# MDM2-recruiting PROTAC Offers Superior, Synergistic Anti-proliferative Activity via Simultaneous Degradation of BRD4 and Stabilization of p53

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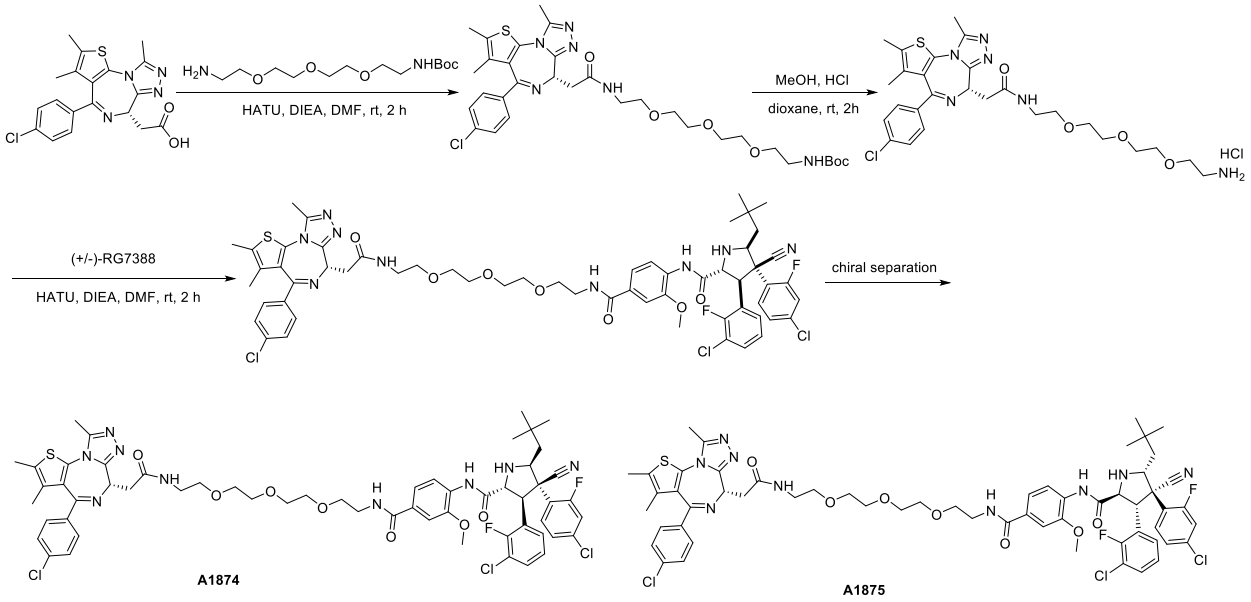
## Supplementary Materials and Methods – Chemistry

### Syntheses and Validation of PROTACs A1874, A1875 and A743

**Scheme 1:** Synthetic route of preparing **A1874** and **A1875**.



**Preparation of (2R,3S,4R,5S)-3-(3-chloro-2-fluorophenyl)-4-(4-chloro-2-fluorophenyl)-N-[4-[(2-[2-[2-(2-[2-[(9S)-7-(4-chlorophenyl)-4,5,13-trimethyl-3-thia-1,8,11,12-tetraazatricyclo[8.3.0.0^[2,6]]trideca-2(6),4,7,10,12-pentaen-9-yl]acetamido]ethoxy)ethoxy]ethoxy]ethyl)carbamoyl]-2-methoxyphenyl]-4-cyano-5-(2,2-dimethylpropyl)pyrrolidine-2-carboxamide (A1874) & (2S,3R,4S,5R)-3-(3-chloro-2-fluorophenyl)-4-(4-chloro-2-fluorophenyl)-N-[4-[(2-[2-[2-(2-[2-[(9S)-7-(4-chlorophenyl)-4,5,13-trimethyl-3-thia-1,8,11,12-tetraazatricyclo[8.3.0.0^[2,6]]trideca-2(6),4,7,10,12-pentaen-9-yl]acetamido]ethoxy)ethoxy]ethoxy]ethyl)carbamoyl]-2-methoxyphenyl]-4-cyano-5-(2,2-dimethylpropyl)pyrrolidine-2-carboxamide (A1875).**

Into a 100-mL round-bottom flask, was placed 2-[(9S)-7-(4-chlorophenyl)-4,5,13-trimethyl-3-thia-1,8,11,12-tetraazatricyclo[8.3.0.0^[2,6]]trideca-2(6),4,7,10,12-pentaen-9-yl]acetic acid (70 mg, 0.17 mmol, 1.0 eq), *tert*-butyl N-(2-[2-[2-(2-aminoethoxy)ethoxy]ethoxy]ethyl)carbamate (51 mg, 0.17 mmol, 1.0 eq), O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*,*N*-tetramethyluronium hexafluorophosphate (79.8 mg, 0.21 mmol, 1.30 eq), *N*,*N*-diisopropylethylamine (0.1 mL, 3.0 eq), and *N*,*N*-dimethylformamide (1 mL). The resulting solution was stirred for 1 h at room temperature. The resulting solution was diluted with water (5 mL). The resulting solution was extracted with ethyl acetate (3x5 mL) and the organic layers were combined and concentrated under vacuum. The residue was applied onto a silica gel column eluted with dichloromethane/methanol (10/1). This resulted in 120.0 mg (crude) of *tert*-butyl N-(2-[2-[2-(2-[2-[(9S)-7-(4-chlorophenyl)-4,5,13-trimethyl-3-thia-1,8,11,12-tetraazatricyclo [8.3.0.0^[2,6]]trideca-2(6),4,7,10,12-pentaen-9-yl]acetamido]ethoxy)ethoxy]ethoxy]ethyl) carbamate as brown oil.

The above oily material (120 mg, 0.18 mmol) was placed inside a 100-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, then methanol (10.0 mL) and hydrogen chloride (4N) in dioxane (3.0 mL) was added. The resulting solution was stirred for 1.0 h at room temperature. The resulting mixture was concentrated under vacuum. This resulted in 118.0 mg (crude) of N-(2-[2-[2-(2-aminoethoxy)ethoxy]ethoxy]ethyl)-2-[(9S)-7-(4-chlorophenyl)-4,5,13-trimethyl-3-thia-1,8,11,12-tetraazatricyclo[8.3.0.0^[2,6]]trideca-2(6),4,7,10,12-pentaen-9-yl]acetamide as a white solid.

The solid intermediate (87 mg, 0.15 mmol) was placed into a 100-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, and (+/-) RG7388 (80 mg, 0.13 mmol, 1.0 eq) was added followed by the addition of HATU (60 mg, 0.16 mmol, 1.20 eq ), DIEA (0.5 mL, 3.0 eq ), and *N*,*N*-dimethylformamide (2 mL). The resulting solution was stirred for 1 h at

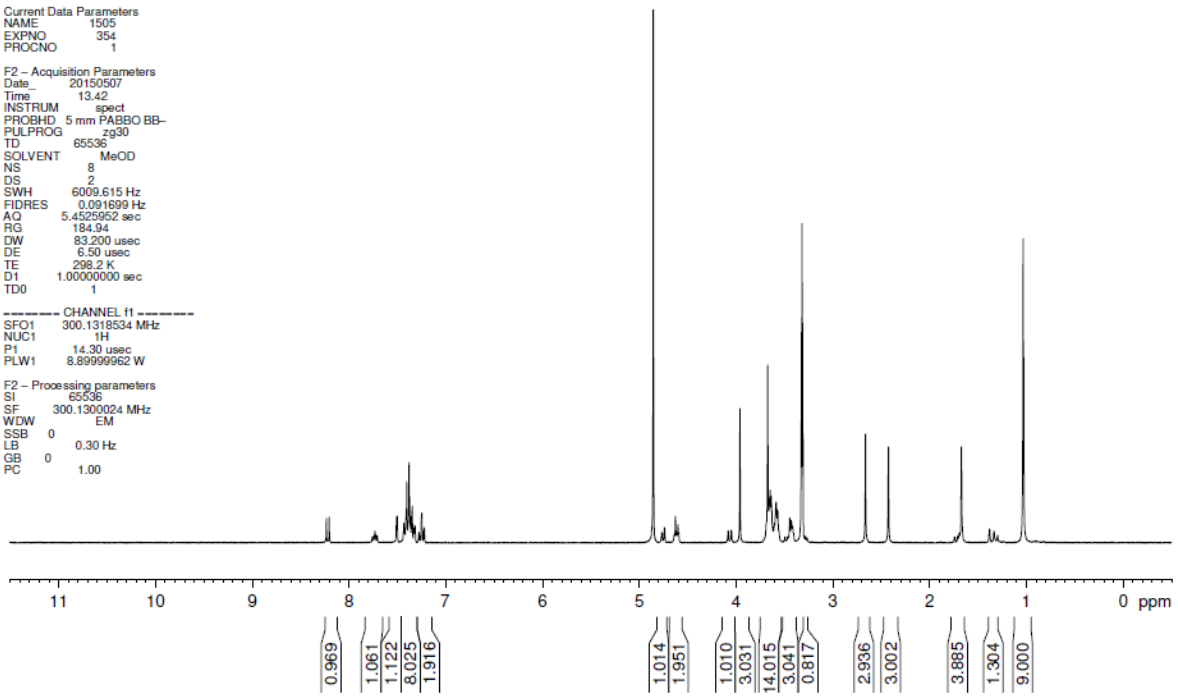
room temperature. The resulting solution was diluted with water (10 mL), extracted with ethyl acetate (3x20 mL) and the organic layers were combined. The resulting mixture was washed with water (2x20 mL) and brine (1x20 mL). This solution was concentrated under vacuum. The crude product was purified by preparative HPLC with the following conditions: column, XBridge Shield RP18 OBD column, 5 um, 19x150 mm; mobile phase, water with 10 mmol $NH_4HCO_3$ and acetonitrile (35.0% acetonitrile up to 95.0% in 10 min); detector, UV 254 nm. The purification resulted in 51 mg (24%) of (2R,3S,4R,5S)/(2S.3R,4S,5R)-3-(3-chloro-2-fluorophenyl)-4-(4-chloro-2-fluorophenyl)-N-[4-[(2-[2-[2-[(2-[2-[(9S)-7-(4-chlorophenyl)-4,5,13-trimethyl-3-thia-1,8,11,12-tetraazatricyclo[8.3.0.0^[2,6]]trideca-2(6),4,7,10,12-pentaen-9-yl]acetamido]ethoxy)ethoxy]ethoxy]ethyl)carbamoyl]-2-methoxyphenyl]-4-cyano-5-(2,2-dimethylpropyl)pyrrolidine-2-carboxamide as a white solid. LC-MS ($ES^+$) calcd for $C_{58}H_{62}Cl_3F_2N_9O_7S$ [m/z] 1171.35 and 1173.35, obsd 1174.40 [$M+H^+$]; $^1H$ NMR (300 MHz, $CD_3OD$): δ 8.23 and 8.10 (2d, 1H), 7.80-7.70 (m, 1 H), 7.53 (br s, 1 H), 7.45-7.32 (m, 8 H), 7.29-7.22 (m, 2 H), 4.80-4.70 (m, 1 H), 4.68-4.58 (m, 2 H), 4.07 (br d, 1 H), 3.97 and 3.95 (2s, 3 H), 3.71-3.54 (m, 14 H), 3.52-3.41 (m, 3 H), 3.40-3.25 (m, 1H), 2.67 and 2.64 (2s, 3 H), 2.44 (s, 3 H), 1.75-1.65 (m, 4 H), 1.40-1.25 (m, 1 H), 0.98 (s, 9 H).

The above purified diastereomeric compound (60 mg, 0.05 mmol) was further separated by preparative -HPLC using a chiral column with the following conditions: column, Chiralpak IC, 2x25 cm, 5 um; mobile phase, methanol and dichloromethane (hold 20.0% DCM in 14 min); detector, UV 254 and 220 nm. This resulted in 19.6 mg (33%) of (2R,3S,4R,5S)-3-(3-chloro-2-fluorophenyl)-4-(4-chloro-2-fluorophenyl)-N-[4-[(2-[2-[2-[(2-[2-[(9S)-7-(4-chlorophenyl)-4,5,13-trimethyl-3-thia-1,8,11,12-tetraazatricyclo[8.3.0.0^[2,6]]trideca-2(6),4,7,10,12-pentaen-9-yl]acetamido]ethoxy)ethoxy]ethoxy]ethyl)carbamoyl]-2-methoxyphenyl]-4-cyano-5-(2,2-dimethylpropyl)pyrrolidine-2-carboxamide (**A1874**) and 16.6 mg (28%) of (2S,3R,4S,5R)-3-(3-chloro-2-fluorophenyl)-4-(4-chloro-2-fluorophenyl)-N-[4-[(2-[2-[2-[(2-[2-[(9S)-7-(4-chlorophenyl)-4,5,13-trimethyl-3-thia-1,8,11,12-tetraazatricyclo[8.3.0.0^[2,6]]trideca-2(6),4,7,10,12-pentaen-9-yl]acetamido]ethoxy)ethoxy]ethoxy]ethyl)carbamoyl]-2-methoxyphenyl]-4-cyano-5-(2,2-dimethylpropyl)pyrrolidine-2-carboxamide (**A1875**). **A1874:** LC-MS ($ES^+$) calcd for $C_{58}H_{62}Cl_3F_2N_9O_7S$ [m/z] 1171.35 and 1173.35, obsd 587.50 [$(M+2H^+)/2$] and 1195.05 [$M+Na^+$]; $^1H$ NMR (300 MHz, $CD_3OD$): δ 8.20 (d, 1H), 7.80-7.70 (m, 1 H), 7.50 (s, 1 H), 7.45-7.30 (m, 8 H), 7.29-7.20 (m, 2 H), 4.75 (br d, 1 H), 4.65-4.55 (m, 2H), 4.07 (d, 1 H),

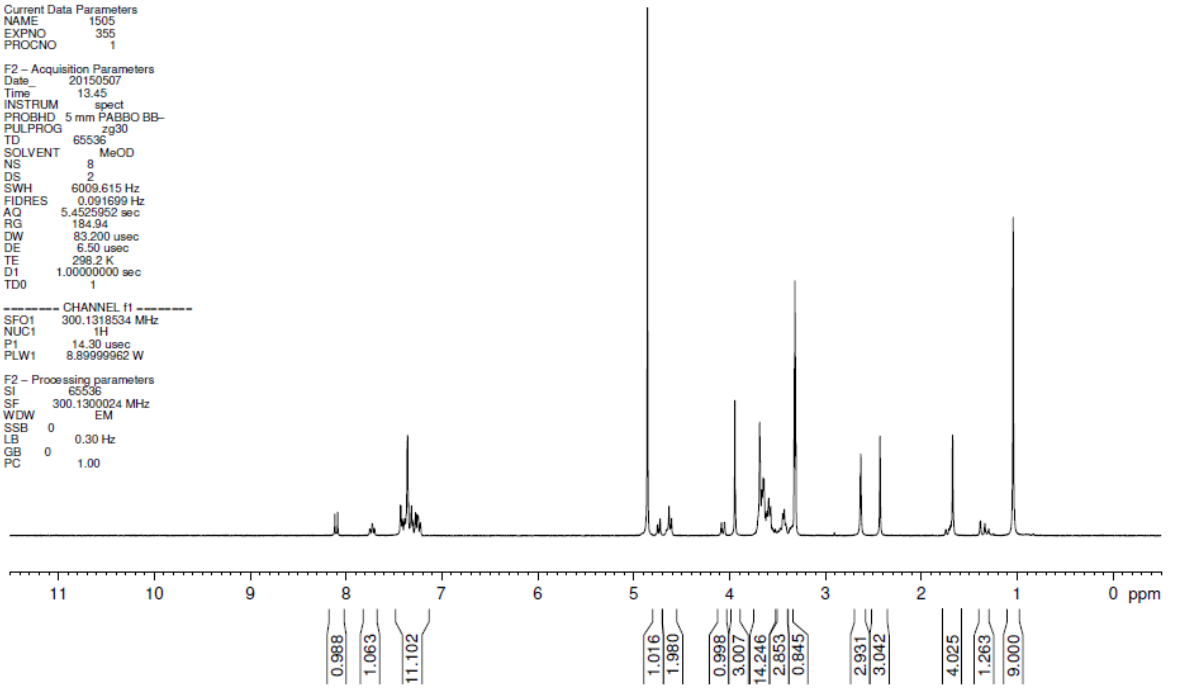
3.97 (s, 3 H), 3.71-3.54 (m, 14 H), 3.52-3.40 (m, 3 H), 3.30 -3.25 (m, 1 H), 2.67 (s, 3 H), 2.42 (s, 3 H), 1.80-1.62 (m, 4 H), 1.40-1.25 (m, 1 H), 0.98 (s, 9 H); **A1875:** LC-MS ($ES^+$) calcd for $C_{58}H_{62}Cl_3F_2N_9O_7S$ [m/z] 1171.35 and 1173.35, obsd 587.50 [$(M+2H^+)/2$] and 1174.30 [$M+H^+$]; $^1H$ NMR (300 MHz, $CD_3OD$): δ8.11 (d, 1H), 7.78-7.68 (m, 1 H), 7.46-7.19 (m, 11H), 4.75 (br d, 1 H), 4.68-4.58 (m, 2 H), 4.07 (d, 1H), 3.96 (s, 3 H), 3.72-3.52 (m, 14 H), 3.49-3.40 (m, 3 H), 3.39 -3.30 (m, 1 H), 2.65 (s, 3 H), 2.43 (s, 3 H), 1.78-1.62 (m, 4 H), 1.40-1.25 (m, 1 H), 0.98 (s, 9

H).

[1]H-NMR of **A1874**

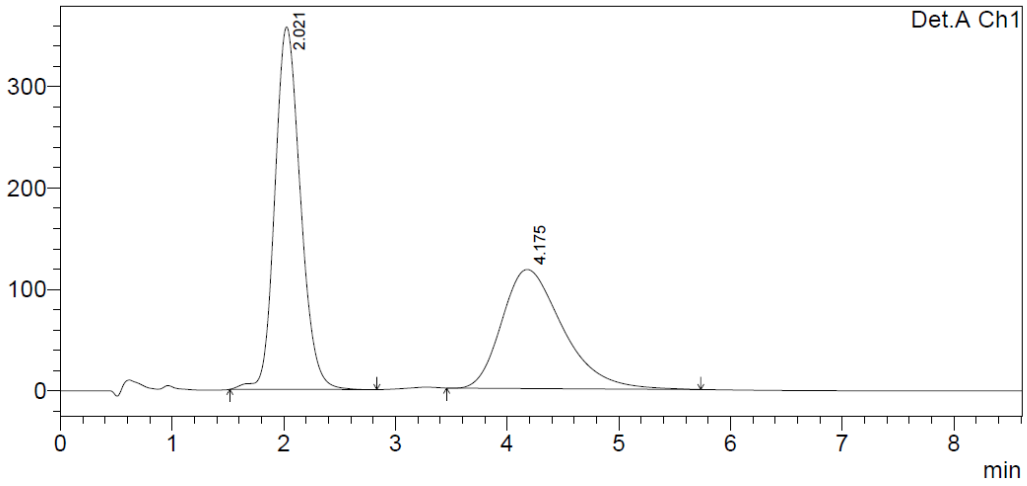



[1]H-NMR of **A1875**

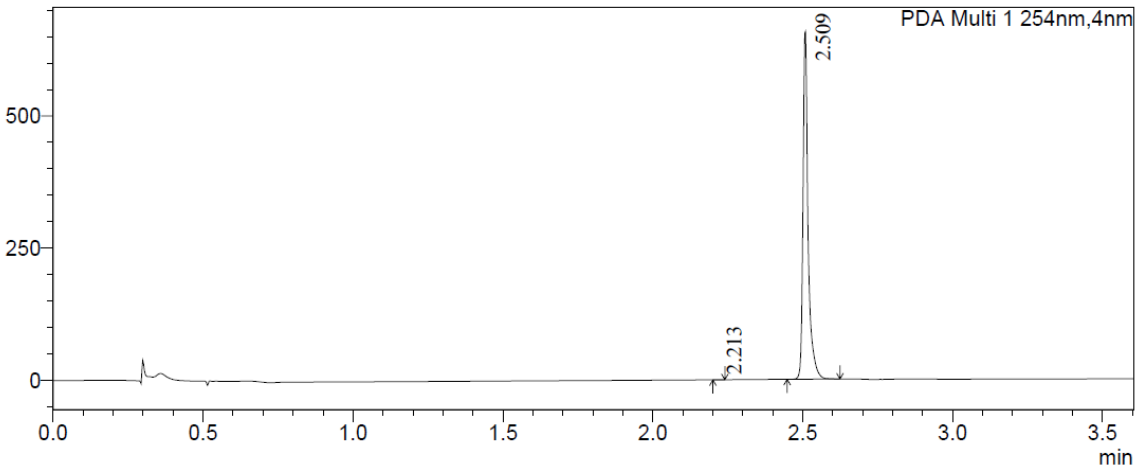


Liquid chromatography analysis of **A1874** and **A1875** mixture using a chiral column (Lux

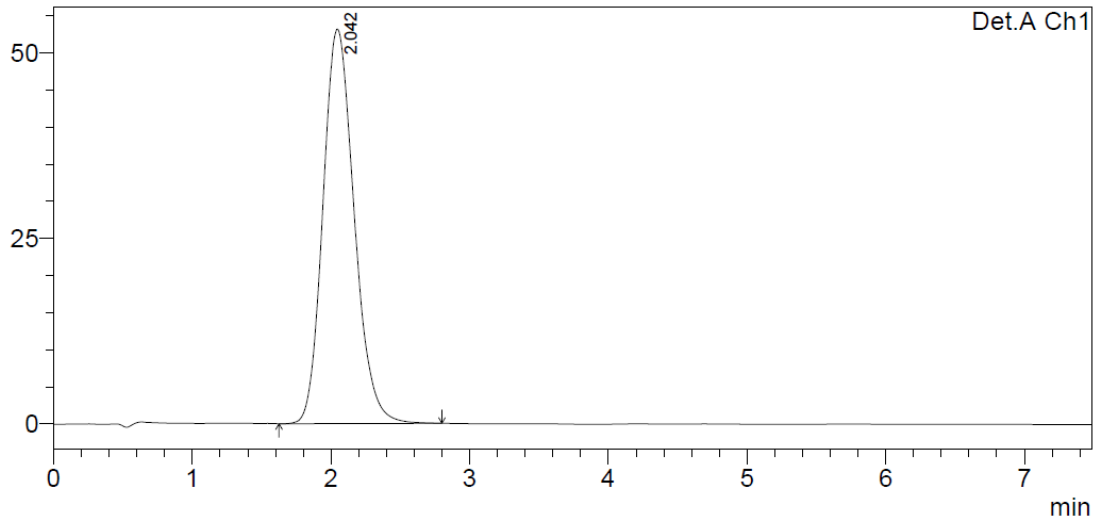
Cellulose-4).



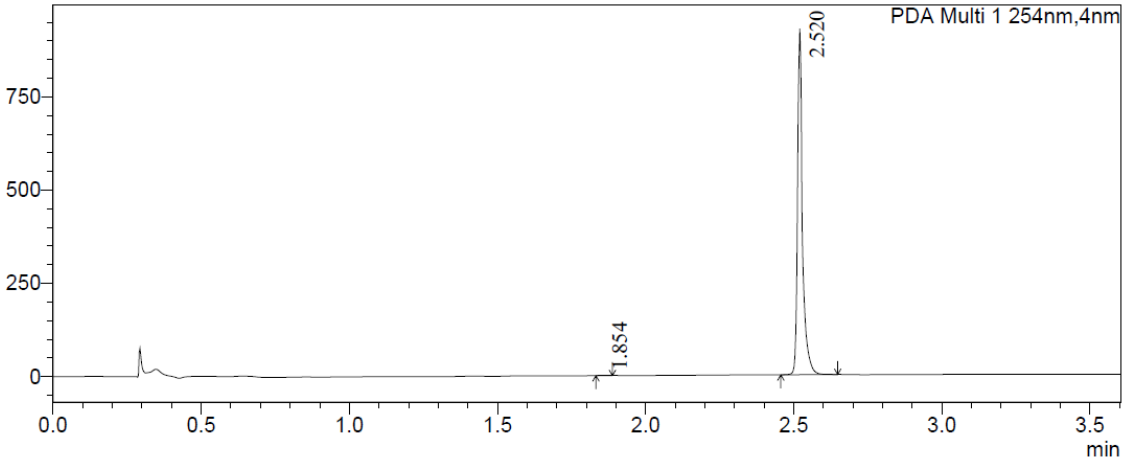

Liquid chromatography analysis of **A1874.**



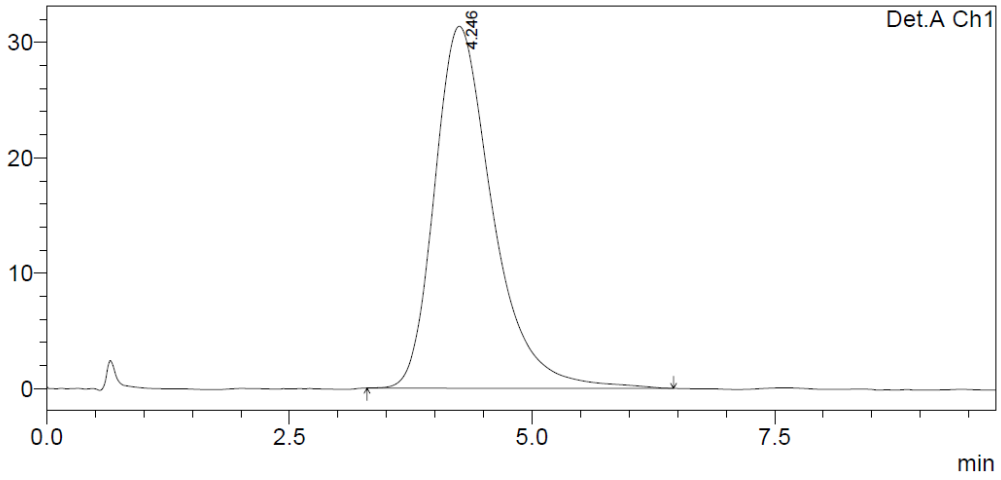
Liquid chromatography analysis of **A1874** using a chiral column (Lux Cellulose-4).



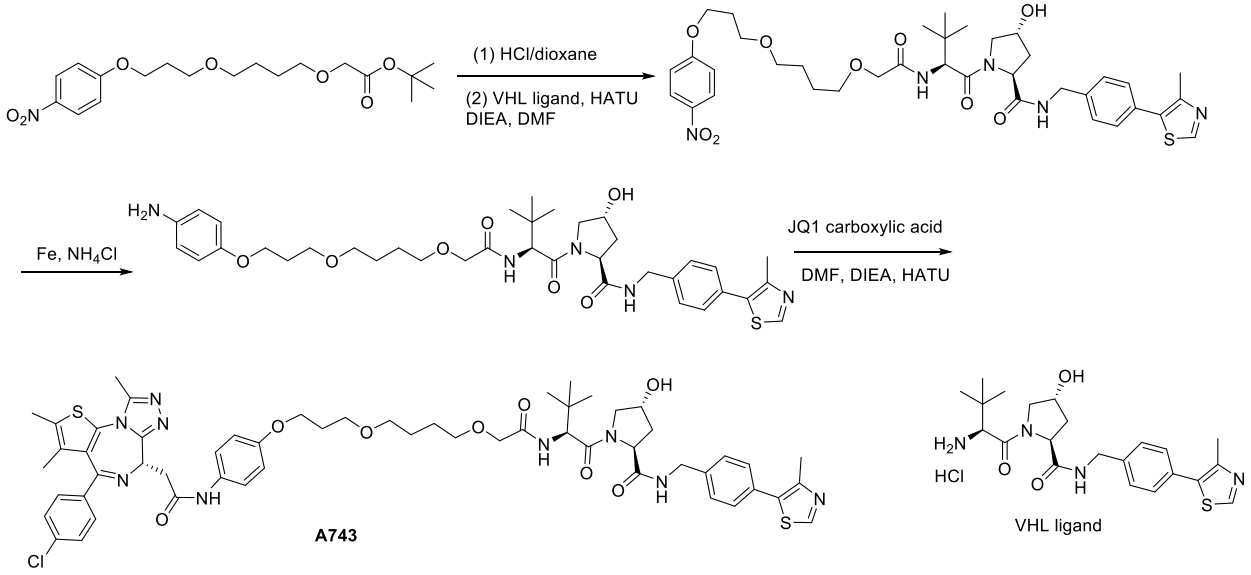

Liquid chromatography analysis of **A1875.**



Liquid chromatography analysis of **A1875** using a chiral column (Lux Cellulose-4).




**Scheme 2:** Synthetic route of preparing **A743**.



**Preparation of (2S,4R)-1-((S)-2-(2-(4-(3-(4-(2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl -6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamido)phenoxy)propoxy)butoxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (A743).**

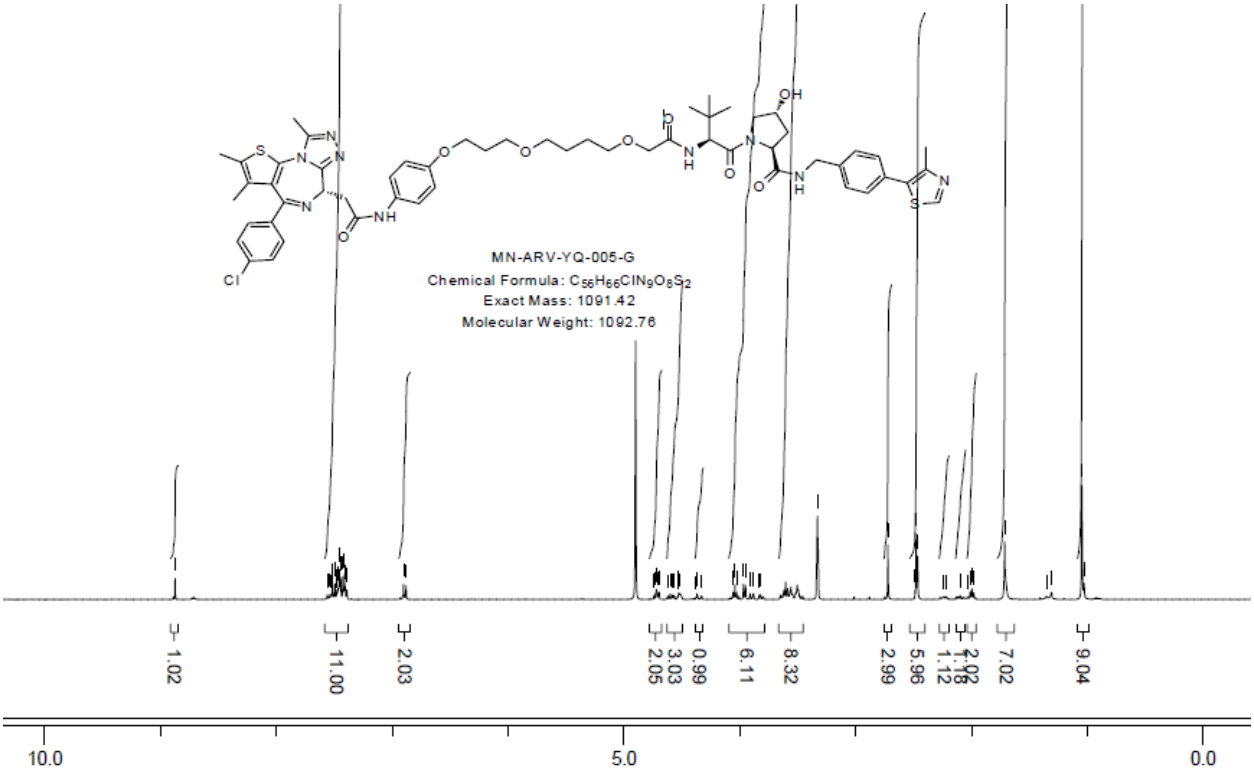
A solution of *tert*-butyl 2-(4-(3-(4-nitrophenoxy)propoxy)butoxy)acetate (150 mg, 0.4 mmol) in hydrochloride/dioxane (3N, 6 mL) was stirred at room temperature for 1 hour. The volatiles were removed under reduced pressure. The residue was re-dissolved in dry N,N-dimethylformamide (2 mL), followed by sequential addition of (2S,4R)-1-((S)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide hydrochloric acid salt (234 mg, 0.5 mmol), N-ethyl-N-isopropylpropan-2-amine (200 mg, 1.48 mmol), and HATU (280 mg, 0.74 mmol) at room temperature. The resulting mixture was stirred at room temperature for 20 minutes. The reaction mixture was poured into water (20 mL), extracted with ethyl acetate (25 ml x 3). The combined organic phase was washed with brine (20 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give a crude residue which was purified by silica gel flash chromatography (eluted with 1-5% methanol in dichloromethane) to afford (2S,4R)-1-((S)-3,3-dimethyl-2-(2-(4-(3-(4-nitrophenoxy)propoxy)butoxy)acetamido)butanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (260 mg, 88%) as light yellow solid. LC/MS ($ES^+$) calcd for $C_{37}H_{49}N_5O_9S$ [m/z] 739.35, obsd 740.40 [$M+H^+$].

The obtained carboxamide (260 mg, 0.36 mmol) was mixed with iron powder (160 mg, 2.86 mmol), and ammonium chloride (150 mg, 2.8 mmol) in ethanol (10 mL) and water (5mL). The mixture was stirred at refluxing temperature for 2.5 hours. The mixture was cooled to room temperature, and the solid precipitate was filtered off. The solid cake was washed with ethyl acetate (10 mL x 2), and the combined filtrates were partitioned between ethyl acetate (150 mL) and water (30 mL). The organic phase was separated, washed with brine (30 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give a crude residue which was purified by silica gel flash column chromatography (eluted with 3-5% methanol in dichloromethane) to afford (2S,4R)-1-((S)-2-(2-(4-(3-(4-aminophenoxy)propoxy)butoxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-

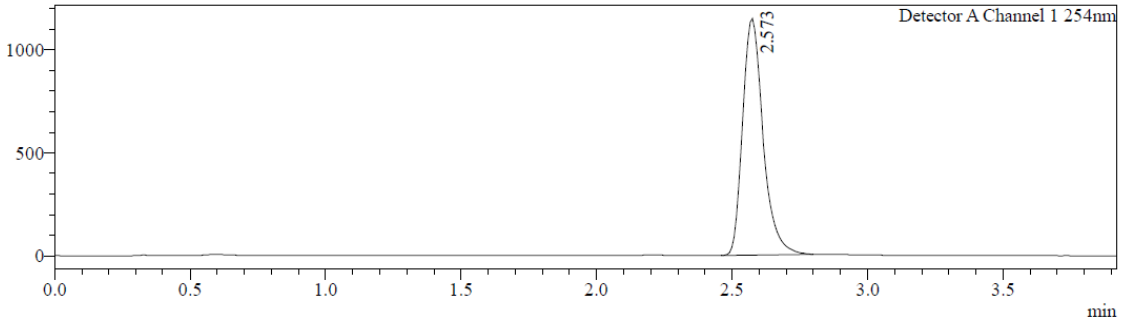
methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (170 mg, 68%) as light yellow solid. LC/MS ($ES^+$) calcd for $C_{37}H_{51}N_5O_7S$ [m/z] 709.35, obsd 710.40 [$M+H^+$].

To solid amine (86 mg, 0.12 mmol) was mixed with (S)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetic acid (JQ1 carboxylic acid, 50 mg, 0.12 mmol), N-ethyl-N-isopropylpropan-2-amine (54 mg, 0.42 mmol) in anhydrous N,N-dimethylformamide (1.5 mL). To this solution was added HATU (91 mg, 0.24 mmol) at 0°C, and the resulting mixture was warmed to room temperature and further stirred at room temperature for 20 min. The mixture was partitioned between ethyl acetate (60 mL) and water (15 mL). The organic layer was collected, washed with brine (10 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give a crude residue which was purified by prep-TLC to afford (2S,4R)-1-((S)-2-(2-(4-(3-(4-(2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamido)phenoxy)propoxy)butoxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (33.5 mg, 27.9%) as pink solid of **A743**. LC/MS ($ES^+$) calcd for $C_{56}H_{66}ClN_9O_8S_2$ [m/z] 1091.42, obsd 1092.40 [$M+H^+$]; $^1H$ NMR (400 MHz, $CD_3OD$): δ 1.05 (s, 9 H), 1.71 (br, 7 H), 1.98-2.13 (m, 3 H), 2.21-2.27 (m, 1 H), 2.47-2.49 (m, 6 H), 2.72 (s, 3 H), 3.46-3.65 (m, 8 H), 3.80-4.09 (m, 6 H), 4.33-4.38 (m, 1 H), 4.51-4.62 (m, 3 H), 4.70-4.74 (m, 2 H), 6.89 (d, 2 H), 7.39-7.56 (m, 10 H), 8.87 (s, 1 H).

$^{1}$H-NMR of **A743**.




Liquid chromatography analysis of **A743**.



Liquid chromatography analysis of **A743** using a chiral column (CHIRALPAK AD).