# Supporting Information

## Novel Autotaxin Inhibitor for the Treatment of Idiopathic Pulmonary Fibrosis: A Clinical Candidate Discovered Using DNA-Encoded Chemistry

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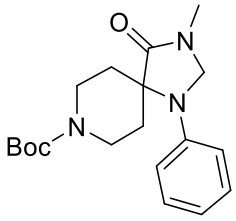
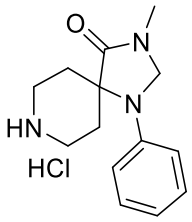
**Synthesis of intermediates**



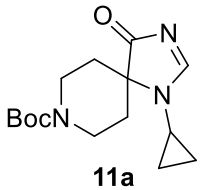

**5a**

*tert-butyl 3-methyl-4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]decane-8-carboxylate* (**5a**) To a solution of *tert*-butyl 4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]decane-8-carboxylate (900 mg, 2.72 mmol) in THF (10 mL) was added NaH (218 mg, 60% in mineral oil, 5.44 mmol) at 0 $^{o}$C. The mixture was stirred at 0 $^{o}$C for 1 h before the addition of methyl iodide (772 mg, 5.44 mmol). After stirring at RT for 2h, the reaction was diluted with ethyl acetate (50 mL) and quenched with aq. citric acid. The organic layer was separated, washed with brine (50 mL, 2 times), dried over anhydrous $Na_2SO_4$, and concentrated under vacuum to give crude product **5a** (800 mg, 88%) as a yellow solid. LCMS (ESI): *m/z* 346 $(M+1)^+$. The compound was used for the next step without further purification.



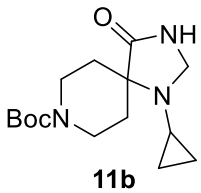

**5b**

*(R)-8-(2-amino-2-cyclohexylacetyl)-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one* **(5b)** HCl (gas) was bubbled into MeOH (30 mL) at 0 $^{o}$C for 1h. **5a** (600 mg, 1.24 mmol) was added and the mixture was stirred at RT for 3h. The resulting solution was concentrated under vacuum to give crude product **5b** (420 mg, 90 %) as a yellow solid. LCMS (ESI): *m/z* 246 $(M+1)^+$.




**11a**

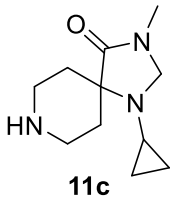
*tert-butyl 1-cyclopropyl-4-oxo-1,3,8-triazaspiro[4.5]dec-2-ene-8-carboxylate* (**11a**) A solution of *tert*-butyl 4-carbamoyl-4-(cyclopropylamino)piperidine-1-carboxylate (250 mg, 0.882 mmol) in trimethoxymethane (3 mL) and AcOH (0.3 mL) was irradiated by microwave in a sealed reactor to 100 $^{o}$C for 20 mins. Upon cooling, aqueous ammonia (10 mL) was added. The reaction mixture was extracted with $CH_2Cl_2$ (20 mL, 2 times). The combined organic extracts were washed with brine (20 mL, 2 times), dried over anhydrous $Na_2SO_4$ and concentrated under vacuum. The residue was purified using silica gel column chromatography ($CH_2Cl_2$/MeOH = 80:1 to 60:1 to 40:1) to give product **11a** (237 mg, 91%) as a white solid. LCMS (ESI): *m/z* 294 $(M+1)^+$.




**11b**

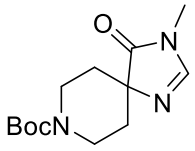
*tert-butyl 1-cyclopropyl-4-oxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate* (**11b)** To a solution of **11a** (237 mg, 0.808 mmol) in MeOH (10 mL) was added $NaBH_4$ (46 mg, 1.212 mmol). The resulting mixture was stirred at RT for 2h. The solvent was then evaporated, and the residue was dissolved in $CH_2Cl_2$ (15 mL) and 12% aqueous ammonia (15 mL). The mixture was stirred at RT for 2h. The organic layer was separated, and the aqueous phase was extracted with $CH_2Cl_2$ (10 mL, 2 times). The combined organic phase was washed with brine (50 mL, 2 times), dried over

anhydrous $Na_2SO_4$, and concentrated under vacuum to give crude product **11b** (264 mg) as a white solid. LCMS (ESI): *m/z* 296 $(M+1)^+$.



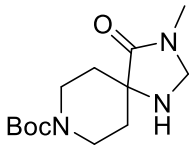

**11c**

*1-cyclopropyl-3-methyl-1,3,8-triazaspiro[4.5]decan-4-one* (**11c**) To a solution of **11b** (264 mg, 0.894 mmol) in THF (5 mL) was added NaH (107 mg, 60% in mineral oil, 2.682 mmol) at 0 $^oC$. The resulting mixture was stirred at 0 $^oC$ for 0.5 h, followed by the addition of methyl iodide (254 mg, 1.788 mmol), and was stirred at RT for an additional 2h. The reaction mixture was diluted with ethyl acetate (20 mL), quenched with aq. citric acid, washed with brine (20 mL, 2 times), dried over anhydrous $Na_2SO_4$, and concentrated under vacuum. The residue was purified using silica gel column chromatography ($CH_2Cl_2$/MeOH = 60:1) to give the desired product *tert*-butyl 1-cyclopropyl-3-methyl-4-oxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate (200 mg, 72 %) as a yellow oil. LCMS (ESI): *m/z* 310 $(M+1)^+$. The compound (200 mg, 0.647 mmol) was added to HCl/Dioxane (4M, 6 mL) at RT, and the mixture was stirred for 3h. The resulting solution was concentrated under vacuum to give crude product **11c** (200 mg) as a white solid, and was used in the next step without further purification. LCMS (ESI): *m/z* 210 $(M+1)^+$.



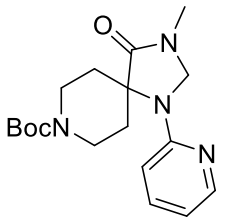

**12a**

*tert-butyl 3-methyl-4-oxo-1,3,8-triazaspiro[4.5]dec-1-ene-8-carboxylate* **(12a)** A solution of *tert*-butyl 4-amino-4-(methylcarbamoyl)piperidine-1-carboxylate (1 g, 3.9 mmol) in trimethoxymethane (10 mL) and AcOH (1 mL) was irradiated by microwave in a sealed reactor at 100 $^{o}$C for 20 min. Upon cooling, $NH_3H_2O$ (20 mL) was added. The reaction mixture was diluted with $CH_2Cl_2$ (30 mL), washed with brine (30 mL, 2 times), dried over anhydrous $Na_2SO_4$, and concentrated under vacuum to give crude product **12a** (1.2 g, 99%) as a white solid. LCMS (ESI): *m/z* 268 $(M+1)^+$.



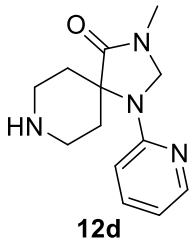

**12b**

*tert-butyl 3-methyl-4-oxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate* **(12b)** To a solution of **12a** (1.2 g, 3.7 mmol) in MeOH (15 mL) was added $NaBH_4$ (400 mg, 11.2 mmol). The resulting mixture was stirred at RT for 1h. The solvent was evaporated, and the residue was dissolved in $CH_2Cl_2$ (15 mL) and 12% aqueous ammonia (15 mL). The mixture was stirred at RT for 2h. The organic layer was separated, and the aqueous phase was extracted with $CH_2Cl_2$ (30 mL, 2 times). The combined organic phase was washed with brine (50 mL, 2 times), dried over anhydrous $Na_2SO_4$, and concentrated under vacuum. The residue was purified using silica gel column chromatography ($CH_2Cl_2$/MeOH = 40:1) to give product **12b** (0.78 g, 71%) as a white solid. LCMS (ESI): *m/z* 270 $(M+1)^+$.



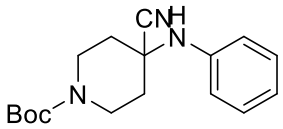

**12c**

*tert-butyl 3-methyl-4-oxo-1-(pyridin-2-yl)-1,3,8-triazaspiro[4.5]decane-8-carboxylate* **(12c)** A solution of **12b** (0.4 g, 1.49 mmol), 2-bromopyridine (0.47 g, 2.97 mmol), *t*-BuOK (0.66 g, 5.9 mmol) and $Pd_2(dba)_3$ (0.13 g, 0.149 mmol), X-Phos (0.17 g, 0.297 mmol) in toluene (20 mL) was irradiated by microwave in a sealed reactor at 130 $^oC$ for 35 min. Upon cooling the reaction mixture was filtered, diluted with $CH_2Cl_2$ (30 mL), washed with brine (30 mL, 2 times), dried over anhydrous $Na_2SO_4$, and concentrated under vacuum to give crude product **12c** (0.9 g, crude) as a black oil. LCMS (ESI): *m/z* 347 $(M+1)^+$.



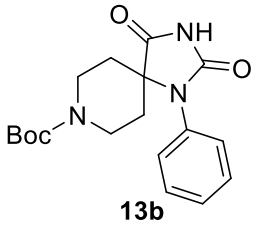

**12d**

*3-methyl-1-(pyridin-2-yl)-1,3,8-triazaspiro[4.5]decan-4-one* **(12d)** Compound **12c** (900 mg, 1.49 mmol) was added to 4M HCl in Dioxane (10 mL) and the mixture was stirred at RT for 3h. The resulting solution was concentrated under vacuum. The residue was basified with aq. sat. $NaHCO_3$ and extracted with ethyl acetate (30 mL, 3 times). The combined organic layer was concentrated and purified using silica gel column chromatography ($CH_2Cl_2$/MeOH = 40:1) to give product **12d** (280 mg, 50%, 2 steps) as a black oil. LCMS (ESI): *m/z* 247 $(M+1)^+$.




**13a**

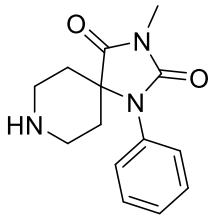
*tert-butyl 4-cyano-4-(phenylamino)piperidine-1-carboxylate* (**13a**) To a solution of aniline (0.5 g, 5 mmol) in acetic acid (20 mL) was added *t*-butyl 4-oxopiperidine-1-carboxylate (1.0 g, 5 mmol). The resulting mixture was cooled to 0°C before TMSCN (0.5 g, 5.1 mmol) was added and stirred at RT overnight. The reaction mixture was then diluted with water (20 mL) and extracted with EtOAc(50 mL, 3 times). The combined organic layer was washed with $Na_2CO_3$ (aq. 10%, 50mL), dried over anhydrous $Na_2SO_4$ and the solvent was removed under reduced pressure. The residue was purified using silica gel column chromatography (1:1 petroleum ether:$CH_2Cl_2$) to give the desired product (1.3 g, 86%) as a white solid. LCMS (ESI): *m/z* 302 $(M+1)^+$.




**13b**

*tert-butyl 3-methyl-2,4-dioxo-1-phenyl-1,3,8-triazaspiro[4.5]decane-8-carboxylate* (**13b**) To a solution of tert-butyl 4-cyano-4-(phenylamino)piperidine-1-carboxylate (**13a**, 0.10 g, 0.33 mmol) in $CH_2Cl_2$ (30 mL) was added $NCOSO_2Cl$ (70 mg, 0.49 mmol) at 0°C. After stirring for 60 minutes at 0°C, the reaction was quenched by the addition of 5% aqueous hydrochloric acid solution (10 mL). The solvent was removed under reduced pressure and ethanol (50 mL) was added. The mixture was refluxed for 30 minutes before solvent was removed under reduced pressure and THF (20 mL) was added. The pH of the solution was then adjusted to 11 by the

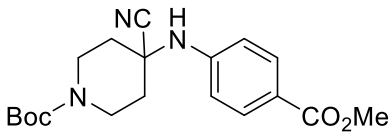
addition of 15% aqueous potassium carbonate before $(Boc)_2O$ (1.0g, 4.6 mmol) in THF (10 mL) was added. After stirring at RT overnight, the solvent was removed under reduced pressure and the residue was purified using silica gel column chromatography (1:1 petroleum ether:EtOAc) to afford **13b** (20 mg, 17%) as a white solid. LCMS (ESI): *m/z* 346 $(M+1)^+$.




**13d**

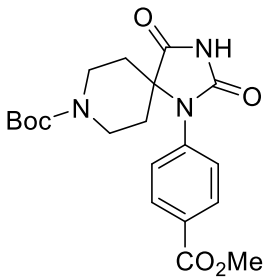
To a solution of tert-butyl 3-methyl-2,4-dioxo-1-phenyl-1,3,8-triazaspiro[4.5]decane-8-carboxylate (**13b**, 0.2 g, 0.57 mmol) in DMF (5 mL) was added NaH (60% in mineral oil, 0.6 mmol) and methyl iodide (83 mg, 0.57 mmol). After stirring at RT for 2 hour, the reaction was quenched by the addition of iced-water (5 mL). The mixture was extracted with ethyl acetate (10 mL, 3 times) and the combined organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified using Combi-flash (1:1 petroleum ether:EtOAc) to afford the desired product as a white solid (140 mg, 67%). LCMS (ESI): *m/z* 360.1 $(M+1)^{+\cdot}$

A solution of tert-butyl 3-methyl-2,4-dioxo-1-phenyl-1,3,8-triazaspiro[4.5]decane-8-carboxylate (140 mg, 0.4 mmol) in 4M hydrochloric acid in dioxane and EtOAc (10 mL/50 mL) was stirred at RT for 2h. The mixture was concentrated in vacuo to afford the desired product **13d** (92 mg, crude) as a white solid, and was used for the next step without further purification. LCMS (ESI): *m/z* 260.1 $(M+1)^+$.




**21a**

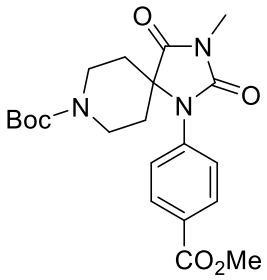
*tert-butyl 4-cyano-4-((4-(methoxycarbonyl)phenyl)amino)piperidine-1-carboxylate* (**21a**) To a solution of methyl 4-aminobenzoate (6.0 g, 39.7 mmol) in acetic acid (100 mL) was added *t*-butyl 4-oxopiperidine-1-carboxylate (9.49 g, 47.7 mmol). The resulting mixture was cooled to 0°C and TMSCN (5.11 g, 51.7 mmol) was added. The solution was stirred at RT overnight. The reaction mixture was diluted with water (200 mL) and filtered. The solid was washed with sat. aq. $NH_4Cl$ and water, dried in vacuo to give the crude product as a white solid (14.0g, crude). LCMS (ESI): *m/z* 360 $(M+1)^+$.




**21b**

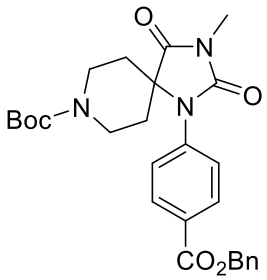
*tert-butyl 1-(4-(methoxycarbonyl)phenyl)-2,4-dioxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate* (**21b**) To a solution of **21a** (6.0 g, 16.7 mmol) in $CH_2Cl_2$ (30 mL) was added $NCOSO_2Cl$ (4.0 g, 28.4 mmol) and stirred at 0°C for 20 mins. The reaction was quenched by the addition of water (10 mL), 15% aqueous hydrochloric acid solution (15 mL) and EtOH (40 mL). The mixture was refluxed for 30 minutes before the solvents were removed under reduced pressure. THF (50 mL) was added to the residue, and the pH was adjusted to 11 by the addition of 15% aqueous

potassium carbonate solution. $(Boc)_2O$ (6.2g, 28.4 mmol) in THF (50 mL) was then added. After stirring at RT for 3 hours, the solvent was removed under reduced pressure and the residue was purified using silica gel column chromatography (5:1 petroleum ether:EtOAc) to afford **21b** (4.0 g, 60%, 2 steps) as a white solid. LCMS (ESI): *m/z* 404 $(M+1)^+$.




**21c**

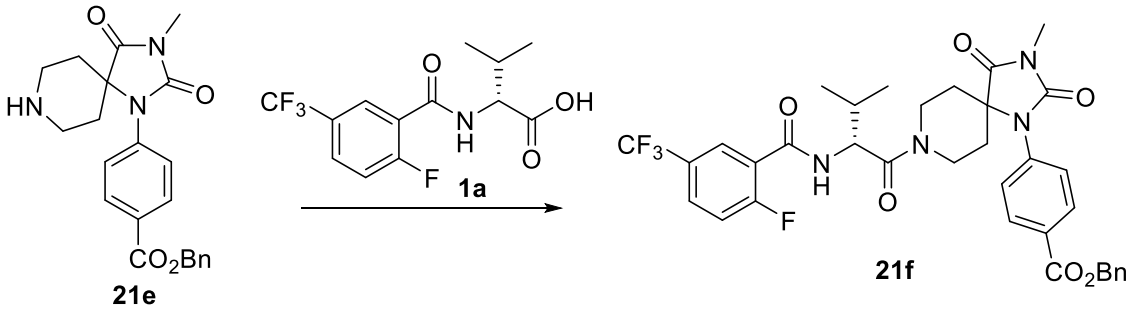
*tert-butyl 1-(4-(methoxycarbonyl)phenyl)-3-methyl-2,4-dioxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate* (**21c**) To a solution of **21b** (3.45 g, 8.56 mmol) in DMF (40 mL), was added NaH (685 mg, 60% in mineral oil, 17.2 mmol) and methyl iodide (1.82 g, 12.8 mmol). The resulting mixture was stirred at RT for 3 hours before it was carefully quenched with water (30 mL). The solution was extracted with EtOAc (10 mL, 4 times), and the combined organic layer was washed with water, brine (30 mL), dried over $Na_2SO_4$ and filtered. The filtrate was concentrated in vacuo and purified using silica gel chromatography (4:1 petroleum ether:EtOAc) to give **21c** (3.0 g, 84%) as a white solid. LCMS (ESI): *m/z* 418 $(M+1)^+$.




**21d**

*tert-butyl 1-(4-((benzyloxy)carbonyl)phenyl)-3-methyl-2,4-dioxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate* (**21d**) To a solution of **21c** (1.30 g, 3.12 mmol) in MeOH (25 mL), was added 15% aq. NaOH (7 mL). The solution was heated to reflux for 30 mins when LC-MS showed the completion of the reaction. The solvent was removed under reduced pressure and the pH of the residue was adjusted to 3 by the addition of conc. HCl before it was extracted with EtOAc (15 mL, 3 times). The combined organic layer was washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo to give the desired product (1.15 g, crude) as a white solid. LCMS (ESI): *m/z* 404 $(M+1)^+$.

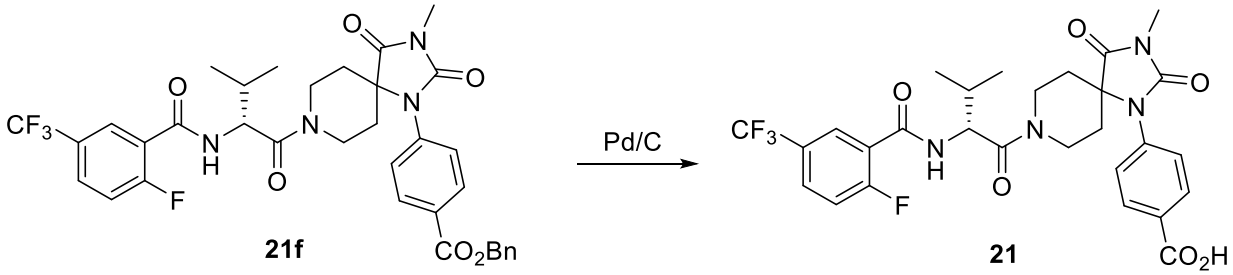
To a solution of the crude product (400 mg, 0.993 mmol) in DMF (6 mL), was added $K_2CO_3$ (274 mg solid, 2.0 mmol) and benzyl bromide (177 mg, 1.29 mmol). The reaction mixture was stirred at RT for 3 hours before it was quenched with water, and extracted with EtOAc (10 mL, 3 times. The combined organic layer was washed with water, brine (10 mL), dried over $Na_2SO_4$ and filtered. The filtrate was concentrated in vacuo and purified using silica gel chromatography (5:1 petroleum ether:EtOAc) to give **21d** (500 mg, 99%) as a white solid. LCMS (ESI): *m/z* 494 $(M+1)^+$.




*benzyl 4-(8-((2-fluoro-5-(trifluoromethyl)benzoyl)-D-valyl)-3-methyl-2,4-dioxo-1,3,8-triazaspiro[4.5]decan-1-yl)benzoate* (**21f**) To a 100 mL round bottom flask, was added **21d** (120 mg, 0.243 mmol) and HCl in dioxane (4M, 4 mL). The mixture was stirred at RT for 30 mins

when LC-MS showed the completion of the reaction, and was concentrated and dried in vacuo. The resulting **21e** was used for the next step without further purification.

To the residue, was added (2-fluoro-5-(trifluoromethyl)benzoyl)-D-valine (**1a**, 76 mg, 0.243 mmol), DCM (3 mL), HATU (139 mg, 0.364 mmol) and DIPEA (95 mg, 0.729 mmol). The resulting mixture was stirred at RT for 2 hours when LC-MS showed the completion of the reaction. The solution was quenched with water (5 mL), and extracted with DCM (10 mL, 3 times). The combined organic layer was washed with brine, dried over anhydrous $Na_2SO_4$ and filtered. The filtrate was concentrated and purified using prep-TLC to give the desired product **21f** as a colorless oil (150 mg, 91%). LCMS (ESI): *m/z* 683 $(M+1)^+$.




*4-(8-((2-fluoro-5-(trifluoromethyl)benzoyl)-D-valyl)-3-methyl-2,4-dioxo-1,3,8-triazaspiro[4.5]decan-1-yl)benzoic acid* (**21**) To a solution of **21f** (150 mg, 0.219 mmol) in MeOH (40 mL), was added Pd/C (10%, 20 mg). The mixture was stirred under $H_2$ atmosphere at RT for 20 mins. Pd/C was filtered off, and the filtrate was concentrated and purified using prep-HPLC to give the desired product **21** (140 mg, 61% yield) as a white solid.

**NMR spectra and LCMS profile**

**Figure S1.** $^{1}$H NMR of Compound **1**

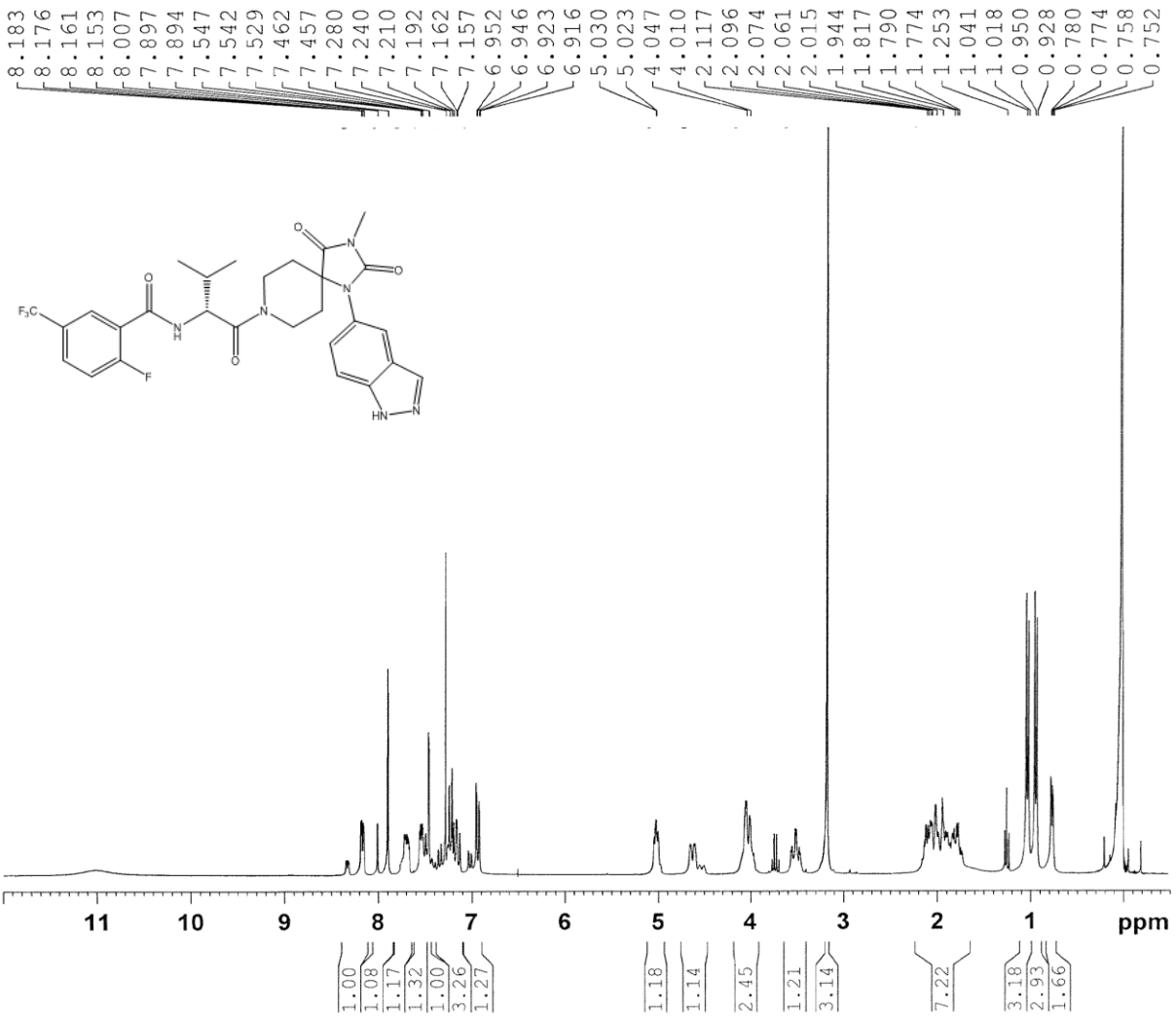


**Figure S2.** $^{13}C$ NMR of Compound **1**

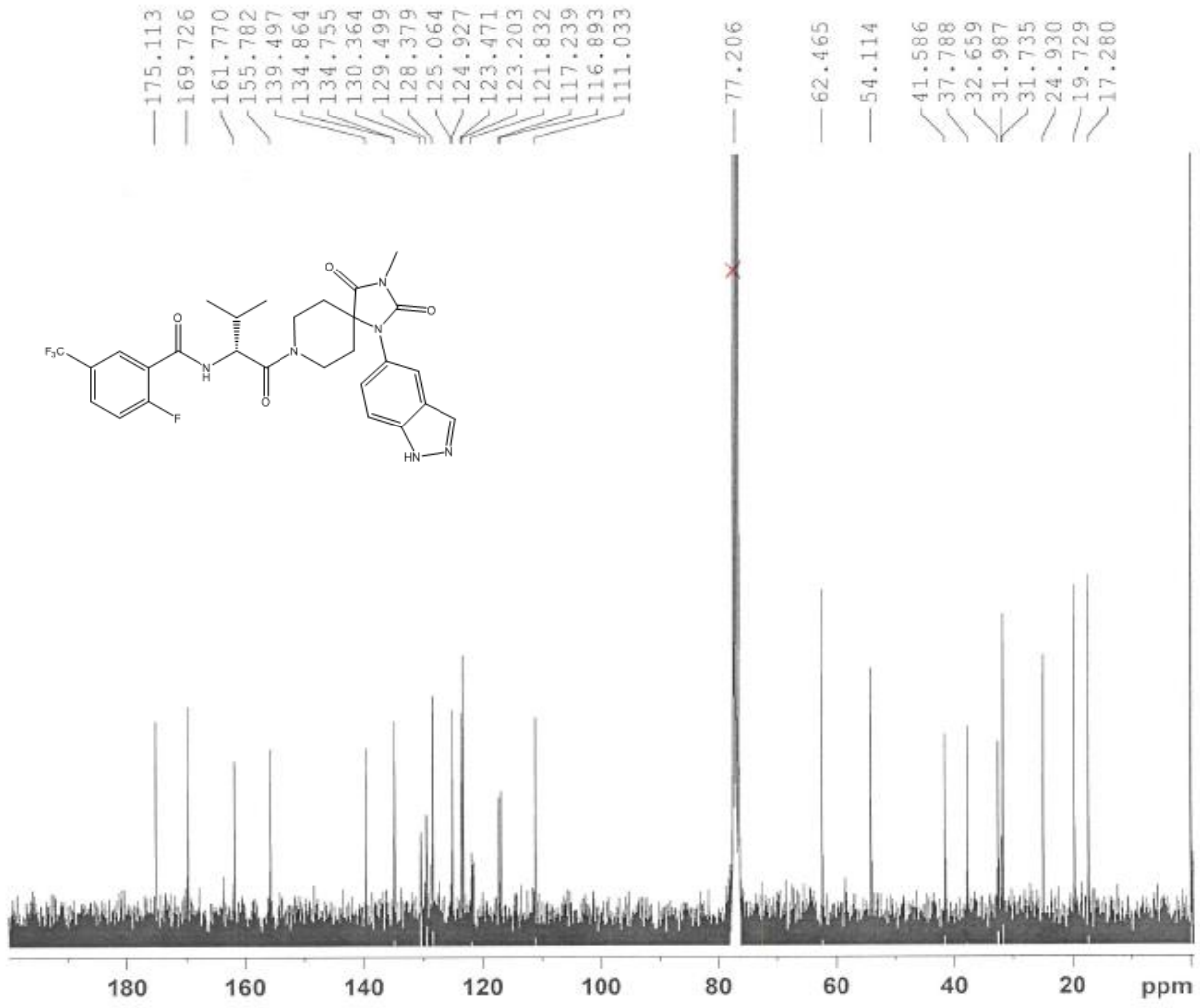
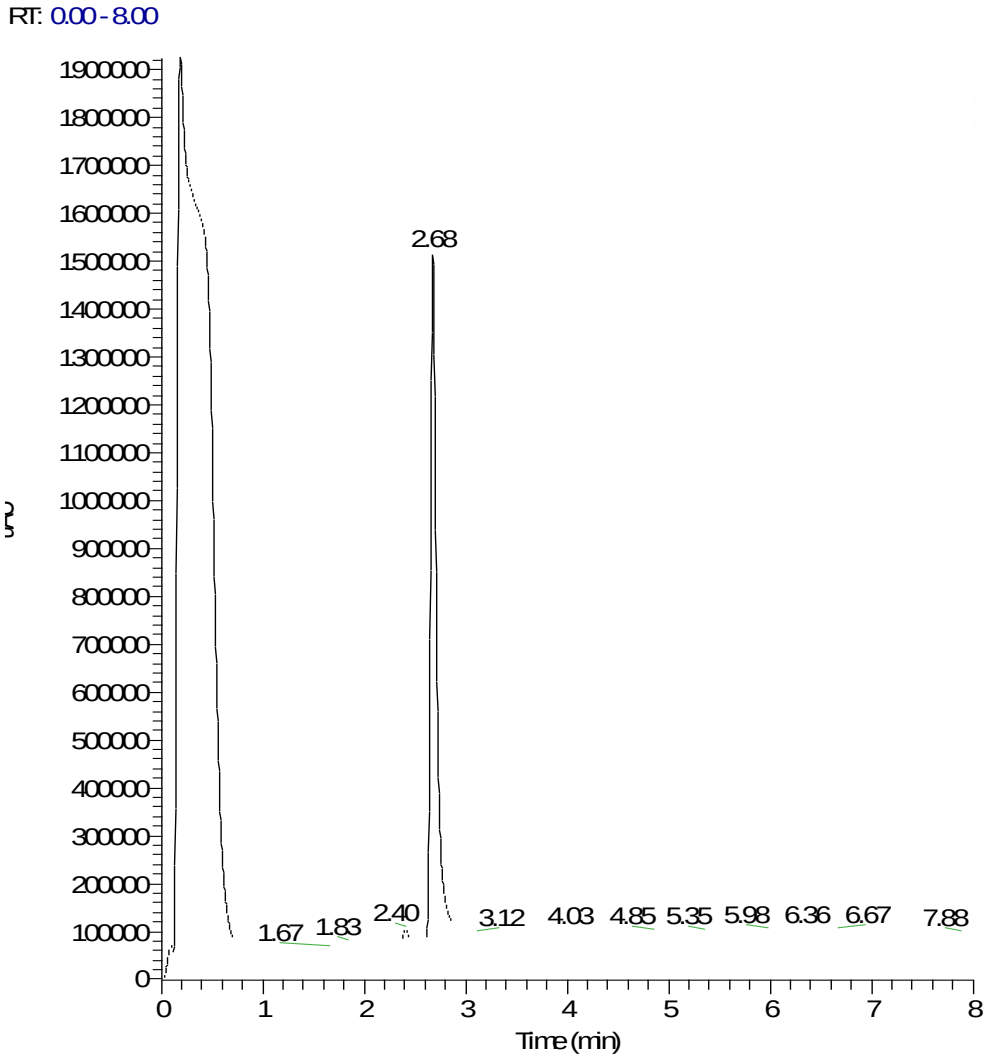
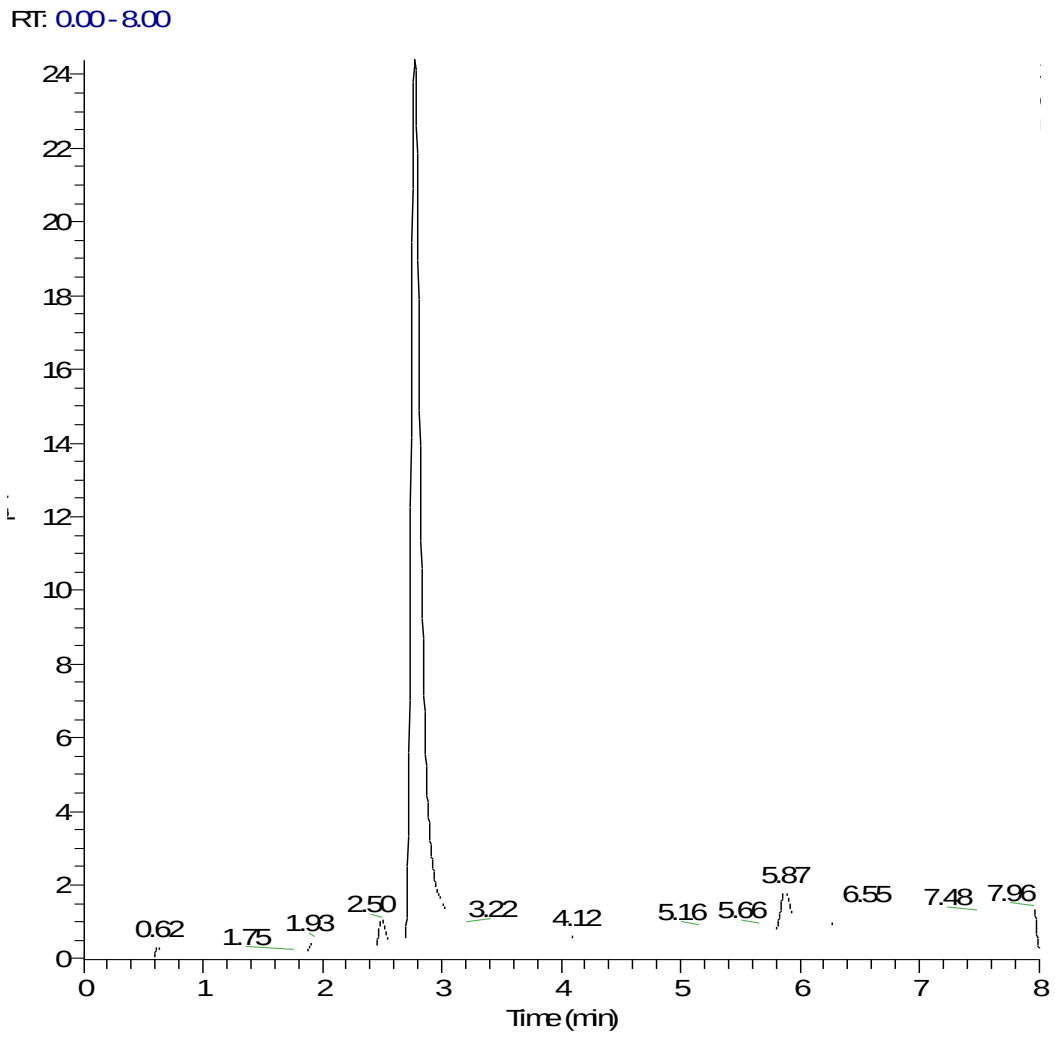
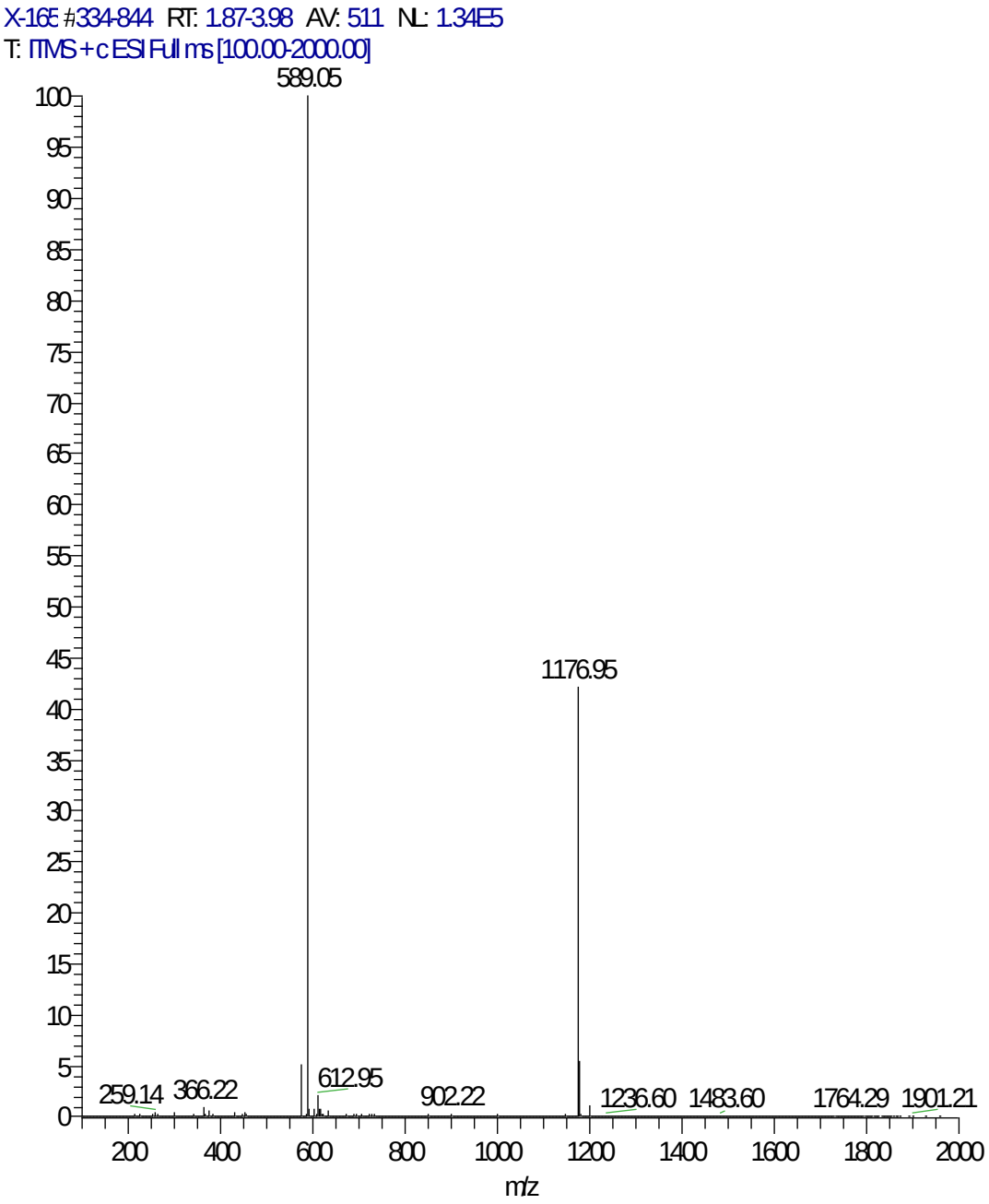


**Figure S3.** Compound **1** LCMS (*Thermo UltiMate 3000* instrument, *Phenomenex* XB-C18 2.6 µM 2.1 x 30 mm column, mobile phase A: 99.9 water/0.1 formic acid; mobile phase B: 99.9 acetonitrile/0.1 formic acid. Elution was effective via a 7.0 min gradient beginning at 90:10 A/B and ending with 100% B, then holding at 100% B for 1.0 min) with compound identified by UV = 212 nM and ESI positive ion mass spectrometry.




RT: 0.00 - 8.00
24
22
20
18
16
14
12
10
8
6
4
2
0
0.62
1.75
1.93
2.50
3.22
4.12
5.16
5.66
5.87
6.55
7.48
7.96
0
1
2
3
4
5
6
7
8
Time (min)

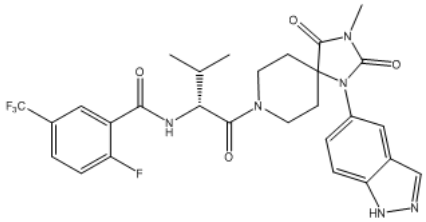



Compound **1**

Chemical Formula: $C_{28}H_{28}F_4N_6O_4$

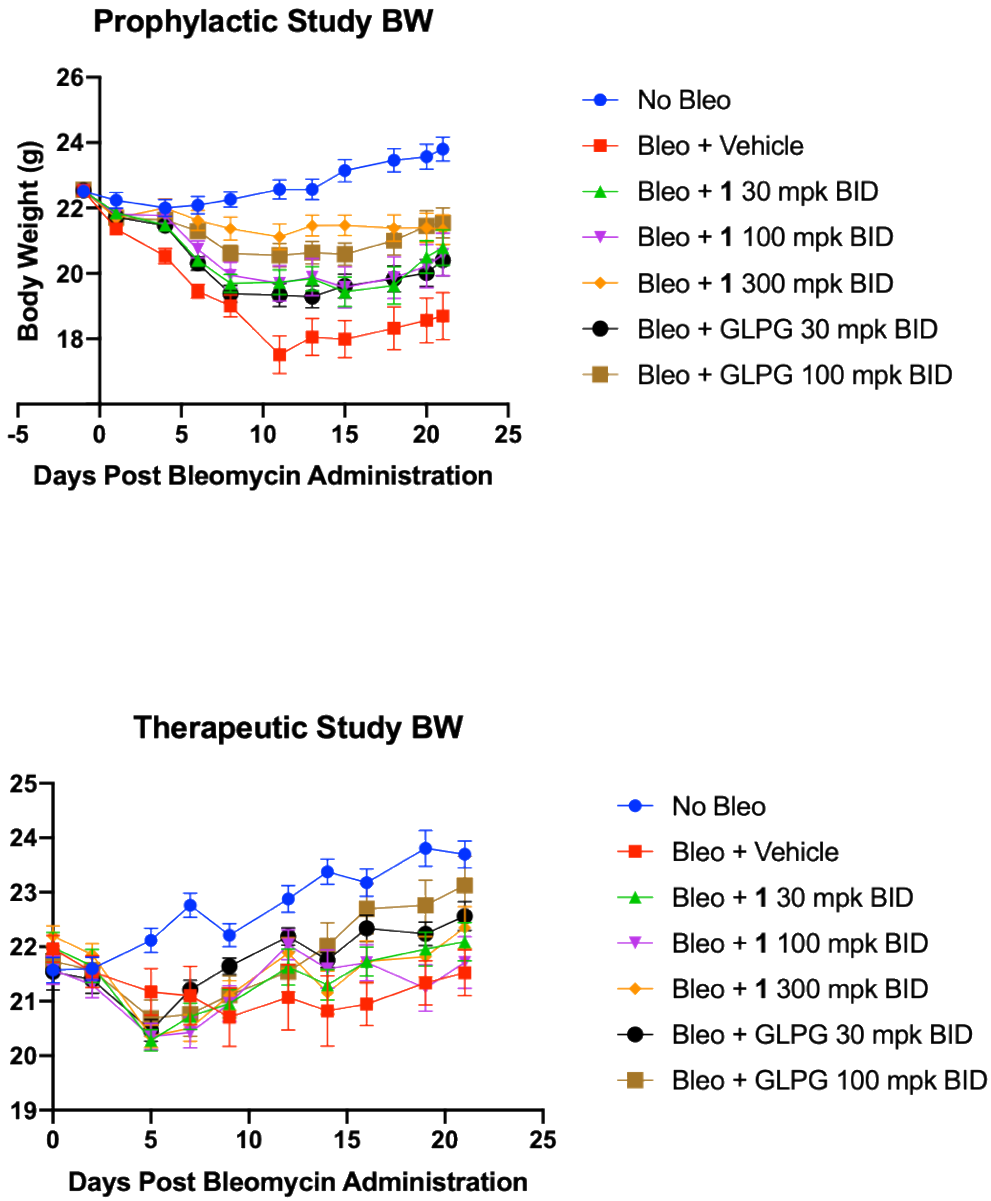
Exact Mass: 588.21

Molecular Weight: 588.56



**Animal model supplemental data**

Figure S4. Body weights of mice over the course of the prophylactic and therapeutic 21 day bleomycin studies (Mean ± SEM).



**Cocrystallization protocol**

Human autotaxin was produced in HEK293 cells as described in the manuscript and was used in crystallization trials employing both a standard screen with approximately 1200 different conditions as well as crystallization conditions identified using literature data. Conditions initially obtained were optimized using standard strategies, systematically varying parameters critically influencing crystallization, such as temperature, protein concentration, drop ratio, and others. These conditions were also refined by systematically varying pH or precipitant concentrations. A cryo-protocol was established using PROTEROS Standard Protocols. Crystals were flash-frozen and measured at a temperature of 100 K. The X-ray diffraction data were collected from complex crystals of hATX with the ligand X-165 at the SWISS LIGHT SOURCE (SLS, Villigen, Switzerland) using cryogenic conditions. The structure was solved and refined to a final resolution of 1.98 Å. The crystals belong to space group P 21. Data were processed using the programs XDS and XSCALE. The phase information necessary to determine and analyze the structure was obtained by molecular replacement. A previously solved structure of hATX was used as a search model. Subsequent model building and refinement was performed according to standard protocols with the software packages REFMAC5 and COOT. The ligand parameterization and generation of the corresponding library files were carried out with CORINA.