Efficient Synthesis of 3-Amino-cyclobut-2-en-1-ones: Squaramide Surrogates as Potent VLA-4 Antagonists

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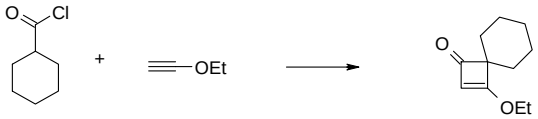
Supporting Information

General. All manipulations were performed under a dry nitrogen atmosphere using Sureseal™ solvents purchased from Aldrich chemical company. All commercially available chemicals were used without further purification. Flash chromatographic separations were performed using 230-400 mesh silca gel 60. LCMS was performed on a Hewlett-Packard 1100 using a C18 column. All compounds described were >98% by LCMS. ^{1}H NMR spectra were recorded using a Bruker DPX400.

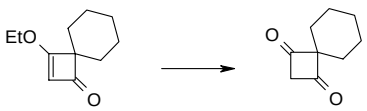
Representative procedure for the cycloaddition of ethoxyacetylene with a ketene. Preparation of 7e.



Name (7e)

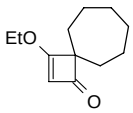
A 500ml two-necked round-bottomed flask fitted with an efficient condenser was charged with diethylether (150ml), cyclohexane carbonyl chloride (11.0g, 75.1mmol) and ethoxyacetylene (21.0g, 50%w/w in hexanes, 150mmol). The stirred solution was then treated dropwise at rt with triethylamine (15.1ml, 112mmol). After 30min the suspension was heated to reflux and stirred for a further 24h. The resulting turbid mixture was allowed to cool prior to removal of triethlammonium chloride by filtration and the filtrate was concentrated *in vacuo* to give a brown oil. Chromatography (SiO_2, 95:5 hexanes:ethyl acetate) gave the title compound as a brown oil (10.9g, 61mmol, 81%). DATA was in accordance with literature.

Representative procedure for the conversion of an 3-alkoxy-cyclobut-2ene-1-one to a cyclobuta-1,3-dione by hydrolysis. Preparation of 8e.



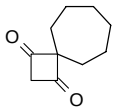
(8e)

A mixture of **7e** (3.2g, 17.8mmol) and 2M hydrochloric acid (25ml) was stirred vigorously at rt for 24h. The resulting solid was recovered by filtration, washed with water and dried in a vacuum oven at 40° to give the title compound as an off-white powder (2.49g, 16.4mmol, 92%).



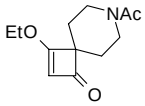
(7f) 3-Ethoxyspiro[3.6]decan-1-one.

A solution of cycloheptyl carbonyl chloride (10.0g, 0.062mol) and ethoxyacetylene (40%w/w solution in hexanes, 6.0g, 0.083mol, 12ml) in diethylether (50ml) was treated dropwise with triethylamine (20ml, 0.14mol) and the reaction stirred for 48h at reflux temperature. Filtration and concentration of the filtrate *in vacuo* followed by chromatography (SiO_2, 5:1 EtOAc:hexanes) gave the title compound as a pale yellow oil (10.5g, 0.054mol, 87%). δH ($CDCl_3$, 300K) 4.78 (1H, s), 4.20 (2H, q J 7.1Hz), 1.94-1.87 (2H, m), 1.83-1.77 (2H, m), 1.71-1.66 (2H, m), 1.63-1.52 (6H, m), 1.45 (3H, t J 7.1Hz). m/z (ES^+, 70V) 194.9 (MH^+).



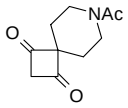
(8f) Spiro[3.6]decane-1,3-dione.

Intermediate **7f** (8.5g, 0.044mol) and 2M hydrochloric acid (30ml) was stirred vigorously for 24h at room temperature. The resulting slurry was extracted with EtOAc (3 x 100ml), the extracts combined and concentrated *in vacuo*, and the resulting solid was recrystallised from diethyl ether to give the title compound as an off-white powder (7.1g, 0.043mol, 95%). δH (DMSO d^6, 300K) 4.58 (2H, s), 1.75-1.29 (12H, m). m/z (ES^+, 70V) 166.9 (MH^+).



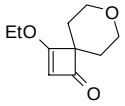
(7g) 7-Acetyl-3-ethoxy-7-azaspiro[3.5]non-2-en-1-one.

A solution of 1-acetyl piperidine-4-carbonyl chloride (5.0g, 26.4mmol) and ethoxyacetylene (4.0g, 55.5mmol) in THF (60ml) was treated dropwise with triethylamine (7.6ml, 55.0mmol). The resulting slurry was stirred at room temperature for 5d prior to filtration and concentration of the filtrate *in vacuo*. Chromatography (SiO_2, 100% EtOAc to 95:5 EtOAc:MeOH) gave the title compound as a white powder (3.97g, 17.8mmol, 67%). δH ($CDCl_3$, 300K) 4.79 (1H, s), 4.17 (2H, q, J 7.1Hz), 3.87-3.81 (1H, m), 3.56-3.42 (3H, m), 2.02 (3H, s), 1.85-1.67 (4H, m), 1.39 (3H, t 7.1Hz). m/z (ES^+, 70V) 223.9 (MH^+).



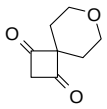
(8g) 7-Acetyl-7-azaspiro[3.5]nonane-1,3-dione.

Intermediate **7g** (700mg, 0.31mmol) and hydrochloric acid (2M, 5ml) were stirred at room temperature for 4h. Concentration of the resulting straw-coloured solution in vacuo gave the title compound as a pale brown water-soluble powder which was used without further purification (535mg, 0.27mmol, 87%). m/z (ES^+, 70V) 195.9 (MH^+).



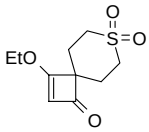
(7h) 3-Ethoxy-7-oxaspiro[3.5]non-2-en-1-one

Tetrahydropyranyl-4-carboxylic acid (14.7g, 0.11mol) and DMF (0.5ml) in DCM (150ml) was treated dropwise with oxalyl chloride (1.1eq, 10.9ml, 0.12mol). After 1h the reaction mixture was concentrated *in vacuo* and the residual slurry was diluted with Et_2O (200ml) and the resulting precipitate removed by filtration. The filtrate was treated with ethoxyacetylene (40%w/w solution in hexanes, 1.3eq, 18ml) followed dropwise with triethylamine (25ml, 0.19mol) and the reaction stirred for 11d at 45ºC. Filtration and concentration of the filtrate *in vacuo* followed by chromatography (SiO_2, 5:1 EtOAc:hexanes) gave the title compound as a pale yellow oil (12.1g, 66.5mmol 59%). δH ($CDCl_3$, 300K) 4.85 (1H, s), 4.23 (2H, q, J 7.1Hz), 3.89-3.75 (4H, m), 1.88-1.79 (4H, m), 1.47 (3H, t, J 7.1Hz). m/z (ES^+, 70V) 182.9 (MH^+).



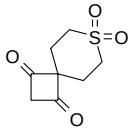
(8h) 7-Oxaspiro[3.5]nonane-1,3-dione

Intermediate **7h** (12.1g, 0.67mol) and 2M hydrochloric acid (26ml) were stirred vigorously for 24h at room temperature. The resulting solution was concentrated to dryness and the residual slurry was washed with Et_2O (25ml) to give the title compound as an off-white powder (8.93g, 58.0mmol, 87%). δH (DMSO d^6, 300K) 4.80 (2H, s), 3.78 (4H, t, J 5.5Hz), 2.62 (4H t J 5.5Hz). m/z (ES^+, 70V) 154.9 (MH^+).



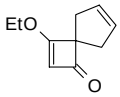
(7i) 3-Ethoxy-7,7-dioxo-7λ^6-thia-spiro[3.5]non-2-en-1-one

A solution of 1,1-dioxo-hexahydro-1λ^6-thiopyran-4-carboxylic acid (10.2g, 57.3mmol) [Prepared according to the procedure of *Org. Prep. Proc. Int.* 1977, 94] and DMF (0.3ml) in DCM (120ml) at rt, was treated dropwise with oxalyl chloride and the resulting slurry stirred for 3d. The crude reaction was then concentrated *in vacuo* to give an oil which was redissolved in THF (100ml), treated with ethoxyacetylene (10ml, 50%w/w) and triethylamine (10ml) and the resulting slurry stirred for 10d at rt. Filtration and concentration of the filtrate *in vacuo* gave a crude oil which was purified by chromatography (SiO2, 30% EtOAc:hexanes) to give the title compound as a yellow oil (8.9g, 38.6mmol, 67%). δH ($CDCl_3$, 300K) 4.88 (1H, s), 4.27 (2H, q, J 7.1Hz), 3.44-3.37 (2H, m), 3.13-3.05 (2H, m), 2.47-2.40 (2H, m), 2.35-2.29 (2H, m), 1.48 (3H, t, J 7.1Hz). m/z (ES^+, 70V) 230.9 (MH^+).



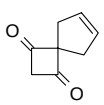
(8i) 3-Hydroxy-7,7-dioxo-7λ^6-thia-spiro[3.5]non-2-en-1-one

Intermediate **7i** (8.6g, 37.4mmol) was stirred with 1M HCl (100ml) for 3d and the resulting solution concentrated *in vacuo*. The residual solid was triturated with EA to give the title compound as an off-white water soluble solid which was used without further purification (5.1g, 25.2mmol, 68%). m/z (ES^+, 70V) 202.9 (MH^+).



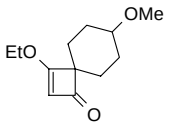
(7j) 3-Ethoxy-spiro[3.4]octa-2,6-dien-1-one

Cyclopent-3-ene carboxylic acid (4.0g, 36.0mmol) and DMF (0.25ml) in DCM (30ml) was treated dropwise with oxalyl chloride (3.5ml, 39.0mmol). After 2h at rt the reaction mixture was concentrated *in vacuo* and the residual slurry was diluted with Et_2O (100ml) and the resulting precipitate removed by filtration and concentrated *in vacuo*. The residual oil was diluted with diethylether (50ml), treated with ethoxyacetylene (40%w/w solution in hexanes, 10ml) followed dropwise with triethylamine (6ml, 44.0mmol) and the reaction stirred for 7d at 45ºC. Filtration and concentration of the filtrate *in vacuo* followed by chromatography (SiO_2, 5:1 EtOAc:hexanes) gave the title compound as a pale yellow oil (4.3g, 26.2mmol, 73%). m/z (ES^+, 70V) 164.9 (MH^+).



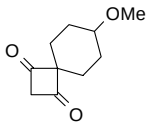
(8j) 3-Hydroxy-spiro[3.4]octa-2,6-dien-1-one

Intermediate **7j** (2.0g, 12.0mmol) and 2M hydrochloric acid (5ml) were stirred vigorously for 24h at room temperature. The resulting solution was extracted with EtOAc (25ml), the extracts dried (MgSO4) and concentrated *in vacuo* to give the title compound as a pale brown powder (1.07g, 7.9mmol, 65%). δH (DMSO d^6, 300K) 5.54 (4H, s), 4.57 (2H, s), 2.52 (2H, s). m/z (ES^+, 70V) 136.9 (MH^+).



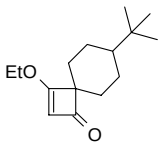
(7k) 3-Ethoxy-7-methoxyspiro[3.5]non-2-en-1-one

Was prepared from 4-methoxy cyclohexanecarbonyl chloride (10g, 52.1mmol) and ethoxyacetylene (7.5g, 0.10mol) according to the method of **7e** to give the title compound as an approx. 1:1 mixture of isomers, as a pale yellow oil (7.2g, 34.4mmol, 65%). δH ($CDCl_3$, 300K) 4.81-4.79 (1H, s), 4.22-4.20 (2H q, J 7.1Hz), 3.34-3.32 (3H, s), 3.31-3.22 (1H, m), 2.04-1.56 (8H, m), 1.44-1.43 (3H t, J 7.1Hz). m/z (ES^+, 70V) 211.0 (MH^+).



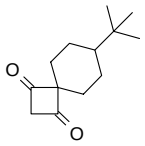
(8k) 7-Methoxyspiro[3.5]nonane-1,3-dione.

Intermediate **7k** (5.0g, 23.9mmol) and 2M hydrochloric acid (20ml) were stirred at room temperature for 18h. The resulting slurry was then diluted with water (50ml) and extracted with EtOAc (3 x 25ml), the extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Recrystallisation from diethylether gave the title compound as an off-white powder (4.06g, 22.4mmol, 94%). δH ($CDCl_3$, 300K) 3.81 (2H, s), 3.25 (4H, m) 1.96-1.90 (2H, m), 1.86-1.79 (2H, m), 1.73-1.66 (2H, m), 1.64-1.56 (2H, m). m/z (ES^+, 70V) 182.9 (MH^+).



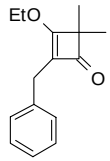
(7l) 3-Ethoxy-7-*tert*butyl[3.5]non-2-en-1-one

Was prepared from 4-*tert*butyl cyclohexanecarbonyl chloride (7.5g, 37.0mmol) and ethoxyacetylene (14.0g, 0.2mol) according to the method of **7e** for 96h to give the title compound as an approx. 1:1 mixture of isomers, as a viscous yellow oil (7.34g, 31.1mmol, 84%). m/z (ES^+, 70V) 2 peaks at 237.0 (MH^+).



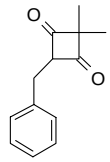
(8l) 7-*tert*Butyl-spiro[3.5]nonane-1,3-dione.

Intermediate **7l** (7.1g, 30.0mmol) and 2M hydrochloric acid (75ml) were stirred at room temperature for 3d. The resulting slurry was then diluted with water (50ml) and the residue collected by filtration, dried *in vacuo* to give the title compound as an off-white powder (5.64g, 27.1mmol, 90%). δH ($CDCl_3$, 300K) 3.78 (2H, s), 2.03-1.99 (2H, m), 1.69-1.65 (2H, m), 1.44 (2H, td, J 3.4Hz, 13.4Hz), 1.30 (2H, qd, J 3.3Hz, 8.4Hz), 0.90 (1H, tt, J 3.3Hz, 11.9Hz), 0.79 (9H, s)m/z (ES^+, 70V) 209.0 (MH^+).



(10a) (+/-) 4-Benzyl-3-ethoxy-4-methyl-2-cyclobuten-1-one

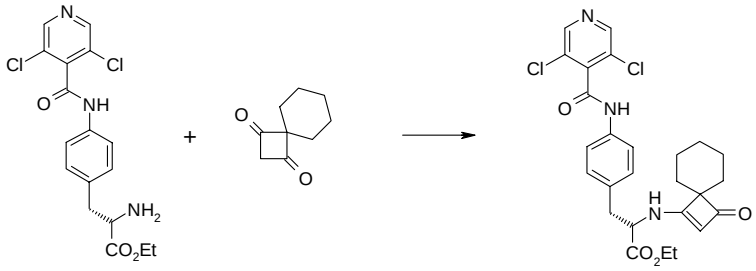
Triethylamine (20ml) was added to a stirred solution containing α-methyl tetrahydro-cinnamoyl chloride (5g, 27.5mmol) and ethyl ethynylether (6g, 40% soln. in hexanes, 85.7mmol) and the resulting slurry heated to 45ºC for 3d. The crude reaction mixture was then filtered and the residue concentrated *in vacuo*. The residual oil was chromatographed (SiO_2, EtOAc 20: hexanes 80) to give the title compound as a pale brown oil (4.91g, 22.7mmol 86%). δH ($CDCl_3$, 300K) 7.19-7.05 (5H, m), 4.56 (1H, s), 4.09-4.00 (1H, m), 3.97-3.89 (1H, m), 2.86 (1H, d, J 14.0Hz), 2.86 (1H, d, J 14.0Hz), 1.30 (3H, t, J 7.1Hz), 1.24 (3H, s). m/z (ES^+, 70V) 216.9 (MH^+).



(11a) (+/-) 4-Benzyl-3-hydroxy-4-methyl-2-cyclobuten-1-one.

Intermediate 10a (4.5g, 20.9mmol) and hydrochloric acid (6M, 10ml) were stirred at room temperature for 48h. Filtration of the resulting slurry and washing of the residue with water (3 x 15ml) gave the title compound as a pale brown powder (3.92g, 20.8mmol, 99%). δH ($CDCl_3$, 300K) 7.03-6.83 (5H, m), 4.24 (1H, s), 2.52 (2H, s), 0.94 (3H, s). m/z (ES^+, 70V) 189.1 (MH^+).

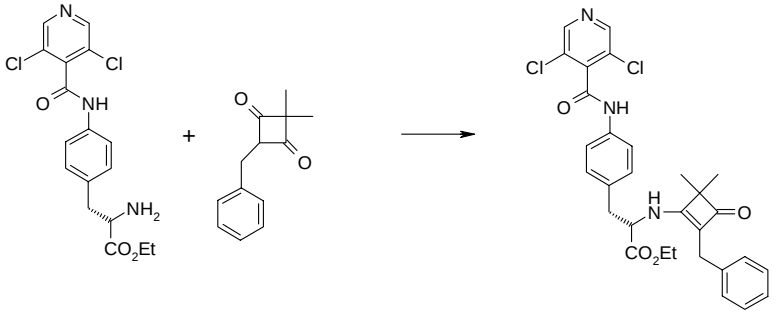
Representative procedure for the condensation of amino ester 12 with a C2-unsubstituted cyclobuta-1,3-dione [Scheme 3, *conditions a*] Preparation of 13e.



(13e) Ethyl (2*S*)-2-[(3-oxospiro[3.5]non-1-en-1-yl)amino]-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate

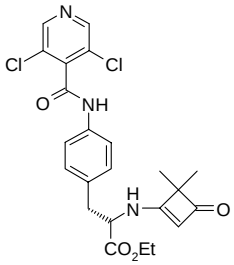
A solution of amine **12** (400mg, 1.04mmol) in THF (15ml) at rt was treated portionwise with 1-keto-3-hydroxyspiro[3,5]-non-2-ene (**8e**) (174mg, 1.14mmol) and the reaction was stirred for 24h. Volatiles were removed *in vacuo* and the residue subjected to chromatography (SiO_2, 1:1 hexane:EtOAc) to give the title compound as a white powder (512mg, 0.99mmol, 95%). δH ($CDCl_3$, 300K) 10.86 (1H, s), 8.78 (2H, s), 8.34 (1H, d, J 8.5Hz), 7.56 (2H, d, J 8.5Hz), 7.25 (2H, d, J 8.5Hz), 4.36 (1H, s), 4.20-4.11 (3H, m), 3.13 (1H, dd, J 13.8, 5.3Hz), 3.00 (1H, dd, J 9.2, 13.8Hz), 1.67-1.19 (10H, m), 1.17 (3H, t, J 4.1Hz). m/z (ES^+, 70V) 516.0 and 518.0 (MH^+).

Representative procedure for the condensation of amino ester 12 with a C2-substituted cyclobuta-1,3-dione [Scheme 3, *conditions b*] Preparation of 13m.



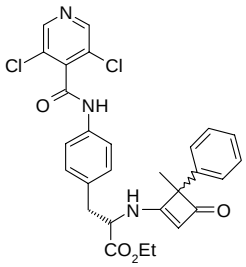
(13m) Ethyl (2*S*) 2-(2-benzyl-4,4-dimethyl-3-oxo-cyclobut-1-enylamino)-3-{4-[(3,5-dichloro-isonicotinoyl)amino]-phenyl} propanoate

A solution of amine **12** (0.82g, 2.1mmol) and **11a** (0.48g, 2.3mmol, 1.1eq.) in nitromethane (8ml) was treated with acetic acid (1 drop). The resulting mixture was heated at 100^{o} for 48h. and then partitioned between EtOAc (50ml) and water (25ml), the organics were separated, washed with water (25ml), Na_2CO_3 (25ml, sat. aq.), brine (25ml), dried (Na_2SO_4), filtered and concentrated *in vacuo* to give a crude foam. This was purified by column chromatography (SiO_2, gradient elution 1:1, hexane:EtOAc to EtOAc) to give the title compound as a white solid (0.71g, 1.25mmol, 59%). δH (d^{6}DMSO, 300K) 10.87 (1H, s), 8.81 (2H, s), 8.38 (1H, d, J 9.3Hz), 7.53 (2H, d, J 8.4Hz), 7.33 (2H, m), 7.15 (2H, m), 7.09 (2H, d, J 8.5Hz), 7.03 (2H, d, J 7.2Hz), 4.15 (1H, m), 4.04 (2H, dq, J 1.6, 7.1Hz), 3.19 (2H, m), 3.04 (1H, dd, J 13.8, 5.0Hz), 2.89 (1H, dd, J 9.5, 4.8Hz), 1.02-1.26 (8H, m). m/z (ESI, 70V) 566.0 (MH^+).



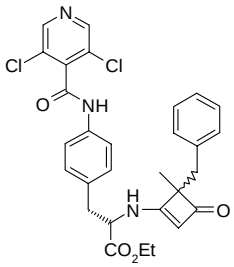
(13a) Ethyl (2*S*)-2-[(4,4-dimethyl-3-oxo-1-cyclobutenyl)amino]-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate

A solution of 3-hydroxy-4,4-dimethyl-2-cyclobutenone **8a** (58mg, 0.52mmol) and amine **12** (200mg, 0.52mmol), in THF (5ml), was stirred at room temperature for 24h. The volatiles were removed *in vacuo* and the residue chromatographed (SiO_2; EtOAc) to give the title compound as a white solid (230mg, 0.48mmol, 93%). δH ($CDCl_3$, 300K) 8.48 (2H, s), 8.10 (1H, s), 7.51 (2H, d, J 8.2Hz), 7.04 (2H, d, 8.2Hz), 5.91 (1H, s), 4.43 (1H, s), 4.22 (2H, q, J 7.1Hz), 3.17 (1H, dd, J 14.0, 5.1Hz), 3.05 (1H, dd, J 14.0, 5.8Hz), 1.28 (3H, t, J 7.1Hz), 1.15 (3H, s), 1.14 (3H, s). m/z (ES$^+$, 70V) 476.0 and 478.0 (MH^+).



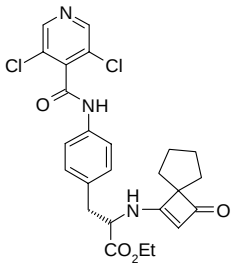
(13b) (2*S*)-Ethyl-2-[(4-methyl-3-oxo-4-phenyl-cyclobut-1-enyl)amino]-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl]-propionate

Prepared from **8b** (300mg, 1.72mmol) and amine **12** (400mg, 1.04mmol), in a similar manner to the compound of example **13e**, to give the title compound as a white powder (329mg, 0.61mmol, 59%) as a mixture of diastereomers (approx. 1:1.3). δH ($CDCl_3$, 300K) 10.95 (1H, s), 10.88 (1H, s), 8.89 (1H, d, J 8.6Hz), 8.81 (2H, s), 8.80 (2H, s), 8.74 (1H, d, J 8.8Hz), 7.63 (2H, d, J 8.5Hz), 7.59 (2H, d, J 8.5Hz), 7.34-7.10 (5H, m), 6.89 (2H, d, J 8.5Hz), 6.85 (2H, d, J 8.5Hz), 4.71 and 4.66 (1H, s), 4.48-4.42 and 4.38-4.33 (1H, m), 4.21 (2H, t, J 7.1Hz), 3.31-3.01 (2H, m), 1.52 and 1.42 (3H, s), 1.25 (3H, t, J 7.1Hz). m/z (ES^+, 70V) 537.9 (MH^+).



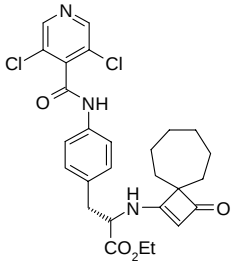
(13c) Ethyl (2*S*)-2-[(4*R,S*)-4-benzyl-4-methyl-3-oxo-1-cyclobutenyl]amino-3-4-[(3,5-dichloroisonicotinoyl)amino]phenylpropanoate

Prepared from **8c** (185mg, 0.98mmol) and amine **12** (300mg, 0.79mmol), in a similar manner to the compound of example **13e** to give the title compound as a white powder (387mg, 0.70mmol, 89%) as a mixture of diastereomers (approx 1:1.25). δH ($CDCl_3$, 300K) 9.36 and 9.31 (1H, s), 8.36 and 8.35 (2H, s), 7.54 and 7.45 (1H, d, J 8.4Hz), 7.19-7.02 (8H, m), 6.09-6.03 (1H, m), 4.31 and 4.20 (1H, s), 4.22-4.01 (3H, m), 3.07-2.92 (2H, m), 2.76-2.63 (2H, m), 1.35-1.15 (2H, m), 1.09 and 1.08 (3H, s). m/z (ES^+, 70V) 551.9 and 553.9 (MH^+).



(13d) (2*S*)-2-[(3-oxo-spiro[3.4]oct-1-en-1-yl)amino]-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}-propionate

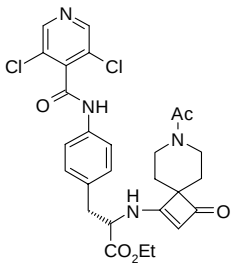
A solution of 3-hydroxy-spiro[3.4]oct-2-en-1-one **8d** (330mg, 2.39mmol) and amine **12** (911mg, 2.39mmol), in DCM (5ml), was stirred at rt for 24h. The volatiles were removed *in vacuo* and the residue chromatographed (SiO_2; EtOAc) to give the title compound as a white solid (1.03g, 2.05mmol, 86%). δH ($CDCl_3$, 300K) 8.97 (1H, s), 8.41 (2H, s), 7.51 (2H, d, J 8.5Hz), 7.01 (2H, d, J 8.5Hz), 5.89 (1H, d, J 7.5Hz), 4.39 (1H, s), 4.21 (3H, obscured m), 3.15 (1H, dd, J 5.3, 14.0Hz), 3.03 (1H, dd, J 5.8, 14.0Hz), 1.74-1.49 (10H, m), 1.27 (3H, t, J 7.1Hz). m/z (ES^+, 70V) 502.0 (MH^+).



(13f) Ethyl (2*S*)- 2-[(3-oxospiro[3.6]dec-1-en-1-yl)amino]-3-{4-[(3,5-dichloro-isonicotinoyl)amino]phenyl}propanoate

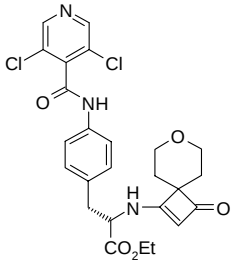
Prepared from **8f** (400mg, 2.4mmol) and amine **12** (920mg, 2.4mmol) in a similar manner to the method of example **13e**, to give the title compound as a white powder (1.1g, 2.07mmol, 86%). δH ($CDCl_3$, 300K) 8.57 (2H, s), 8.28 (1H, s), 7.61 (2H, d J 8.5Hz), 7.14 (2H, d J 8.5Hz), 5.76 (1H, d J 7.5Hz), 4.33-4.23 (3H, m), 3.25 (1H, dd J 5.3, 14.0Hz),

3.12 (1H, dd J 5.7, 13.9Hz), 1.95-1.89 (2H, m), 1.79-1.70 (4H, m), 1.71-1.50 (6H, m), 1.36 (3H, t J 7.1Hz). m/z (ES^+, 70V) 530.0 (MH^+).



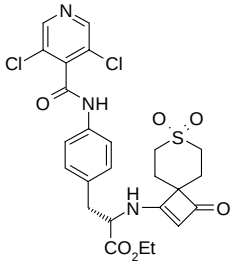
(13g) Ethyl (2*S*)-2-[(3-oxo-7-acetyl-7-azaspiro[3.5]non-1-en-1-yl)amino]-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate.

Prepared from **8g** (150mg, 0.77mmol) and amine **12** (150mg, 0.39mmol) in a similar manner to the method of example **13e**, to give the title compound as a white powder (143mg, 0.26mmol, 67%). δH (d^6 DMSO, 300K) 10.89 (1H, s), 8.89 (2H, s), 8.55-8.48 (1H, m), 7.58 (2H, d, J 7.9Hz), 7.25 (2H, d, J 7.9Hz), 4.47 (1H, s), 4.29-4.23 (1H, m), 4.16 (2H, q, J 7.1Hz), 3.76-3.72 (1H, m), 3.15 (1H, dd, J 13.8, 5.2Hz), 3.01-2.89 (2H, m), 2.00 (3H, s), 1.90-1.37 (6H, m), 1.21 (3H q J 7.1Hz). m/z (ES^+, 70V) 559.0 (MH^+).



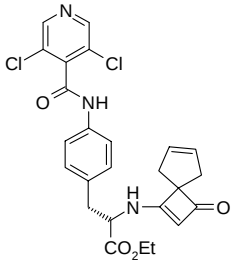
(13h) Ethyl (2*S*)-2-[(3-oxo-7-oxaspiro[3.5]non-1-en-1-yl)amino]-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate

Prepared from 7-oxaspiro[3.5]nonane-1,3-dione **8h** (1.2g, 7.8mmol) and amine **12** (2.67g, 7.0mmol) in a similar manner to the method of example **13e**, to give the title compound as a white powder (3.31g, 6.38mmol, 91%). δH ($CDCl_3$, 300K) 8.61 (1H, s), 8.33 (2H, s), 7.41 (2H, d J 5Hz), 6.94 (2H, d J 8.5Hz), 6.30 (1H, s br), 4.35 (1H, s), 4.11 (2H, q J 7.1Hz) and (1H, m obscured), 5.72 (4H, m), 3.07 (1H, dd J 14.0, 5.0Hz), 2.94 (1H, dd J 14.0, 6.6Hz), 1.75-1.66 (2H, m), 155-1.48 (2H, m), 1.17 (3H, t J 7.1Hz). m/z (ES^+, 70V) 517.9 (MH^+).



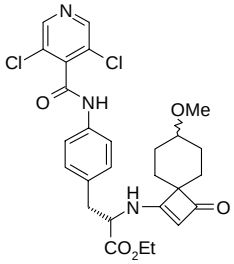
(13i) (2*S*)-Ethyl-2-[(3,7,7-trioxo-7lambda*6*-thia-spiro[3.5]non-1-en-1-yl)amino]-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}-propionate

Prepared from **8i** (1.1g, 5.4mmol) and amine **12** (2.08mg, 5.5mmol), in a similar manner to the compound of example **13e** to give the title compound as a white powder (712mg, 1.25mmol, 23%). δH ($CDCl_3$, 300K) 8.51 (1H, s), 8.33 (2H, s), 7.37 (2H, d, J 8.2Hz), 6.96 (2H, d, J 8.2Hz), 4.25 (1H, s), 4.10 (2H q, J 7.1Hz), 4.01 (1H, m), 3.40-3.33 (2H, m), 3.06 (1H, dd, J 4.5, 14.2Hz), 2.90 (1H, dd, J 14.1, 8.0Hz), 2.79-2.75 (2H, m), 2.38-2.31 (2H, m), 1.99-1.96 (1H, m), 1.86-1.81 (1H, m), 1.16 (3H, t, J 7.1Hz). m/z (ES^+, 70V) 565.9 (MH^+).



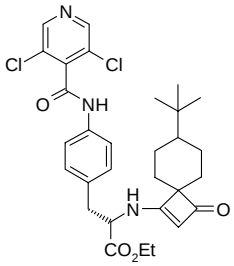
(13j) (*2S*)-Ethyl-2-[(3-oxo-spiro[3.4]octa-1,6-dien-1-yl)amino]-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propionate

A solution of **8j** (1.0g, 7.3mmol) and amine **12** (2.48g, 7.3mmol), in DCM (25ml), was stirred at rt for 48h. The resulting precipitate was removed by filtration and washed with diethylether (2x10ml) and dried *in vacuo* to give the title compound as a white solid (2.14g, 4.28mmol, 59%). δH ($CDCl_3$, 300K) 9.02 (1H, s), 8.38 (2H, s), 7.49 (2H, d, 8.5Hz), 7.00 (2H, d, J 8.5Hz), 6.03 (1H, d, J 7.8Hz), 5.54 (2H, s), 4.41 (1H, s), 4.21 (2H, q, J 7.1Hz), 4.20 (1H, m), 3.15 (1H, dd, J 5.2, 14.0Hz), 3.03 (1H, dd, J 6.1, 14.0Hz), 1.56-1.51 (2H, m), 2.38-2.34 (2H, m), 1.18 (3H, t, J 7.1Hz). m/z (ES^+, 70V) 500.0 (MH^+).



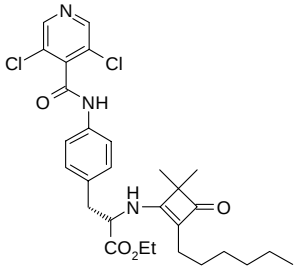
(13k) Ethyl (2*S*)-2-[(7-methoxy-3-oxospiro[3.5]non-1-en-1-yl)amino]-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate

Prepared from **8k** (500mg, 2.77mmol) and amine **12** (980mg, 2.6mmol) in a similar manner to the method of example **13e**, to give the title compound as an inseparable mixture of isomers (1:1.5) (1.23g, 2.25mmol, 87%). δH ($CDCl_3$, 300K, 2 isomers) 9.12/8.99 (1H, s), 8.51/8.50 (2H, s), 7.59/7.56 (2H, d, J 8.5Hz), 7.08 (2H, d, J 8.5Hz), 6.21/5.98 (1H, d, J 7.9Hz/7.6Hz), 4.46/4.43 (1H, s), 4.29/4.10 (3H, m), 3.13-3.08 (1H, m), 3.39 (1H, m), 3.30/3.29 (3H, s), 3.23-3.18 (1H, m), 3.13-3.08 (1H, m), 1.97-1.58 (8H, m), 1.35-1.34 (3H, t, J 7.1Hz). m/z (ES^+, 70V) 546.0 (MH^+).



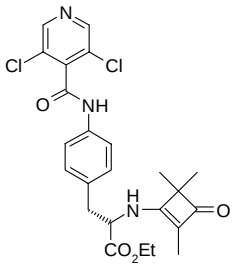
(13l)

Prepared from **8l** (274mg, 1.32mmol) and amine **12** (460mg, 1.2mmol) in a similar manner to the method of example **13e**, to give the title compound as a single diastereoisomer (592mg, 1.03mmol, 86%). δH ($CDCl_3$, 300K) 8.47 (2H, s), 7.52 (2H, d, J 8.4Hz), 7.02 (2H, d, J 8.4Hz), 5.78 (1H, d, J 6.4Hz), 4.39 (1H, s), 4.20 (2H, q, J 7.1Hz), 3.14 (1H, dd, J 5.0Hz 13.9Hz), 3.02 (1H, dd, J 5.7Hz 13.9Hz), 1.78-1.34 (8H, m), 1.27 (3H, t, 7.1Hz), 0.82-0.78 (1H, m), 0.77 (9H, s). m/z (ES^+, 70V) 572.0, 573.0 (MH^+).



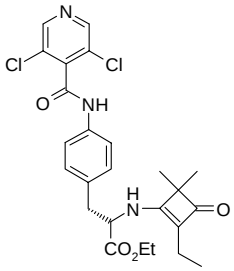
(13n) Ethyl (2*S*)-2-[(4,4-dimethyl-3-oxo-2-hexyl-1-cyclobutenyl)amino]-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate

Prepared from **11b** (200mg, 1.0mmol) and amine **12** (200mg, 0.52mmol), in a similar manner to the compound of example **13m** to give the title compound as a white powder (201mg, 0.42mmol, 72%). δH ($CDCl_3$, 300K) 8.99 (1H, s), 8.42 (2H, s), 7.52 (2H, d, J 8.4Hz), 7.02 (2H, d, J 7.6Hz), 5.54 (1H, s), 4.34 (1H, s), 4.19 (2H, q, J 7.1Hz), 3.07 (2H, br s), 1.95-1.81 (2H, br s), 1.27-0.77 (17H, m). m/z (ES^+, 70V) 560.0 and 562.0 (MH^+).



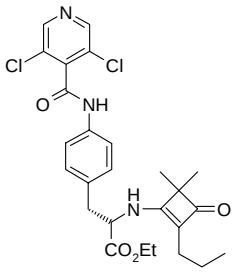
(13o) Ethyl (*2S*) 3-{4-[(3,5-dichloroisonicotnoyl)amino]phenyl}-2-(2,4,4-trimethyl-3-oxo-cyclobut-1-enylamino) propanoate

Prepared from **11c** (300mg, 2.4mmol) and amine **12** (905mg, 2.36mmol), in a similar manner to the compound of example **13m** to give the title compound as a white powder (810mg, 1.65mmol, 70%). δH ($CDCl_3$, 300K) 8.78 (1H, br s), 8.44 (2H, s), 7.52 (2H, d, J 8.4 Hz), 7.04 (2H, d, J 8.3Hz), 5.55 (1H,d, J 9.0Hz), 4.39 (1H, m), 4.20 (2H, q, J 7.1Hz), 3.07 (2H, m), 1.41 (3H, s), 1.26 (3H, t, J 7.1Hz), 1.05 (6H, s). m/z (ES^+, 70V) 490.0 (MH^+).



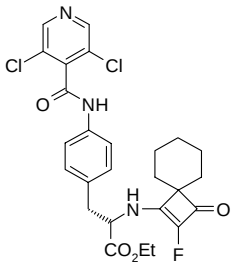
(13p) Ethyl (*2S*) 3-{4-[(3,5-Dichloroisonicotinoyl)amino]phenyl}-2-(2-ethyl-4,4-dimethyl-3-oxo-cyclobut-1-enylamino) propanoate

Prepared from **11d** (366mg, 2.61mmol) and amine **12** (1.0g, 2.61mmol), in a similar manner to the compound of example **13m** to give the title compound as a white powder (955mg, 1.89mmol, 73%). δH (d^6 DMSO, 300K) 8.78 (2H, s), 8.13 (1H, d, J 9.0Hz), 7.59 (2H, d, J 8.4Hz), 7.30 (2H, d, J 8.3Hz), 4.25 (1H, m), 4.17 (2H, q, J 7.0Hz), 3.12 (1H, m), 3.00 (1H, m), 1.83 (2H, m), 1.20 (4H, m), 1.06 (3H, m), 0.98 (3H, s), 0.84 (3H, t, J 7.5Hz). m/z (ES^+, 70V) 504.0 (MH^+).



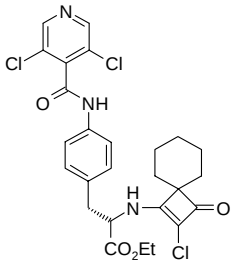
(13q) Ethyl (2S) 3-{4-[(3,5-Dichloroisonicotinoyl)amino]phenyl}-2-(4,4-dimethyl-3-oxo-2-propyl-cyclobut-1-enylamino) propanoate

Prepared from **11e** (460mg, 2.98mmol) and amine **12** (1.14g, 2.98mmol), in a similar manner to the compound of example **13m** to give the title compound as an off-white powder (1.16mg, 2.24mmol, 75%). δH (d^6 DMSO, 300K) 8.80 (2H, s), 8.11 (1H, d, J 9.3Hz), 7.59 (2H, d, J 8.3Hz), 7.30 (2H, d, J 8.3Hz), 4.26 (1H, m), 4.15(2H, q, J 7.1Hz), 3.12 (1H, m), 3.00 (1H, m), 1.75 (2H, m), 1.23 (2H,m), 1.07 (3H, s), 0.99 (3H,s), 0.73 (3H, t, J 7.3Hz). m/z (ES^+, 70V) 518.0 (MH^+).



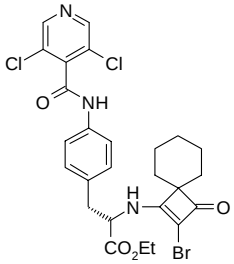
(14a) (2*S*)-Ethyl-2-[(2-fluoro-3-oxo-spiro[3.5]non-1-en-1-yl)amino]-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}-propionate

A solution of **13e** (2.02g, 3.91mmol) in THF (55ml) was treated with Selectfluor™ (1.38g, 3.89mmol) and heated to 70degC. After 48h the reaction was diluted with EtOAc (300ml) and washed with saturated aqueous sodium hydrogencarbonate solution (50ml). The organic phase was dried (MgSO4), filtered and concentrated *in vacuo*. Chromatography (SiO2, 60% EtOAc:hexanes) gave the title compound as a white powder (1.87g, 3.50mmol, 89%). δH (DMSO d^6, 390K) 10.89 (1H, s), 8.81 (2H, s), 8.47 (1H, d, J 8.7Hz), 7.59 (12H, d, J 8.5Hz), 7.27 (2H, d, J 8.5Hz), 4.26 (1H, m), 4.19 (2H, q, J 7.1Hz), 3.21 (1H, dd, J 4.9, 13.8Hz), 2.98 (1H, dd, J 9.8, 13.8Hz), 1.70-1.38 (10H, m), 1.22 (3H, t, J 7.1Hz). m/z (ES^+, 70V) 534.1 (MH^+).



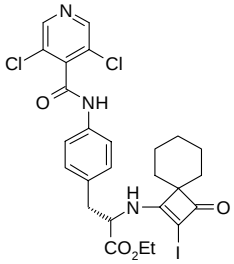
(14b) Ethyl (2*S*)-2-[(2-chloro-3-oxospiro[3.5]non-1-en-1-yl)amino]-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate

A solution of **13e** (366mg, 0.71mmol) in THF (25ml), at room temperature, was treated portionwise with N-chloro succinimide (100mg, 0.75mmol). After 30min the reaction mixture was poured into a mixture of EtOAc (150ml) and saturated aqueous $NaHCO_3$ solution (50ml). The organic layer was extracted and washed with brine (25ml), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Chromatography (SiO_2; 70% EtOAc:hexanes) gave the title compound as a white powder (312mg, 0.56mmol, 80%). δH ($CDCl_3$) 8.50 (2H, s), 7.73 (1H, s), 7.53 (1H, d, J 8.4Hz), 7.04 (2H, d, J 8.4Hz), 5.73 (1H, d, J 8.0Hz), 4.88-4.81 (1H, m), 4.21 (2H, q, J 7.1Hz), 3.21-3.16 (2H, m), 1.79-1.65 (4H, m), 1.51-1.36 (6H, m), 1.28 (3H, t, J 7.1Hz). m/z (ES^+,70V) 550.0 (MH^+).



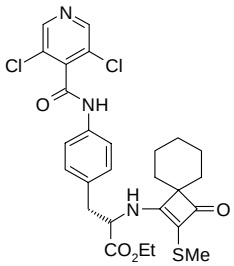
(14c) Ethyl (2*S*)-2-[(2-bromo-3-oxospiro[3.5]non-1-en-1-yl)amino]-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate

A solution of **13e** (500mg, 0.97mmol) and triethylamine (2eq, 270µl) in THF (10ml) at 0^{o} was treated dropwise with a solution of bromine (1.1eq, 170mg) in THF (5ml). After 20mins the reaction was allowed to warm to room temperature prior to dilution with EtOAc (100ml). The crude reaction mixture was washed with saturated aqueous $NaHCO_3$ (20ml) and brine (20ml), dried ($MgSO_4$) filtered and concentrated *in vacuo*. The residual foam was chromatographed (SiO_2; EtOAc) to give the title compound as a white powder (511mg, 0.86mmol, 95%). δH ($CDCl_3$, 300K) 8.48 (2H, s), 8.05 (1H, s br), 7.52 (2H, d J 8.4Hz), 7.04 (2H, d J 8.5Hz), 5.81 (1H, d br, J 8.3Hz), 4.98-4.91 (1H, m), 4.21 (2H, q, J 7.1Hz), 3.21 (2H, d J 5.3Hz), 1.70-1.66 (4H, m), 1.53-1.44 (4H, m), 1.28 (3H, t J 7.1Hz), 1.20-1.16 (2H, m). m/z (ES$^+$, 70V) 597.9 and 595.0 (MH^+).



(14d) Ethyl (2*S*)-2-[(2-iodo-3-oxospiro[3.5]non-1-en-1-yl)amino]-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propanoate

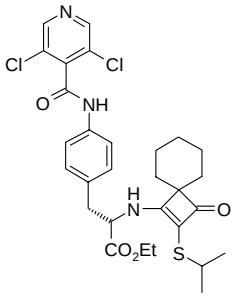
To a stirred solution of **13e** (1.0g, 1.9mmol) in THF (10ml) at room temperature was added N-iodosuccinamide (460mg, 2.0mmol) in one portion. After 5 minutes the mixture was concentrated *in vacuo* and the residue triturated with a mixture of ether (10ml) and water (10ml), filtered and washed with ether and water. Oven drying gave the title compound as a yellow powder (802mg, 66%) as a yellow solid. δH (d^6 DMSO, 300K) 8.90 (1H, d, J 9.1Hz), 8.78 (2H, s), 7.58 (2H, d, J 8.5Hz), 7.25 (2H, d, J 8.5Hz), 4.91 (1H, m), 4.20 (2H, q, J 7.1Hz), 3.30-3.00 (2H, m), 1.80-1.24 (10H, m), 1.21 (3H, t, J 7.1Hz). m/z (ES$^+$, 70V) 642.0 (MH^+).



(14e) (*2S*)-Ethyl-2-[(2-methylsulfanyl-3-oxo-spiro[3.5]non-1-en-1-yl)amino]-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}propionate

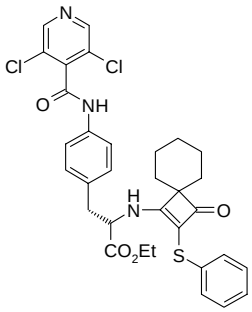
A solution of **13e** (1.0g, 1.94mmol) in THF (25ml) at rt was treated dropwise with a 1.0M solution of methanesulfenyl chloride in DCM [prepared according to the method of Still, I. W. J., *et al*. J. Org. Chem., *1982*, 47, 560] (2.13ml, 1.0M). After 20min the reaction was diluted with EtOAc (100ml) and washed with saturated aqueous sodium hydrogencarbonate solution (50ml). The organic phase was dried (MgSO4), filtered and concentrated *in vacuo*. Chromatography (SiO2, 60% EtOAc:hexanes) gave the title compound as a white powder (1.03g, 1.83mmol,

94%). δH (DMSO d^6, 390K) 10.86 (1H, s), 8.78 (2H, s), 8.70 (1H, d, J 9.2Hz), 7.57 (2H, d, J 8.4Hz), 7.26 (2H, d, J 8.4Hz), 5.11 (1H, m), 4.18 (2H, q, J 7.1Hz), 3.20 (1H, dd, J 4.6, 13.9Hz), 3.00 (1H, dd, J 9.8, 13.9Hz), 1.93 (3H, s), 1.66-1.33 (10H, m), 1.21 (3H, t, J 7.1Hz) . m/z (ES^+, 70V) 562.1 (MH^+).



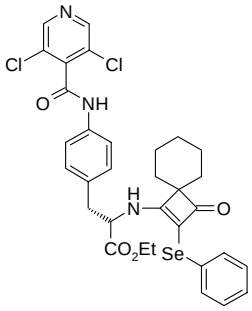
(14f) (2*S*)-Ethyl-2-[(2-Isopropylsulfanyl-3-oxo-spiro[3.5]non-1-en-1-yl)amino]-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}-propionate

A stirred solution of **13e** (1.0g, 1.93mmol) in THF (50ml) at rt was treated dropwise with a solution of isopropyl sulfenyl chloride in DCM (approx 2M) until a yellow colouration of the reaction just persisted. The reaction was then diluted with EtOAc (200ml) and washed with saturated aqueous sodium hydrogencarbonate solution (50ml). The organic phase was then dried (MgSO4), filtered and concentrated *in vacuo*. Chromatography (SiO2, 100% EtOAc) gave the title compound as a pale yellow powder (987mg, 1.67mmol, 87%). δH (DMSO d^6, 390K) 10.85 (1H, s), 8.79 (2H, s), 8.73 (1H, d, J 9.5Hz), 7.56 (2H, d, J 8.5Hz), 7.25 (2H, d, J 8.5Hz), 5.20 (1H, m), 4.17 (2H, q, J 7.1Hz), 3.18 (1H, dd, J 4.3, 13.8Hz), 2.97 (1H, dd, J 10.2, 13.8Hz), 2.65 (1H, m), 1.73-1.57 (8H, m), 1.36-1.33 (1H, m), 1.21 (3H, t, J 7.1Hz), 1.17-1.14 (1H, m), 1.02 (6H, d, J 6.6Hz). m/z (ES^+, 70V) 590.0 (MH^+).



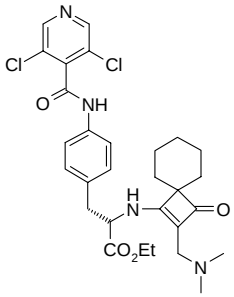
(14g) (2*S*)-Ethyl-2-[(2-phenylsulfanyl-3-oxo-spiro[3.5]non-1-en-1-yl)amino]-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}-propionate

Prepared from **13e** (1.5g, 2.91mmol) and phenylsulphenyl chloride (510mg, 3.5mmol), in a similar manner to the compound of example **14f**, to give the title compound as a white powder (1.47g, 2.35mmol, 81%). δH (DMSO d^6, 390K) 10.87 (1H, s), 9.16 (1H, d, J 9.1Hz), 8.80 (2H, s), 7.51 (2H, d, J 8.3Hz), 7.25-7.21 (2H, m), 7.13-7.07 (3H, m), 6.99 (2H, d, J 7.3Hz), 4.98-4.96 (1H, m), 3.98 (3H, q, J 7.1Hz), 3.11 (1H, dd, J 4.7Hz 13.9Hz), 2.94 (1H, dd, J 9.9Hz, 13.9Hz), 1.85-1.17 (10H, m), 1.16 (3H, t, J 7.1Hz). m/z (ES^+, 70V) 624.0 and 626.0 (MH^+).



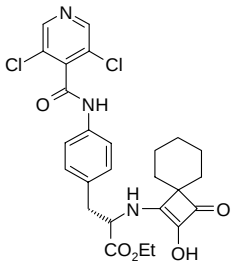
(14h) (2*S*)-Ethyl-2-[(2-phenylselenenyl-3-oxo-spiro[3.5]non-1-en-1-yl)amino]-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}-propionate

Prepared from **13e** (1.0g, 1.93mmol) and phenylselenenyl chloride (400mg, 2.12mmol), in a similar manner to the compound of example **14f**, to give the title compound as a white powder (1.13g, 1.68mmol, 87%). δH (DMSO d^6, 390K) 10.86 (1H, s), 9.07 (1H, d, J 9.2Hz), 8.81 (2H, s), 7.53 (2H, d, J 8.5Hz), 7.21 (2H, d, J 8.5Hz), 7.14-7.11 (5H, m), 5.14-5.08 (1H, m), 4.01 (2H, q, J 7.1Hz), 3.12 (1H, dd, J 4.7Hz 13.9Hz), 2.95 (1H, dd, J 9.9Hz 13.9Hz), 1.87-1.45 (10H, m br), 1.08 (3H, t, J 7.1Hz). m/z (ES^+, 70V) 670.0, 671.0, 672.0, 674.0 (IsotopicMH$^+$).



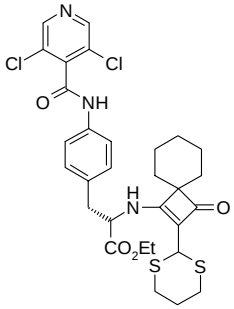
(14i) (2*S*)-Ethyl-2-[(2-dimethylaminomethyl-3-oxo-spiro[3.5]non-1-en-1-yl)amino]-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}-propionate

A solution of **13e** (1.61g, 3.11mmol) and Eschenmoser's salt (630mg, 3.42mmol) in THF (20ml) was stirred at rt for 24h. The reaction was then diluted with EtOAc (100ml) and washed with water (10ml) followed by saturated aqueous sodium carbonate (100ml), brine (50ml) and the organics dried ($MgSO_4$), filtered and concentrated *in vacuo*. The resulting oil was then subjected to chromatography (SiO2, 100% EtOAc) to give the title compound as a white powder (1.58g, 2.76mmol, 89%). δH (DMSO d^6, 390K) 8.75 (2H, s), 8.11 (1H, d, J 8.1Hz), 7.51 (2H, d, J 8.5Hz), 7.25 (2H, d, J 8.5Hz), 5.01-4.97 (1H, m), 4.14 (2H, q, J 7.1Hz), 3.14 (1H, dd, J 4.6Hz 13.9Hz), 2.95 (1H, dd, J 9.8Hz 13.9Hz), 2.53 (1H, d, J 13.2Hz), 2.40 (1H, d, J 13.2Hz), 2.00 (3H, s), 1.99 (3H, s), 1.85-1.50 (10H, m br), 1.09 (3H, t, J 7.1Hz). m/z (ES^+, 70V) 573.0, 574.0 (MH^+).



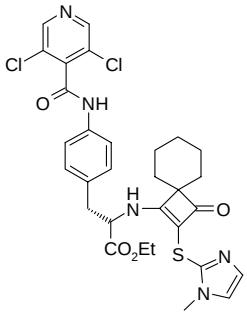
(14j) Ethyl (2*S*)-3-{4-[(3,5-dichloroisonicotinoyl)amino]phenyl}-2-(2-hydroxy-3-oxo-spiro[3.5]non-1-en-1-ylamino)-propionic acid ethyl ester

To a solution of **13e** (1.0g, 1.9mmol) in DCM (40ml) at –40°C was added lead tetraacetate (0.94g, 2.1mmol). The mixture was allowed to warm to 0°C and stirred at this temperature for 8h. The reaction mixture was partitioned between EtOAc (200ml) and water (100ml), the organics were separated, washed with water (2x100ml), brine (50ml) and dried ($MgSO_4$), filtered and concentrated *in vacuo* to give a crude oil. The crude was dissolved in ethanol (10ml) and treated with NaH (100mg). The mixture was stirred at room temperature until TLC analysis indicated that all acetoxy intermediate had been consumed. The reaction was quenched by the addition of NH_4Cl (5ml, sat. aq.). Extraction with EtOAc (2x20ml) followed by washing of the combined extracts with water (10ml), brine (10ml), drying ($MgSO_4$), filtering and concentration *in vacuo* gave a crude oil which was subjected to chromatography (SiO_2, 1:1EtOAc:Hexane) to give the title compound as a white foam (0.89g, 1.67mmol, 87%). δH (DMSO d^6, 300K) 10.83 (1H, br), 8.78 (2H, s), 7.51 (2H, d, J 8.5Hz), 7.12 (2H, d, J 8.5Hz), 4.94 (1H, dd, J 11.4, 5.0Hz), 4.10 (2H, m), 3.33 (1H, dd, J 14.1, 4.9Hz), 3.14 (1H, dd, J 14.0, 11.4Hz), 1.40-1.63 (4H, m), 1.19-1.33 (6H, m), 1.16 (3H, t, J 7.1Hz). m/z (ES^+, 70V) 532 (MH^+).



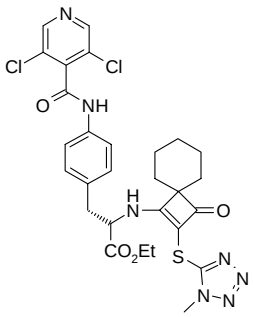
(14k) Ethyl 3-{4-[(3,5-Dichloroisonicotinoyl)amino]phenyl}-2-(2-[1,3]dithian-2-yl-3-oxo-spiro[3.5]non-1-en-1-ylamino) propanoate

A solution of **13e** (1.5g, 2.9mmol) in DCM (100ml) was treated portionwise with 1,3-dithienium tetrafluorborate (3g, 14mmol) [prepared by the method of Paterson I; Price L.G. Tet. Lett.1981, 22 (29), 2829]. The mixture was stirred overnight and then partitioned between EtOAc (200ml) and sodium carbonate (100ml, sat. aq.), the organics were separated, washed with water (3 x 50ml), brine (50ml), dried (Na_2SO_4), filtered and concentrated *in vacuo* to give a crude product which was purified by column chromatography (SiO_2: 4:1, EtOAc: hexane) to give the title compound as a pale yellow solid (1.6g, 2.52mmol, 87%). δH NMR (d6 DMSO, 300K) 8.67 (2H, s), 8.15 (1H, d, J 9.5Hz), 7.67 (2H, d, J 8.5Hz), 7.12 (2H, d, J 8.5Hz), 5.06 (1H, m), 4.65 (1H, s),1.10 (1H, m), 4.08 (2H, t, J 7.1Hz), 3.17-2.72 (3H, m), 2.65 (2H, m), 1.95 (1H, m), 1.87 (1H, m), 1.78-1.46 (11H, m), 1.25 (1H, d, J 12.3Hz), 1.08 (3H, t, J 7.1Hz). m/z (ESI, 70V) 634.0 (MH^+)



(14l) NAME

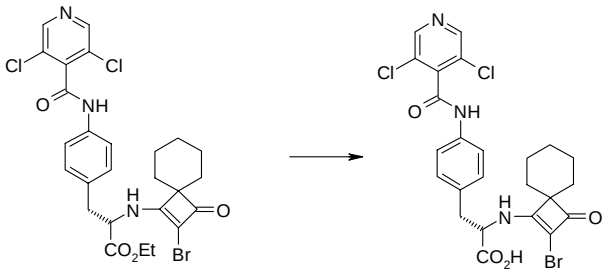
A well-stirred slurry of N-methyl-2-mercaptoimidazole (340mg, 2.98mmol) in THF (10ml) was treated portionwise with NCS (386mg, 2.89mmol) at rt. After approx. 90min the reaction was then treated dropwise with a solution containing **13e** (1.5g, 2.89mmol) in THF (10ml). The slurry was stirred at rt for a further 12h then diluted with 50ml of THF and warmed to 50°C prior to removal of solids by filtration. The clear filtrate was concentrated *in vacuo* and subjected to chromatography (SiO_2: 4:1, EtOAc: hexane) to give the title compound as a white powder (1.63g, 2.59mmol, 89%). δH NMR (d6 DMSO, 300K) 10.86 (1H, s), 9.05 (1H, d, J 9.0Hz), 8.80 (2H, s), 7.53 (2H, d, J 8.5Hz), 7.28 (1H, s), 7.22 (2H, d, J 8.5Hz), 7.00 (1H, s), 5.42-5.36 (1H, m), 4.15 (2H, q, J 7.1Hz), 3.65 (3H, s), 3.25 (1H, dd, J 5.0Hz 14.1Hz), 3.05 (1H, dd, J 9.4Hz 14.1Hz), 1.73-1.11 (10H, m), 1.15 (3H, t, J 7.1Hz). m/z (ESI, 70V) 629.0, 630.0, 632.0 (MH^+).



(14m) NAME

Prepared from **13e** (1.0g, 1.93mmol), 1N-methyl 2-mercapto tetrazole (246mg, 2.12mmol) and NCS (255mg, 1.93mmol), in a similar manner to the compound of example **14l**, to give the title compound as a white powder (1.08g, 1.71mmol, 89%). δH (DMSO d^6, 390K) 10.87 (1H, s), 9.39 (1H, d, J 9.1Hz), 8.80 (2H, s), 7.55 (2H, d, J 8.5Hz), 7.20 (2H, d, J 8.5Hz), 5.05-5.01 (1H, m), 4.09 (2H, q, J 7.1Hz), 3.94 (3H, s), 3.21 (1H, dd, J 5.1Hz 14.0Hz), 3.03 (1H, dd, J 9.3Hz 14.0Hz), 1.77-1.41 (10H, m), 1.15 (3H, t, J 7.1Hz). m/z (ES^+, 70V) 632.0, 634.0 (IsotopicMH$^+$).

Representative procedure for hydrolysis of 14 to 15. Preparation of 15c.



(15c) (2*S*)-2-[(2-bromo-3-oxospiro[3.5]non-1-en-1-yl)amino]-3-{4-[(3,5dichloroisonicotinoyl)amino] phenyl} propanoic acid

A solution of **14c** (1.0g, 1.68mmol) in THF (10ml) at rt was treated with 1M LiOH (2.0ml, 2.0mmol) and the reaction stirred for approx 24h. Volatiles were then removed *in vacuo* and the resulting residue taken up into water (25ml) and acidified with 1M HCl to a pH of around 3. The resulting white precipitate was then removed by filtration, washed several times with water and dried *in vacuo* to give the title compound as a white powder (901mg, 1.59mmol, 94%).

1H NMR spectrum of 14c: When observed at approx. rt the 1H NMR spectra of compounds **13a-q** and **14a-m** indicate presence of a minor rotamer (usually <5%). These become resolved to a single conformer when heated to 398K. See below for a diagrammatic example. INSERT HERE

Cell Assay procedure: [VLA-4 ($\alpha_4\beta_1$) Integrin-dependent Jurkat cell adhesion to VCAM-Ig]

96 well NUNC plates were coated with $F(ab)_2$ fragment goat anti-human IgG Fcγ-specific antibody [Jackson Immuno Research 109-006-098: 100 µl at 2 µg/ml in 0.1M $NaHCO_3$, pH 8.4], overnight at 4^o. The plates were washed (3x) in phosphate-buffered saline (PBS) and then blocked for 1h in PBS/1% BSA at room temperature on a rocking platform. After washing (3x in PBS) 9 ng/ml of purified 2d VCAM-Ig diluted in PBS/1% BSA was added and the plates left for 60 minutes at room temperature on a rocking platform. The plates were washed (3x in PBS) and the assay then performed at 37^o for 30 min in a total volume of 200 µl containing 2.5 x 10^5 Jurkat cells in the presence or absence of titrated test compounds.

Each plate was washed (2x) with medium and the adherent cells were fixed with 100□l methanol for 10 minutes followed by another wash. 100µl 0.25% Rose Bengal (Sigma R4507) in PBS was added for 5 minutes at room temperature and the plates washed (3x) in PBS. 100µl 50% (v/v) ethanol in PBS was added and the plates left for 60min after which the absorbance (570nm) was measured.