Supporting Information

Selective Stepwise Arylation of Unprotected Peptides by Pt$^{IV}$ Complexes

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# Supporting Information

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1. General information

The synthetic manipulations involving air-sensitive compounds were performed in a nitrogen-filled Innovative Technology glove box. All chemicals were purchased from Sigma Aldrich, Alfa Aesar, Acros, Chem Impex International and used without further purification. Aminopeptidase M, porcine kidney (3.4 mg/ml, 16.5 U/mg) was purchased from EMD Millipore Corp. All solvents were degassed and stored under high-purity nitrogen and activated 4Å molecular sieves. All deuterated solvents were stored under high-purity nitrogen on 3Å molecular sieves. Compounds 2a, 2’a, 2’b and 2c were prepared according to the literature procedure.1 The NMR spectra were recorded on Bruker Avance 400MHz spectrometer. 1H and 13C NMR signals are reported in ppm downfield from TMS. 1H signals are referenced to the residual proton of a deuterated solvent 7.26 ppm for CDCl3, 2.50 ppm for (CD3)2SO and 7.15 ppm for C6D6. 13C signals are referenced to the solvent signal at 77.36 ppm for CDCl3 and 128.00 ppm for C6D6. 19F chemical shifts are reported in ppm downfield from CClF3. Mass Spectra were recorded on a VG-Autospec M-250 instrument. The LCMS spectra were acquired using liquid chromatography (LC) (Acquity-UPLC, Waters Inc.) coupled with an UV detector (Acquity - TUV detector, Waters Inc.) and ESI mass spectrometer. The stationary phase consisted of a C18 (1.7 µm, 2.1 × 100 mm) column (Waters Inc.) and the mobile phase compositions were: A: 100% H2O + 0.1% Formic acid; B: 100% CH3CN + 0.1% Formic acid. The elution gradient was as follows: 100% A for 10s, followed 100% B for 9.5min at flow rate = 0.5 mL/min and return to 100% A for additional 30s. The temperature in the sample chamber was pre-set to 40°C. The UV detector was set to 260 nm. Mass spectra were acquired over the scan range m/z 750 – 2000. 7b, 7ba, 7baa, 7baaaa, 8b, 8ba, and 8bc were analyzed in the negative ion mode. Products of the reactions with 9 were analyzed both, in the negative and positive ion modes.
2. Experimental section

2.1 Synthesis of Pt complexes

Complex 2*a.

In a glove box, a freshly prepared solution of N-fluoropyridinium tetrafluoroborate (127.3 mg, 0.06883 mmol, 2.5 equiv.) in DCM (2 mL) was added slowly during 10 min to a DCM (2 mL) solution of (O^P)Pt(4-FC_6H_4)(Py)_1 (210.0 mg, 0.02753 mmol, 1 equiv.) at RT and the mixture was stirred for 45 min. The ^19F NMR analysis showed the formation of 2*a as the only product. The reaction mixture was filtered and the filtrate was concentrated to 0.5mL. Hexane (10 mL) was added to precipitate the Pt(IV) complex 2*a. The light yellow solid was collected and was dried under vacuum.

(248.3 mg, yield 95%) ^31P NMR (162 MHz, CDCl_3) δ 40.03 (s, J = 2601.7 Hz). ^1H NMR (400 MHz, CDCl_3) δ 8.89 (s, 2H), 7.71 (d, J = 7.3 Hz, 2H), 7.37 (s, 2H), 7.27 – 7.19 (m, 4H), 7.14 (s, 2H), 6.74 (s, 1H), 6.73 (dd, J = 21.9, 10.7 Hz, 2H), 6.51 (t, J = 8.6 Hz, 1H), 2.80 (s, 3H), 2.54 (s, 3H), 2.15 (s, 3H), 2.01 (s, 3H), 1.87 (d, J = 10.4 Hz, 3H), 1.80 (d, J = 9.2 Hz, 3H), 1.73 (s, 3H), 1.58 (s, 9H). ^19F NMR (377 MHz, MeOD) δ -120.15 (s), -303.02 (s, J = 1213.9 Hz). ^13C NMR (101 MHz, MeOD) δ 174.28 (s), 162.64 (d, J = 245.4 Hz), 149.27 (s), 147.44 (s), 143.17 (bs), 141.02 (s), 134.55 (s), 133.14 (s), 131.5 (m), 126.98 (s), 126.58 (s), 119.95 (d, J = 6.9 Hz), 118.66 (d, J = 7.2 Hz), 114.09 (d, J = 20.3 Hz), 49.64 (d, J = 15.8 Hz), 49.20 (s), 40.06 (s), 38.19 (s), 35.57 (d, J = 8.8 Hz), 29.27 (d, J = 9.6 Hz), 28.65 (d, J = 9.1 Hz).

Complex 2*b. In a glove box, a freshly prepared solution of N-fluoropyridinium tetrafluoroborate (82.9 mg, 0.4482 mmol, 2.5 equiv.) in DCM (3 mL) was added to a DCM (3 mL) solution of (O^P)Pt(3,5-F_2C_6H_3)(Py)_1 (140.0 mg, 0.1793 mmol, 1 equiv.) in a Schlenk at RT and the mixture was heated at 40°C with vigorous stirring for 10h. The reaction mixture was filtered and the filtrate was concentrated to 0.5mL. Hexane (12 mL) was added to precipitate the Pt(IV) complex 2*b. The light yellow solid was collected and was dried under vacuum.
Complex 2c.

2-(4-iodobenzyl)-1H-benzo[d]isoquinoline-1,3(2H)-dione (S1)

A mixture of 1,8-naphthalic anhydride (396.4 mg, 2.0 mmol), (4-iodophenyl)methanamine (932.2 mg, 4.0 mmol), and imidazole (2.86 g, 42.0 mmol) was refluxed in CH$_3$Cl (12 mL). After
2 h, the solvent was removed in vacuo. The residue was taken up in absolute ethanol and the resulting suspension was sonicated for 15 min. A light yellow solid was filtered and dried under vacuum to afford the product.

\[ \text{Product (710.4 mg, yield 86%)} \]

\( ^1\text{H NMR (400 MHz, CDCl}_3\) \( \delta \) 8.61 (d, \( J = 7.2 \text{ Hz, 2H} \)), 8.21 (d, \( J = 8.2 \text{ Hz, 2H} \)), 7.75 (t, \( J = 7.7 \text{ Hz, 2H} \)), 7.63 (d, \( J = 7.7 \text{ Hz, 2H} \)), 7.31 (d, \( J = 7.7 \text{ Hz, 2H} \)), 5.32 (s, 2H). \( ^{13}\text{C NMR (101 MHz, CDCl}_3\) \( \delta \) 164.34 (s), 137.70 (s), 137.15 (s), 134.36 (s), 131.69 (s), 131.22 (s), 127.16 (s), 122.68 (s), 93.30 (s), 43.25 (s). HRMS (ESI): calcd. for [\( \text{C}_{19}\text{H}_{12}\text{INO}_2 + H\)]: 413.9991, found: 413.9992.

2-(4-(trimethylstannyl)benzyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (S2)

To a solution of 2-(4-iodobenzyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (S1) (341.5 mg, 0.827 mmol, 1.0 equiv.) in anhydrous dioxane (6 mL) was added hexamethylditin (413.1 \( \mu\text{L}, 1.98 \text{ mmol, 2.4 equiv.} \)) followed by Pd(PPh\(_3\))\(_2\)Cl\(_2\) (15.0 mg, 2.5% mmol) and the reaction mixture was heated for 3 h under reflux. The mixture was filtered through celite and purified by column chromatography using hexanes/ethyl acetate (9/1) as eluent to afford the product as a light yellow solid.
(232.2 mg, yield 62.4%) ^1^H NMR (400 MHz, CDCl$_3$) δ 8.61 (d, $J$ = 7.1 Hz, 2H), 8.19 (d, $J$ = 8.1 Hz, 2H), 7.74 (t, $J$ = 7.6 Hz, 2H), 7.54 (d, $J$ = 6.8 Hz, 2H), 7.44 (d, $J$ = 7.2 Hz, 2H), 5.38 (s, 2H), 0.24 (s, 9H).

$^{13}$C NMR (101 MHz, CDCl$_3$) δ 141.51 (s), 137.48 (s), 136.12 (s), 134.17 (s), 131.75 (s), 131.59 (s), 128.76 (s), 128.36 (s), 127.10 (s), 122.86 (s), 43.73 (s), -9.46 (s).

HRMS (ESI): calcd. for [C$_{22}$H$_{21}$NO$_2$Sn + H]$^+$: 448.0668, found: 448.0660.

**Complex S3**

In a glove box, compound **S2** (193.9 mg, 0.431 mmol, 1.2 equiv.) was mixed with PtI$_2$(COD) (200.0 mg, 0.359 mmol, 1 equiv.) in anhydrous CH$_3$CN (5 mL) in a Schlenk tube containing a magnetic stirring bar. The reaction mixture was heated at 75°C for 5h and was filtered to collect the solid. The solid was washed with hexane and dried under vacuum.

(162.0 mg, yield 63%) ^1^H NMR (400 MHz, CDCl$_3$) δ 8.60 (d, $J$ = 7.2 Hz, 2H), 8.19 (t, $J$ = 7.5 Hz, 2H), 7.73 (t, $J$ = 6.9 Hz, 2H), 7.50 (d, $J$ = 7.5 Hz, 1H), 7.41 (d, $J$ = 7.5 Hz, 1H), 7.31 (d, $J$ = 7.8 Hz, 1H), 7.18 (d, $J$ = 7.9 Hz, 1H), 5.78 (t, $J$ = 16.0 Hz, 1H), 5.35 (t, $J$ = 12.0 Hz, 2H), 5.30 (s, 4H), 4.68 (t, $J$ = 40.0 Hz, 1H), 2.54 – 2.40 (m, 2H), 2.38 – 2.23 (m, 1H), 2.13 – 1.98 (m, 1H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 164.36 (s), 136.50 (s), 135.07 (s), 134.29 (s), 134.09 (d, $J$ = 15.0 Hz), 131.53 (d, $J$ = 12.9 Hz), 129.37 (s), 128.87 (s), 127.09 (m), 113.22 (t, $J$ = 27 Hz), 93.78 (s), 53.59 (s), 43.67 (s), 43.27 (s), 31.94 (s), 28.82 (s). HRMS (ESI): calcd. for [C$_{27}$H$_{24}$NO$_2$Pt + H]$^+$: 716.0557, found: 716.0559.

**Complex S4**

In a glove box, NaH (4.0 mg, 0.163 mmol, 1.8 equiv.) was added to a solution of the (O^P) ligand (53.7 mg, 0.136 mmol, 1.5 equiv.) in anhydrous THF and the mixture was stirred for 20
min. The solution was filtered and the filtrate was added to a solution of S₃ (65.0 mg, 0.091 mmol, 1.0 equiv.) in THF (5 mL). The reaction was heated in the presence of 10 equiv. pyridine (73.1µL, 0.907 mmol, 10.0 equiv.) at 65°C for overnight. The ³¹P NMR spectrum of the reaction mixture indicated the formation of a new complex. The solution was evaporated and the residue was dissolved in DCM (1 mL). the Pt(IV) complex was purified by TLC (THF/Hexane, 1/1) to afford the product as light yellow solid.

(46.7 mg, yield 54%) ³¹P NMR (162 MHz, CDCl₃) δ 42.99 (s, J = 4165 Hz). ¹H NMR (400 MHz, CDCl₃) δ 8.65 (d, J = 2.2 Hz, 2H), 8.56 (d, J = 7.1 Hz, 2H), 8.11 (d, J = 8.1 Hz, 2H), 7.69 (t, J = 7.7 Hz, 2H), 7.64 (t, J = 7.5 Hz, 1H), 7.56 (d, J = 7.7 Hz, 2H), 7.43 (t, J = 7.4 Hz, 1H), 7.18 – 7.15 (m, 5H), 6.83 (dd, J = 7.9, 3.8 Hz, 1H), 6.48 (t, J = 7.0 Hz, 1H), 5.27 (s, 2H), 2.37 (d, J = 10.9 Hz, 6H), 2.06 (d, J = 10.8 Hz, 6H), 1.89 (s, 6H), 1.68 (d, J = 11.8 Hz, 6H), 1.61 (d, J = 11.4 Hz, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 164.36(s), 150.13 (s), 140.02 (s), 137.75 (s), 134.62 (s), 133.93 (s), 132.43 (s), 131.67 (s), 131.31 (s), 130.86(s), 128.23 (s), 127.98 (s), 127.05 (s), 124.89 (s), 123.05 (s), 119.69 (d, J = 7.9 Hz), 113.53 (d, J = 6.7 Hz), 43.71 (s), 42.26 (d, J = 27.2 Hz), 40.43 (s), 36.78 (s), 28.66 (d, J = 9.2 Hz). HRMS (ESI): calcd. for [C₅₀H₅₁N₂O₃P₂Pt + H]^+: 953.3342, found: 953.3339.

**Complex 2c**

In a glove box, a freshly prepared solution of XeF₂ (3.4 mg, 0.02 mmol) in DCM (0.5 mL) was quickly added to a DCM solution of S₄ (19.1 mg, 0.02 mmol) in a plastic vial at RT. The ³¹P NMR and ¹⁹F NMR analysis immediately showed the formation of complex 2c as the major product. Removal of solvent produced light solid which could be used with further purification.
(19.6 mg, yield 98%) $^{31}$P NMR (162 MHz, CDCl$_3$) $\delta$ 41.13 (s, $J = 2629.3$ Hz). $^{19}$F NMR (377 MHz, DMSO) $\delta$ -217.3 (d, $J = 75.4$ Hz, 1F), -306.0 (d, $J = 79.2$ Hz, 2H), 8.18 (d, $J = 8.1$ Hz, 2H), 7.76 (m, 4H), 7.61 (d, $J = 8.4$ Hz, 1H, 7.49 – 7.43 (m, 2H), 7.26 – 7.15 (m, 3H), 7.09 (d, $J = 8.1$ Hz, 1H), 6.93 (d, $J = 8.2$ Hz, 1H), 6.77 – 6.66 (m, 2H), 5.21 (dd, $J = 40.9$, 13.8 Hz, 2H), 2.63 (s, 3H), 2.30 (d, $J = 7.4$ Hz, 3H), 2.21 (d, $J = 10.1$ Hz, 3H), 1.97 (s, 3H), 1.79 (s, 3H), 1.59 (s, 9H), 1.36 (d, $J = 11.7$ Hz, 3H), 1.20 (d, $J = 11.2$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 164.06 (s), 145.66 (s), 139.62 (s), 135.73 (s), 134.18 (s), 133.55 (s), 132.11 (s), 131.70 (s), 131.41 (s), 127.77 (d, $J = 18.7$ Hz), 127.07 (s), 125.58 (s), 122.75 (s), 118.72 (dd, $J = 28.7$, 7.4 Hz), 49.25 (d, $J = 17.3$ Hz), 47.33 (d, $J = 17.4$ Hz), 40.03 (s), 38.54 (s), 36.43 (s), 35.82 (s), 29.18 (s, $J = 9.3$ Hz), 28.83 (d, $J = 9.3$ Hz). HRMS (ESI): calcd. for [C$_{50}$H$_{51}$F$_2$N$_2$O$_3$PPt]$^+$: 991.3253, found: 991.3254.

2.2 Synthesis of protected amino acids

O-Methyl-N$^\alpha$-(4-fluorophenyl)tryptophane (5)

L-tryptophan (204.2 mg, 1.0 mmol) was dissolved in MeOH (5 mL) and the solution was cooled down to 0°C. Thionyl chloride (72.6 µL, 1.0 mmol) was added dropwise over a period of 15 min. The reaction mixture was heated at 80°C for 3h and then cooled down to RT. The solvent was removed under vacuum to give the L-tryptophan methyl ester HCl salt as a white solid. The product can be used in the next step without further purification. To an ice-cooled solution of L-Tryptophan methyl ester hydrochloride (300.5 mg, 1.18 mmol) in dry DCM was added NEt$_3$ (160 µL, 1.17 mmol). To this stirred solution, 4-fluorobenzoyl chloride (280.5 mg, 1.77 mmol) was added dropwise over a period of 1 h. After 12h, the reaction mixture was washed sequentially with 0.2 N H$_2$SO$_4$, NaHCO$_3$ solution and water. The organic layer was dried over MgSO$_4$, filtered off, evaporated and purified by column
chromatography using ethyl acetate and hexane to give 5 as white solid.\(^4\) (247.2 mg, yield 61.5%)

\[ 5 \]

\(^{19}\)F NMR (377 MHz, CDCl\(_3\)) \(\delta -108.14\) (s, 1F). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 8.53\) (s, 1H), 7.66 (dd, \(J = 8.3, 5.4\) Hz, 2H), 7.54 (d, \(J = 7.9\) Hz, 1H), 7.33 (d, \(J = 8.0\) Hz, 1H), 7.18 (t, \(J = 7.5\) Hz, 1H), 7.08 (t, \(J = 7.4\) Hz, 1H), 7.01 (t, \(J = 8.4\) Hz, 2H), 6.97 (s, 1H), 6.70 (s, 1H), 5.14 (dd, \(J = 12.7, 5.3\) Hz, 1H), 3.72 (s, 3H), 3.53 – 3.32 (m, 2H). \(^1\)H NMR (400 MHz, DMSO-d\(_6\)) \(\delta 10.93\) (s, 1H), 8.94 (d, \(J = 6.8\) Hz, 1H), 7.99 (s, 2H), 7.65 (d, \(J = 7.4\) Hz, 1H), 7.40 (dd, \(J = 16.9, 8.0\) Hz, 3H), 7.29 (s, 1H), 7.15 (t, \(J = 8.0\) Hz, 1H), 7.08 (t, \(J = 8.0\) Hz, 1H), 4.77 (d, \(J = 5.2\) Hz, 1H), 3.73 (s, 3H), 3.40 – 3.26 (m, 2H). \(^13\)C NMR (101 MHz, CDCl\(_3\)) \(\delta 172.60\) (s), 166.28 (s), 165.01 (d, \(J = 248.5\) Hz), 136.39 (s), 130.13 (s), 129.65 (d, \(J = 9.0\) Hz), 127.83 (s), 122.79 (d, \(J = 64.6\) Hz), 122.47 (s), 119.88 (s), 118.70 (s), 115.75 (d, \(J = 21.9\) Hz), 111.63 (s), 109.96 (s), 53.84 (s), 52.66 (s), 27.77 (s). HRMS (ESI): calcd. for \([C_{19}H_{17}FN_2O_3 + H]^+\): 341.1301, found: 341.1295.

**O-methyl proline (S5)**

Thionyl chloride (87.2 µL, 1.2 mmol) was added dropwise to anhydrous methanol (5 mL) at 0°C. The solution was stirred at 0°C for 30 min and L-Proline (115.1 mg, 1.0 mmol) was added. The reaction mixture was refluxed for 5h and TLC (CH\(_2\)Cl/MeOH, 9/1) indicated complete disappearance of L-Proline. The reaction mixture was evaporated under reduced pressure and the yellow oil residue was pure enough for further reaction (129.0 mg, yield 100%).

\[ S5 \]

\(^1\)H NMR (400 MHz, MeOD) \(\delta 4.47\) (t, \(J = 7.7\) Hz, 1H), 3.89 (s, 3H), 3.42 (dd, \(J = 16.4, 7.5\) Hz, 2H), 2.46 (td, \(J = 14.0, 7.1\) Hz, 1H), 2.23 – 2.05 (m, 3H). LCMS (ESI) m/z : 130.2 (M + H)^+.
**O-methyl phenylalanine (6-Phe)**

Thionyl chloride (726.3 µL, 10.0 mmol) was slowly added to a solution of L-phenylalanine (826.0 mg, 5.0 mmol) in anhydrous methanol at 0°C. The reaction mixture was heated at 65°C for 5h with stirring. The solvent was removed and saturated Na₂CO₃ solution was added until pH = 9. Extract with EtOAc (3 x 50 mL) and the combined organic layer was dried over anhydrous MgSO₄. Filter and evaporate under reduced pressure to obtain the product.²

\[
\begin{align*}
\text{6-Phe} & \quad \text{OMe} \\
\text{NH}_2 & \quad \text{Ph}
\end{align*}
\]

¹H NMR (400 MHz, CDCl₃) δ 7.31 (t, J = 7.2 Hz, 2H), 7.25 (d, J = 7.2 Hz, 1H), 7.19 (d, J = 7.2 Hz, 2H), 3.78 – 3.72 (m, 1H), 3.71 (s, 3H), 3.09 (dd, J = 13.5, 5.0 Hz, 1H), 2.86 (dd, J = 13.5, 7.9 Hz, 1H). GC-MS (EI) m/z : 179.0 (M⁺)

### 2.3 Synthesis of peptides 7 and 8

The peptides 7 and 8 were synthesized on a 0.25 mmol scale using the CEM Liberty Blue™ automated microwave peptide synthesizer by the 9-fluorenylmethoxycarbonyl/tert-butyl (Fmoc/tBu) chemistry on Fmoc-Ser(OtBu)-Wang resin (0.51 meq/g substitution) and Fmoc-Arg(Pbf)-Wang resin (0.33 meq/g substitution), respectively. Side chains of trifunctional amino acids were protected with 2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-sulfonyl (Pbf) for Arg; tert-butyl (tBu) for Tyr, Thr, Asp, Glu; tert-butyloxycarbonyl (Boc) for Trp, Lys; trityl (Trt) for Cys (Tzamal D-Chem, Pethah Tikva, Israel). Deprotection was performed with 20% piperidine in DMF. Coupling reactions were performed in 5-fold excess of 0.2 M Fmoc-AA-OH with 1.0 M DIC and 1.0 M Oxyma Pure in DMF. Cleavage was performed using the cleavage cocktail 92.5:2.5:2.5 TFA/H₂O/TIS/DODT at RT for three hours. Following cleavage, the peptide was precipitated with Et₂O and lyophilized overnight.
The peptides were analyzed on a Waters Autopurification system analytical module with PDA detector equipped with an Waters XSelect Peptide CSH C18 column (5µm, 4.6mm x 100mm) using a 10 minute gradient from 95:5 water : acetonitrile (both with 0.1% formic acid) to acetonitrile (0.1% formic acid). The Autopurification system was connected to a Waters SQD2 MS detector for structural determination. Purification was performed with a gradient elution of 0.1% FA in (i) H₂O and (ii) MeCN with XSelect CSH C18 Prep Column (5µm, 19mm x 250mm).

7: ¹H NMR (400 MHz, DMSO-d₆) δ 10.97 (s, 1H), 8.55 (d, J = 7.7 Hz, 1H), 8.37 – 8.25 (m, 2H), 8.19 (d, J = 7.7 Hz, 1H), 8.12 (d, J = 6.6 Hz, 1H), 7.68 (d, J = 7.7 Hz, 1H), 7.47 (d, J = 5.3 Hz, 1H), 7.43 (d, J = 7.9 Hz, 1H), 7.28 (s, 1H), 7.19 – 7.13 (m, 1H), 7.10 – 7.03 (m, 1H), 4.48 (d, J = 6.0 Hz, 1H), 4.42 – 4.32 (m, 4H), 4.24 (s, 1H), 3.84 (d, J = 4.9 Hz, 2H), 3.75 (s, 2H), 3.63 (m), 3.58 – 3.50 (m), 3.24 (d), 2.84 (m, 6H), 2.16 – 1.98 (m, 2H), 1.83 (m, 2H), 1.72 – 1.41 (m, 12H), 1.23 (d, J = 6.4 Hz, 4H), 1.04 – 0.77 (m, 14H).

8: ¹H NMR (400 MHz, DMSO-d₆) δ 10.85 (s, 1H), 10.17 (s, 1H), 9.09 (s, 1H), 8.41 (d, J = 23.2 Hz, 4H), 8.25 (s, 2H), 8.07 (d, J = 42.1 Hz, 4H), 7.61 (d, J = 7.2 Hz, 2H), 7.46 (s, 1H), 7.40 (d, J = 7.8 Hz, 2H), 7.28 (bs, 1H), 7.23 – 7.18 (s, 3H), 7.11 (d, J = 6.5 Hz, 4H), 7.07 – 6.99 (m, 2H), 6.72 (d, J = 7.5 Hz, 2H), 4.61 (s, 3H), 4.47 (s, 1H), 4.35 (s, 9H), 4.15 (d, J = 16.3 Hz, 2H), 4.00 (s, 1H), 3.21 – 2.99 (m, 6H), 2.82 (m, 10H), 2.30 (s, 5H), 1.83 (s, 9H), 1.63 (m, 24H), 1.37 – 1.28 (m, 17H), 1.14 (s, 10H), 0.87 (d, J = 28.3 Hz, 26H).
3. Arylation of protected amino acids, synthetic peptides and insulin

3.1 Arylation of protected amino acids with 2a

The reactions were performed using 16.7 mM concentration of an amino acid derivative as a limiting reagent. To a solution of 2a or 2’a in CH₃CN or DMSO in an NMR tube was added perfluorobenzene (C₆F₆) as internal reference. The ratio between the Pt(IV) reagent and C₆F₆ were verified by the ¹⁹F NMR spectroscopy prior to the addition of an amino acid derivative. Amino acid derivative was added, along with 1-2 equiv. of NEt₃ (if indicated), and the reaction mixture was kept at RT or 38°C for a reported time (cf. Scheme 1).

| Amino acid derivative | Amino acid (equiv.) | Pt(IV) reagent | Time (h) | Temp. (°C) | Yield (%) |
|-----------------------|---------------------|----------------|----------|------------|-----------|
| 3                     | 1                   | 1°             | 1 min    | 25         | 100°      |
| 4                     | 1                   | 1abc           | 6        | 38         | 46°       |
| 5                     | 1                   | 1.5abc         | 8        | 38         | 99°       |
| 6-Lys                 | 1                   | 1°             | 1        | 38         | 94°       |
| 55                    | 1                   | 1bdc           | 15       | 38         | 74°       |
| 6a-Gly                | 1                   | 3bdc           | 11       | 38         | 73°       |

*a 2a in CH₃CN. b 2’a and 2.5 equiv. of NEt₃ in DMSO. c 1 equiv. of NEt₃ was added. d yield based on the amount of 2a or 2’a. e yield based on the amount of amino acid derivative 5 or 6a-Gly.

3.2 Characterization of arylated amino acid derivatives

![](image)

¹⁹F NMR (377 MHz, CDCl₃) δ -113.46 (s, 1F). ¹H NMR (400 MHz, CDCl₃) δ 7.52 – 7.26 (m, 2H), 7.06 – 6.87 (m, 2H), 5.33 (d, J = 4.5 Hz, 1H), 4.53 (bs, 1H), 3.56 (s, 3H), 3.31 (s, 2H), 1.41 (s, 9H).
\[^{13}\text{C}\] NMR (101 MHz, CDCl\(_3\)) \(\delta\) 171.07 (s), 162.39 (d, \(J = 247.6\) Hz), 155.02 (s), 134.13 (d, \(J = 8.1\) Hz), 125.61 (s), 116.23 (d, \(J = 21.9\) Hz), 53.40 (s), 52.45 (s), 38.40 (s), 30.42 (s), 28.36 (s). GC-MS (EI) \(m/z\) : 329.1 (M\(^+\)).

\[^{19}\text{F}\] NMR (377 MHz, CDCl\(_3\)) \(\delta\) -123.78 (s, 2F).

\[^{1}\text{H}\] NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.79 (d, \(J = 7.6\) Hz, 1H), 7.54 (t, \(J = 6.9\) Hz, 1H), 7.46 (t, \(J = 7.2\) Hz, 1H), 6.93 (t, \(J = 8.4\) Hz, 2H), 6.86 (d, \(J = 4.6\) Hz, 2H), 6.66 (d, \(J = 7.0\) Hz, 1H), 4.84 (dd, \(J = 12.9, 6.3\) Hz, 1H), 3.77 (s, 1H), 3.58 (t, \(J = 7.3\) Hz, 1H), 2.06 − 1.91 (m, 1H), 1.85 − 1.74 (m, 1H), 1.66 (d, \(J = 6.3\) Hz, 1H), 1.50 − 1.34 (m, 1H). \[^{13}\text{C}\] NMR (101 MHz, CDCl\(_3\)) \(\delta\) 173.21 (s), 167.11 (s), 159.27 (s), 144.66 (s), 132.05 (s), 128.84 (s), 127.21 (s), 122.42 (d, \(J = 7.7\) Hz), 116.11 (d, \(J = 22.3\) Hz), 52.71 (s), 52.58 (s), 32.80 (s), 27.23 (s), 22.98 (s). GC-MS (EI) \(m/z\) : 452.2 (M\(^+\)).

\[^{19}\text{F}\] NMR (377 MHz, CDCl\(_3\)) \(\delta\) -108.00 (s, 1F), -115.33 (s, 1F).

\[^{1}\text{H}\] NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.71 (dd, \(J = 8.3, 5.4\) Hz, 1H), 7.59 (d, \(J = 7.9\) Hz, 1H), 7.45 (d, \(J = 8.3\) Hz, 1H), 7.40 (dd, \(J = 8.6, 4.7\) Hz, 1H), 7.21 (dd, \(J = 17.7, 9.0\) Hz, 1H), 7.14 (t, \(J = 7.4\) Hz, 1H), 7.10 − 7.03 (m, 1H), 6.68 (d, \(J = 7.4\) Hz, 1H), 5.19 (dd, \(J = 12.5, 5.2\) Hz, 1H), 3.77 (s, 1H), 3.51 (qd, \(J = 14.8, 5.1\) Hz, 1H). \[^{13}\text{C}\] NMR (101 MHz, CDCl\(_3\)) \(\delta\) 172.48 (s), 166.08 (s), 165.07 (d, \(J = 253.5\) Hz), 136.51 (s), 135.68 (s), 129.66 (d, \(J = 8.9\) Hz), 129.13 (s), 126.77 (s), 126.25 (d, \(J = 8.4\) Hz), 123.09 (s), 120.61 (s), 119.25 (s), 116.70 (d, \(J = 22.8\) Hz), 115.79 (d, \(J = 22.0\) Hz), 111.37 (s), 110.59 (s), 53.80 (s), 52.72 (s), 27.72 (s).

HRMS (ESI): calcd. for \([\text{C}_{25}\text{H}_{30}\text{F}_{2}\text{N}_{2}\text{O}_{3} + \text{H}]^{+}\): 435.1520, found: 435.1518.
$^{19}$F NMR (377 MHz, CDCl$_3$) $\delta$ -129.52 (s, 1F). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 6.97 (t, $J$ = 8.7 Hz, 2H), 6.52 (dd, $J$ = 8.8, 4.1 Hz, 2H), 4.25 (d, $J$ = 8.5 Hz, 1H), 3.75 (s, 3H), 3.59 (m, 1H), 3.37 (dd, $J$ = 15.3, 7.5 Hz, 1H), 2.41 – 2.30 (m, 1H), 2.24 – 2.15 (m, 2H), 2.14 – 2.08 (m, 1H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 115.84 (d, $J$ = 22.2 Hz), 112.91 (d, $J$ = 7.1 Hz), 61.50 (s), 52.29 (s), 49.07 (s), 31.05 (s), 24.08 (s). GC-MS (EI) m/z : 223.1 (M$^+$)

$^{19}$F NMR (377 MHz, CDCl$_3$) $\delta$ -129.83 (s, 1F). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.81 (d, $J$ = 7.4 Hz, 1H), 7.54 (t, $J$ = 7.2 Hz, 1H), 7.46 (t, $J$ = 7.5 Hz, 1H), 6.87 (t, $J$ = 8.7 Hz, 1H), 6.70 (d, $J$ = 7.6 Hz, 1H), 6.52 (dd, $J$ = 8.8, 4.3 Hz, 1H), 4.88 (dd, $J$ = 12.9, 7.5 Hz, 1H), 3.80 (s, 2H), 3.08 (t, $J$ = 6.8 Hz, 1H), 2.03 (ddd, $J$ = 15.1, 10.4, 5.6 Hz, 1H), 1.88 – 1.78 (m, 1H), 1.75 – 1.62 (m, 1H), 1.59 – 1.42 (m, 1H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 173.26 (s), 167.23 (s), 157.11 (s), 144.80 (s), 134.05 (s), 132.02 (s), 128.83 (s), 127.21 (s), 115.79 (d, $J$ = 22.4 Hz), 113.70 (d, $J$ = 7.3 Hz), 52.73 (s), 52.46 (s), 44.51 (s), 32.86 (s), 29.13 (s), 23.03 (s). GC-MS (EI): m/z 358.2 (M$^+$)

$^{19}$F NMR (377 MHz, CDCl$_3$) $\delta$ -129.81 (s, 1F). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 6.91 (t, $J$ = 8.6 Hz, 2H), 6.60 (dd, $J$ = 8.7, 4.2 Hz, 2H), 3.90 (s, 2H), 3.78 (s, 3H). GC-MS (EI): m/z 183.1 (M$^+$).
\[ \text{6aa-Gly} \]

\[ 1^9\text{F NMR (377 MHz, CDCl}_3\text{)} \delta -121.95 \text{ (s, 2F).} \]

\[ 1^1\text{H NMR (400 MHz, CDCl}_3\text{)} \delta 6.95 \text{ (m, 8H), 4.38 (s, 2H), 3.75 (s, 3H).} \]

\[ 1^{13}\text{C NMR (101 MHz, CDCl}_3\text{)} \delta 171.27 \text{ (s), 158.53 (d, } J = 242.4 \text{ Hz), 143.95 (s), 122.19 (d, } J = 7.8 \text{ Hz), 116.22 (d, } J = 22.4 \text{ Hz), 54.47 (s), 52.29 (s). GC-MS (EI): m/z 277.1 (M\(^+\)).} \]

\[ \text{6a-Phe} \]

\[ 1^9\text{F NMR (377 MHz, CDCl}_3\text{)} \delta -127.78 \text{ (s, 1F).} \]

\[ 1^1\text{H NMR (400 MHz, CDCl}_3\text{)} \delta 7.34 - 7.27 \text{ (m, 1H), 7.17 (d, } J = 7.0 \text{ Hz, 1H), 6.88 (t, } J = 8.6 \text{ Hz, 1H), 6.55 (dd, } J = 8.6, 4.1 \text{ Hz, 1H), 4.29 (t, } J = 6.2 \text{ Hz, 1H), 3.67 (s, 1H), 3.13 (qd, } J = 13.5, 6.3 \text{ Hz, 1H). GC-MS (EI) m/z : 273.2 (M\(^+\)).} \]

### 3.3 Arylation of peptide 7.

#### 3.31. Preparation of 7ba.

In a NMR tube, 2\(^b\) (6.3 mg, 1.3 equiv, 0.007 mmol) was added to a freshly prepared solution of 7 (4.0 mg, 0.005mmol in 0.6mL DMSO, 1 equiv.), and C\(_6\)F\(_6\) (0.005mmol, 1 equiv.) as reference was added. The reaction mixture was left at 30°C for 40min. The \(^9\text{F NMR and LCMS analysis showed the formation of the S- modified product 7b (Fig. S64, S68). Without further purification, 2\(^a\) (6.2mg, 1.3 equiv. 0.007 mmol) and NEt\(_3\) (0.9 µL, 1.3 equiv., 0.007 mmol) were added to the reaction mixture. The reaction mixture was left at 35°C for 2h, and another portion of 2\(^a\) (6.2 mg, 1.3 equiv., 0.007 mmol) was added. After 5h at 35°C, the \(^9\text{F NMR and LCMS analysis showed the formation of the N terminus modified product 7ba. The solution was filtered and the filtrate was stirred for 10 min with 3.4 mg of DTT and transferred to a 15mL centrifuge tube. 10mL H\(_2\)O was added and the precipitated solid was separated by centrifuge (8000r/min, 15min, 0°C). The aqueous phase was removed and the solid was washed with 5 mL DCM. The solid was separated by centrifuge again under} \]
the same condition and dried by lyophilization. The solid could be used for further arylation reactions to prepare 7baaaa (below) without purification.

**Purification of 7ba**

In a glass vial, the 7ba was dissolved in 1 mL DMSO. NaBH₄ (0.8 mg, 4.0 equiv., 0.02 mmol) was added followed by 20 µL H₂O. The reaction was stirred in the glove box for 3h, tightly capped. The LCMS analysis showed the 7ba as the major product and disappearance of the by-product 7ba-Pt(P-O) (retention time 8.77min). 12 mL H₂O was added and trifluoroacetic acid was added to adjust to the pH ~7. The precipitated solid was separated by centrifuge and washed with 2 x 5 mL of cold H₂O. The solid was dried by lyophilization. Further purification of 7ba was achieved using prep-HPLC and the compound was isolated as a white solid.

7b (¹⁹F NMR yield 88%, vs. internal reference C₆F₆) ¹⁹F NMR (377 MHz, DMSO) δ -109.4 (s, 2F). LC-MS analysis showed for [C₄₃H₆₁F₂N₉O₉S - H]⁻ m/z 916.6, calculated for [C₄₃H₆₁F₂N₉O₉S - H]⁻ m/z 916.4.

7ba (¹⁹F NMR yield 95% vs. the 3,5-F₂C₆H₃ group) ¹⁹F NMR (377 MHz, DMSO) δ -109.40 (s, 2F), -129.37 (s, 1F). LC-MS analysis found for [C₄₉H₆₄F₃N₉O₉S - H]⁻ m/z 1010.8, calculated for [C₄₉H₆₄F₃N₉O₉S - H]⁻ m/z 1010.4 (M-H⁻). ¹H NMR (400 MHz, DMSO-d₆) δ 10.88 (s, 1H), 8.51 (d, J = 7.4 Hz, 1H), 8.28 (d, J = 6.6 Hz, 1H), 8.21 (d, J = 8.5 Hz, 1H), 8.13 (d, J = 7.3 Hz, 1H), 8.07 (d, J = 7.1 Hz, 1H), 8.01 (d, J = 8.1 Hz, 1H), 7.79 (s, 2H), 7.70 (d, J = 7.7 Hz, 1H), 7.39 (d, J = 7.8 Hz,
1H), 7.27 (s, 1H), 7.17 (d, \( J = 6.5\) Hz, 2H), 7.15 – 7.09 (m, 2H), 7.04 (t, \( J = 7.1\) Hz, 1H), 6.89 (t, \( J = 8.4\) Hz, 2H), 6.66 (s, 2H), 4.55 (d, \( J = 6.7\) Hz, 1H), 4.47 (s, 1H), 4.34 (d, \( J = 5.5\) Hz, 5H), 3.83 – 3.74 (m, 2H), 3.70 (s, 2H), 3.27 – 3.01 (m, 6H), 2.83 (s, 4H), 2.03 (d, \( J = 12.9\) Hz, 1H), 1.72 (s, 3H), 1.59 (d, \( J = 16.9\) Hz, 6H), 1.41 (s, 3H), 1.27 (d, \( J = 6.4\) Hz, 3H), 1.00 – 0.85 (m, 14H).

3.32. Preparation of 7baaa. In a NMR tube, the modified peptide 7ba (0.005 mmol, 1equiv.) and 2a (8.0 mg, 0.01 mmol, 2 equiv.) were mixed in 0.6 mL of anhydrous DMSO. After 2h at 38°C, the reaction was analyzed by \(^{19}\)F NMR showing ca. 50% conversion to 7baaa.

\[ \text{7baaa} \]

\(^{19}\)F NMR (377 MHz, DMSO) \( \delta \) -109.57 (s, 2F), -123.02 (s, 2F), -129.48 (s, 1F). MS found for \([C_{61}H_{70}F_{5}N_{9}O_{9}S - H]\) m/z 1198.8 (M-H), calculated for \([C_{61}H_{70}F_{5}N_{9}O_{9}S - H]\) m/z 1198.5 (M-H).

After that, complex 2*a (23.7 mg, 0.025 mmol, 5 equiv.) was added along with NEt\(_3\) (3.5 µL, 0.025 mmol) and the reaction was allowed to stay at 38°C for overnight. \(^{19}\)F NMR and LCMS showed the formation of 7baaaa as the major product. After separation by centrifuge, 7baaaa was treated with DTT and NaBH\(_4\) using the same procedure as reported for 7ba. Further purification of 7baaaa was achieved using prep-HPLC and the compound was isolated as a white solid.

\[ \text{7baaaa} \]

\(^{19}\)F NMR (471 MHz, DMSO) \( \delta \) -109.02 (s, 2F), -116.12 (s, 1F), -122.58 (s, 2F), -128.75 (s, 1F). LC-MS found m/z 1292.6 (M-H), calculated
m/z 1292.5 (M-H). 1H NMR (400 MHz, DMSO-d6) δ 8.52 (s, 2H), 8.29 (s, 2H), 7.99 (s, 4H), 7.80 (d, J = 6.8 Hz, 2H), 7.64 (s, 2H), 7.57 – 7.49 (m, 2H), 7.47 – 7.39 (m, 2H), 7.25 (s, 2H), 7.20 – 7.13 (m, 4H), 7.08 (s, 2H), 7.00 (s, 3H), 6.90 (d, J = 7.8 Hz, 2H), 6.73 (s, 3H), 5.83 (d, J = 8.2 Hz, 2H), 4.54 (s, 1H), 4.37 (d, J = 25.7 Hz, 13H), 2.06 (d, J = 35.3 Hz, 4H), 1.72 (bs, 5H), 1.57 (bs, 6H), 1.33 (s, 7H), 1.27 – 1.25 (s, 4H), 1.20 – 1.15 (m, 2H), 0.97 – 0.77 (m, 14H).

3.4 Arylation of peptide 8.

3.4.1 Reactions in DMSO / HEPES buffer (10 mM, 4:1, 1.2 mL)

3.4.1.1 Protocol for the preparation of HEPES buffer

| Reagent   | Amount to add (for 1L) | Final concentration |
|-----------|------------------------|---------------------|
| NaCl      | 6.72g                  | 115mM               |
| CaCl₂     | 133mg                  | 1.2mM               |
| MgCl₂     | 114mg                  | 1.2mM               |
| K₂HPO₄    | 418mg                  | 2.4mM               |
| HEPES     | 4.77g                  | 20mM                |
| H₂O       | to 1L                  |                     |

Adjust the pH to 7.4 with HCl or NaOH. HEPES buffer can be stored refrigerated for several weeks. (doi:10.1101/pdb.rec12259 Cold Spring Harb Protoc. 2010)

For a 4:1 DMSO-HEPES solution, 2mL HEPES buffer (20mM) and 2 ml of deionized H₂O were mixed and added to 16mL of DMSO.

3.4.1.2 Preparation of 8ba

Peptide 8 (4.6 mg, 0.0025 mmol) was dissolved in DMSO/HEPES buffer (10 mM/L, 4:1, 1.2 mL) and C₆F₆ (0.0025 mmol) was added as a reference. After the peptide was completely dissolved, complex 2⁺b (3.1 mg, 0.003 mmol, 1.3 equiv.) was added. The reaction was left at 35°C for 3h. 1⁹F NMR spectroscopy and LCMS analysis showed the S- modified peptide 8b as the major product. The crude mixture was filtered to remove the precipitated Pt(II) salt and 2⁺a (6.0 mg, 0.0063 mmol, 2.5 equiv.) was added. The reaction was left at 38°C for 1 day. 1⁹F NMR spectroscopy and LCMS analysis showed the modified peptide 8ba as the major product.
8b: $^{19}$F NMR (377 MHz, DMSO) δ -109.3 (s, 2F). LC-MS analysis found for [C$_{87}$H$_{129}$F$_{2}$N$_{23}$O$_{24}$S]: m/z 974.5 (M)$^+$, calculated for [C$_{87}$H$_{129}$F$_{2}$N$_{23}$O$_{24}$S]: m/z 974.5 (M)$^+$.  

8ba (19F NMR yield 99% vs. the 3,5-F$_2$C$_6$H$_3$ group) $^{19}$F NMR (377 MHz, DMSO) δ -109.6 (s, 2F), -129.77 (s, 1F). LC-MS analysis found for [C$_{93}$H$_{132}$F$_{3}$N$_{23}$O$_{24}$S]: m/z 1021.4 (M)$^+$, calculated for [C$_{93}$H$_{132}$F$_{3}$N$_{23}$O$_{24}$S]: m/z 1021.5 (M)$^+$. Both, 8b and 8ba were independently prepared in pure DMSO and showed identical NMR and LCMS spectra.

3.4.2 Preparation of 8bc

Peptide 8 (9.2 mg, 0.005 mmol) was dissolved in DMSO (0.6 mL), and 2'b (6.2 mg, 0.007 mmol, 1.3 equiv.) was added. The reaction was left at 35°C for 2h. $^{19}$F NMR and LCMS analysis showed the S-modified product 8b as the major product. Then, 2c (6.4 mg, 0.007 mmol, 1.3 equiv.) was added along with NEt$_3$ (0.9 µL, 0.0065 mmol, 1.3 equiv.). After 2h, another portion of 2c (6.4 mg, 0.007 mmol, 1.3 equiv.) was added. The reaction was left at 35°C for 5h. $^{19}$F NMR spectroscopy and LCMS analysis showed the formation of peptide 8bc.

The product was purified with DTT and NaBH$_4$ using the same procedure as described for 7ba. Further purification of 8bc was achieved using prep-HPLC and the compound was isolated as a white solid.
**8bc** ($^{19}$F NMR yield in DMSO 97%) $^{19}$F NMR (377 MHz, DMSO) δ -109.65 (s, 2F). LC-MS analysis found for [C$_{106}$H$_{140}$F$_2$N$_2$O$_{26}$S]: m/z 1117.4 (M)$^+$, calculated for [C$_{106}$H$_{140}$F$_2$N$_2$O$_{26}$S]: m/z 1116.99 (M)$^+$.  

### 3.4.3 Attempted enzymatic degradation of 8bc

A 0.5 mM solution of 8bc (10 µl) was added to 10 mM of HEPES buffer to reach total volume of 200 µl. Commercial solution of aminopeptidase M (3.4 mg/ml) was diluted 100 times with 10 mM of HEPES buffer to the concentration of 0.034 mg/ml. The solution of the enzyme (1 µl) was added to the solution of 8bc and the reaction was monitored by LCMS. No changes in the spectra were observed after 15 min or 3 days. A control reaction with 8b under the same conditions showed the disappearance of 8 after 15 min.

### 3.5 Arylation of insulin, 9

Cf. Scheme 4: in a NMR tube, human recombinant insulin 9 (14.5 mg, 0.0025 mmol) was dissolved in DMSO. The solution was kept at 35°C for several minutes, until all the solid was completely dissolved. Then, 2'a (7.1 mg, 0.0075 mmol, 3 equiv.) and C$_6$F$_6$ (internal reference, 0.0025 mmol) were added. The mixture was left at 35°C for 3h. The $^{19}$F NMR analysis showed
the formation of \(9\text{aa}\).

For the LCMS analysis, a 1/4 solution of the solution was taken out and DTT (2.4 mg, 25.0 equiv.) and PPh\(_3\) (3.4 mg, 20.0 equiv.) were added. The mixture was stirred at 40\(^\circ\)C for 5h and then DCM (6 mL) and H\(_2\)O (6 mL) were added resulting in the formation of solid between the two phases. This solid was collected after centrifugation and dried by lyophilization.

For the complete arylation, to the crude solution of \(9\text{aa}\), \(2^*\text{a}\) (9.5 mg, 0.01 mmol, 4 equiv.) was added along with NEt\(_3\) (0.7\(\mu\)L, 2 equiv.) and, after 4h, another portion of \(2^*\text{a}\) (9.5 mg, 0.01 mmol, 4 equiv.) was added along with NEt\(_3\) (0.7\(\mu\)L, 2 equiv.). The reaction mixture was left at 38\(^\circ\)C for additional 4h. The \(^{19}\text{F} \) NMR analysis showed the formation of \(9\text{aaaa}\) in a quantitative yield.

The compound was separated by centrifugation and reacted with DTT and PPh\(_3\) to get \(9\text{Aaa}\) and \(9\text{Baaa}\) (as described for \(9\text{aa}\)).

\(9\text{aa}\) \(^{19}\text{F} \) NMR yield 85\% vs. internal C\(_6\)F\(_6\) \(^{19}\text{F} \) NMR (377 MHz, DMSO) \(\delta\) -129.12 (s, overlapped, 1F), -129.31 (s, overlapped, 1F).

HRMS : calculated for 9\text{Aa}[C\(_{105}\)H\(_{158}\)FN\(_{25}\)O\(_{35}\)S\(_{4}\)]\(^{2+}\) : 1237.5045; Found : 1237.5055.

calculated for 9\text{Ba}[C\(_{164}\)H\(_{237}\)FN\(_{40}\)O\(_{42}\)S\(_{2}\)]\(^{2+}\) : 1760.8480; Found : 1760.8423.

calculated for 9\text{Ba}[C\(_{164}\)H\(_{237}\)FN\(_{40}\)O\(_{42}\)S\(_{2}\)]\(^{3+}\) : 1173.5627; Found : 1173.5601.
**F NMR (377 MHz, DMSO) δ -129.08 (s, 1F), -122.71 (s, 2F), -122.45 (s, 2F).**

HRMS: calculated for 9Aaa [C_{111}H_{161}F_{25}N_{25}O_{35}S_{4}]^{2+} : 1284.5155; Found : 1284.5117.

calculated for 9Baaa [C_{176}H_{243}F_{3}N_{40}O_{42}S_{2}]^{2+} : 1856.8856; Found : 1856.8798.

calculated for 9Baaa [C_{176}H_{243}F_{3}N_{40}O_{42}S_{2}]^{3+} : 1238.2596; Found : 1238.2594.

calculated for 9Baaa [C_{176}H_{243}F_{3}N_{40}O_{42}S_{2}]^{4+} : 928.9467; Found : 928.9430.
4. NMR spectra and LCMS data

4.1 NMR spectra of Pt complexes

Figure S1. $^{31}$P NMR spectrum of 2a

Figure S2. $^{19}$F NMR spectrum of 2a

Figure S3. $^1$H NMR spectrum of 2a
Figure S4. $^{13}$C NMR spectrum of $2^\text{a}$

Figure S5. DEPT135 NMR spectrum of $2^\text{a}$

Figure S6. $^{31}$P NMR spectrum of $2^\text{b}$
Figure S7. $^{19}$F NMR spectrum of 2'b

Figure S8. $^1$H NMR NMR spectrum of 2'b
Figure S9. $^{13}$C NMR spectrum of $2'b$

Figure S10. $^1$H NMR spectrum of S1

Figure S11. $^{13}$C NMR spectrum of S1
Figure S12. $^1$H NMR spectrum of S2

Figure S13. $^{13}$C NMR S2

Figure S14. $^1$H NMR spectrum of S3
Figure S15. $^{13}$C NMR spectrum of S3

Figure S16. DEPT135 NMR spectrum of S3

Figure S17. $^{31}$P NMR spectrum S4
Figure S18. $^1$H NMR spectrum of S4

Figure S19. $^{13}$C NMR spectrum of S4

Figure S20. DEPT135 spectrum of S4
Figure S21. $^{31}$P NMR spectrum of 2c

Figure S22. $^{19}$F NMR spectrum of 2c

Figure S23. $^1$H NMR spectrum of 2c
4.2 NMR and MS spectra of substrates and products

4.2.1. Spectra of the starting amino acid derivatives

Figure S24. $^{13}$C NMR spectrum of 2c

Figure S25. DEPT135 NMR spectrum of 2c

Figure S26. $^{19}$F NMR spectrum of 5
Figure S27. $^1$H NMR spectrum of 5 in CDCl$_3$ (left) and DMSO-d$_6$ (right)

Figure S28. $^{13}$C NMR spectrum of 5

Figure S29. $^1$H NMR spectrum of S5
Figure S30. $^1$H NMR spectrum of 6-Phe

Figure S31. $^{13}$C NMR spectrum of 6-Phe
4.2.2. LCMS spectra of peptides 7 and 8

Figure S32. LCMS spectra of peptide 7.

Detected Mass: ES mode +: 404.28 [M+2H/2] and 806.65 [M+H].

Figure S33. LCMS spectra of peptide 8.
Detected Mass: ES mode +: 461.03 [M+4H/4]; 614.37 [M+3H/3]; 920.83 [M+2H/2] and 1840.64 [M+H].

4.2.3. Spectra of the arylation products

Figure S34. $^{19}$F NMR spectrum of 3a

Figure S35. $^1$H NMR spectrum of 3a
Figure S36. $^{13}$C NMR spectrum of 3a

Figure S37. $^{19}$F NMR spectrum of 6a-Lys

Figure S38. $^1$H NMR spectrum of 6a-Lys
Figure S39. $^{13}$C NMR spectrum of 6a-Lys

Figure S40. $^{19}$F NMR spectrum of 4aa

Figure S41. $^1$H NMR spectrum of 4aa
Figure S42. $^{13}$C NMR spectrum of 4aa

Figure S43. DEPT 135 NMR spectrum of 4aa

Figure S44. $^{19}$F NMR spectrum of 5a
Figure S45. $^1$H NMR spectrum of 5a

Figure S46. $^{13}$C NMR spectrum of 5a

Figure S47. $^{19}$F NMR spectra of O-methyl -N- (4-fluorophenyl)phenylalanine (6a-Phe)
Figure S48. $^1$H NMR spectrum of O-methyl -N- (4-fluorophenyl)phenylalanine (6a-Phe)

Figure S49. $^{19}$F NMR spectrum of S5a

Figure S50. $^1$H NMR spectrum of S5a
Figure S51. $^{13}$C NMR spectrum of S5a

Figure S52. $^{19}$F NMR spectrum of O-methyl-N- (4-fluorophenyl)glycine (6a-Gly)

Figure S53. $^1$H NMR spectrum of O-methyl-N- (4-fluorophenyl)glycine (6a-Gly)
Figure S54. GC-MS spectrum of O-methyl-N- (4-fluorophenyl)glycine (6a-Gly)

Figure S55. $^{19}$F NMR spectrum of O-methyl-N- (di-(4-fluorophenyl))glycine (6aa-Gly)

Figure S56. $^1$H NMR spectrum of O-methyl -N- (di-(4-fluorophenyl))glycine (6aa-Gly)
**Figure S57.** $^{13}$C NMR spectrum of O-methyl -N- (di-(4-fluorophenyl))glycine (6aa-Gly)

**Figure S58.** GC-MS spectrum of O-methyl-N- (di-(4-fluorophenyl))glycine (6aa-Gly)

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S43
4.3 NMR and MS Analysis of the Arylation Reactions

4.3.1 Reactions with amino acid derivatives

Figure S59a. $^{19}$F NMR analysis of the reaction between 3 and 2a (before the reaction)

![Figure S59a](image1)

Figure S59b. $^{19}$F NMR analysis of the reaction between 3 and 2a (after the reaction)

![Figure S59b](image2)

Figure S60. MS spectrum of 3a

![Figure S60](image3)

Figure S61a. $^{19}$F NMR analysis of the reaction between 6-Lys and 2a (before the reaction)

![Figure S61a](image4)
Figure S61b. $^{19}$F NMR analysis of the reaction between 6-Lys and 2a (after the reaction)

Figure S62. MS spectrum of O-methyl N$^6$-benzoyl-N$^2$-(4-fluorophenyl)lysine (6a-Lys)

Figure S63a. $^{19}$F NMR analysis of the reaction between 4 and 2a (before the reaction)
Figure S63b. $^{19}$F NMR analysis of the reaction between 4 and 2a (after the reaction)

Figure S64. MS spectrum of 4aa

Figure S65a. $^{19}$F NMR analysis of the reaction between 5 and 2a (before the reaction)
Figure S65b. $^{19}$F NMR analysis of the reaction between 5 and 2a (after the reaction)

Figure S66. MS spectrum of 5a

Figure S67a. $^{19}$F NMR analysis of the reaction between 55 and 2a (before the reaction)
Figure S67b. $^{19}$F NMR analysis of the reaction between S5 and 2a (after the reaction)

Figure S68. MS spectrum of S5a.

Figure S69a. $^{19}$F NMR analysis of the reaction between 6a-Gly and 2’a (before the reaction)
Figure S69b. $^{19}$F NMR analysis of the reaction between 6a-Gly and 2'a (after the reaction).

Figure S70. MS spectrum of 6aa-Gly.
4.3.2 Reactions with peptides 7, 8 and insulin 9

Figure S71. $^{19}$F NMR spectrum of the crude reaction mixture between 7 and 2b

Figure S72. $^{19}$F NMR spectrum of the crude reaction mixture between 7b and 2a (same pot reaction)

Figure S73. $^{19}$F NMR spectrum of purified 7ba
Figure S74. Comparative $^1$H NMR spectra of 7 (left) and purified 7ba (right) in DMSO-$d_6$.

Figure S75. $^{19}$F NMR spectrum of the crude 7baaaa (same pot reaction)

Figure S76. $^{19}$F NMR spectrum of purified 7baaaa
Figure S77. $^1$H NMR spectrum of purified 7baaaa

Figure S78. LCMS follow up of the arylation of 7.

Figure S79. Preparative HPLC purification of 7ba.
Figure S80. HRMS of 7ba.
HRMS : calculated for 7ba [C_{49}H_{64}F_{3}N_{9}O_{9}S + H]^{+}: 1012.4578; Found : 1012.4627.

Figure S81. Calibration graph and calculation of the reaction yield for 7ba.
Area expected for 0.0025 mmol/ml – 150000; Area obtained – 126224. Yield – 84.15%

Figure S82. Preparative HPLC purification of 7baaa.
Figure S83. HRMS of 7baaaa.
HRMS : calculated for 7baaaa [C_{49}H_{64}F_{3}N_{9}O_{9}S + H]^+ : 1012.4578 ; Found : 1012.4627.

Figure S84. ^19F NMR spectrum of 8b in DMSO/0.1M HEPES (4:1) crude reaction mixture

Figure S85. ^19F NMR spectrum of 8ba in DMSO/0.1M HEPES (4:1) crude reaction mixture (same pot)
Figure S86. $^{19}$F NMR spectrum of 8ba in DMSO after the workup

Figure S87. $^{19}$F NMR spectrum of crude 8b prior to the reaction with 2c.

Figure S88. $^{19}$F NMR spectrum of crude 8bc.
Figure S89. $^{19}$F NMR spectrum of purified 8bc in DMSO.

Figure S90. Comparative $^1$H NMR spectra of 8 and purified 8bc in DMSO-d$_6$.

Figure S91. UV-Fluorescence spectra of 8bc.
Figure S92. LCMS spectra of 8b.

Figure S93. LCMS spectra of 8ba.

Total TIC area – 123981; TIC (8ba) – 110747. Yield – 89.3%

Figure S94. LCMS spectra of 8bc.
Total TIC area – 171621; TIC (8bc) – 149981. Yield – 87.4%

Figure S95. Preparative HPLC-MS separation of 8bc.

Figure S96. HRMS of 8bc

HRMS : calculated for 8bc [C_{107}H_{141}F_{23}N_{23}O_{28}S + H]^+ : 1119.0186; Found : 1119.0150.
Figure S97. $^{19}$F NMR spectrum of crude 9aa prior to the reaction with 2’a.

Figure S98. $^{19}$F NMR spectrum of crude 9aaaa.

Figure S99. $^{19}$F NMR spectrum of purified 9aaaa.
Figure S100. LCMS spectrum of 9 after the reaction with DTT (reference reaction, formation of 9A and 9B)

Figure S101. LCMS spectrum of 9aa after the reaction with DTT (formation of 9Aa and 9Ba)
Figure S102. HRMS of the mixture of 9Aa (top) and 9Ba (bottom)

Figure S103. LCMS spectrum of 9aaaa after the reaction with DTT (formation of 9Aaa and 9Baaa)
Figure S104. HRMS of the mixture of 9Aaa (top) and 9Baaa (bottom)
5 References

1. I.S. Dubinsky-Davidchik, I. Goldberg, A. Vigalok, A. N. Vedernikov, Angew. Chem. Int. Ed., 2015, 54, 12447-12451.
2. S. Fan, Y. Ding, X. Chen, Y. Gao, L. Fu, S. Li, G. Li, J. Org. Chem., 2019, 84, 13003-13012.
3. S. Kumar, C. C. Malakar, V. Singh, ChemistrySelect, 2021, 6, 4005-4010.
4. V. Haridas, P. P. P. Kumar, I. Bhardwaj, P. Venugopalan, ChemistrySelect, 2017, 2, 130-135.
5. Z. Chen, Y. Zhou, X. Bu, T. Zhang, M. He, Des. Monomers Polym., 2014, 17, 701-716.
6 B. Xu, N. Wang, W. Pan, J. Qiu, P. Cao, M. Zhu, Y. Feng, G. Liang, Bioorg. Chem., 2014, 56, 34-40.
7. K. Gu, L. Bi, M. Zhao, C. Wang, J. Ju, S. Peng, Bioorg. & Med. Chem., 2007, 15, 6273-6290.
8. M. Diéguez, O. Pàmies, Chem. – Eur. J., 2008, 14, 3653-3669.