Supplementary Materials

Experimental Sections

Bioactive Conformation Hypotheses Prediction Using FieldTemplater (Forge)

Due to the lack of structural information for BMS-626529 in its target-bound state, FieldTemplater (Forge, Cresset, UK) was used to determine the most likely 3D conformation(s) adopted in binding to the target, using field and shape information from BMS-626529,[1, 2] BMS-488043, [3-5] and BMS-378806[5, 6] to calculate potential bioactive conformation hypotheses. Bioactive conformations of molecules that bind to the same active site are expected to display a similar shape, distribution of charge and hydrophobic groups.

In the FieldTemplater[7] experiment (conducted within Forge[7]), each of the three compounds was conformationally populated using Cresset's Xedex conformational search method (embedded within Forge). The 3D field point pattern for each conformation of each template molecule was calculated and used to cross-compare to each other in field / shape space, with no dependence on chemical structure. The calculation of field points and their use in comparing molecules has been described in detail in previous literature, [8] and a brief description is included in **Figure 3** of the main manuscript. It is noteworthy that these field point patterns are generated from the whole molecule, and not reliant on local or isolated features such as hydrogen bonds or aromatic moieties, thus providing an extremely concise, high content representation of the molecule's pharmacophoric features.

Using 3D field point patterns, the conformations of the three BMS molecules were exhaustively compared in a pair-wise fashion until field point patterns common to all three molecules were identified. The resulting templates (*i.e.*, binding mode hypotheses) each consist of one conformation of each of the three molecules (trios).

It is postulated and experimentally supported[8-12] that trios reported by the experiment are likely to include the correct alignment and bioactive conformation.

The trios from the FieldTemplater experiment using the BMS compounds were visually inspected and one conformation of BMS-626529 was selected as the query molecule for the Blaze (Cresset, UK) field based virtual screening experiments. The selected conformation of BMS-626529 appeared in several of the top-scoring templates.

Blaze Virtual Screening Using BMS-626529 Template

The 3D field point pattern for the proposed bioactive conformation of BMS-626529 selected from the FieldTemplater experiment was used to query a database of approximately 6 million commercially available compounds (Blaze Database) using Blaze (Cresset, UK). Each of these 6 million compounds was previously conformationally populated using Cresset's conformational hunter, XedeX (http://www.cresset-group.com/products/xedtools/#overview) which employs Cresset's XED forcefield.[13] In addition to conformational hunting, each conformation has its three-dimensional field point pattern calculated and stored with the structure.

The Blaze search procedure has been demonstrated to be a practical, efficient method of ligand-based virtual screening that employs both shape and field based molecular comparisons that retrieve actives that are both structurally novel and lead-like.[14-17]

The Blaze virtual screening procedure is comprised of the following steps:

(1) Identification of an active ligand and its 3D bioactive conformation (single conformation). This structure is known as the search molecule.

(2) The field point pattern for this conformation of the search molecule is produced, and it is that arrangement of field points in 3 dimensions that provides the Blaze Query.

(3) The Blaze Query is compared to the field point pattern of every conformation of all of the molecules in the Blaze Database.
(4) The results are returned and contain a similarity score (by default, based on 50% shape / 50% fields) for each molecule, a 3D alignment of the best scoring conformation, and a 2D representation.

The Blaze experiment resulted in the rank ordering of the top 1000 commercially available compounds whose field point patterns had similarities to that of BMS-626529 in the proposed bioactive conformation from the template. Based on visual inspection of field point pattern comparisons and combined shape-field similarity scores (50:50), fifty compounds were chosen and purchased for biological testing using the single-round infection assay.[18]

Spark Bioisosteric Group Replacement

The use of Cresset's field point technology has been employed for field based scaffold hopping and bioisosteric replacement since 2005 [11] and has been well discussed in two recent reviews. [19, 20] These experiments were conducted using Spark (Cresset, UK).

To summarize the method, a section a molecule in its bioactive conformation (not necessary, but 3D binding structure is desirable) is selected and designated for replacement. When that moiety is clipped away, replacement candidates from Spark's fragment databases are fit into the molecule and scored (more details are provided below).

Spark's native fragment databases consist of fragments generated from commercially available screening compounds, ChEMBL[21], and VeHICLE[22] databases.

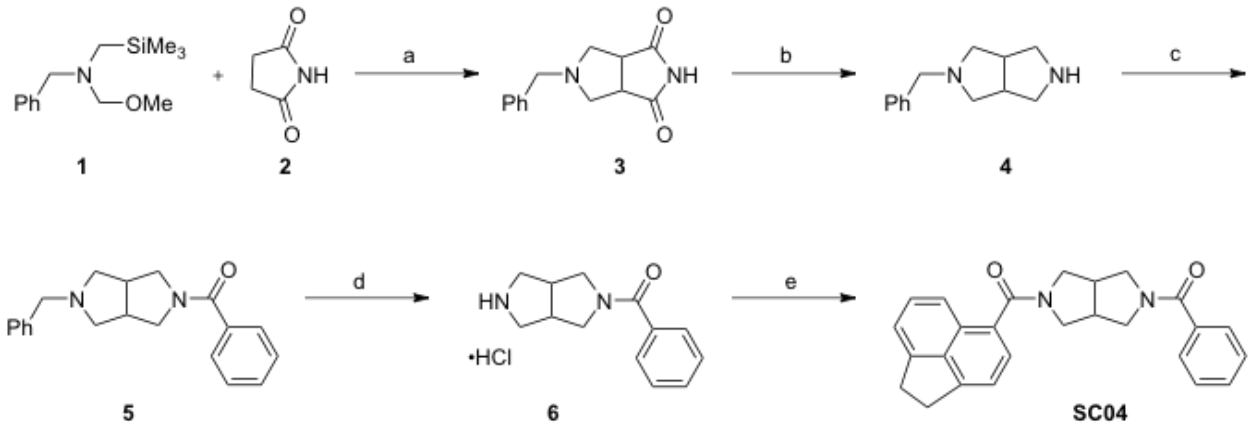
Fragments are generated by the method outlined in the Spark user manual [7] which entails breaking all “breakable” bonds in a molecule, then reconnecting sets of pieces to form bigger fragments. Fragments, by default, may contain up to 15 heavy atoms and 5 rotatable bonds, with a molecular weight maximum of 250. Each fragment has up to 30 conformations generated and stored, with field point patterns calculated for each conformation. Along with field point patterns, the relative orientations and positions of fragment attachment points are recorded.

In the course of the Spark experiment, a portion of the molecule to be replaced is selected; the number of bonds that were broken along with the distances and angles between broken bonds are recorded. The first pass through the Spark fragment databases involves finding fragments which have the same number of attachment points, and approximately the same geometry (‘bite size’ and ‘bite angle’). Each one of these first-pass fragments is fit into the original molecule; fields and field points are calculated for the resulting whole molecules. The resulting molecules are minimized using the XED forcefield, then the full field and shape of the molecule is scored against the full field and shape of starting molecule (or other designated reference molecule, or combination thereof). By default, 50% shape and 50% fields are used in score calculations.

Screening chemicals*.* All chemicals for screening were purchased from Enamine, Ltd., and were 95% pure or greater.

De novo syntheses. Synthesis of novel compounds was performed by Enamine Ltd. (Kiev, Ukraine), AsisChem, Inc. (Watertown, MA), HD Biosciences Co., Ltd. (Shanghai, China), or WuXi Apptec (Shanghai, China) according to the schemes and procedures outlined below.

Scheme 1. Synthesis of **SC04**[a]



[a]Reagent and conditions: (a) TFA, CH_2Cl_2. (b) LAH, THF. (c) PhCOCl, Et_3N, CH_2Cl_2. (d) H2, Pd/C, MeOH. (e) Acenaphtene-5-carboxylic acid, EDC, Et_3N, CH_2Cl_2.

Synthesis of SC04: Trifiuoroacetic acid (TFA, 1.48 g, 0.013 mol) was added to a cold (0ºC) solution of compound **2** 12.6 g, 0.13 mol) in dichloromethane (250 mL) under nitrogen. A solution of compound **1** (31 g, 0.13 mol) in dichloromethane (200 mL) was added drop-wise over 45 min. After the addition was complete, the mixture was warmed slowly to ambient temperature and stirred for 16 h. The mixture was concentrated and the resulting residue was dissolved in dichloromethane (200 mL) and washed with saturated aqueous sodium bicarbonate (2 x 100 mL). The aqueous layer was separated and extracted with dichloromethane (2 x 150 mL). The combined dichloromethane extracts were washed with brine (150 mL), dried over anhydrous magnesium sulphate, filtered and concentrated to give 30 g (75 percent yield) of compound **3** as a light yellow solid.

The crude compound **3** (30 g, 0.13 mol) was dissolved in dry tetrahydrofuran (THF) (500 mL) under nitrogen atmosphere, and then lithium aluminium hydride (14.8 g, 0.39 mol) was slowly added at 0°C over 30 min. The resulting mixture was stirred at ambient temperature for 30 min and then warmed to reflux for 7 h. The mixture was then cooled to 0 ºC and quenched by the slow addition of an excess of H_2O/THF solution (1/10). The mixture was warmed to ambient temperature and stirred for 1 h. The solids were filtered and the residue was washed with ethyl acetate (3 x 150 mL). The combined filtrates were concentrated to give 27 g of a crude material.

Purification of the crude compound **4** was successfully performed by column chromatography with EtOAc/hexene (3/1 + 10 % TEA) as an eluent. 19.2 g (73 percent yield) of the pure material were obtained.

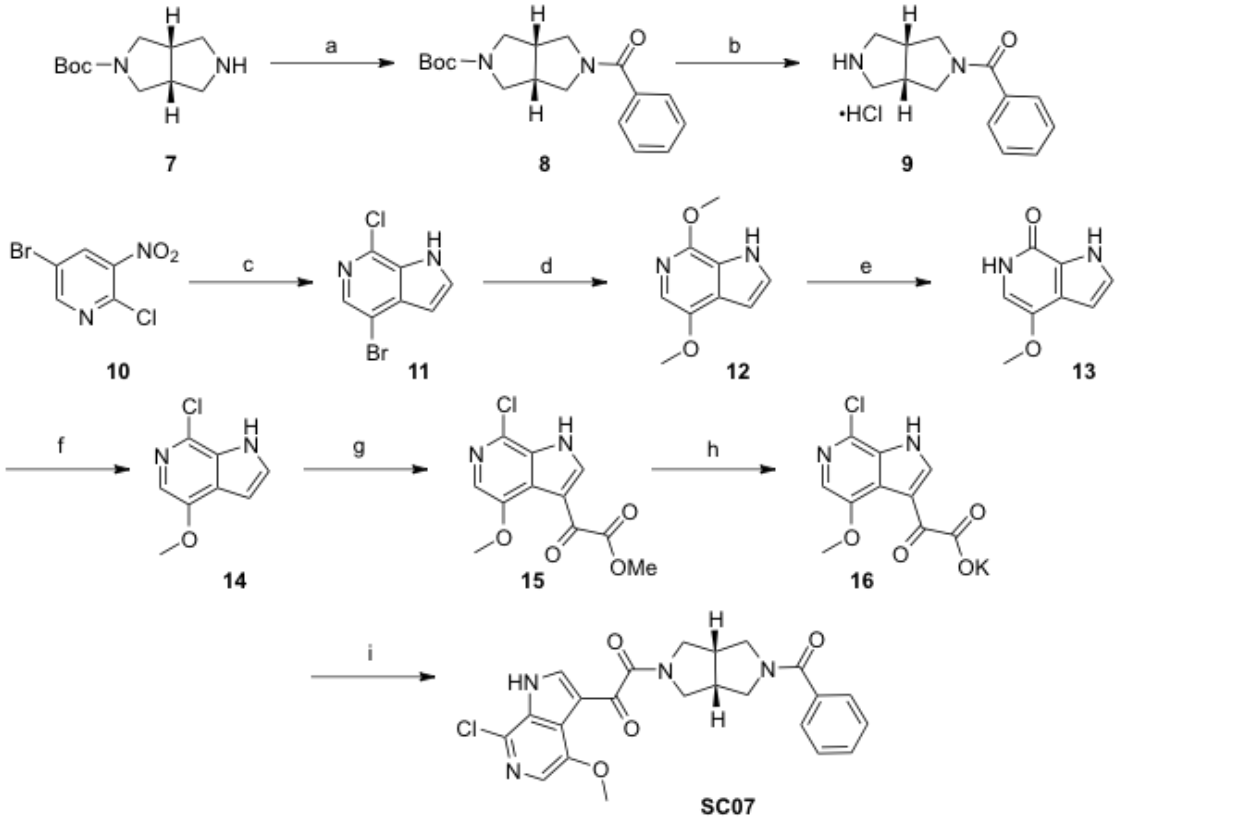
A solution of benzoyl chloride (6.6 g, 0.047 mol) in DCM (100 mL) was being added dropwise to a stirred mixture of compound **4** (9.5 g, 0.047 mol) and Et_3N (20 g, 0.15 mol) in DCM (150 mL) at room temperature during 30 min. After the addition was complete, the reaction mixture was stirred at room temperature for 5 h and then washed with water (2 x 100 mL), 1 N aq. HCl (2 x 50 mL), water (2 x 50 mL), saturated aq. $NaHCO_3$ (2 x 75 mL), and brine (2 x 50 mL). After drying over $MgSO_4$, the solvent was evaporated under reduced pressure to give the compound **5** (13.3 g, 0.043 mol, 93 percent yield) as a yellow oil.

Compound **5** (10.3 g, 0.033 mol) was added to the suspension of 10% Pd/C (1.5 g) in anhydrous methanol (250 mL). This mixture was hydrogenated at 20 atm and RT for 10 h. The catalyst was filtered off through a pad of Celite, which was then washed with anhydrous methanol (200 mL). The solvent was evaporated under reduced pressure to afford the crude product **6** as yellow oil. Obtained material were dissolved in 0.5 N aq. HCl (50 mL), treated with charcoal, and filtered. The filtrate was evaporated to dryness to give 7 g (0.027 mol, 83 percent yield) of compound **6***HCl as a white solid.

Acenaphthene-5-carboxylic acid (0.3 g, 1.5 mmol) was added to a mixture of compound **6***HCl (0.38 g, 1.5 mmol) and TEA (0.41 g, 4 mmol) in CH_2Cl_2 (25 mL) at the room temperature. Then 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.3 g, 1.6 mmol) was added and the mixture was stirred at the room temperature for 16 h. The organic phase was then washed with water (2 x 200 mL), 1 N aq. HCl (2 x 150 mL), water (2 x 200 mL), saturated aq. $NaHCO_3$ (2 x 200 mL), and brine (2 x 150 mL). After drying over $MgSO_4$, the solvent was evaporated under reduced pressure to give the crude compound **7** (0.52 g) in a form of a yellow oil. The crude material was purified three times by column chromatography with DCM/MeOH (10/2) as an eluent. Final

purification was performed by preparative HPLC to provide 40 mg (0.6 mmol, 8 percent yield) of the pure compound **7** as a light yellow solid.

Scheme 2. Synthesis of **SC07**[a]



[a]Reagent and conditions: (a) PhCOCl, Et_3N, CH_2Cl_2, 0 °C to rt. (b) HCl (4N), Et_2O. (c) vinylmagnesium bromide THF, -78 °C. (d) 1) MeOK, toluene, reflux; 2) Cu (cat.), toluene, reflux. (e) HCl, NMP-H_2O, 100 °C. (f) $POCl_3$, 100 °C. (g) $ClCOCO_2Me$, $AlCl_3$, CH_2Cl_2, 0 °C to rt. (h) K_2CO_3, MeOH. (i) **9**, EDCI, DiEA, DMF.

Synthesis of SC07: To a solution of compound **7** (212.3 mg; 1 mmol) in DCM (30 ml) was added TEA (325 mg; 3.2 mmol). The resulted solution was cooled to 0 °C and a solution of benzoyl chloride (140 mg; 1 mmol) in DCM (5 ml) was added dropwise. The reaction mixture was stirred at room temperature for 2 h, and then water (100 ml) with $NaHCO_3$ sat. (10 ml) were added. Organic layer was separated, washed with water and dried over Na_2SO_4. DCM was evaporated to afford compound **8** (160 mg; 50%) as yellow oil. To a solution of compound **8** (1.1 g; 3.5 mmol) in dry Et_2O (5 ml) under argon was added 4N HCl in Et_2O (5 ml). The mixture

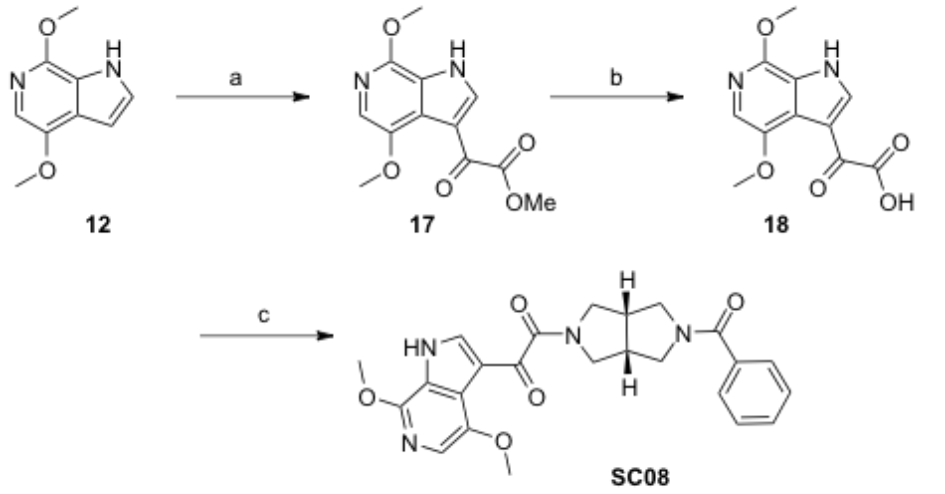
was stirred for 3 h and the formed precipitate was filtered, washed with Et_2O and stored into a desiccator to give compound **9** (0.67 g, 89%) as a white powder.

To a solution of compound **10** (250 mg; 1.05 mmol) in dry THF (10 ml) under argon at -78 °C was added dropwise a 1.42 M solution of vinylmagnesium bromide in THF (6 ml, 8.4 mmol; prepared by dilution of 1.7 M solution of in THF (50 ml) with THF (12 ml)). The reaction mixture was stirred at the same temperature for another 1.5 h and then it was quenched by addition of NH_4Cl sat. (30 ml). The THF-layer was separated and the aqueous layer was extracted with EtOAc (2×10 ml). Combined organic layers (THF with EtOAc extracts) were dried over Na_2SO_4 and concentrated to afford 270 mg of yellow oily material which was treated with DCM (10 ml). Light-yellow solid was filtered and dried in oven (45 °C) to give compound **11** (70 mg, 29%).

To a solution of compound **11** (3.8 g; 16.4 mmol), celite (6.16 g) in dry toluene (60 ml) was added a solution of potassium (6.39 g; 164 mmol) in MeOH (50 ml). The reaction mixture was heated at reflux (with Dean-Stark trap) for 12 h, then it was cooled to ambient temperature and mixture of toluene and methanol (1/1, 50 ml) was added. Then the reaction mixture was heated again and when the temperature was 60°C, CuI (3.43 g; 18 mmol) was added. The mixture was held at 115°C for 1.5h while collecting a mixture of MeOH and toluene. The mixture was cooled to ambient temperature and water (60 ml) was added. A precipitate formed was filtered off and washed with EtOAc (100 ml), and then the aqueous layer was extracted with EtOAc (50 ml). Combined EtOAc-layers were dried over Na_2SO_4 and concentrated to afford **compound 12** (2.4 g, 82%) as light-yellow solid. To a solution of compound **12** (0.8 g; 4.5 mmol) in *N*-methylpyrrolidone (5 ml), water (0.5 ml) and hydrochloric acid (5-6 drops) were added. The mixture was stirred at 100°C for 6 h. The mixture was cooled to room temperature and water (100 ml) was added. Then $NaHCO_3$ sat. (20 ml) was added, the product was extracted with EtOAc (5×20 ml). Combined extracts were dried over Na_2SO_4 and concentrated to afford **compound 13** (0.6 g, 81%) as pink solid. A solution of compound **13** (1.8 g; 11 mmol) in $POCl_3$ (20 ml) was stirred at 100 °C for 12 h. Then $POCl_3$ was evaporated under reduced pressure. To

a residue ice water (100 ml) and then $NaHCO_3$ sat. (25 ml) were added, the product was extracted with EtOAc (3×35 ml). Combined extracts were dried over Na_2SO_4 and concentrated to afford compound **14** (0.8 g, 40%) as light-yellow solid. To a 0 °C suspension of ethyl 2-chloro-2-oxoacetate (222.7 mg; 1.63 mmol) and $AlCl_3$ (237 mg; 1.78 mmol) in DCM (5 ml) was added dropwise a suspension of compound **14** (165 mg; 0.92 mmol) and $AlCl_3$ (122 mg; 0.92 mmol) in DCM (5 ml). The reaction mixture was stirred at room temperature for 2 h. Then the mixture was cooled again and water (50 ml) with $NaHCO_3$ sat. (10 ml) were added maintaining the temperature 0 °C. Organic layer was separated, dried over Na_2SO_4 and concentrated. Crude product was purified by column chromatography (EtOAc/hexane 1:1) to afford compound **15** (50 mg, 20%) as yellow crystals. Compound **15** (350 mg; 1.24 mmol) was dissolved in aqueous methanol (1/1, 14 ml) and K_2CO_3 (0.34 g; 2.48 mmol) was added to the solution. The mixture was stirred at room temperature for 8 h. The solvent was removed under vacuo to afford crude compound 16 (361 mg) as white powder which used for the next step without further purification. A solution of compound 16 (361 mg; 1.23 mmol), EDCI (353 mg; 1.85 mmol) and DIPEA (635 mg; 4.92 mmol) in dry DMF (10 ml) was stirred for 1 h. Then compound **9** (311 mg; 1.23 mmol) was added and the reaction mixture was stirred at 20 °C for another 18 h. Then water (100 ml) with $NaHCO_3$ sat. (10 ml) were added. Target compound was extracted with DCM (3×30 ml). Combined extracts were dried over Na_2SO_4 and concentrated. Crude material was purified by column chromatography (EtOAc/MeOH 9:1) to afford **SC07 (**28 mg, 4.6%) as a yellow powder.

Scheme 3. Synthesis of **SC08**[a]

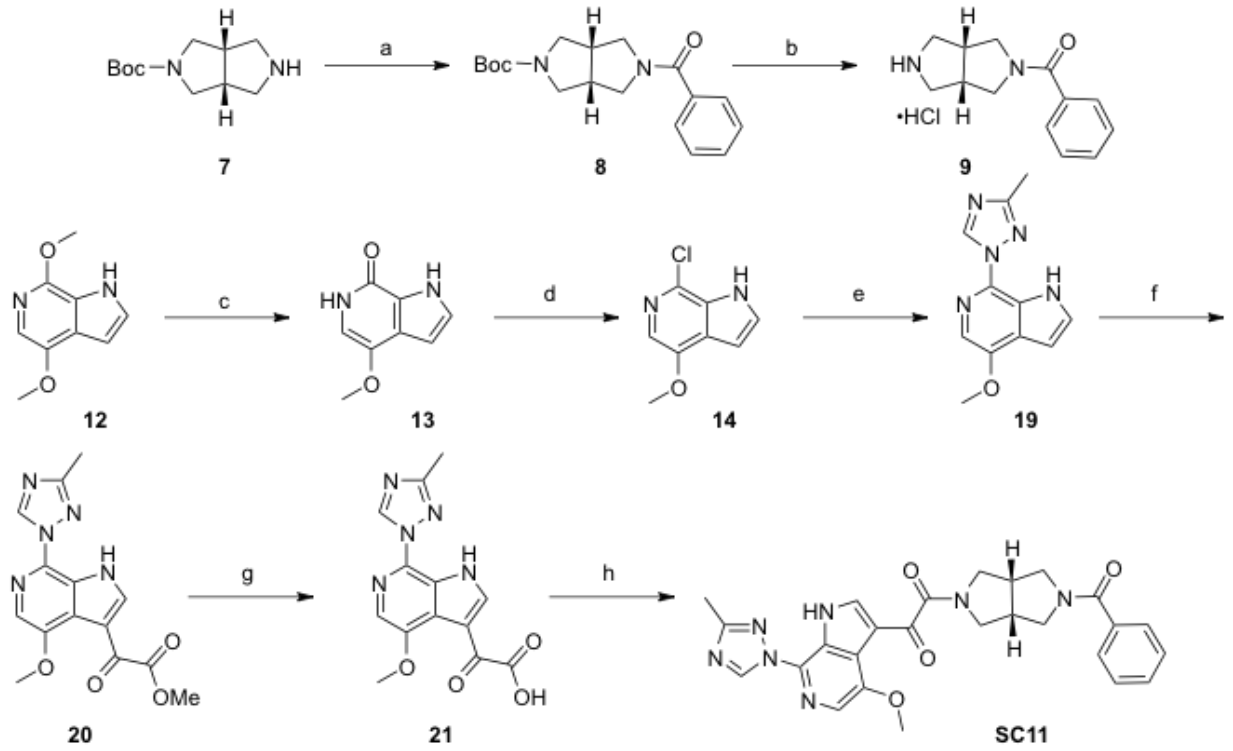


[a]Reagent and conditions: (a) $ClCOCO_2Me$, $AlCl_3$, CH_2Cl_2, 0 ºC to rt. (b) K_2CO_3, MeOH. (c) **9**, EDCI, DiEA, DMF.

Synthesis of SC08: A solution of compound **12** (300 mg; 1.69 mmol) and $AlCl_3$ (230 mg; 1.75 mmol) in DCM (10 ml) was added dropwise to a 0 °C suspension of ethyl 2-chloro-2-oxoacetate (600 mg; 4.3 mmol) and $AlCl_3$ (600 mg; 4.6 mmol) in DCM (10 ml). The reaction mixture was stirred at room temperature for 18 h. Then the mixture was cooled again and water (50 ml) with $NaHCO_3$ sat. (25 ml) were added maintaining the temperature 0°C. Organic layer was separated, dried over Na_2SO_4 and concentrated. Crude product was purified by column chromatography (EtOAc/hexane 1:2) to afford compound **17** (144 mg, 30%) as a yellow powder. K_2CO_3 (0.839 g; 6.08 mmol) was added to a solution of compound **17** (845 mg; 3.04 mmol) in aqueous methanol (1/1; 14 ml). The mixture was stirred at room temperature for 8 h. A precipitate obtained was filtered and washed with MeOH to give compound **18** (500 mg, 57%) as a white powder. A solution of compound **18** (288 mg; 1 mmol), EDCI (287 mg; 1.5 mmol) and DIPEA (516 mg; 4 mmol) in dry DMF (10 ml) was stirred for 1 h. Then compound **9** (252 mg; 1 mmol) was added and the reaction mixture was stirred at 20 °C for another 18 h. Then water (100 ml) with $NaHCO_3$ sat. (10 ml) were added. Target compound was extracted with DCM (3×30 ml). Combined extracts were dried over Na_2SO_4 and concentrated. Crude material

was purified by column chromatography (EtOAc/MeOH 9:1) to afford **SC08** (190 mg, 42%) as a pale green solid.

Scheme 4. Synthesis of **SC11**[a]



[a]Reagent and conditions: (a) $PhCO_2H$, HBTU, Et_3N, CH_2Cl_2, rt. (b) HCl (4N), dioxane. (c) 1) HCl (4N), dioxane; 2) NMP-H_2O, 90 °C. (d) $POCl_3$, 95 °C. (d) 3-methyl-1*H*-1,2,4-triazole, 143 °C. (e) $ClCOCO_2Me$, $AlCl_3$, CH_2Cl_2, rt. (g) NaOH, MeOH•H_2O. (h) **9**, DEPBT, DiEA, DMF.

Synthesis of SC11: To a stirred solution of benzoic acid (116 mg, 0.94 mmol) in DCM (3 mL) were added compound **7** (200 mg, 0.94 mmol), TEA (0.4 mL, 2.82 mmol) and HBTU (536 mg, 1.39 mmol). The resulting mixture was stirred at r.t. overnight before quenched with sat. $NaHCO_3$. The layers were separated and the aqueous layer was extracted with DCM (10 mL x 3). The combined organic layers was dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (silica gel, 0 ~ 50% ethyl acetate in petroleum ether with 0.5% TEA) to provide compound **8** (164 mg, 55 %) as a yellow solid. Compound **9** (303 mg, 100 %) was obtained as a yellow oil by treating compound **8** (345 mg, 1.09 mmol) with 4N HCl/Dioxane (3 mL).

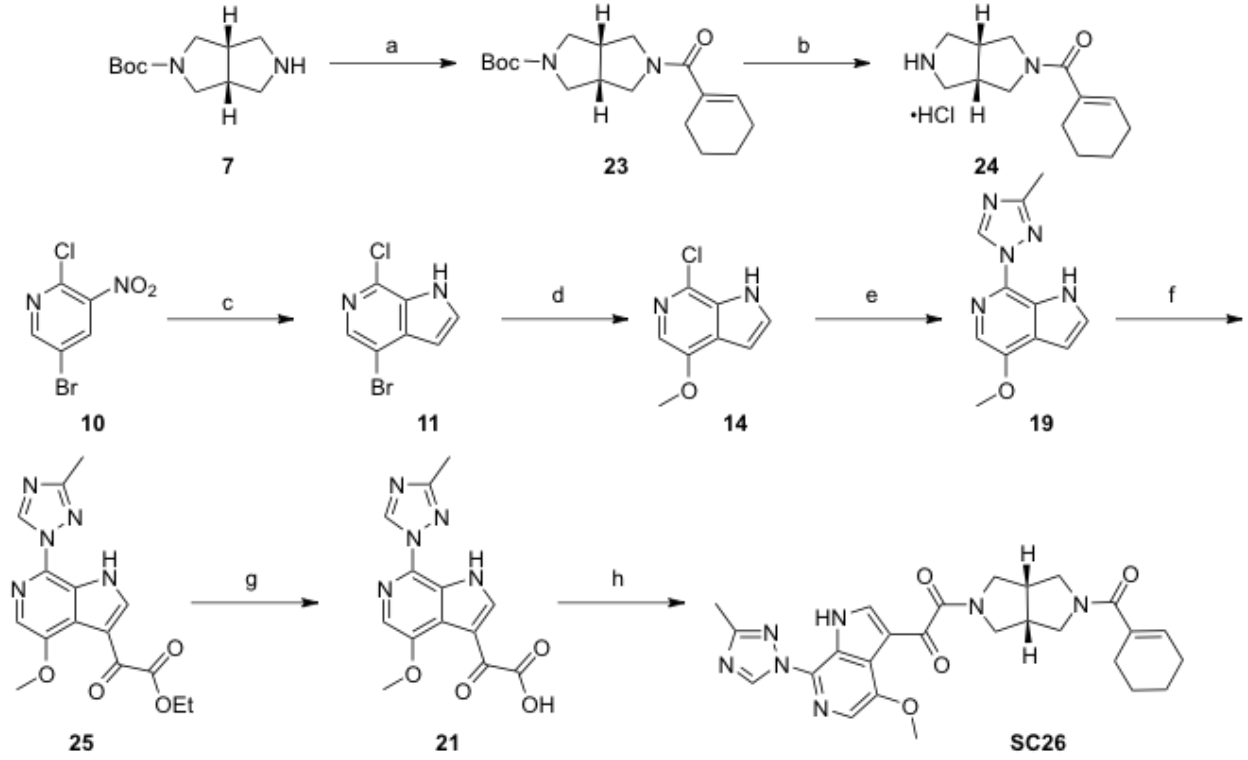
To a solution of compound **12** (0.8 g; 4.5 mmol) in *N*-methylpyrrolidone (5 ml), water (0.5 ml)

and hydrochloric acid (5-6 drops) were added. The mixture was stirred at 100°C for 6 h. The mixture was cooled to room temperature and water (100 ml) was added. Then $NaHCO_3$ sat. (20 ml) was added, the product was extracted with EtOAc (5×20 ml). Combined extracts were dried over Na_2SO_4 and concentrated to afford compound **13** (0.6 g, 81%) as pink solid. A solution of compound **13** (1.8 g; 11 mmol) in $POCl_3$ (20 ml) was stirred at 100 °C for 12 h. Then $POCl_3$ was evaporated under reduced pressure. To a residue ice water (100 ml) and then $NaHCO_3$ sat. (25 ml) were added, the product was extracted with EtOAc (3×35 ml). Combined extracts were dried over Na_2SO_4 and concentrated to afford compound **14** (0.8 g, 40%) as light-yellow solid

At 143°C, to a melt 3-methyl-1*H*-1,2,4-triazole (744 mg, 8.95 mmol) under N_2 atmosphere was added compound 14 (392 mg, 1.79 mmol). After stirring for 20 hrs, the reaction mixture was cooled down to r.t. and partitioned between H_2O (20 mL) and EA (20 mL). The layers were separated and the aqueous layer was extracted with EA (20 mL x 3). The combined organic layers was dried over Na_2SO_4, filtered and concentrated. The residue was purified by prep. HPLC (C18, 10% to 89% acetonitrile in water (0.1% formic acid)) to compound 19 (182 mg, 44 %) as a white solid. To a stirred solution of $AlCl_3$ (1.588 g, 11.9 mmol) in DCM (10 mL) was added methyl 2-chloro-2-oxoacetate (389 mg, 3.18 mmol) and the resulting mixture was stirred at r.t. until it became a clear solution before introduction of compound 19 (182 mg, 0.79 mmol, in 1 mL DCM). The stirring was continued at r.t. overnight. After completion of the reaction, the mixture was quenched with saturated $NaHCO_3$ and extracted with DCM (10 mL x 3). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (silica gel, 0 ~ 90% ethyl acetate in petroleum ether) to provide compound **20** (58 mg, 23 %) as a yellow oil. To a stirred solution of compound **20** (58 mg, 0.18 mmol) in MeOH/H_2O (1 mL/2 mL) was added NaOH (15 mg, 0.37 mmol). After stirring at r.t. for 15 min, the reaction mixture was neutralized with 1N HCl, concentrated and lyophilized to provide compound **21** (73 mg, 95 %, mixed with NaCl) as a green solid.

Compound **21** (55 mg, 0.18 mmol) in DMF and compound **9** (59 mg, 0.27 mmol) were added to DIPEA (0.28 mL, 1.63 mmol) and DEPBT (325 mg, 1.09 mmol). After stirring at r.t. overnight, the reaction mixture was concentrated to remove DMF under the reduced pressure. The residue was partitioned between with EA (10 mL) and saturated $NaHCO_3$ (10 mL). The layers were separated and the aqueous layer was extracted with EA (10 mL x 2). The combined organic layers was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by preparative HPLC to give compound **SC11** (22 mg, 24 %).

Scheme 6. Synthesis of **SC11**[a]



[a]Reagent and conditions: (a) cyclohexene-1-carboxylic acid, HATU, DIEA, DMF, rt. (b) HCl, EtOAc. (c) vinylmagnesium bromide THF, -78 ºC to -40 ºC. (d) NaOMe, CuI, MeOH, microwave, 110 ºC. (e) 3-methyl-1*H*-1,2,4-triazole, copper powder, KOHm, 107-175 ºC. (f) $ClCOCO_2Et$, EtMgBr, py, THF, -45 ºC- -10 ºC. (g) NaOH, MeOH•H_2O. (h) **24**, HATU, DiEA, DMF.

Synthesis of SC26: To a solution of cyclohexene-1-carboxylic acid (0.15 g, 1.19 mmol) in DMF (5 mL) was added compound **7** (0.21 g, 0.99 mmol), HATU (0.45 g, 1.19 mmol, 1.2 eq.) and DIEA (0.26 g, 1.98 mmol). Then the mixture was stirred at 25^{o}C for 16hrs. The crude product

was purified by pre-HPLC to give tert-butyl compound **23** (0.21 g, 0.67 mmol, 67%). HCl/AcOEt (20 mL) was added compound 23 (0.21 g, 0.67 mmol), the mixture was stirred at 25^{o}C for 2 hrs, TLC showed the S.M was consumed completely. The mixture was concentrated to give compound **24** (0.15 g, 0.58 mmol) as crude product.

To a solution of compound **21** (0.03 g, 0.99 mmol) in DMF (2 mL) was added compound **24** (0.026 g, 0.12 mmol), HATU (0.045 g, 0.12 mmol) and DIEA (0.026 g, 0.2 mmol). Then the mixture was stirred at 25°C for 16 hrs. The crude product was purified by pre-HPLC to give **SC26** (15 mg, 30%).

Cells. Human embryonic kidney 293T cells were cultured in Dulbecco modified Eagle medium supplemented with 10% heat-inactivated fetal bovine serum, L-glutamine, and antibiotics. Human astroglioma U87 cells stably transfected for the expression of CD4 and CXCR4 (obtained from HongKui Deng and Dan Littman, through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH) [23] were cultured in Dulbecco modified Eagle medium supplemented as above plus puromycin and G418 (Geneticin, Gibco BRL Life Technologies, Grand Island, NY).

Production of pseudotypes. Single-round infectious envelope-pseudotyped luciferase-reporter viruses were produced in 293T cells co-transfected by calcium phosphate precipitation (Profection Mammalian Transfection System) with the envelope-deficient HIV-1 NL4-3 vector (pNL4-3-Luc$R^{+}E^{-}$; a gift of N. R. Landau, New York University),[24] which carries the luciferase-reporter gene; and either the HIV-1 YU-2, JR-CSF, HxBc2-envelope expressing vector (obtained from Kathleen Page and Dan Littman through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH).[25] After 6 h of incubation at 37°C, the DNA-containing medium was removed, cells were washed, and fresh medium was added.

Supernatants containing the envelope-pseudotyped viruses were collected 2 days later, clarified by centrifugation, aliquoted, and stored at -80°C until use. Pseudotype stocks were quantified by $p24^{gag}$ content (Advanced Bioscience Laboratories, Kensington, MD).

Single-round infection assays. U87-CD4/CCR5 or U87-CD4/CXCR4 cells were seeded at a density of $1.2x10^4$ cells per well in 96 well luminometer-compatible tissue culture plates. The following day, cells were infected with 20 ng of p24 of pseudotyped virus (described above) per well with compounds or DMSO. Prior to addition of virus, compounds were diluted between six and nine times (1:10 or 1:5 dilution, respectively). Each dilution of compound was applied on a plate in triplicate. Forty eight hours later, cells were lysed with 50 µl of reporter lysis buffer (Promega) and luciferase activity was measured in GloMax 96 Microplate Luminometer (Promega). Percent infection was determined by the ratio of luciferase activity in compound treated wells versus DMSO treated wells.

Anti-HIV efficacy evaluation in human peripheral blood mononuclear cells. Viral isolates were obtained from the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH, as follows: HIV-1 group M isolate 92UG037 (subtype A, CCR5-tropic; Cat# 1743) from the UNAIDS Network for HIV Isolation and Characterization;[26] HIV-1 group M isolate 91US004 (subtype B, CCR5-tropic; also referred to as “US4”, “GS 007”, or 91US_4; Cat# 7689) from Dr. Nelson Michael ; [27-29] HIV-1 Group M isolates 94US_33931N (Subtype B, CCR5-tropic; also referred to as “33931N”; Cat# 11250), 98US_MSC5016 (Subtype C, CCR5-tropic; also referred to as “56313”; Cat# 11253), and 99UG_A07412M1 (Subtype D, CCR5-Tropic; also referred to as “A07412M1”; Cat# 11261) from Dr. Victoria Polonis; [30] and HIV-2 isolate CDC310319 (Cat# 3930) from Dr. Stefan Wiktor and Dr. Mark Rayfield.[31] Antiviral evaluations in human PBMCs were performed as described previously. [32-34]

References

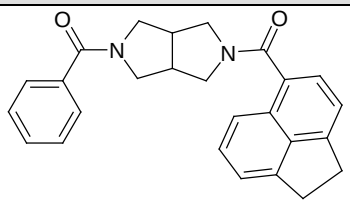
1. Li, Z.; Zhou, N.; Sun, Y.; Ray, N.; Lataillade, M.; Hanna, G. J.; Krystal, M. *Antimicrobial agents and chemotherapy* **2013**.
2. Nowicka-Sans, B.; Gong, Y. F.; McAuliffe, B.; Dicker, I.; Ho, H. T.; Zhou, N.; Eggers, B.; Lin, P. F.; Ray, N.; Wind-Rotolo, M.; Zhu, L.; Majumdar, A.; Stock, D.; Lataillade, M.; Hanna, G. J.; Matiskella, J. D.; Ueda, Y.; Wang, T.; Kadow, J. F.; Meanwell, N. A.; Krystal, M. *Antimicrobial agents and chemotherapy* **2012,** *56*, 3498.
3. Hanna, G. J.; Lalezari, J.; Hellinger, J. A.; Wohl, D. A.; Nettles, R.; Persson, A.; Krystal, M.; Lin, P.; Colonno, R.; Grasela, D. M. *Antimicrobial agents and chemotherapy* **2011,** *55*, 722.
4. Wang, T.; Yin, Z. W.; Zhang, Z. X.; Bender, J. A.; Yang, Z.; Johnson, G.; Yang, Z.; Zadjura, L. M.; D'Arieno, C. J.; Parker, D. D.; Gesenberg, C.; Yamanaka, G. A.; Gong, Y. F.; Ho, H. T.; Fang, H.; Zhou, N. N.; McAuliffe, B. V.; Eggers, B. J.; Fan, L.; Nowicka-Sans, B.; Dicker, I. B.; Gao, Q.; Colonno, R. J.; Lin, P. F.; Meanwell, N. A.; Kadow, J. F. *Journal of medicinal chemistry* **2009,** *52*, 7778.
5. Yang, Z.; Zadjura, L. M.; Marino, A. M.; D'Arienzo, C. J.; Malinowski, J.; Gesenberg, C.; Lin, P. F.; Colonno, R. J.; Wang, T.; Kadow, J. F.; Meanwell, N. A.; Hansel, S. B. *Journal of pharmaceutical sciences* **2010,** *99*, 2135.
6. Lin, P. F.; Blair, W.; Wang, T.; Spicer, T.; Guo, Q.; Zhou, N.; Gong, Y. F.; Wang, H. G.; Rose, R.; Yamanaka, G.; Robinson, B.; Li, C. B.; Fridell, R.; Deminie, C.; Demers, G.; Yang, Z.; Zadjura, L.; Meanwell, N.; Colonno, R. *Proceedings of the National Academy of Sciences of the United States of America* **2003,** *100*, 11013.
7. Cresset. New Cambridge House, Bassingbourn Road, Litlington, Cambridgeshire, UK SG8 0SS.
8. Cheeseright, T.; Mackey, M.; Rose, S.; Vinter, A. *Journal of chemical information and modeling* **2006,** *46*, 665.
9. Cheeseright, T. J.; Mackey, M. D.; Scoffin, R. A. *Current computer-aided drug design* **2011,** *7*, 190.
10. Collins, J. C.; Armstrong, A.; Chapman, K. L.; Cordingley, H. C.; Jaxa-Chamiec, A. A.; Judd, K. E.; Mann, D. J.; Scott, K. A.; Tralau-Stewart, C. J.; Low, C. M. R. *Medchemcomm* **2013,** *4*, 1148.
11. Low, C. M.; Buck, I. M.; Cooke, T.; Cushnir, J. R.; Kalindjian, S. B.; Kotecha, A.; Pether, M. J.; Shankley, N. P.; Vinter, J. G.; Wright, L. *Journal of medicinal chemistry* **2005,** *48*, 6790.
12. Low, C. M.; Vinter, J. G. *Journal of medicinal chemistry* **2008,** *51*, 565.
13. Vinter, J. G. *Journal of computer-aided molecular design* **1996,** *10*, 417.
14. Cheeseright, T. J.; Holm, M.; Lehmann, F.; Luik, S.; Gottert, M.; Melville, J. L.; Laufer, S. *Journal of medicinal chemistry* **2009,** *52*, 4200.
15. Cheeseright, T. J.; Mackey, M. D.; Melville, J. L.; Vinter, J. G. *Journal of chemical information and modeling* **2008,** *48*, 2108.
16. Mackey, M. D.; Melville, J. L. *Journal of chemical information and modeling* **2009,** *49*, 1154.
17. Webster, S. P.; Binnie, M.; McConnell, K. M.; Sooy, K.; Ward, P.; Greaney, M. F.; Vinter, A.; Pallin, T. D.; Dyke, H. J.; Gill, M. I.; Warner, I.; Seckl, J. R.; Walker, B. R. *Bioorganic & medicinal chemistry letters* **2010,** *20*, 3265.

18. Biorn, A. C.; Cocklin, S.; Madani, N.; Si, Z.; Ivanovic, T.; Samanen, J.; Van Ryk, D. I.; Pantophlet, R.; Burton, D. R.; Freire, E.; Sodroski, J.; Chaiken, I. M. *Biochemistry* **2004,** *43*, 1928.
19. Ertl, P.; Lewis, R. *Journal of computer-aided molecular design* **2012,** *26*, 1207.
20. Schuffenhauer, A. *Wires Comput Mol Sci* **2012,** *2*, 842.
21. Gaulton, A.; Bellis, L. J.; Bento, A. P.; Chambers, J.; Davies, M.; Hersey, A.; Light, Y.; McGlinchey, S.; Michalovich, D.; Al-Lazikani, B.; Overington, J. P. *Nucleic acids research* **2012,** *40*, D1100.
22. Pitt, W. R.; Parry, D. M.; Perry, B. G.; Groom, C. R. *Journal of medicinal chemistry* **2009,** *52*, 2952.
23. Bjorndal, A.; Deng, H.; Jansson, M.; Fiore, J. R.; Colognesi, C.; Karlsson, A.; Albert, J.; Scarlatti, G.; Littman, D. R.; Fenyo, E. M. *J Virol* **1997,** *71*, 7478.
24. Connor, R. I.; Chen, B. K.; Choe, S.; Landau, N. R. *Virology* **1995,** *206*, 935.
25. Page, K. A.; Landau, N. R.; Littman, D. R. *J Virol* **1990,** *64*, 5270.
26. Gao, F.; Yue, L.; Craig, S.; Thornton, C. L.; Robertson, D. L.; McCutchan, F. E.; Bradac, J. A.; Sharp, P. M.; Hahn, B. H. *AIDS research and human retroviruses* **1994,** *10*, 1359.
27. Jagodzinski, L. L.; Wiggins, D. L.; McManis, J. L.; Emery, S.; Overbaugh, J.; Robb, M.; Bodrug, S.; Michael, N. L. *Journal of clinical microbiology* **2000,** *38*, 1247.
28. Michael, N. L.; Herman, S. A.; Kwok, S.; Dreyer, K.; Wang, J.; Christopherson, C.; Spadoro, J. P.; Young, K. K.; Polonis, V.; McCutchan, F. E.; Carr, J.; Mascola, J. R.; Jagodzinski, L. L.; Robb, M. L. *Journal of clinical microbiology* **1999,** *37*, 2557.
29. Vahey, M.; Nau, M. E.; Barrick, S.; Cooley, J. D.; Sawyer, R.; Sleeker, A. A.; Vickerman, P.; Bloor, S.; Larder, B.; Michael, N. L.; Wegner, S. A. *Journal of clinical microbiology* **1999,** *37*, 2533.
30. Brown, B. K.; Darden, J. M.; Tovanabutra, S.; Oblander, T.; Frost, J.; Sanders-Buell, E.; de Souza, M. S.; Birx, D. L.; McCutchan, F. E.; Polonis, V. R. *Journal of virology* **2005,** *79*, 6089.
31. Owen, S. M.; Ellenberger, D.; Rayfield, M.; Wiktor, S.; Michel, P.; Grieco, M. H.; Gao, F.; Hahn, B. H.; Lal, R. B. *Journal of virology* **1998,** *72*, 5425.
32. Kortagere, S.; Madani, N.; Mankowski, M. K.; Schon, A.; Zentner, I.; Swaminathan, G.; Princiotto, A.; Anthony, K.; Oza, A.; Sierra, L. J.; Passic, S. R.; Wang, X.; Jones, D. M.; Stavale, E.; Krebs, F. C.; Martin-Garcia, J.; Freire, E.; Ptak, R. G.; Sodroski, J.; Cocklin, S.; Smith, A. B., 3rd. *Journal of virology* **2012**.
33. Zentner, I.; Sierra, L. J.; Fraser, A. K.; Maciunas, L.; Mankowski, M. K.; Vinnik, A.; Fedichev, P.; Ptak, R. G.; Martin-Garcia, J.; Cocklin, S. *ChemMedChem* **2013,** *8*, 426.
34. Zentner, I.; Sierra, L. J.; Maciunas, L.; Vinnik, A.; Fedichev, P.; Mankowski, M. K.; Ptak, R. G.; Martin-Garcia, J.; Cocklin, S. *Bioorganic & medicinal chemistry letters* **2013,** *23*, 1132.

PROJECT SC04

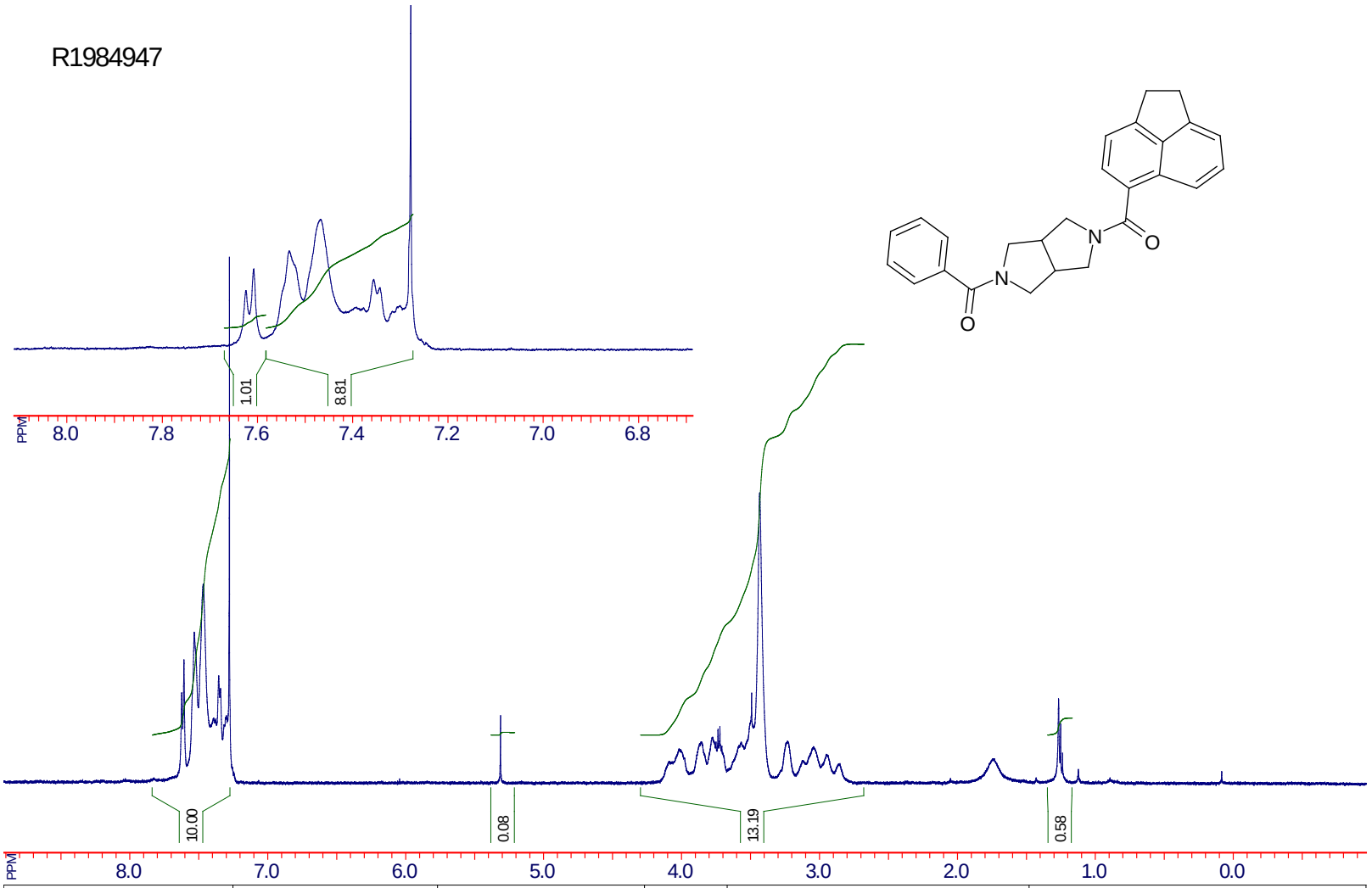
Final Report

1. Material prepared:

No.	Product Code	Structure	Amount delivered, g	Purity, %	Comments
1	**SC03**		0.041	>96%	

2. Analytical characteristics

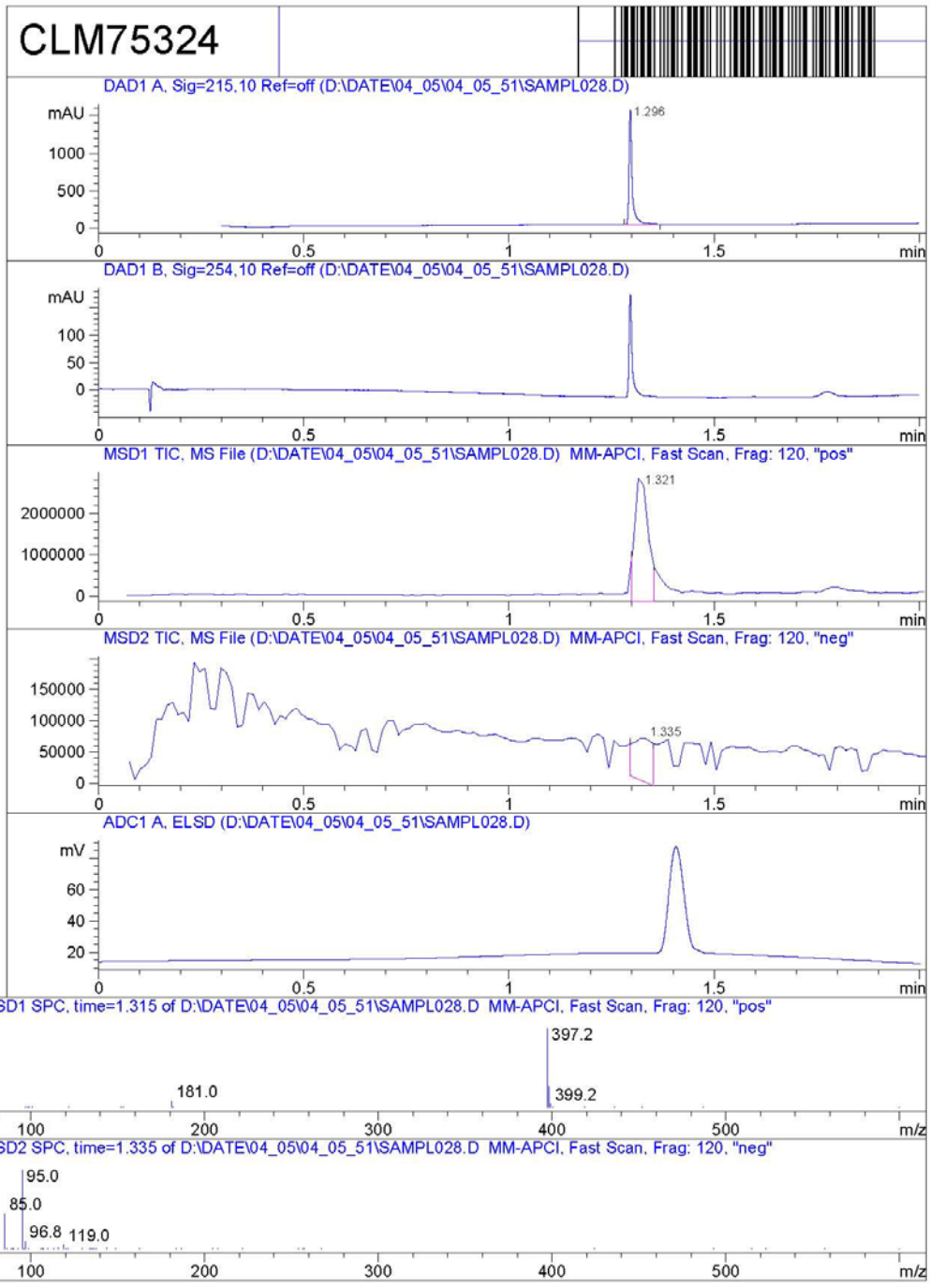
R1984947



File name: R1984947	Operator: Reutova	SF: 500.0680 MHz	NSC: 1	PW: 0.00 usec, RG: 24	SI: 32768
Date: 19-Mar-2012	Solvent: CDCl3	SW: 8503 Hz	TE: 0 K	AQ: 1.93 sec, RD: 0.00 sec	*R1984947*

MaxPeak: 100.00%
Ret_Time: 1.296 min

CLM75324



#	Time	Area%
1	1.296	100.00

Inj.Date 4/5/2012 L P2-D-01 RR Acq. Method C:\CHEM32\-> ->

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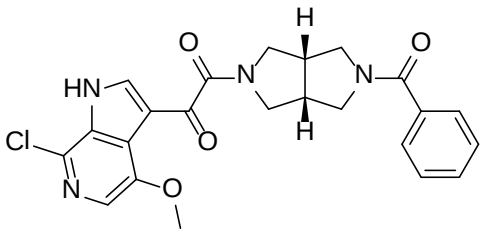
Compound Data Sheet



Molecular Weight: 452.89

DREX-006-01

Compound Information

Batch:	ASG CC-0833
Salt:	None
Amount:	10 mg
Appearance:	Yellow solid

Standard QC Analytical Report

Purity:	97.22% (335 nm) by HPLC	
^{1}H NMR:	See attached spectra	
For $C_{23}H_{21}ClN_4NaO_4$ ($M+Na^+$):	MS (ES+)	
	Calculated:	475.11
	Found:	475.1145
For $C_{23}H_{21}ClKN_4O_4$ ($M+K^+$):	MS (ES+)	
	Calculated:	491.09
	Found:	491.0872
For $C_{46}H_{42}Cl_2N_8NaO_8$ ($2M+Na^+$):	MS (ES+)	
	Calculated:	927.24
	Found:	927.2392

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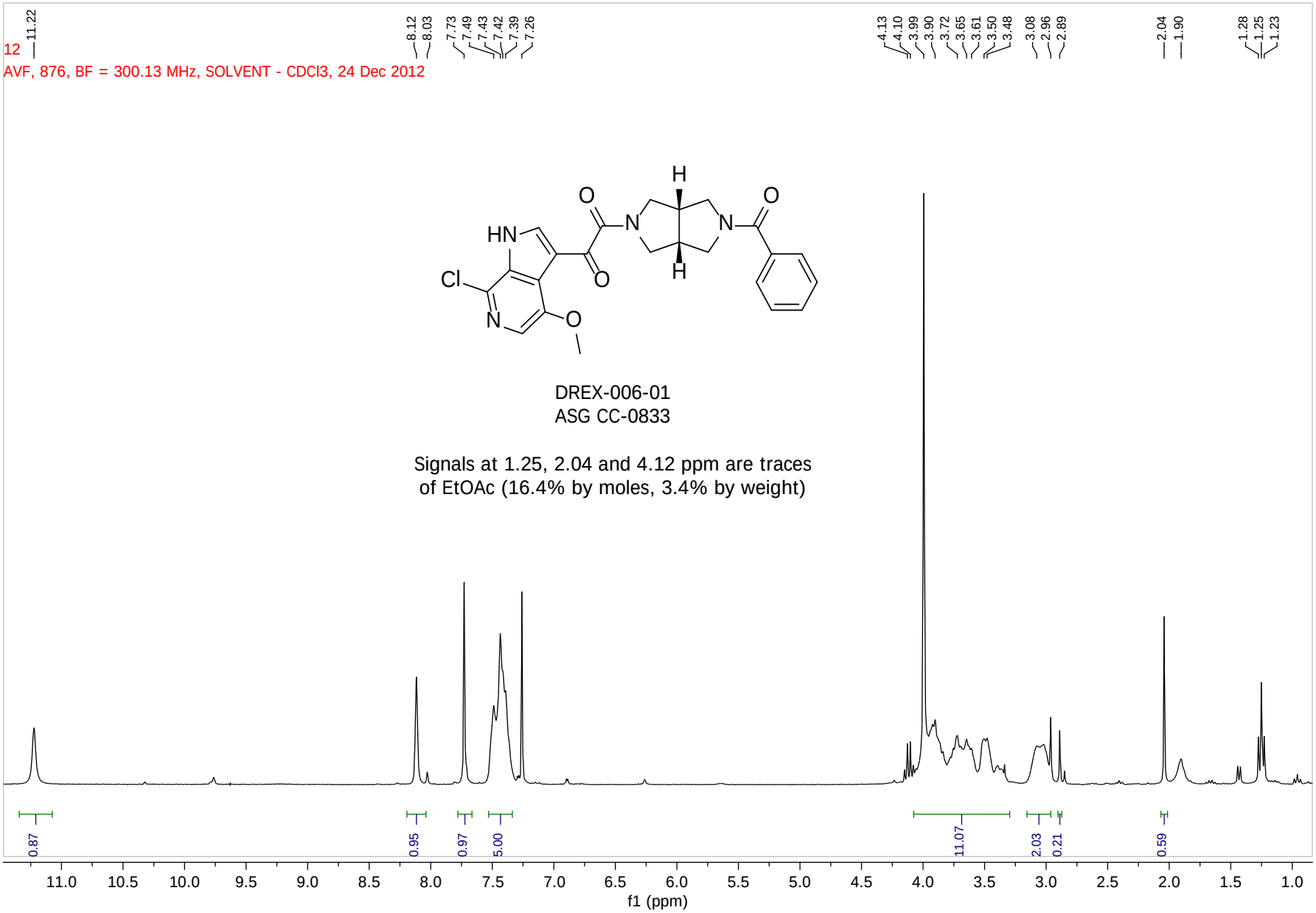
12
AVF, 876, BF = 300.13 MHz, SOLVENT - CDCl3, 24 Dec 2012
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8.03
7.73
7.49
7.43
7.42
7.39
7.26
4.13
4.10
3.99
3.90
3.72
3.65
3.61
3.50
3.48
3.08
2.96
2.89
2.04
1.90
1.28
1.25
1.23
HN
Cl
N
O
O
O
N
N
O
H
H
DREX-006-01
ASG CC-0833
Signals at 1.25, 2.04 and 4.12 ppm are traces
of EtOAc (16.4% by moles, 3.4% by weight)
0.87
0.95
0.97
5.00
11.07
2.03
0.21
0.59
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

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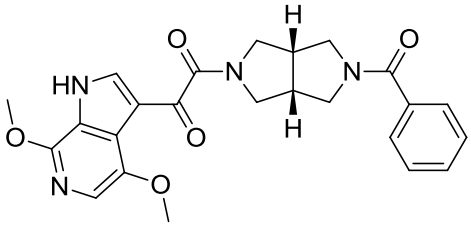
Compound Data Sheet



Molecular Weight: 448.47

DREX-006-02-FB

Compound Information

Batch:	ASG CC-0804
Salt:	None
Amount:	19 mg
Appearance:	Pale-pale green solid

Standard QC Analytical Report

Purity:	98.02% (254 nm) by HPLC	
^{1}H NMR:	See attached spectra	
For $C_{24}H_{25}N_4O_5$ ($M+H^+$):	MS (ES+)	
	Calculated:	449.18
	Found:	449.1840
For $C_{24}H_{24}N_4NaO_5$ ($M+Na^+$):	MS (ES+)	
	Calculated:	471.16
	Found:	471.1666
For $C_{24}H_{24}KN_4O_5$ ($M+K^+$):	MS (ES+)	
	Calculated:	487.14
	Found:	487.1393
For $C_{48}H_{49}N_8O_{10}$ ($2M+H^+$):	MS (ES+)	
	Calculated:	897.36
	Found:	897.3604
For $C_{48}H_{48}N_8NaO_{10}$ ($2M+Na^+$):	MS (ES+)	
	Calculated:	919.34
	Found:	919.3435
For $C_{48}H_{48}KN_8O_{10}$ ($2M+K^+$):	MS (ES+)	
	Calculated:	935.31
	Found:	935.3151

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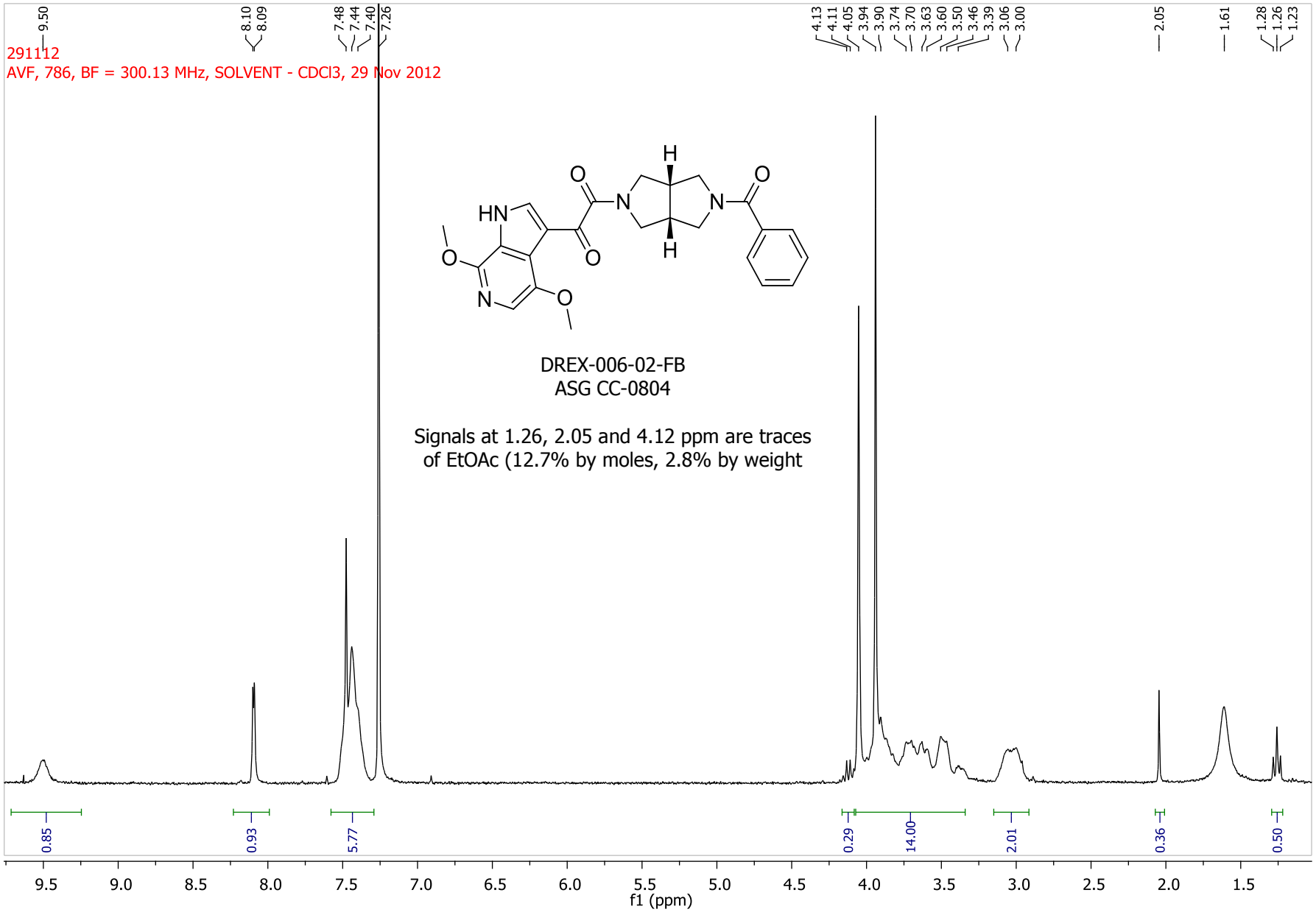
291112
AVF, 786, BF = 300.13 MHz, SOLVENT - CDCl3, 29 Nov 2012
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8.09
7.48
7.44
7.40
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4.13
4.11
4.05
3.94
3.90
3.74
3.70
3.63
3.60
3.50
3.46
3.39
3.06
3.00
2.05
1.61
1.28
1.26
1.23
DREX-006-02-FB
ASG CC-0804
Signals at 1.26, 2.05 and 4.12 ppm are traces of EtOAc (12.7% by moles, 2.8% by weight
0.85
0.93
5.77
0.29
14.00
2.01
0.36
0.50
f1 (ppm)

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LCMS Analysis Report

Compound ID: SC-11(HDBA0527-18-1)

Pump A : 0.1% formic acid in 100% water

Pump B : 0.1% formic acid in 100% acetonitrile

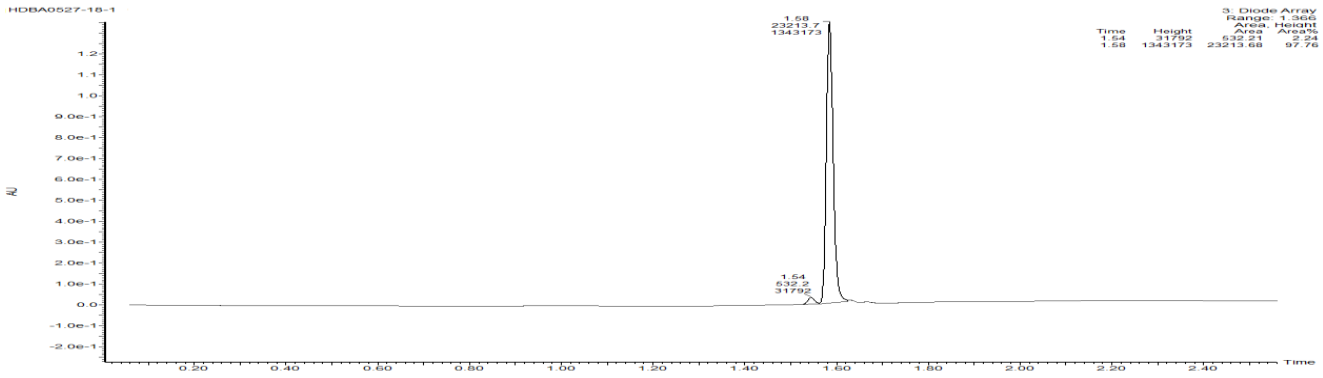
Total Flow : 0.3ml/min

Volume : 0.2ul

wave-length : 190-500nm

Gradient:	**Time**	**A**	**B**
	0.01	**80%**	**20%**
	2.50	**0%**	**100%**
	2.60	**80%**	**20%**

Column: Waters BEH C18 2.1x50mm 1.7um



Peak number	Retention time(min)	Height(Au)	Area(Au*s)	Area%
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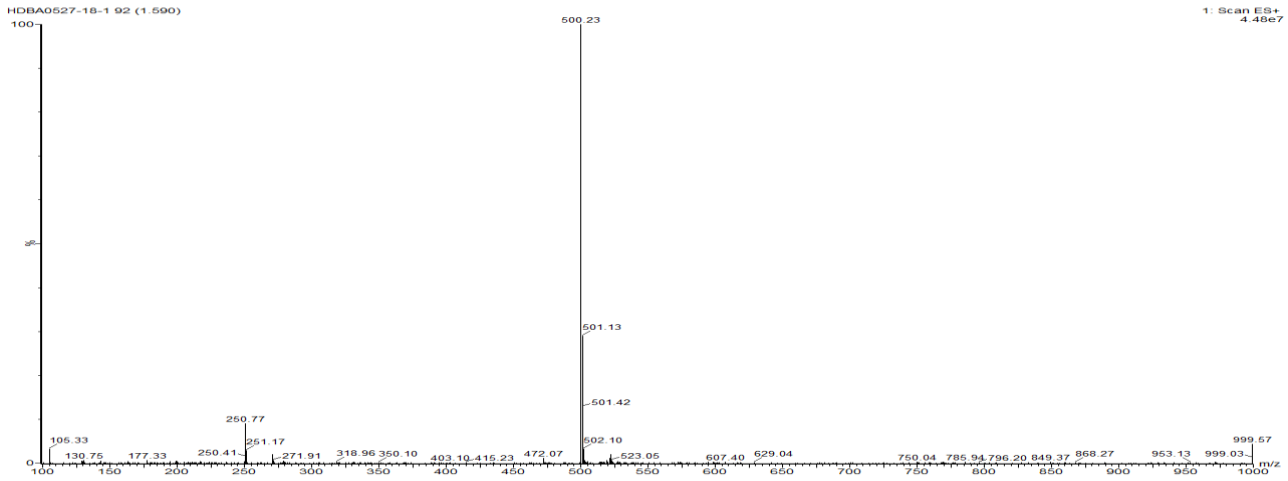
2	1.58	1343173	23213.68	97.76



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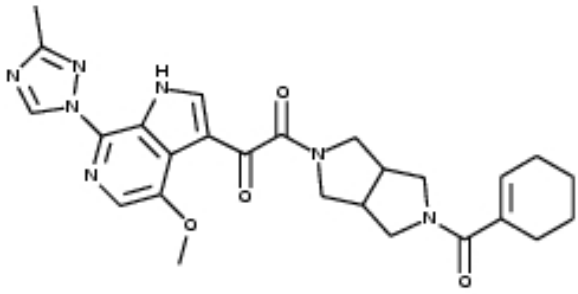
QP-140126-SC26_001 MW:503.6



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Inj. Vol. : 2ul
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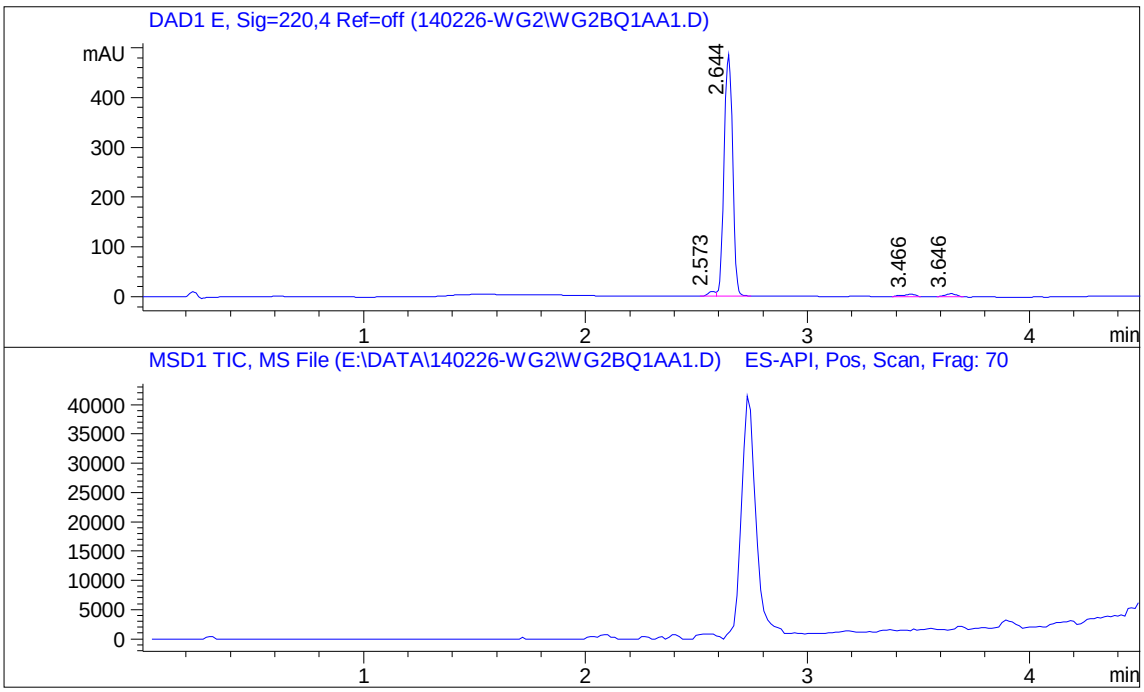
LCMS-W



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Report

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Signal 1 : DAD1 E, Sig=220,4 Ref=off

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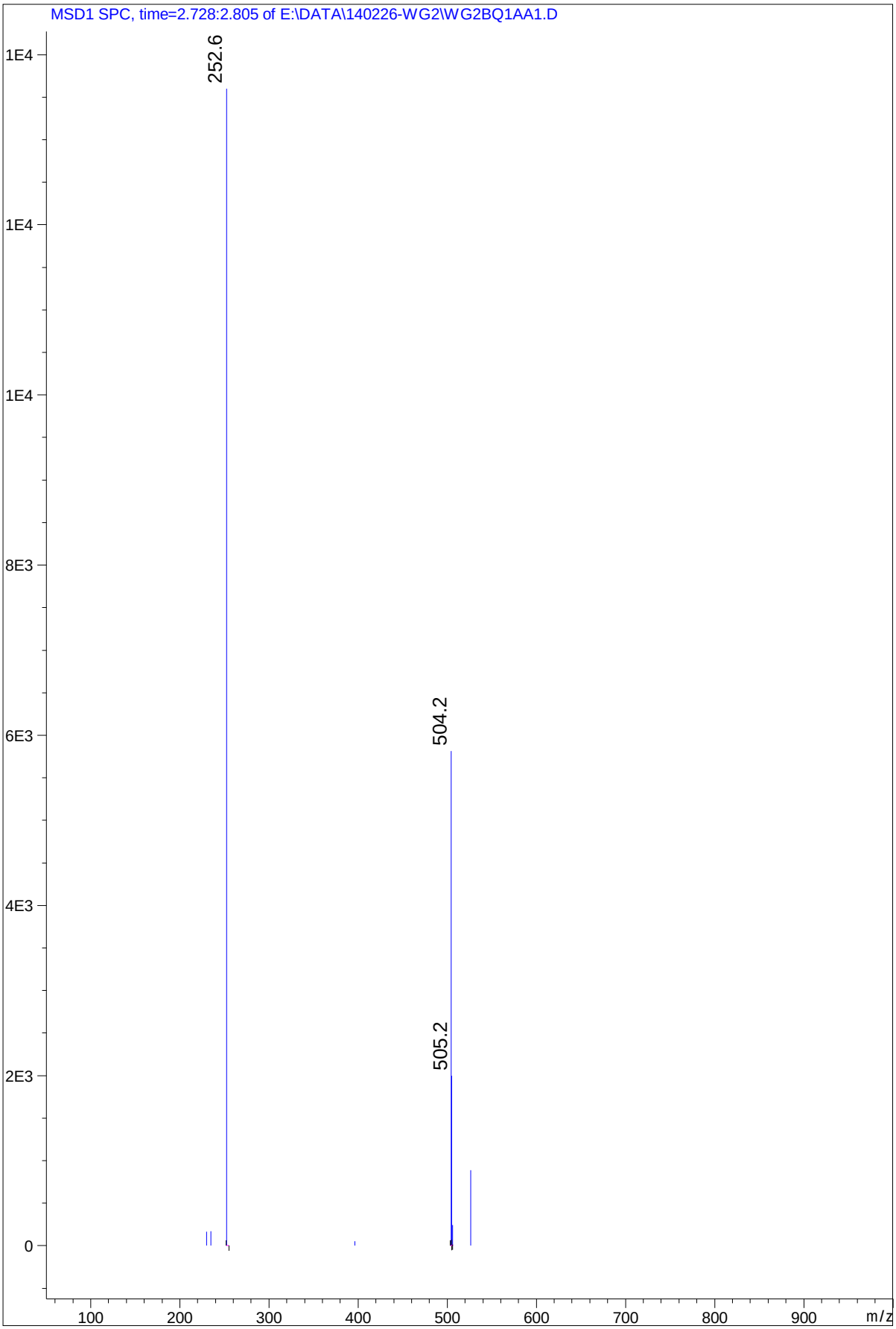
2	2.644	1299.946	484.754	95.754	0.043	95.120
3	3.466	22.216	5.468	1.080	0.068	1.626
4	3.646	21.815	6.306	1.246	0.058	1.596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6E3
4E3
2E3
0
100
200
300
400
500
600
700
800
900
m/z