Supporting information

Solid phase methods for the preparation of epoxysuccinate-based inhibitors of cysteine proteases

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Experimental Section

General methods. Unless otherwise noted, all resins and reagents were purchased from commercial suppliers and used without further purification. All solvents used were of HPLC grade. Reverse-phase HPLC was conducted on a C18 column using an ÄKTA explorer 100 (Amersham Pharmacia Biotech). LCMS data were acquired using a API 150EX LC/MS system (Applied Biosystems).

General solid phase synthesis method. Solid phase reactions were conducted in polypropylene cartridges (Applied Separations, Allentown, PA) with 3-Way Nylon Stopcocks (BioRad Laboratories, Hercules, CA). The cartridges were connected to a 20 port vacuum manifold (Waters, Milford, MA) that was used to drain solvent and reagents from the cartridge. The resin was gently shaken on a rotating shaker during solid-phase reactions.

Method A:

Safety Catch resin. The desired Fmoc-protected amino acid (3 eq.; relative to resin loading indicated by supplier) and DIEA (6 eq.) in DCM (0.5 M with respect to the amino acid) were added to the resin. The mixture was cooled at -20° C for 20 minutes. Next, PyBop (3 eq.) was added to the reaction. After shaking at -20° C for 8 hours, the reaction mixture was drained and the resin was washed with DCM (3x). Fmoc quantification revealed a loading of 0.6 mmol/g of the first amino acid onto the resin. After Fmoc deprotection using 20% piperdine in DMF (15 min) and wash with DMF (3x), the epoxysuccinate **6** (3eq.; relative to resin loading), PyBop (3eq.) and DIEA (6 eq.) in DMF (0.375 M with respect to **6**) were added to the resin. The reaction mixture

was shaken for 3.5 hours. After wash with DMF (3x), and DCM (3x), tyramine (3eq.) was coupled onto the epoxysuccinate using PyBop and DIEA as described in the previous step.

Cleavage from the Safety Catch resin and purification. A 1 M solution of DIEA (5 eq.) in anhydrous NMP was added to the resin. Next, iodoacetonitrile (20 eq.; filtered through a basic alumina plug) was added and the reaction mixture was shaken in the dark for 24 h. After the activation process, the resin was washed with NMP (5x 10 min.), DCM (3x), and a solution of the corresponding amine (5 eq.; 0.5 M in THF) was added. After 4 hours, the solution was collected and the resin was washed with THF. A drop of acetic acid was added to the combined THF layers, after which solvent was removed *in vacuo*. The product was purified by RP-HPLC.

Method B:

BAL resin. The resin was solvated in DMF for 15 minutes. The desired amine for the R^3 position in DMF (0.8 M; 10 eq.; relative to aldehyde resin loading reported by the vendor) was added and the reaction was shaken for 15 minutes. $NaBH_3CN$ (10 eq.) was added and the mixture was shaken for an additional 20 hours. The solution was drained and the resin was washed with DMF (3x), DCM (3x) and MeCN (3x). The desired amino acid (10 eq.), HATU (10 eq.) and DIEA (20 eq.) in DMF (0.2 M with respect to the amino acid) were added to the resin. The reaction was shaken for 2 hours. After washing the resin with DMF (3x) and DCM (3x), Fmoc quantification showed 0.5mmol/g loading of the first amino acid on the resin. After Fmoc deprotection using 20% piperdine in DMF and wash with DMF (3x) and DCM (3x), epoxysuccinate **5** (3eq.; 0.3 M in DMF) was added. After 2 hours, the resin was washed as described above and the resin was

treated with 1 M KOH in ethanol/THF, 1/3, for 3 hours. Biphenylethylamine (3eq.) was coupled to the epoxysuccinate using PyBop and DIEA as described in method A.

Cleavage from the BAL resin and purification. A solution of 95% TFA/2.5% TIS/2.5% H_2O was added to the resin. After standing for 1h, the cleavage mixture was collected, and the resin was washed with the fresh cleavage solution. The combined fractions were evaporated to dryness and the product was purified by RP-HPLC. Fractions containing product were pooled and lyophilized.

Inhibitor Evaluation. 25µl of rat liver homogenate (0.8 mg/ml) in reaction buffer (50mM sodium acetate, 2 mM DTT, 5 mM $MgCl_2$, pH 5.5) was incubated for 20 minutes with the inhibitors at indicated concentrations. Subsequently, 1 µl of radiolabeled ^{125}I-DCG-04 (10^6 cpm) was added and the samples were incubated for 1 hour. Proteins were resolved by SDS-PAGE and labeled cathepsin activities were visualized by autoradiography.

AMS17: Synthesized on safety catch resin according to the above described protocol. 12% yield. ESI-MS: *m/z* 469.3 $[M+H]^+$ ^{1}H NMR: (500 MHz, dmso-d_6): δ 9.22 (bs, 1H), 8.62 (s, 1H), 8.56 (d, 1H, *J* = 8.2 Hz), 8.45 (t, 1H, *J* = 5.6 Hz), 8.20 (t, 1H, *J* = 5.6 Hz), 8.11 (bs, 1H), 7.50 (bs, 2H), 6.99 (d, 2H, *J* = 8.4 Hz), 6.80 (d, 2H, *J* = 8.4 Hz), 4.25- 4.20 (m, 1H), 3.57 (d, 1H, *J* = 1.8 Hz), 3.49-3.29 (m, 3H), 3.28-3.23 (m, 2H), 2.30- 2.97 (m, 2H), 2.62- 2.53 (m, 2H), 1.49- 1.33 (m, 3H), 0.84 (d, 3H, *J* = 6.6 Hz), 0.80 (d, 3H, *J* = 6.6 Hz). HRMS: found $[M+H]^+$ 469.2424. $C_{25}H_{33}N_4O_5^+$ requires 469.2451.

AMS20: Synthesized on safety catch resin according to the above described protocol. 13% yield. ESI-MS: *m/z* 504.4 $[M+H]^+$ ^{1}H NMR: (500 MHz, dmso-d_6): δ 8.69-8.62 (m,

2H), 8.46 (t, 1H, *J* = 5.5 Hz), 8.06-8.01 (m, 1H), 7.96-7.94 (m, 1H), 7.85 (d, 1H, *J* = 8.1 Hz), 7.58-7.52 (m, 2H), 7.47 (t, 1H, *J* = 7.5 Hz), 7.42 (d, 1H, *J* = 7.0 Hz), 6.99 (d, 2H, *J* = 8.3 Hz), 6.68 (d, 2H, *J* = 8.3 Hz), 4.73 (d, 2 H, *J* = 5.6 Hz), 4.44-4.37 (m, 1H), 3.61 (d, 1H, *J* = 1.6 Hz), 3.49 (d, 1H, *J* = 1.6 Hz), 3.31-3.20 (m, 2H), 2.62 (t, 2H, *J* = 7.4 Hz), 1.62-1.52 (m, 2H), 1.51-1.44 (m, 1H), 0.88 (d, 3H, *J* = 6.2 Hz), 0.82 (d, 3H, *J* = 6.2 Hz). HRMS: found $[M+H]^+$ 504.2561. $C_{29}H_{33}N_3O_5^+$ requires 504.2420.

AMS27: Synthesized on safety catch resin according to the above described protocol. 20% yield. ESI-MS: *m/z* 420.3 $[M+H]^+$ ^{1}H NMR: (500 MHz, dmso-d_6): δ 9.2 (s, 1H), 8.56 (d, 1H, *J* = 8.1 Hz), 8.44 (t, 1H, *J* = 5.5 Hz), 8.09-8.05 (m, 1H), 6.98 (d, 2H, *J* = 8.2 Hz), 6.67 (d, 2H, *J* = 8.3 Hz), 4.35-4.31 (m, 1H), 3.59 (d, 1H, *J* = 1.78 Hz), 3.46 (d, 1H, *J* = 1.70 Hz), 3.27- 3.22 (m, 2H), 2.93-2.78 (m, 2H), 2.64-2.59 (m, 2H), 1.69-1.64 (m, 1H), 1.56-1.50 (m, 2H), 1.49-1.40 (m, 3H), 0.90-0.81 (m, 12H). HRMS: found $[M+H]^+$ 420.2565. $C_{22}H_{34}N_3O_5^+$ requires 420.2498.

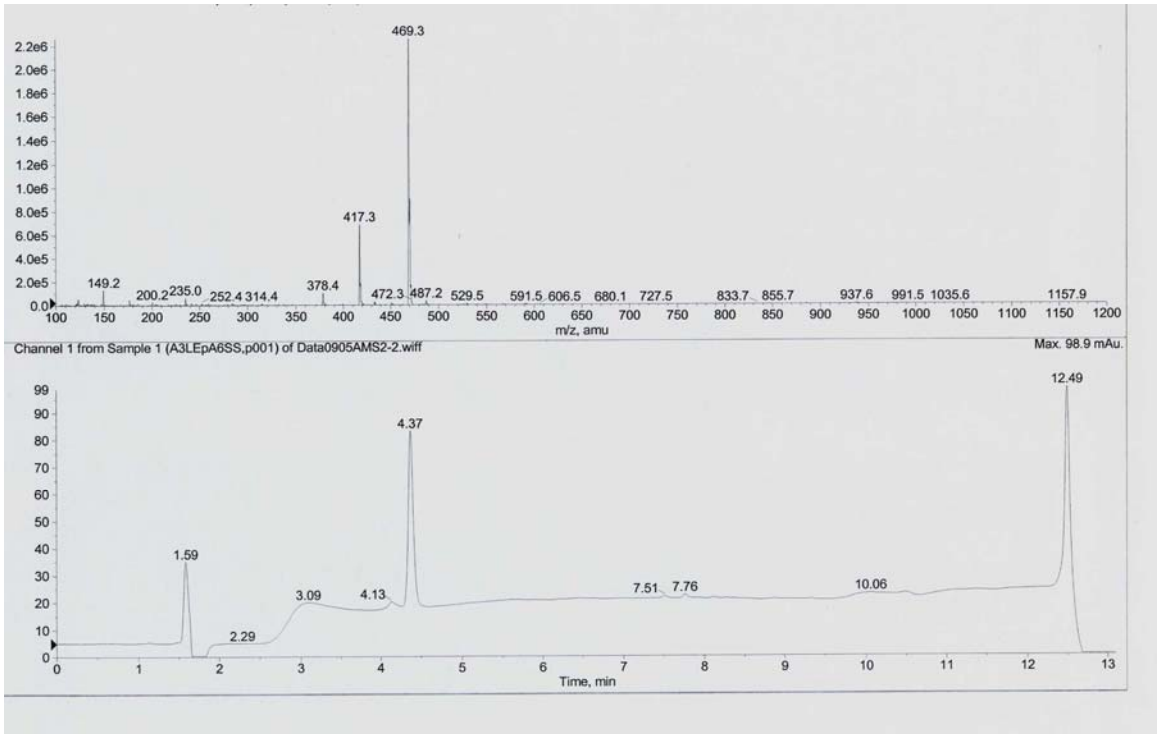
AMS33: Synthesized on the BAL resin according to the above described protocol. 5% yield. ESI-MS: *m/z* 550.3 $[M+H]^+$ ^{1}H NMR: (500 MHz, dmso-d_6): δ 8.67-8.63 (m, 2H), 8.55 (t, 1H, *J* = 5.9 Hz), 8.05-8.02 (m, 1H), 7.97-7.94 (m, 1H), 7.85 (d,1H, *J* = 7.9 Hz), 7.65 (d, 2H, *J* = 8.4 Hz), 7.61 (d, 2H, *J* = 8.1 Hz), 7.55-7.53 (m, 2H), 7.49-7.42 (m, 4H), 7.36-7.30 (m, 3H), 4.80-4.68 (m, 2H), 4.38-4.33 (m,1H), 3.65 (d, 1H, *J* = 1.7 Hz), 3.51 (d, 1H, *J* = 1.8 Hz), 2.79 (t, 2H, *J* = 7.3 Hz), 1.65-1.56 (m, 2H), 1.32-1.24 (m, 2H), 0.84 (t, 3H, *J* = 7.3 Hz). HRMS: found $[M+H]^+$ 550.2486. $C_{34}H_{36}N_3O_4^+$ requires 550.2706.

AMS34: Synthesized on the BAL resin according to the above described protocol. 42% yield. ESI-MS: *m/z* 364.4 $[M+H]^+$ ^{1}H NMR: (500 MHz, dmso-d_6): δ 8.65 (s, 1H), 8.6 (d, 1H, *J* = 8.1 Hz), 8.20 (t, 1H, *J* = 5.6 Hz), 8.06 (bs, 1H), 7.53 (bs, 2H), 4.22-4.14 (m, 3H),

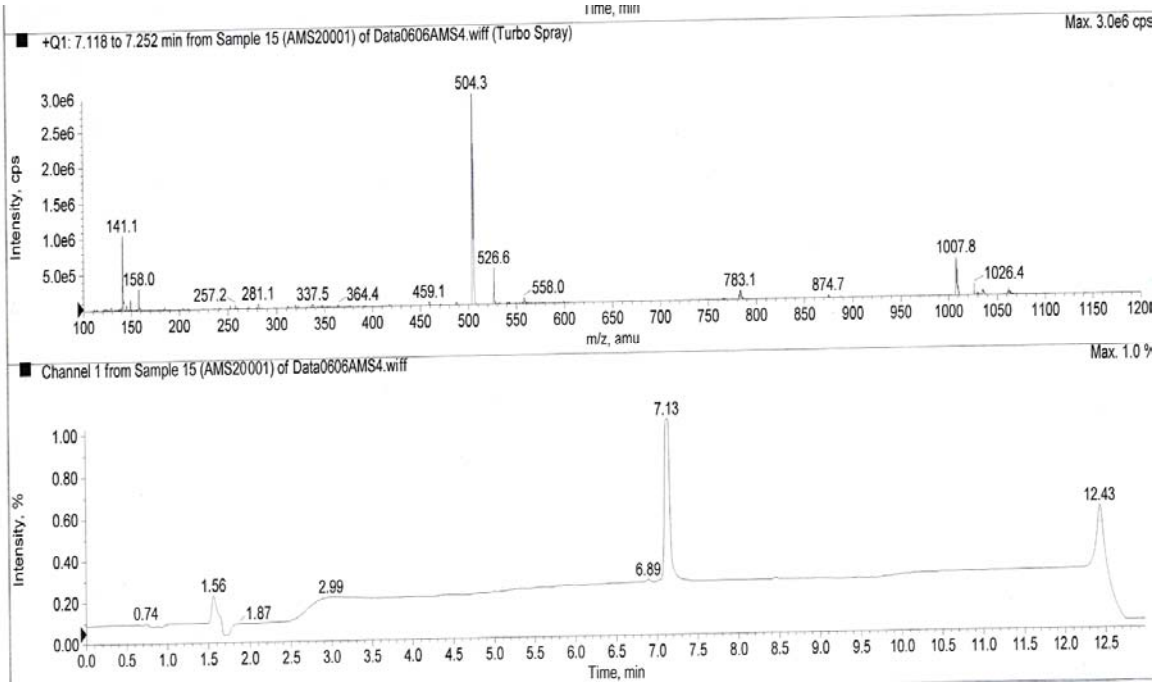
3.72 (d, 1H, *J* = 1.7 Hz), 3.59 (d, 1H, *J* = 1.8), 3.53-3.49 (m, 1H), 3.42-3.38 (m, 1H), 2.98 (t, 2H, *J* = 6.5 Hz), 1.55-1.49 (m, 1H), 1.47-1.42 (m, 1H), 1.23 (t, 3H, *J* = 7.1 Hz), 1.20-1.13 (m, 2H), 0.82 (t, 3H, *J* = 7.3 Hz). HRMS: found $[M+H]^+$ 364.1799. $C_{18}H_{26}N_3O_5^+$ requires 364.1872.

AMS35: Synthesized on the BAL resin according to the above described protocol. 37% yield. ESI-MS: *m/z* 371.1 $[M+H]^+$ ^{1}H NMR: (500 MHz, dmso-d_6): δ 8.68 (d, 1H, *J* = 8.1 Hz), 8.65 (t, 1H, *J* = 5.5 Hz) 8.05-8.00 (m, 1H), 7.98-7.94 (m, 1H), 7.85 (d, 1H, *J* = 7.8 Hz), 7.59-7.54 (m, 2H), 7.50-7.45 (m, 1H) 7.43 (t, 1H, *J* = 5.3 Hz), 4.78-4.70 (m, 2H), 4.38-4.34 (m, 1H), 3.70 (d, 1H, *J* = 1.8 Hz), 3.47 (d, 1H, *J* = 1.8 Hz), 1.67-1.50 (m, 3H), 1.30- 1.23 (m, 2H), 0.85-0.79 (m, 3H). HRMS: found $[M+H]^+$ 371.1734. $C_{20}H_{23}N_2O_5^+$ requires 371.1607.

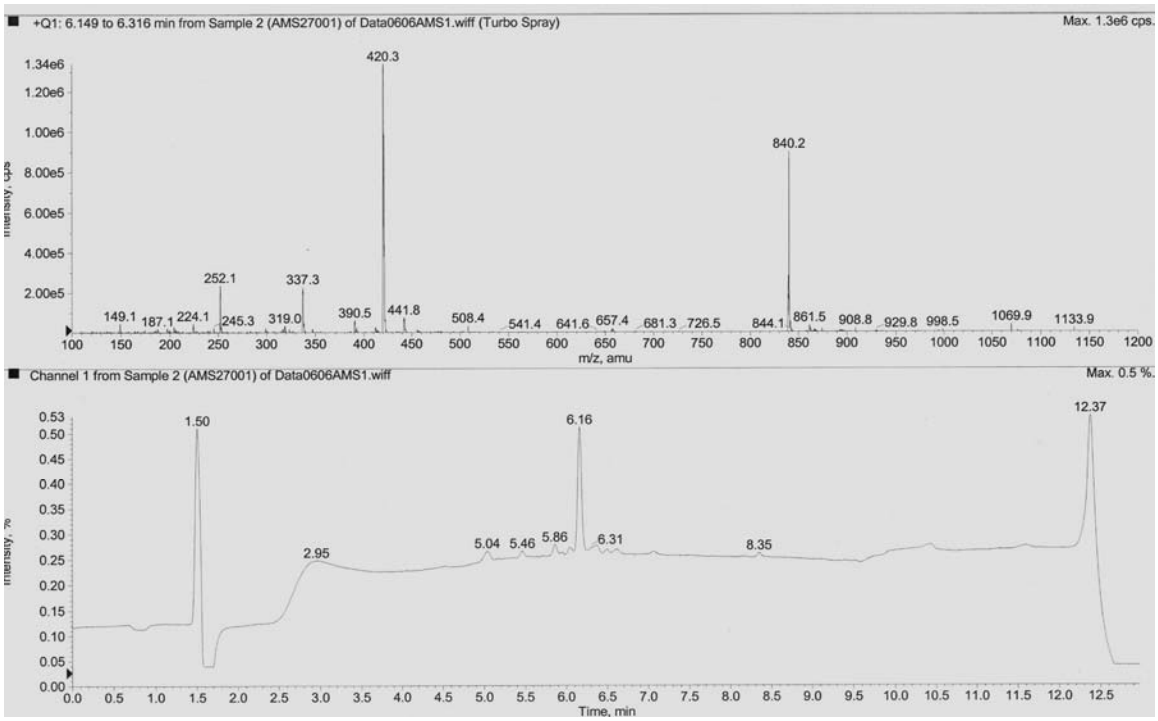
LC-MS spectrum of AMS17



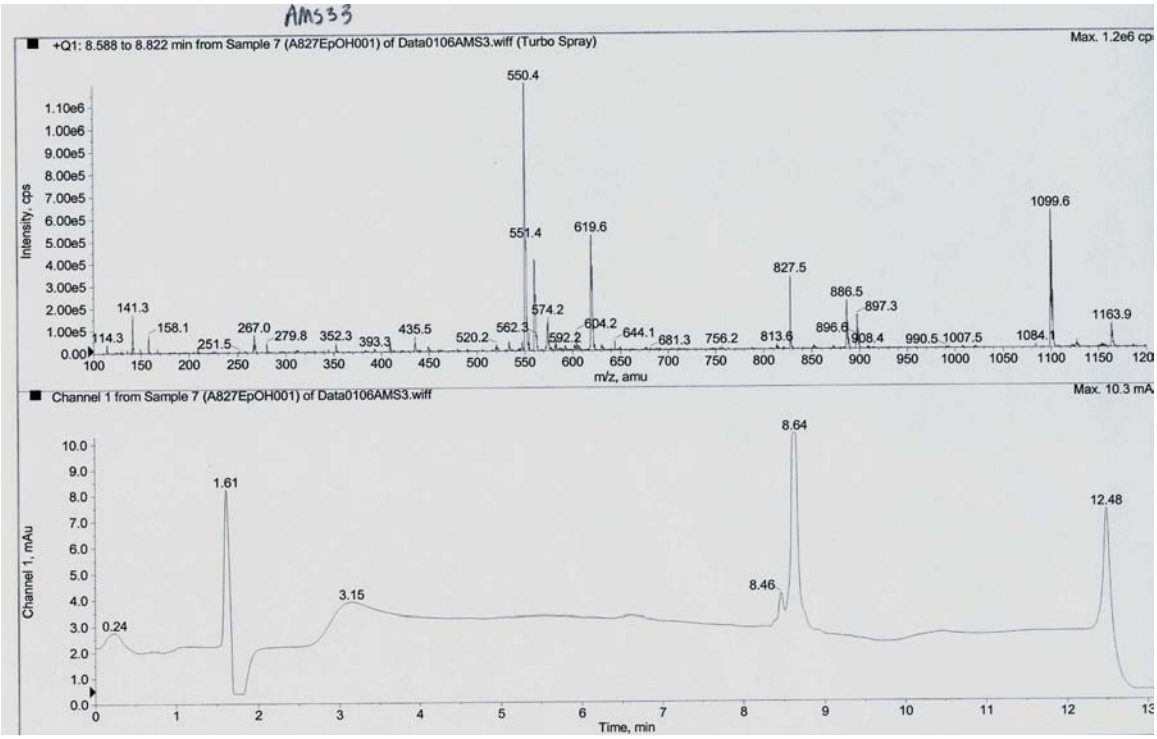
LCMS spectrum of AMS20



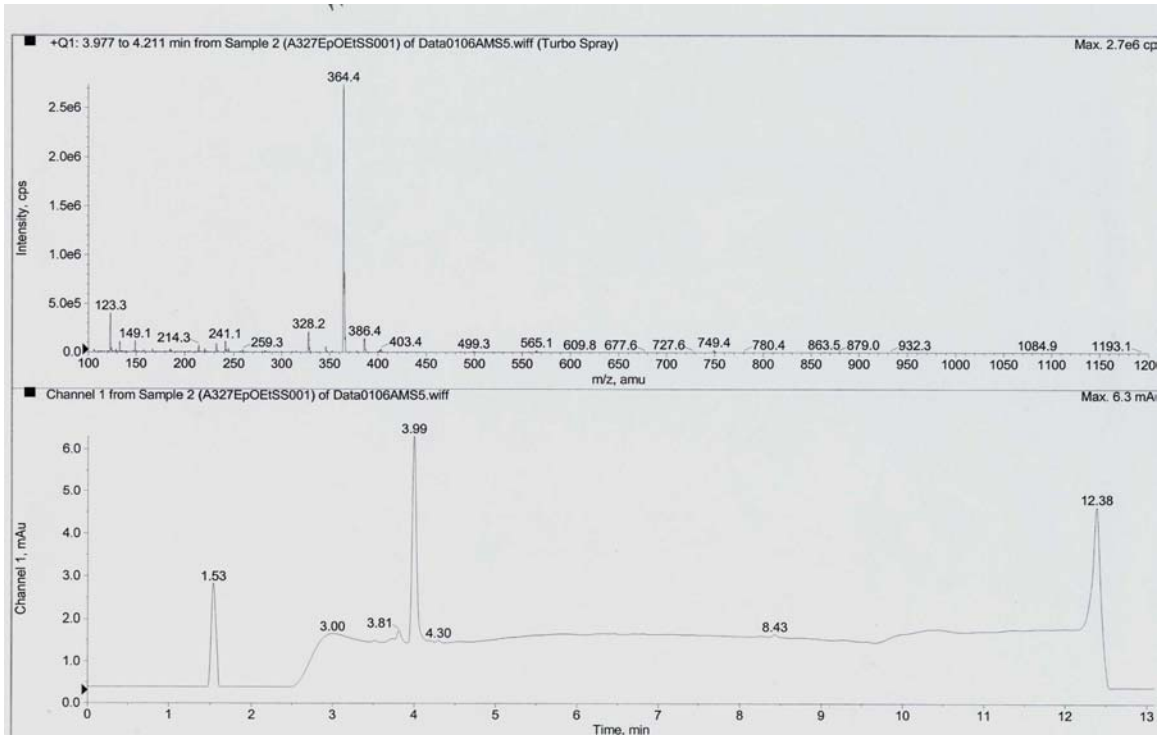
LCMS spectrum of AMS27



LCMS spectrum of AMS33



LCMS spectrum of AMS34



LCMS spectrum of AMS35