Synthesis, Structure and SAR of Pyran-based LpxC Inhibitors

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Supporting Information

Biochemical inhibition assay of compounds with *P. aeruginosa* LpxC

LpxC enzyme assays were run in buffer consisting of 50 mM NaH_2PO_4, pH 7.3, 0.2 mg/mL BSA, 0.5 M sucrose. Compound dilutions were preincubated with 200 pM concentrations of *Pseudomonas aeruginosa* LpxC for 20 minutes in assay buffer. Reactions were then initiated by addition of an equal volume of substrate (UDP-3-*O*-(*R*-3-hydroxydecanoyl)-*N*-acetylglucosamine, NuRx, Alberta, Canada) in assay buffer to reach a final substrate concentration of 5 μM. A reference inhibitor (CH-90) was used as a 100% inhibition control and 2% DMSO was used as a 0% inhibition control. Reactions were allowed to proceed for 20 minutes prior to quenching with 1.5x volume of 2% acetic acid. Assays were measured by direct product detection using LC/MS/MS. For LC/MS/MS analysis, 10 ul aliquots of quenched reaction mixture were injected onto a Shimadzu LC-20 HPLC system interfaced to an API-4000 triple quadrupole mass spectrometer operating in MRM mode. Reaction product and unreacted substrate were eluted into the mass

spectrometer with a gradient starting at 5% acetonitrile, 95% 10mM ammonium acetate/Water to 90% acetonitrile, 10% 10 mM ammonium acetate/water. Deacetylated substrate was quantified during the HPLC run by monitoring abundance of m/z 185 fragment ions of the parent ion at m/z 734 produced in the mass spectrometer. Quantification of unreacted substrate was performed by monitoring fragment ions produced from the corresponding parent ion at m/z 776. Percent inhibition was measured across a 10 point 2-fold dilution series such that final concentrations of compound ranged from 200 to 0.39 nM. Dose-response data were fit to a standard 4-parameter logistic model.

Cloning, expression and purification of *A. aeolicus* LpxC

The *A. aeolicus* LpxC gene encoding the first 271 amino acids out of 282 was synthesized *in vitro* (GenScript, Piscataway, NJ) to match the *A. aeolicus* VF5 strain protein sequence. The tagless construct contained the point mutation C181A. The sequence was codon-optimised for *E. coli* expression. The synthesised DNA fragment was restriction-cloned into pET28a expression vector (Novagen Biosciences, Madison, WI). The resulting plasmid was designated pJT1104.

For protein overproduction, the plasmid was transformed into BL21(DE3) (EMD Chemicals, Gibbstown, NJ) and plated on Luria-Bertani (LB) medium containing 25 μg/mL kanamycin at 37 °C overnight. A single colony of BL21(DE3)/pJT1104 was inoculated into a 100-mL culture of LB containing 25 μg/mL kanamycin and grown overnight at 37 °C. The overnight culture was diluted to OD_{600}=0.1 in 2 x 1-liter of LB containing 25 μg/mL kanamycin and grown at 30 °C with aeration to mid-logarithmic phase (OD_{600} = 0.5). IPTG was then added to a final concentration in each culture of 0.5 mM. After 3 hours induction at 30 °C, the cells

were harvested by centrifugation at 5,000 x g for 15 min at 25 °C. Cell pastes were stored at –20 °C.

Frozen cell pastes were suspended in 20 mL of Lysis Buffer [25 mM Tris-HCl, pH 8.0, 0.1 mM $ZnSO_4$, 5% glycerol, 1 Protease inhibitor cocktail tablet (Roche Diagnostics, Indianapolis, IN)]. Cells were disrupted by passing them twice through a French press operated at 18,000 psi, and the crude extract was centrifuged at 30,000 rpm (45Ti rotor, Beckman-Coulter, Brea, CA) for 30 min at 4 ºC. The supernatant was loaded at a flow rate of 2.0 mL/min onto a 20 mL Q-Sepharose HP (HR16/10) column (GE Healthcare Life Sciences, Piscataway, NJ) pre-equilibrated with Buffer A (25 mM Tris-HCl, pH 8.0, 0.1 mM $ZnSO_4$, 5% glycerol). The column was then washed with Buffer A, and the protein was eluted by a linear gradient from 0 to 1 M NaCl in Buffer A. Fractions containing LpxC were pooled and concentrated to 5 mL by Amicon® Ultracel-10K (Millipore, Billerica, MA). The 5 mL sample was applied at a flow rate of 1.5 mL/min to a 120 mL Superdex 200 (HR 16/60) (GE Healthcare Life Sciences) pre-equilibrated with Buffer C (25 mM Tris-HCl, pH 8.0, 0.15 M NaCl, 0.1 mM $ZnSO_4$, 5% glycerol). The fractions containing LpxC were pooled and concentrated by Amicon® Ultracel-10K (Millipore). The protein concentration was determined by the method of Bradford and characterized by SDS-PAGE analysis. The protein was stored at –80 ºC.

Crystallization, X-ray Data Collection and Structure Determination

Stored protein at 16 mg/mL concentration was thawed on ice. Either compound was dissolved from solid in DMSO to make a 100 mM solution. 2 μL of compound solution was mixed with 100 μL of protein solution and equilibrated on ice for 30 minutes prior to

dispensing into crystallization trays where equal volumes of protein and well solution were combined to form a sitting drop in a vapor diffusion experimental plate. *A. aeolicus* LpxC crystals were grown in a well solution containing 15% PEG 550 MME, 15% PEG 20K, 50 mM imidazole and 50 mM MES (Morpheus buffer 1), pH 6.5, 2% 1,6-hexanediol; 2% 1-butanol 1,2-propanediol (racemic); 2% 2-propanol; 2% 1,4-butanediol; 2% 1,3-propanediol. Crystals were harvested approximately five days after setting the crystallization experiment and directly immersed in liquid nitrogen while mounted on the goniometer pin. X-ray diffraction data was collected on the IMCACAT (17ID) beamLine at the Advanced Photon Source of Argonne National Labs and subsequently reduced and analyzed using the autoPROC[1] suite of programs. Phases were calculated by molecular replacement with the program PHASER[2] and using the unliganded coordinates of PDB: 2GO4[3] as the starting model. Stereochemistry libraries were calculated using GRADE[4] and compound the compound model fitted into electron density with the program RHOFIT[5]. *Coot* [6]was used to inspect the model and electron density and BUSTER[7] was used for macromolecular refinement calculations.

Table S1. X-ray data collection and structure refinement statistics.

Data collection	Compound 2	Compound 9
Resolution (Å)[a]	133.6 – 1.34 (1.36 – 1.34)	133 – 1.25 (1.31 – 1.25)
Space Group	P61	P61
Unit Cell (Å)	65.9 x 65.9 x 133.6 90°, 90°, 120°	65.9 x 65.9 x 133.3 90°, 90°, 120°
# of reflection (measured/unique)	559217/71559	958795/89070
% Completness	98.2 (89.4)	97.8 (94.9)
R_{merge}[a,b]	0.047 (0.44)	0.071 (0.537)
<I/σI>	23.5 (3.3)	20.9 (4.3)
Redundancy	7.8 (5.6)	10.8 (10.8)
Refinement		
R_{work}[c]	0.165	0.158
R_{free}[d]	0.184	0.168
Bond Length RMSD (Å)	0.010	0.010
Bond Angle RMSD (Deg)	1.13	1.09
Ramachandran plot distribution[e]	87.4, 11.7, 0.9	88.7, 10.4, 0.9
PDB code[f]	4U3B	4U3D

[a]Values in parenthesis are for the highest resolution shell.
[b]$R_{merge} = \sum_j \sum_h |I_{hj} - \langle I_h \rangle| / \sum_j \sum_h \langle I_h \rangle$
[c]$R_{work} = \sum_{hkl} ||F_o| - |F_c|| / \sum_{hkl} |F_o|$, where F_o and F_c are observed and calculated structure factors, respectively.
[d]5% of the data was set aside for calculating R_{free}.
[e]Values are % of residues in core, allowed and generously allowed as analyzed in a Ramachandran plot, respectively.
[f]Accession code in the Research Collaboratory for Structural Bioinformatics: *www.rcsb.org.*

Docking

The *P. aeruginosa* LpxC protein structure was obtained from 4G3D.pdb. The structure was prepared using Maestro 8.5 protein preparation wizard (Schrodinger, LLC, 2008, New York, NY). Hydrogens were added, waters were deleted, and bond orders were assigned. A restrained minimization was performed using the default settings. The Glide SP scoring protocol was used to obtain a single ligand conformation**.**

Minimum Inhibitory Concentration (MIC) testing

Minimum Inhibitory Concentration (MIC) testing for each organism/drug combination was conducted according to Clinical and Laboratory Standards Institute Guidelines (CLSI documents M100-S24, 2014 and M07-A9, 2012). Each test included reference compounds and quality control reference strains to validate both the compound preparation and experimental methodologies used.

Clinical and Laboratory Standards Institute. M07-A9. 2012. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard. Ninth edition. Wayne, PA. Volume 32. Number 2.

Clinical and Laboratory Standards Institute. M100-S24. 2014. Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement. Wayne, PA. Volume 34. Number 1.

Synthesis of Compounds 9, 11a – 11f, 18a – 18i, 23, 25, 27a, 27b

All commercial reagents and anhydrous solvents were obtained from commercial sources and were used without further purification, unless otherwise specified.

LC-MS conditions: Samples were analyzed by reversed phase LC-MS using Acquity HSS T3, 2.1 mm x 50 mm, 1.8 μm particle size columns. A Waters Acquity UPLC (Milford, MA, USA) LC system was used with a gradient elution profile of 5–95% B over 2 min at 1 mL/min, then re-equilibration at 5% B and 1 mL/min to 2.5 minutes. Injection volume was 1 μL and column temperature was 30 °C. Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile. Detection was based on electrospray ionization (ESI) in positive and negative polarity using Waters Quattro Micro mass spectrometer (Milford, MA, USA) and diode-array UV detector from 210 to 400 nm.

^{1}H NMR spectra (δ, ppm) were recorded using Bruker Advance Ultrashield 300 MHz or Bruker DPX 400 MHz instruments.

Column chromatography was performed using Silcycle FLHR10030B Silisep cartridges (12–330 g). Preparative reversed phase HPLC chromatography was carried out using a Waters Atlantis T3-C18 column, 19 mm × 100 mm, 5 μm, a linear gradient from 10% to 90% CH_3CN in H_2O over 12 min (0.1% trifluoroacetic acid), and a flow rate of 20 mL/min.

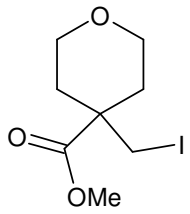
The purity of the final compounds was assessed on the basis of analytical LC-MS, and the results were greater than 95% unless specified otherwise.

DMF	*N,N*-dimethylformamide;
EtOAc	ethyl acetate;
TFA	trifluoroacetic acid;
DMSO	dimethylsulphoxide;
DIPEA	*N*,*N*,-diisopropylethylamine (Hunig's Base);
HATU	*N*,*N*,*N*',*N*'-tetramethyl-*O*-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate;
HBTU	2-(1*H*-benzotriazol-1-yl)-1,1,3,3,-tetramethyluronium hexafluorophosphate;
HOBt	1-hydroxybenzotriazole hydrate;
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride;
DCM	dichloromethane;
THF	tetrahydrofuran;
MeOH	methanol;
Et_3N	triethylamine;
MeCN	Acetonitrile;
BOC	*tert*-butoxycarbonyl;
PTSA	*p*-toluenesulfonic acid
PPTS	pyridinium 4-toluenesulfonate
LDA	lithium di-isopropylamine
DMAP	4-(Dimethylamino)pyridine
MTBE	Methyl *tert*-butylether
TMEDA	*N,N,N',N'*-Tetramethylethylenediamine

Example 1

N-Hydroxy-4-[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenoxy) methyl]tetrahydro-2*H*-pyran-4-carboxamide (**9**)

A. Methyl 4-(iodomethyl)tetrahydro-2*H*-pyran-4-carboxylate (**4**)



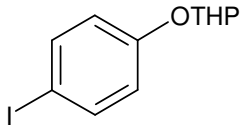
To the solution of methyl tetrahydro-2*H*-pyran-4-carboxylate (5 g, 0.035 mol), in THF (100 mL) at -40 °C was added LDA (26 mL, 0.052 mol, 2M solution in THF) and the reaction was stirred for 1 h at -40 °C. To the reaction mixture was added CH_2I_2 (9.3 g, 0.035 mol) and the reaction mixture was allowed to warm to room temperature and the reaction mixture was stirred for 2 h at room temperature. Water was added to the reaction solution and it was extracted 3 times with ethyl acetate. The pooled organics were washed with sat. NaCl solution, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel chromatography (5% ethylacetate in hexanes) to afford the desired product (7.4 g, 80%) as a pale yellow oil.

LC-MS: $[M+1]^+$ 285.1

Mass: calculated for $C_8H_{13}IO_3$, 284.09

^{1}H NMR (400 MHz, $CDCl_3$) δ: ppm 3.83 (m, 2H); 3.70 (s, 3H); 3.50 (m, 2H); 3.30 (s, 2H); 2.17 (d, 2H); 1.59 (m, 2H).

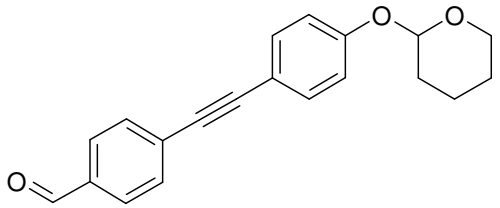
B. 2-(4-Iodophenoxy)tetrahydro-2*H*-pyran



4-Iodophenol (100 g, 452 mmol) was dissolved in diethyl ether (1 L) and cooled to 0 °C. To this was added 3,4-dihydro-2*H*-pyran (160 mL, 1742 mmol) and PTSA (5 g, 17 mmol) and stirred for 1.5 h. Solid K_2CO_3 (18 g, 248 mmol) was added and the reaction was stirred for another 3 h. 1N NaOH (500 mL) was added to the reaction mixture and it was extracted with diethyl ether. The organic layer was washed with water, brine, dried over sodium sulphate and concentrated. Re-crystallization from ethanol gave the desired product (110 g, 80%) as a pale yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ: ppm 7.56 (dd, 2H); 6.84 (dd, 2H); 5.38 (t, 1H); 3.86 (m, 1H); 3.60 (m, 1H); 1.87 (m, 1H); 1.69 (m, 2H).

C. 4-{[4-(tetrahydro-2*H*-pyran-2-yloxy)phenyl]ethynyl}benzaldehyde (**6**)

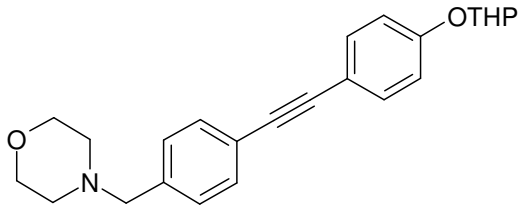


2-(4-Iodophenoxy)tetrahydro-2*H*-pyran (10 g, 33 mmol) and 4-ethynyl benzaldehyde (4.2 g, 33 mmol) were taken in acetonitrile (50 mL) and the solution was purged with Argon for 15 min. To this solution was added bis(triphenylphosphine)palladium(II)chloride $Pd(PPh_3)_2Cl_2$ (2.3 g, 3.3 mmol), Et_3N (13.7 mL, 99 mmol) and copper(I) iodide (0.3 g, 1.6 mmol) and stirred at 60 °C for 4 h. The solvents from the reaction mixture were removed under vacuum. The crude material was purified by silica gel chromatography (5-20% EtOAc in Hexane) to afford 8 g of the desired product (79%).

LC-MS: $[M+1]^+$ 307.3

Mass: calculated for $C_{20}H_{18}O_3$, 306.35

D. 4-(4-{[4-(Tetrahydro-2*H*-pyran-2-yloxy)phenyl]ethynyl}benzyl)morpholine

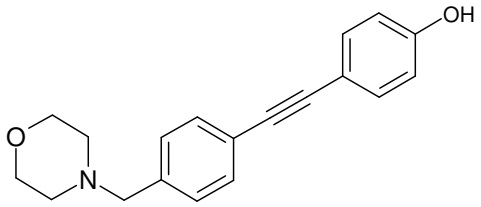


To the solution of 4-{[4-(tetrahydro-2*H*-pyran-2-yloxy)phenyl]ethynyl} benzaldehyde (2 g, 6.5 mmol) in dichloroethane (20 mL) was added morpholine (0.62 g, 7.15 mmol) and acetic acid (0.4 mL) at room temperature and stirred for 30 min. The reaction mixture was cooled to 0 °C and sodium triacetoxyborohydride (2 g, 9.75 mmol) was added portion-wise and the reaction mixture was allowed to stir at room temperature for 2 h. TLC showed the disappearance of the starting material. Water was added to the reaction mixture and it was extracted with DCM. The residue was taken for the next step without further purification.

LC-MS: $[M+1]^+$ 378.5

Mass: calculated for $C_{24}H_{27}NO_3$, 377.47

E. 4-{[4-(Morpholin-4-ylmethyl)phenyl]ethynyl}phenol (**7**)



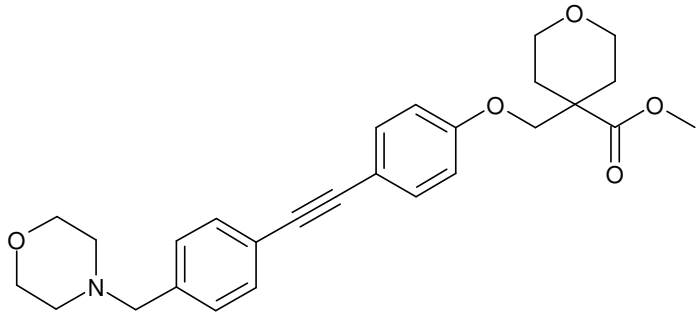
To the solution of 4-(4-{[4-(tetrahydro-2*H*-pyran-2-yloxy)phenyl]ethynyl}benzyl) morpholine (1.8 g, 4.71 mmol) in methanol was added 4N HCl (2 mL) and stirred at room temperature for 1 h. The solvents were removed and the residue obtained was washed with water. The residue was re-precipitated in methanol:water mixture and filtered. 1.2 g of the residue thus obtained, showed >95% purity by UPLC and was taken to the next step without further purification.

LC-MS: $[M+1]^+$ 294.3

Mass: calculated for $C_{19}H_{19}NO_2$, 293.35

^{1}H NMR (400 MHz, $CDCl_3$) δ: ppm 7.57 (s, 2H); 7.33 (m, 4H); 6.79 (m, 2H); 4.49 (s, 1H); 4.33 (m, 1H); 3.92 (m, 1H); 3.69 (m, 1H); 3.09 (m, 4H).

F. Methyl 4-[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenoxy)methyl] tetrahydro-2*H*-pyran-4-carboxylate (**8**)

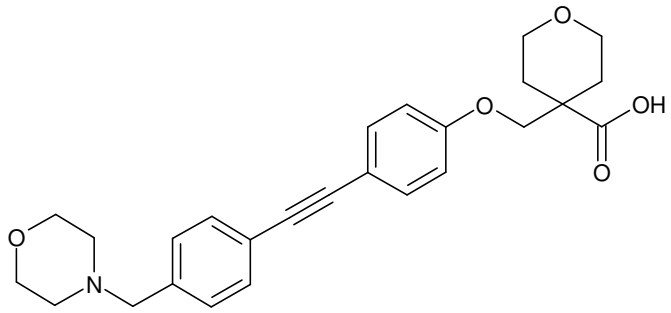


To the solution of methyl 4-(iodomethyl)tetrahydro-2*H*-pyran-4-carboxylate (2 g, 7 mmol) and K_2CO_3 (1.94 g, 14 mmol) in DMF (10 mL), was added 4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenol (2 g, 7 mmol) and stirred at 120 °C for 5 h. Water was added to the reaction mixture and it was extracted 3 times with ethyl acetate. The pooled organics were washed with sat. NaCl solution, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel chromatography (5% ethylacetate in hexanes) to afford the desired product (1.5 g) as an off-white solid (51% for three steps).

LC-MS: $[M+1]^+$ 450.5

Mass: calculated for $C_{27}H_{31}NO_5$, 449.53

G. 4-[(4-{[4-(Morpholin-4-ylmethyl)phenyl]ethynyl}phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxylic acid

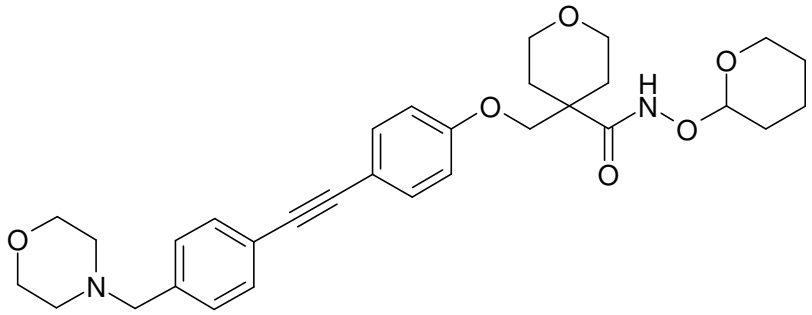


To a solution of methyl 4-[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxylate (1 g, 2.2 mmol) in methanol:THF:Water (1:1:1, 10 mL) was added $LiOH \cdot H_2O$ (273 mg, 6.6 mmol) and the reaction was heated to 60 °C for 14 h. The organic solvents were removed under vacuum and the resulting aqueous solution was acidified with 1N HCl to pH 2 at 0 °C. The precipitate that formed was filtered and dried under vacuum to give the desired product (750 mg, 95%) as an off-white solid.

LC-MS: $[M+1]^+$ 436.5

Mass: calculated for $C_{26}H_{29}NO_5$, 435.51

H. 4-[(4-{[4-(Morpholin-4-ylmethyl)phenyl]ethynyl}phenoxy)methyl]-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide



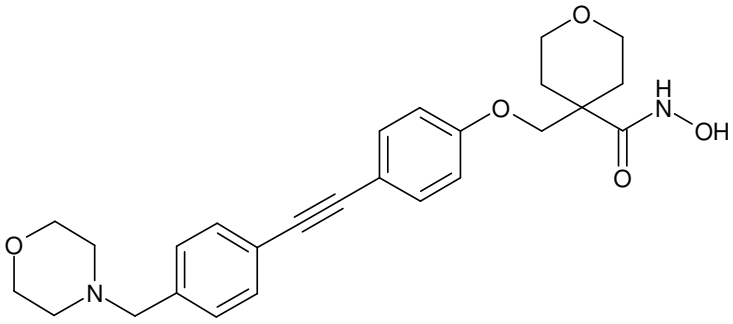
To a solution of 4-[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxylic acid (500 mg, 1.14 mmol) in DCM (10 mL) was added NH_2-OTHP (270 mg, 2.3 mmol) and diethyl cyanophosphonate (280 mg, 1.71 mmol) and cooled to 0 °C. Et_3N (0.4 mL, 2.85 mmol) was added and the reaction was allowed to stir at room temperature for 2 h. After this time, LC-MS indicated formation of the desired

product. The reaction mixture was diluted with DCM (50 mL) and washed with water, brine, dried over Na_2SO_4, filtered, and concentrated. The residue was taken to the next step without further purification.

LC-MS: $[M+1]^+$ 535.4

Mass: calculated for $C_{31}H_{38}N_2O_6$, 534.64

I. *N*-Hydroxy-4-[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenoxy) methyl]tetrahydro-2*H*-pyran-4-carboxamide (**9**)



To the solution of 4-[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenoxy) methyl]-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide (1 g, 1.8 mmol) in methanol (10 mL) was added 4N HCl (1 mL) and stirred at room temperature for 1 h. The solvents were removed and the residue obtained was washed with water. The crude residue was taken up in DMSO for reverse phase purification. The clean fractions were pooled, concentrated and lyophilized overnight to afford 450 mg of the desired product as an off-white solid (88% over two steps).

LC-MS: $[M+1]^+$ 451.2

HRMS: $[M+1]^+$ 451.2234

Mass: calculated for $C_{26}H_{30}N_2O_5$, 450.52

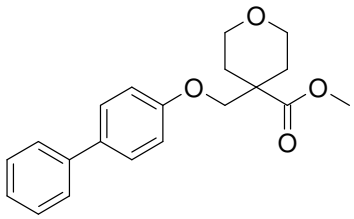
^{1}H NMR (400 MHz, DMSO) δ: ppm 10.63 (s, 1H); 8.80 (bs, 1H); 7.56 (m, 6H); 6.95 (m, 2H); 4.34 (m, 2H); 3.95 (m, 4H); 3.69 (m, 4H); 3.41 (m, 2H); 3.20 (m, 2H); 3.10 (m, 2H); 2.02 (d, 2H); 1.58 (m, 2H).

^{13}C NMR (500 MHz, DMSO) δ: ppm 169.5, 158.9, 133.0, 131.8, 131.4, 127.9, 126.3, 115.1, 114.1, 90.6, 27.4, 73.4, 63.8, 63.0, 58.5, 50.7, 43.5, 30.3

Example 2

4-[(Biphenyl-4-yloxy)methyl]-*N*-hydroxytetrahydro-2*H*-pyran-4-carboxamide (**11a**)

A. Methyl 4-[(biphenyl-4-yloxy)methyl]tetrahydro-2*H*-pyran-4-carboxylate

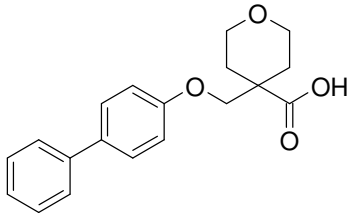


The title compound was synthesized as described for Example 1, Step F from methyl 4-(iodomethyl)tetrahydro-2*H*-pyran-4-carboxylate (**4**) and 4-phenylphenol (**10a**).

LC-MS: $[M+1]^+$ 327.4

Mass: calculated for $C_{20}H_{22}O_4$, 326.38

B. 4-[(Biphenyl-4-yloxy)methyl]tetrahydro-2*H*-pyran-4-carboxylic acid

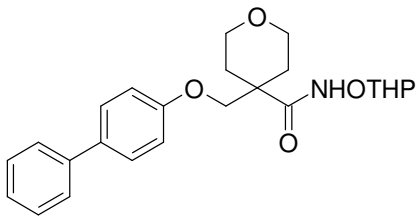


The title compound was synthesized as described for Example 1, Step G from methyl 4-[(biphenyl-4-yloxy)methyl]tetrahydro-2*H*-pyran-4-carboxylate.

LC-MS: $[M-1]^-$ 311.4

Mass: calculated for $C_{19}H_{20}O_4$, 312.35

C. 4-[(Biphenyl-4-yloxy)methyl]-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide

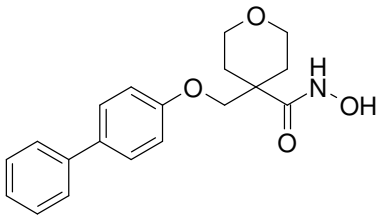


The title compound was synthesized as described for Example 1, Step H from 4-[(biphenyl-4-yloxy)methyl]tetrahydro-2*H*-pyran-4-carboxylic acid and *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine.

LC-MS: $[M+1]^+$ 412.5

Mass: calculated for $C_{24}H_{29}NO_5$, 411.49

D. 4-[(Biphenyl-4-yloxy)methyl]-*N*-hydroxytetrahydro-2*H*-pyran-4-carboxamide (**11a**)



The title compound was synthesized as described for Example 1, Step I from 4-[(biphenyl-4-yloxy)methyl]-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide.

LC-MS: $[M+1]^+$ 328.5

HRMS: $[M+1]^+$ 328.1559

Mass: calculated for $C_{19}H_{21}NO_4$, 327.37

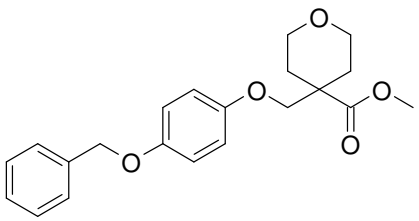
^{1}H NMR (400 MHz, DMSO) δ: ppm 10.63 (bs, 1H); 7.58 (m, 4H); 7.41 (m, 2H); 7.29 (m, 1H); 6.99 (d, 2H); 4.16 (s, 2H); 3.80 (m, 2H); 3.42 (m, 2H); 1.59 (m, 2H); 1.21 (m, 2H).

^{13}C NMR (500 MHz, DMSO) d: ppm 169.6, 158.2, 139.7, 132.7, 128.8, 127.7, 126.7, 126.1, 115.1, 73.5, 63.9, 43.6, 30.4.

Example 3

4-{[4-(Benzyloxy)phenoxy]methyl}-*N*-hydroxytetrahydro-2*H*-pyran-4-carboxamide (**11b**)

A. Methyl 4-{[4-(benzyloxy)phenoxy]methyl}tetrahydro-2*H*-pyran-4-carboxylate

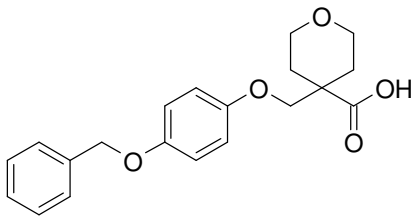


The title compound was synthesized as described for Example 1, Step F from methyl 4-(iodomethyl)tetrahydro-2*H*-pyran-4-carboxylate (**4**) and 4-benzyloxyphenol (**10b**).

LC-MS: $[M+1]^+$ 357.5

Mass: calculated for $C_{21}H_{24}O_5$, 356.41

B. 4-{[4-(Benzyloxy)phenoxy]methyl}tetrahydro-2*H*-pyran-4-carboxylic acid

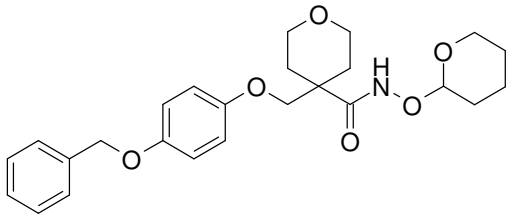


The title compound was synthesized as described for Example 1, Step G from methyl 4-{[4-(benzyloxy)phenoxy]methyl}tetrahydro-2*H*-pyran-4-carboxylate.

LC-MS: $[M-1]^-$ 341.4

Mass: calculated for $C_{20}H_{22}O_5$, 342.38

C. 4-{[4-(Benzyloxy)phenoxy]methyl}-*N*-(tetrahydro-2*H*-pyran-2-yloxy) tetrahydro-2*H*-pyran-4-carboxamide

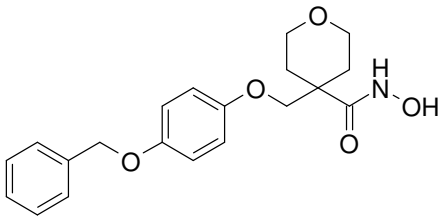


The title compound was synthesized as described for Example 1, Step H from 4-{[4-(benzyloxy)phenoxy]methyl}tetrahydro-2*H*-pyran-4-carboxylic acid and *O*-(Tetrahydro-2H-pyran-2-yl)hydroxylamine.

LC-MS: $[M+1]^+$ 442.5

Mass: calculated for $C_{25}H_{31}NO_6$, 441.51

D. 4-{[4-(Benzyloxy)phenoxy]methyl}-*N*-hydroxytetrahydro-2*H*-pyran-4-carboxamide (**11b**)



The title compound was synthesized as described for Example 1, Step I from 4-{[4-(benzyloxy)phenoxy]methyl}-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide.

LC-MS: $[M+1]^+$ 358.5

HRMS: $[M+1]^+$ 358.1667

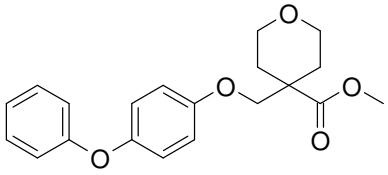
Mass: calculated for $C_{20}H_{23}NO_5$, 357.40

^{1}H NMR (400 MHz, DMSO) δ: ppm δ 10.57 (s, 1H); 8.79 (bs, 1H); 7.38 (m, 4H); 7.31 (m, 1H); 6.91 (d, 2H); 6.80 (d, 2H); 5.01 (s, 2H); 3.84 (s, 2H); 3.69 (m, 2H); 3.40 (m, 2H); 1.99 (m, 2H); 1.55 (m, 2H).

Example 4

N-Hydroxy-4-[(4-phenoxyphenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxamide (**11c**)

A. Methyl 4-[(4-phenoxyphenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxylate

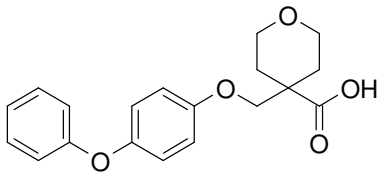


The title compound was synthesized as described for Example 1, Step F, from methyl 4-(iodomethyl)tetrahydro-2*H*-pyran-4-carboxylate (**4**) and 4-phenoxyphenol (**10c**).

LC-MS: $[M+1]^+$ 343.4

Mass: calculated for $C_{20}H_{22}O_5$, 342.38

B 4-[(4-Phenoxyphenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxylic acid

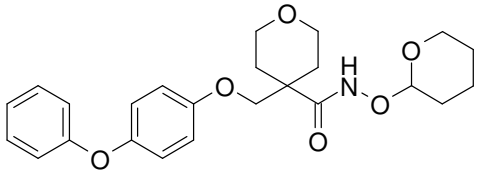


The title compound was synthesized as described for Example 1, Step G from methyl 4-[(4-phenoxyphenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxylate.

LC-MS: $[M-1]^-$ 327.4

Mass: calculated for $C_{19}H_{20}O_5$, 328.35

C. 4-[(4-Phenoxyphenoxy)methyl]-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide

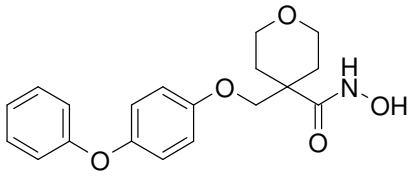


The title compound was synthesized as described for Example 1, Step H from 4-[(4-phenoxyphenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxylic acid and *O*-(Tetrahydro-2H-pyran-2-yl)hydroxylamine.

LC-MS: $[M+1]^+$ 428.5

Mass: calculated for $C_{24}H_{29}NO_6$, 427.49

D. *N*-Hydroxy-4-[(4-phenoxyphenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxamide (**11c**)



The title compound was synthesized as described for Example 1, Step I from 4-[(4-phenoxyphenoxy)methyl]-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide.

LC-MS: $[M+1]^+$ 344.5

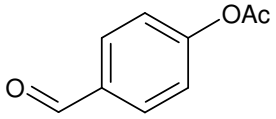
HRMS: $[M+1]^+$ 344.1504

Mass: calculated for $C_{19}H_{21}NO_5$, 343.37

^{1}H NMR (400 MHz, DMSO) δ: ppm 10.61 (s, 1H); 8.82 (s, 1H); 7.34 (m, 2H); 7.00 (m, 1H); 6.97 (m, 6H); 3.92 (s, 2H); 3.71 (m, 2H); 3.42 (m, 2H); 2.00 (m, 2H); 1.57 (m, 2H).
^{13}C (500 MHz, DMSO) d: ppm 169.6, 157.9, 154.8, 149.6, 129.9, 122.6, 120.6, 117.3, 115.9, 73.8, 63.9, 43.6, 30.4

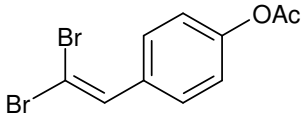
N-Hydroxy-4-{[4-(4-phenylbuta-1,3-diyn-1-yl)phenoxy]methyl}tetrahydro-2*H*-pyran-4-carboxamide (**11d**)

A. 4-formylphenyl acetate



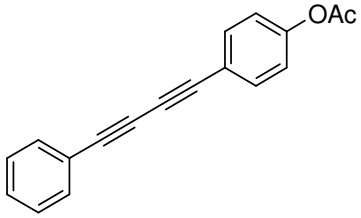
To a solution of 4-hydroxybenzaldehyde (**12**, 1 g, 8.19 mmol) and Et_3N (1.24 g, 12.3 mmol) in DCM (10 mL) at 0 °C was added drop-wise acetyl chloride (1.29 g, 16.3 mmol) and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was quenched with sat. $NaHCO_3$ solution (5 mL) and extracted with DCM (20 mL x 2). The pooled organics were washed with sat. NaCl solution, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel chromatography (5% ethylacetate in hexanes) to afford 1 g of the desired product (74%).
Mass: calculated for $C_9H_8O_3$, 164.15
^{1}H NMR (400 MHz, $CDCl_3$) δ: ppm 10.0 (s, 1H); 7.93 (m, 2H); 7.29 (d, 2H); 2.34 (s, 3H).

B. 4-(2,2-Bibromoethenyl)phenyl acetate



To a solution of 4-formylphenyl acetate (1 g, 6.09 mmol) and carbon tetrabromide (3 g, 9.1 mmol) in DCM (20 mL) at 0 °C was added drop-wise a solution of triphenylphosphine (4.7 g, 18.3 mmol) in DCM (20 mL). The reaction mixture was stirred at room temperature for 4 h. DCM was removed under vacuum and the residue was purified by silica gel chromatography (5-15% ethylacetate in hexanes) to afford 0.55 g of the desired product (28%).
GC-MS: 320
Mass: calculated for $C_{10}H_8Br_2O_2$, 319.97

C. 4-(4-Phenylbuta-1,3-diyn-1-yl)phenyl acetate

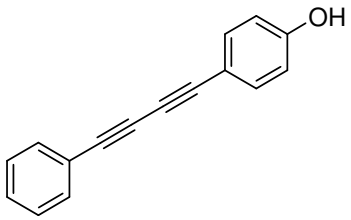


A solution of 4-(2,2-dibromoethenyl)phenyl acetate (0.5 g, 1.56 mmol), ethynylbenzene (0.22 g, 2.1 mmol), Tris(dibenzylideneacetone)dipalladium (0) (58 mg, 0.06 mmol) and Tris(4-methoxyphenyl)phosphine (17 mg, 0.05 mmol) in DMF (5 mL) was degassed by purging with argon for 10 min. To this solution was added Et_3N (0.5 mL, 3.8 mmol) and the reaction mixture was heated to 85 °C and stirred for 2 h. The reaction was cooled to room temperature and diluted with ethylacetate (30 mL), washed with water (20 mL x 3), washed with sat. NaCl solution, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel chromatography (5-20% ethylacetate in hexanes) to afford 245 mg of the desired product (60%).

LC-MS: $[M+1]^+$ Not seen by LCMS

Mass: calculated for $C_{18}H_{12}O_2$, 260.28

D. 4-(4-Phenylbuta-1,3-diyn-1-yl)phenol (**10d**)

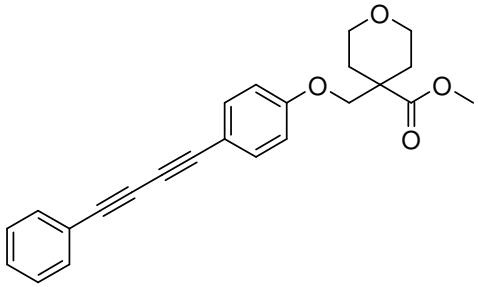


To a solution of 4-(4-phenylbuta-1,3-diyn-1-yl)phenyl acetate (250 mg, 0.96 mmol) in methanol:THF:water (1:1:1, 5 mL) was added $LiOH \cdot H_2O$ (121 mg, 2.8 mmol) and stirred at room temperature for 14 h. The organic solvents were removed under vacuum and the resulting aqueous solution was acidified with 1 N HCl solution at 0 °C. The precipitate formed was filtered and dried under vacuum to give 180 mg of the desired product (80%).

LC-MS: $[M-1]^-$ 217.2

Mass: calculated for $C_{16}H_{10}O$, 218.25

E. Methyl 4-{[4-(4-phenylbuta-1,3-diyn-1-yl)phenoxy]methyl}tetrahydro-2*H*-pyran-4-carboxylate

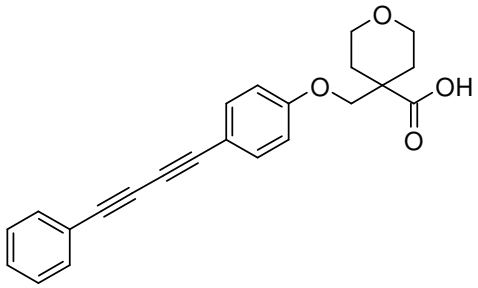


To the solution of methyl 4-(iodomethyl)tetrahydro-2*H*-pyran-4-carboxylate (0.5 g, 1.7 mmol, Example 4, Step A) and K_2CO_3 (0.36 g, 2.62 mmol) in DMF (5 mL), was added 4-(4-phenylbuta-1,3-diyn-1-yl)phenol (0.38 g, 1.75 mmol) and stirred at 120 °C for 5 h. Water was added to the reaction solution and it was extracted 3 times with ethyl acetate. The pooled organics were washed with sat. NaCl solution, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel chromatography (5% ethylacetate in hexanes) to afford 0.3 g of the desired product (46%).

LC-MS: $[M+1]^+$ Not seen by LCMS

Mass: calculated for $C_{24}H_{22}O_4$, 374.42

F. 4-{[4-(4-Phenylbuta-1,3-diyn-1-yl)phenoxy]methyl}tetrahydro-2*H*-pyran-4-carboxylic acid

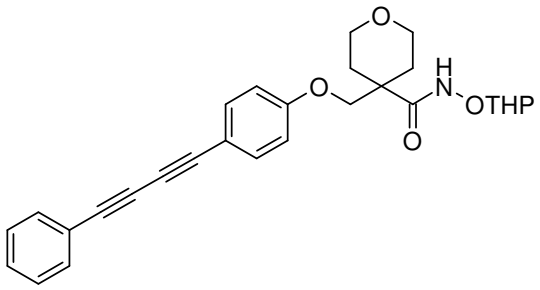


To a solution of methyl 4-{[4-(4-phenylbuta-1,3-diyn-1-yl)phenoxy] methyl}tetrahydro-2*H*-pyran-4-carboxylate (300 mg, 0.8 mmol) in methanol:THF:water (1:1:1, 5 mL) was added $LiOH \cdot H_2O$ (100 mg, 2.4 mmol) and heated to 60 °C for 14 h. The organic solvents were removed under vacuum and the resulting aqueous solution was acidified with 1 N HCl solution at 0 °C. The precipitate that formed was filtered and dried under vacuum to give 200 mg of the desired product as a white solid (69%).

LC-MS: $[M-1]^-$ 359.4

Mass: calculated for $C_{23}H_{20}O_4$, 360.40

G. 4-{[4-(4-Phenylbuta-1,3-diyn-1-yl)phenoxy]methyl}-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide

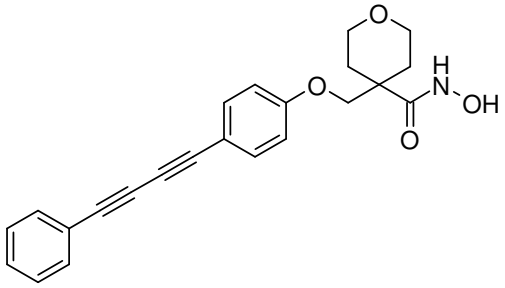


To a solution of 4-{[4-(4-phenylbuta-1,3-diyn-1-yl)phenoxy]methyl}tetrahydro-2*H*-pyran-4-carboxylic acid (200 mg, 0.55 mmol) in DCM (5 mL) was added *O*-(Tetrahydro-2H-pyran-2-yl)hydroxylamine (120 mg, 1 mmol), *N*-methyl-2-chloropyridinium iodide (255 mg, 1 mmol), DMAP (6 mg, 0.05 mmol), DIPEA (0.1 mL, 0.60 mmol) and the reaction was allowed to stir at room temperature for 1 h. After this time, UPLC indicated formation of the desired product. The reaction mixture was concentrated and the crude residue was purified by silica gel (60-120 mesh) column chromatography using ethylacetate-hexanes (5%-40% of ethylacetate in Hexanes). The clean fractions were pooled, concentrated to afford 50 mg of the desired product as a pale yellow solid (20%).

LC-MS: $[M+1]^+$ 460.5

Mass: calculated for $C_{28}H_{29}NO_5$, 459.53

H. *N*-Hydroxy-4-{[4-(4-phenylbuta-1,3-diyn-1-yl)phenoxy]methyl}tetrahydro-2*H*-pyran-4-carboxamide (**11d**)



To the solution of 4-{[4-(4-phenylbuta-1,3-diyn-1-yl)phenoxy]methyl}-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide (50 mg, 0.1 mmol) in methanol (1 mL) was added aq. HCl (10%, 0.2 mL) and stirred at room temperature for 30 min. The reaction mixture was concentrated and the crude residue was purified by re-crystallization using methanol-water to afford 25 mg of the desired product (67%).

LC-MS: $[M+1]^+$ 376.4

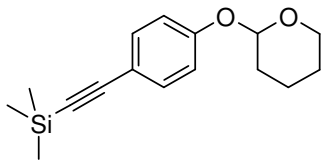
HRMS: $[M+1]^+$ 376.1555

Mass: calculated for $C_{23}H_{21}NO_4$, 375.41

^{1}H NMR (400 MHz, DMSO-d_6) δ: ppm 10.62 (s, 1H); 8.81 (bs, 1H); 7.59 (m, 3H); 7.44 (m, 5H); 6.96 (d, 2H); 3.99 (s, 2H); 3.70 (m, 2H); 3.41 (m, 2H); 2.02 (m, 2H); 1.55 (m, 2H).

N-Hydroxy-4-{[4-(5-hydroxy-5-methylhexa-1,3-diyn-1-yl)phenoxy]methyl} tetrahydro-2*H*-pyran-4-carboxamide (**11e**)

A. Trimethyl{[4-(tetrahydro-2*H*-pyran-2-yloxy)phenyl]ethynyl}silane

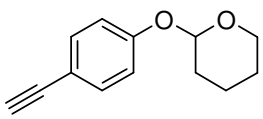


2-(4-Iodophenoxy)tetrahydro-2*H*-pyran (**5**) (0.5 g, 1.64 mmol) and trimethylsilylacetylene (0.32 g, 3.28 mmol) were diluted with acetonitrile (5 mL) and the solution was purged with argon for 15 min. To this solution was added Bis(triphenylphosphine)palladium**(II)** dichloride (0.06 g, 0.08 mmol), Et_3N (0.44 mL, 3.3 mmol) and copper(I) iodide (0.015 g, 0.08 mmol). The mixture was stirred at room temperature for 14 h. The solvents from the reaction mixture were removed under vacuum. The crude material was purified by silica gel chromatography (5-20% ethyl acetate in hexanes) to give 0.3 g of the desired product (67%).

LC-MS: $[M+1]^+$ 275.4

Mass: calculated for $C_{16}H_{22}O_2Si$, 274.43

B. 2-(4-Ethynylphenoxy)tetrahydro-2*H*-pyran (**14**)



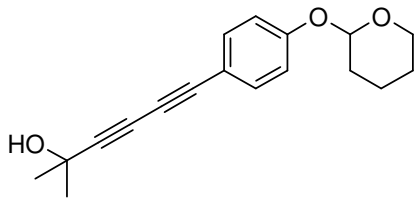
To a solution of trimethyl{[4-(tetrahydro-2*H*-pyran-2-yloxy)phenyl]ethynyl} silane (0.3 g, 1.09 mmol) in methanol (3 mL) was added solid K_2CO_3 (0.15 g, 1.09 mmol). The mixture was stirred at room temperature for 4 h. The reaction mixture was filtered and washed with methanol. The filtrate was concentrated *in vacuo* to give a solid which was re-crystallized from methanol and MTBE to give the desired product 0.16 g as a pale yellow solid (73%).

LC-MS: $[M+1]^+$ 203.3

Mass: calculated for $C_{13}H_{14}O_2$, 202.24

^{1}H NMR (400 MHz, $CDCl_3$) δ: ppm 7.41 (d, 2H); 6.98 (d, 2H); 5.44 (t, 1H); 3.85 (m, 1H); 3.59 (m, 1H); 1.98 (m, 3H); 1.87 (m, 2H); 1.63 (m, 3H).

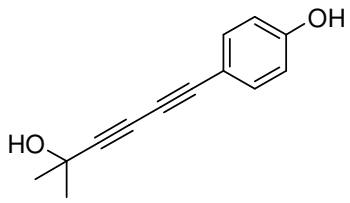
C. 2-Methyl-6-[4-(tetrahydro-2*H*-pyran-2-yloxy)phenyl]hexa-3,5-diyn-2-ol



CuI (2.35 mg, 0.012 mmol) and $NiCl_2.6H_2O$ (1.6 mg, 0.012 mmol) were dissolved in THF (2 mL) and stirred for 10 min. TMEDA (5.7 mg, 0.049 mmol) was added and the solution was stirred for 5 min at RT. 2-Methylbut-3-yn-2-ol (104 mg, 1.23 mmol) and 2-(4-ethynylphenoxy)tetrahydro-2*H*-pyran (50 mg, 0.25 mmol) were added subsequently and the reaction mixture was stirred for 72 h at RT. The mixture was concentrated *in vacuo* and the residue was purified by silica gel chromatography (ethyl acetate-hexanes 20%) to afford 20 mg (28%) of desired product.

^{1}H NMR (400 MHz, $CDCl_3$) δ: ppm 7.41 (d, 2H); 6.97 (d, 2H); 5.44 (t, 1H); 3.83 (m, 1H); 3.59 (m, 1H); 1.68 (m, 6H); 1.58 (s, 6H).

D. 4-(5-Hydroxy-5-methylhexa-1,3-diyn-1-yl)phenol (**10e**)



To a solution of 2-methyl-6-[4-(tetrahydro-2*H*-pyran-2-yloxy)phenyl]hexa-3,5-diyn-2-ol (0.125 g, 0.4 mmol) in methanol was added PPTS (0.11 g, 0.4 mmol). The mixture

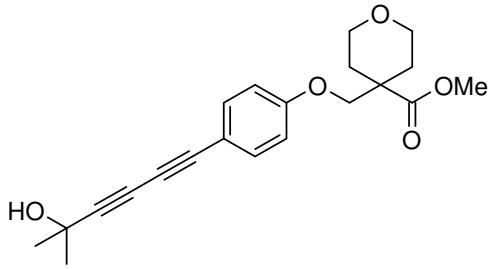
was stirred at RT for 4 h. The solvents were removed and the residue obtained was dissolved in ethyl acetate and washed with water, brine, and dried over anhydrous sodium sulphate. The organic layer was filtered and the filtrate was concentrated. The residue thus obtained was used without further purification.

LC-MS: $[M-1]^-$ 199.3

Mass: calculated for $C_{13}H_{12}O_2$, 200.23

^{1}H NMR (400 MHz, $CDCl_3$) δ: ppm 7.38 (m, 2H); 6.77 (m, 2H); 5.07 (s, 1H); 1.58 (s, 6H).

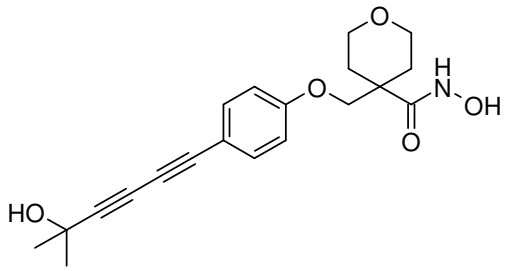
E. Methyl 4-{[4-(5-hydroxy-5-methylhexa-1,3-diyn-1-yl)phenoxy]methyl} tetrahydro-2*H*-pyran-4-carboxylate



To a solution of methyl 4-(iodomethyl)tetrahydro-2*H*-pyran-4-carboxylate (**4**) and K_2CO_3 (0.15 g, 1.14 mmol) in DMF (2 mL) was added 4-(5-hydroxy-5-methylhexa-1,3-diyn-1-yl)phenol (0.29 g, 1.05 mmol). The mixture was stirred at 80 °C for 5 h. The reaction mixture was cooled to RT and ice-cold water was added and the mixture was extracted 3 times with ethyl acetate. The pooled organics were washed with sat. NaCl solution, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel chromatography (5% ethyl acetate in hexanes) to afford the desired product 0.1 g as an off-white solid (70% over two steps).

^{1}H NMR (300 MHz, $CDCl_3$) δ: ppm 7.40 (d, 2H); 6.80 (d, 2H); 3.99 (s, 2H); 3.87 (m, 2H); 3.74 (s, 3H); 3.54 (m, 2H); 2.17 (m, 2H); 1.71 (m, 2H); 1.57 (s, 6H).

F. *N*-Hydroxy-4-{[4-(5-hydroxy-5-methylhexa-1,3-diyn-1-yl)phenoxy]methyl} tetrahydro-2*H*-pyran-4-carboxamide (**11e**)



To a solution of methyl 4-{[4-(5-hydroxy-5-methylhexa-1,3-diyn-1-yl)phenoxy] methyl}tetrahydro-2*H*-pyran-4-carboxylate (100 mg, 0.28 mmol) in THF:Methanol (1:1, 2 mL), aqueous hydroxylamine (50wt%, 2 mL) and KOH (47 mg, 0.8 mmol) were added. The reaction was allowed to stir at RT for 14 h. The reaction mixture was concentrated and the crude residue was taken up in DMSO for reverse phase purification. The clean fractions were pooled, concentrated and lyophilized to afford 20 mg of the desired product as an off-white solid (20%).

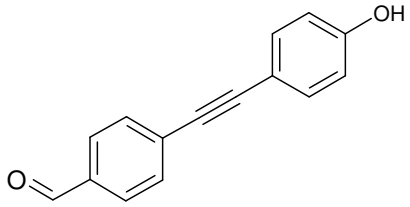
LC-MS: $[M-1]^-$ 356.3

Mass: calculated for $C_{20}H_{23}NO_5$, 357.40

^{1}H NMR (400 MHz, DMSO) δ: ppm 10.60 (bs, 1H); 8.82 (s, 1H); 7.48 (d, 2H); 6.94 (d, 2H); 5.63 (s, 1H); 3.98 (s, 2H); 3.70 (m, 2H); 3.42 (m, 2H); 2.01 (m, 2H); 1.60 (m, 2H); 1.41 (s, 6H).

4-{[4-({4-[(4-Acetylpiperazin-1-yl)methyl]phenyl}ethynyl)phenoxy]methyl}-*N*-hydroxytetrahydro-2*H*-pyran-4-carboxamide (**18a**)

A. 4-[(4-Hydroxyphenyl)ethynyl]benzaldehyde (**15**)



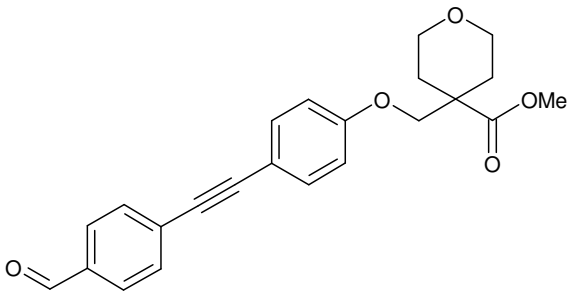
To the solution of 4-{[4-(tetrahydro-2*H*-pyran-2-yloxy)phenyl]ethynyl} benzaldehyde (**6**, 2 g, 6.5 mmol) in methanol was added 4N HCl (2 mL) and stirred at room temperature for 1 h. The solvents were removed and the residue obtained was washed with water. The residue was re-precipitated in methanol:water mixture and filtered. 1.2 g of the residue thus obtained, showed >95% purity by UPLC and was taken to the next step without further purification (83%).

LC-MS: $[M+1]^+$ 223.2

Mass: calculated for $C_{15}H_{10}O_2$, 222.23

^{1}H NMR (400 MHz, DMSO) δ: ppm 10.05 (s, 1H); 10.02 (s, 1H); 7.92 (d, 2H); 7.71 (d, 2H); 7.42 (d, 2H); 6.82 (d, 2H).

B. Methyl 4-({4-[(4-formylphenyl)ethynyl]phenoxy}methyl)tetrahydro-2*H*-pyran-4-carboxylate (**16**)



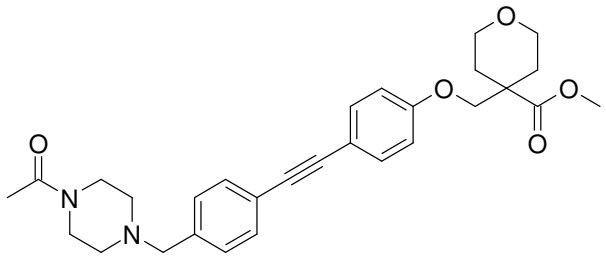
To the solution of methyl 4-(iodomethyl)tetrahydro-2*H*-pyran-4-carboxylate (**4**, 0.65 g, 2.2 mmol) and K_2CO_3 (0.45 g, 3.3 mmol) in DMF (5 mL), was added 4-[(4-hydroxyphenyl)ethynyl]benzaldehyde (0.55 g, 2.5 mmol) and stirred at 120 °C for 5 h. Water was added to the reaction solution and it was extracted 3 times with ethyl acetate. The pooled organics were washed with sat. NaCl solution, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel chromatography (5% ethylacetate in hexanes) to afford 0.5 g of the desired product as off-white solid (53%).

LC-MS: $[M+1]^+$ 379.4

Mass: calculated for $C_{23}H_{22}O_5$, 378.41

^{1}H NMR (300 MHz, DMSO) δ: ppm 10.01 (s, 1H); 7.92 (d, 2H); 7.72 (d, 2H); 7.50 (d, 2H); 7.00 (d, 2H); 4.10 (s, 2H); 3.74 (m, 2H); 3.65 (s, 3H); 3.38 (m, 2H); 2.00 (m, 2H); 1.61 (m, 2H).

C. Methyl 4-{[4-({4-[(4-acetylpiperazin-1-yl)methyl]phenyl}ethynyl)phenoxy] methyl}tetrahydro-2*H*-pyran-4-carboxylate (**17a**)

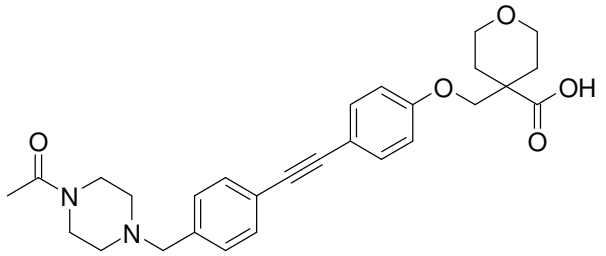


The title compound was synthesized as described for Example 1 step D from methyl 4-({4-[(4-formylphenyl)ethynyl]phenoxy}methyl)tetrahydro-2*H*-pyran-4-carboxylate and *N*-acetylpiperazine.

LC-MS: $[M+1]^+$ 491.5

Mass: calculated for $C_{29}H_{34}N_2O_5$, 490.59

D. 4-{[4-({4-[(4-Acetylpiperazin-1-yl)methyl]phenyl}ethynyl)phenoxy] methyl}tetrahydro-2*H*-pyran-4-carboxylic acid

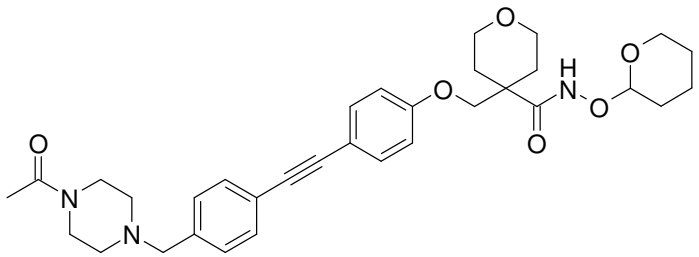


The title compound was synthesized as described for Example 1 step G from methyl 4-{[4-({4-[(4-acetylpiperazin-1-yl)methyl]phenyl}ethynyl)phenoxy]methyl} tetrahydro-2*H*-pyran-4-carboxylate.

LC-MS: $[M+1]^+$ 477.5

Mass: calculated for $C_{28}H_{32}N_2O_5$, 476.56

E. 4-{[4-({4-[(4-Acetylpiperazin-1-yl)methyl]phenyl}ethynyl)phenoxy]methyl}-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide

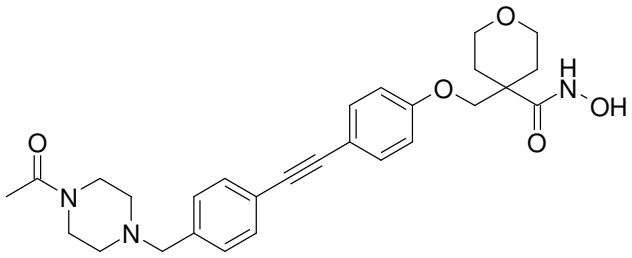


The title compound was synthesized as described for Example 1 step H from 4-{[4-({4-[(4-acetylpiperazin-1-yl)methyl]phenyl}ethynyl)phenoxy]methyl}tetrahydro-2*H*-pyran-4-carboxylic acid.

LC-MS: $[M+1]^+$ 576.5

Mass: calculated for $C_{33}H_{41}N_3O_6$, 575.69

F. 4-{[4-({4-[(4-Acetylpiperazin-1-yl)methyl]phenyl}ethynyl)phenoxy]methyl}-*N*-hydroxytetrahydro-2*H*-pyran-4-carboxamide (**18a**)



The title compound was synthesized as described for Example 1 step I from 4-{[4-({4-[(4-acetylpiperazin-1-yl)methyl]phenyl}ethynyl)phenoxy]methyl}-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide.

LC-MS: $[M+1]^+$ 492.5

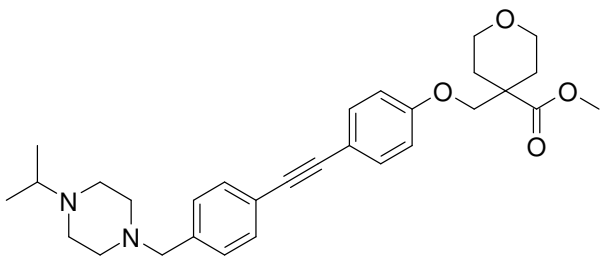
HRMS: $[M+1]^+$ 492.2491

Mass: calculated for $C_{28}H_{33}N_3O_5$, 491.57

^{1}H NMR (400 MHz, DMSO) δ: ppm 10.63 (m, 1H); 10.01 (d, 1H); 7.61 (s, 2H); 7.48 (s, 4H); 6.97 (m, 2H); 3.99 (s, 2H); 3.40 (m, 4H); 3.15 (m, 8H); 2.06 (s, 3H); 1.59 (m, 3H); 1.24 (m, 1H).

N-Hydroxy-4-({4-[(4-{[4-(propan-2-yl)piperazin-1-yl]methyl}phenyl) ethynyl]phenoxy}methyl)tetrahydro-2*H*-pyran-4-carboxamide (**18b**)

A. Methyl 4-({4-[(4-{[4-(propan-2-yl)piperazin-1-yl]methyl}phenyl)ethynyl] phenoxy}methyl)tetrahydro-2*H*-pyran-4-carboxylate (**17b**)

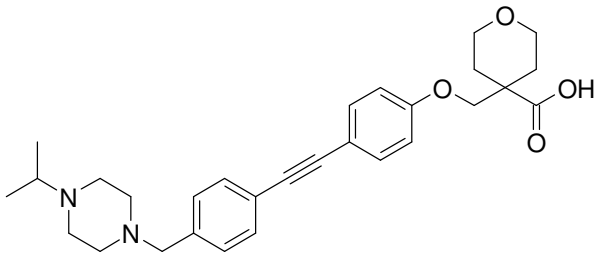


The title compound was synthesized as described for Example 1 step D from methyl 4-({4-[(4-formylphenyl)ethynyl]phenoxy}methyl)tetrahydro-2*H*-pyran-4-carboxylate (**16**) and *N*-isopropylpiperazine.

LC-MS: $[M+1]^+$ 491.5

Mass: calculated for $C_{30}H_{38}N_2O_4$, 490.63

B. 4-({4-[(4-{[4-(Propan-2-yl)piperazin-1-yl]methyl}phenyl)ethynyl] phenoxy}methyl)tetrahydro-2*H*-pyran-4-carboxylic acid

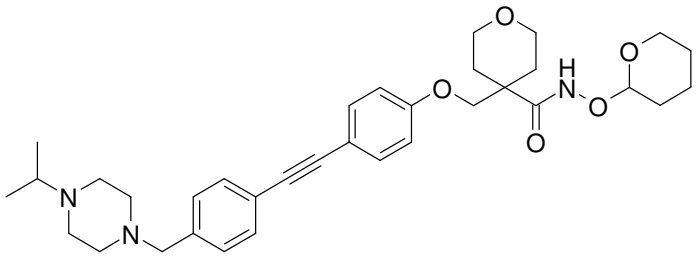


The title compound was synthesized as described for Example 1 step G from methyl 4-({4-[(4-{[4-(propan-2-yl)piperazin-1-yl]methyl}phenyl)ethynyl]phenoxy} methyl)tetrahydro-2*H*-pyran-4-carboxylate.

LC-MS: $[M+1]^+$ 477.5

Mass: calculated for $C_{29}H_{36}N_2O_4$, 476.60

C. 4-({4-[(4-{[4-(Propan-2-yl)piperazin-1-yl]methyl}phenyl)ethynyl]phenoxy} methyl)-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide

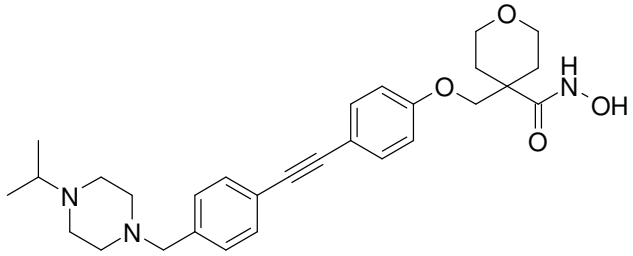


The title compound was synthesized as described for Example 1 step H from 4-({4-[(4-{[4-(propan-2-yl)piperazin-1-yl]methyl}phenyl)ethynyl]phenoxy} methyl)tetrahydro-2*H*-pyran-4-carboxylic acid.

LC-MS: $[M+1]^+$ 576.5

Mass: calculated for $C_{34}H_{45}N_3O_5$, 575.73

D. *N*-Hydroxy-4-({4-[(4-{[4-(propan-2-yl)piperazin-1-yl]methyl}phenyl) ethynyl]phenoxy}methyl)tetrahydro-2*H*-pyran-4-carboxamide (**18b**)



The title compound was synthesized as described for Example 1 step I from 4-({4-[(4-{[4-(propan-2-yl)piperazin-1-yl]methyl}phenyl)ethynyl]phenoxy}methyl)-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide.

LC-MS: $[M+1]^+$ 492.5

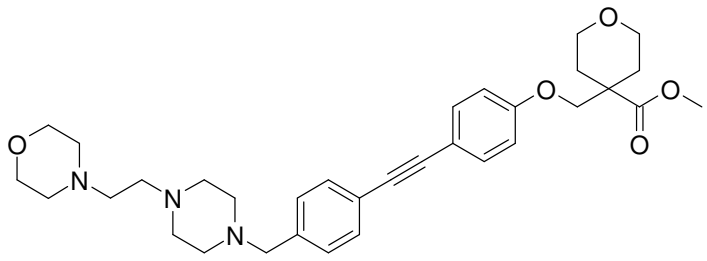
HRMS: $[M+1]^+$ 492.2853

Mass: calculated for $C_{29}H_{37}N_3O_4$, 491.62

^{1}H NMR (400 MHz, DMSO) δ: ppm 7.46 (m, 3H); 7.37 (m, 2H); 7.21 (s, 1H); 6.96 (d, 2H); 3.98 (s, 2H); 3.70 (m, 2H); 3.08 (m, 8H); 2.03 (m, 2H); 1.50 (m, 2H); 1.07 (m, 6H).

N-Hydroxy-4-[(4-{[4-({4-[2-(morpholin-4-yl)ethyl]piperazin-1-yl}methyl)phenyl]ethynyl}phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxamide (**18c**)

A. Methyl 4-[(4-{[4-({4-[2-(morpholin-4-yl)ethyl]piperazin-1-yl}methyl)phenyl] ethynyl}phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxylate (**17c**)

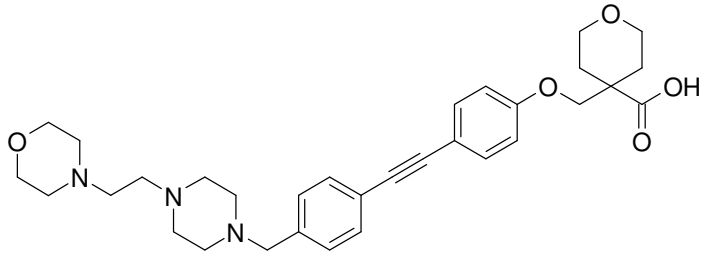


The title compound was synthesized as described for Example 1 step D from methyl 4-({4-[(4-formylphenyl)ethynyl]phenoxy}methyl)tetrahydro-2*H*-pyran-4-carboxylate (**4**) and 4-[2-(piperazin-1-yl)ethyl]morpholine.

LC-MS: $[M+1]^+$ 562.5

Mass: calculated for $C_{33}H_{43}N_3O_5$, 561.71

B. 4-[(4-{[4-({4-[2-(Morpholin-4-yl)ethyl]piperazin-1-yl}methyl)phenyl] ethynyl}phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxylic acid

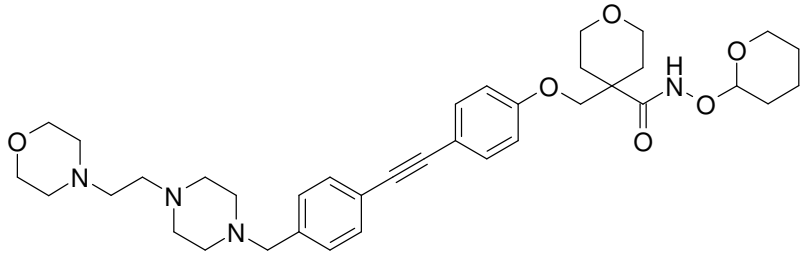


The title compound was synthesized as described for Example 1 step G from methyl 4-[(4-{[4-({4-[2-(morpholin-4-yl)ethyl]piperazin-1-yl}methyl)phenyl] ethynyl}phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxylate.

LC-MS: $[M+1]^+$ 548.5

Mass: calculated for $C_{32}H_{41}N_3O_5$, 547.68

C. 4-[(4-{[4-({4-[2-(Morpholin-4-yl)ethyl]piperazin-1-yl}methyl)phenyl] ethynyl}phenoxy)methyl]-N-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2H-pyran-4-carboxamide

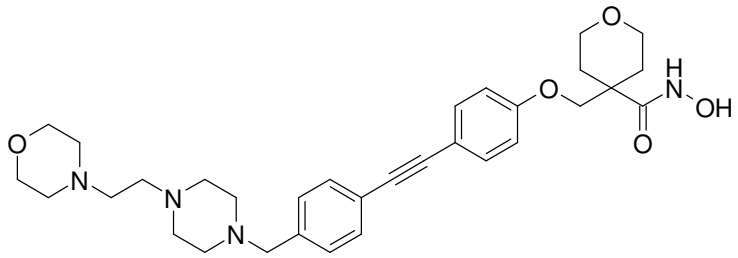


The title compound was synthesized as described for Example 1 step H from 4-[(4-{[4-({4-[2-(morpholin-4-yl)ethyl]piperazin-1-yl}methyl)phenyl]ethynyl}phenoxy) methyl]tetrahydro-2*H*-pyran-4-carboxylic acid.

LC-MS: $[M+1]^+$ 647.5

Mass: calculated for $C_{37}H_{50}N_4O_6$, 646.81

D. *N*-Hydroxy-4-[(4-{[4-({4-[2-(morpholin-4-yl)ethyl]piperazin-1-yl}methyl)phenyl]ethynyl}phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxamide (**18c**)



The title compound was synthesized as described for Example 1 step IS from 4-[(4-{[4-({4-[2-(morpholin-4-yl)ethyl]piperazin-1-yl}methyl)phenyl]ethynyl}phenoxy) methyl]-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide.

LC-MS: $[M+1]^+$ 563.6

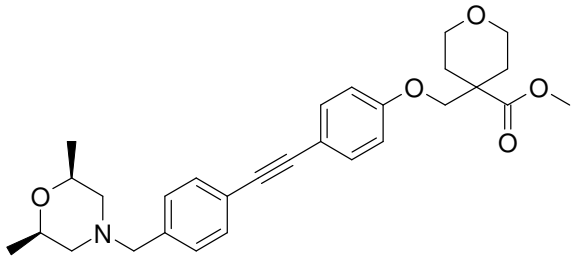
HRMS: $[M+1]^+$ 563.3229

Mass: calculated for $C_{32}H_{42}N_4O_5$, 562.69

^{1}H NMR (400 MHz, DMSO) δ: ppm 10.61 (s, 1H); 10.13 (bs, 1H); 7.59 (m, 3H); 7.47 (d, 2H); 6.96 (d, 2H); 4.34 (s, 2H); 3.97 (s, 2H); 3.83 (bs, 3H); 3.70 (m, 2H); 3.40 (m, 4H); 3.25 (m, 5H); 3.02 (m, 4H); 2.72 (m, 2H); 2.39 (m, 2H); 2.01 (m, 2H); 1.58 (m, 2H).

4-({4-[(4-{[(2*R*,6*S*)-2,6-Dimethylmorpholin-4-yl]methyl}phenyl)ethynyl] phenoxy}methyl)-*N*-hydroxytetrahydro-2*H*-pyran-4-carboxamide (**18d**)

A. Methyl 4-({4-[(4-{[(2*R*,6*S*)-2,6-dimethylmorpholin-4-yl]methyl}phenyl) ethynyl]phenoxy}methyl)tetrahydro-2*H*-pyran-4-carboxylate (**17d**)

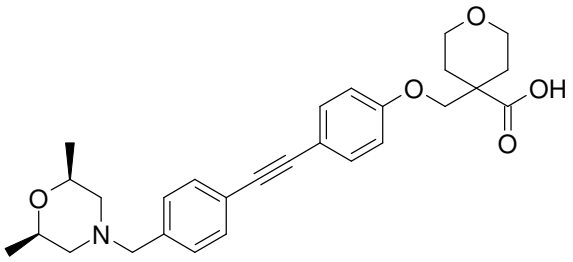


The title compound was synthesized as described for Example 1 step D from methyl 4-({4-[(4-formylphenyl)ethynyl]phenoxy}methyl)tetrahydro-2*H*-pyran-4-carboxylate (**4**) and *cis*-2,6-dimethylmorpholine.

LC-MS: $[M+1]^+$ 478.5

Mass: calculated for $C_{29}H_{35}NO_5$, 477.59

B. 4-({4-[(4-{[(2*R*,6*S*)-2,6-Dimethylmorpholin-4-yl]methyl}phenyl)ethynyl] phenoxy}methyl)tetrahydro-2*H*-pyran-4-carboxylic acid

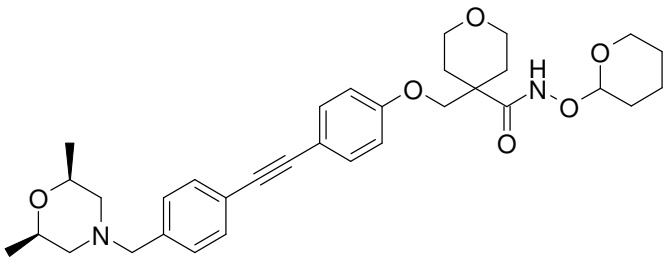


The title compound was synthesized as described for Example 1 Step G from methyl 4-({4-[(4-{[(2*R*,6*S*)-2,6-dimethylmorpholin-4-yl]methyl}phenyl)ethynyl]phenoxy}methyl)tetrahydro-2*H*-pyran-4-carboxylate.

LC-MS: $[M+1]^+$ 464.5

Mass: calculated for $C_{28}H_{33}NO_5$, 463.56

C. 4-({4-[(4-{[(2*R*,6*S*)-2,6-Dimethylmorpholin-4-yl]methyl}phenyl)ethynyl]phenoxy}methyl)-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide

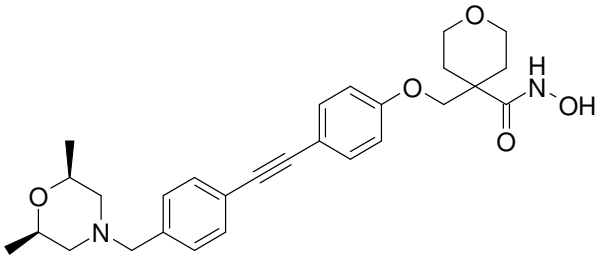


The title compound was synthesized as described for Example 1 Step H from 4-({4-[(4-{[(2*R*,6*S*)-2,6-dimethylmorpholin-4-yl]methyl}phenyl)ethynyl]phenoxy}methyl)tetrahydro-2*H*-pyran-4-carboxylic acid.

LC-MS: $[M+1]^+$ 563.6

Mass: calculated for $C_{33}H_{42}N_2O_6$, 562.69

D. 4-({4-[(4-{[(2*R*,6*S*)-2,6-Dimethylmorpholin-4-yl]methyl}phenyl)ethynyl]phenoxy}methyl)-*N*-hydroxytetrahydro-2*H*-pyran-4-carboxamide (**18d**)



The title compound was synthesized as described for Example 1 Step I from 4-({4-[(4-{[(2*R*,6*S*)-2,6-dimethylmorpholin-4-yl]methyl}phenyl)ethynyl]phenoxy}methyl)-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide.

LC-MS: $[M+1]^+$ 479.5

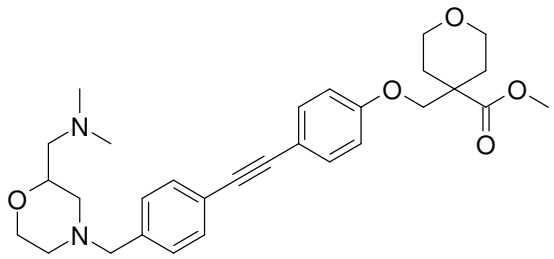
HRMS: $[M+1]^+$ 479.2538

Mass: calculated for $C_{28}H_{34}N_2O_5$, 478.57

^{1}H NMR (400 MHz, DMSO) δ: ppm 10.62 (s, 1H); 7.62 (d, 2H); 7.51 (m, 4H); 6.97 (d, 2H); 4.32 (m, 2H); 3.98 (s, 2H); 3.71 (m, 4H); 3.20 (m, 3H); 2.59 (m, 2H); 2.02 (m, 2H); 1.58 (m, 2H); 1.10 (d, 6H).

4-[(4-{[4-({2-[(dimethylamino)methyl]morpholin-4-yl}methyl)phenyl]ethynyl}phenoxy)methyl]-*N*-hydroxytetrahydro-2*H*-pyran-4-carboxamide (**18e**)

A. Methyl 4-[(4-{[4-({2-[(dimethylamino)methyl]morpholin-4-yl}methyl)phenyl]ethynyl}phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxylate (**17e**)

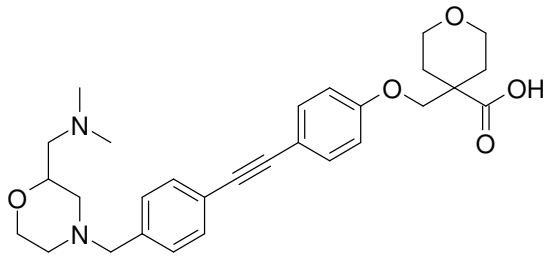


The title compound was synthesized as described for Example 1 step D from methyl 4-({4-[(4-formylphenyl)ethynyl]phenoxy}methyl)tetrahydro-2*H*-pyran-4-carboxylate (Example 14 step B) and Dimethyl-morpholin-2-ylmethylamine.

LC-MS: $[M+1]^+$ 507.5

Mass: calculated for $C_{30}H_{38}N_2O_5$, 506.63

B. 4-[(4-{[4-({2-[(Dimethylamino)methyl]morpholin-4-yl}methyl)phenyl]ethynyl}phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxylic acid

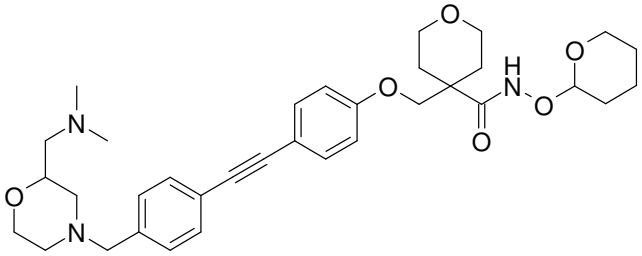


The title compound was synthesized as described for Example 1 step G from methyl 4-[(4-{[4-({2-[(dimethylamino)methyl]morpholin-4-yl}methyl)phenyl] ethynyl}phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxylate.

LC-MS: $[M+1]^+$ 493.5

Mass: calculated for $C_{29}H_{36}N_2O_5$, 492.60

C. 4-[(4-{[4-({2-[(Dimethylamino)methyl]morpholin-4-yl}methyl)phenyl] ethynyl}phenoxy)methyl]-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide

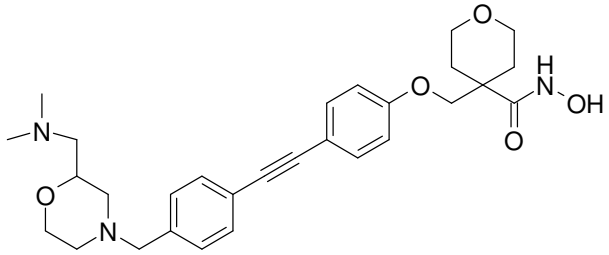


The title compound was synthesized as described for Example 1 step H from 4-[(4-{[4-({2-[(dimethylamino)methyl]morpholin-4-yl}methyl)phenyl]ethynyl} phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxylic acid.

LC-MS: $[M+1]^+$ 592.6

Mass: calculated for $C_{34}H_{45}N_3O_6$, 591.73

D. 4-[(4-{[4-({2-[(Dimethylamino)methyl]morpholin-4-yl}methyl)phenyl] ethynyl}phenoxy)methyl]-*N*-hydroxytetrahydro-2*H*-pyran-4-carboxamide (**18e**)



The title compound was synthesized as described for Example 1 step I from 4-[(4-{[4-({2-[(dimethylamino)methyl]morpholin-4-yl}methyl)phenyl]ethynyl} phenoxy)methyl]-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide.

LC-MS: $[M+1]^+$ 508.5

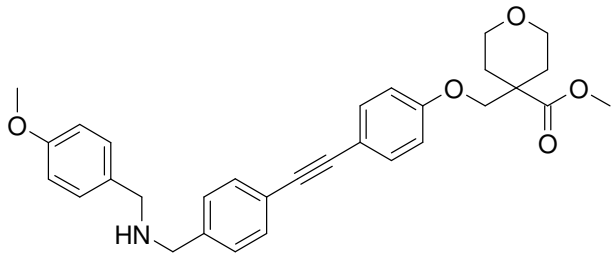
HRMS: $[M+1]^+$ 508.2816

Mass: calculated for $C_{29}H_{37}N_3O_5$, 507.62

^{1}H NMR (400 MHz, DMSO) δ: ppm 8.25 (s, 1H); 7.46 (m, 4H); 7.34 (d, 2H); 6.96 (d, 2H); 3.98 (s, 2H); 3.73 (m, 4H); 3.50 (m, 7H); 2.73 (m, 2H); 2.60 (m, 2H); 2.23 (d, 2H); 2.11 (s, 6H); 2.03 (m, 4H); 1.76 (m, 2H); 1.59 (m, 2H).

N-Hydroxy-4-({4-[(4-{[(4-methoxybenzyl)amino]methyl}phenyl)ethynyl] phenoxy}methyl)tetrahydro-2*H*-pyran-4-carboxamide (**18f**)

A. Methyl 4-({4-[(4-{[(4-methoxybenzyl)amino]methyl}phenyl)ethynyl] phenoxy}methyl)tetrahydro-2*H*-pyran-4-carboxylate (**17f**)

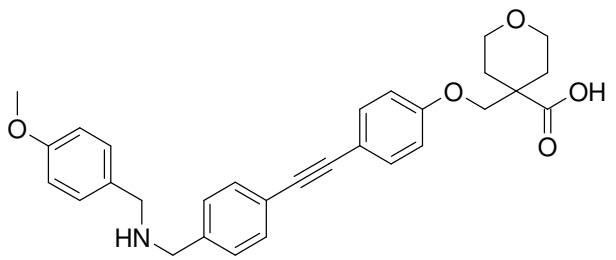


To the solution of methyl 4-({4-[(4-formylphenyl)ethynyl]phenoxy}methyl) tetrahydro-2*H*-pyran-4-carboxylate (**16**, 0.5 g, 1.32 mmol) in dichloroethane (5 mL) was added 4-methoxybenzylamine (0.2 g, 1.45 mmol) and acetic acid (0.1 mL) at room temperature and stirred for 30 min. The reaction mixture was cooled to 0 °C and sodium triacetoxy borohydride (0.42 g, 1.98 mmol) was added portion-wise and the reaction mixture was allowed to stir at room temperature for 2 h. TLC showed the disappearance of the starting material. Water (5 mL) was added to the reaction mixture and it was extracted with DCM. The residue was taken for the next step without further purification.

LC-MS: $[M+1]^+$ 500.6

Mass: calculated for $C_{31}H_{33}NO_5$, 499.59

B. 4-({4-[(4-{[(4-Methoxybenzyl)amino]methyl}phenyl)ethynyl]phenoxy} methyl)tetrahydro-2*H*-pyran-4-carboxylic acid

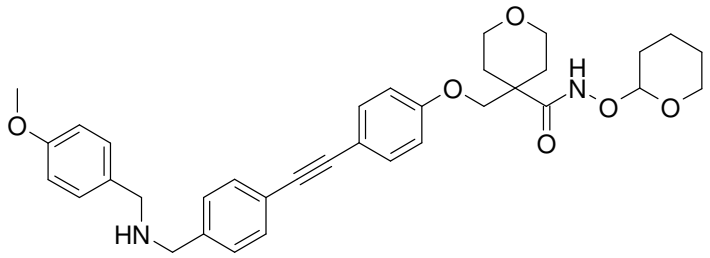


The title compound was synthesized as described for Example 1, Step G from methyl 4-({4-[(4-{[(4-methoxybenzyl)amino]methyl}phenyl)ethynyl]phenoxy} methyl)tetrahydro-2*H*-pyran-4-carboxylate.

LC-MS: $[M-1]^-$ 484.6

Mass: calculated for $C_{30}H_{31}NO_5$, 485.57

C. 4-({4-[(4-{[(4-Methoxybenzyl)amino]methyl}phenyl)ethynyl]phenoxy} methyl)-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide

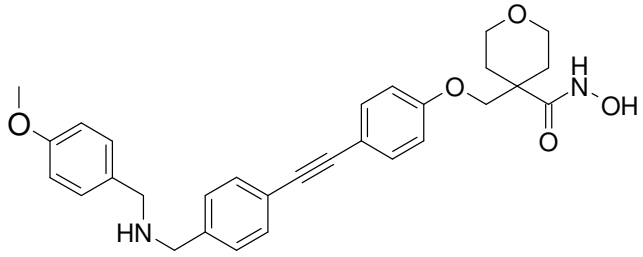


The title compound was synthesized as described for Example 1, Step H from 4-({4-[(4-{[(4-methoxybenzyl)amino]methyl}phenyl)ethynyl]phenoxy}methyl)tetrahydro-2*H*-pyran-4-carboxylic acid and *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine.

LC-MS: $[M+1]^+$ 585.5

Mass: calculated for $C_{35}H_{40}N_2O_6$, 584.70

D. *N*-Hydroxy-4-({4-[(4-{[(4-methoxybenzyl)amino]methyl}phenyl)ethynyl] phenoxy}methyl)tetrahydro-2*H*-pyran-4-carboxamide (**18f**)



The title compound was synthesized as described for Example 1, Step I from 4-({4-[(4-{[(4-methoxybenzyl)amino]methyl}phenyl)ethynyl]phenoxy}methyl)-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide.

LC-MS: $[M+1]^+$ 501.5

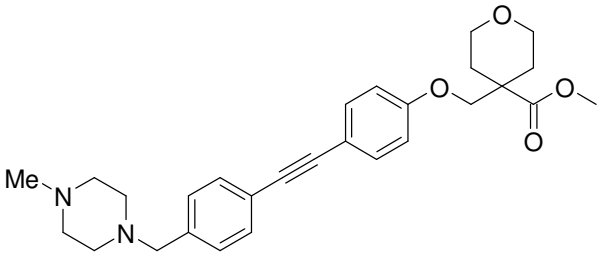
HRMS: $[M+1]^+$ 501.2382

Mass: calculated for $C_{30}H_{32}N_2O_5$, 500.58

^{1}H NMR (400 MHz, DMSO) δ: ppm δ 10.62 (s, 1H); 9.29 (bs, 1H); 7.58 (m, 2H); 7.52 (m, 2H); 7.45 (d, 2H); 7.42 (d, 2H); 6.98 (m, 4H); 4.04 (m, 2H); 4.14 (m, 2H); 3.97 (s, 2H); 3.74 (s, 3H); 3.69 (m, 2H); 3.40 (m, 2H); 2.00 (m, 2H); 1.55 (m, 2H).

N-Hydroxy-4-{[4-({4-[(4-methylpiperazin-1-yl)methyl]phenyl}ethynyl)phenoxy] methyl}tetrahydro-2*H*-pyran-4-carboxamide (**18g**)

A. Methyl 4-{[4-({4-[(4-methylpiperazin-1-yl)methyl]phenyl}ethynyl) phenoxy]methyl}tetrahydro-2*H*-pyran-4-carboxylate (**17g**)

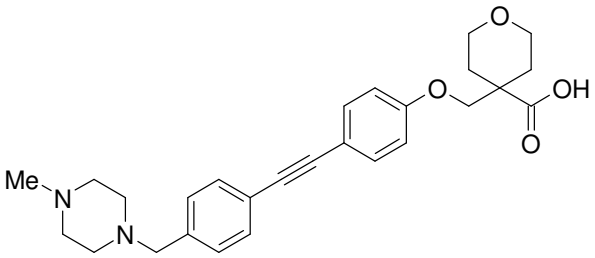


To a solution of methyl 4-({4-[(4-formylphenyl)ethynyl]phenoxy}methyl) tetrahydro-2*H*-pyran-4-carboxylate (**16**) in dichloroethane (2.5 mL) was added 1-methylpiperazine (0.072 g, 0.72 mmol) and acetic acid (0.5 mL). The mixture was stirred at room temperature for 30 min. The reaction mixture was cooled to 0 °C and sodium triacetoxyborohydride (0.16 g, 0.79 mmol) was added in portions. The reaction mixture was allowed to stir at room temperature for 2 h. Ice-cold water (5 mL) was added to the reaction mixture and it was extracted with DCM. The organic layer was washed with 1N HCl (5 mL) and the aqueous layer was basified with $NaHCO_3$ solution. The aqueous layer was then extracted with DCM (20 mL) and the organic layer was concentrated. The residue was used without further purification.

LC-MS: $[M+1]^+$ 463.5

Mass: calculated for $C_{28}H_{34}N_2O_4$, 462.58

B. 4-{[4-({4-[(4-Methylpiperazin-1-yl)methyl]phenyl}ethynyl)phenoxy]methyl}tetrahydro-2*H*-pyran-4-carboxylic acid

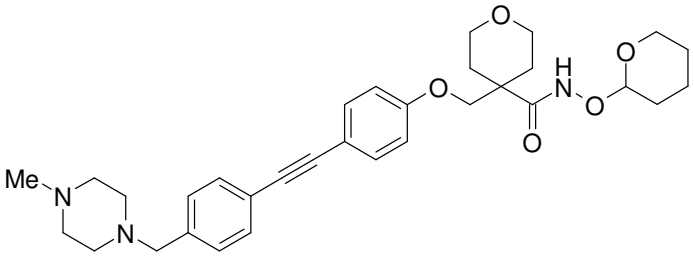


To a solution of methyl 4-{[4-({4-[(4-methylpiperazin-1-yl)methyl]phenyl} ethynyl)phenoxy]methyl}tetrahydro-2*H*-pyran-4-carboxylate (0.25 g, 0.5 mmol) in methanol:THF:Water (1:1:1, 10 mL) was added LiOH•H_2O (38 mg, 1.6 mmol) and the mixture was heated to 60 °C for 14 h. The organic solvents were removed under vacuum and the resulting aqueous solution was neutralized with 1N HCl at 0 °C. The resultant precipitate was filtered and dried under vacuum to give 0.2 g of the desired product as an off-white solid (62% over two steps).

LC-MS: $[M+1]^+$ 449.5

Mass: calculated for $C_{27}H_{32}N_2O_4$, 448.55

C. 4-{[4-({4-[(4-Methylpiperazin-1-yl)methyl]phenyl}ethynyl)phenoxy]methyl}-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide

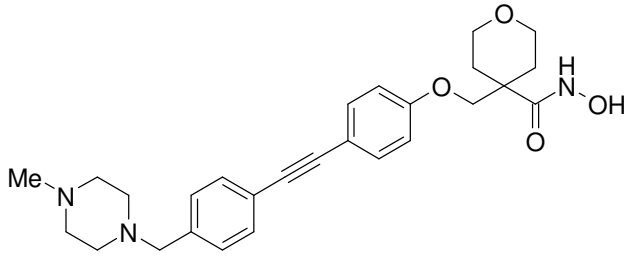


To a solution of 4-{[4-({4-[(4-methylpiperazin-1-yl)methyl]phenyl} ethynyl)phenoxy]methyl}tetrahydro-2*H*-pyran-4-carboxylic acid (250 mg, 0.55 mmol) in DCM (2.5 mL) was added NH_2-OTHP (195 mg, 1.6 mmol) and 2-chloro-1-methyl-pyridinium iodide (280 mg, 1.1 mmol). The mixture was cooled to 0 °C. DIPEA (0.08 mL, 1.6 mmol) and DMAP (6 mg, 0.05 mmol) were added, and the reaction was allowed to stir at room temperature for 14 h. The reaction mixture was diluted with DCM (10 mL) and washed with water, brine, dried over Na_2SO_4, filtered and concentrated. The residue was used without further purification.

LC-MS: $[M+1]^+$ 548.5

Mass: calculated for $C_{32}H_{41}N_3O_5$, 547.68

D. *N*-Hydroxy-4-{[4-({4-[(4-methylpiperazin-1-yl)methyl]phenyl}ethynyl) phenoxy]methyl}tetrahydro-2*H*-pyran-4-carboxamide (**18g**)



To a solution of 4-{[4-({4-[(4-methylpiperazin-1-yl)methyl]phenyl}ethynyl) phenoxy]methyl}-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide (0.1 g, 0.18 mmol) in methanol (10 mL) was added 4N HCl (1 mL) and the mixture was stirred at room temperature for 1 h. The solvents were removed and the residue obtained was washed with water. The crude residue was taken up in DMSO for reverse phase purification. The clean fractions were pooled, concentrated and lyophilized overnight to afford 6 mg of the desired product as a pale brown solid (2% over two steps).

LC-MS: $[M+1]^+$ 464.5

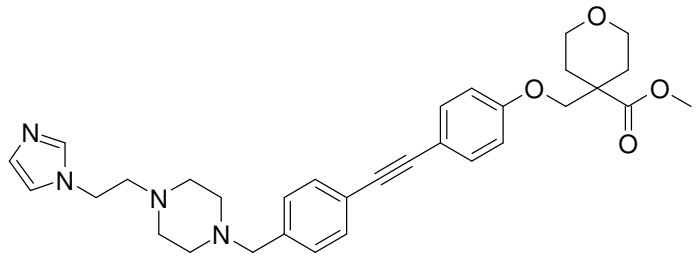
HRMS: $[M+1]^+$ 464.2549

Mass: calculated for $C_{27}H_{33}N_3O_4$, 463.25

^{1}H NMR (400 MHz, DMSO) δ: ppm 10.61 (s, 1H); 9.50 (bs, 1H); 8.90 (bs, 1H); 7.47 (m, 3H); 7.36 (d, 2H); 7.21 (s, 1H); 7.09 (s, 1H); 6.96 (m, 2H); 3.98 (s, 2H); 3.40 (m, 3H); 2.95 (m, 4H); 2.76 (s, 3H); 2.04 (m, 2H); 1.61 (m, 2H).

N-Hydroxy-4-[(4-{[4-({4-[2-(1*H*-imidazol-1-yl)ethyl]piperazin-1-yl}methyl)phenyl]ethynyl}phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxamide (**18h**)

A. Methyl 4-[(4-{[4-({4-[2-(1*H*-imidazol-1-yl)ethyl]piperazin-1-yl}methyl)phenyl]ethynyl}phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxylate (**17h**)

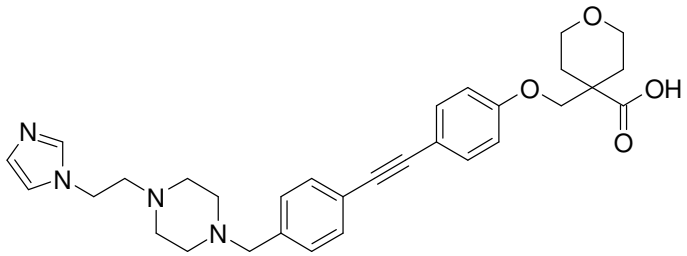


The title compound was synthesized as described for Example 1 step D from methyl 4-({4-[(4-formylphenyl)ethynyl]phenoxy}methyl)tetrahydro-2*H*-pyran-4-carboxylate (Example 14 step B) and 1-[2-(1*H*-imidazol-1-yl)ethyl]piperazine.

LC-MS: $[M+1]^+$ 543.5

Mass: calculated for $C_{32}H_{38}N_4O_4$, 542.66

B. 4-[(4-{[4-({4-[2-(1*H*-Imidazol-1-yl)ethyl]piperazin-1-yl}methyl)phenyl] ethynyl}phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxylic acid

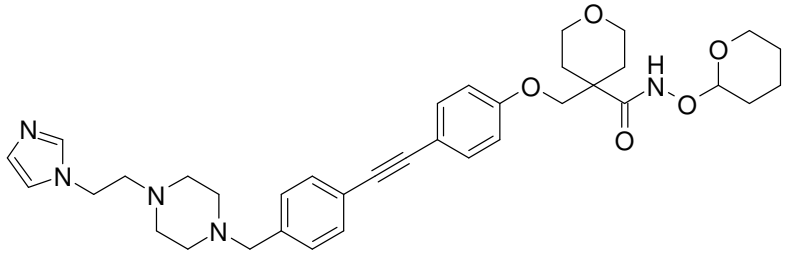


The title compound was synthesized as described for Example 1 step G from methyl 4-[(4-{[4-({4-[2-(1*H*-imidazol-1-yl)ethyl]piperazin-1-yl}methyl)phenyl]ethynyl} phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxylate.

LC-MS: $[M+1]^+$ 529.5

Mass: calculated for $C_{31}H_{36}N_4O_4$, 528.64

C. 4-[(4-{[4-({4-[2-(1*H*-Imidazol-1-yl)ethyl]piperazin-1-yl}methyl)phenyl] ethynyl}phenoxy)methyl]-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide

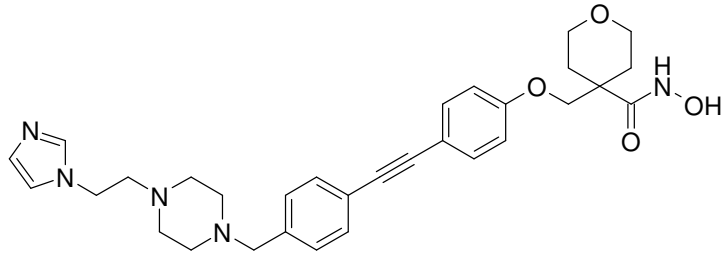


The title compound was synthesized as described for Example 1 step H from 4-[(4-{[4-({4-[2-(1*H*-imidazol-1-yl)ethyl]piperazin-1-yl}methyl)phenyl]ethynyl}phenoxy) methyl]tetrahydro-2*H*-pyran-4-carboxylic acid.

LC-MS: $[M+1]^+$ 628.5

Mass: calculated for $C_{36}H_{45}N_5O_5$, 627.77

D. *N*-Hydroxy-4-[(4-{[4-({4-[2-(1*H*-imidazol-1-yl)ethyl]piperazin-1-yl}methyl)phenyl]ethynyl}phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxamide



The title compound was synthesized as described for Example 1 step I from 4-[(4-{[4-({4-[2-(1*H*-imidazol-1-yl)ethyl]piperazin-1-yl}methyl)phenyl]ethynyl}phenoxy)methyl]-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide.

LC-MS: $[M+1]^+$ 544.5

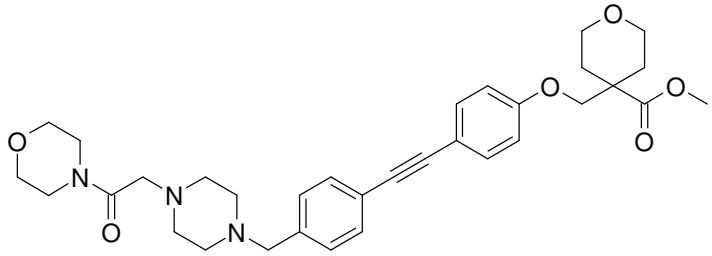
HRMS: $[M+1]^+$ 544.2934

Mass: calculated for $C_{31}H_{37}N_5O_4$, 543.65

^{1}H NMR (400 MHz, DMSO) δ: ppm 10.60 (bs, 1H); 8.84 (bs, 1H); 7.60 (s, 1H); 7.47 (m, 4H); 7.31 (d, 2H); 7.16 (s, 1H); 6.95 (d, 2H); 6.84 (s, 1H); 4.05 (m, 2H); 3.97 (s, 2H); 3.71 (m, 2H); 3.44 (m, 4H); 2.59 (m, 2H); 2.33 (m, 8H); 2.03 (m, 2H); 1.60 (m, 2H).

N-hydroxy-4-[(4-{[4-({4-[2-(morpholin-4-yl)-2-oxoethyl]piperazin-1-yl}methyl)phenyl]ethynyl}phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxamide (**18i**)

A. Methyl 4-[(4-{[4-({4-[2-(morpholin-4-yl)-2-oxoethyl]piperazin-1-yl}methyl)phenyl]ethynyl}phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxylate (**17i**)

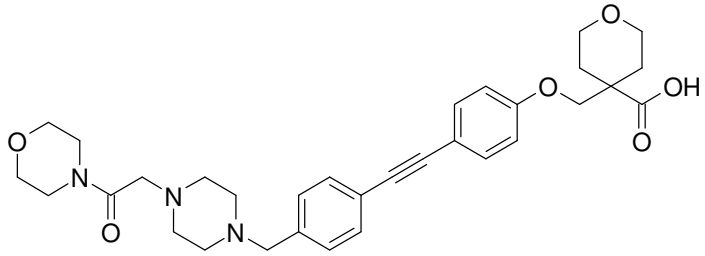


The title compound was synthesized as described for Example 1 step D from methyl 4-({4-[(4-formylphenyl)ethynyl]phenoxy}methyl)tetrahydro-2*H*-pyran-4-carboxylate (Example 14 step B) and 1-(morpholin-4-yl)-2-(piperazin-1-yl)ethanone.

LC-MS: $[M+1]^+$ 576.6

Mass: calculated for $C_{33}H_{41}N_3O_6$, 575.69

B. 4-[(4-{[4-({4-[2-(Morpholin-4-yl)-2-oxoethyl]piperazin-1-yl}methyl)phenyl]ethynyl}phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxylic acid

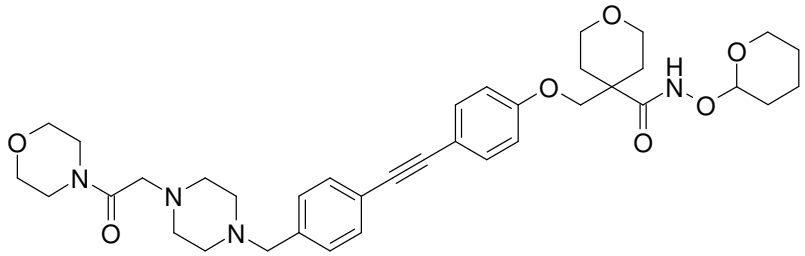


The title compound was synthesized as described for Example 1 step G from methyl 4-[(4-{[4-({4-[2-(morpholin-4-yl)-2-oxoethyl]piperazin-1-yl}methyl)phenyl]ethynyl}phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxylate

LC-MS: $[M+1]^+$ 562.5

Mass: calculated for $C_{32}H_{39}N_3O_6$, 561.66

C. 4-[(4-{[4-({4-[2-(Morpholin-4-yl)-2-oxoethyl]piperazin-1-yl}methyl)phenyl]ethynyl}phenoxy)methyl]-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide

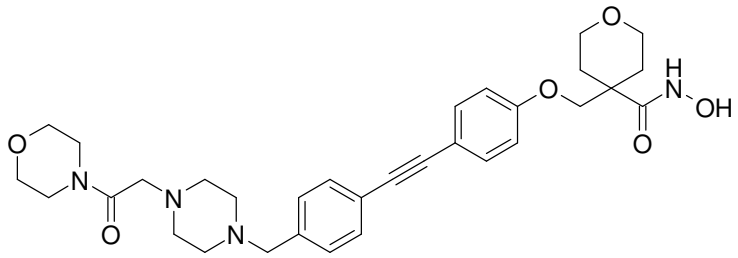


The title compound was synthesized as described for Example 1 step G from 4-[(4-{[4-({4-[2-(morpholin-4-yl)-2-oxoethyl]piperazin-1-yl}methyl)phenyl]ethynyl}phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxylic acid.

LC-MS: $[M+1]^+$ 661.6

Mass: calculated for $C_{37}H_{48}N_4O_7$, 660.79

D. *N*-Hydroxy-4-[(4-{[4-({4-[2-(morpholin-4-yl)-2-oxoethyl]piperazin-1-yl}methyl)phenyl]ethynyl}phenoxy)methyl]tetrahydro-2*H*-pyran-4-carboxamide (**18i**)



The title compound was synthesized as described for Example 1 step I from 4-[(4-{[4-({4-[2-(morpholin-4-yl)-2-oxoethyl]piperazin-1-yl}methyl)phenyl]ethynyl} phenoxy)methyl]-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide.

LC-MS: $[M+1]^+$ 577.5

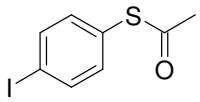
HRMS: $[M+1]^+$ 577.3024

Mass: calculated for $C_{32}H_{40}N_4O_6$, 576.68

^{1}H NMR (400 MHz, DMSO) δ: ppm 7.52 (m, 4H); 7.38 (d, 2H); 6.99 (d, 2H); 3.60 (m, 2H); 3.53 (m, 2H); 3.48 (m, 8H); 2.39 (m, 8H); 2.05 (m, 2H); 1.30 (m, 2H).

N-Hydroxy-4-{[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenyl)sulfanyl] methyl}tetrahydro-2*H*-pyran-4-carboxamide (**23**)

A. *S*-(4-Iodophenyl)ethanethioate (**20**)



To a stirred suspension of zinc powder (0.76 g, 11.6 mmol) and dichlorodimethylsilane (1.36 mL, 11.3 mmol) in 1,2-dichloroethane (10 mL) was added a solution of 4-iodobenzenesulfonylchloride (**19**, 1 g, 3.3 mmol) and *N*,*N*-dimethylacetamide (0.9 mL, 9.9 mmol) in dichloroethane (10 mL). The mixture was stirred at 75 °C for 2 h until the zinc powder was no longer visible. The reaction mixture was cooled to 50 °C, acetyl chloride (0.32 g, 4.17 mmol) was added and the mixture was stirred at 50 °C for 15 min. The mixture was poured into water and extracted with DCM (3 x 20 mL). The combined organic layers were dried over anhydrous sodium sulphate, filtered and concentrated *in vacuo.*

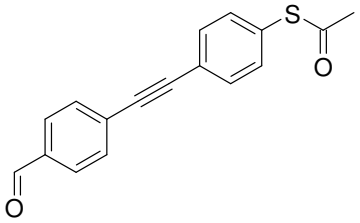
Purification by silica gel column chromatography (5% of ethyl acetate in hexanes) gave 0.6 g of the desired product (65%).

LC-MS: $[M+1]^+$ 279.5

Mass: calculated for C_8H_7IOS, 278.11

^1H NMR (400 MHz, $CDCl_3$) δ: ppm 7.74 (d, 2H); 7.12 (d, 2H); 2.43 (s, 3H).

B. *S*-{4-[(4-Formylphenyl)ethynyl]phenyl}ethanethioate



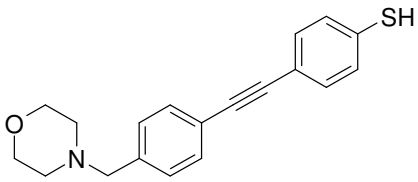
S-(4-Iodophenyl)ethanethioate (0.1 g, 0.36 mmol) and 4-ethynylbenzaldehyde (0.056 g, 0.43 mmol) were diluted with acetonitrile (2 mL) and the solution was purged with argon for 15 min. To this solution was added bis(triphenylphosphine)palladium**(II)** dichloride (25 mg, 0.035 mmol), Et_3N (0.097 mL, 0.72 mmol) and copper(I)iodide (3.4 mg, 0.018 mmol). The mixture was stirred at 60 °C for 2 h. The solvents from the reaction mixture were removed under vacuum. The crude material was purified by silica gel chromatography (5-10% of ethyl acetate in hexanes) to give 0.075 g of the desired product (74%).

LC-MS: $[M+1]^+$ 281.3

Mass: calculated for $C_{17}H_{12}O_2S$, 280.34

^1H NMR (400 MHz, $CDCl_3$) δ: ppm 10.03 (s, 1H); 7.88 (d, 2H); 7.67 (d, 2H); 7.59 (d, 2H); 7.43 (d, 2H); 2.45 (s, 3H).

C. 4-{[4-(Morpholin-4-ylmethyl)phenyl]ethynyl}benzenethiol (**21**)



To a solution of *S*-{4-[(4-formylphenyl)ethynyl]phenyl}ethanethioate (100 mg, 0.35 mmol) in dichloroethane (2 mL) was added morpholine (62 mg, 0.72 mmol) and acetic acid (0.2 mL). The mixture was stirred at RT for 30 min. The reaction mixture was cooled to 0 °C and sodium triacetoxyborohydride (0.23 g, 1.07 mmol) was added in portions. The

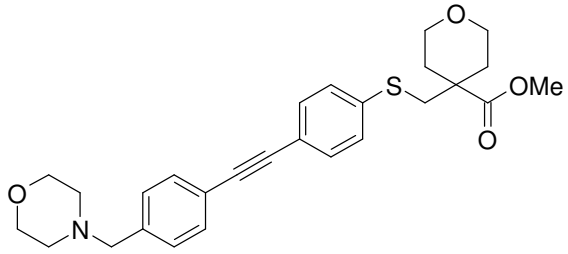
reaction mixture was allowed to stir at RT for 1 h. An ice-cold solution of $NaHCO_3$ (5 mL) was added and the reaction mixture was extracted with DCM. The organic layer was washed with water, brine, dried over Na_2SO_4, filtered and concentrated. The residue was used without further purification.

LC-MS: $[M+1]^+$ 310.4

Mass: calculated for $C_{19}H_{19}NOS$, 309.42

^1H NMR (400 MHz, $CDCl_3$) δ: ppm 7.47 (d, 2H); 7.39 (d, 2H); 7.32 (d, 2H); 7.24 (d, 2H); 3.72 (m, 4H); 3.50 (s, 2H); 2.45 (m, 4H).

D. Methyl 4-{[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenyl)sulfanyl] methyl}tetrahydro-2*H*-pyran-4-carboxylate (**22**)



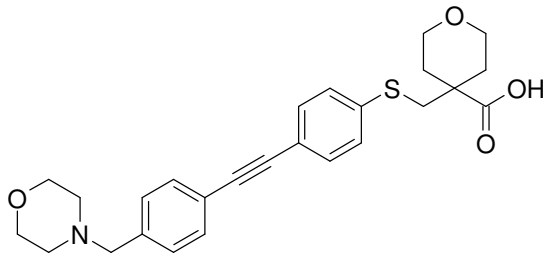
To a solution of methyl 4-(iodomethyl)tetrahydro-2*H*-pyran-4-carboxylate (**4**) (0.63 g, 2.25 mmol) and solid K_2CO_3 (0.31 g, 2.25 mmol) in DMF (6 mL) was added 4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}benzenethiol (**21**) (0.58 g, 1.87 mmol). The mixture was stirred at 80 °C for 3 h. The reaction mixture was cooled to room temperature, ice-cold water was added, and the reaction mixture was extracted 3 times with ethyl acetate. The pooled organics were washed with sat. NaCl solution, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel chromatography (5% ethyl acetate in hexanes) to afford the desired product (0.6 g) as an off-white solid (57%).

LC-MS: $[M+1]^+$ 466.4

Mass: calculated for $C_{27}H_{31}NO_4S$, 465.60

^1H NMR (400 MHz, DMSO) δ: ppm 7.48 (m, 4H); 7.37 (m, 4H); 3.73 (m, 2H); 3.57 (t, 4H); 3.49 (s, 4H); 3.29 (s, 3H); 2.34 (m, 4H); 2.00 (m, 2H); 1.58 (m, 2H).

E. 4-{[(4-{[4-(Morpholin-4-ylmethyl)phenyl]ethynyl}phenyl)sulfanyl] methyl}tetrahydro-2*H*-pyran-4-carboxylic acid

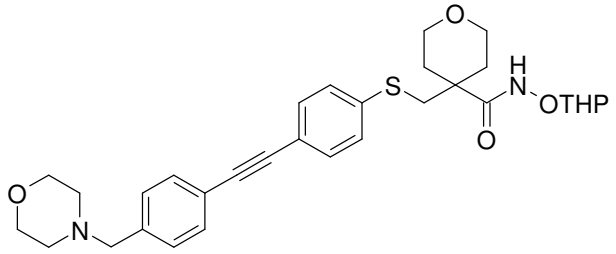


To a solution of methyl 4-{[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenyl)sulfanyl]methyl}tetrahydro-2*H*-pyran-4-carboxylate (0.2 g, 0.43 mmol) in methanol:THF:water (4:4:1, 2 mL) was added LiOH•H_2O (54 mg, 1.2 mmol). The mixture was heated to 60 °C for 2 h. The reaction mixture was neutralized with 1N HCl at 0 °C and the solvents were removed under vacuum. The resultant precipitate was filtered and dried under vacuum to give 0.16 g of the desired product as an off-white solid (82%).

LC-MS: $[M+1]^+$ 452.4

Mass: calculated for $C_{26}H_{29}NO_4S$, 451.57

F. 4-{[(4-{[4-(Morpholin-4-ylmethyl)phenyl]ethynyl}phenyl)sulfanyl]methyl}-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide

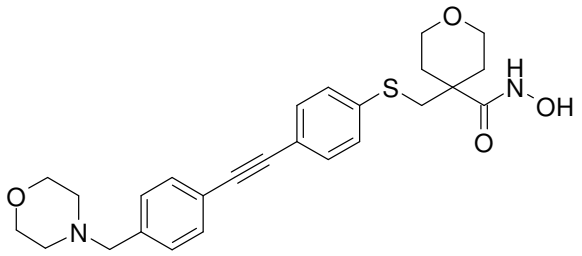


To a solution of 4-{[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenyl)sulfanyl]methyl}tetrahydro-2*H*-pyran-4-carboxylic acid (0.16 g, 0.35 mmol) in DCM (2 mL) was added NH_2-OTHP (82 mg, 0.71 mmol) and 2-chloro-1-methyl-pyridinium iodide (180 mg, 0.71 mmol). The mixture was cooled to 0 °C and DIPEA (0.14 mL, 1.06 mmol) and DMAP (3 mg, 0.025 mmol) were added. The reaction was allowed to stir at RT for 14 h. The reaction mixture was diluted with DCM (10 mL) and washed with water, brine, dried over Na_2SO_4, filtered and concentrated. The residue was used without further purification.

LC-MS: $[M+1]^+$ 551.5

Mass: calculated for $C_{31}H_{38}N_2O_5S$, 550.70

G. *N*-Hydroxy-4-{[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenyl)sulfanyl]methyl}tetrahydro-2*H*-pyran-4-carboxamide (**23**)



To a solution of 4-{[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenyl) sulfanyl]methyl}-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide (0.1 g, 0.18 mmol) in methanol (2 mL) was added 4 N HCl (1 mL) and the mixture was stirred at RT for 30 min. The solvents were removed and the residue obtained was washed with water. The crude residue was taken up in DMSO for reverse phase purification. The clean fractions were pooled, concentrated and lyophilized to afford 6 mg of the desired product as a pale brown solid (4% over two steps).

LC-MS: $[M+1]^+$ 467.4

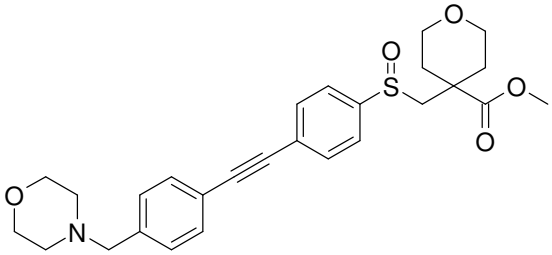
HRMS: $[M+1]^+$ 467.202

Mass: calculated for $C_{26}H_{30}N_2O_4S$, 466.59

^{1}H NMR (400 MHz, DMSO) δ: ppm 10.65 (s, 1H); 8.80 (s, 1H); 7.47 (m, 4H); 7.35 (d, 4H); 3.68 (m, 2H); 3.57 (m, 4H); 3.48 (s, 2H); 3.32 (m, 4H); 3.07 (s, 2H); 2.34 (m, 4H); 2.01 (m, 2H); 1.55 (m, 2H).

(±)-*N*-Hydroxy-4-{[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenyl)sulfinyl] methyl}tetrahydro-2*H*-pyran-4-carboxamide (**25**)

A. (±)-Methyl 4-{[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenyl)sulfinyl] methyl}tetrahydro-2*H*-pyran-4-carboxylate (**24**)



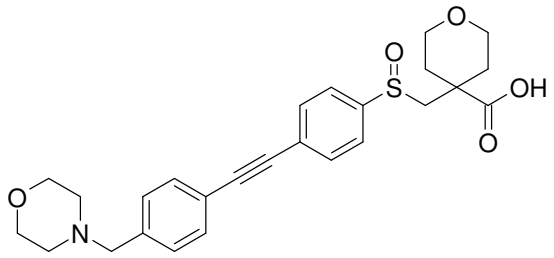
To a solution of (±)-methyl 4-{[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl} phenyl)sulfanyl]methyl}tetrahydro-2*H*-pyran-4-carboxylate (**22**) (0.4 g, 0.86 mmol) in acetic acid (2 mL) at 0 °C was added H_2O_2 (aq. 50%, 0.049 mL, 0.86 mmol) and the mixture was stirred at 0 °C for 1 h. The reaction mixture was poured into ice-cold solution of $NaHCO_3$

and was extracted with ethyl acetate (20 mL X 3). The organic layer was washed with water, brine and dried over anhydrous sodium sulphate. The organic layer was filtered and the filtrate was concentrated. The residue thus obtained was subjected to silica gel column chromatography (230-400 mesh) using 6-8% of methanol in DCM to afford 0.22 g of the desired product (53%).

LC-MS: $[M+1]^+$ 482.5

Mass: calculated for $C_{27}H_{31}NO_5S$, 481.60

B. (±)-4-{[(4-{[4-(Morpholin-4-ylmethyl)phenyl]ethynyl}phenyl)sulfinyl] methyl}tetrahydro-2*H*-pyran-4-carboxylic acid

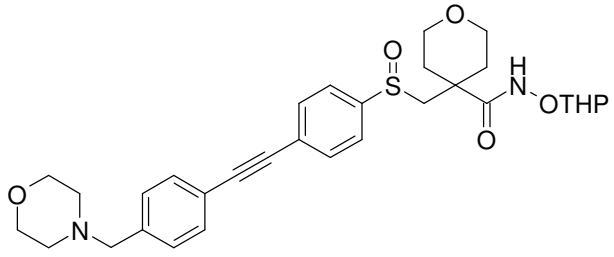


The title compound was synthesized as described for Example 1 step G from (±)-methyl 4-{[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenyl)sulfinyl]methyl} tetrahydro-2*H*-pyran-4-carboxylate.

LC-MS: $[M+1]^+$ 468.4

Mass: calculated for $C_{26}H_{29}NO_5S$, 467.57

C. (±)-4-{[(4-{[4-(Morpholin-4-ylmethyl)phenyl]ethynyl}phenyl)sulfinyl]methyl}-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide

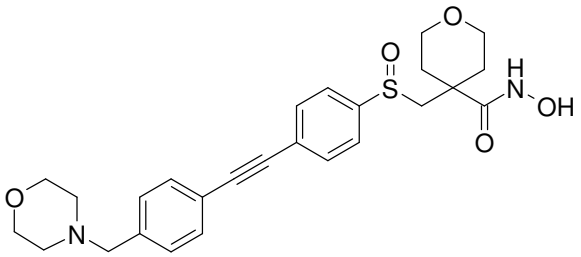


The title compound was synthesized as described for Example 1 step H from (±)-4-{[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenyl)sulfinyl]methyl}tetrahydro-2*H*-pyran-4-carboxylic acid and NH_2OTHP.

LC-MS: $[M+1]^+$ 567.4

Mass: calculated for $C_{31}H_{38}N_2O_6S$, 566.70

D. (±)-*N*-Hydroxy-4-{[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenyl) sulfinyl]methyl}tetrahydro-2*H*-pyran-4-carboxamide (**25**)



The title compound was synthesized as described for Example 1 step I from 4-{[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenyl)sulfinyl]methyl}-*N*-(tetrahydro-2*H*-pyran-2-yloxy)tetrahydro-2*H*-pyran-4-carboxamide.

LC-MS: $[M+1]^+$ 483.4

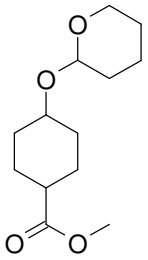
HRMS: $[M+1]^+$ 483.195

Mass: calculated for $C_{26}H_{30}N_2O_5S$, 482.59

^{1}H NMR (400 MHz, MeOD) δ: ppm 8.43 (bs, 2H); 7.73 (m, 4H); 7.53 (d, 2H); 7.41 (d, 2H); 3.78 (m, 2H); 3.64 (m, 4H); 3.59 (m, 4H); 2.50 (m, 4H); 2.30 (m, 1H); 2.12 (m, 1H); 1.93 (m, 1H); 1.79 (m, 1H); 1.32 (m, 2H).

N,4-Dihydroxy-1-[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenoxy) methyl]cyclohexanecarboxamide (**27a**)

A. Methyl 4-(tetrahydro-2*H*-pyran-2-yloxy)cyclohexanecarboxylate (**26a**)

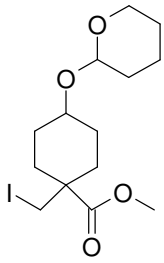


The title compound was synthesized as described for Example 3 step A from methyl 4-hydroxycyclohexanecarboxylate.

GCMS: 158 [-THP cleaved]

Mass: calculated for $C_{13}H_{22}O_4$, 242.31

B. Methyl 1-(iodomethyl)-4-(tetrahydro-2*H*-pyran-2-yloxy)cyclohexane carboxylate

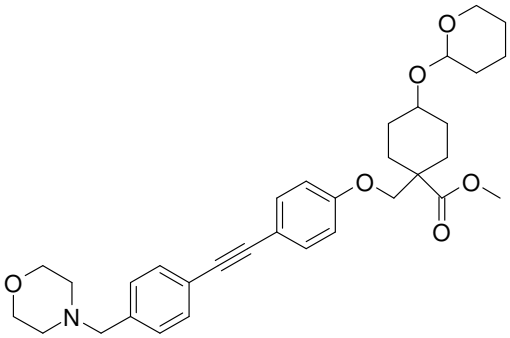


The title compound was synthesized as described for Example 1 step A from methyl 4-(tetrahydro-2*H*-pyran-2-yloxy)cyclohexanecarboxylate.

GCMS: 299.2 [-THP cleaved]

Mass: calculated for $C_{14}H_{23}IO_4$, 382.23

C. Methyl 1-[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenoxy)methyl]-4-(tetrahydro-2*H*-pyran-2-yloxy)cyclohexanecarboxylate

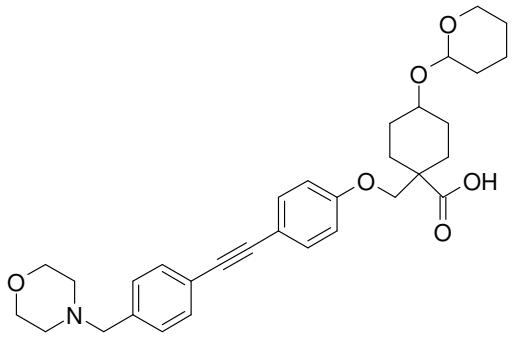


The title compound was synthesized as described for Example 1 step F from methyl 1-(iodomethyl)-4-(tetrahydro-2*H*-pyran-2-yloxy)cyclohexanecarboxylate and 4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenol (**7**).

LC-MS: $[M+1]^+$ 548.5

Mass: calculated for $C_{33}H_{41}NO_6$, 547.68

D. 1-[(4-{[4-(Morpholin-4-ylmethyl)phenyl]ethynyl}phenoxy)methyl]-4-(tetrahydro-2*H*-pyran-2-yloxy)cyclohexanecarboxylic acid

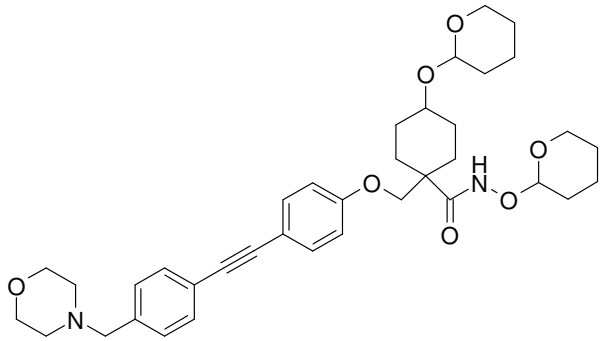


The title compound was synthesized as described for Example 1 step G from methyl 1-[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenoxy)methyl]-4-(tetrahydro-2*H*-pyran-2-yloxy)cyclohexanecarboxylate.

LC-MS: $[M+1]^+$ 534.5

Mass: calculated for $C_{32}H_{39}NO_6$, 533.65

E. 1-[(4-{[4-(Morpholin-4-ylmethyl)phenyl]ethynyl}phenoxy)methyl]-*N*,4-bis(tetrahydro-2*H*-pyran-2-yloxy)cyclohexanecarboxamide

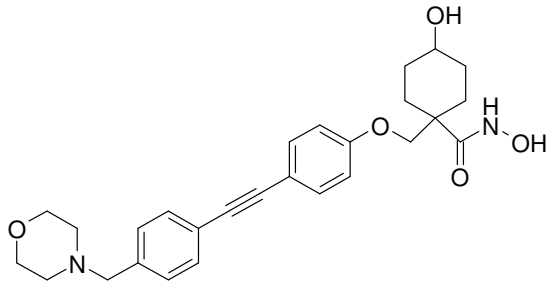


The title compound was synthesized as described for Example 1 step H from 1-[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenoxy)methyl]-4-(tetrahydro-2*H*-pyran-2-yloxy)cyclohexanecarboxylic acid.

LC-MS: $[M+1]^+$ 633.6

Mass: calculated for $C_{37}H_{48}N_2O_7$, 632.78

F. *N*,4-dihydroxy-1-[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenoxy) methyl]cyclohexanecarboxamide (**27a**)



The title compound was synthesized as described for Example 1 step I from 1-[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenoxy)methyl]-*N*,4-bis(tetrahydro-2*H*-pyran-2-yloxy)cyclohexanecarboxamide.

LC-MS: $[M+1]^+$ 465.5

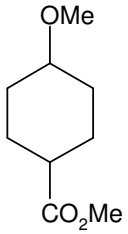
Mass: calculated for $C_{27}H_{32}N_2O_5$, 464.55

^{1}H NMR (400 MHz, DMSO) δ: ppm 7.46 (m, 4H); 7.35 (d, 2H); 6.95 (d, 2H); 3.70 (m, 4H); 3.54 (s, 2H); 2.47 (m, 4H); 1.92 (m, 4H); 1.78 (m, 2H); 1.58 (m, 2H).

N-Hydroxy-4-({4-[(4-{[4-(propan-2-yl)piperazin-1-yl]methyl}phenyl)ethynyl] phenoxy}methyl)tetrahydro-2*H*-pyran-4-carboxamide

N-Hydroxy-4-methoxy-1-[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl} phenoxy)methyl]cyclohexanecarboxamide (**27b**)

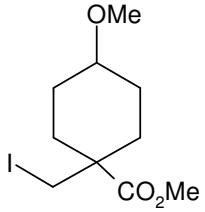
A. Methyl 4-methoxycyclohexanecarboxylate (**26b**)



To a solution of 4-methoxycyclohexanecarboxylic acid (3.5 g, 0.02 mol) in methanol (35 mL) was added concentrated sulfuric acid (0.5 mL) and the mixture was refluxed at 70 °C for 14 h. The reaction was cooled to RT. The solvents were removed under vacuum and the residue was dissolved in ethyl acetate (250 mL). The organic layer was washed with 10% $NaHCO_3$ solution, sat. NaCl, dried over Na_2SO_4, filtered and concentrated to give 3.2 g of the desired product as a colorless liquid (93%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: ppm 3.66 (s, 3H); 3.59 (m, 1H); 3.3 (s, 3H); 2.37 (m, 1H); 1.88 (m, 4H)); 1.66 (m, 2H); 1.52 (m, 2H).

B. Methyl 1-(iodomethyl)-4-methoxycyclohexanecarboxylate

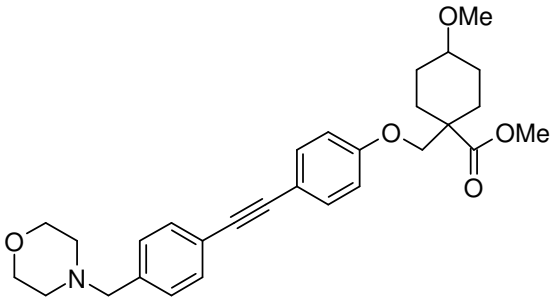


To a solution of methyl 4-methoxycyclohexanecarboxylate (5 g, 0.029 mol) in THF (100 mL) at -100 °C was added LDA (29 mL, 0.058 mol, 2M solution in THF). The mixture was stirred for 1 h at -100 °C. Next, CH_2I_2 (7.7 g, 0.029 mol) was added and the reaction mixture was allowed to warm up to RT and stir for 2 h at RT. Water was added to the reaction solution and it was extracted with ethyl acetate (250 mL x 3). The pooled organics were washed with sat. NaCl solution, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel chromatography (0-10% ethyl acetate in hexanes) to afford 7.8 g of the desired product as a pale yellow liquid (86%).

GCMS: 312

Mass: calculated for $C_{10}H_{17}IO_3$, 312.14

C. Methyl 4-methoxy-1-[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl} phenoxy)methyl]cyclohexanecarboxylate



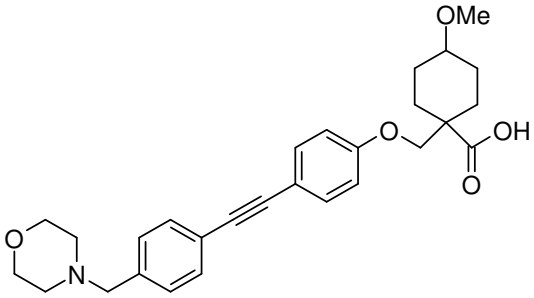
To a solution of methyl 1-(iodomethyl)-4-methoxycyclohexanecarboxylate (2 g, 6.4 mmol) and solid K_2CO_3 (1.3 g, 9 mmol) in DMF (10 mL), was added 4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenol (**7**, 1.9 g, 6 mmol). The mixture was stirred at 120 °C for 5 h. Water was added to the reaction mixture and it was extracted with ethyl acetate (50 mL x 3). The pooled organics were washed with sat. NaCl solution, dried over Na_2SO_4, filtered

and concentrated. The crude product was purified by silica gel chromatography (5-50% ethyl acetate in hexanes) to afford the desired product (1.7 g) as an off-white solid (56%).

LC-MS: $[M+1]^+$ 478.5

Mass: calculated for $C_{29}H_{35}NO_5$, 477.59

D. 4-Methoxy-1-[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenoxy) methyl]cyclohexanecarboxylic acid

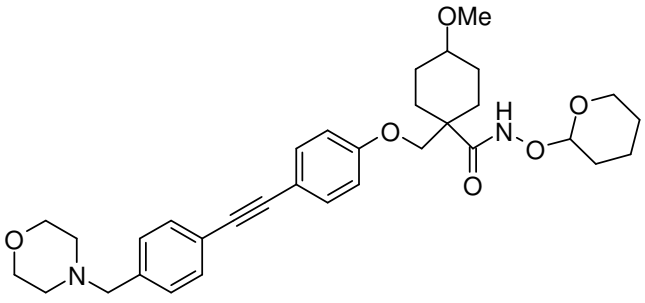


To a solution of methyl 4-methoxy-1-[(4-{[4-(morpholin-4-ylmethyl)phenyl] ethynyl}phenoxy)methyl]cyclohexanecarboxylate (1 g, 2.09 mmol) in methanol:THF:Water (1:1:1, 10 mL) was added $LiOH•H_2O$ (264 mg, 6.2 mmol). The mixture was heated to 60 °C for 14 h. The organic solvents were removed under vacuum and the resulting aqueous solution was acidified at 0 °C with 1N HCl to pH 2. The resultant precipitate was filtered and dried under vacuum to give 0.8 g of the desired product as an off-white solid (>98%).

LC-MS: $[M+1]^+$ 464.5

Mass: calculated for $C_{28}H_{33}NO_5$, 463.56

E. 4-Methoxy-1-[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl}phenoxy) methyl]-*N*-(tetrahydro-2*H*-pyran-2-yloxy)cyclohexanecarboxamide

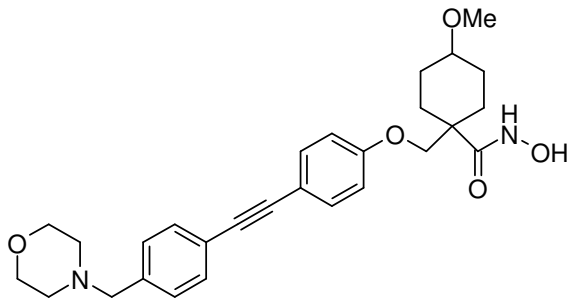


To a solution of 4-methoxy-1-[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl} phenoxy)methyl]cyclohexanecarboxylic acid (500 mg, 1.07 mmol) in DCM (10 mL) was added NH_2-OTHP (252 mg, 2.1 mmol) and 2-chloro-1-methyl-pyridinium iodide (584 mg, 2.1 mmol). The mixture was cooled to 0 °C and DIPEA (0.56 mL, 3.2 mmol) and DMAP (13 mg, 0.1 mmol) were added. The reaction was allowed to stir at RT for 2 h. The reaction mixture was diluted with DCM (50 mL) and washed with water, brine, dried over Na_2SO_4, filtered and concentrated. The residue was used without further purification.

LC-MS: $[M+1]^+$ 563.5

Mass: calculated for $C_{33}H_{42}N_2O_6$, 562.69

F. *N*-Hydroxy-4-methoxy-1-[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl} phenoxy)methyl]cyclohexanecarboxamide (**27b**)



To a solution of 4-methoxy-1-[(4-{[4-(morpholin-4-ylmethyl)phenyl]ethynyl} phenoxy)methyl]-*N*-(tetrahydro-2*H*-pyran-2-yloxy)cyclohexanecarboxamide (0.5 g, 0.88 mmol) in methanol (10 mL) was added 4N HCl (1 mL) and the mixture was stirred at RT for 1 h. The solvents were removed and the residue obtained was washed with water. The crude residue was taken up in DMSO for reverse phase purification. The clean fractions were pooled, concentrated and lyophilized to afford 70 mg of the desired product as an off-white solid (14% over two steps).

LC-MS: $[M+1]^+$ 479.5

HRMS: $[M+1]^+$ 479.2544

Mass: calculated for $C_{28}H_{34}N_2O_5$, 478.57

^{1}H NMR (400 MHz, DMSO) δ: ppm 10.50 (s, 1H); 8.18 (s, 1H); 7.45 (m, 3H); 7.33 (d, 2H); 6.93 (d, 2H); 3.88 (s, 2H); 3.57 (m, 4H); 3.47 (s, 2H); 3.21 (s, 3H); 3.12 (m, 2H); 2.34 (m, 4H); 2.18 (m, 2H); 1.82 (m, 2H); 1.31 (m, 4H).

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