Supporting Information for

Homoserine and Threonine Peptide Assembly

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General procedures for synthesizing activated esters.

**Protocol A:** The acetyl AA (1 equiv) is dissolved in dichloromethane (0.1 M) and set to stir at 0 °C. EDCI (1.2 equiv), HOBt (1.2 equiv) and 1,1,1,3,3,3-hexafluoroisopropanol (10 equiv) were added to the reaction mixture. The mixture was stirred at 0 °C for 30 min, then for 4 h at room temperature. The solvent was removed in vacuo, and the residue was dissolved in ethyl acetate and washed with 1 N HCl and sat. NaHCO₃. The organic phase was dried over sodium sulfate, filtered, and concentrated in vacuo to afford a crude product that was purified by column chromatography.

**Protocol B:** The acetyl AA (1 equiv) was dissolved in acetonitrile (0.1 M) and set to stir at 0 °C. Triethylamine (2.0 equiv) was added followed by chloroacetonitrile (1.5 equiv). The mixture was stirred at 0 °C for 30 min, then for 4 h at room temperature. The solvent was removed in vacuo to afford a crude product that was purified by column chromatography.

**Protocol C:** The acetyl AA (1 equiv) was dissolved in acetonitrile (0.1 M) and set to stir at 0 °C. Cesium carbonate (2.0 equiv) was added followed by a triflate (TFE/PFP/HIP, 1.5 equiv). The mixture was stirred at 0 °C for 30 min, then for 16 h at room temperature. The solvent was removed in vacuo, and the residue was dissolved in ethyl acetate and washed with 1 N HCl and sat. aqueous NaHCO₃. The organic phase was dried over sodium sulfate, filtered, and concentrated in vacuo to afford a crude product that was purified by column chromatography.

**Acetyl alanine HIP ester.** The general procedure for the synthesis of activated esters, protocol A, was followed using acetyl alanine. The crude product was
purified by column chromatography (SiO₂, acetone/dichloromethane gradient, 1:9 to 2:3) to give a white solid (1.9862 g, 92%). Mp: 69-71 °C. \([\alpha]^{D}_{25} – 46.8 \ (c 1.01, \text{MeOH})\). IR (neat): 3252, 3073, 2996, 2973, 1798, 1788, 1641, 1545, 1460, 1383, 1362, 1312, 1284, 1260, 1248, 1232, 1194, 1174, 1122, 1106, 1077, 1052, 1013, 961, 930, 900, 732, 688, 646, 594, 552, 536, 514, 451 cm⁻¹. \(^1\)H NMR (500 MHz, CDCl₃) \(\delta 6.64 \ (s, \text{NH})\), 5.88 – 5.61 \(\text{m, 1H}\), 4.82 – 4.57 \(\text{m, 1H}\), 2.01 \(\text{s, } J = 7.5 \text{ Hz, 3H}, 1.45 \ (\text{d, } J = 7.3 \text{ Hz, 3H})\). \(^{13}\)C NMR (101 MHz, CDCl₃) \(\delta 171.0, 170.4, 124.6, 121.8, 119.0, 116.2, 67.9, 67.5, 67.2, 66.8, 66.5, 48.2, 22.4, 17.1\). \(^{19}\)F NMR (376 MHz, CDCl₃) \(\delta -76.15, -76.17, -76.20, -76.22, -76.24, -76.35, -76.37, -76.40, -76.42, -76.44\). HRMS calcd. for C₈H₉F₆NO₃Na \([\text{M+Na}^+]\) 304.0379, found 304.0431.

Acetyl alanine TFE ester. The general procedure for the synthesis of activated esters protocol C was followed, using acetyl alanine and TFE triflate. The crude product was purified by column chromatography (SiO₂, acetone/dichloromethane gradient, 1:9 to 2:3) to give a white solid (0.8109 g, 83%). Mp: 60-63 °C. \([\alpha]^{D}_{25} – 33.2 \ (c 1.01, \text{MeOH})\). IR (neat): 3298, 3077, 2981, 2962, 1751, 1644, 1546, 1454, 1422, 1374, 1345, 1284, 1268, 1201, 1156, 1075, 1036, 959, 925, 893, 837, 752, 694, 642, 612, 598, 515, 436, 408 cm⁻¹. \(^1\)H NMR (400 MHz, CDCl₃) \(\delta 6.34 \ (s, \text{NH})\), 4.71 – 4.53 \(\text{m, 2H}\), 4.49-4.34 \(\text{m, 1H}\), 2.01 \(\text{s, 3H}, 1.43 \ (\text{d, } J = 7.3 \text{ Hz, 3H})\). \(^{13}\)C NMR (101 MHz, CDCl₃) \(\delta 171.9, 170.2, 127.0, 124.3, 121.5, 118.7, 61.5, 61.1, 60.8, 60.4, 48.1, 23.0, 17.9\). \(^{19}\)F NMR (376 MHz, CDCl₃) \(\delta -76.98, -77.01, -77.03\). HRMS calcd. for C₇H₁₉F₃NO₃Na \([\text{M+Na}^+]\) 236.0505, found 236.0466.
**Acetyl alanine CM ester.** The general procedure for the synthesis of activated esters, protocol B, was followed using acetyl alanine. The crude product was purified by column chromatography (SiO₂, acetone/dichloromethane gradient, 1:9 to 2:3) to give a white solid (0.7455 g, 94%). Mp: 62-64 °C. [α]$_{D}^{25}$ –84.1 (c 1.00, MeOH). IR (neat): 3254, 3061, 2994, 2942, 1771, 1758, 1639, 1542, 1455, 1420, 1375, 1351, 1304, 1277, 1262, 1193, 1143, 1058, 1015, 996, 954, 927, 917, 861, 838, 685, 596, 554, 497, 488 cm$^{-1}$. $^1$H NMR (500 MHz, CDCl$_3$) δ 6.81 (d, $J$ = 7.0 Hz, NH), 4.76 (d, $J$ = 15.8 Hz, 1H), 4.70 (d, $J$ = 15.8 Hz, 1H), 4.51 (p, $J$ = 7.1 Hz, 1H), 1.95 (s, 3H), 1.37 (d, $J$ = 7.3 Hz, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$) δ 171.7, 170.4, 114.3, 49.0, 47.8, 22.6, 17.3. HRMS calcd. for C$_7$H$_{11}$N$_2$O$_3$[M+H]$^+$ 171.0764, found 171.0778.

**Acetyl alanine PFP ester.** The general procedure for the synthesis of activated esters, protocol C, was followed using acetyl alanine and PFP triflate. The crude product was purified by column chromatography (SiO₂, acetone/dichloromethane gradient, 1:9 to 2:3) to give a clear oil (1.0636 g, 88%). [α]$_{D}^{25}$ –38.9 (c 1.00, MeOH). IR (neat): 3310, 3066, 3007, 2944, 1760, 1654, 1538, 1456, 1376, 1353, 1335, 1308, 1280, 1196, 1144, 1107, 1058, 997, 976, 967, 949, 939, 899, 879, 797, 780, 752, 718, 655, 619, 588, 522, 463, 428 cm$^{-1}$. $^1$H NMR (400 MHz, CDCl$_3$) δ 6.43 (d, $J$ = 41.9 Hz, NH), 4.79 – 4.55 (m, 2H), 4.46 (q, $J$ = 12.8 Hz, 1H), 1.99 (s, $J$ = 2.9 Hz, 3H), 1.40 (d, $J$ = 7.3 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 171.8, 170.3, 120.3, 120.0, 119.6, 117.5, 117.1, 116.8, 114.8, 114.37, 112.2, 111.8, 109. 7, 109.3, 60.0, 59.7, 59.5, 48.1, 22.8, 17.7. $^{19}$F NMR (376 MHz,
CDCl₃ δ -86.86, -86.90, -86.94, -126.53, -126.56, -126.59, -126.63, -126.66.

HRMS calcd. for C₈H₁₁F₅NO₃ [M+H]^+ 264.0654, found 264.0640.

**Acetyl valine HIP ester.** The general procedure for the synthesis of activated esters, protocol A, was followed using acetyl valine. The crude product was purified by column chromatography (SiO₂, acetone/dichloromethane gradient, 1:9 to 2:3) to give a white solid (2.1678 g, 93%). Mp: 54-58 °C. [α]D<sup>25</sup> = -24.7 (c 1.01, MeOH). IR (neat): 3354, 3327, 3298, 2976, 2941, 2882, 1774, 1680, 1652, 1530, 1471, 1447, 1372, 1357, 1294, 1268, 1254, 1215, 1195, 1158, 1105, 1076, 1045, 1017 998, 947, 926, 906, 884, 873, 837, 811, 793, 779, 766, 713, 691, 637, 609, 598, 575, 529, 521, 478, 431 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ 6.57 (s, NH), 5.75 (hept, J = 5.9 Hz, 1H), 4.74 – 4.61 (m, 1H), 2.28 – 2.13 (m, 1H), 2.03 (s, 3H), 0.97 (d, J = 6.9 Hz, 3H), 0.92 (d, J = 6.9 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 170.9, 169.4, 124.6, 121.9, 119.0, 116.2, 68.0, 67.7, 67.3, 67.0, 66.6, 66.3, 65.9, 57.3, 30.8, 22.7, 18.8, 17.5. ¹⁹F NMR (376 MHz, CDCl₃) δ -76.09, -76.10, -76.18, -76.20.

HRMS calcd. for C₁₀H₁₄F₆NO₃ [M+H]^+ 310.0872, found 310.0850.

**Acetyl valine CM ester.** The general procedure for the synthesis of activated esters, protocol B, was followed using acetyl valine. The crude product was purified by column chromatography (SiO₂, acetone/dichloromethane gradient, 1:9 to 2:3) to give a white solid (0.1517 g, 90%). Mp: 80-82 °C. [α]D<sup>25</sup> = -58.0 (c 0.506, MeOH). IR (neat): 3306, 3068, 3018, 2972, 2937, 2915, 2878, 1754, 1650, 1538, 1471, 1427, 1376, 1346, 1334, 1306, 1293, 1273, 1249, 1183, 1147, 1115, 1102, 1058, 1034, 1013, 979, 942, 915, 887, 836, 754, 719, 636, 600, 567, 516, 464, 414 cm⁻¹. ¹H NMR (500 MHz, CDCl₃) δ 6.20 (d, J = 7.4 Hz, NH), 4.83 (d, J = 15.7 Hz, 1H).
4.70 (d, J = 15.7 Hz, 1H), 4.64 – 4.46 (m, 1H), 2.31 – 2.10 (m, 1H), 2.03 (s, 3H), 0.97 (d, J = 6.8 Hz, 3H), 0.94 (d, J = 6.8 Hz, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$) δ 171.0, 170.5, 114.2, 57.1, 48.9, 31.1, 23.1, 19.1, 18.0. HRMS calcd. for C$_9$H$_{14}$N$_2$O$_3$Na [M+Na]$^+$ 221.0897, found 221.0889.

**Acetyl phenylalanine HIP ester.** The general procedure for the synthesis of activated esters, protocol A, was followed using acetyl phenylalanine. The crude product was purified by column chromatography (SiO$_2$, acetone/dichloromethane gradient, 1:9 to 2:3) to give a white solid (0.5776 g, 96%). Mp: 96-98 °C. $[\alpha]^{25}_D$–11.2 (c 1.01, MeOH). IR (neat): 3339, 3066, 3034, 2976, 2943, 1777, 1647, 1534, 1497, 1456, 1443, 1384, 1357, 1272, 1240, 1191, 1158, 1141, 1108, 1092, 1031, 1014, 963, 919, 895, 855, 777, 749, 734, 697, 677, 612, 589, 555, 535, 524, 479, 462, 434 cm$^{-1}$. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.32 – 7.14 (m, 3H), 7.06 (d, J = 6.7 Hz, 2H), 5.88 (s, NH), 5.68 (hept, J = 6.0 Hz, 1H), 4.93 (dd, J =13.5, 7.1 Hz, 1H), 3.15 (dd, J = 14.2, 5.9 Hz, 1H), 3.00 (dd, J = 14.2, 7.0 Hz, 1H), 1.89(s, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 170.2, 169.2, 134.8, 129.3, 129.1, 127.8, 121.9, 119.1, 68.1, 67.7, 67.4, 67.0, 66.7, 53.1, 37.3, 22.9. $^{19}$F NMR (376 MHz, CDCl$_3$) δ -76.00, -76.02, -76.03, -76.05, -76.12, -76.14, -76.16, -76.18. HRMS calcd. for C$_{14}$H$_{13}$F$_6$NO$_3$Na [M+Na]$^+$ 380.0692, found 380.0690.

**Acetyl phenylalanine CM ester.** The general procedure for the synthesis of activated esters, protocol B, was followed using acetyl phenylalanine. The crude product was purified by column chromatography (SiO$_2$, acetone/dichloromethane gradient, 1:9 to 2:3) to give a white solid (0.2335 g, 93%). Mp: 100-103 °C. $[\alpha]^{25}_D$–4.1 (c 1.03, MeOH). IR (neat): 3308, 3087, 3067,
Acetyl leucine HIP ester. The general procedure for the synthesis of activated esters, protocol A, was followed using acetyl leucine. The crude product was purified by column chromatography (SiO$_2$, acetone/dichloromethane gradient, 1:9 to 2:3) to give a clear oil (0.9398 g, 98%). [$\alpha$]$^D_{25}$-29.6 ($c$ 0.996, MeOH). IR (neat): 3271, 3076, 2965, 2877, 1786, 1656, 1546, 1472, 1441, 1384, 1358, 1288, 1269, 1228, 1199, 1109, 1033, 981, 923, 906, 880, 843, 761, 717, 687, 600, 538, 519, 451 cm$^{-1}$. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 6.37 (d, $J$ = 7.3 Hz, NH), 5.73 (hept, $J$ = 6.0 Hz, 1H), 4.77 - 4.63 (m, 1H), 2.02 (s, $J$ =6.4 Hz, 3H), 1.79 - 1.50 (m, 3H), 1.00 - 0.90 (m, 6H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$170.8, 170.3, 124.7, 121.8, 119.0, 116.2, 67.8, 67.5, 67.1, 66.8, 66.4, 51.0, 40.6, 25.0, 22.7, 21.7. $^{19}$F NMR (376 MHz, CDCl$_3$) $\delta$ -76.18, -76.20, -76.22, -76.24, -76.26, -76.29, -76.30, -76.32. HRMS calcd. for C$_{11}$H$_{16}$F$_6$NO$_3$ [M+H]$^+$ 324.1029, found 324.1046.

Acetyl leucine CM ester. The general procedure for the synthesis of activated esters, protocol B, was followed using acetyl leucine. The crude product was purified by column chromatography (SiO$_2$, acetone/dichloromethane gradient,
1:4 to 2:3) to give a clear oil (0.4745 g, 96%). [α]D_{25}^{45} = -56.7 (c 0.988, MeOH). IR (neat): 3385, 3286, 3064, 2960, 2873, 1758, 1654, 1536, 1470, 1431, 1371, 1274, 1226, 1144, 1012, 919, 821, 693, 595, 506, 466 cm\(^{-1}\). \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 6.42 (s, NH), 4.79 (d, \(J = 15.8\) Hz, 1H), 4.69 (d, \(J = 15.7\) Hz, 1H), 4.57 (dd, \(J = 13.2, 8.2\) Hz, 1H), 1.99 (s, 3H), 1.74 – 1.47 (m, 3H), 0.92 (t, \(J = 6.8\) Hz, 6H). \(^13\)C NMR (126 MHz, CDCl\(_3\)) \(\delta\) 171.9, 170.6, 114.3, 50.7, 49.0, 40.7, 24.9, 22.9, 22.8, 21.8. HRMS calcd. for C\(_{10}\)H\(_{16}\)N\(_2\)O\(_3\)Na [M+Na\(^+\)] 235.1053, found 235.1029.

**Acetyl isoleucine HIP ester.** The general procedure for the synthesis of activated esters, protocol A, was followed using acetyl isoleucine. The crude product was purified by column chromatography (SiO\(_2\), acetone/dichloromethane gradient, 1:9 to 2:3) to give a clear oil (0.8547 g, 90%). [α]D_{25}^{45} = -11.0 (c 1.00, MeOH). IR (neat): 3292, 3058, 2974, 2942, 2884, 1785, 1651, 1539, 1463, 1386, 1361, 1285, 1270, 1226, 1191, 1127, 1109, 1075, 970, 941, 904, 869, 772, 724, 692, 671, 595, 524, 502, 467, 454, 432, 417 cm\(^{-1}\). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 5.76 (hept, \(J = 6.0\) Hz, 1H), 4.76 (dd, \(J = 8.4, 5.0\) Hz, 1H), 2.05 (s, 3H), 2.03 – 1.90 (m, 1H), 1.49 – 1.36 (m, 1H), 1.30 – 1.14 (m, 1H), 0.99 – 0.93 (m, 6H). \(^13\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 170.5, 169.4, 124.8, 122.0, 119.1, 116.3, 67.8, 67.4, 67.1, 7, 66.4, 56.7, 37.7, 25.2, 23.1, 15.5, 11.6. \(^19\)F NMR (376 MHz, CDCl\(_3\)) \(\delta\) -76.08, -76.09, -76.11, -76.14, -76.16, -76.18, -76.20, -76.22. HRMS calcd. for C\(_{11}\)H\(_{16}\)F\(_6\)NO\(_3\) [M+H\(^+\)] 324.1029, found 324.1036.

**Acetyl isoleucine CM ester.** The general procedure for the synthesis of activated esters, protocol B, was followed using acetyl isoleucine. The crude product was purified by column chromatography (SiO\(_2\),
acetone/dichloromethane gradient, 1:9 to 2:3) to give a white solid (0.4869 g, 98%). Mp: 78-81 °C. [α]D25 –36.2 (c 1.01, MeOH). IR (neat): 3294, 3057, 3012, 2969, 2937, 2882, 1752, 1651, 1536, 1457, 1432, 1371, 1345, 1293, 1273, 1231, 1177, 1142, 1106, 1032, 1002, 932, 910, 883, 781, 746, 724, 639, 602, 523, 473, 424 cm\(^{-1}\).

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 6.11 (d, \(J = 7.9\) Hz, NH), 4.84 (d, \(J = 15.6\) Hz, 1H), 4.70 (d, \(J = 15.7\) Hz, 1H), 4.62 (dd, \(J = 8.4, 5.4\) Hz, 1H), 2.03 (s, \(J = 4.9\) Hz, 3H), 1.97 – 1.85 (m, 1H), 1.52 – 1.38 (m, 1H), 1.29 – 1.14 (m, 1H), 0.96 – 0.91 (m, 6H).

\(^13\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 171.0, 170.4, 114.1, 56.4, 48.8, 37.8, 25.4, 23.2, 15.6, 11.6. HRMS calcd. for C\(_{10}\)H\(_{16}\)N\(_2\)O\(_3\)Na \([M+Na]^+\) 235.1053, found 235.1036.

**Acetyl proline HIP ester.** The general procedure for the synthesis of activated esters, protocol A, was followed using acetyl proline. The crude product was purified by column chromatography (SiO\(_2\), acetone/dichloromethane gradient, 1:9 to 2:3) to give a clear oil (0.7619 g, 77%). [α]D25 –63.0 (c 0.994, MeOH). IR (neat): 2969, 2884, 1789, 1649, 1633, 1419, 1384, 1285, 1264, 1226, 1198, 1182, 1132, 1102, 999, 960, 935, 905, 893, 841, 751, 736, 686, 616, 580, 539, 523, 479, 462, 407 cm\(^{-1}\). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 5.74 (hept, \(J = 6.1\) Hz, 1H), 4.60 (dd, \(J = 8.8, 4.1\) Hz, 1H), 3.71 – 3.62 (m, 1H), 3.61 – 3.51 (m, 1H), 2.37 – 2.23 (m, 1H), 2.09 (s, 3H), 2.09 – 1.96 (m, 4H). \(^{13}\)C NMR(101 MHz, CDCl\(_3\)) \(\delta\) 167.0, 169.5, 124.7, 121.9, 119.1, 116.3, 67.7, 67.3, 67.0, 66.7, 66.3, 58.3, 47.8, 29.4, 25.1, 22.0. \(^{19}\)F NMR (376 MHz, CDCl\(_3\)) \(\delta\) -76.16, -76.17, -76.19, -76.21, -76.23, -76.35, -76.37, -76.39, -76.41, -76.44. HRMS calcd. for C\(_{10}\)H\(_{11}\)F\(_6\)NO\(_3\)Na[M+Na]\(^+\) 330.0535, found 330.0567.
Acetyl proline CM ester. The general procedure for the synthesis of activated esters, protocol B, was followed using acetyl proline. The crude product was purified by column chromatography (SiO$_2$, acetone/dichloromethane gradient, 1:9 to 2:3) to give a clear oil (0.4698 g, 94%). [α]$^D_{25}$ –105.1 (c 0.995, MeOH). IR (neat): 3474, 2960, 2883, 1757, 1630, 1417, 1380, 1357, 1273, 1239, 1152, 1099, 1072, 1035, 1010, 953, 916, 874, 819, 693, 619, 605 cm$^{-1}$. $^1$H NMR (500 MHz, CDCl$_3$) δ 4.80 (d, $J = 15.7$ Hz, 1H), 4.68 (d, $J = 15.7$ Hz, 1H), 3.62 (dd, $J = 13.6$, 8.4 Hz, 1H), 3.50 (dd, $J = 16.2$, 7.0 Hz, 1H), 2.29 – 2.16 (m, 1H), 2.14 – 1.90 (m, 6H). $^{13}$C NMR (126 MHz, CDCl$_3$) δ 171.0, 169.7, 114.4, 58.2, 48.9, 47.8, 29.31, 25.0, 22.2. HRMS calcd. for C$_9$H$_{12}$N$_2$O$_3$Na [M+Na]$^+$ 219.0740, found 219.0713.

General procedures for peptide assembly.

Ligation protocol A: The hydroxyl amino acid or peptide (1 equiv) was dissolved in organic solvent (1 M) and set to stir at room temperature. The activated amino acid or peptide ester (HIP/TFE/CN, 1.5 equiv) was added, followed by AcOH (0.2 equiv). The reaction was monitored by TLC. After reaction completion, the mixture was concentrated in vacuo to afford a crude product that was purified by column chromatography.

Ligation protocol B: The hydroxyl amino acid or peptide (1 equiv) was added to a microwave reaction vessel and dissolved in organic solvent (1 M). The activated amino acid or peptide ester (HIP/TFE/CN, 1.5 equiv) was added, followed by AcOH (0.2 equiv) and sealed under air. The reaction was irradiated at 70 °C (300W, 5 min ramp time, 250 psi max, with stirring on). The reaction
mixture was concentrated in vacuo to afford a crude product that was purified by column chromatography.

**Threonine peptide assembly.** Ligations with threonine methyl ester and the acetyl amino acid esters were performed with varying activators, solvents and utilizing both ligation protocols A and B. The crude products were purified by column chromatography (SiO$_2$, acetone/dichloromethane gradient, 1:4 to 4:1) to give the title compounds. The experimental procedures reported are for the optimized reaction conditions utilizing the HIP activator and THF solvent.

**Ac-Ala-Thr-OMe (3).** The general procedure for threonine peptide assembly was followed, to get a white solid product. Ligation protocol A: 16 h room temperature (0.1508 g, 96%). Ligation protocol B: 4 h at 70 °C (0.1606 g, 96%). Mp: 146-149 °C. [α]$^D_{25}$ –56.0 (c 0.519, MeOH). IR(neat): 3302, 3158, 3086, 2996, 2950, 2916, 2868, 2758, 1753, 1641, 1556, 1528, 1440, 1379, 1338, 1313, 1267, 1209, 1177, 1151, 1121, 1082, 1065, 1040, 1007, 992, 953, 937, 917, 896, 844, 778, 750, 699, 649, 602, 529, 460, 449 cm$^{-1}$. $^1$H NMR (400 MHz, CDCl$_3$) δ 6.62 (d, J = 7.3 Hz, NH), 4.68 – 4.52 (m, 2H), 4.41 – 4.31 (m, 1H), 3.78 (s, J = 6.2 Hz, 3H), 2.01 (s, J = 8.4 Hz, 3H), 1.92 (br s, OH), 1.45 (d, J = 7.0 Hz, 3H), 1.22 (d, J = 6.5 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 173.0, 171.4, 170.8, 68.3, 58.0, 52.8, 49.3, 23.3, 20.2, 19.0. HRMS calcd. for C$_{10}$H$_{19}$N$_2$O$_5$ [M+H]$^+$ 247.1288, found 247.1286.

**Ligation protocol A + TBD instead of AcO$_2$H:** 19 h room temperature (0.0796 g, 39%). [α]$^D_{25}$ –18.7 (c 0.254, MeOH). IR (neat): 3291, 3067, 2980, 2937, 1743, 1647, 1533, 1437, 1374, 1274, 1207, 1162, 1063, 1016, 972, 869, 844, 688, 590, 539, 467 cm$^{-1}$. $^1$H NMR (500 MHz, CD$_3$OD) δ 5.47 – 5.36 (m, 1H), 4.52-4.40 (m, 2H), 4.40-4.32
(m, 1H), 3.72 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.41-1.33 (m, 6H), 1.25 (d, J = 6.2 Hz, 3H). $^1$C NMR (126 MHz, CD$_3$OD) δ 175.9, 173.3, 173.3, 172.5, 171.2, 72.4, 68.5, 59.3, 56.9, 50.5, 22.5, 20.4, 17.9, 17.3, 17.1. HRMS calcd. for C$_{13}$H$_{26}$N$_3$O$_7$ [M+H]$^+$ 360.1749, found 360.1765.

**Ac-Val-Thr-OMe (5a).** The general procedure for threonine peptide assembly was followed, to get a white solid product. Ligation protocol B: 5 h at 70 °C (0.1162 g, 62%). Mp: 160-164 °C. $[^{[\alpha]}]$D $^{25}$ = 27.5 (c 0.361, MeOH). IR (neat): 3285, 3078, 2960, 2935, 2873, 1721, 1635, 1545, 1435, 1380, 1346, 1288, 1269, 1224, 1156, 1125, 1083, 1020, 964, 943, 923, 883, 855, 786, 716, 668, 629, 603, 543, 497, 456, 422 cm$^{-1}$. $^1$H NMR (400 MHz, CD$_3$OD) δ 4.46 (d, J = 3.1 Hz, 1H), 4.34 – 4.26 (m, 1H), 3.73 (s, 3H), 2.14 – 2.05 (m, 1H), 2.00 (s, 3H), 1.18 (d, J = 6.4 Hz, 3H), 0.99 (t, J = 7.0 Hz, 6H). $^{13}$C NMR (101 MHz, CD$_3$OD) δ 174.5, 173.5, 172.5, 68.5, 60.6, 59.4, 52.8, 31.8, 22.5, 20.4, 19.8, 18.9. HRMS calcd. for C$_{12}$H$_{22}$N$_2$O$_5$Na [M+Na]$^+$ 297.1421, found 297.1415.

**Ac-Pro-Thr-OMe (5b).** The general procedure for threonine peptide assembly was followed, to get a clear oil product. Ligation protocol B: 5 h at 70 °C (0.1537 g, 83%). $[^{[\alpha]}]$D $^{25}$ = 72.0 (c 0.518, MeOH). IR (neat): 3311, 3272, 3069, 2976, 2951, 2887, 1747, 1651, 1623, 1543, 1438, 1339, 1304, 1205, 1142, 1119, 1081, 1036, 998, 920, 897, 886, 866, 778, 692, 651, 627, 605, 534, 494, 453, 416 cm$^{-1}$. $^1$H NMR (400 MHz, CDCl$_3$) δ 4.46 (dd, J = 9.0, 2.8 Hz, 1H), 4.28 – 4.20 (m, 1H), 3.69 (s, 3H), 3.64 – 3.54 (m, 1H), 3.48 – 3.39 (m, 1H), 2.30 – 2.09 (m, 2H), 2.04 (s, 3H), 2.02 – 1.82 (m, 3H), 1.14 (d, J = 6.5 Hz, 3H). $^{13}$C NMR (101 MHz, CD$_3$OD) δ 175.2,
Ac-Phe-Thr-OMe (5c). The general procedure for threonine peptide assembly was followed, to get a white solid product. Ligation protocol B: 5 h at 70 °C (0.1953 g, 90%). Mp: 147-150 °C. [α]$^D_{25}$ 1.7 (c0.507, MeOH). IR (neat): 3266, 3064, 3032, 2953, 2929, 2859, 1753, 1638, 1545, 1498, 1454, 1437, 1380, 1341, 1304, 1284, 1262, 1209, 1193, 1155, 1131, 1080, 1024, 996, 957, 903, 871, 833, 763, 743, 719, 698, 633, 599, 499, 464, 419 cm$^{-1}$. $^1$H NMR (400 MHz, CD$_3$OD) δ 7.30 – 7.25 (m, 4H), 7.24 – 7.18 (m, 1H), 4.72 (dd, $J$ = 9.4, 5.4 Hz, 1H), 4.45 (d, $J$ = 3.1 Hz, 1H), 4.32 – 4.24 (m, 1H), 3.71 (s, 3H), 3.18 (dd, $J$ = 14.0, 5.4 Hz, 1H), 2.89 (dd, $J$ = 14.0, 9.4 Hz, 1H), 1.89 (s, 3H), 1.17 (d, $J$ = 6.5 Hz, 3H). $^{13}$C NMR(101 MHz, CD$_3$OD) δ 174.4, 173.4, 172.4, 138.6, 130.4, 129.6, 127.9, 68.6, 59.4, 56.2, 53.0, 38.8, 22.5, 20.3. HRMS calcd. for C$_{16}$H$_{23}$N$_2$O$_5$ [M+H]$^+$ 323.1604, found 323.1604.

Ac-Ile-Thr-OMe (5d). The general procedure for threonine peptide assembly was followed, to get 5d as a white solid product. Ligation protocol B: 5 h at 70 °C (0.1319 g, 67%). Mp: 172-176 °C. [α]$^D_{25}$–28.0 (c 0.306, MeOH). IR (neat): 3276, 3072, 2970, 2958, 2932, 2875, 1723, 1633, 1543, 1455, 1437, 1376, 1286, 1251, 1214, 1156, 1128, 1082, 1015, 995, 961, 942, 917, 885, 865, 784, 747, 710, 669, 627, 605, 556, 501, 478 cm$^{-1}$. $^1$H NMR (400 MHz, CD$_3$OD) δ 4.46 (d, $J$ = 3.1 Hz, 1H), 4.31 – 4.27 (m, 2H), 3.73 (s, $J$ = 2.9 Hz, 3H), 1.99 (s, $J$ = 2.2 Hz, 3H), 1.91 – 1.81 (m, 1H), 1.62 – 1.52 (m, 1H), 1.26 – 1.20 (m, 1H), 1.18 (d, $J$= 6.5 Hz, 3H), 0.98 (d, $J$ = 6.8 Hz, 3H), 0.93 (t, $J$ = 7.5 Hz, 3H). $^{13}$C NMR (101 MHz, CD$_3$OD) δ 174.6, 173.5,
172.4, 68.5, 59.6, 59.4, 52.8, 38.0, 26.1, 22.5, 20.4, 16.0, 11.5. HRMS calcd. for C_{13}H_{24}N_{2}O_{5}Na [M+Na]^+ 311.1577, found 311.1553.

**Ac-Leu-Thr-OMe (5e).** The general procedure for threonine peptide assembly was followed, to get 5e as a clear oil product. Ligation protocol B: 5 h at 70 °C (0.1256 g, 70%). [α]_{D}^{25} = 32.7 (c 0.513, MeOH). IR (neat): 3280, 3071, 2956, 2935, 2872, 1742, 1536, 1436, 1373, 1341, 1281, 1207, 1160, 1108, 1083, 1020, 995, 943, 898, 868, 830, 696, 597, 461, 417 cm$^{-1}$. $^{1}$H NMR (400 MHz, CD$_{3}$OD) δ 4.53 - 4.44 (m, 2H), 4.35 - 4.26 (m, 1H), 3.74 (s, $J = 6.0$ Hz, 3H), 1.99 (s, 3H), 1.79 - 1.65 (m, 1H), 1.65 - 1.53 (m, 2H), 1.17 (d, $J = 6.5$ Hz, 3H), 0.98 (d, $J = 6.5$ Hz, 3H), 0.94 (d, $J = 6.5$ Hz, 3H). $^{13}$C NMR (101 MHz, CD$_{3}$OD) δ 175.4, 173.4, 172.4, 68.5, 59.2, 53.4, 52.9, 41.9, 26.0, 23.5, 22.5, 22.2, 20.4. HRMS calcd. for C_{13}H_{24}N_{2}O_{5}Na [M+Na]^+ 311.1577, found 311.1568.

**Boc-Phenylalanine tert-butyl amide.** N-Boc phenylalanine (3.077 g, 11.6 mmol) was dissolved in dichloromethane (100 mL) and set to stir at 0 °C. HATU (5.2934 g, 13.9 mmol) was added to the reaction mixture, followed by DIPEA (2.4 mL, 14.0 mmol) and tert-butylamine (1.8 mL, 17.4 mmol). The mixture was stirred at 0 °C for 30 min, then for 4 h at room temperature. The solvent was removed in vacuo, and the residue was dissolved in ethyl acetate (50 mL) and washed with 1 N HCl (20 mL) and sat. NaHCO$_{3}$ (20 mL). The organic phase was dried over sodium sulfate, filtered, and concentrated in vacuo to afford a crude product that was purified by column chromatography (SiO$_{2}$, acetone/dichloromethane gradient, 1:9 to 1:4) to give the title compound as a white solid (3.6301 g, 98%). Mp: 133-135 °C. [α]_{D}^{25} 9.5 (c 1.01, MeOH). IR
(neat): 3303, 3064, 3038, 2972, 2930, 2868, 1685, 1654, 1537, 1498, 1453, 1391, 1363, 1300, 1273, 1250, 1223, 1171, 1083, 1048, 1021, 963, 932, 915, 884, 860, 791, 733, 696, 621, 570, 553, 509, 470, 439, 411 cm\(^{-1}\). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.34 – 7.26 (m, 2H), 7.24 (t, \(J = 7.8\) Hz, 3H), 4.20 – 4.10 (m, 1H), 3.11 (dd, \(J = 13.5, 5.7\) Hz, 1H), 2.91 (dd, \(J = 13.1, 8.7\) Hz, 1H), 1.43 (s, 9H), 1.20 (s, 9H). \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 170.1, 155.4, 137.3, 129.7, 128.8, 127.1, 80.1, 56.7, 51.4, 39.4, 28.7, 28.5. HRMS calcd. for C\(_{18}\)H\(_{29}\)N\(_2\)O\(_3\) [M+H]\(^+\) 321.2173, found 321.2201.

**Boc-Hse(OBn)-Phe tert-butyl amide (7).** N-Boc-Hse(OBn)-OH (1.1796 g, 3.81 mmol) was dissolved in dichloromethane (40 mL) and set to stir at 0 °C. HOBt (0.7592 g, 4.96 mmol) was added to the reaction mixture, followed by EDCI (0.9510 g, 4.96 mmol) and phenylalanine tert-butylamide (1.2678 g, 5.75 mmol). The mixture was stirred at 0 °C for 30 min, then for 6 h at room temperature. The solvent was removed in vacuo, and the residue was dissolved in ethyl acetate (25 mL) and washed with 1 N HCl (10 mL) and sat. NaHCO\(_3\) (10 mL). The organic phase was dried over sodium sulfate, filtered, and concentrated in vacuo to afford a crude product that was purified by column chromatography (SiO\(_2\), acetone/dichloromethane gradient, 1:9 to 2:3) to give 7 as a white solid (1.9274 g, 98%). Mp: 48-51 °C. [\(\alpha\)]\(^D\)\(_{25}\) -24.6 (c 1.02, MeOH). IR (neat): 3305, 3064, 3030, 2973, 2930, 2867, 1762, 1644, 1496, 1454, 1391, 1364, 1247, 1225, 1162, 1100, 1029, 912, 863, 738, 697, 607, 519, 494 cm\(^{-1}\). \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.40 – 7.29 (m, 5H), 7.29 – 7.14 (m, 5H), 6.82 (d, \(J = 7.0\) Hz, NH), 5.96 (s, NH), 5.83 (s, NH), 4.55 (dd, \(J = 13.0, 6.4\) Hz, 1H), 4.46 (s, 2H), 4.16 (dd, \(J = 11.5, 5.6\) Hz, 1H), 3.56 (d, \(J = 4.1\) Hz, 2H), 3.19 (dd, \(J = 13.3, 4.5\) Hz, 1H), 2.88 (dd, \(J =
13.7, 7.3 Hz, 1H), 2.11 – 1.96 (m, 2H), 1.38 (s, 9H), 1.23 (s, 9H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 171.4, 169.4, 156.0, 137.8, 136.8, 129.5, 128.8, 128.6, 128.0, 127.8, 127.1, 80.2, 73.5, 68.3, 55.0, 54.4, 51.5, 38.1, 31.5, 31.5, 28.6, 28.4. HRMS calcd. for C$_{29}$H$_{42}$N$_3$O$_5$ [M+H]$^+$ 512.3119, found 512.3078.

Boc-Hse-Phe tert-butyl amide. A suspension of peptide 7 (3.2500 g, 6.35 mmol) and 10% palladium on activated carbon (0.1385 g) in MeOH/AcOH (50 mL, 9:1) was hydrogenated, stirring the reaction mixture at room temperature under H$_2$ (1 atm) for 20 h. The reaction mixture was filtered through celite and concentrated in vacuo. The crude product was dissolved in ethyl acetate (50 mL) and washed with 1 N HCl (20 mL) and sat. NaHCO$_3$ (20 mL). The organic phase was dried over sodium sulfate, filtered, and concentrated in vacuo to afford the title compound as a white solid (2.5536, 95%). Mp: 62-66 °C. $[\alpha]^{25}_D$ –3.3 (c 1.02, MeOH). IR (neat): 3299, 3088, 3064, 3030, 2971, 2930, 1644, 1497, 1454, 1391, 1365, 1247, 1225, 1164, 1052, 1031, 904, 865, 742, 699, 647, 598, 521, 494, 426 cm$^{-1}$. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.37 – 7.29 (m, 2H), 7.29 – 7.22 (m, 3H), 7.05 (br s, NH), 5.63 (br s, NH), 5.57(br s, NH), 4.52 (dd, $J$ = 14.4, 7.9 Hz, 1H), 4.31 (d, $J$ = 5.3 Hz, 1H), 3.77 – 3.58 (m, 2H), 3.16 (dd, $J$ = 13.4, 5.6 Hz, 1H), 2.97 (dd, $J$ = 13.6, 8.2 Hz, 1H), 2.05 – 1.91 (m, 2H), 1.82(br s, OH), 1.44 (s, 9H), 1.22 (s, 9H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 172.0, 169.6, 156.4, 137.0, 129.7, 128.9, 127.3, 80.6, 59.3, 55.2, 52.9, 51.7, 38.7, 35.5, 28.6, 28.5. HRMS calcd. for C$_{22}$H$_{35}$N$_3$O$_5$Na [M+Na]$^+$ 444.2469, found 444.2428.

H$_2$N-Hse-Phe tert-butyl amide (8). The preceding dipeptide (2.5536 g, 6.06 mmol) was dissolved in a minimum amount of MeOH (1 mL) and set to stir
at 0 °C. A solution of hydrochloric acid in ether (2 N, 15 mL) was added slowly, and the stirring was continued for 1 h. The reaction mixture was concentrated in vacuo without heat and used crude in the next step. The reaction mixture was dissolved in water and ethyl acetate (1:1, 12 mL) and set to stir at 0 °C. Sodium bicarbonate (3.0587 g, 36.4 mL) was added slowly to the reaction mixture, followed by addition of benzyl chloroformate (1.28 mL, 9.09 mmol). The reaction was stirred at 0 °C for 15 min, then for 14 h at room temperature. The organic phase was separated, and the aqueous phase was extracted with ethyl acetate (4 x 10 mL). The organic portions were combined, dried over sodium sulfate, filtered, and concentrated in vacuo to afford a crude product that was purified by column chromatography (SiO₂, acetone/dichloromethane gradient, 1:9 to 2:3) to give the N-Cbz protected dipeptide as a white solid (1.6069 g, 58%). Mp: 157-159 °C. [α]D25 –38.7 (c 0.506, MeOH). IR (neat): 3371, 3343, 3226, 3063, 3028, 2976, 2961, 2934, 2885, 2868, 1711, 1663, 1643, 1585, 1538, 1499, 1447, 1415, 1392, 1364, 1330, 1317, 1286, 1259, 1232, 1187, 1167, 1154, 1115, 1095, 1080, 1046, 1030, 994, 944, 902, 890, 845, 789, 754, 743, 716, 699, 625, 586, 575, 512, 502, 466, 450, 412 cm⁻¹. ¹H NMR (500 MHz, CDCl₃) δ 7.39 – 7.31 (m, 5H), 7.31 – 7.26 (m, 2H), 7.25 – 7.18 (m, 3H), 5.09 (q, J = 12.2 Hz, 2H), 4.53 (dd, J = 14.9, 7.6 Hz, 1H), 4.44 (dd, J = 12.4, 6.6 Hz, 1H), 3.72 – 3.63 (m, 1H), 3.63 – 3.55 (m, 1H), 3.07 (dd, J =13.5, 6.3 Hz, 1H), 2.99 (dd, J = 13.2, 8.4 Hz, 1H), 2.81 (br s, OH), 2.06 – 1.89 (m, 1H), 1.88 – 1.77 (m, 1H), 1.20 (s, 9H). ¹³C NMR (126 MHz, CDCl₃) δ 171.7, 169.7, 156.8,
A suspension of Cbz-Hse-Phe tert-butyl amide (1.0047 g, 2.21 mmol) and 10% palladium on activated carbon (51.0 mg) in MeOH (5 mL) was hydrogenated, stirring the reaction mixture at room temperature under H₂ (1 atm) for 1 h. The reaction mixture was filtered through celite and concentrated in vacuo to give 8 as a white solid (0.6905 g, 97%). Mp: 104-107 °C. [α]²⁵D 7.4 (c 0.497, MeOH). IR ( neat): 3286, 3084, 3031, 2969, 2925, 2871, 1638, 1549, 1497, 1454, 1392, 1363, 1299, 1250, 1222, 1157, 1117, 1055, 1032, 962, 913, 876, 846, 732, 696, 667, 574, 494, 436 cm⁻¹. ¹H NMR (400 MHz, CD₃OD) δ 7.34 – 7.14 (m, 5H), 4.53 (t, J = 7.5 Hz, 1H), 3.68 – 3.55 (m, 2H), 3.43 (dd, J = 7.8, 5.5 Hz, 1H), 3.02 (dd, J = 13.5, 7.3 Hz, 1H), 2.91 (dd, J = 13.5, 7.8 Hz, 1H), 1.92 – 1.76 (m, 1H), 1.71 – 1.56 (m, 1H), 1.23 (s, 9H). ¹³C NMR (101 MHz, CD₃OD) δ 177.2, 172.5, 138.4, 130.7, 129.6, 127.9, 60.2, 56.3, 54.1, 52.3, 39.8, 38.6, 28.9. HRMS calcd. for C₁₇H₂₈N₃O₃ [M+H]+ 322.2125, found 322.2102.

**Homoserine peptide assembly.** The ligation with homoserine dipeptide 8 and the acetyl amino acid esters were performed with varying activators and utilizing both ligation protocols A and B. The crude products were purified by column chromatography (SiO₂, acetone/dichloromethane gradient, 2:3 to 4:1) to give the title compounds. The experimental procedures reported below are for the optimized reaction conditions utilizing the HIP activator and DMF.

**Ac-Ala-Hse-Phe tert-butyl amide (9a).** The general procedure for homoserine peptide assembly was followed, with the use of Ac-alanine CM ester in place
of the HIP ester to get a white solid product. Ligation protocol A: 30 h room temperature (91.2 mg, 89%). Ligation protocol B: 2 h at 70 °C (76.8 mg, 62%).

Mp: 233 °C, dec. [α]D 25 −63.2 (c 0.519, MeOH). IR (neat): 3284, 3089, 3030, 2970, 2930, 2873, 1686, 1621, 1531, 1435, 1393, 1363, 1276, 1224, 1159, 1050, 977, 930, 882, 847, 741, 696, 602, 569, 520, 496, 451, 438, 427, 411 cm⁻¹. ¹H NMR (400 MHz, CD₃OD) δ 7.33 – 7.17 (m, 5H), 4.51 (dd, J = 9.1, 5.9 Hz, 1H), 4.32 (dd, J = 7.2, 5.3 Hz, 1H), 4.20 (q, J = 7.2 Hz, 1H), 3.56 – 3.44 (m, 1H), 3.35 – 3.26 (m, 1H), 2.94 (dd, J = 13.7, 9.2 Hz, 1H), 2.03 (s, 3H), 1.94 – 1.82 (m, 1H), 1.79 – 1.67 (m, 1H), 1.34 (d, J = 7.2 Hz, 3H), 1.29 (s, J = 6.5 Hz, 9H). ¹³C NMR (101 MHz, CD₃OD) δ 175.7, 174.1, 173.8, 172.6, 138.9, 130.5, 129.6, 127.9, 59.7, 56.8, 53.9, 52.6, 51.7, 39.0, 34.6, 29.0, 22.7, 17.5. HRMS calcd. for C₂₂H₃₅N₄O₅ [M+H]+ 435.2602, found 435.2627.

**Ac-Phe-Hse-Phe tert-butyl amide (9b).** The general procedure for homoserine peptide assembly was followed, to get a white solid product. Ligation protocol A: 12 h room temperature (76.0 mg, 53%). Ligation protocol B: 2 h at 70 °C (66.8 mg, 52%). Mp: 245 °C, dec. [α]D 25 −28.5 (c 0.50, MeOH). IR (neat): 3285, 3088, 3063, 3029, 2967, 2927, 1681, 1622, 1549, 1496, 1453, 1393, 1363, 1264, 1224, 1128, 1051, 1031, 956, 913, 885, 843, 823, 741, 697, 598, 578, 520, 499, 427, 411 cm⁻¹. ¹H NMR (500 MHz, CD₃OD) δ 7.29 – 7.16 (m, 10H), 4.54 (dd, J = 9.6, 4.9 Hz, 1H), 4.50 (dd, J = 8.2, 6.7 Hz, 1H), 4.37 (dd, J = 7.8, 5.3 Hz, 1H), 3.52 (dt, J = 10.9, 5.4 Hz, 1H), 3.44 – 3.35 (m, 1H), 3.16 – 3.04 (m, 2H), 2.97 – 2.82 (m, 2H), 1.92 (s, 3H), 1.91 – 1.85 (m, 1H), 1.80 – 1.71 (m, 1H), 1.27 (s, 9H). ¹³C NMR (126 MHz, CD₃OD) δ 174.1, 173.8, 173.6, 172.4, 138.6, 130.6, 130.3, 129.6, 129.6, 127.9,
127.9, 59.6, 56.8, 56.7, 53.4, 52.4, 39.2, 38.5, 35.1, 28.9, 22.6. HRMS calcd. for C_{28}H_{39}N_{4}O_{5} [M+H]^+ 511.2915, found 511.2909.

**Ac-Val-Hse-Phe tert-butyl amide (9c).** The general procedure for homoserine peptide assembly was followed, to get a white solid product. Ligation protocol A: 84 h room temperature (59.2 mg, 46%). Ligation protocol B: 2 h at 70 °C (59.4 mg, 46%). Mp: 254 °C, dec. [α]_{D}^{25} –55.5 (c 0.418, MeOH). IR (neat): 3281, 3088, 3031, 2965, 2932, 2874, 1686, 1623, 1531, 1454, 1392, 1363, 1264, 1225, 1120, 1054, 952, 887, 741, 694, 600, 528, 499, 427, 412 cm^{-1}. \textsuperscript{1}H NMR (500 MHz, CD_{3}OD) δ 7.35 – 7.13 (m, 5H), 4.50 (dd, J = 8.5, 6.4 Hz, 1H), 4.40 (dd, J = 7.7, 5.4 Hz, 1H), 4.07 (d, J = 6.6 Hz, 1H), 3.58 – 3.49 (m, 2H), 3.42 – 3.34 (m, 1H), 3.09 (dd, J = 13.7, 6.4 Hz, 1H), 2.98 – 2.90 (m, 1H), 2.04 (s, 3H), 1.95 – 1.86 (m, 1H), 1.81 – 1.71 (m, 1H), 1.26 (s, J = 5.8 Hz, 9H), 0.95 (t, J = 7.3 Hz, 6H). \textsuperscript{13}C NMR (126 MHz, CD_{3}OD) δ 174.2, 174.1, 172.3, 167.3, 138.7, 130.6, 129.5, 127.8, 127.3, 126.8, 53.3, 52.4, 39.2, 35.1, 31.5, 28.9, 22.6, 19.9, 18.9. HRMS calcd. for C_{24}H_{39}N_{4}O_{5}[M+H]^+ 463.2912, found 463.2912.

**Ac-Pro-Hse-Phe tert-butyl amide (9d).** The general procedure for homoserine peptide assembly was followed, to get a clear oil film. Ligation protocol B: 2 h at 70 °C (83.5 mg, 76%). [α]_{D}^{25} –68.8 (c 0.50, MeOH). IR (neat): 3291, 3087, 3063, 3030, 2964, 2929, 2875, 1624, 1526, 1450, 1420, 1393, 1362, 1300, 1224, 1119, 1058, 1033, 997, 917, 876, 847, 743, 699, 661, 620, 597, 543, 498, 451, 423, 411 cm^{-1}. \textsuperscript{1}H NMR (400 MHz, CD_{3}OD) δ 7.32 – 7.16 (m, 5H), 4.57 – 4.48 (m, 1H), 4.36 – 4.29 (m, 2H), 3.72 – 3.56 (m, 2H), 3.56 – 3.47 (m, 1H), 3.17 (dd, J = 13.8, 5.5 Hz, 1H), 2.94 (dd, J = 13.2, 10.1 Hz, 1H), 2.29 – 2.18 (m, 1H), 2.15 (s, 3H), 2.05 – 1.92 (m, 4H),
1.87 (dd, J = 8.7, 5.1 Hz, 1H), 1.77 – 1.65 (m, 1H), 1.30 (s, 9H). 13C NMR (101 MHz, CD3OD) δ 175.0, 173.8, 173.1, 172.6, 138.9, 130.5, 129.5, 127.8, 62.2, 59.7, 56.8, 56.7, 53.9, 52.5, 39.0, 34.4, 31.0, 29.0, 26.0, 22.7. HRMS calcd. for C24H37N4O5 [M+H]+ 462.2789, found 462.2816.

**Ac-Leu-Hse-Phe tert-butyl amide (9e).** The general procedure for homoserine peptide assembly was followed, to get a white solid product. Ligation protocol B: 2 h at 70 °C (39.2 mg, 34%). Mp: 271 °C, dec. [α]D25 = –58.2 (c 0.253, MeOH). IR (neat): 3273, 3087, 2965, 2931, 2876, 1686, 1622, 1536, 1455, 1393, 1364, 1286, 1265, 1225, 1154, 1054, 949, 912, 888, 741, 696, 600, 498, 427 cm⁻¹. 1H NMR (400 MHz, CD3OD) δ 7.31 – 7.14 (m, 5H), 4.50 (t, J = 7.4 Hz, 1H), 4.32 (t, J = 6.2 Hz, 1H), 4.26 (t, J = 7.5 Hz, 1H), 3.56 – 3.46 (m, 1H), 3.13 (dd, J = 13.7, 6.0 Hz, 1H), 2.93 (dd, J = 13.6, 9.2 Hz, 1H), 2.03 (s, 3H), 1.94 – 1.83 (m, 1H), 1.80 – 1.65 (m, 2H), 1.56 (t, J = 7.3 Hz, 2H), 1.28 (s, 9H), 0.97 (d, J = 6.5 Hz, 3H), 0.93 (d, J = 6.4 Hz, 3H). 13C NMR (101 MHz, CD3OD) δ 175.4, 174.3, 173.8, 172.5, 138.8, 130.6, 129.6, 127.9, 59.7, 56.8, 54.3, 53.8, 52.6, 41.5, 39.1, 34.7, 29.0, 26.1, 23.5, 22.6, 22.0. HRMS calcd. for C25H41N4O5 [M+H]+ 477.3071, found 477.3097.

**Ac-Ile-Hse-Phe tert-butyl amide (9f).** The general procedure for homoserine peptide assembly was followed to get a white solid product. Ligation protocol B: 2 h at 70 °C (75.1 mg, 56%). Mp: 243 °C, dec. [α]D25 = –45.9 (c 0.30, MeOH). IR (neat): 3273, 3087, 2965, 2931, 2876, 1686, 1622, 1536, 1455, 1393, 1364, 1286, 1265, 1225, 1154, 1054, 949, 912, 888, 741, 696, 600, 498, 427 cm⁻¹. 1H NMR (400 MHz, CD3OD) δ 7.29 – 7.17 (m, 5H), 4.49 (dd, J = 8.5, 6.3 Hz, 1H), 4.43 – 4.34
(m, 1H), 4.16 – 4.09 (m, 1H), 3.59 – 3.45 (m, 1H), 3.39 – 3.33 (m, 1H), 3.20 – 3.06 (m, 1H), 3.02 – 2.89 (m, 1H), 2.04 (s, 3H), 1.94 – 1.78 (m, 2H), 1.78 – 1.69 (m, 1H), 1.27 (s, 9H), 1.26 (d, J = 20.4 Hz, 2H), 0.98 – 0.88 (m, 6H). \(^{13}\text{C} \text{NMR} \) (101 MHz, CD\textsubscript{3}OD) δ 174.3, 174.1, 173.7, 172.4, 138.8, 130.6, 129.6, 127.9, 60.3, 59.6, 56.9, 53.4, 52.4, 39.1, 37.8, 34.9, 28.9, 26.4, 22.6, 16.2, 11.8. HRMS calcd. for C\textsubscript{25}H\textsubscript{40}N\textsubscript{4}O\textsubscript{5}Na [M+Na]\(^{+}\) 499.2891, found 499.2907.

**General procedure for homoserine peptide oxidation.** The homoserine peptide (1 equiv) was dissolved in 3 ml acetone/DMF (2:1) and set to stir at 0 °C. An aqueous 15% solution of NaHCO\textsubscript{3} (0.5 mL) was added, followed by NaBr (0.05 equiv) and TEMPO (0.01 equiv). TCICA (2 equiv) was added over 20 min (0.5 equiv portions every 5 min). After the addition of TCICA, the reaction mixture was stirred for 2 h and 2-propanol (2 mL) was added and stirring was continued for 15 min. The reaction mixture was concentrated in vacuo. The mixture was dissolved with sat. aqueous NaHCO\textsubscript{3} (5 mL) and was washed with ethyl acetate (15 mL). The aqueous phase was acidified to pH 2 using 1N hydrochloric acid, and extracted with ethyl acetate (4 × 15 mL). The combined organic extract was dried over anhydrous sodium sulfate and concentrated to afford the oxidized peptide.

**Ac-Val-Asp-Phe tert-butyl amide (10b).** The general procedure for homoserine peptide oxidation was followed, to get a white solid (0.594 g, 75%). Mp: 250 °C, dec. \([ \alpha ]^{\text{D}}_{25} – 27.2 \) (c 0.20, MeOH). IR (neat): 3205, 3059, 2972, 2916, 2884, 2831, 2779, 1778, 1754, 1693, 1535, 1454, 1416, 1397, 1220, 1061, 1050, 914, 843, 780, 759, 740, 691, 530, 446 cm\(^{-1}\). \(^{1}\text{H} \text{NMR} \) (400 MHz, CD\textsubscript{3}OD) δ 7.32 – 7.15 (m,
5H), 4.50 (t, $J = 6.2$ Hz, 1H), 4.42 (t, $J = 7.1$ Hz, 1H), 4.11 (d, $J = 5.9$ Hz, 1H), 3.09 (dd, $J = 13.8$, 6.3 Hz, 1H), 2.97 (dd, $J = 13.5$, 8.2 Hz, 1H), 2.50 (dd, $J = 18.6$, 6.3 Hz, 1H), 2.18 – 2.08 (m, 1H), 2.06 (s, 3H), 1.26 (s, 9H), 0.94 (d, $J = 6.5$ Hz, 6H). $^{13}$C NMR (101 MHz, MeOD) $\delta$ 178.0, 174.4, 173.9, 173.8, 172.5, 138.8, 130.6, 129.6, 127.8, 61.3, 57.0, 53.1, 52.4, 40.0, 39.1, 31.5, 29.0, 22.7, 19.9, 18.6. HRMS calcd. for C$_{24}$H$_{40}$N$_5$O$_6$ [M+NH$_4$]$^+$ 494.2973, found 494.3007.

**Ac-Phe-Asp-Phe tert-butyl amide (10c).** The general procedure for homoserine peptide oxidation was followed, to get a white solid product (43.7 mg, 62%).

Mp: 253 °C, dec. $[\alpha]^D_{25}$ –8.1 (c 0.40, MeOH). IR (neat): 3272, 3206, 3078, 3061, 3037, 2974, 2923, 2826, 2779, 1692, 1632, 1538, 1495, 1454, 1416, 1396, 1365, 1293, 1221, 1050, 952, 913, 844, 782, 741, 698, 608, 581, 529, 445 cm$^{-1}$. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 7.31 – 7.11 (m, 10H), 4.63 (t, $J = 6.6$ Hz, 1H), 4.58 – 4.51 (m, 1H), 4.49 – 4.42 (m, 1H), 3.11 – 2.98 (m, 2H), 2.92 (dd, $J = 13.7$, 8.0 Hz, 1H), 2.86 – 2.76 (m, 1H), 1.89 (s, 3H), 1.26 (s, 9H). $^{13}$C NMR (101 MHz, CD$_3$OD) $\delta$ 174.0, 173.9, 173.6, 172.5, 172.2, 138.6, 138.0, 130.7, 130.6, 130.4, 129.6, 128.0, 127.9, 56.8, 56.4, 52.4, 51.5, 49.2, 39.6, 39.1, 38.7, 36.3, 28.9, 22.5 HRMS calcd. for C$_{28}$H$_{37}$N$_4$O$_6$ [M+H]$^+$ 525.2708, found 525.2758.

**Fmoc-Leu-Lys(Boc)-Ala-Met-Asp(OtBu)-Pro.** A general procedure for Fmoc solid phase peptide synthesis on chlorotrityl resin was followed on a 600 $\mu$mol scale to obtain this protected peptide as a white solid (0.4746 g, 75%). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 172.5, 172.3, 171.8, 171.4, 171.1, 170.5, 168.7, 168.5, 135.9, 135.5, 128.5, 128.3, 128.2, 128.1, 127.9, 95.2, 77.8, 77.7, 66.9, 66.7, 65.3, 65.3, 61.1, 59.6, 59.4, 59.3, 58.1, 52.2, 51.9, 51.1, 47.6, 47.4, 47.3, 46.8, 41.1, 38.8, 31.6,

HRMS calcd. for C\textsubscript{53}H\textsubscript{78}N\textsubscript{7}O\textsubscript{13}S [M+H]\textsuperscript{+} 1052.5373, found 1052.5402.

**Fmoc-Thr-Pro-Pro-Leu.** A general procedure for Fmoc solid phase peptide synthesis on chlorotrityl resin was followed on a 150 \( \mu \)mol scale to obtain this protected peptide as a white solid (0.0846 g, 87%). \( ^{13} \)C NMR (101 MHz, CD\textsubscript{3}OD) \( \delta \) 176.1, 174.4, 172.6, 171.6, 158.7, 145.3, 142.7, 128.9, 128.3, 126.3, 121.1, 68.5, 68.2, 61.4, 60.0, 59.7, 52.3, 41.8, 30.4, 29.5, 26.0, 23.5, 22.1, 20.0. HRMS calcd. for C\textsubscript{35}H\textsubscript{45}N\textsubscript{4}O\textsubscript{8} [M+H]\textsuperscript{+} 649.3232, found 649.3219.

**Fmoc-Leu-Lys(Boc)-Ala-Met-Asp(OtBu)-Pro-OHIP (11).** The peptide (0.4355 g, 0.414 mmol) was dissolved in dichloromethane (4 mL) and set to stir at 0 °C. EDCI (95.9 mg, 0.500 mmol), HOBt (68.3 mg, 0.505 mmol) and 1,1,1,3,3,3-hexafluoroisopropanol (0.44 mL, 4.18 mmol) were added to the reaction mixture. The mixture was stirred at 0 °C for 30 min, then for 6 h at room temperature. The solvent was removed in vacuo, and the residue was dissolved in ethyl acetate and washed with 1 N HCl and sat. NaHCO\textsubscript{3}. The organic phase was dried over sodium sulfate, filtered, and concentrated in vacuo to afford a crude product that was purified by column chromatography (SiO\textsubscript{2}, acetone/dichloromethane gradient, 1:9 to 2:3) to give 11 as a white solid (0.4033 g, 81%). \( ^{13} \)C NMR (101 MHz, CDCl\textsubscript{3}) \( \delta \) 173.8, 172.8, 172.1, 171.4, 169.9, 169.0, 168.9, 156.8, 156.1, 144.5, 144.1, 141.4, 127.8, 127.2, 125.6, 125.4, 124.7, 121.9, 120.0, 119.1, 116.3, 81.1, 78.8, 67.2, 66.8, 66.5, 58.6, 56.1, 53.8, 52.1, 47.2, 42.3, 40.4, 38.6, 34.6, 30.0, 29.0, 28.5, 28.0, 25.2, 24.9, 23.0, 21.2, 15.3. \( ^{19} \)F NMR (376 MHz,
CDCl₃ δ -76.13, -76.27. HRMS calcd. for C₅₆H₇₇F₆N₇O₁₃Na [M+Na]⁺ 1224.5096, found 1224.5036.

Fmoc-Thr-Pro-Pro-Leu-OBn. The peptide (0.2172 g, 0.335 mmol) was dissolved in acetonitrile (5 mL) and set to stir at room temperature. Cesium carbonate (0.1322 g, 0.406 mmol) and benzyl bromide (0.10 mL, 0.836 mmol) were added to the reaction mixture and stirred for 4 h. The solvent was removed in vacuo to afford a crude product that was purified by column chromatography (SiO₂, acetone/dichloromethane gradient, 1:4 to 2:3) to give the title compound as a white solid (0.2071 g, 84%). ¹³C NMR (101 MHz, CD₃OD) δ 174.5, 174.0, 172.9, 172.6, 171.6, 158.7, 145.3, 142.7, 137.3, 129.7, 129.5, 128.9, 128.3, 126.3, 121.1, 68.5, 68.2, 68.0, 61.2, 60.0, 59.7, 52.6, 41.4, 30.4, 29.5, 26.1, 25.9, 23.4, 22.1, 20.0. HRMS calcd. for C₄₂H₅₀N₄O₈Na [M+Na]⁺ 761.3521, found 761.3452.

H₂N-Thr-Pro-Pro-Leu-OBn (12). The preceding peptide (0.4779 g, 0.647 mmol) was dissolved in THF (7 mL) and set to stir at room temperature. 1-Octanethiol (1.2 mL, 6.92 mmol) was added to the reaction mixture, followed by DBU (10 µL, 66.9 µmol). The reaction mixture was stirred for 1 h at room temperature and concentrated in vacuo to afford a crude product that was purified by column chromatography (SiO₂, acetone/dichloromethane gradient, 1:9 to 9:1) to give 12 as a white solid (0.3322 g, 99%). ¹³C NMR (126 MHz, CDCl₃) δ 172.5, 172.3, 171.7, 171.4, 171.1, 170.5, 168.7, 168.5, 135.9, 135.5, 128.5, 128.3, 128.2, 128.1, 127.9, 95.3, 95.2, 77.8, 77.7, 67.0, 66.7, 65.32, 65.26, 61.1, 59.6, 59.3, 58.1, 52.2, 51.1, 47.6, 47.4, 47.3, 46.8, 41.1, 38.8, 31.6, 31.5, 29.3, 28.5, 28.1, 27.7, 27.1, 25.2,
25.0, 24.8, 24.7, 23.2, 22.9, 22.3, 21.9, 21.1, 19.4, 19.3. HRMS calcd. for C_{27}H_{41}N_{4}O_{6} [M+H]^+ 517.3021, found 517.3033.

**LTNF-10 threonine peptide assembly.** The ligations of 11 and 12 were performed with varying solvents and concentrations utilizing both ligation protocols A and B. The crude products were purified by column chromatography (SiO₂, acetone/dichloromethane gradient, 2:3 to 9:1) to give the title compound. The experimental procedures reported below are for the optimized reaction conditions for both ligation protocols A and B.

**Fmoc-Leu-Lys(Boc)-Ala-Met-Asp(OtBu)-Pro-Thr-Pro-Pro-Leu-OBn (13).** The general procedure for LTNF-10 threonine peptide assembly was followed, to get a white solid product. Ligation protocol A: 5 days room temperature in 0.33 M DMF (72.9 mg, 30%). Ligation protocol B: 5 h at 70 °C in 0.25 M THF (18.9 mg, 10%). $^{13}$C NMR (126 MHz, CDCl₃) δ 172.7, 171.9, 171.7, 170.0, 157.2, 156.7, 143.8, 141.5, 135.7, 128.7, 128.5, 128.4, 128.0, 127.4, 125.3, 120.2, 67.4, 67.1, 65.9, 61.3, 60.6, 59.9, 59.5, 59.2, 54.8, 51.2, 47.7, 47.3, 45.5, 41.2, 38.6, 38.1, 29.9, 29.7, 28.7, 28.2, 25.4, 25.0, 23.2, 23.1, 22.9, 22.4, 22.0, 21.4, 19.3. HRMS calcd. for C_{80}H_{115}N_{11}O_{18}SNa [M+Na]^+ 1572.8034, found 1572.8057.

**LTNF-10: Leu-Lys-Ala-Met-Asp-Pro-Thr-Pro-Pro-Leu.** A suspension of peptide 13 (72.3 mg, 46.6 μmol) and 10% palladium on activated carbon (25.9 mg) in MeOH/AcOH (5 mL, 9:1) was hydrogenated, stirring the reaction mixture at room temperature under H₂ (1 atm) for 24 h. The reaction mixture was filtered through celite and concentrated in vacuo. The crude mixture was precipitated in ether and filtered to obtain the crude peptide as a white solid
used in the subsequent deprotection reaction. HRMS calcd. for C\textsubscript{73}H\textsubscript{109}N\textsubscript{11}O\textsubscript{18}SnNa [M+Na]\textsuperscript{+} 1482.7565, found 1482.7693.

The crude peptide was treated with 20% 4-methylpiperidine in dichloromethane (2 mL) and stirred for 1 h at room temperature. The reaction mixture was concentrated in vacuo and the crude mixture was precipitated in ether and filtered to obtain the crude peptide as a white solid used in the subsequent deprotection reaction. HRMS calcd. for C\textsubscript{58}H\textsubscript{100}N\textsubscript{11}O\textsubscript{16}S [M+H]\textsuperscript{+} 1238.7065, found 1238.7015.

The crude peptide was treated with the deprotection cocktail of TFA/water/triethylsilane (5 mL, 95/2.5/2.5) and stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuo and the crude peptide was precipitated in ether and filtered to obtain the TFA salt as a white solid (33.1 mg, 54% over three steps). \(^{13}\text{C}\) NMR (101 MHz, CD\textsubscript{3}OD) \(\delta\) 176.1, 175.1, 174.5, 174.3, 174.2, 173.4, 172.7, 171.9, 170.9, 170.8, 68.6, 62.0, 61.4, 60.1, 57.9, 54.6, 53.8, 53.0, 52.3, 50.8, 41.8, 40.6, 36.9, 32.8, 32.6, 31.1, 30.7, 30.4, 29.6, 28.2, 26.0, 25.8, 25.5, 23.5, 23.3, 22.2, 22.0, 19.9, 17.9, 15.4. HRMS calcd. for C\textsubscript{49}H\textsubscript{84}N\textsubscript{11}O\textsubscript{14}S [M+H]\textsuperscript{+} 1082.5914, found 1082.5881.
Reverse-Phase HPLC analysis of synthesized LTNF-10. A solution of LTNF-10 was manually injected onto an Agilent Eclipse Plus C18 RP-HPLC column, and eluted with 5% acetonitrile/95% water (solutions containing 0.1% TFA) isocratic for five min, followed by a gradient elution to 50% acetonitrile/50% water (solutions contain 0.1% TFA) over 30 min, with a flow rate of 1 mL/min. The peak around 2 min corresponds to solvent and the peak around 21 min corresponds to LTNF-10. The DAD chromatogram is of absorbance at 220 nm.
Ac-Ala-OHIP
19F CDCl₃
(IS=Hexafluorobenzene)
Ac-Ala-OTFE
1H CDCl₃
Ac-Ala-OTFE
13C CDCl₃
(IS=Hexafluorobenzene)
Ac-Ala-OTFE
19F CDCl₃
(IS=Hexafluorobenzene)

\[
\text{O} \quad \text{N} \quad \text{O} \quad \text{C}_3\text{F}_3
\]
Ac-Ala-OCM
1H CDCl₃

![Diagram of a chemical structure with peaks at 0.96, 2.00, 2.95, and 3.00 ppm.]

-7.27
Ac-Ala-OCM

$^{13}$C CDCl$_3$
Ac-Ala-OPFP
1H CDCl₃

![Chemical Structure](image)
Ac-Ala-OPFP
13C CDCl₃
(IS=Hexafluorobenzene)
Ac-Ala-OPFP
19F CDCl₃
(IS=Hexafluorobenzene)
Ac-Val-OHIP
1H CDCl₃

![Chemical Structure]
Ac-Val-GHIP
13C CDCl3
(IS=Hexafluorobenzene)
Ac-Val-OHIP
19F CDCl₃
(IS=Hexafluorobenzene)

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{N} & \quad \text{CF}_3
\end{align*}
\]
Ac-Val-OCM
1H CDCl₃

Chemical structure and NMR spectrum of Ac-Val-OCM in 1H CDCl₃.
Ac-Val-OCM
13C CDCl₃

\[
\begin{array}{c}
\text{O} & \text{C} & \text{N} \\
\text{H} & \text{O} & \text{C} \\
\hline
\text{O} & \text{C} & \text{N}
\end{array}
\]

- 171.03
- 170.52
- 114.15
- 57.13
- 48.85
- 31.07
- 23.10
- 19.05
- 18.02

f1 (ppm)
Ac-Phe-OHIP
1H CDCl₃
Ac-Phe-OHIP
13C CDCl₃
(IS=Hexafluorobenzene)
Ac-Phe-OHIP
19F CDCl₃
(IS=Hexafluorobenzene)
Ac-Phe-OCM
1H CDCl₃

![NMR Spectrum](image)

- f1 (ppm) values: 3.00, 2.17, 2.17, 1.09, 2.06, 1.18, 3.16, 3.00
Ac-Phe-OCM
13C CDCl₃

![Chemical structure](image)

<table>
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<tr>
<th>f1 (ppm)</th>
<th>135.20</th>
<th>129.28</th>
<th>129.07</th>
<th>122.70</th>
<th>113.96</th>
<th>53.16</th>
<th>49.64</th>
<th>37.72</th>
<th>23.08</th>
</tr>
</thead>
</table>

f1 (ppm) range: -1 to 200
Ac-Leu-OHIP
1H CDCl₃

[Chemical structure image]

[Graph of NMR spectrum with peaks at 0.99, 1.00, 1.02, 3.24, 3.29, and 6.48 ppm]
Ac-Leu-OH\text{IP}
13C CDCl$_3$
(IS=Hexafluoro benzene)
Ac-Leu-OHIP
19F CDCl₃
(IS=Hexafluorobenzene)
Ac-Leu-OCM
1H CDCl₃
Ac-Leu-OCM
13C CDCl₃
Ac-Ile-CHIP
1H CDCl₃
Ac-Ile-CHIP
13C CDCl₃

\[ \text{f1 (ppm)} \]

-124.75
-121.95
-119.14
-116.32
-67.77
-67.42
-67.07
-66.72
-66.38
-56.69
-37.65
-25.16
-23.07
-15.47
-11.56
Ac-Ile-CHIP
19F CDCl3
(IS=Hexafluorobenzene)

\[
\begin{array}{c}
\text{O} \\
\text{H} \\
\text{CH}_3 \\
\text{CO} \\
\text{O-CF}_3
\end{array}
\]
Ac-Pro-OHIP
1H CDCl₃

![Chemical Structure]
Ac-Pro-OHIP

19F CDCl₃

(IS=Hexafluorobenzene)
Ac-Ala-Thr-OMe (3)  
1H CDCl₃  

-7.27

\[ \text{Ac-Ala-Thr-OMe} \]

\[ \text{1H CDCl₃} \]

\[ \text{-7.27} \]
Ac-Ala-Thr-OMe (3)
13C CDCl₃

![NMR Spectrum of Ac-Ala-Thr-OMe (3)]
Ac-Val-Thr-OMe (5a)
1H CD$_3$OD

![Chemical structure image]
Ac-Val-Thr-OMe (5a)
13C CD$_3$OD
Ac-Pro-Thr-OMe (5b)

1H CDCl₃
Ac-Pro-Thr-OMe (5b)

13C CD$_3$OD

![Chemical structure of Ac-Pro-Thr-OMe (5b)](image)
Ac-Phe-Thr-OMe (5c)
1H CD$_3$OD
Ac-Phe-Thr-OMe (5c)
13C CD$_3$OD

![Chemical structure](image)
Ac-Ile-Thr-OME (5d)
1H CD$_3$OD
Ac-Ile-Thr-OMe (5d)
13C CD$_3$OD

\[ \text{f1 (ppm)} \]

-10 -5 -1 -130 -140 -150 -160 -170 -180 -190 0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190

174.55 173.49 172.44 15.95 20.35 22.49 26.13 38.03 52.84 59.37 59.59 68.54 172.44 173.49 174.55
Ac-Leu-Thr-OMe (5e)
1H CD$_3$OD
Ac-Leu-Thr-OMe (5e)
1H CD$_3$OD

[Chemical structure image]
Boc-Phe tert-butyl amide
13C CDCl₃
Boc-Hse(OBn)-Phe tert-butyl amide (7)
1H CDCl₃
Boc-Hse(Obn)-Phe tert-butyl amide (7)
13C CDCl₃
Boc-Hse-Phe tert-butyl amide
1H CDCl₃
Boc-Hse-Phe tert-butyl amide
13C CDCl₃

![Chemical structure](image)

- 171.95
- 169.56
- 156.38
- 137.04
- 129.65
- 128.90
- 127.25
- 59.26
- 55.21
- 51.65
- 38.68
- 35.53
- 28.64
- 28.46
Cbz-Hse-Phe tert-butyl amide
1H CDCl₃
Cbz-Hse-Phe tert-butyl amide
13C CDCl₃

\[
\text{CbzHN} \quad \begin{array}{c}
\text{O} \\
\text{H} \\
\text{O}
\end{array}
\quad \text{N} \quad \text{H}
\text{CbzHN}
\]

f1 (ppm)
Hse-Phe tert-butyl amide (8)

1H CD$_3$OD
Hse-Phe tert-butyl amide (8)
13C CD$_3$OD

![Chemical structure of Hse-Phe tert-butyl amide (8)](image-url)
Ac-Ala-Hse-Phe *tert*-butyl amide (9a)
1H CD₃OD

![Chemical Structure](image)
Ac-Ala-Hse-Phe tert-butyl amide (9a)

13C CD$_3$OD
Ac-Phe-Hse-Phe tert-butyl amide (9b)
1H CD$_3$OD

![Chemical Structure](image)
Ac-Phe-Hse-Phe tert-butyl amide (9b)
13C CD$_3$OD
Ac-Val-Hse-Phe tert-butyl amide (9c)
1H CD$_3$OD
Ac-Val-Hse-Phe *tert*-butyl amide (9c)

$^{13}$C CD$_3$OD
Ac-Pro-Hse-Phe **tert**-butyl amide (9d)

1H CD$_2$OD
Ac-Pro-Hse-Phe tert-butyl amide (9d)
13C CD$_3$OD
Ac-Leu-Hse-Phe *tert*-butyl amide (9e)
1H CD$_3$OD
Ac-Leu-Hse-Phe tert-butyl amide (9e)
13C CD$_2$OD

\[
\begin{align*}
\text{f1 (ppm)} & \quad \text{175.43} & \quad \text{174.25} & \quad \text{173.75} & \quad \text{172.53} & \quad -138.83 & \quad -130.56 & \quad -129.58 & \quad -127.88 \\
\text{127.88} & \quad \text{129.58} & \quad \text{130.56} & \quad \text{138.83} & \quad 172.53 & \quad 173.75 & \quad 174.25 & \quad 175.43 \\
\end{align*}
\]
Ac-Ile-Hse-Phe tert-butyl amide (9f)
1H CD$_3$OD

\[
\begin{align*}
\text{f1 (ppm)}: & \quad 6.01 \quad 11.10 \quad 1.17 \quad 2.10 \quad 3.00 \\
\text{tert-} \quad & \quad 5.78 \quad 1.07 \quad 1.04 \quad 1.06 \quad 1.22 \quad 1.47 \quad 3.00 \quad 2.10 \quad 1.17 \quad 1.01
\end{align*}
\]
Ac-Ile-Hse-Phe tert-butyl amide (9f)
13C CD3OD
Ac-Val-Asp-Phe tert-butyl amide (10b)
1H CD$_3$OD
Ac-Val-Asp-Phe tert-butyl amide (10b)
13C CD$_3$OD
Ac-Phe-Asp-Phe tert-butyl amide (10c)
1H CD$_3$OD
Ac-Phe-Asp-Phe tert-butyl amide (10c)
13C CD$_3$OD

![Chemical structure image]
Fmoc-Leu-Lys(Boc)-Ala-Met-Asp(OtBu)-Pro
1H CDCl₃

f₁ (ppm)

3.31
Fmoc-Leu-Lys(Boc)-Ala-Met-Asp(OtBu)-Pro
13C CDCl₃
Fmoc-Thr-Pro-Pro-Leu

$^{13}$C CD$_3$OD
Fmoc-Leu-Lys(Boc)-Ala-Met-Asp(OtBu)-Pro-OHIP (11)
1H CDCl₃
Fmoc-Leu-Lys(Boc)-Ala-Met-Asp(OtBu)-Pro-OH (11)
13C CD3OD
Fmoc-Leu-Lys(Boc)-Ala-Met-Asp(OtBu)-Pro-C6HIP \((\text{11})\)

19F CD$_3$OD (IS=Hexafluorobenzene)
Fmoc-Thr-Pro-Pro-Leu-OBn
1H CD₃OD

-3.31
Fmoc-Thr-Pro-Pro-Leu-OBn
13C CD$_3$OD

![Chemical Structure]

- $^{1}$H: 174.52, 173.55, 172.89, 172.58, 171.60, 158.68
- $^{13}$C: 145.33, 142.69, 137.32, 129.68, 128.92, 126.33, 121.08
- $^{19}$F: 68.48, 68.15, 67.99, 61.16, 60.00, 59.74, 52.55
- $^{31}$P: 41.42, 30.38, 29.49, 26.06, 25.94, 23.38, 22.08, 19.97
Thr-Pro-Pro-Leu-OBn (12)
1H CDCl₃
Thr-Pro-Pro-Leu-OBn (12)
13C CDCl₃
Fmoc-Leu-Lys(Boc)-Ala-Met-Asp(OrBu)-Pro-Thr-Pro-Pro-Leu-OBn \textbf{(13)}

$1\text{H} \text{CDCl}_3$
Fmoc-Leu-Lys(Boc)-Ala-Met-Asp(OtBu)-Pro-Thr-Pro-Pro-Leu-OBn (13)
13C CDCl₃

FmocH

NHBOc
LTNF-10: Leu-Lys-Ala-Met-Asp-Pro-Thr-Pro-Pro-Leu
1H CD$_3$OD

![Chemical structure image]
LTNF-10: Leu-Lys-Ala-Met-Asp-Pro-Thr-Pro-Pro-Leu
13C CD$_3$OD