Supporting Information

Potent and Selective Small-Molecule Inhibitors of cIAP1/2 Proteins Reveal that the Binding of Smac Mimetics to XIAP BIR3 is not Required for Their Effective Induction of Cell Death in Tumor Cells

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Scheme S1. Synthesis of Smac mimetics.

The synthesis of compounds 3-23 is shown in Scheme S1. Compound 25, was prepared in four steps from 24 as a mixture of two isomers with the desired cis isomer as the major product. Removal of the Boc protecting group from 25 gave two amines which, condensed with L-Boc-Dap(Z)-OH in the presence of EDC and HOBT, led to a single isomer of the amide 26. Under these conditions, the trans amine fails to react with the acid and can be recovered intact. Ozonolysis of the methylene double bond in 26 and subsequent reduction with Et$_3$N furnished the aldehyde 27. Cleavage of the carbobenzoxy
protecting group in 27, followed by intramolecular reductive amination of the resulting amino aldehyde and Pd-catalyzed hydrogenation in methanol led to the key intermediate 28. Condensation of the secondary amine in 28 with phenylacetyl chloride furnished an amide 29 and hydrolysis of the methyl ester group in 29 gave an acid, condensation of which with benzyl alcohol yielded the benzyl ester 30. We found if the L-N-Boc-N-methyl-alanine is introduced without first converting the methyl ester to a benzyl ester, subsequent hydrolysis of the methyl ester with LiOH can cause partial isomerization of the chiral center through which the ester is linked to the 5-membered ring. Accordingly, the amine that was produced by removal of the Boc protecting group from 30 was condensed with L-N-Boc-N-methyl-alanine to give the amide 31, hydrogenolysis of which furnished an acid. Condensation of this acid with various amines yielded a series of amides, and removal of the Boc protecting from these amides provided the designed compounds 3-23.

**General Methods.** $^1$H NMR spectra were acquired at 300 MHz and $^{13}$C spectra at 75 MHz. $^1$H chemical shifts are reported relative to internal standards CDCl$_3$ (7.27 ppm) or HDO (4.70 ppm) and $^{13}$C chemical shifts are reported relative to CDCl$_3$ (77.00 ppm) as the internal standard. The synthetic Smac mimetics were purified by a C$_{18}$ reverse phase semi-preparative HPLC column with solvent A (0.1% of TFA in H$_2$O) and solvent B (0.1% of TFA in CH$_3$CN) as eluents.
HCl solution (10 mL, 4N in 1,4-dioxane) was added to a solution of 25 (1.35 g, 5 mmol) in MeOH (20 mL). The solution was stirred at room temperature overnight and then concentrated. The residue was suspended in CH₂Cl₂ (20 mL). To this mixture was added L-Boc-Dap(Z)-OH (2.0 g, 6 mmol), EDC (1.16 g, 6 mmol), HOBt (0.81 g, 6 mmol) and N,N-di-isopropylethylamine (5 mL). The mixture was stirred at room temperature overnight and then concentrated. The residue was purified by chromatography to give 26 (1.52 g, 62% yield over two steps). Rf value at EtOAc/Hexane 1:2 is 0.3. The column was run using EtOAc and hexane as eluents from EtOAc/Hexane 1:4 to EtOAc/Hexane 1:1.5. ¹H NMR (300 MHz, CDCl₃, TMS) δ 7.34-7.28 (m, 5H), 5.78 (m, 1H), 5.59 (m, 1H), 5.36-5.33 (m, 2H), 5.19-5.01 (m, 4H), 4.67-4.62 (m, 1H), 4.47-4.44 (m, 1H), 3.75 (m, 1H), 3.74-3.71 (m, 2H), 2.31 (m, 1H), 2.14 (m, 1H), 1.99-1.95 (m, 2H), 1.42 (s, 9H); ESI MS: m/z 490.3 (M + H)⁺.

A solution of 26 (1.4 g, 2.86 mmol) in CH₂Cl₂ (30 mL) was cooled to -78 °C then O₃ was bubbled into this solution until the color turned pale blue. The passage of O₃ was continued for a further 5 min and then air was bubbled into the solution for 10 min to remove excess O₃. After dropwise addition of Et₃N (3 mL), the solution was warmed to room temperature and then concentrated. The residue was purified by chromatography to give 27 (1.12 g, 80%). Rf value at EtOAc/Hexane 1:1 is 0.3. The column was run using EtOAc and hexane as eluents from EtOAc/Hexane 1:3 to EtOAc/Hexane 1:1. ¹H NMR (300 MHz, CDCl₃, TMS) δ 9.72 (m, 1H), 7.53-7.32 (m, 5H), 5.44 (s, 1/2 H), 5.32 (s, 1/2
H), 5.15-5.06 (m, 2H), 4.64 (m, 1H), 4.40-4.39 (m, 1H), 3.78-3.76 (s, 3/2 H), 3.76-3.74 (s, 3/2H), 3.48-3.42 (m, 3H), 2.78-2.52 (m, 1H), 2.30 (m, 1H), 2.16 (m, 2H), 1.95 (m, 1H), 1.44-1.43 (m, 9H); ESI MS: m/z 492.2 (M + H)^+.

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\text{\begin{align*}
\text{H}, & \text{ 5.15-5.06 (m, 2H), 4.64 (m, 1H), 4.40-4.39 (m, 1H), 3.78-3.76 (s, 3/2 H), 3.76-3.74 (s, 3/2H), 3.48-3.42 (m, 3H), 2.78-2.52 (m, 1H), 2.30 (m, 1H), 2.16 (m, 2H), 1.95 (m, 1H), 1.44-1.43 (m, 9H); ESI MS: m/z 492.2 (M + H)^+.}
\end{align*}}
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10% Pd-C (100 mg) was added to a solution of 27 (1.1 g, 2.2 mmol) in MeOH (100 mL). The mixture was stirred under H\(_2\) for 24 h and then filtered through celite. The filtrate was concentrated and the residue was purified by chromatography to give 28 (260 mg, 34%). Rf value at EtOAc/methanol 5:1 is 0.3. The column was run using EtOAc and methanol as eluents from pure EtOAc to EtOAc/methanol 4:1. \(^1\)H NMR (300 MHz, CDCl\(_3\), TMS) \(\delta\) 5.45 (brd, \(J = 8.0\) Hz, 1H), 4.67 (m, 1H), 4.52 (t, \(J = 9.0\) Hz, 1H), 4.23 (m, 1H), 3.74 (s, 3H), 3.20 (m, 2H), 2.94 (m, 1H), 2.74 (dd, \(J = 13.6, 10.9\) Hz, 1H), 2.35 (m, 1H), 2.14 (m, 1H), 1.99 (m, 1H), 1.86-1.74 (m, 3H), 1.66 (m, 1H), 1.43 (brs, 9H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\), TMS) \(\delta\) 173.4, 170.7, 155.2, 79.7, 59.5, 58.4, 55.1, 52.5, 52.4, 46.8, 37.6, 32.2, 28.3, 27.0; ESI MS: m/z 342.2 (M + H)^+.

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\text{\begin{align*}
\text{Phenylacetyl chloride (0.15 mL) was added dropwise to a solution of 28 (240 mg, 0.7 mmol) in CH\(_2\)Cl\(_2\) (10 mL) and N,N-diisopropylethylamine (0.5 mL) at 0^\circ\text{C}. The solution was stirred at room temperature overnight and then concentrated. The residue was}
\end{align*}}
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\text{\begin{align*}
\text{Phenylacetyl chloride (0.15 mL) was added dropwise to a solution of 28 (240 mg, 0.7 mmol) in CH\(_2\)Cl\(_2\) (10 mL) and N,N-diisopropylethylamine (0.5 mL) at 0^\circ\text{C}. The solution was stirred at room temperature overnight and then concentrated. The residue was}
\end{align*}}
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purified by chromatography to give 29 (295 mg, 91%). Rf value at EtOAc/Hexane 1:1 is 0.3. The column was run using EtOAc and hexane as eluents from EtOAc/Hexane 1:4 to EtOAc/Hexane 1.5:1. £H NMR (300 MHz, CDCl$_3$, TMS) δ 7.40-7.20 (m, 5H), 5.80 (brs, J = 7.0 Hz, 1H), 4.55-4.45 (m, 2H), 4.30-4.15 (m, 2H), 4.15-3.80 (m, 3H), 3.70 (s, 3H), 3.20 (m, 1H), 3.02 (m, 1H), 2.50-2.20 (m, 2H), 2.20-1.60 (m, 4H), 1.50 (brs, 9H); £C NMR (75 MHz, CDCl$_3$, TMS) δ 172.7, 171.8, 168.8, 155.0, 135.2, 128.7, 128.6, 126.7, 80.1, 59.6, 56.1, 54.7, 53.9, 52.4, 46.7, 41.1, 32.4, 31.2, 28.3, 26.6; ESI MS: m/z 460.3 (M + H)$^+$.  

2N LiOH (2 mL) was added to a solution of 7 (230 mg, 0.5 mmol) in 1,4-dioxane (4 mL). The mixture was stirred at room temperature for 3 h, and then neutralized with 1N HCl solution until the pH reached 5. The mixture was extracted with EtOAc (3x20 mL) and the combined organic layers were dried over Na$_2$SO$_4$. After concentration, the crude acid was redissolved in CH$_2$Cl$_2$ (15 mL) and EDC (116 mg, 0.6 mmol), HOB$^t$ (80 mg), benzyl alcohol (0.3 mL) and 0.5 mL of N,N-diisopropylethylamine (0.5 mL) were added. The mixture was stirred at room temperature overnight and then concentrated to give a residue which was purified by chromatography to give 30 (230 mg, 85% over two steps). Rf value at EtOAc/Hexane 1:1.5 is 0.3. The column was run using EtOAc and hexane as eluents from EtOAc/Hexane 1:4 to EtOAc/Hexane 1:1. £H NMR (300 MHz, CDCl$_3$, TMS) δ 7.40-7.20 (m, 10H), 5.90 (brs, J = 7.0 Hz, 1H), 5.20 (brs, 2H), 4.65-4.45 (m, 2H), 4.30-4.10 (m, 2H), 4.02-3.80 (m, 3H), 3.05 (m, 1H), 2.95 (m, 1H), 2.50-2.20 (m, 2H),
2.25-1.75 (m, 3H), 1.70 (m, 1H), 1.50 (brs, 9H); $^{13}$C NMR (75 MHz, CDCl$_3$, TMS) δ 171.9, 170.9, 168.8, 155.0, 135.4, 135.2, 128.8, 128.7, 128.6, 128.5, 128.2, 126.7, 80.1, 67.1, 59.9, 56.1, 54.6, 53.8, 46.6, 41.0, 32.3, 31.1, 28.3, 26.6, 14.0; ESI MS: m/z 536.3 (M + H)$^+$. 

TFA (3 mL) was added to a solution of 30 (215 mg, 0.4 mmol) in CH$_2$Cl$_2$ (5 mL). The solution was stirred at room temperature overnight and then concentrated. The residue was partitioned between CH$_2$Cl$_2$ (100 mL) and 1N LiOH (20 mL), and the organic layer was dried over Na$_2$SO$_4$, then concentrated. A mixture of this residue, L-N-Boc-N-methylalanine (102 mg, 0.5 mmol), EDC (98 mg, 0.5 mmol) and HOBt (68 mg, 0.5 mmol) was suspended in CH$_2$Cl$_2$ (15 mL). To this mixture was added N,N-diisopropylethylamine (0.5 mL) and then the mixture was stirred at room temperature overnight. After concentration, the residue was purified by chromatography to give 31 (196 mg, 79% over two steps). Rf value at EtOAc/Hexane 2:1 is 0.3. The column was run using EtOAc and hexane as eluents from EtOAc/Hexane 1:2 to EtOAc/Hexane 2:1. $^1$H NMR (300 MHz, CDCl$_3$, TMS) δ 7.45-7.20 (m, 11H), 5.30-5.20 (m, 2H), 4.70-4.50 (m, 3H), 4.35-4.10 (m, 2H), 4.10-3.80 (m, 3H), 3.05 (m, 1H), 2.95 (m, 1H), 2.75 (s, 3H), 2.50-2.20 (m, 2H), 2.20-1.90 (m, 2H), 1.80 (m, 1H), 1.65 (m, 1H), 1.50 (brs, 9H), 1.45 (d, J = 7.2 Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$, TMS) δ 172.0, 171.9, 171.3, 168.3, 135.3, 135.1, 128.8, 128.7, 128.6, 128.5, 128.2, 126.7, 80.7, 67.1, 59.8, 56.0, 53.9, 53.1, 46.7, 40.8, 32.3, 31.1, 30.3, 28.3, 26.6; ESI MS: m/z 621.3 (M + H)$^+$. 

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10% Pd-C was added to a solution of 31 in MeOH. The mixture was stirred under H₂ overnight and then filtered through celite. The filtration was concentrated to give an acid which was used in the next step directly without purification. To a solution of 1 eq of this acid was added 1 eq of a corresponding amine, 1.1 eq of EDC, 1.1 eq of HOBT and 4 eq of N,N-diisopropylethylamine. The mixture was stirred at room temperature overnight and then concentrated. The residue was purified by chromatography to give an amide.

HCl solution (4N in 1,4-dioxane) was added to a solution of this amide in MeOH (5 mL). The solution was stirred at room temperature overnight and then concentrated to give crude product which was purified by semi-preparative HPLC on a C18 reverse phase column to provide pure Smac mimetic. The yield for two steps is between 85-90%. The purity of each final compound was determined by reverse phase analytical HPLC to be >95%.

**Compound 2:** Eluent was ran from 80% of A (1% of TFA in H₂O) and 20% of B (1% of TFA in CH₃CN) to 60% of A and 40% of B in 40 min. ¹H NMR (300 MHz, D₂O) δ 7.35-7.10 (m, 10H), 4.50-4.20 (m, 4H), 4.10 (m, 1H), 3.90-3.30 (m, 7H), 2.60 (s, 3H), 2.25 (m, 1H), 2.50-1.70 (m, 5H), 1.42 (d, J = 8.0 Hz, 3H); ESI MS: m/z 520.3 (M + H)+.

**Compound 3:** Eluent was ran from 80% of A (1% of TFA in H₂O) and 20% of B (1% of TFA in CH₃CN) to 60% of A and 40% of B in 40 min. ¹H NMR (300 MHz, D₂O) δ 7.30-7.10 (m, 7H), 7.05-6.95 (m, 2H), 4.50-4.25 (m, 4H), 4.10 (m, 1H), 3.95-3.30 (m, 7H),
2.55 (s, 3H), 2.25 (m, 1H), 2.20-1.70 (m, 5H), 1.45 (d, J = 8.0 Hz, 3H); ESI MS: m/z 538.3 (M + H)+.

**Compound 4:** Eluent was ran from 80% of A (1% of TFA in H\textsubscript{2}O) and 20% of B (1% of TFA in CH\textsubscript{3}CN) to 60% of A and 40% of B in 40 min. \textsuperscript{1}H NMR (300 MHz, D\textsubscript{2}O) δ 7.40-7.08 (m, 9H), 4.55 (m, 1H), 4.50-4.25 (m, 3H), 4.20 (m, 1H), 4.02-3.38 (m, 7H), 2.62 (s, 3H), 2.40-1.80 (m, 6H), 1.50 (d, J = 8.2 Hz, 3H); ESI MS: m/z 554.3 (M + H)+.

**Compound 5:** Eluent was ran from 80% of A (1% of TFA in H\textsubscript{2}O) and 20% of B (1% of TFA in CH\textsubscript{3}CN) to 60% of A and 40% of B in 40 min. \textsuperscript{1}H NMR (300 MHz, D\textsubscript{2}O) δ 7.50-7.40 (m, 2H), 7.30-7.05 (m, 7H), 4.60-4.20 (m, 4H), 4.15 (m, 1H), 3.95-3.30 (m, 7H), 2.60 (s, 3H), 2.23 (m, 1H), 2.20-1.70 (m, 5H), 1.45 (d, J = 8.0 Hz, 3H); ESI MS: m/z 598.2 (M + H)+.

**Compound 6:** Eluent was ran from 80% of A (1% of TFA in H\textsubscript{2}O) and 20% of B (1% of TFA in CH\textsubscript{3}CN) to 60% of A and 40% of B in 40 min. \textsuperscript{1}H NMR (300 MHz, D\textsubscript{2}O) δ 7.35-7.10 (m, 9H), 4.52-4.25 (m, 4H), 4.20 (m, 1H), 4.02-3.35 (m, 7H), 2.60 (s, 3H), 2.35-1.70 (m, 9H), 1.45 (d, J = 8.0 Hz, 3H); ESI MS: m/z 534.3 (M + H)+.

**Compound 7:** Eluent was ran from 80% of A (1% of TFA in H\textsubscript{2}O) and 20% of B (1% of TFA in CH\textsubscript{3}CN) to 60% of A and 40% of B in 40 min. \textsuperscript{1}H NMR (300 MHz, D\textsubscript{2}O) δ 7.60-7.50 (m, 2H), 7.45-7.30 (m, 2H), 7.30-7.02 (m, 5H), 4.55-4.25 (m, 4H), 4.20 (m, 1H), 3.95-3.30 (m, 7H), 2.60 (s, 3H), 2.40-1.70 (m, 6H), 1.45 (d, J = 8.0 Hz, 3H); ESI MS: m/z 588.3 (M + H)+.

**Compound 8:** Eluent was ran from 80% of A (1% of TFA in H\textsubscript{2}O) and 20% of B (1% of TFA in CH\textsubscript{3}CN) to 60% of A and 40% of B in 40 min. \textsuperscript{1}H NMR (300 MHz, D\textsubscript{2}O) δ 7.35-7.10 (m, 9H), 4.52-4.20 (m, 4H), 4.15 (m, 1H), 3.95-3.32 (m, 7H), 2.60 (s, 3H), 2.50 (q, J
= 7.2 Hz, 2H), 2.25 (m, 1H), 2.20-1.70 (m, 5H), 1.45 (d, J = 8.0 Hz, 3H), 1.05 (t, J = 7.2 Hz, 3H); ESI MS: m/z 548.3 (M + H)⁺.

**Compound 9:** Eluent was ran from 80% of A (1% of TFA in H₂O) and 20% of B (1% of TFA in CH₃CN) to 60% of A and 40% of B in 40 min. ¹H NMR (300 MHz, D₂O) δ 7.35-7.10 (m, 9H), 4.52-4.20 (m, 4H), 4.15 (m, 1H), 3.95-3.32 (m, 7H), 2.82 (m, 1H), 2.60 (s, 3H), 2.25 (m, 1H), 2.20-1.70 (m, 5H), 1.45 (brd, J = 8.0 Hz, 3H), 1.15-1.05 (m, 6H); ESI MS: m/z 562.3 (M + H)⁺.

**Compound 11:** Eluent was ran from 80% of A (1% of TFA in H₂O) and 20% of B (1% of TFA in CH₃CN) to 60% of A and 40% of B in 40 min. ¹H NMR (300 MHz, D₂O) δ 7.40-7.30 (m, 2H), 7.30-7.10 (m, 7H), 4.50 (m, 1H), 4.50-4.20 (m, 3H), 4.13 (m, 1H), 3.95-3.30 (m, 7H), 2.60 (s, 3H), 2.23 (m, 1H), 2.15-1.70 (m, 5H), 1.45 (brd, J = 8.0 Hz, 3H), 1.15 (brs, 9H); ESI MS: m/z 576.4 (M + H)⁺.

**Compound 11:** Eluent was ran from 80% of A (1% of TFA in H₂O) and 20% of B (1% of TFA in CH₃CN) to 60% of A and 40% of B in 40 min. ¹H NMR (300 MHz, D₂O) δ 7.45-6.75 (m, 14H), 4.50-3.98 (m, 5H), 3.95-3.20 (m, 7H), 2.65-2.55 (m, 3H), 2.25-1.40 (m, 9H); ESI MS: m/z 596.3 (M + H)⁺.

**Compound 12:** Eluent was ran from 80% of A (1% of TFA in H₂O) and 20% of B (1% of TFA in CH₃CN) to 60% of A and 40% of B in 40 min. ¹H NMR (300 MHz, D₂O) δ 7.30-7.15 (m, 7H), 6.90-6.80 (m, 2H), 4.50 (m, 1H), 4.45-4.20 (m, 3H), 4.18 (m, 1H), 3.95-3.30 (m, 10H), 2.60 (s, 3H), 2.25 (m, 1H), 2.20-1.70 (m, 5H), 1.45 (brd, J = 8.0 Hz, 3H); ESI MS: m/z 550.3 (M + H)⁺.

**Compound 13:** Eluent was ran from 80% of A (1% of TFA in H₂O) and 20% of B (1% of TFA in CH₃CN) to 60% of A and 40% of B in 40 min. ¹H NMR (300 MHz, D₂O) δ
7.50-7.35 (m, 4H), 7.30-7.15 (m, 4H), 7.02-6.90 (m, 1H), 4.90-4.70 (m, 1H), 4.50-4.30 (m, 3H), 4.22 (m, 1H), 3.98-3.35 (m, 7H), 3.15 (s, 2H), 2.98 (s, 4H), 2.60 (s, 3H), 2.40-1.65 (m, 6H), 1.45 (d, J = 8.0 Hz, 3H); ESI MS: m/z 563.3 (M + H)⁺.

**Compound 14:** Eluent was ran from 80% of A (1% of TFA in H₂O) and 20% of B (1% of TFA in CH₃CN) to 60% of A and 40% of B in 40 min. ¹H NMR (300 MHz, D₂O) δ 8.20-8.02 (m, 2H), 7.50-7.38 (m, 2H), 7.30-7.02 (m, 5H), 4.60 (m, 1H), 4.50-4.30 (m, 3H), 4.15 (m, 1H), 4.02-3.40 (m, 7H), 2.60 (s, 3H), 2.25 (m, 1H), 2.20-1.70 (m, 5H), 1.45 (d, J = 8.0 Hz, 3H); ESI MS: m/z 565.3 (M + H)⁺.

**Compound 15:** Eluent was ran from 80% of A (1% of TFA in H₂O) and 20% of B (1% of TFA in CH₃CN) to 60% of A and 40% of B in 40 min. ¹H NMR (300 MHz, D₂O) δ 7.75-7.65 (m, 2H), 7.40-7.25 (m, 2H), 7.30-7.02 (m, 5H), 4.60 (m, 1H), 4.50-4.25 (m, 3H), 4.20 (m, 1H), 3.95-4.35 (m, 7H), 2.62 (s, 3H), 2.25 (m, 1H), 2.20-1.70 (m, 5H), 1.45 (brd, J = 8.0 Hz, 3H); ESI MS: m/z 563.3 (M + H)⁺.

**Compound 16:** Eluent was ran from 80% of A (1% of TFA in H₂O) and 20% of B (1% of TFA in CH₃CN) to 60% of A and 40% of B in 40 min. ¹H NMR (300 MHz, D₂O) δ 7.30-6.98 (m, 9H), 4.50-4.20 (m, 4H), 4.16 (m, 1H), 3.95-3.30 (m, 7H), 2.60 (s, 3H), 2.30-1.70 (m, 6H), 1.45 (brd, J = 8.0 Hz, 3H); ESI MS: m/z 538.3 (M + H)⁺.

**Compound 17:** Eluent was ran from 80% of A (1% of TFA in H₂O) and 20% of B (1% of TFA in CH₃CN) to 60% of A and 40% of B in 40 min. ¹H NMR (300 MHz, D₂O) δ 7.35-7.05 (m, 6H), 7.05-6.80 (m, 3H), 4.55-4.20 (m, 4H), 4.10 (m, 1H), 3.95-3.30 (m, 7H), 2.59 (s, 3H), 2.23 (m, 1H), 2.20-1.70 (m, 5H), 1.45 (d, J = 8.0 Hz, 3H); ESI MS: m/z 538.3 (M + H)⁺.
**Compound 18:** Eluent was ran from 80% of A (1% of TFA in H₂O) and 20% of B (1% of TFA in CH₃CN) to 60% of A and 40% of B in 40 min. ¹H NMR (300 MHz, D₂O) δ 7.40-7.03 (m, 9H), 4.55-4.25 (m, 4H), 4.15 (m, 1H), 3.95-3.35 (m, 7H), 2.60 (s, 3H), 2.40-1.70 (m, 6H), 1.45 (d, J = 8.0 Hz, 3H); ESI MS: m/z 554.3 (M + H)⁺.

**Compound 19:** Eluent was ran from 80% of A (1% of TFA in H₂O) and 20% of B (1% of TFA in CH₃CN) to 60% of A and 40% of B in 40 min. ¹H NMR (300 MHz, D₂O) δ 7.30-7.03 (m, 9H), 4.50 (m, 1H), 4.40-4.25 (m, 3H), 4.20 (m, 1H), 3.95-3.30 (m, 7H), 2.60 (s, 3H), 2.30-1.70 (m, 6H), 1.45 (d, J = 8.0 Hz, 3H); ESI MS: m/z 554.3 (M + H)⁺.

**Compound 20:** Eluent was ran from 80% of A (1% of TFA in H₂O) and 20% of B (1% of TFA in CH₃CN) to 60% of A and 40% of B in 40 min. ¹H NMR (300 MHz, D₂O) δ 7.50 (m, 1H), 7.35-7.02 (m, 8H), 4.60-4.22 (m, 4H), 4.15 (m, 1H), 3.95-3.35 (m, 7H), 2.60 (s, 3H), 2.25 (m, 1H), 2.20-1.70 (m, 5H), 1.45 (d, J = 8.0 Hz, 3H); ESI MS: m/z 598.2 (M + H)⁺.

**Compound 21:** Eluent was ran from 80% of A (1% of TFA in H₂O) and 20% of B (1% of TFA in CH₃CN) to 60% of A and 40% of B in 40 min. ¹H NMR (300 MHz, D₂O) δ 7.50-7.03 (m, 9H), 4.65 (m, 1H), 4.45-4.20 (m, 3H), 4.18 (m, 1H), 3.90-3.35 (m, 7H), 2.60 (s, 3H), 2.25 (m, 1H), 2.20-1.70 (m, 5H), 1.45 (brd, J = 8.0 Hz, 3H); ESI MS: m/z 598.2(M + H)⁺.

**Compound 22:** Eluent was ran from 80% of A (1% of TFA in H₂O) and 20% of B (1% of TFA in CH₃CN) to 60% of A and 40% of B in 40 min. ¹H NMR (300 MHz, D₂O) δ 7.70-7.60 (m, 1H), 7.60-7.02 (m, 8H), 4.60-4.25 (m, 4H), 4.18 (m, 1H), 3.95-3.35 (m, 7H), 2.60 (s, 3H), 2.27 (m, 1H), 2.20-1.70 (m, 5H), 1.45 (d, J = 8.0 Hz, 3H); ESI MS: m/z 588.3 (M + H)⁺.
**Compound 23:** Eluent was ran from 80% of A (1% of TFA in H₂O) and 20% of B (1% of TFA in CH₃CN) to 60% of A and 40% of B in 40 min. $^1$H NMR (300 MHz, D₂O) δ 7.60-7.40 (m, 4H), 7.30-7.02 (m, 5H), 4.60-4.22 (m, 4H), 4.18 (m, 1H), 3.95-3.38 (m, 7H), 2.60 (s, 3H), 2.40-1.70 (m, 6H), 1.45 (brd, J = 8.0 Hz, 3H); ESI MS: m/z 588.3 (M + H)^+.