 Hz, 1H), 4.30 (q, J = 5.8 Hz, 1H), 3.67 (s, 3H), 3.02 (dt, J = 13.9, 6.6 Hz, 2H), 2.37 (t, J = 7.1 Hz, 2H), 2.08 (dt, J = 13.4, 6.5 Hz, 1H), 2.00 (s, 3H), 1.89 (dd, J = 14.4, 7.1 Hz, 1H), 1.37 (s, 9H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 171.83, 171.01, 166.76, 155.41, 151.28, 149.82, 134.94, 133.97, 131.56, 130.44, 122.22, 116.80, 116.43, 109.52, 80.50, 55.69, 52.59, 51.60, 37.29, 31.52, 29.74, 28.29, 15.41. HRMS (ESI) cald. for (M+Na)$^+$ $C_{25}$H$_{31}$N$_3$O$_7$Na: 540.1775, found, 540.1780.

4-((S)-2-((S)-2-acetamido-3-(1H-indol-3-yl)propanamido)-3-methoxy-3-oxopropyl)phenyl 2-aminobenzoate (3ah): yellow oil (Yield: 70%, 113.78 mg). $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 8.65 (s, 1H), 8.08 (dd, J = 8.3, 1.5 Hz, 1H), 7.72 (d, J = 7.7 Hz, 1H), 7.36 (ddd, J = 8.5, 7.3, 1.5 Hz, 1H), 7.29 (d, J = 7.9 Hz, 1H), 7.17 – 7.09 (m, 2H), 7.04 (d, J = 8.5 Hz, 2H), 6.94 – 6.90 (m,
2H), 6.76 – 6.71 (m, 2H), 6.57 (d, J = 2.2 Hz, 1H), 6.41 (d, J = 7.5 Hz, 1H), 6.16 (d, J = 7.5 Hz, 1H), 5.80 (s, 2H), 4.76 (dtd, J = 15.6, 8.4, 7.8, 4.7 Hz, 2H), 3.68 (s, 3H), 3.24 (dd, J = 14.3, 4.4 Hz, 1H), 3.05 (dd, J = 14.3, 9.1 Hz, 1H), 2.95 (dd, J = 14.4, 4.7 Hz, 1H), 2.80 (dd, J = 14.4, 8.1 Hz, 1H), 1.98 (s, 3H). ¹³C NMR (101 MHz, Chloroform-d) δ 171.57, 171.31, 169.98, 167.74, 151.52, 149.80, 136.36, 135.38, 133.13, 131.75, 130.23, 127.06, 123.64, 122.36, 122.16, 119.66, 119.31, 116.97, 116.60, 111.26, 110.37, 109.08, 53.46, 52.84, 52.41, 36.55, 28.89, 23.35. HRMS (ESI) cald. for (M+H)+ C₃₀H₃₁N₄O₆: 543.2238, found, 543.2240.

**4-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)pent-4-ynamido)-3-methoxy-3-oxopropyl)phenyl 2-aminobenzoate (3ai):** White oil (Yield: 60 %, 91.75 mg), ¹H NMR (400 MHz, Chloroform-d) δ 8.04 (dd, J = 8.3, 1.6 Hz, 1H), 7.32 (ddd, J = 8.6, 7.2, 1.6 Hz, 1H), 7.17 (d, J = 8.5 Hz, 2H), 7.09 (d, J = 8.5 Hz, 2H), 6.88 (d, J = 6.8 Hz, 1H), 6.72 – 6.67 (m, 2H), 5.78 (s, 2H), 5.34 (s, 1H), 4.87 (q, J = 5.8 Hz, 1H), 4.29 (s, 1H), 3.73 (s, 3H), 3.16 (qd, J = 14.0, 5.8 Hz, 2H), 2.78 (d, J = 15.3 Hz, 1H), 2.61 – 2.52 (m, 1H), 2.09 (t, J = 2.6 Hz, 1H), 1.45 (s, 9H). ¹³C NMR (101 MHz, Chloroform-d) δ 171.41, 170.05, 166.77, 155.34, 151.33, 149.92, 134.94, 133.28, 131.55, 130.43, 122.18, 116.81, 116.38, 109.45, 80.60, 79.31, 72.09, 53.39, 52.84, 52.48, 37.18, 28.27, 22.26. HRMS (ESI) cald. for (M+H)+ C₂₇H₃₂N₃O₇: 510.2235, found, 510.2238.

**3aj**

**(R)-4-2-((tert-butoxycarbonyl)amino)-3-(3-methoxy-3-oxoprop-1-en-2-yl)amino)-3-oxopropylphenyl 2-aminobenzoate (3aj):** Light yellow oil (Yield: 63 %, 89.30 mg), ¹H NMR (400 MHz, Chloroform-d) δ 8.03 (dd, J = 8.3, 1.6 Hz, 1H), 7.30 (ddd, J = 8.6, 7.2, 1.6 Hz, 1H), 7.18 (d, J = 8.5 Hz, 2H), 7.09 (d, J = 8.5 Hz, 2H), 6.88 (d, J = 6.8 Hz, 1H), 6.72 – 6.67 (m, 2H), 5.78 (s, 2H), 5.34 (s, 1H), 4.87 (q, J = 5.8 Hz, 1H), 4.29 (s, 1H), 3.73 (s, 3H), 3.16 (qd, J = 14.0, 5.8 Hz, 2H), 2.78 (d, J = 15.3 Hz, 1H), 2.61 – 2.52 (m, 1H), 2.09 (t, J = 2.6 Hz, 1H), 1.45 (s, 9H). ¹³C NMR (101 MHz, Chloroform-d) δ 171.41, 170.05, 166.77, 155.34, 151.33, 149.92, 134.94, 133.28, 131.55, 130.43, 122.18, 116.81, 116.38, 109.45, 80.60, 79.31, 72.09, 53.39, 52.84, 52.48, 37.18, 28.27, 22.26. HRMS (ESI) cald. for (M+H)+ C₂₇H₃₂N₃O₇: 510.2235, found, 510.2238.
MHz, Chloroform-d) \( \delta \) 8.26 (s, 1H), 8.05 (dd, \( J = 8.3, 1.6 \text{ Hz}, 1H \)), 7.33 (ddd, \( J = 8.5, 7.1, 1.6 \text{ Hz}, 1H \)), 7.26 – 7.23 (m, 2H), 7.12 (d, \( J = 8.5 \text{ Hz}, 2H \)), 6.72 – 6.67 (m, 2H), 6.61 (s, 1H), 5.90 (d, \( J = 1.1, 1H \)), 5.76 (s, 2H), 5.03 (s, 1H), 4.46 (s, 1H), 3.81 (s, 3H), 3.14 (d, \( J = 6.2 \text{ Hz}, 2H \)), 1.43 (s, 9H). \(^{13}\text{C} \) NMR (101 MHz, Chloroform-d) \( \delta \) 170.13, 166.72, 164.02, 155.43, 151.29, 149.89, 134.92, 133.77, 131.56, 130.63, 130.30, 122.30, 116.80, 116.42, 109.55, 109.47, 80.71, 56.40, 52.97, 37.37, 28.25. HRMS (ESI) cald. for (M+H)+ \( C_{25}H_{30}N_3O_7 \): 484.2078, found, 484.2079.

(R)-4-((tert-butoxycarbonyl)amino)-3-((4-ethoxy-4-oxobutyl)amino)-3-oxopropyl)phenyl 2-aminobenzoate (3ak): White oil (Yield: 60%, 92.30 mg), \(^1\text{H} \) NMR (400 MHz, Chloroform-d) \( \delta \) 7.98 (dd, \( J = 8.4, 1.6 \text{ Hz}, 1H \)), 7.26 (td, \( J = 7.7, 7.1, 1.6 \text{ Hz}, 1H \)), 7.20 – 7.16 (m, 2H), 7.04 (d, \( J = 8.5 \text{ Hz}, 2H \)), 6.66 – 6.59 (m, 2H), 6.17 (t, \( J = 5.7 \text{ Hz}, 1H \)), 5.72 (s, 2H), 5.11 (d, \( J = 6.3 \text{ Hz}, 1H \)), 4.24 (s, 1H), 4.03 (q, \( J = 7.1 \text{ Hz}, 2H \)), 3.73 – 3.63 (m, 2H), 3.15 (q, \( J = 6.8, 6.3 \text{ Hz}, 2H \)), 2.99 (hept, \( J = 7.8, 7.1 \text{ Hz}, 2H \)), 2.18 (t, \( J = 7.3 \text{ Hz}, 2H \)), 1.81 – 1.74 (m, 2H), 1.66 (p, \( J = 7.0 \text{ Hz}, 2H \)), 1.35 (s, 9H), 1.16 (t, \( J = 7.1 \text{ Hz}, 3H \)). \(^{13}\text{C} \) NMR (101 MHz, Chloroform-d) \( \delta \) 173.25, 171.21, 166.76, 155.43, 151.31, 149.75, 134.90, 134.31, 131.55, 130.36, 122.18, 116.35, 109.48, 80.23, 67.97, 60.51, 55.99, 38.86, 38.03, 31.56, 28.31, 25.61, 24.50, 14.21. HRMS (ESI) cald. for (M+H)+ \( C_{27}H_{36}N_3O_7 \): 514.2547, found, 514.2551.

### 3.4 Polypeptide scope and characterization

**General procedure for product (3ba-f):** In an oven-dried undivided three-necked bottle (25 mL) equipped with a stir bar, isatin (0.45 mmol), tripeptide (0.3 mmol) and \( \# \)Bu₄NF·3H₂O (0.3 mmol) were combined and added. Then, CH₃CN (6 mL) were injected into the tubes via syringes. The bottle was equipped with platinum plate (15 mm×15 mm×0.3 mm) as the anode and plumbum...
plate (15 mm×15 mm×0.3 mm) as the cathode. The reaction mixture was stirred and electrolysis at a constant current of 5 mA under room temperature for 4 h. After completion of the reaction, as indicated by TLC and LC-MS, the pure product was obtained by flash column chromatography on silica gel (eluent: DCM/MeOH).

**3ba**

4-((R)-2-((R)-2-acetamido-3-phenylpropanamido)-3-((4-ethoxy-4-oxobutyl)amino)-3-oxopropyl)phenyl 2-aminobenzoate (3ba): light yellow oil (Yield: 68 %, 123.02 mg), ¹H NMR (400 MHz, DMSO-d6) δ 8.24 (d, J = 8.1 Hz, 1H), 8.14 (d, J = 8.1 Hz, 1H), 7.91 (dt, J = 15.4, 6.4 Hz, 2H), 7.34 – 7.15 (m, 8H), 7.10 (t, J = 7.4 Hz, 2H), 6.83 (d, J = 8.4 Hz, 1H), 6.73 (s, 2H), 6.59 (t, J = 7.5 Hz, 1H), 4.53 – 4.40 (m, 2H), 4.03 (q, J = 7.0 Hz, 2H), 3.09 – 2.59 (m, 6H), 2.25 (q, J = 7.8 Hz, 2H), 1.75 (s, 3H), 1.62 (dq, J = 14.0, 7.0 Hz, 2H), 1.16 (d, J = 18.7 Hz, 3H). ¹³C NMR (101 MHz, DMSO-d6) δ 173.12, 171.64, 171.01, 169.81, 166.44, 152.60, 149.54, 138.43, 135.56, 135.26, 131.43, 130.62, 129.59, 128.44, 126.64, 122.16, 117.13, 115.40, 108.07, 60.21, 54.64, 54.50, 38.29, 37.77, 37.51, 31.28, 24.81, 22.90, 14.56. HRMS (ESI) cald. for (M+H)+ C₃₃H₃₉N₄O₇: 603.2774, found, 603.2769

**3bb**

Methyl(6R,9R,12R)-9-((2-aminobenzoyl)oxy)benzyl)-2,2,12-trimethyl-6-(2-(methylthio)ethyl)-4,7,10-trioxo-3-oxa-5,8,11-triazatridecan-13-oate (3bb): yellow oil (Yield: 63 %, 115.72 mg), ¹H NMR (400 MHz, DMSO-d6) δ 9.73 (d, J = 8.3 Hz, 1H), 8.80 (d, J = 6.3 Hz, 1H),
7.90 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 7.9 Hz, 2H), 7.32 (t, J = 7.5 Hz, 1H), 7.15 (d, J = 8.1 Hz, 2H),
6.83 (d, J = 8.4 Hz, 1H), 6.72 (s, 2H), 6.65 (d, J = 7.4 Hz, 1H), 6.59 (t, J = 7.5 Hz, 1H), 4.66 – 4.60
(m, 1H), 4.54 – 4.49 (m, 1H), 4.35 – 4.30 (m, 1H), 3.64 (s, 3H), 3.16 (d, J = 13.5 Hz, 1H), 2.96
(dt, J = 22.7, 9.5 Hz, 2H), 2.84 – 2.75 (m, 1H), 2.11 (s, 3H), 2.00 (dq, J = 20.2, 11.5, 11.0 Hz, 2H),
1.44 (s, 9H), 1.34 (d, J = 7.3 Hz, 3H).\(^{13}\)C NMR (101 MHz, DMSO-d6) \(\delta\)
172.86, 169.75, 165.98, 156.45, 152.16, 152.07, 149.21, 134.83, 134.80, 131.01, 130.07, 121.82, 116.67, 114.93, 107.58,
79.81, 54.45, 51.97, 47.84, 44.19, 39.93, 35.97, 31.29, 29.04, 27.79, 16.77, 14.79. HRMS (ESI)
cald. for (M+Na)+ C\(_{30}\)H\(_{40}\)N\(_{4}\)O\(_{8}\)Na: 639.2459, found, 639.2463.

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\text{4-((R)-2-((R)-2-acetamido-4-methylpentanamido)-3-(((R)-1-methoxy-3-methyl-1-oxobutan-2-yl)amino)-3-oxopropyl)phenyl 2-aminobenzoate: yellow oil (Yield: 55\%, 93.82 mg),}\]
\(^{1}\)H NMR (400 MHz, Methanol-d4) \(\delta\)
7.97 (dd, J = 8.2, 1.5 Hz, 1H), 7.27 – 7.25 (m, 1H), 7.25 – 7.23 (m, 2H), 7.10 (d, J = 8.5 Hz, 2H), 6.79 (dd, J = 0.8, 8.4 Hz, 1H), 6.63 (ddd, J = 8.1, 7.1, 1.1 Hz, 1H), 4.70 – 4.62 (m, 2H), 3.69 (s, 3H), 3.18 (dd, J = 13.9, 5.6 Hz, 1H), 3.11 – 2.99 (m, 2H), 2.82 (dd, J = 13.9, 9.1 Hz, 1H), 2.23 – 2.11 (m, 2H), 2.06 – 2.01 (m, 1H), 1.88 (s, 3H), 1.00 (d, J = 4.0 Hz, 3H), 0.92 – 0.91 (m, 3H), 0.83 (d, J = 6.6 Hz, 3H), 0.79 (d, J = 6.0 Hz, 3H). \(^{13}\)C NMR (101 MHz, Methanol-d4) \(\delta\)
172.12, 171.66, 171.57, 171.54, 166.69, 152.23, 149.90, 134.09, 130.90, 130.04, 128.87, 121.69, 116.45, 115.23, 108.52, 57.35, 54.45, 53.67, 53.53, 41.13, 37.37, 29.97, 22.93, 22.34, 20.98. HRMS (ESI) cald. for (M+H)+ C\(_{30}\)H\(_{41}\)N\(_{4}\)O\(_{7}\): 569.2931, found, 569.2933.
4-((R)-2-((R)-2-acetamido-3-phenylpropanamido)-3-((R)-1-methoxy-3-methyl-1-oxobutan-2-yl)amino)-3-oxopropyl)phenyl 2-aminobenzoate (3bd): yellow liquid (Yield: 60 %, 108.52 mg), $^1$H NMR (400 MHz, DMSO-d6) δ 8.40 (d, J = 7.2 Hz, 1H), 7.98 – 7.92 (m, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.32 (d, J = 7.8 Hz, 2H), 7.23 (dd, J = 16.5, 7.4 Hz, 5H), 7.10 (d, J = 7.6 Hz, 2H), 6.96 (d, J = 8.4 Hz, 1H), 6.84 (d, J = 8.4 Hz, 1H), 6.73 (s, 2H), 6.60 (t, J = 7.4 Hz, 1H), 4.49 (d, J = 6.9 Hz, 1H), 4.40 (d, J = 7.7 Hz, 1H), 4.18 (t, J = 8.9 Hz, 1H), 3.57 (s, 3H), 3.00 (dp, J = 22.2, 7.9, 6.9 Hz, 3H), 2.78 – 2.69 (m, 1H), 1.61 (dd, J = 12.6, 6.3 Hz, 1H), 1.31 (s, 3H), 0.87 (dd, J = 6.2, 16.0 Hz, 6H). $^{13}$C NMR (101 MHz, DMSO-d6) δ 172.54, 172.22, 171.79, 166.51, 155.77, 152.60, 149.45, 137.53, 136.11, 135.25, 131.44, 130.61, 129.47, 128.70, 126.98, 122.07, 117.15, 115.42, 108.12, 56.13, 53.95, 52.25, 51.14, 37.61, 36.96, 24.40, 23.48, 22.21. HRMS (ESI) cald. for (M+H)+ C$_{33}$H$_{39}$N$_4$O$_7$: 603.2774, found, 603.2771.

4-((S)-2-((S)-2-acetamido-3-phenylpropanamido)-4-methylpentanamido)-3-methoxy-3-oxopropyl)phenyl 2-aminobenzoate (3be): yellow oil (Yield: 71 %, 129.32 mg), $^1$H NMR (400 MHz, DMSO-d6) δ 8.40 (d, J = 7.2 Hz, 1H), 7.92 (dd, J = 16.9, 8.2 Hz, 2H), 7.32 (d, J = 7.8 Hz, 2H), 7.23 (dd, J = 16.5, 7.4 Hz, 5H), 7.10 (d, J = 7.6 Hz, 2H), 6.96 (d, J = 8.4 Hz, 1H), 6.84 (d, J = 8.4 Hz, 1H), 6.60 (t, J = 7.4 Hz, 1H), 4.49 (q, J = 7.3, 6.7 Hz, 1H), 4.40 (q, J = 7.5 Hz, 1H), 4.18 (t, J = 9.0 Hz, 1H), 3.57 (s, 3H), 2.99 (dtt, J = 20.6, 15.1, 14.5, 6.5 Hz, 3H), 2.80 –
2.69 (m, 1H), 1.62 (dt, J = 12.6, 6.5 Hz, 1H), 1.31 (s, 3H), 1.29 – 1.21 (m, 2H), 0.87 (dd, J = 15.8, 6.1 Hz, 6H). $^{13}$C NMR (101 MHz, DMSO-d6) δ 172.54, 172.22, 171.79, 166.51, 155.77, 152.60, 149.45, 137.53, 136.11, 135.25, 131.44, 130.61, 129.47, 128.70, 126.98, 122.07, 117.15, 115.42, 108.12, 56.13, 53.95, 52.25, 51.14, 41.73, 37.61, 36.96, 24.40, 23.48, 22.21. HRMS (ESI) cald. for (M+H)+ C$_{34}$H$_{41}$N$_4$O$_7$: 617.2931, found, 617.2926.

4-((S)-2-(2-((S)-2-acetamido-3-phenylpropanamido)acetamido)-3-methoxy-3-oxopropyl)phenyl 2-aminobenzoate (3bf): yellow oil (Yield: 67%, 93.82 mg), $^1$H NMR (400 MHz, DMSO-d6) δ 8.34 (p, J = 7.3, 6.3 Hz, 2H), 8.20 (d, J = 8.0 Hz, 1H), 7.90 (d, J = 8.1 Hz, 1H), 7.36 – 7.29 (m, 2H), 7.28 – 7.24 (m, 5H), 7.20 – 7.16 (m, 1H), 7.13 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 8.3 Hz, 1H), 6.74 (s, 2H), 6.60 (t, J = 7.5 Hz, 1H), 4.49 (dp, J = 9.9, 6.3, 4.3 Hz, 2H), 3.74 (ddd, J = 15.8, 5.8 Hz, 2H), 3.62 (s, 3H), 3.08 – 2.92 (m, 3H), 2.78 – 2.69 (m, 1H), 1.75 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-d6) δ 172.76, 172.63, 171.11, 168.00, 166.29, 156.48, 156.10, 135.31, 133.16, 131.92, 130.67, 130.38, 127.87, 127.64, 122.97, 115.51, 111.58, 108.33, 107.27, 54.37, 52.13, 49.05, 36.48, 32.94, 28.66. HRMS (ESI) cald. for (M+H)+ C$_{30}$H$_{33}$N$_4$O$_7$: 561.2306, found, 561.2310.

**General procedure for bioconjugation of polypeptides (3bg-j):** In an oven-dried undivided three-necked bottle (15 mL) equipped with a stir bar, polypeptides (5 mg), isatin (10 mg), n$^4$Bu$_4$NF 3H$_2$O(10 mg), CH$_3$CN (0.75 mL) and phosphate buffer solution (0.75 mL, pH= 7.4) were combined and added. The bottle was equipped platinum plate (10 mm×10 mm×0.1 mm) as the anode and plumbum plate (10 mm×10 mm×0.3 mm) as the cathode. The reaction mixture was stirred and electrolysis at constant current of 5 mA under room temperature for 30 min. After completion of the reaction, the solution was analyzed by LC-MS spectroscopy. The reaction was
analyzed by reverse phase HPLC using a gradient of 60% to 50% buffer B over 20 minutes on an Agilent Zorbax SB-Aq 5μm column of 250 mm length. HPLC analysis used buffers A (water) and B (acetonitrile + 0.1% TFA). Conversion reported as a % conversion as determined.

**bioconjugated product 3bg:**

![Chemical structure of 3bg](image)

HPLC: >99% conversion.

After the reaction finished, there are four peaks that elute at 50% buffer B (acetonitrile + 0.1% TFA) with retention times of 1.722 min, 3.415 min, 4.415 min and 5.236 min. Polypeptide 2g is a peak that elutes at 50% buffer B (acetonitrile + 0.1% TFA) with a retention time of 3.204 min.
HPLC Spectra:

HRMS (ESI-TOF) calcd for C_{39}H_{48}N_{6}O_{9}, [M+Na]^+, 767.3375, found 767.3371.

**bioconjugated product 3bh :**

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\text{bioconjugated product 3bh :}
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HPLC: >99% conversion.

After the reaction finished, there are four peaks that elute at 50% buffer B (acetonitrile + 0.1% TFA) with retention times of 1.809 min, 3.629 min, 6.382 min and 7.171 min. Polypeptide
2h is a peak that elutes at 50% buffer B (acetonitrile + 0.1% TFA) with a retention time of 3.032 min.

HPLC Spectra:


bioconjugated product 3bi:

HPLC: >99% conversion.

After the reaction finished, there are four peaks that elute at 50% buffer B (acetonitrile + 0.1% TFA) with retention times of 1.720 min, 1.911 min, 3.387 min and 6.531 min. Polypeptide
2i is a peak that elutes at 50% buffer B (acetonitrile + 0.1% TFA) with a retention time of 4.723 min.

HPLC Spectra:

HRMS (ESI-TOF) calcd for C_{46}H_{59}N_{7}O_{10s}, [M+H]^+, 892.4255, found 892.4257.

bioconjugated product 3bj:

HPLC: >99% conversion.
After the reaction finished, there are four peaks that elute at 50% buffer B (acetonitrile + 0.1% TFA) with retention times of 1.939 min, 2.728 min, 3.146 min and 4.513 min. Polypeptide 2j is a peak that elutes at 50% buffer B (acetonitrile + 0.1% TFA) with a retention time of 4.048 min.

HPLC Spectra:

HRMS (ESI-TOF) calcd for C_{55}H_{69}N_{9}O_{11}S, [M+H]^+, 1086.4735, found 1086.4731

3.5 Drug molecules and Natural products scope and characterization

Detailed descriptions for products:
2-methoxyphenyl 2-aminobenzoate (3ca): yellow oil (Yield: 78%, 57.10 mg), $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 8.12 (d, J = 8.1 Hz, 1H), 7.33 (t, J = 7.7 Hz, 1H), 7.27 – 7.22 (m, 1H), 7.16 – 7.12 (m, 1H), 7.03 – 6.97 (m, 2H), 6.72 (t, J = 8.6 Hz, 2H), 5.74 (s, 2H), 3.82 (s, 3H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 166.39, 151.53, 151.13, 139.79, 134.75, 131.91, 126.87, 123.26, 120.85, 116.71, 116.42, 112.51, 109.74, 55.95. HRMS (ESI) calcd. for (M+H)+ C$_{14}$H$_{14}$NO$_3$: 244.0968, found, 244.0966.

5-isopropyl-2-methylphenyl 2-aminobenzoate (3cb): yellow oil (Yield: 73%, 58.91 mg), $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 8.12 (dd, J = 8.4, 1.4 Hz, 1H), 7.38 – 7.31 (m, 1H), 7.19 (d, J = 7.8 Hz, 1H), 7.05 (dd, J = 7.8, 1.4 Hz, 1H), 6.97 (d, J = 1.3 Hz, 1H), 6.76 – 6.69 (m, 2H), 5.78 (s, 2H), 2.89 (dd, J = 11.4, 4.2 Hz, 1H), 2.19 (s, 3H), 1.25 (d, J = 6.9 Hz, 6H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 166.59, 151.23, 149.29, 148.13, 134.78, 131.62, 130.91, 127.65, 124.10, 120.15, 116.79, 116.43, 109.76, 33.61, 23.96, 15.90. HRMS (ESI) calcd. for (M+H)+ C$_{17}$H$_{20}$NO$_2$: 270.1488, found, 270.1490.

4-allyl-2-methoxyphenyl 2-aminobenzoate (3cc): light yellow oil (Yield: 75%, 63.67 mg), $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 8.12 (dd, J = 8.0, 1.3 Hz, 1H), 7.36 – 7.30 (m, 1H), 7.06 (d, J = 7.9 Hz, 1H), 6.83 (d, J = 9.8 Hz, 2H), 6.74 – 6.68 (m, 2H), 5.99 (ddt, J = 16.8, 10.0, 6.7 Hz, 1H), 5.75 (s, 2H), 5.17 – 5.09 (m, 2H), 3.81 (s, 3H), 3.41 (d, J = 6.7 Hz, 2H). $^{13}$C NMR (101 MHz,
Chloroform-d) $\delta$ 166.52, 151.28, 151.11, 138.97, 137.99, 137.18, 134.72, 131.90, 122.96, 120.79, 116.70, 116.40, 116.16, 112.85, 109.79, 55.93, 40.17. HRMS (ESI) cald. for (M+H)+ $\text{C}_{17}\text{H}_{18}\text{NO}_3$: 284.1281, found, 284.1278.

4-acetamidophenyl 2-aminobenzoate (3cd): light yellow solid (Yield: 74%, 59.94 mg), $^1$H NMR (400 MHz, Methanol-d4) $\delta$ 7.97 (dd, $J = 8.1$, 1.4 Hz, 1H), 7.60 (d, $J = 8.9$ Hz, 2H), 7.29 (ddd, $J = 8.5$, 7.1, 1.5 Hz, 1H), 7.12 (d, $J = 8.9$ Hz, 2H), 6.78 (d, $J = 8.4$ Hz, 1H), 6.66 – 6.59 (m, 1H), 2.13 (s, 3H). $^{13}$C NMR (101 MHz, Methanol-d4) $\delta$ 170.23, 166.83, 152.19, 146.97, 136.11, 134.49, 130.96, 121.93, 120.69, 116.48, 115.29, 108.56, 22.42. HRMS (ESI) cald. for (M+H)+ $\text{C}_{15}\text{H}_{15}\text{N}_2\text{O}_3$: 271.1077, found, 271.1081.

4-(1-(1H-indole-3-carboxamido)-2-methoxy-2-oxoethyl)phenyl 2-aminobenzoate (3ce): yellow oil (Yield: 74%, 98.55 mg), $^1$H NMR (400 MHz, Methanol-d4) $\delta$ 8.05 (d, $J = 8.1$ Hz, 1H), 7.99 (dd, $J = 8.8$, 1.3 Hz, 2H), 7.49 – 7.46 (m, 1H), 7.41 (d, $J = 8.2$ Hz, 2H), 7.33 (t, $J = 7.6$ Hz, 1H), 7.20 (dt, $J = 19.4$, 7.4 Hz, 4H), 6.82 (d, $J = 8.4$ Hz, 1H), 6.64 (t, $J = 7.6$ Hz, 1H), 4.90 (dd, $J = 9.1$, 5.6 Hz, 1H), 3.77 (s, 3H). $^{13}$C NMR (101 MHz, Methanol-d4) $\delta$ 172.77, 166.63, 166.33, 152.30, 149.88, 136.62, 135.11, 134.70, 131.14, 130.15, 128.33, 125.96, 122.35, 121.98, 120.88, 120.71, 116.56, 115.27, 111.75, 110.02, 108.42, 53.96, 51.57. HRMS (ESI) cald. for (M+H)+ $\text{C}_{25}\text{H}_{22}\text{N}_3\text{O}_5$: 444.1554, found, 444.1551.
2-methyl-4-oxo-4\textit{H}-pyran-3-yl 2-aminobenzoate (3cf): light yellow solid (Yield: 67%, 49.25 mg), $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 8.04 (dd, J = 8.5, 1.5 Hz, 1H), 7.71 (d, J = 5.7 Hz, 1H), 7.32 (td, J = 7.7, 7.3, 1.5 Hz, 1H), 6.70 – 6.65 (m, 2H), 6.45 (d, J = 5.7 Hz, 1H), 5.74 (s, 2H), 2.31 (s, 3H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 172.42, 164.78, 159.53, 154.19, 151.51, 138.66, 135.22, 131.90, 116.92, 116.44, 108.59, 15.12. HRMS (ESI) cald. for (M+H)$^+$ C$_{13}$H$_{12}$NO$_4$: 246.0761, found, 246.0759.

Benzo[d][1,3]dioxol-5-yl 2-aminobenzoate (3cg): yellow oil (Yield: 72%, 55.52 mg), $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 8.05 (d, J = 8.1 Hz, 1H), 7.33 (t, J = 7.7 Hz, 1H), 6.82 (d, J = 8.3 Hz, 1H), 6.74 – 6.66 (m, 3H), 6.63 (dd, J = 8.3, 2.0 Hz, 1H), 6.00 (s, 2H), 5.76 (s, 2H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 167.10, 151.25, 148.09, 145.38, 145.09, 135.90, 131.56, 116.79, 116.42, 114.35, 109.55, 108.06, 104.16, 101.72. HRMS (ESI) cald. for (M+H)$^+$ C$_{14}$H$_{12}$NO$_4$: 258.0761, found, 258.0766.

2-(hydroxymethyl)phenyl 2-aminobenzoate (3ch): yellow oil (Yield: 70%, 51.05 mg), $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.96 (d, J = 5.3 Hz, 1H), 7.37 – 7.28 (m, 1H), 7.18 (t, J = 7.5 Hz, 1H), 7.04 (s, 1H), 6.97 (s, 1H), 6.90 – 6.80 (m, 2H), 6.75 – 6.63 (m, 2H), 5.75 (s, 2H), 4.82 (s, 2H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 169.90, 155.99, 150.51, 134.16, 129.46, 129.25, 128.04, 124.91, 120.06, 116.89, 116.46, 116.17, 113.12, 64.30. HRMS (ESI) cald. for (M+H)$^+$
C\textsubscript{14}H\textsubscript{14}NO\textsubscript{3}: 244.0968, found, 244.0967.

\((S)-4-(2-((\text{tert}-\text{butoxycarbonyl})\text{amino})-3\text{-methoxy}-3\text{-oxopropyl})\text{2,3-dimethyl 6-iodoquinoline-2,3,4-tricarboxylate (3ci)}: \text{light yellow oil (Yield: 60\%, 70.03 mg),} ^1\text{H NMR (400 MHz, Chloroform-d)} \delta 8.07 (d, J = 8.2 Hz, 1H), 7.34 (d, J = 8.2 Hz, 2H), 6.99 – 6.90 (m, 2H), 6.71 (t, J = 7.1 Hz, 2H), 5.77 (s, 2H), 2.98 – 2.90 (m, 2H), 2.57 – 2.40 (m, 2H), 2.32 (t, J = 8.7 Hz, 1H), 2.21 – 1.94 (m, 4H), 1.56 (ddq, J = 43.2, 20.1, 11.2, 10.4 Hz, 6H), 0.92 (s, 3H). ^{13}\text{C NMR (101 MHz, Chloroform-d)} \delta 220.88, 167.14, 151.22, 148.68, 138.08, 137.36, 134.82, 131.60, 126.47, 122.00, 119.21, 116.78, 116.40, 109.76, 50.47, 47.99, 44.21, 38.06, 35.89, 31.59, 29.45, 26.39, 25.80, 21.62, 13.86. \text{HRMS (ESI) cald. for (M+H)+ C}_{25}\text{H}_{28}\text{NO}_{3}: 390.2064, \text{found, 390.2065.}

\((8R,9S,13S,14S)-17\text{-hydroxy-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-3-yl 2-aminobenzoate (3cj): white oil (Yield: 57\%, 66.85 mg),} ^1\text{H NMR (400 MHz, Chloroform-d)} \delta 8.07 (dd, J = 8.5, 1.6 Hz, 1H), 7.36 – 7.29 (m, 2H), 6.94 (dd, J = 8.4, 2.5 Hz, 1H), 6.89 (d, J = 2.4 Hz, 1H), 6.74 – 6.68 (m, 2H), 5.77 (s, 2H), 3.74 (t, J = 8.5 Hz, 1H), 3.64 (s, 1H), 2.93 – 2.84 (m, 2H), 2.39 – 2.21 (m, 2H), 2.16 – 2.08 (m, 1H), 2.01 – 1.94 (m, 1H), 1.90 (ddt, J = 11.3, 5.6, 3.0 Hz, 1H), 1.71 (dddt, J = 13.1, 10.3, 6.4, 3.0 Hz, 1H), 1.61 – 1.15 (m, 7H), 0.79 (s, 3H). ^{13}\text{C NMR (101 MHz, Chloroform-d)} \delta 167.18, 151.20, 148.50, 138.33, 137.97, 134.78, 131.62, 126.47, 121.91, 119.01, 116.77, 116.40, 109.83, 81.92, 50.09, 44.19, 43.24, 38.52, 36.70, 30.60, 29.59, 27.08, 26.20, 23.15, 11.07. \text{HRMS (ESI) cald. for (M+H)+}
3.6 Other substrates expansion

**General procedure for product (3da-dl):** In an oven-dried undivided three-necked bottle (25 mL) equipped with a stir bar, isatin derivatives (0.3 mmol), Tyrosine residue (0.6 mmol) and NBu₄NF·3H₂O (0.3 mmol) were combined and added. Then, solvent (6 mL) were injected into the tubes via syringes. The bottle was equipped with platinum plate (15 mm×15 mm×0.3 mm) as the anode and plumbum plate (15 mm×15 mm×0.3 mm) as the cathode. The reaction mixture was stirred and electrolysis at a constant current of 5 mA under room temperature for 4 h. After completion of the reaction, as indicated by TLC and LC-MS, the pure product was obtained by flash column chromatography on silica gel.

**Detailed descriptions for products:**

(S)-4-(2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyl 2-aminobenzoate (3a): light yellow oil (Yield: 75%, 93.15 mg), ¹H NMR (400 MHz, Chloroform-d) δ 8.06 (dd, J = 8.3, 1.5 Hz, 1H), 7.33 (ddd, J = 8.5, 7.2, 1.6 Hz, 1H), 7.18 (d, J = 8.4 Hz, 2H), 7.12 (d, J = 8.5 Hz, 2H), 6.73 – 6.68 (m, 2H), 5.79 (s, 2H), 5.04 (d, J = 8.1 Hz, 1H), 4.64 – 4.55 (m, 1H), 3.73 (s, 3H), 3.11 (qd, J = 13.9, 6.0 Hz, 2H), 1.43 (s, 9H). ¹³C NMR (101 MHz, Chloroform-d) δ 172.28, 166.76, 155.15, 151.32, 149.84, 134.92, 133.59, 131.58, 130.34, 122.10, 116.81, 116.39, 109.52, 80.06, 54.42, 52.33, 37.70, 28.33. HRMS (ESI) cald. for (M+H)+ C₂₂H₂₇N₂O₆: 415.1864, found, 415.1867.
(S)-4-(2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyl 2-amino-5-methylbenzoate (3da): light yellow oil (Yield: 67%, 86.03 mg), $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.86 (d, J = 1.1 Hz, 1H), 7.20 – 7.15 (m, 3H), 7.11 (d, J = 8.5 Hz, 2H), 6.64 (d, J = 8.4 Hz, 1H), 5.63 (s, 2H), 5.02 (d, J = 8.0 Hz, 1H), 4.60 (q, J = 6.1 Hz, 1H), 3.73 (s, 3H), 3.11 (qd, J = 13.9, 5.9 Hz, 2H), 2.27 (s, 3H), 1.43 (s, 9H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 171.23, 165.72, 154.08, 148.83, 148.14, 135.09, 132.49, 130.02, 129.29, 124.52, 121.06, 115.90, 108.37, 79.01, 53.35, 51.28, 36.66, 27.28, 19.28. HRMS (ESI) cald. for (M+H)$^+$ C$_{23}$H$_{29}$N$_2$O$_6$: 429.2020, found, 429.2018.

(S)-4-(2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyl 2-amino-3,5-dimethylbenzoate (3db): light yellow oil (Yield: 67%, 83.53 mg), $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.78 (s, 1H), 7.19 (d, J = 8.4 Hz, 2H), 7.12 (dd, J = 5.5, 2.9 Hz, 3H), 5.72 (s, 2H), 5.02 (d, J = 8.0 Hz, 1H), 4.60 (q, J = 6.0 Hz, 1H), 3.73 (s, 3H), 3.11 (qd, J = 14.0, 6.0 Hz, 2H), 2.26 (s, 3H), 2.18 (s, 3H), 1.44 (s, 9H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 172.28, 167.24, 155.13, 149.95, 147.76, 137.02, 133.48, 130.33, 128.94, 124.81, 123.29, 122.14, 108.90, 80.06, 54.40, 52.34, 37.69, 28.33, 20.34, 17.45. HRMS (ESI) cald. for (M+H)$^+$ C$_{24}$H$_{31}$N$_2$O$_6$: 443.2177, found, 443.2176.
(S)-4-(2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyl 2-amino-5-chlorobenzenate (3dc): light yellow solid (Yield: 73%, 98.31 mg), \(^1\)H NMR (400 MHz, Chloroform-d) \(\delta\) 7.98 (d, \(J = 8.6\) Hz, 1H), 7.18 (d, \(J = 8.4\) Hz, 2H), 7.10 (d, \(J = 8.5\) Hz, 2H), 6.71 (d, \(J = 1.9\) Hz, 1H), 6.67 (dd, \(J = 8.6, 2.0\) Hz, 1H), 5.86 (s, 2H), 5.02 (d, \(J = 8.1\) Hz, 1H), 4.60 (q, \(J = 6.1\) Hz, 1H), 3.73 (s, 3H), 3.11 (qd, \(J = 13.9, 5.9\) Hz, 2H), 1.43 (s, 9H). \(^{13}\)C NMR (101 MHz, Chloroform-d) \(\delta\) 172.26, 166.20, 155.13, 151.92, 149.62, 140.96, 133.76, 132.97, 130.39, 122.00, 116.94, 116.05, 108.11, 54.39, 52.36, 37.72, 28.33. HRMS (ESI) cald. for (M+H)+ C\(_{22}\)H\(_{26}\)ClN\(_2\)O\(_6\): 449.1474, found, 449.1476.

(S)-4-(2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyl 2-amino-4-chlorobenzenate (3dd): light yellow solid (Yield: 78%, 104.81 mg), \(^1\)H NMR (400 MHz, Chloroform-d) \(\delta\) 7.98 (d, \(J = 8.6\) Hz, 1H), 7.19 (d, \(J = 8.3\) Hz, 2H), 7.11 (d, \(J = 8.4\) Hz, 2H), 6.71 (d, \(J = 1.8\) Hz, 1H), 6.67 (dd, \(J = 8.6, 1.9\) Hz, 1H), 5.88 (s, 2H), 5.04 (d, \(J = 8.1\) Hz, 1H), 4.60 (q, \(J = 6.1\) Hz, 1H), 3.73 (s, 3H), 3.11 (qd, \(J = 13.9, 5.9\) Hz, 2H), 1.43 (s, 9H). \(^{13}\)C NMR (101 MHz, Chloroform-d) \(\delta\) 172.26, 166.20, 155.14, 151.96, 149.63, 140.94, 133.76, 132.96, 130.38, 122.00, 116.89, 116.06, 108.08, 80.09, 54.40, 52.35, 37.72, 28.33. HRMS (ESI) cald. for (M+H)+ C\(_{22}\)H\(_{26}\)ClN\(_2\)O\(_6\): 449.1474, found, 449.1476.
(S)-4-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyl 2-amino-5-flurobenzoate (3de): light yellow oil (Yield: 60%, 79.81 mg), $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.72 (dd, $J = 9.0$, 3.6 Hz, 1H), 7.19 (d, $J = 8.3$ Hz, 1H), 7.10 (d, $J = 8.5$ Hz, 1H), 6.95 (d, $J = 8.5$ Hz, 2H), 6.75 (d, $J = 8.1$ Hz, 2H), 5.73 (s, 2H), 5.15 (d, $J = 8.2$ Hz, 1H), 4.52 (q, $J = 6.1$ Hz, 1H), 3.68 (s, 3H), 2.99 (tt, $J = 14.2$, 6.7 Hz, 2H), 1.41 (s, 9H). $^{19}$F NMR (376 MHz, Chloroform-d) $\delta$ -128.04. $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 172.83, 166.03, 155.53 (d, $J = 8.9$ Hz), 152.59, 149.58, 148.07, 130.29, 126.91 (d, $J = 14.5$ Hz), 123.12 (d, $J = 23.4$ Hz), 122.00, 118.18 (d, $J = 7.1$ Hz), 116.16 (d, $J = 23.2$ Hz), 109.14, 80.37, 54.72, 52.34, 37.39, 28.30. HRMS (ESI) cald. for (M+H)$^+$ C$_{22}$H$_{26}$FN$_2$O$_6$: 433.1769, found, 433.1771.

(S)-4-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyl 2-amino-3-(trifluoromethyl)benzoate (3df): light yellow solid (Yield: 50%, 72.31 mg), $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 8.29 (d, $J = 7.9$ Hz, 1H), 7.68 (d, $J = 7.6$ Hz, 1H), 7.20 (d, $J = 8.2$ Hz, 2H), 7.11 (d, $J = 8.4$ Hz, 2H), 6.76 (t, $J = 7.9$ Hz, 1H), 6.49 (s, 2H), 5.03 (d, $J = 8.0$ Hz, 1H), 4.60 (d, $J = 7.3$ Hz, 1H), 3.73 (s, 3H), 3.11 (qd, $J = 13.9$, 6.0 Hz, 2H), 1.43 (s, 9H). $^{19}$F NMR (376 MHz, Chloroform-d) $\delta$ -63.44. $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 171.19, 165.27, 154.08, 148.48, 147.72, 134.87, 132.96, 131.67 (q, $J = 5.0$ Hz), 120.89, 113.88, 110.12, 79.08, 53.37, 51.30, 36.74, 28.68, 27.28. HRMS (ESI) cald. for (M+H)$^+$ C$_{23}$H$_{26}$F$_3$N$_2$O$_6$: 483.1737, found, 483.1734.
(S)-4-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyl 5-((acetoxyethyl)carbamoyl)-2-aminobenzoate (3dg): light yellow oil (Yield: 65%, 103.16 mg), \(^1\)H NMR (400 MHz, Methanol-d4) \(\delta\) 8.58 (d, J = 2.1 Hz, 1H), 7.80 – 7.73 (m, 1H), 7.23 (d, J = 8.1 Hz, 2H), 7.09 (d, J = 8.2 Hz, 2H), 6.78 (d, J = 8.8 Hz, 1H), 4.35 (dd, J = 8.9, 5.6 Hz, 1H), 4.04 (s, 2H), 3.67 (d, J = 11.9 Hz, 6H), 3.08 (dd, J = 13.8, 5.4 Hz, 1H), 2.90 (dd, J = 13.7, 9.1 Hz, 1H), 1.36 (s, 9H). \(^{13}\)C NMR (101 MHz, Methanol-d4) \(\delta\) 172.77, 170.91, 168.18, 166.22, 156.44, 154.73, 149.66, 134.72, 133.09, 131.60, 129.95, 121.66, 119.89, 116.15, 107.58, 79.33, 55.14, 51.33, 51.29, 40.99, 36.64, 27.33. HRMS (ESI) cald. for (M+H)+ C\(_{26}\)H\(_{32}\)N\(_3\)O\(_9\): 530.2133, found, 530.2131.

4-((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyl 5-(((S)-1-acetoxyethyl)carbamoyl)-2-aminobenzoate (3dh): light yellow oil (Yield: 55%, 89.61 mg), \(^1\)H NMR (400 MHz, DMSO-d6) \(\delta\) 8.63 (d, J = 6.9 Hz, 1H), 8.53 (d, J = 7.6 Hz, 1H), 8.10 (d, J = 8.5 Hz, 1H), 7.87 (dd, J = 8.8, 2.2 Hz, 1H), 7.31 (d, J = 8.5 Hz, 2H), 7.25 (d, J = 2.4 Hz, 2H), 7.19 (s, 2H), 6.86 (d, J = 8.8 Hz, 1H), 4.59 – 4.43 (m, 2H), 3.63 (s, 3H), 3.60 (s, 3H), 2.97 (dt, J = 13.9, 3.9 Hz, 2H), 1.73 (s, 9H), 1.38 (d, J = 7.3 Hz, 3H). \(^{13}\)C NMR (101 MHz, Methanol-d4) \(\delta\) 173.56, 173.32, 168.75, 166.78, 156.95, 155.20, 150.19, 135.23, 133.77, 132.38, 130.47, 122.20, 120.64, 116.54, 108.06, 79.83, 59.32, 55.65, 51.66, 37.15, 30.79, 27.84, 18.84. HRMS (ESI) cald. for (M+H)+ C\(_{27}\)H\(_{34}\)N\(_3\)O\(_9\): 544.2295, found, 544.2290.
4-((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenyl 5-(((S)-1-acetoxy-2-methylpropyl)carbamoyl)-2-aminobenzoate (3di): yellow oil (Yield: 55%, 97.64 mg), $^1$H NMR (400 MHz, Methanol-d$_4$) $\delta$ 8.62 (d, $J = 2.1$ Hz, 1H), 7.82 (dd, $J = 8.8, 2.1$ Hz, 1H), 7.29 (d, $J = 8.3$ Hz, 2H), 7.15 (d, $J = 8.3$ Hz, 2H), 6.83 (d, $J = 8.8$ Hz, 1H), 4.46 (d, $J = 7.1$ Hz, 1H), 4.39 (dd, $J = 8.8, 5.6$ Hz, 1H), 3.72 (d, $J = 9.0$ Hz, 6H), 3.14 (dd, $J = 13.8, 5.5$ Hz, 1H), 2.95 (dd, $J = 13.7, 9.1$ Hz, 1H), 2.24 (dq, $J = 13.7, 6.8$ Hz, 1H), 1.40 (s, 9H), 1.01 (dd, $J = 11.1, 6.8$ Hz, 6H). $^{13}$C NMR (101 MHz, Methanol-d$_4$) $\delta$ 172.74, 168.28, 166.27, 156.46, 154.72, 149.70, 134.73, 133.24, 131.86, 129.94, 121.66, 120.15, 115.99, 107.56, 79.29, 58.81, 55.13, 51.29, 51.09, 36.64, 30.27, 27.30, 18.29, 17.95. HRMS (ESI) calcd. for (M+H)$^+$ C$_{29}$H$_{39}$N$_3$O$_9$: 572.2608, found, 572.2605.

4-((R)-2-acetamido-3-(((R)-1-methoxy-3-methyl-1-oxobutan-2-yl)amino)-3-oxopropyl)phenyl 2-amino-5-(((R)-1-methoxy-3-methyl-1-oxobutan-2-yl)carbamoyl)benzoate (3dj): light yellow oil (Yield: 56%, 102.82 mg), $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 8.57 – 8.50 (m, 2H), 8.45 (d, $J = 7.7$ Hz, 1H), 8.10 (d, $J = 8.5$ Hz, 1H), 7.90 (dd, $J = 8.8, 2.1$ Hz, 1H), 7.31 (d, $J = 8.5$ Hz, 2H), 7.27 – 7.24 (m, 2H), 7.18 (s, 2H), 6.87 (d, $J = 8.8$ Hz, 1H), 4.54 (dq, $J = 14.4, 7.7, 6.5$ Hz, 2H), 4.26 (t, $J = 7.8$ Hz, 1H), 3.62 (d, $J = 13.8$ Hz, 6H), 3.13 – 2.92 (m, 2H), 2.70 (dd, $J = 13.8, 10.0$ Hz, 1H), 2.16 (dq, $J = 13.7, 6.8$ Hz, 1H), 1.74 (s, 3H), 1.06 (dd, $J = 10.7, 4.7$ Hz, 6H), 0.94 (dd, $J = 19.4, 6.7$ Hz, 6H). $^{13}$C NMR (101 MHz, DMSO-d$_6$) $\delta$ 172.57, 171.70, 171.65, 169.07, 165.99, 165.82, 154.12, 149.19, 134.67, 133.83, 131.90, 130.18, 129.15, 128.01, 120.15, 116.01,
58.67, 53.61, 53.57, 51.91, 51.56, 37.51, 35.93, 29.47, 22.43, 19.30, 19.22. HRMS (ESI) cald. for (M+H)+ C_{31}H_{41}N_{4}O_{9}: 613.2829, found, 613.2833.

(R)-4-((tert-butoxycarbonyl)amino)-3-((4-ethoxy-4-oxobutyl)amino)-3-oxopropyl)phenyl 2-amino-5-((2-methoxy-2-oxoethyl)carbamoyl)benzoate (3dk): light yellow oil (Yield: 60%, 113.19 mg), $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 8.58 (d, J = 6.3 Hz, 1H), 8.25 (d, J = 7.4 Hz, 1H), 7.87 (d, J = 11.7 Hz, 1H), 7.30 (d, J = 7.3 Hz, 1H), 6.99 (d, J = 7.8 Hz, 2H), 6.74 (s, 2H), 6.67 (d, J = 7.9 Hz, 2H), 6.20 (d, J = 18.2 Hz, 1H), 4.39 (q, J = 8.0, 7.5 Hz, 1H), 4.04 – 3.93 (m, 4H), 3.58 (s, 3H), 2.93 – 2.84 (m, 3H), 2.82 – 2.74 (m, 1H), 2.07 (t, J = 5.5 Hz, 2H), 1.59 – 1.50 (m, 2H), 1.37 (s, 9H), 1.21 (s, 3H). $^{13}$C NMR (101 MHz, DMSO-d$_6$) $\delta$ 172.76, 172.61, 171.10, 166.28, 166.21, 156.50, 156.09, 154.64, 135.31, 131.92, 130.38, 127.63, 120.38, 110.64, 115.51, 110.64, 107.25, 77.95, 54.37, 52.12, 49.05, 36.47, 32.94, 28.66, 26.23. HRMS (ESI) cald. for (M+H)+ C_{31}H_{41}N_{4}O_{10}: 629.2778, found, 629.2780.

4-((R)-2-acetamido-3-(((R)-1-methoxy-3-methyl-1-oxobutan-2-yl)amino)-3-oxopropyl)phenyl 2-amino-5-(((R)-1-methoxy-1-oxopropan-2-yl)carbamoyl)benzoate (3dl): yellow oil (Yield: 60%, 105.13 mg), $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 8.64 (d, J = 6.9 Hz, 1H), 8.58 – 8.52 (m, 2H), 8.10 (d, J = 8.5 Hz, 1H), 7.87 (dd, J = 8.8, 2.2 Hz, 1H), 7.32 (s, 2H), 7.27 – 7.24 (m, 2H), 7.20 (s, 2H), 6.87 (d, J = 8.8 Hz, 1H), 4.54 (dt, J = 15.3, 6.2 Hz, 2H), 4.48 – 4.41 (m, 1H), 3.62 (d, J = 10.4 Hz, 6H), 3.08 – 2.95 (m, 2H), 2.73 – 2.67 (m, 1H), 1.74 (s, 3H), 1.38 (d, J = 7.3 Hz, 3H),
0.99 (dd, J = 8.3, 4.3 Hz, 6H). $^{13}$C NMR (101 MHz, DMSO-d6) δ 173.94, 172.15, 172.11, 169.52, 166.26, 165.81, 154.59, 149.64, 132.04, 130.63, 130.47, 129.60, 128.46, 120.43, 115.53, 54.41, 54.01, 52.36, 52.27, 48.67, 37.97, 36.39, 22.88, 19.10, 17.26. HRMS (ESI) cald. for (M+H)+ $^{1}$C$_{29}$H$_{37}$N$_{4}$O$_{9}$: 585.2482, found, 585.2484.

### 3.7 Additional application of electrochemical bioconjugation

#### 3.7.1 Bioconjugation of Biotin$^{[5]}$

**Synthesis of compound 5:** In a round bottomed flask, add an excess of ethylene diamine (10 mL) to a solution of methyl biotinate (2.5 mmol) in methanol (10 mL). Keep the solution at 60 °C for 48 hours, and then, remove the excess ethylene diamine and methanol under reduced pressure. Obtain the product as light yellow solid biotin (2-amino-ethyl)-amide) and use directly for the next experiment. $^{[5]}$

In a round bottomed flask, equipped with a stir bar, Dioxoindoline-5-carboxylic Acid (2.0 mmol), HOBT (3.0 mmol), HBTU (3.0 mmol), dichloromethane (40 mL) and triethylamine (2.4 mmol) were combined and added. The mixture was stirred for 30 min at room temperature. And then, biotin (2-amino-ethyl)-amide) (2.0 mmol) was added to the solution. The reaction was stirred overnight. After regular workup, the reaction mixture washed with saturated NaHCO$_3$ solution (40 mL x 3), 2M hydrochloric acid solution (40 mL x 3) and H$_2$O (40 mL x 3). The organic layers were combined, dried over Na$_2$SO$_4$, and concentrated. Evaporation of the solvent that was dissolved in DMSO. Then a crude product recrystallize by methanol.

In an oven-dried undivided three-necked bottle (15 mL) equipped with a stir bar, polypeptides 2g (5 mg), compound 4 (20 mg), nBu$_4$NF-3H$_2$O (10 mg), CH$_3$CN (1.0 mL) and phosphate buffer solution (0.75 mL, pH= 7.4) were combined and added. The bottle was equipped platinum plate (10 mm×10 mm×0.1 mm) as the anode and plumbum plate (10 mm×10 mm×0.3 mm) as the cathode. The reaction mixture was stirred and electrolysis at constant current of 5 mA under room
temperature for 30 min. After completion of the reaction, the solution was analyzed by LC-MS spectroscopy. The reaction was analyzed by reverse phase HPLC using a gradient of 60% to 50% buffer B over 20 minutes on an Agilent Zorbax SB-Aq 5μm column of 250 mm length. HPLC analysis used buffers A (water) and B (acetonitrile + 0.1% TFA). Conversion reported as a % conversion as determined.

Detailed descriptions for products 5:

HPLC: >99% conversion.

After the reaction finished, there are four peaks that elute at 50% buffer B (acetonitrile + 0.1% TFA) with retention times of 1.828 min, 2.541 min, 3.077 min and 4.055 min. Polypeptide
2g is a peak that elutes at 50% buffer B (acetonitrile + 0.1% TFA) with a retention time of 3.204 min.

HRMS (ESI-TOF) calcd for C\textsubscript{52}H\textsubscript{68}N\textsubscript{10}O\textsubscript{12}SNa, [M+Na]+, 1079.4631, found 1079.4629

### 3.7.2 Bioconjugation of Oxytocin

**Synthesis of compound 6**: In an oven-dried undivided three-necked bottle (15 mL) equipped with a stir bar, Oxytocin (10 mg), 1a (15 mg), \( n\text{Bu}_4\text{NF} \cdot 3\text{H}_2\text{O} \) (10 mg), CH\textsubscript{3}CN (2.0 mL) were combined and added. The bottle was equipped platinum plate (10 mm×10 mm×0.1 mm) as the anode and plumbum plate (10 mm×10 mm×0.3 mm) as the cathode. The reaction mixture was stirred and electrolysis at constant current of 2 mA under room temperature for 30 min. After completion of the reaction, the solution was analyzed by LC-MS spectroscopy. The reaction was analyzed by reverse phase HPLC using a gradient of 60% to 50% buffer B over 20 minutes on an Agilent Zorbax SB-Aq 5\( \mu \)m column of 250 mm length. HPLC analysis used buffers A (water) and B (acetonitrile + 0.1% TFA). Conversion reported as a % conversion as determined.

![Diagram of Oxytocin and 1a](image)

**Detailed descriptions for products 6**:  
HPLC: 50% conversion.

After the reaction finished, there are three peaks that elute at 50% buffer B (acetonitrile + 0.1% TFA) with retention times of 2.028 min, 4.543 min, 5.09 min. Oxytocin is a peak that elutes at 50% buffer B with a retention time of 3.98 min.
HRMS (ESI-TOF) calcd for C$_{50}$H$_{72}$N$_{13}$O$_{13}$S$_2$, [M+H]$^+$, 1126.4814, found 1126.4823.

3.7.3 Bioconjugation of Protein

Synthesis of 3n: In an oven-dried undivided three-necked bottle (10 mL) equipped with a stir bar, Myoglobin (5 mg), 1a (10 mg), $n$Bu$_4$NF·3H$_2$O (10 mg), CH$_3$CN (1.0 mL) and phosphate buffer solution (0.75 mL, pH= 7.4) were combined and added. The bottle was equipped platinum plate (10 mm×10 mm×0.1 mm) as the anode and plumbum plate (10 mm×10 mm×0.3 mm) as the cathode. The reaction mixture was stirred and electrolysis at constant current of 2 mA under -20°C for 10 min. After completion of the reaction, the solution was analyzed by Maldi-Tof MS.
Effect of anthranilic acyl modification on structure of Myoglobin

Comparison of CD spectra between Myoglobin and 3n sample (100μg/mL in PBS buffer).

Synthesis of 3o : In an oven-dried undivided three-necked bottle (10 mL) equipped with a stir bar, Cytochrome C (5 mg), 1a (7 mg), 4 Bu₄NF·3H₂O (5 mg), CH₃CN (1.0 mL) and phosphate buffer solution (0.75 mL, pH= 7.4) were combined and added. The bottle was equipped platinum plate (10 mm×10 mm×0.1 mm) as the anode and plumbum plate (10 mm×10 mm×0.3 mm) as the cathode. The reaction mixture was stirred and electrolysis at constant current of 2 mA under -10°C for 15 min. After completion of the reaction, the solution was analyzed by Maldi-Tof MS.
Effect of anthranilic acyl modification on structure of Cytochrome C
Comparison of CD spectra between Cytochrome C and 3o sample (100μg/mL in PBS buffer).

Synthesis of 3p: In an oven-dried undivided three-necked bottle (10 mL) equipped with a stir bar, insulin (10 mg), 1a (15 mg), "Bu4NF·3H2O (15 mg), CH3CN (1.0 mL) and phosphate buffer solution (0.75 mL, pH= 7.4) were combined and added. The bottle was equipped platinum plate (10 mm×10 mm×0.1 mm) as the anode and plumbum plate (10 mm×10 mm×0.3 mm) as the cathode. The reaction mixture was stirred and electrolysis at constant current of 2 mA under -15°C for 20 min. After completion of the reaction, the solution was analyzed by Maldi-Tof MS.
Effect of anthranilic acyl modification on structure of insulin

Comparison of CD spectra between insulin and 3p sample (100μg/mL in PBS buffer).
4. References


5. Spectra

5.1 NMR Spectra of Products

![NMR Spectra Image]
NH₂

AcO

MeO₂C

H

H

N

O

O

H

H

3bf
from Guaiacol
3ca
from Carvacrol
3cb
NH₂O from Eugenol 3cc
from Acetaminophen

3cd
from Indoleformic acid
3ce
from Maltol
3cf
O
O
NH$_2$

from Sesamol
3cg

- 72 -
from Salicyl alcohol
3ch
from Estrone 3\text{ci}
from Estradiol 3cj
NH₂

O

MeO₂

2

C

H

N

Boc

Cl

O

3dc

1.00 -

2.02 -

1.01 -

1.99 -

3.08 -

1.98 -

9.06 -

10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

172.26

168.20

155.13

149.62

140.96

132.37

128.04

116.05

108.11

80.09

54.39

52.36

37.72

26.33
5.2 $^1$H NMR spectroscopic investigation

The concentration dependence of isochroman of the chemical shift of 2-H of Boc-Tyr-OMe

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<tr>
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<td>0.1</td>
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<td>6.836 ppm</td>
</tr>
</tbody>
</table>

Stacked $^1$H NMR spectrum of 2-H of Boc-Tyr-OMe