Supplementary Information

Part 1: Supplementary Figures, Legends and Experimental Procedures

A pairwise chemical genetic screen identifies new inhibitors of glucose transport

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Supplemental Figures

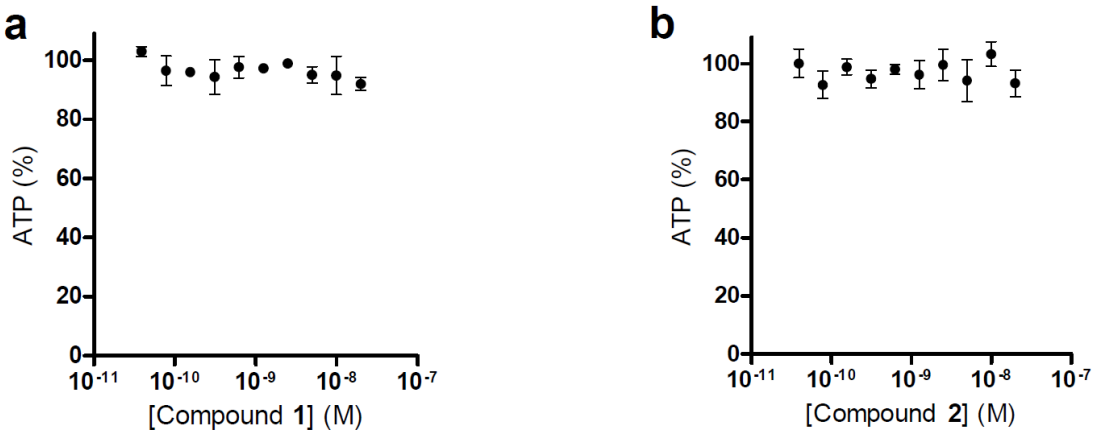


Figure S1 (Supports Figure 1). Effects of leucascandrolide A analog (**1**) and antimycin A (**2**) on intracellular ATP levels in A549 cells.

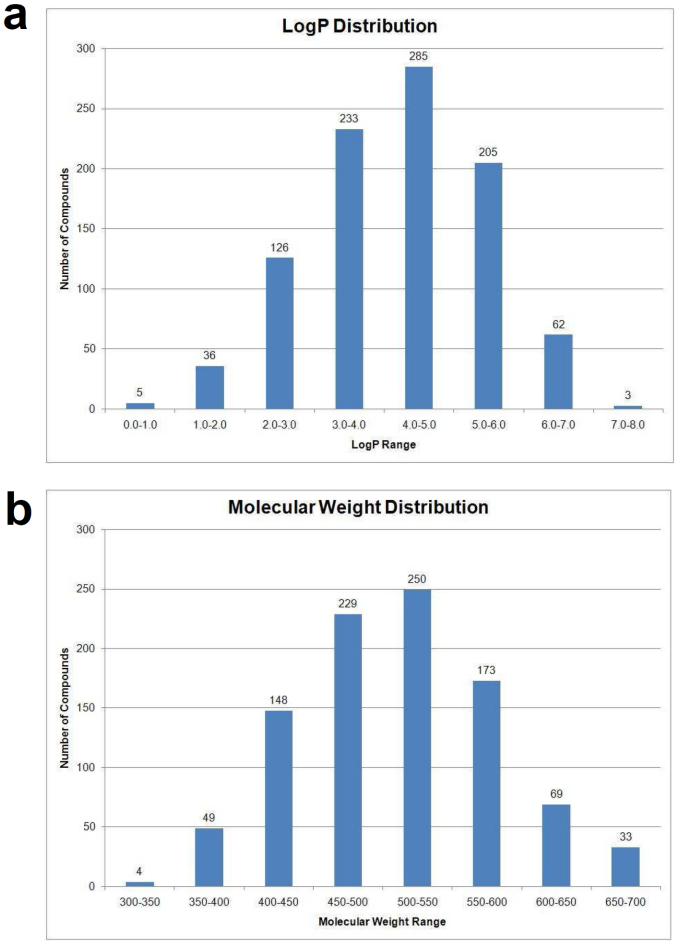


Figure S2 (Supports Figure 2). Distribution of (**a**) LogP and (**b**) molecular weight for the pyrrolidinone-based library. LogPs were calculated using ALOGPS 2.1 software from Virtual Computational Chemistry Laboratory (www.vcclab.org)[1]. Molecular weights were calculated from the SMILES sequences using ChemDraw/Excel.

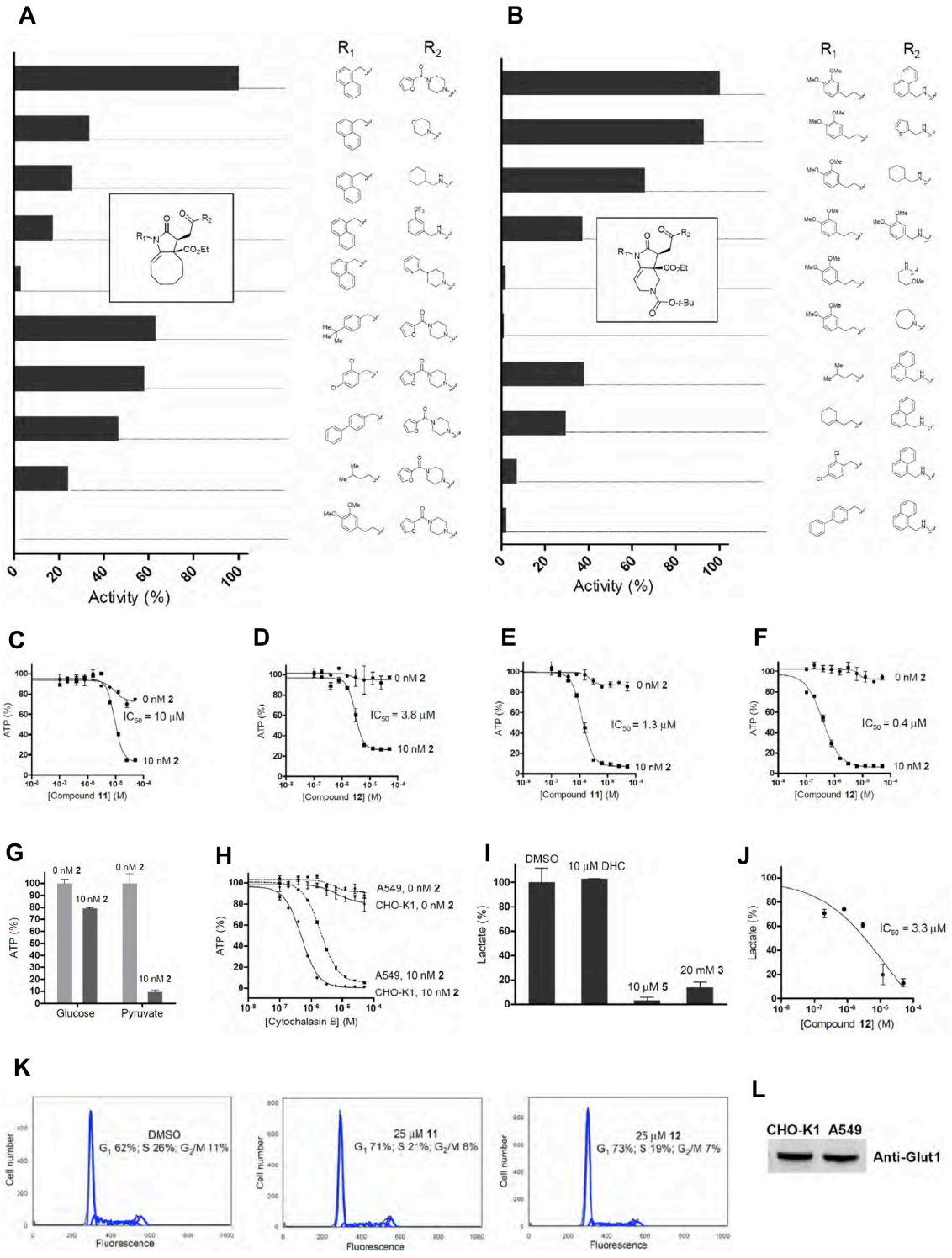


Figure S3 (Supports Figure 3). A. Structure–activity relationship studies on cyclooctapyrrolone derivatives. Relative activities were calculated using primary screening data, which corresponds to the ability of the compound to inhibit intracellular ATP levels in A549 cells in the presence of 10 nM antimycin A; compound **11** was

assigned a relative activity of 100%. B. Structure–activity relationship studies on azaindolone derivatives. Relative activities were calculated using primary screening data, which corresponds to the ability of the compound to inhibit intracellular ATP levels in A549 cells in the presence of 10 nM antimycin A; compound **12** was assigned a relative activity of 100%. C. Effect of **11** on intracellular ATP levels in the presence and absence of mitochondrial inhibitor **2** in PC3 cells. D. Effect of **12** on intracellular ATP levels in the presence and absence of mitochondrial inhibitor **2** in PC3 cells. E. Effect of **11** on intracellular ATP levels in the presence and absence of mitochondrial inhibitor **2** in U373 cells. F. Effect of **12** on intracellular ATP levels in the presence and absence of mitochondrial inhibitor **2** in U373 cells. G. Sensitization of CHO-K1 cells to mitochondrial inhibitor **2** by replacing glucose (3 g/l) with pyruvate (0.22 g/l) in DMEM culture medium. H. Effect of cytochalasin B (**5**) on intracellular ATP levels in the presence and absence of mitochondrial inhibitor **2** in A549 and CHO-K1 cells. I. Effect of dihydrocytochalasin B (DHC), cytochalasin B and 2-deoxy-D-glucose on lactate production in CHO-K1 cells. J. Suppression of lactate production in PC3 cells by compound **12**. K. Cell-cycle regulation by **11** and **12** in A549 cells. Data were approximated using Watson Pragmatic statistical model. L. Expression of glucose transporter isoform 1 (Glut1) in CHO-K1 and A549 cells.

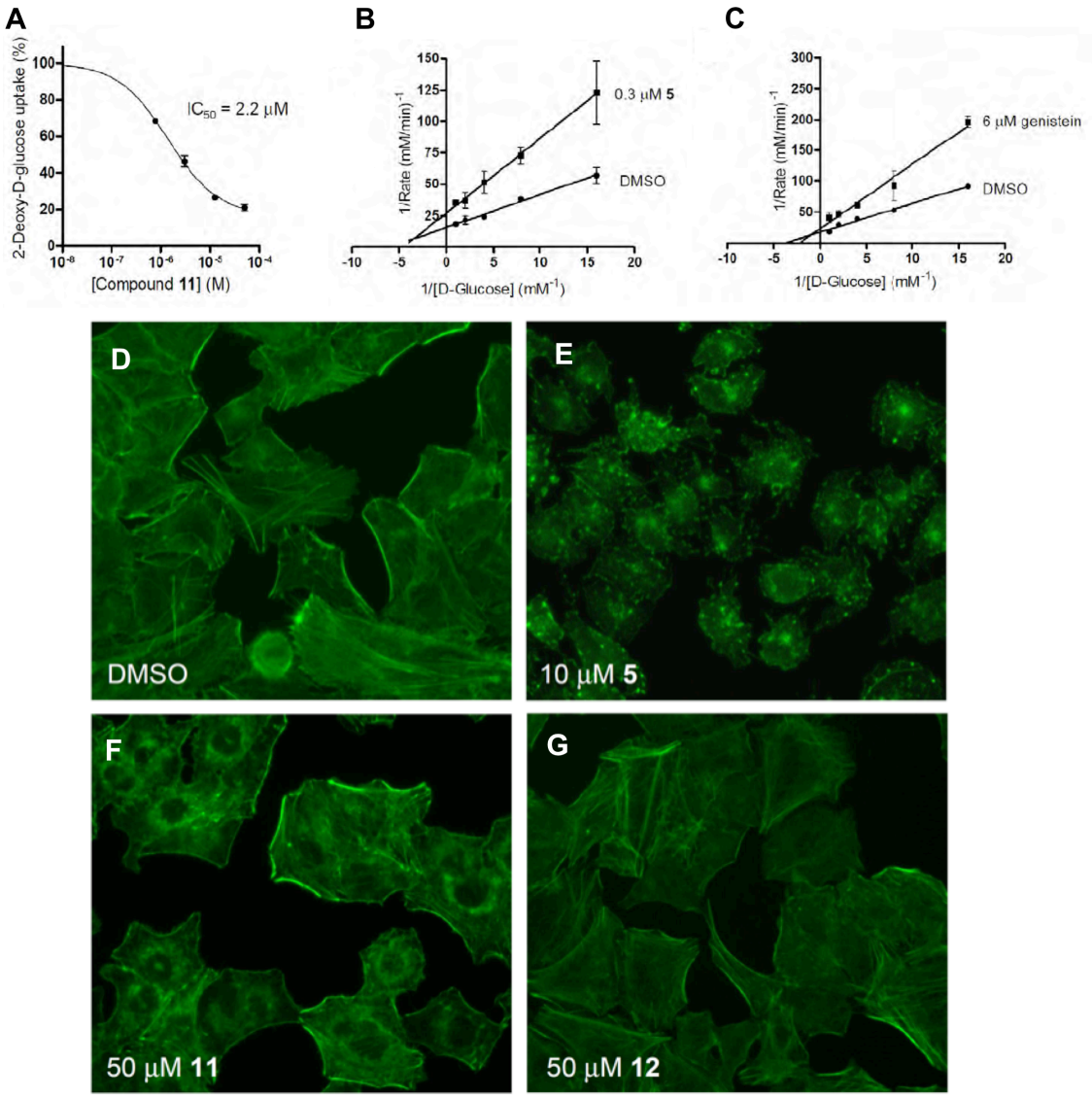


Figure S4 (Supports Figure 4). A. Inhibition of 2-deoxy-D-glucose uptake in U373 cells by compounds **11**. B. Non-competitive inhibition of D-glucose uptake in erythrocyte ghosts by cytochalasin B (**5**). C. Competitive inhibition of D-glucose uptake in erythrocyte ghosts by genistein. D. F-actin staining of A549 cells with Alexa Fluor 488 Phalloidin. E. Cytochalasin B (**5**) depolymerizes F-actin in A549 cells at 10 μM concentration. F. Compound **11** does not depolymerize F-actin in A549 cells at 50 μM concentration. G. Compound **12** does not depolymerize F-actin in A549 cells at 50 μM concentration.

Supplemental Experimental Procedures

General. Dichloromethane (HPLC grade), ethyl acetate (ACS grade), hexanes (ACS grade), diethyl ether (ACS grade) were purchased from Fisher Scientific and used without further purification. Anhydrous tetrahydrofuran was purified by distillation from sodium-benzophenone. Commercially available reagents were used without further purification. Reactions were monitored by thin layer chromatography (TLC) using Whatman precoated glass silica gel plates. Flash column chromatography was performed over Silacycle silica gel (230-400 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DRX-400 or DMX-500 spectrometers using residual solvent peaks as an internal standard. Mass Spectra were recorded with a VG Instruments Model 7070EQ tandem mass spectrometer. All cell lines, media, serum and supplements were purchased from ATCC unless otherwise noted. Antimycin A, 2-deoxyglucose, sodium iodoacetate, cytochalasin B, dihydrocytochalasin B and genistein were purchased from Sigma-Aldrich. Methyl-D-Glucose, 3-O(methyl-^{3}H) (80 Ci/mmol) and Glucose, D-(1-^{3}H) (20 Ci/mmol) were purchased from American Radiolabeled Chemicals. 2-[1,2-(N)-^{3}H] 2-deoxy-D-glucose (33.8 Ci/mmol) was purchased from Perkin Elmer. All other reagents were purchased from Sigma-Aldrich unless otherwise stated. Culture dishes, 96-well plates and all other supplies were purchased from Fisher Scientific.

Cell Culture. PC3 cell line was purchased from ATCC and maintained in F12-K medium. U87 and U373 cell lines were obtained from Dr. Ulasov and were maintained in DMEM medium. All media were supplemented with 10% FBS and 1% Penicillin-Stretptomycin-Glutamine solution.

Cell-cycle analysis. A549 cells were seeded into treated polystyrene dishes at density of 0.5 x 10^{6} cells/dish and incubated overnight. Old medium was removed and cells were treated with different concentrations of small molecules for 24 h. After trypsinization, cells were washed with PBS, fixed in 70% ethanol and stained with 10 mg/ml propidium iodide solution containing 0.5 mg/ml RNase A. DNA content was analyzed by flow cytometry on BD FACScanto flow cytometer. All data were processed on FlowJo software.

Western blotting. After cell lysis, protein samples (50 μg/lane) were separated using SDS-polyacrylamide gel electrophoresis Samples were then transferred to a nitrocellulose membrane, blocked for 1h with 5 % milk and incubated with anti-Glut1 (Millipore, 07-1401, 1:1000 dilution) overnight at +4°C. Next day membranes were stained with goat anti-rabbit Ig-FITC (SouthernBiotech, 4010-01, 1:10000 dilution), followed by visualization of labeled bands.

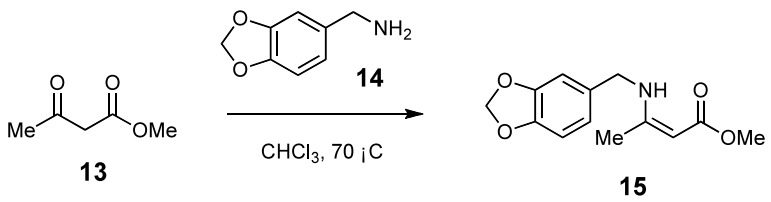
Uptake of 2-deoxy-D-glucose. U373 cells were plated at a concentration of 90,000 cells/well in six-well plates and incubated for 48h. Cells were washed twice with glucose-free buffer (138 mM NaCl, 0.3 mM Na_2HPO_4, 0.4 mM $MgSO_4$, 0.5 mM $MgCl_2$, 5 mM KCl, 0.3 mM KH_2PO_4, 1.3 mM $CaCl_2$), followed by 5 min incubation with small molecules. Cell were next treated with glucose free buffer containing 2-deoxy-D-glucose (100 μM + 1 μCi/ml), and glucose uptake was allowed to proceed for 5 min. Then, cells were washed twice with glucose-free buffer containing 0.25 mM phloretin and dissolved in 0.1 N NaOH. After addition of scintillation liquid, each sample was counted using LS-6000IC scintillation counter. Non-specific uptake was assessed by treatment of cells with 0.1 mM phloretin and was excluded from all samples. All samples were done in duplicate.

F-actin staining. 0.5 x 10^6 A549 cells grown on coverslips were incubated with small molecules for 3.5 h, fixed with 4% formaldehyde and permeabilized with 0.1% Triton X-100. After washing with PBS, cells were stained with Alexa Fluor 488 for 1h. Cellular actin was visualized using Leica DMI6000 microscope. All data were processed on ImageJ software.

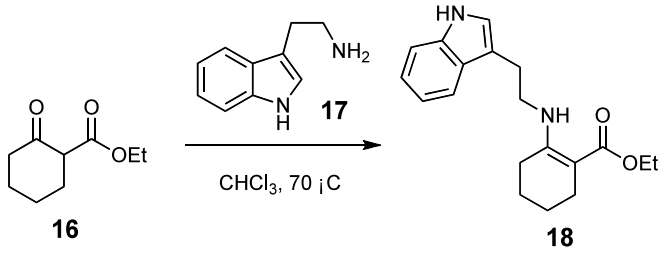
Chemical Library Synthesis. The following procedure represents the synthesis of the first set of 96 compounds. Eight 1.5 ml polypropylene centrifuge tubes were charged with $CHCl_3$ (0.8 ml each), methyl acetoacetate (0.5 mmol each) and treated individually with eight primary amines (**II**, 0.5 mmol) at 70 °C in a sand bath. The vinylogous amides **III** were purified by preparative TLC (ethyl acetate: hexanes = 1:5 to 1:1), dissolved in $CHCl_3$ (0.8 ml) and treated with maleic anhydride (0.3-0.5 mmol) at 20 °C. Upon completion of each reaction, the mixtures were diluted with $CHCl_3$ (1.6 ml) and THF (4.8 ml), followed by treatment with *N*-hydroxysuccinimide (0.38-0.63 mmol) and PS-carbodiimide resin (1.1 mmol/g, 345-573 mg). The reaction mixtures were stirred for 2-4 h at 20 °C, filtered, concentrated and purified by preparative TLC (ethyl acetate: hexanes = 2:1) to give the eight corresponding succinimide esters **IV**, which were diluted with

CH_2Cl_2 to final concentrations of 0.1 M. Aliquots (25 µl) of each resulting stock solutions were transferred into a polypropylene 96-well PCR plate and treated with 12 amines **V** (4 µmol per well) and CH_2Cl_2 (30 µl per well) using the plate maps shown in Part 2 of the Supplementary Information. Upon completion, the reaction mixtures were transferred onto preparative TLC plates using a multichannel pipettor with adjustable gaps. The plates were developed in ethyl acetate/hexanes (3:2). The products **VI** were detected under UV light and removed from TLC plates as circular silica gel pellets. Each compound was eluted from silica gel with ethyl acetate (0.6 ml). Following removal of the solvent *in vacuo*, 12 randomly selected compounds were dissolved in CD_3OD (0.5 ml) and analyzed by ^1H NMR. The amount of material in each sample was determined by integration using residual MeOH as a precalibrated internal standard. This protocol was used next to prepare all the remaining library members.

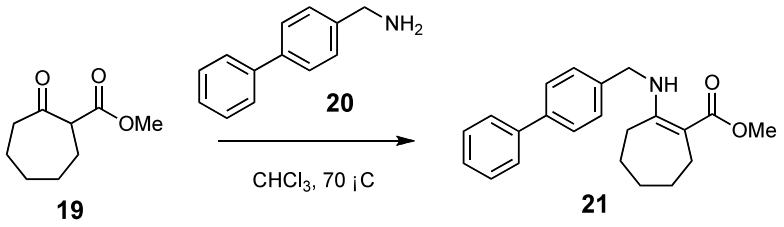
General Protocol A: Preparation of Vinylogous Amides



Ketoester **13** (23.2 mg 0.2 mmol) was dissolved in 0.8 ml of $CHCl_3$ in a 1.5 ml polypropylene Eppendorf centrifuge tube, and treated with amine **14** (45.3 mg, 37.4 µl, 0.3 mmol). The tube was capped and heated to 70 ^oC using a sand bath. The progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was concentrated under reduced pressure and purified by flash chromatography on silica gel (ethyl acetate: hexanes = 1:5 to 1:1) to give 44.8 mg (90%) of vinylogous amide **15**. ^1H NMR (400 MHz, $CDCl_3$) δ 8.85 (br s, 1H), 6.69-6.77 (m, 3H), 4.93 (s, 2H), 4.52 (s, 1H), 4.31 (d, 2H, *J* = 6.4 Hz), 3.62 (s, 3H), 1.91 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$), δ 170.82, 161.71, 148.03, 146.85, 132.48, 119.91, 108.36, 107.36, 101.03, 82.76, 49.94, 46.58, 19.31.; MS (APCI) calculated for $C_{13}H_{15}NO_4$ 249.10 (M^+), found 250.1 (M+H)

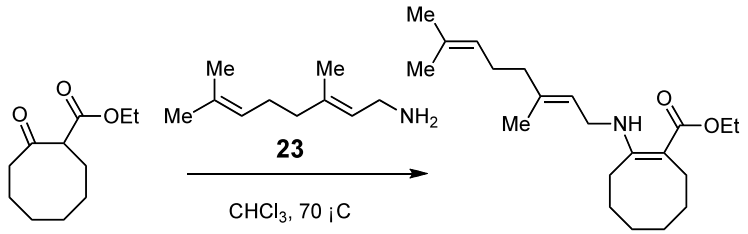


Compound **18** was prepared in 84% yield according to General Protocol A. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.07 (br s, 1H), 8.16 (br s, 1H), 7.58 (d, 1H, *J* = 7.5 Hz), 7.33 (d, 1H, *J* = 8 Hz), 7.20 (t, 1H, *J* = 7 Hz), 7.13 (t, 1H, *J* = 7.5 Hz), 7.04 (d, 1H, *J* = 2.5 Hz), 4.14 (q, 2H, *J* = 7.2 Hz), 3.49 (dt, 2H, *J* = 7 Hz, 6 Hz), 3.02 (t, 2H, *J* = 7.3 Hz), 2.28-2.30 (m, 4H), 1.61-1.63 (m, 2H), 1.54-1.57 (m, 2H), 1.28 (t, 3H, *J* = 7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.88, 159.51, 136.26, 127.15, 122.21, 121.91, 119.22, 118.50, 112.89, 111.20, 89.36, 58.58, 42.81, 26.52, 26.43, 23.80, 22.66, 22.25, 14.65.



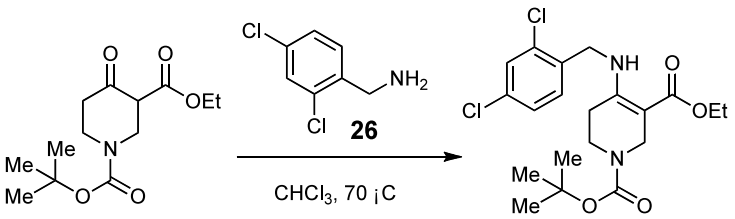
19 **21**

Compound **21** was prepared in 79% yield according to General Protocol A. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.72 (br s, 1H), 7.57-7.60 (m, 4H), 7.43-7.46 (m, 2H), 7.34-7.36 (m, 3H), 4.50 (d, 2H, *J* = 6.5 Hz), 3.69 (s, 3H), 2.48-2.53 (m, 4H), 1.68-1.70 (m, 2H), 1.45-1.50 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.00, 167.45, 140.71, 140.04, 138.69, 128.72, 127.34, 127.21, 127.17, 126.99, 95.07, 50.40, 46.58, 31.78, 28.74, 28.34, 25.86, 25.02.



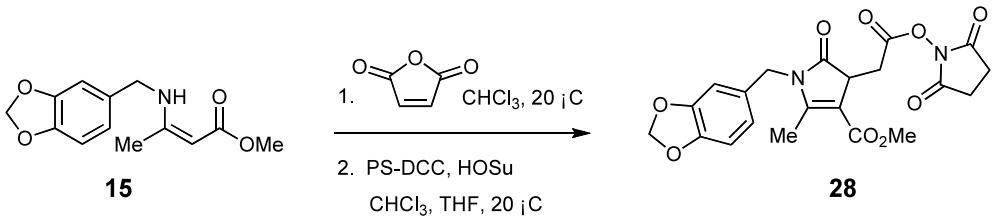
22 **24**

Compound **24** was prepared in 57% yield according to General Protocol A. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (br s, 1H), 5.24-5.26 (m, 1H), 5.07-5.09 (m, 1H), 4.10 (q, 2H, *J* = 7.1 Hz), 3.79-3.82 (m, 2H), 2.48-2.51 (m, 2H), 2.40 (br, 2H), 2.05-2.10 (m, 2H), 1.98-2.01 (m, 2H), 1.59-1.67 (m, 11H), 1.45-1.50 (m, 6H), 1.24 (t, *J* = 7 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.70, 163.20, 138.33, 131.66, 123.88, 121.55, 91.74, 58.42, 40.88, 39.46, 30.62, 28.58, 26.71, 26.34, 26.25, 25.66, 25.51, 17.68, 16.30, 14.70.

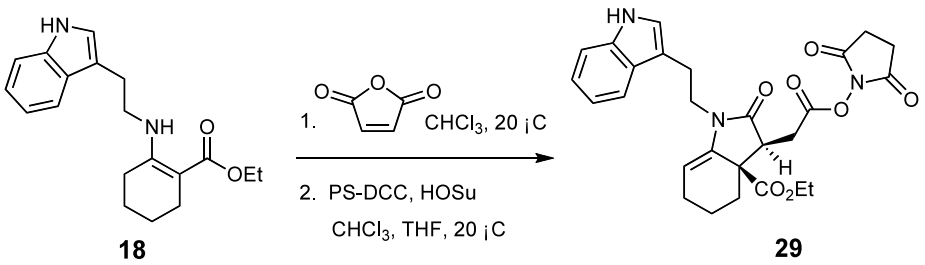


Compound **27** was prepared in 73% yield according to General Protocol A. ^{1}H NMR (500 MHz, CD_3CN, 298 K) δ 9.15 (s, 1H), 7.48 (d, 1H, *J* = 2 Hz), 7.27-7.37 (m, 2H), 4.45 (d, 2H, *J* = 6.5 Hz), 4.10 (q, 2H, *J* = 7 Hz), 4.00 (s, 2H), 3.41 (t, 2H, *J* = 6 Hz), 2.33 (t, 2H, *J* = 6 Hz), 1.43 (s, 9H), 1.22 (t, 3H, *J* = 7.3 Hz). ^{13}C NMR (125 MHz, CD_3CN, 298 K) δ 169.49, 158.77, 155.25, 137.18, 134.25, 134.19, 130.73, 130.07, 128.54, 80.07, 59.82, 44.00, 28.52, 26.26, 14.85.

General Protocol B: Preparation of Activated Esters

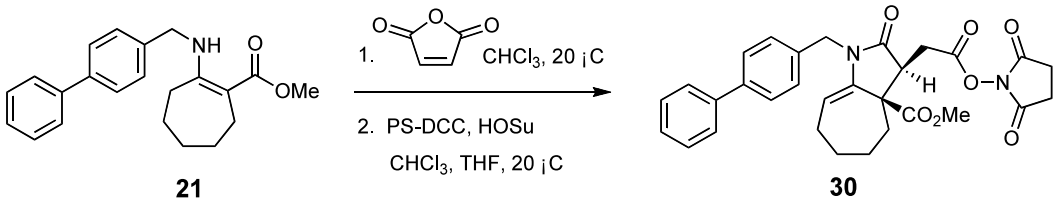


Vinylogous amide **15** (43.1 mg, 0.173 mmol) was dissolved in $CHCl_3$ (0.8 ml) and treated with maleic anhydride (20.3 mg, 1.2eq) at room temperature. The progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was diluted with 0.8 ml of $CHCl_3$ and 2.4 ml of THF, followed by treatment with N-hydroxysuccinimide (27.3 mg, 1.5 eq) and PS-carbodiimide resin (1.1 mmol/g, 157 mg, 1.5 eq). The progress of the reaction was monitored by TLC. The reaction mixture was filtered, concentrated and purified by flash chromatography on silica gel (ethyl acetate: hexanes = 1:1) to give 26.8 mg of activated ester **28** (67% yield for the two steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.67-6.73 (m, 3H), 5.92 (d, 2H, *J* = 4 Hz), 4.62-4.71 (m, 2H), 3.74 (s, 3H), 3.64 (br s, 1H), 3.41 (dd, 1H, *J* = 5.8 Hz, 15.8 Hz), 3.29 (dd, 1H, *J* = 4.3 Hz, 16 Hz), 2.77 (br s, 4H), 2.38 (d, 3H, *J* = 2 Hz); ^{13}C NMR (125 Hz, $CDCl_3$), δ 176.90, 168.56, 165.87, 164.07, 156.41, 148.00, 147.07, 130.11, 120.57, 108.30, 107.79, 104.94, 101.09, 51.08, 43.60, 42.57, 31.29, 25.46, 12.79.; MS (APCI) calculated for $C_{21}H_{20}N_2O_9$ 444.12 (M^+), found 445.0 (M+H)



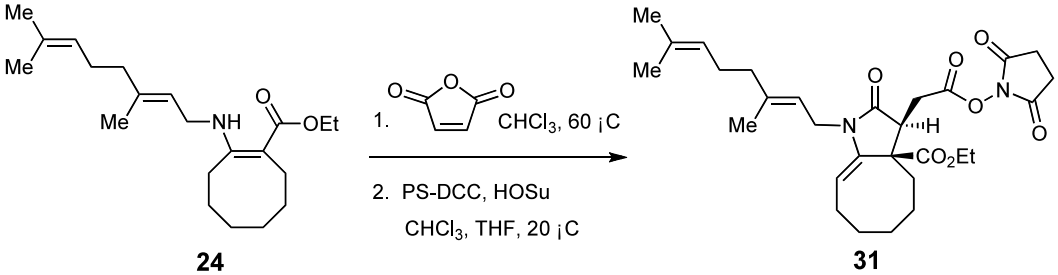
18 **29**

Compound **29** was prepared in 84% yield according to General Protocol B. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (br s, 1H), 7.64 (d, 1H, *J* = 7.5 Hz), 7.36 (d, 1H, *J* = 8 Hz), 7.17-7.20 (m, 1H), 7.11-7.14 (m, 1H), 7.08 (d, 1H, *J* = 2 Hz), 5.11 (m, 1H), 4.12-4.21 (m, 2H), 3.97-4.03 (m, 1H), 3.55-3.60 (m, 1H), 3.26 (dd, 1H, *J* = 5 Hz, 17.5 Hz), 2.93-3.16 (m, 3H), 2.81 (br s, 4H), 2.66-2.68 (m, 1H), 2.60 (dd, 1H, *J* = 9.5 Hz, 17.5 Hz), 2.25-2.31 (m, 1H), 2.10-2.16 (m, 1H), 1.85-1.89 (m, 1H), 1.48-1.60 (m, 2H), 1.23 (t, 3H, *J* = 7.8 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.33, 171.22, 168.80, 167.38, 138.00, 136.19, 127.29, 121.97, 119.31, 118.57, 112.65, 111.19, 101.00, 61.55, 52.32, 47.28, 40.84, 30.57, 28.58, 25.51, 22.75, 22.36, 19.60, 14.04.; MS (APCI) calculated for $C_{27}H_{29}N_3O_7$ 507.20 (M^+), found 508.1 (M+H).



21 **30**

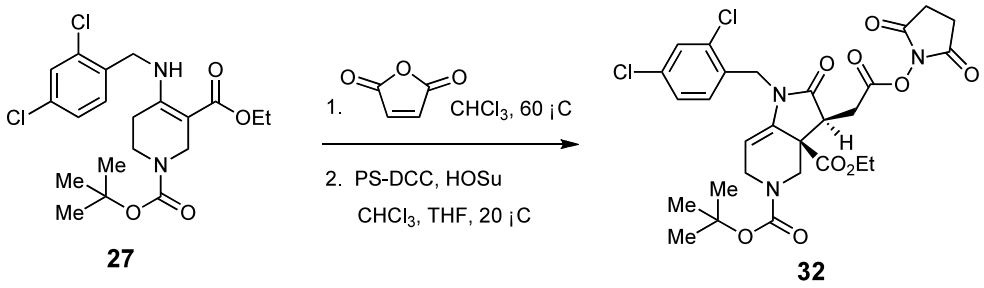
Compound **30** was prepared in 63% yield according to General Protocol B. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54-7.59 (m, 4H), 7.41-7.44 (m, 2H), 7.30-7.35 (m, 3H), 5.16 (dd, 1H, *J* = 4 Hz, 9Hz), 4.85 (d, 1H, *J* = 15.5 Hz), 4.68 (d, 1H, *J* = 15.5 Hz), 3.77 (s, 3H), 3.29 (dd, 1H, *J* = 5.5 Hz, 17.5 Hz), 3.21-3.24 (m, 1H), 2.84 (s, 4H), 2.60-2.84 (m, 2H), 2.12-2.18 (m, 1H), 1.85-1.94 (m, 2H), 1.61-1.72 (m, 3H), 1.19-1.22 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.88, 171.12, 168.72, 167.22, 142.38, 140.68, 140.14, 134.54, 128.71, 127.35, 127.23, 127.18, 126.98, 106.05, 55.68, 52.33, 48.07, 44.03, 36.13, 29.32, 27.68, 27.19, 26.03, 25.55.



24 **31**

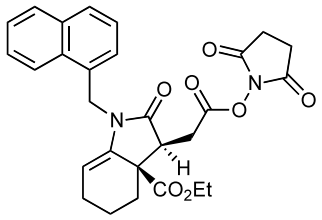
Compound **31** was prepared in 87% yield according to General Protocol B. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.98-5.06 (m, 2H), 4.80 (dd, 1H, *J* = 8 Hz, 5.3 Hz), 4.18-4.28 (m, 3H), 4.07 (dd, 1H, *J* =

6.5 Hz, 7.8 Hz), 4.20 (dd, 1H, *J* = 5 Hz, 9 Hz), 3.02 (dd, 1H, *J* = 4.8 Hz, 4.3 Hz), 4.81 (br s, 4H), (dd, 1H, *J* = 8.8 Hz, 8.9 Hz), 2.59-2.63 (m, 1H), 1.96-2.08 (m, 7H), 1.55-1.74 (m, 15H), 1.36-1.46 (m, 2H), 1.25 (t, 3H, *J* = 7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.33, 171.17, 168.66, 167.25, 140.44, 139.24, 131.66, 123.73, 118.09, 103.28, 61.63, 53.63, 47.92, 39.34, 39.13, 38.67, 29.53, 27.96, 26.31, 25.81, 25.62, 25.50, 22.96, 22.58, 17.62, 16.47, 13.98.



Compound **32** was prepared according to General Protocol B. Due to the low stability, this activated ester was used directly for the next amidation step.

X-Ray Crystallographic Characterization of Ester 33.



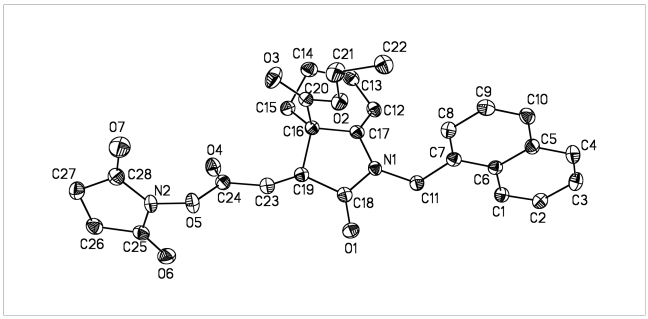
33

Data Collection

An irregular broken fragment (0.16 x 0.16 x 0.16 mm) was selected under a stereo-microscope while immersed in Fluorolube oil to avoid possible reaction with air. The crystal was removed from the oil using a tapered glass fiber that also served to hold the crystal for data collection. The crystal was mounted and centered on a Bruker SMART APEX system at 100 K. Rotation and still images showed the diffractions to be sharp. Frames separated in reciprocal space were obtained and provided an orientation matrix and initial cell parameters. Final cell parameters were obtained from the full data set. A “full sphere” data set was obtained which samples approximately all of reciprocal space to a resolution of 0.75 Å using 0.3o steps in w using 10 second integration times for each frame. Data collection was made at 100 K. Integration of intensities and refinement of cell parameters were done using SAINT. Absorption corrections were applied using SADABS based on redundant diffractions.

Structure solution and refinement. The space group was determined as P1(bar) based on systematic absences and intensity statistics. Direct methods were used to locate most C atoms

and from the E-map. Repeated difference Fourier maps allowed recognition of all expected C, N, and O atoms. Following anisotropic refinement of all non-H atoms, ideal H-atom positions were calculated. Final refinement was anisotropic for all non-H atoms, and isotropic-riding for H atoms. No anomalous bond lengths or thermal parameters were noted. The ORTEP diagram of **33** shown below is drawn with 50% probability ellipsoids.

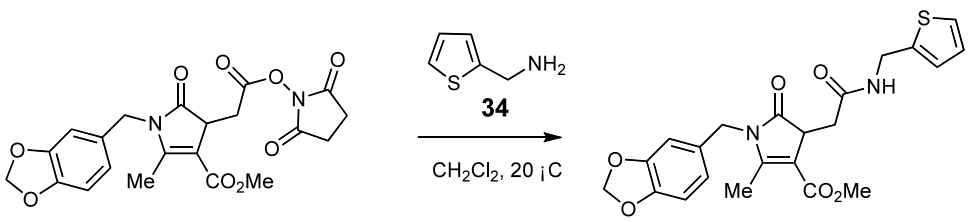


Crystal and structure refinement for **33**.

Identification Code	Jcui01	
Empirical formula	$C_{28}H_{28}N_2O_7$	
Formula weight	504.52	
Temperature	100 K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space Group	P1(bar)	
Unit cell dimensions	*a* = 9.485(3) Å	α = 105.968(4) ^{o}
	b = 10.685(3) Å	β = 106.660(4) ^{o}
	c = 12.690(3) Å	γ = 93.538(5) ^{o}
Volume	1170.7(5) Å^3	
Z	2	
Density (calculated)	1.431 Mg/m^3	
Absorption coefficient	0.104 mm^{-1}	
F(000)	532	
Crystal size, color, habit	0.16 x 0.16 x 0.16 mm, clear, fragment	
Theta range for data collection	2.01 – 28.43 ^{o}	
Index ranges	-12 ≤ h ≤ 12, -14 ≤ k ≤ 14, -16 ≤ l ≤ 16	
Reflections collected	13,789	
Independent reflections	5,535 (R_{int} = 0.0374)	
Reflections with I > 4σ(F_o)	3,878	
Absorption correction	SADABS based on redundant diffractions	

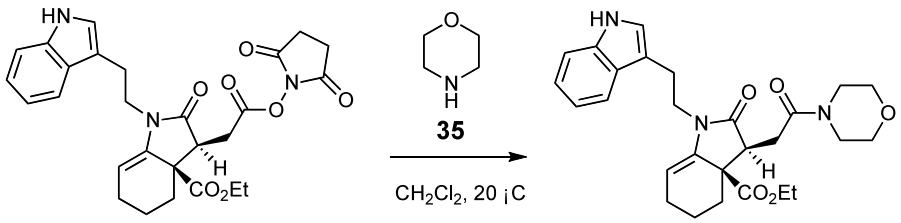
Max. and min. transmission	1.0, 0.742
Refinement method	Full-matrix least squares on F^2
Weighting scheme	$w = q [\sigma^2 (F_o^2) + (aP)^2 + bP]^{-1}$ where:
	$P = (F_o^2 + 2F_c^2)/3$, a = 0.057, b = 0.0, q =1
Data / restraints / parameters	5535 / 0 / 335
Goodness-of-fit on F^2	0.886
Final R indices [I > 2 sigma(I)]	R1 = 0.0470, wR2 = 0.0990
R indices (all data)	R1 = 0.0697, wR2 = 0.1081
Largest diff. peak and hole	0.317, -0.213 eÅ $^{-3}$

General Protocol C: Amine Condensation



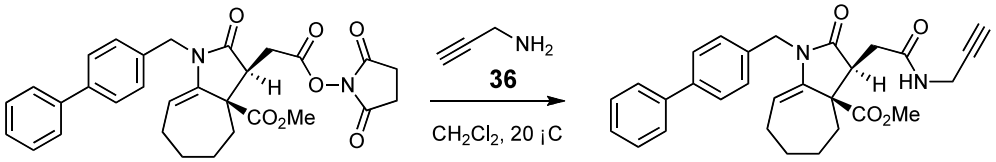
28 **6**

Activated ester **28** (26.8 mg, 60.3 mmol) was dissolved in CH_2Cl_2 (0.5 ml) and treated with amine **34** (8.2 mg, 7.4 μl, 1.2 eq). The reaction mixture was kept at room temperature for 2 h, concentrated under reduced pressure and purified by flash chromatography on silica gel (ethyl acetate: hexanes = 1:1) to give amide **6** in 82% (21.9 mg) yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18-7.19 (m, 1H), 6.90-6.92 (m, 2H), 6.70-6.77 (m, 3H), 6.26 (br s, 1H), 5.93 (s, 2H), 4.69 (d, 1H, *J* = 15.5 Hz), 4.64 (d, 1H, *J* = 16 Hz), 4.59 (dd, 1H, *J* = 6 Hz, 7.8 Hz), 4.49 (dd, 1H, *J* = 5.3 Hz, 7.5 Hz), 3.68 (s, 3H), 3.50-3.53 (m, 1H), 2.98 (dd, 1H, *J* = 3.8 Hz, 7.1 Hz), 2.93 (dd, 1H, *J* = 6 Hz, 7.5 Hz), 2.31 (d, 3H, *J* = 2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.62, 169.17, 164.52, 154.93, 148.08, 147.03, 140.83, 130.18, 126.75, 125.93, 125.01, 120.15, 108.31, 107.51, 106.19, 101.07, 50.90, 43.56, 43.39, 38.05, 35.58, 12.89; MS (APCI) calculated for $C_{22}H_{22}N_2O_6S$ 442.12 (M^+), found 443.0 (M+H).



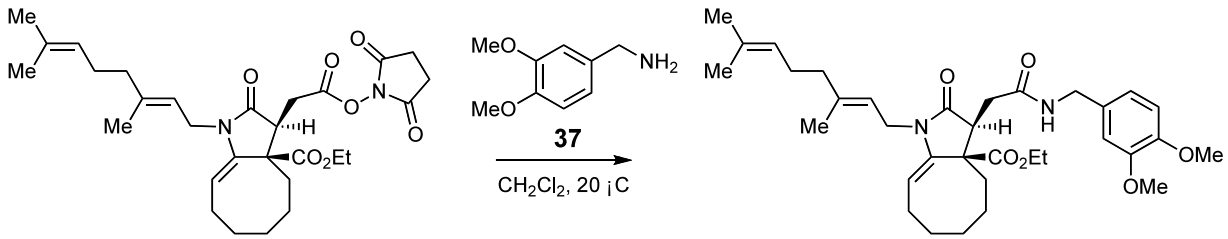
29 **7**

Compound **7** was prepared in 82% yield according to General Protocol C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.29-8.33 (br, 1H), 7.66 (d, 1H, *J* = 7.5 Hz), 7.36 (d, 1H, *J* = 8 Hz), 7.19 (t, 1H, *J* = 7.5 Hz), 7.13 (t, 1H, *J* = 7.3 Hz), 7.08 (s, 1H), 5.11 (t, 1H, *J* = 3.5 Hz), 4.08 (q, 2H, *J* = 7 Hz), 3.98-4.04 (m, 1H), 3.54-3.70 (m, 7H), 3.42-3.45 (m, 2H), 3.28 (dd, 1H, *J* = 4.8 Hz), 3.07-3.14 (m, 1H), 2.97-3.02 (m, 1H), 2.88 (dd, 1H, *J* = 4.5 Hz, 8.3 Hz), 2.61-2.63 (m, 1H), 2.22-2.27 (m, 2H), 2.13-2.17 (m, 1H), 1.83-1.87 (m, 1H), 1.50-1.60 (m, 2H), 1.19 (t, 3H, *J* = 7.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.48, 172.06, 168.96, 138.46, 136.20, 127.29, 121.96, 121.85, 119.29, 118.59, 112.75, 111.15, 100.39, 66.76, 66.40, 61.05, 52.51, 47.54, 45.77, 42.27, 40.60, 30.71, 29.58, 22.84, 22.36, 19.69, 14.13; MS (APCI) calculated for $C_{27}H_{33}N_3O_5$ 479.24 (M^+), found 480.1 (M+H).



30 **8**

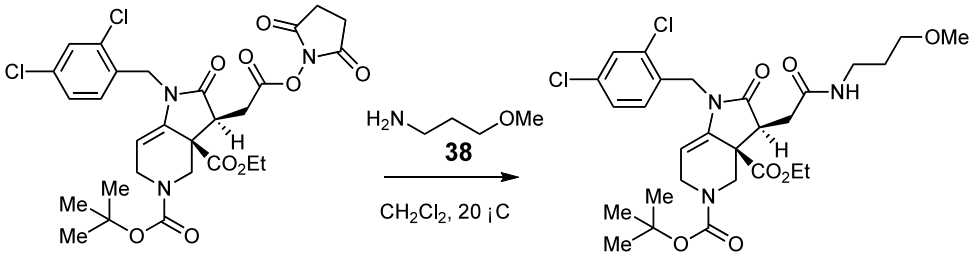
Compound **8** was prepared in quantitative yield according to General Protocol C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54-7.59 (m, 4H), 7.42-7.45 (m, 2H), 7.31-7.36 (m, 3H), 5.14 (dd, 1H, *J* = 4.5 Hz, 9Hz), 4.86 (d, 1H, *J* = 16 Hz), 4.63 (d, 1H, *J* = 16 Hz), 4.11 (ddd, 1H, *J* = 2.5 Hz, 2.8 Hz, 8.8Hz), 4.02 (ddd, 1H, *J* = 2.5 Hz, 2.5 Hz, 8.8 Hz), 3.66 (s, 3H), 3.11 (dd, 1H, *J* = 4.8 Hz, 8.3 Hz), 2.62 (dd, 1H, *J* = 8.3 Hz, 15.3 Hz), 2.47-2.52 (m, 1H), 2.27 (dd, 1H, *J* = 4.8 Hz, 15.3 Hz), 2.22 (t, *J* = 2.5 Hz), 1.48-1.88 (m, 6H), 1.25-1.32 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.34, 171.22, 170.21, 142.26, 140.64, 140.25, 134.58, 128.75, 127.45, 127.30, 127.18, 126.99, 105.95, 79.53, 71.38, 55.52, 52.08, 48.89, 43.98, 35.17, 33.46, 29.31, 27.20, 27.07, 25.99; MS (APCI) calculated for $C_{29}H_{30}N_2O_4$ 470.22 (M^+), found 471.1 (M+H).



31 **9**

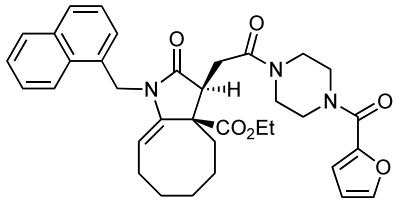
Compound **9** was prepared in 92% yield according to General Procedure C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21 (br, 1H), 6.87 (s, 1H), 6.78-6.87 (m, 2H), 5.02-5.05 (m, 1H), 4.97-4.99 (m, 1H), 4.80 (t, 1H, *J* = 9 Hz), 4.36 (d, 2H, *J* = 2.8 Hz), 4.08-4.13 (m, 4H), 3.87 (s, 3H), 3.84 (s, 3H), 2.97 (dd, 1H, *J* = 4.5 Hz, 4.3 Hz), 2.64 (dd, 1H, *J* = 8.5 Hz, 7.8 Hz), 2.53-2.58 (m, 1H), 2.16 (dd, 1H, *J* = 4.5 Hz, 7.8 Hz), 2.01-2.05 (m, 4H), 1.96-1.99 (m, 2H), 1.57-1.69 (m, 14H), 1.36-1.48 (m, 2H),

1.20 (t, 3H, *J* = 2.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.74, 171.39, 170.42, 149.02, 148.20, 140.32, 139.17, 131.66, 130.97, 123.67, 119.88, 118.01, 111.06, 111.01, 103.65, 61.20, 55.84, 55.81, 54.41, 47.85, 43.46, 39.34, 38.49, 37.19, 34.55, 27.72, 26.37, 26.28, 25.61, 22.97, 22.34, 17.60, 16.40, 14.00. MS (APCI) calculated for $C_{34}H_{48}N_2O_6$ 580.35 (M^+), found 581.3 (M+H).



32 **10**

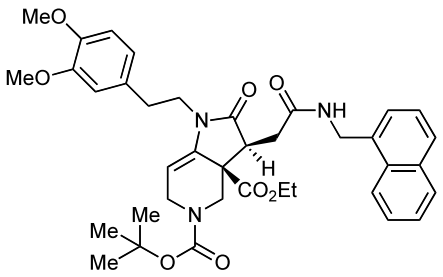
Compound **10** was prepared in 65% yield according to General Protocol C. ^{1}H NMR (500 MHz, C_6D_6, 343K) δ 7.43 (d, 1H, *J* = 8 Hz), 7.24-7.25 (m, 1H), 7.04-7.06 (m, 1H), 5.59 (br, 1H), 5.26 (d, 1H, *J* = 12.5 Hz), 4.95 (d, 1H, *J* = 17 Hz), 4.49 (t, 1H, *J* = 3.3 Hz), 4.42 (d, 1H, *J* = 16.5 Hz), 4.25 (br, 1H), 3.91-4.01 (m, 2H), 3.46 (dd, 1H, *J* = 3.5 Hz, 18 Hz), 3.34-3.38 (m, 2H), 3.26-3.28 (m, 2H), 3.26-3.28 (m, 3H), 2.93 (dd, 1H, *J* = 4.8 Hz, 7.9Hz), 2.79 (d, 1H, *J* = 13 Hz), 2.23 (dd, 1H, *J* = 8.8 Hz, 7.9 Hz), 1.67-1.70 (m, 2H), 1.55 (s, 9H), 1.02 (t, 3H, *J* = 7.3 Hz); ^{13}C NMR (125 MHz, C_6D_6, 343K) δ 173.97, 170.14, 169.53, 154.51, 138.40, 134.12, 133.86, 132.57, 130.15, 129.40, 129.11, 127.91, 96.84, 79.49, 71.27, 61.28, 58.31, 53.37, 48.19, 45.37, 42.28, 41.46, 38.11, 33.48, 30.09, 29.87, 28.51, 14.06; MS (APCI) calculated for $C_{28}H_{37}Cl_2N_3O_7$ 597.20 (M^+), found 634.0 (M+Cl)



11

Compound **11** was prepared according to General Protocols A, B and C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (d, 1H, *J* = 8.5 Hz), 7.88 (d, 1H, *J* = 7.5 Hz), 7.76 (d, 1H, *J* = 8 Hz), 7.56-7.49 (m, 3H), 7.41 (t, 1H, *J* = 3.8 Hz), 7.26-7.24 (m, 2H), 7.05 (d, 1H, *J* = 3.5 Hz), 6.49 (dd, 1H, *J* = 4 Hz, 2 Hz), 5.25 (d, 1H, *J* = 16.5 Hz), 5.19 (d, 1H, *J* = 16.5 Hz), 4.70 (dd, 1H, *J* = 10.5 Hz, 8 Hz), 4.22-4.07 (m, 2H), 3.87-3.78 (m, 5H), 3.61 (br, 2H), 3.54-3.50 (m, 1H), 3.48 (dd, 1H, *J* = 8Hz, 5 Hz), 3.01 (dd, 1H, *J* = 16.5 Hz, 5 Hz), 2.79-2.74 (m, 1H), 2.54 (dd, 1H, *J* = 16.5 Hz, 8 Hz), 1.97-1.90 (m, 2H), 1.80-1.56 (m, 5H), 1.48-1.46 (m, 1H), 1.32-1.22 (m, 2H), 1.26 (t, 2H, *J* = 7 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3, 172.1, 169.2, 159.2, 147.7, 143.9, 141.2, 133.8, 130.9, 130.2, 128.9, 127.8, 126.3, 125.8, 125.2, 123.3, 122.6, 117.1, 111.5, 104.0, 61.2, 54.3, 48.7, 45.5, 42.2, 39.0,

31.6, 27.9, 25.8, 23.0, 22.9, 14.1. MS (APCI) calculated for $C_{35}H_{39}N_3O_6$ 597.28 (M^+), found 598.3 (M+H).



12

Compound **12** was prepared according to General Protocols A, B and C. ^{1}H NMR (500 MHz, d^6-DMSO, 323 K) δ 8.26 (brs, 1H), 8.08 (d, 1H, *J* = 8.5 Hz), 7.93 (d, 1H, *J* = 8 Hz), 7.83 (d, 1H, *J* = 8 Hz), 7.59-7.45 (m, 4H), 6.87-6.86 (m, 2H), 6.77 (dd, 1H, *J* = 8.5 Hz, 2 Hz), 5.16 (brs, 1H), 4.84-4.73 (m, 3H), 4.13 (dd, 1H, *J* = 17.5 Hz, 2.5 Hz), 4.06-3.90 (m, 2H), 3.78-3.70 (m, 2H), 3.76 (s, 3H), 3.73 (s, 3H), 3.60-3.50 (m, 1H), 3.19 (dd, 1H, *J* = 9.5 Hz, 4.5 Hz), 2.86 (d, 1H, *J* = 12.5 Hz), 2.83-2.77 (m, 1H), 2.71 (dd, 1H, *J* = 16.5 Hz, 4.5 Hz), 2.19 (dd, 1H, *J* = 9.5 Hz, 17 Hz), 1.43 (s, 9H), 1.08 (t, 3H, *J* = 7 Hz). ^{13}C NMR (125 MHz, d^6-DMSO, 333 K) δ 173.2, 169.8, 169.7, 154.3, 149.5, 148.2, 138.3, 134.8, 133.8, 131.8, 131.4, 128.8, 127.9, 126.7, 126.1, 126.0, 125.8, 123.9, 121.1, 113.7, 113.1, 96.0, 79.2, 61.2, 56.3, 56.2, 52.6, 44.2, 41.6, 32.3, 32.2, 28.5, 14.1. MS (APCI) calculated for $C_{38}H_{45}N_3O_8$ 671.32 (M^+), found 672.3 (M+H).

Supplemental References

1. Tetko, I. V.; Gasteiger, J.; Todeschini, R.; Mauri, A.; Livingstone, D.; Ertl, P.; Palyulin, V. A.; Radchenko, E. V.; Zefirov, N. S.; Makarenko, A. S.; Tanchuk, V. Y. & Prokopenko, V. V. Virtual computational chemistry laboratory - design and description, *J. Comput. Aid. Mol. Des.* **19**, 453-463 (2005).