Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

Electronic Supplementary Information One

Irreversible inhibition of BoNT/A protease: Proximity-driven reactivity contingent upon a bifunctional approach

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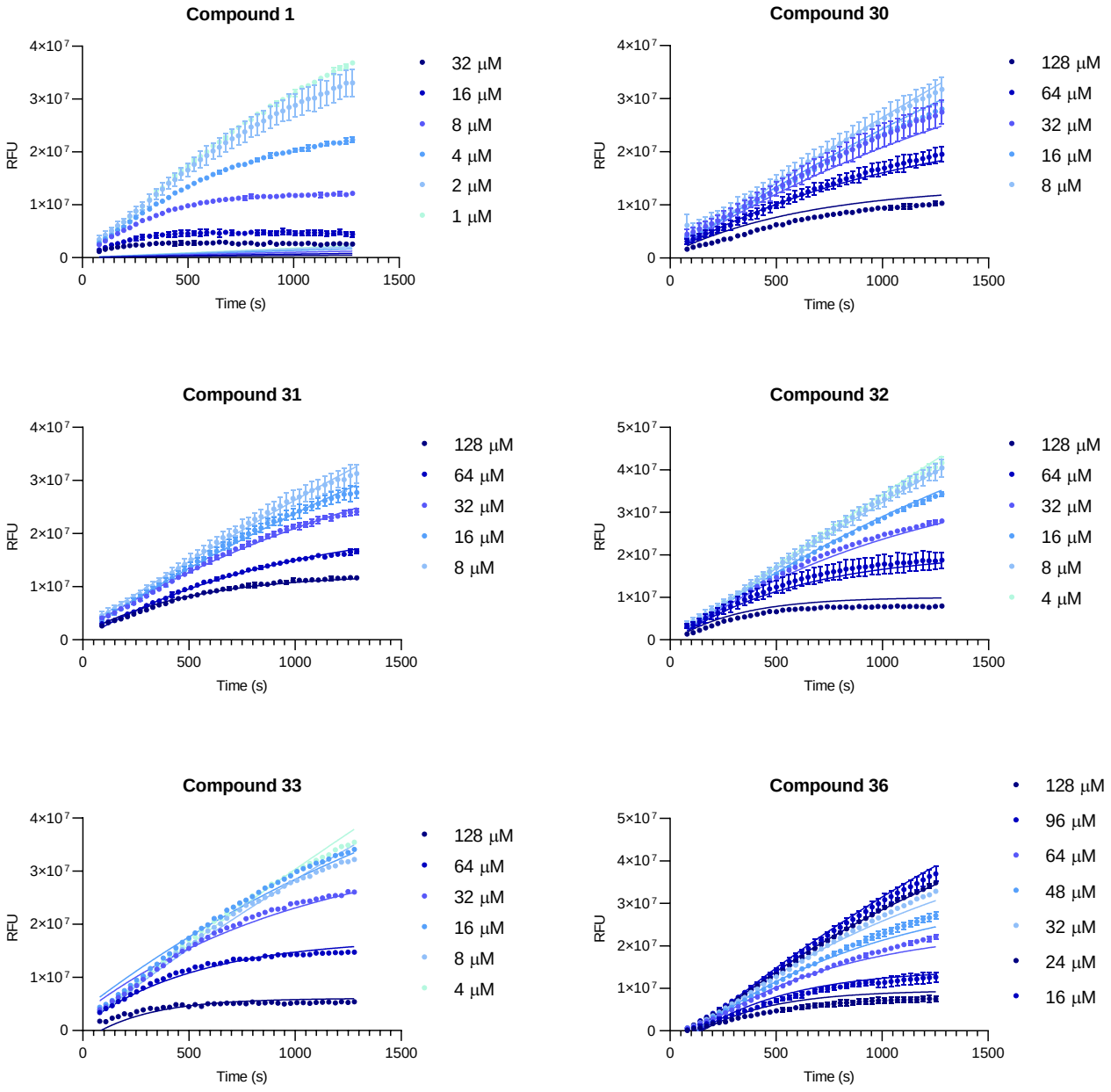
Table of Contents

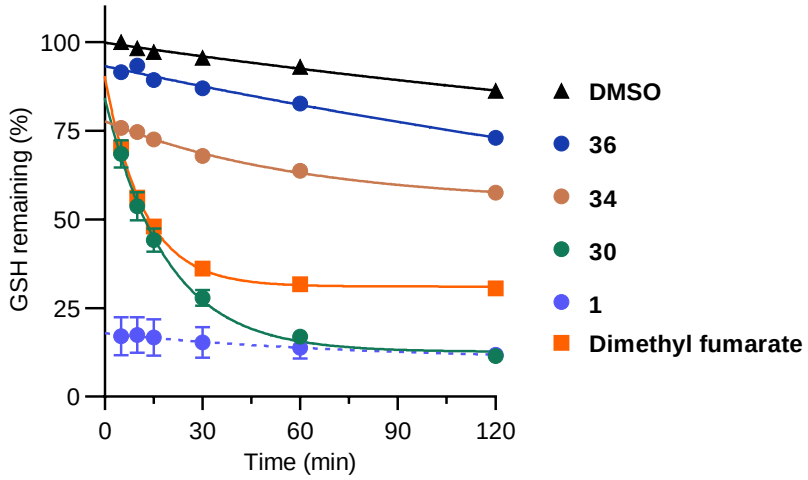
1.0 Supplementary Figures

1.1 Figure S1



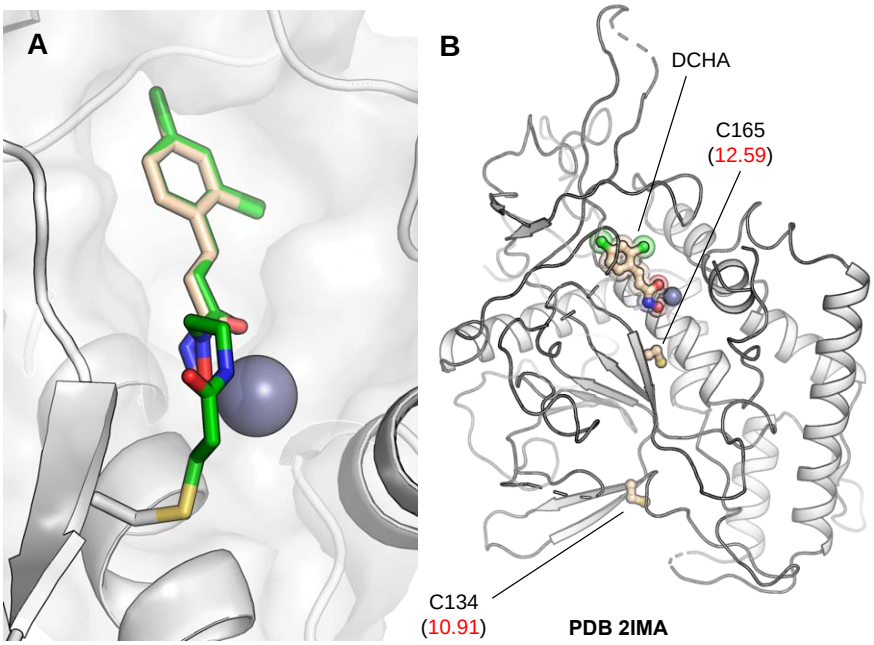
Continuous SNAPtide FRET progression curves for covalent hits. Data sets shown are representative replicates, n = 3–4.

1.2 Figure S2



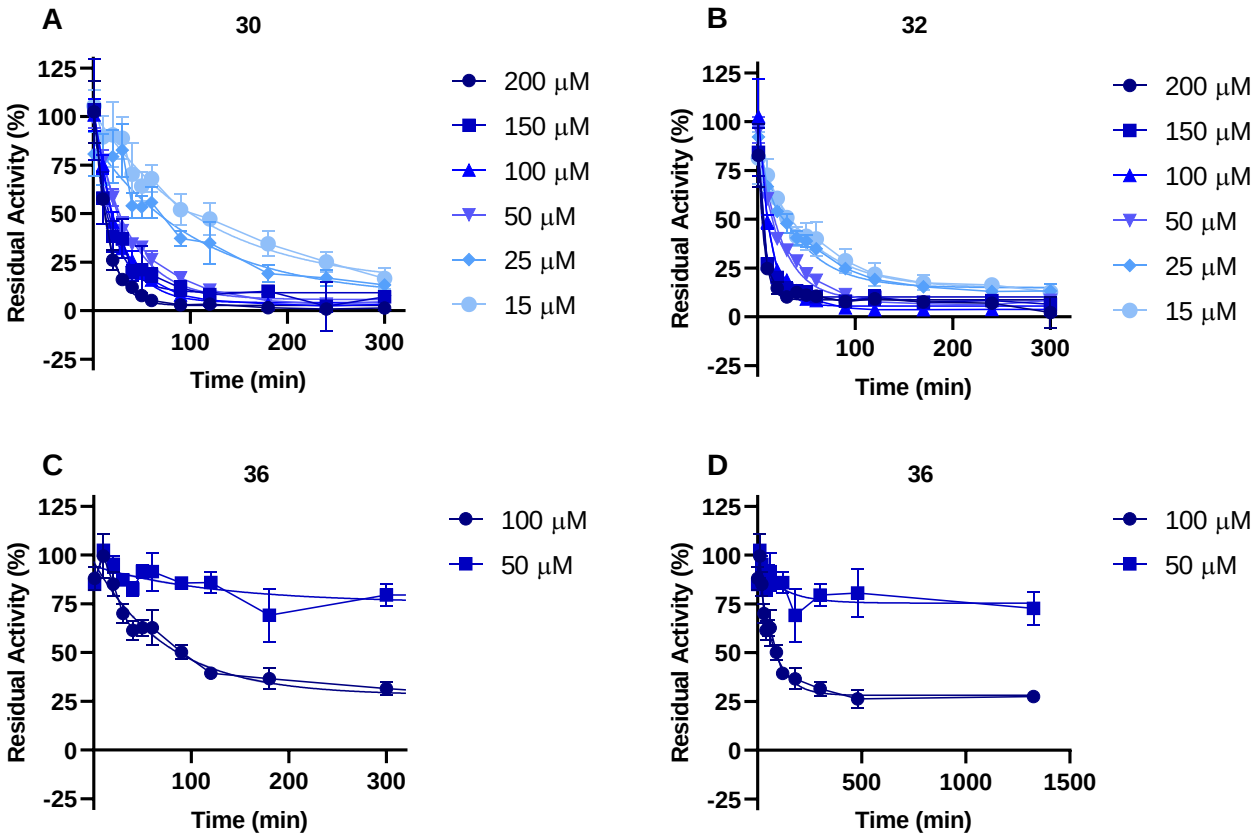
Glutathione reactivity graph. Error bars represent the mean ± SD, n = 3.

1.3 Figure S3



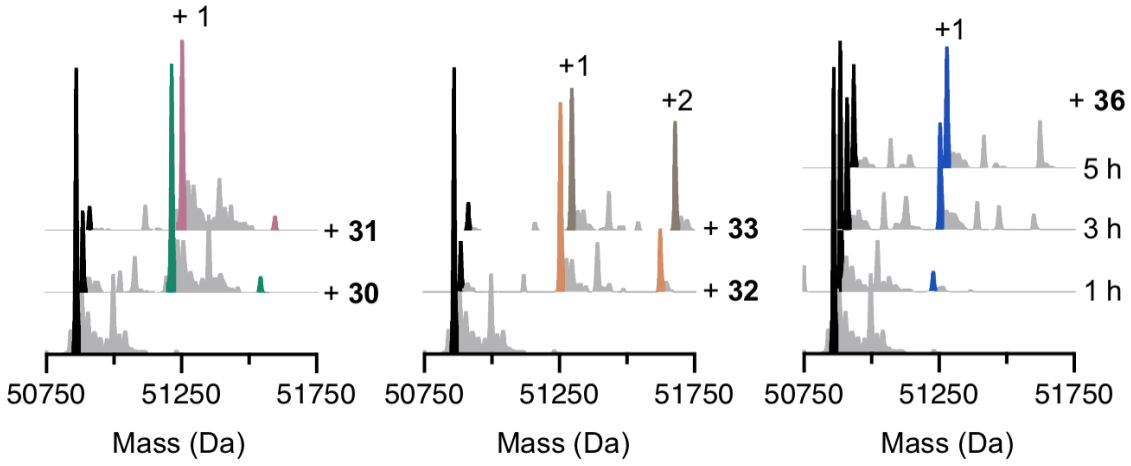
(A) The predicted binding mode derived from covalent docking of compound 2 (green) into the BoNT/A LC active site (PDB 2IMA) superimposed with the binding mode for DCHA (beige). The catalytic zinc metal is rendered as a grey sphere. (B) X-ray co-crystal structure of BoNT/A LC in complex with DCHA (PDB 2IMA)[1] highlighting the two cysteine residues and their predicted pKa values (in red). Values were calculated using PROPKA.[2]

1.4 Figure S4



Preincubation assays for **30**, **32** and **36**. Error bars represent the mean ± SD, n = 3. (A–B) Dose response curves for **30** and **32** showing indicative behavior of covalent inhibition. (C) Dose response inhibition curves for **36** over 5 hours. (D) Dose response inhibition curves over 22 hours.

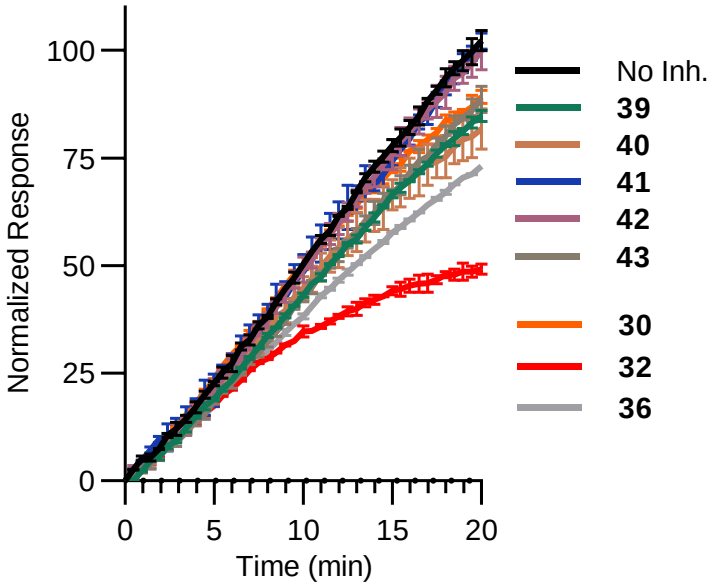
1.5 Figure S5



Deconvoluted HRMS traces of covalent adducts of BoNT/A LC.

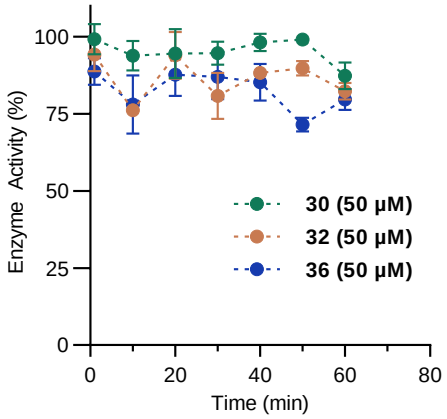
Maleimide compounds **32** and **33** showed double labeling within an hour, however, an excess of compound, coupled with the high reactivity of maleimide warheads, can explain this phenomenon.

1.6 Figure S6



Expansion of Fig 4B showing continuous assay profiles for control compounds **39**–**43** and covalent hits **30**, **32** and **36**.

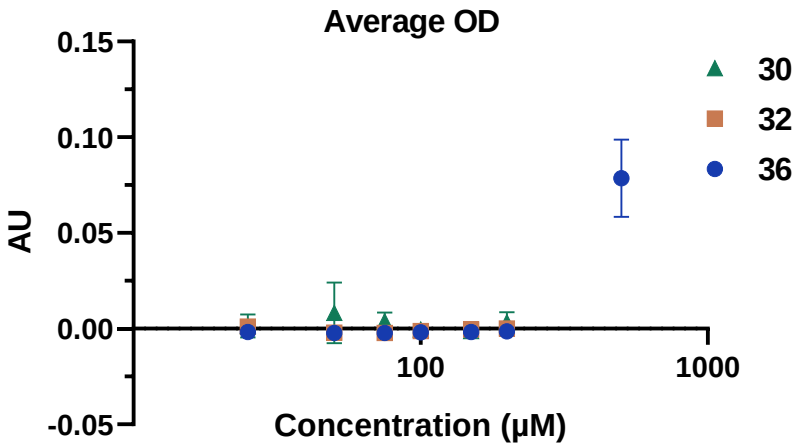
1.7 Figure S7



C165A BoNT/A LC preincubation assay with covalent compounds **30**, **32** and **36** against. Errors represent the mean ± SEM, n = 3.

Enzyme activity for C165A was severely impaired after one-hour incubation, however, there was a drastic difference in time-dependent inhibition when compared to the WT BoNT/A LC.

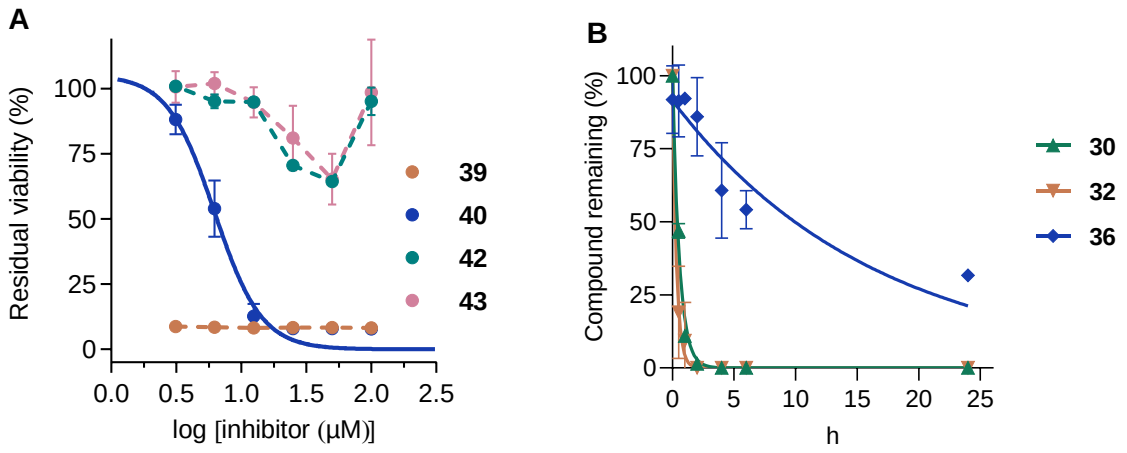
1.8 Figure S8



Absorbance of compound solutions in assay buffer using UV/Vis spectroscopy. Errors represent the mean ± SEM, n = 3.

Compounds **30**, **32** and **36** were soluble in the FRET-based assay buffer at a concentration ≤ 200 µM. Compound **36** at 500 µM was included as a positive control and did exhibit significant precipitation.

1.9 Figure S9



(A) MTT assays of control compounds. Data shown are average residual activity ± SEM, n = 2. (B) Serum stability assay over 24 h. Data shown as the mean ± SEM, n = 3.

2.0 Supplementary Tables

2.1 Table S1. BoNT/A LC percent inhibition values.

%-inhibition

Compound	100 µM	50 µM	25 µM
2–6, **8,9, 12, 17–18*, 20–22, 34, 35, 39, 40**	no inhib.	no inhib.	no inhib.
7	36 ± 2	27 ± 2	17 ± 1
10	53 ± 4	33 ± 5	32 ± 3
11	26 ± 2	no inhib.	no inhib.
13	41 ± 3	20 ± 2	6 ± 2
14	23 ± 3	no inhib.	no inhib.
15	59 ± 2	33 ± 1	17 ± 1
16	94 ± 4	57 ± 2	no inhib.
19	6 ± 1	no inhib.	no inhib.
23	50 ± 4	18 ± 3	16 ± 2
24	10 ± 3	no inhib.	no inhib.
25	18 ± 2	no inhib.	no inhib.
26	46 ± 1	37 ± 1	27 ± 1
27	49 ± 2	33 ± 2	24 ± 2
28	41 ± 1	32 ± 1	22 ± 5
29	53 ± 1	25 ± 4	no inhib.
37	74 ± 1	53 ± 1	22 ± 1
38	5 ± 2	no inhib.	no inhib.

Data are based on 2–3 individual experiments performed in duplicate. *Assays ran at compound concentration of 10, 5 and 2.5 µM, respectively – **17** and **18** were insoluble at higher concentrations.

2.2 Table S2. MMP screening panel

%–inhibition at 25 µM

MMP	30	32	36
1	120	44 ± 19	164 ± 13
2	123 ± 20	59 ± 17	133 ± 21
3	108 ± 3	85 ± 26	107 ± 3
7	109 ± 6	116 ± 9	113 ± 14
8	120 ± 22	50 ± 13	113 ± 23
9	114 ± 34	49.1 ± 0.2	108 ± 21
12	118 ± 1	65 ± 1	121 ± 16
13	149 ± 35	48 ± 15	157 ± 30
14	155 ± 12	34 ± 4	105.4 ± 0.1
19	134 ± 6	85 ± 42	162 ± 26

Data shown are average residual activity ± SD, n = 2–3 with the exception of compound **30**, MMP-1, which represents a single point.

3.0 Materials and Methods

3.1 Biochemical assays

3.1.1 BoNT/A LC reversible inhibition assay

BoNT/A LC 1–425[3] was kindly provided by Dr. Joseph Barbieri and activity assays were conducted as previously reported,[4] with the exception for running the assays at 37 °C.

3.1.2 BoNT/A LC continuous covalent assay

Assays were performed using the same concentrations and setup as the reversible inhibition assay, amended such that substrate and inhibitor were plated first, then enzyme was added to initiate the reaction with no incubation period. Data analysis was performed in GraphPad Prism 8. Background fluorescence of the substrate was subtracted from each time point and fluorescence (RFU) was plotted versus time (s). The data was then fit to the custom equation corresponding to Equation 1 constraining substrate concentration [S], K_M, and inhibitor concentration [I] to defined values. Assays were performed in experimental triplicate with technical duplicates.

$$P = \frac{k_{cat}\frac{[S]}{K_M}}{k_{inact}\frac{[I]}{K_I}} E_T \left(1 - e^{-\frac{k_{inact}\frac{[I]}{K_I}}{1+\frac{[S]}{K_M}+\frac{[I]}{K_I}} \cdot t} \right) \qquad \text{Equation 1}$$

3.1.3 GSH reactivity assay

The GSH reactivity assay was modified from Bohme *et al*.[5] GSH and 2,2'-dinitro-5,5'dithiodibenzoic acid (DTNB) were purchased from Sigma (purity ≥ 98%) and 10 mM stocks prepared in assay buffer (0.1 M sodium dihydrogen phosphate, 1.0 mM EDTA, pH 8.0). 1 mL of each compound (1 mM, in assay buffer, 10% DMSO) was incubated with 1 mL of GSH (1 mM in assay buffer), $[\,]_f$ = 0.5 mM each, 5% DMSO. A negative DMSO control was also included, and samples were incubated at 37 °C. At given time points, 50 µL of the reaction mixture was removed and mixed vigorously in 150 µL of DTNB (0.2 mM), plated in a 96-well plate (Corning; cat. #3370) and the absorbance read in a plate reader (Spectramax i3x, Molecular Devices) at 412 nm. Background absorbance of control wells containing 25 µL of 1 mM compound, 25 µL of assay buffer, and 150 µL of 0.2 mM DTNB were subtracted from readings. A standard curve was constructed by preparing GSH standards in assay buffer containing 5% DMSO at the following concentrations: 0.8, 0.4, 0.2, 0.1, 0.05, 0.025, 0.0125 mM. 50 µL of each standard was immediately diluted into 150 µL of DTNB in assay buffer, and the absorbance read at 412 nm, subtracting a background reading containing only DTNB. The standard curve was constructed in GraphPad Prism 8,

showing high correlation (r^2 = 0.9992, $s_{y.x}$=0.01514). Second order reaction rates were determined by plotting the inverse of the GSH concentration with respect to time and calculating the slope, and then multiplied by the original concentration (*i.e.* 0.5 mM) to calculate theoretical pseudo-first-order reaction rates. The pseudo-first order k_{GSH} of DMSO was subtracted from the calculated pseudo-first order k_{GSH} of compounds to yield the corrected pseudo-first-order k_{GSH}. Compound half-life was then calculated using the first order equation for half-life. All linear and curve fitting was performed in GraphPad Prism 8, with additional arithmetic performed in Microsoft Excel. Assay was performed in experimental quadruplicate.

3.1.4 BoNT/A LC Preincubation covalent activity assay

50 µL of 1 µM BoNT/A LC (reaction concentration = 500 nM) was incubated with 50 µL of 2× compound stock in assay buffer (40 mM HEPES, 0.01% Triton X-100, pH 7.4, 2% DMSO) at 37 °C. At given timepoints, 2 µL of reaction mixture was withdrawn and diluted into 98 µL of SNAPtide substrate flP6 (BoNT/A LC $[\ ]_f$ = 10 nM, substrate $[\ ]_f$ = 4.00 µM) plated in a black half-volume 96-well plate. The assay plate was monitored in fluorescence kinetics mode as described above and the reaction monitored for 5–10 min. For each inhibitor concentration, the relative enzyme activity (normalized to no inhibitor control) was plotted versus time. The data was fit with a one-phase exponential decay to obtain k_{obs}. The obtained k_{obs} values were then plotted versus inhibitor concentration and the resulting curves fit with equation 2 to obtain k_{inact} and K_i. Data was analyzed in Microsoft Excel to obtain relative enzyme activity, then GraphPad Prism 8 for curve fitting. Assays were performed in experimental triplicate.

$$k_{obs} = \frac{k_{inact} \times I}{K_i + I} \qquad \text{Equation 2}$$

3.1.5 Exhaustive dialysis assay

Inhibitors were incubated at a concentration of 100 (**30** and **32**) or 200 µM (**36** and DCHA) with 500 nM BoNT/A LC for 1 h at 37 °C in assay buffer (40 mM HEPES, 0.01% Triton X-100, pH 7.4), 1% DMSO. Control samples containing no inhibitor were also prepared. Enzyme activity was evaluated at 1 h as performed in the preincubation assay described above. The remaining samples (90 µL) were immediately stored at 4 °C and individually injected into pre-equilibrated 100 µL Slide-A-Lyzer MINI dialysis units (10k MWCO, ThermoFisher; cat. #69750) and the samples dialyzed in assay buffer overnight, changing buffer after 2 and 4 hours. Enzyme concentrations were quantified using the BCA Assay kit (ThermoFisher; cat. #23227), and samples were diluted such that enzyme concentrations were equal. The enzyme activity of each sample was then measured using the SNAPtide assay as previously described. Data analysis proceeded as described above, assay was performed in experimental triplicate.

3.1.6 Hi-resolution mass spectrometry of covalent adducts

Inhibitors were incubated with BoNT/A LC WT for 1 h at 37 °C at a concentration of 100 µM inhibitor, 10 µM enzyme in assay buffer (40 mM HEPES, 0.01% Triton X-100, pH 7.4), 1% DMSO. Control samples containing only enzyme were also analysed. Putative masses of the covalent adducts were determined in ChemDraw 19. HRMS analysis of proteins was carried out using an Agilent 6230 TOF LC/MS mass spectrometer with a Dual AJS ESI ion source. Analytical grade reagents were supplied from commercial sources and used without further purification. The difference between the masses of inhibitor-incubated protein and control protein samples were found, and then divided by the covalent adduct mass to give the number of compounds bound.

3.2 C165A mutagenesis, expression and purification

Site-directed mutagenesis of the C165A variant was carried out using QuikChange II XL site-directed mutagenesis kit and the protocol carried out according to the manufacturer's procedure.[6] DNA encoding the C165A mutation was transformed into BL21 (DE3) cells and these used to inoculate LB/CARB overnight culture. This culture was used to inoculate 1.5 L of LB/CARB and the culture incubated at 37 °C, 200 rpm, until OD_{600} was ~0.8. The culture was induced with IPTG (0.75 mM $[\ ]_f$) and then incubated at 16 °C overnight. Cell cultures were centrifuged at 9000 rpm for 20 min and pellets stored at -80 °C. Purification steps were conducted at 4 °C. Cell pellets (~4 g) were resuspended in 4 mL of lysis buffer (20 mM Tris-HCl, pH 7.4, 500 mM NaCl, 10 mM imidazole, 0.05% Tween 20, 1 mM lysozyme, 10 mM BME, 3 EDTA-free protease inhibitor tablets) and the cells lysed using sonication. DNAse I (100 µg/mL) was added and the mixture incubated for 10 min. Cell suspension was centrifuged at 12,000 rpm, 4 °C for 20 m and the supernatant added to pre-equilibrated cobalt TALON® resin (5 mL) and batch bound by rotating for 30 min. The resin was loaded onto a gravity column and the flow through collected and passed over the column twice. Resin was washed with a step gradient using lysis buffer (10 mL) containing: 20, 40, 60 mM imidazole, before eluting protein with 250 mM imidazole. Collected fractions were analyzed by SDS-PAGE and pure fractions pooled accordingly. Protein was dialyzed in PBS, pH 7.4, 10% glycerol for 2 × 2 h and then overnight, followed by dialysis in 20 mM K^+HEPES, pH 7.4, 0.01% Triton X-100 for 2 × 2 h and then overnight. Protein was concentrated to ~1–2 mg/mL and quantified using nanodrop and a calibration curve from a known standard.[4]

3.3 Cell assays

3.3.1 BoNT/A hiPSC cell assay

BoNT/A cell activity assays were performed as previously reported.[4] Human induced pluripotent stem cell (hiPSC)-derived GABA neurons and culture medium (Fujifilm Cellular Dynamics International (Madison, WI) were cultured in 0.01% poly-L-ornithine (Sigma Aldrich) and 8.3 µg/cm^2 matrigel, growth

factor reduced, (BD Biosciences) coated 96-well TPP plates (Midsci) for 12 days prior to the assay. 200 LD_{50} Units of BoNT/A1 (150 kDa purified as described previously,[7] specific activity (1.7×108 U/mg) was added to the cells in 50 µL stimulation medium (modified neurobasal containing 2.2 mM $CaCl_2$ and 56 mM KCl and supplemented with 1x B27 and 1x GlutaMAX (100x-stocks, Life Technologies), and the cells were incubated at 37 °C in a humidified 5% CO_2 atmosphere for 7.5 min. Toxin was removed, cells were washed twice in 300 µL of culture medium, and further incubated in fresh culture medium at 37 °C in a humidified 5% CO_2 atmosphere. At 30 min post first toxin addition, the inhibitors were added at the indicated concentrations and with a final DMSO concentration of 1%. Positive control (+C) was toxin without inhibitor in culture media, and negative control (-C) was culture media, both with 1% DMSO added. Cells were incubated for 7.5 h post toxin addition at 37 ºC, 5% CO_2 to allow for SNAP-25 cleavage. Inhibitor mixtures were then aspirated, and cells lysed in 50 µL of 1× LDS lysis buffer (Invitrogen). Cell lysates were analyzed by Western blot using a monoclonal anti-SNAP-25 antibody (Synaptic Systems, Germany; cat. #111011) as described previously,[8] and bands were visualized using Phosphaglo chemiluminescent reagent (KPL) on an Azure C600 imaging system equipped with a CCD camera (Azure Biosystems). Assays were performed in experimental triplicate.

3.3.2 BoNT/A RSC lysate assay

Primary rat spinal cord (RSC) cells were prepared from E15 Sprague Dawley rat pups and seeded at a density of 75,000 cells/well into the same plates used in the hiPSC assay. The RSC cells were maintained in Neurobasal media (Neurobasal culture media supplemented with 1x B27, 1x GlutaMAX, 50 µg/mL penicillin and streptomycin) with bi-weekly changes of the media. The cells were maintained for at least 19 days in culture before use in a toxin assay. To prepare cell lysates, cells were washed in DPBS (Life Technologies), and 1.5 mL of MPER mammalian cell lysis buffer (Thermo Scientific) containing 5 mM NaCl was added to each well. The cells were removed from the surface of the plate, cell clumps dispersed by pipetting, and cell lysis allowed to proceed for 10 min at r.t. Cell lysates were centrifuged at 14,000×*g* for 5 min to remove any insoluble cell debris, and the supernatant used immediately in the in vitro assay. 40 ng BoNT/A1 (final concentration in reaction: 4.4 nM) was reduced in 15 µL of 4× reaction buffer (150 mM HEPES, pH 7.0, 15 mM NaCl, 30 µM $ZnCl_2$, 2 mM DTT) for 15 min at r.t. After 15 min, 5 uL dilutions of compound **36** (100x) in DMSO were added to 125 uL of the toxin mixture. 15 µL of this solution was immediately mixed with 45 uL of lysate and the mixtures incubated at 37 °C for 75 min. The reactions were stopped by addition of LDS sample buffer (Life technologies) to 1× and heating the samples to 95 °C. All were tested in triplicate. A negative control not containing any toxin was included, and a positive control containing toxin but no compound was included. All samples were analyzed by Western blot as described above.

3.3.3 Cell viability assays (MTT assay[9])

Cell viability assays were performed as previously reported,[4] with data analysis carried out in GraphPad Prism 8.

3.4 Compound solubility assay

Compound solubility was assessed by UV/Vis spectroscopy using an adaptation of a previously reported protocol.[10] DMSO stock solutions of compounds **30**, **32** and **36** ranging from 50 mM to 0.25 mM were diluted 100-fold into the FRET-based assay buffer (1 µL into 99 µL) in a 96-well plate (Costar 3370) to give a final DMSO concentration of 1%. A control containing DMSO-alone was also included. Solutions were incubated at 37 °C with agitation for 2 h before the absorbance was read at 550, 600, 650 and 700 nm on a Spectramax i3x (Molecular Devices) plate reader. Averaged absorbance values from the control were subtracted from each data point and the AU from each wavelength averaged and plotted against compound concentration. Data analysis was conducted in Excel and the data plotted in GraphPad Prism 8. Data was performed in experimental triplicate. Analysis of 200 µM DMSO solutions of compounds **30**, **32** and **36** did not show any absorbance in the measured wavelength range.

3.5 Serum stability assay

Serum stability assays were performed using an adaptation of a previously reported protocol.[11] 400 mL human male serum (Sigma; cat. #H4522) was incubated at 37 °C for 15 min. The serum was spiked with a DMSO-stock solution of the respective inhibitor to reach a final concentration of 150 mM. The mixture was shaken at 750 rpm at 37 °C and samples (45 mL) were taken out at time points (0 min, 30 min, 1 h, 2 h, 4 h, 6 h, and 24 h) and quenched with aqueous urea (6 M, 50 mL) and incubated for 10 min at 4 °C. Ice-cold acetonitrile (100 mL) was added to the sample and incubated for another 10 min at 4 °C. The samples were centrifuged for 90 min at 20,000 g and the supernatant analyzed by HPLC. Half-lives ($t_{1/2}$) were determined by integration of the peak areas of recovered compound over time using GraphPad Prism 8 and fitted to a one-exponential decay equation, assuming first-order kinetics. Each assay was performed in experimental duplicate.

3.6 MMP selectivity assay

MMP inhibitor profiling kit was purchased from Enzo Life Sciences (cat. #BML-AK308-0001) and used per manufacturer instructions as described previously.[4] Each assay was performed in experimental triplicate with outliers excluded by the Grubb's Outlier test.

3.7 Computational modelling

Molecular modelling was performed with modules from the Schrödinger Small Molecule Drug Discovery Suite (Maestro), release 2018–3, using the OPLS3 force field for parameterization.[12] The X-ray co-crystal structure of BoNT/A LC (PDB 2IMA)[1] was imported from the protein data bank and prepared using the Protein Preparation Wizard using default settings.[13] Ligands were imported into and prepared using LigPrep.[13] Covalent docking was carried out in a confined receptor grid built 30 Å around the receptor-bound ligand (DCHA) and simulated using CovDock[14] (Reaction types: epoxide opening, nucleophilic substitution or Michael addition). Figures were generated using PyMol Molecular Graphics System (version 2.3.4., Schrödinger, LLC).

3.8 Interference compound filter

All final compounds were examined using publicly accessible PAINS filter:

Zinc patterns search (http://zinc15.docking.org/patterns/home/)

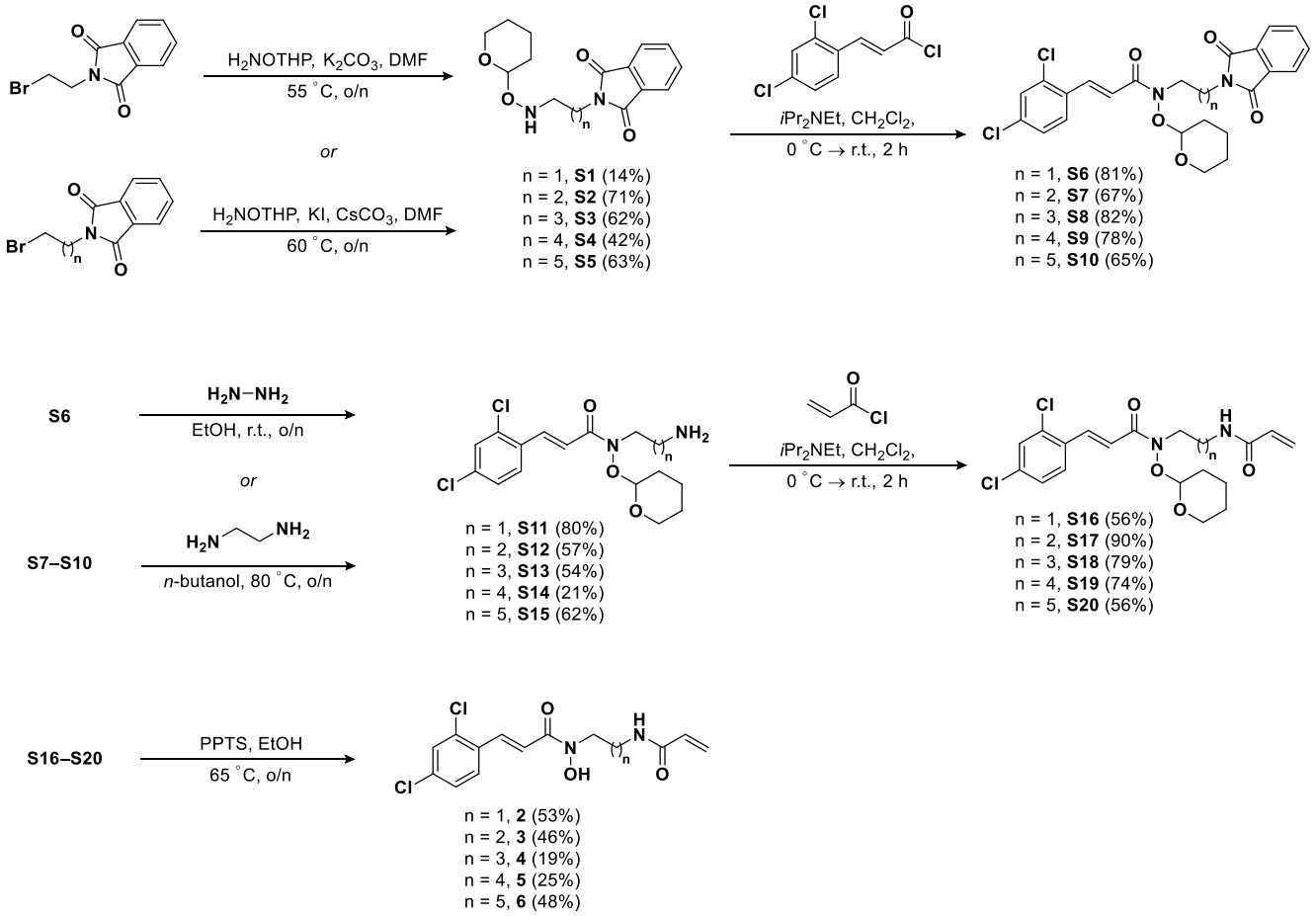
No PAINS compounds were found.

4.0 Chemistry

4.1 Chemical Schemes

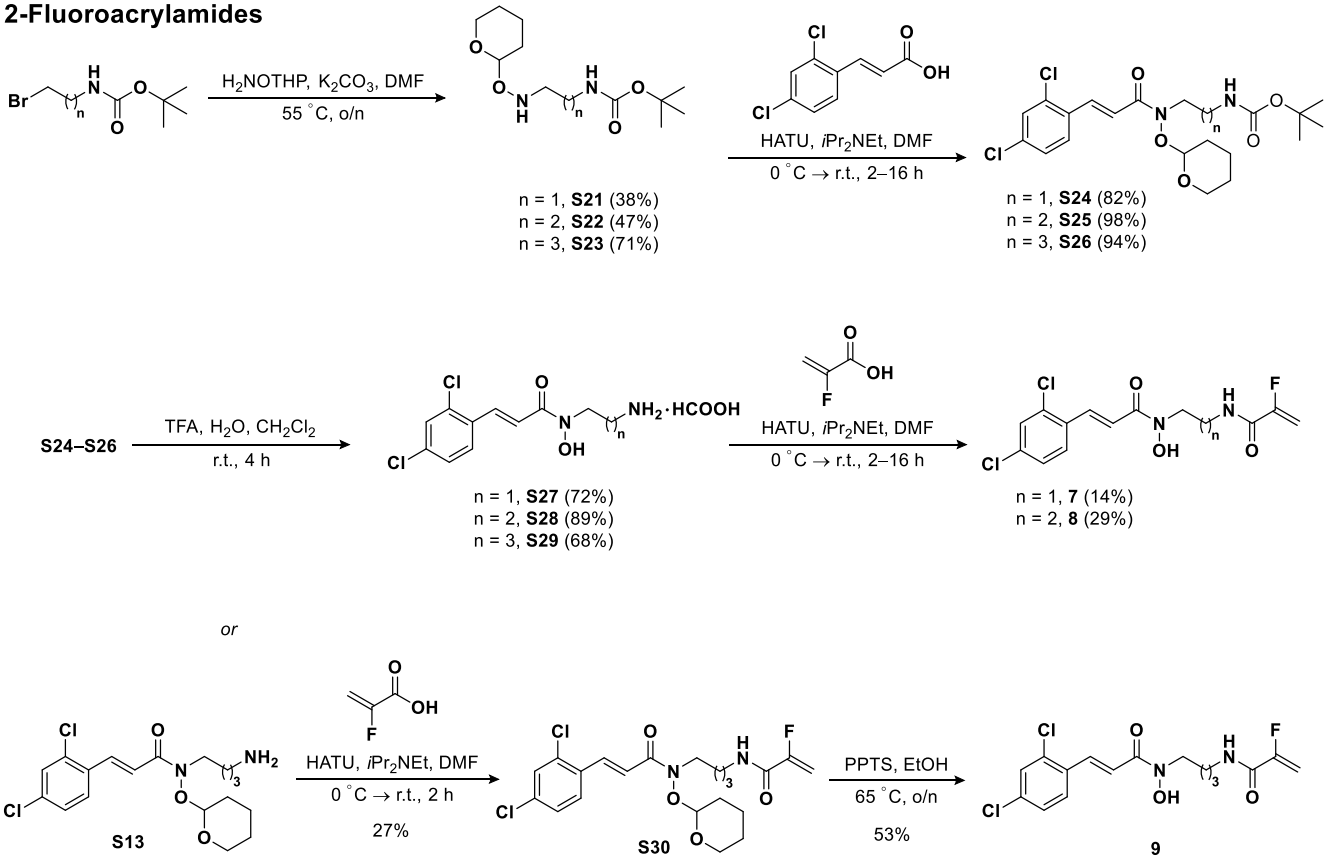
4.1.1 Scheme S1. Synthesis of acrylamide compounds

Acrylamides

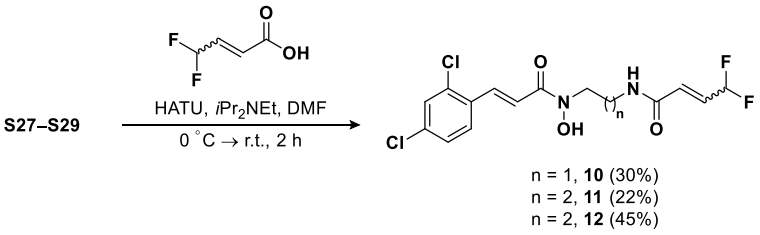


4.1.2 Scheme S2. Synthesis of 2-fluoroacrylamide and 4,4-difluoro-2-butenamide compounds

2-Fluoroacrylamides

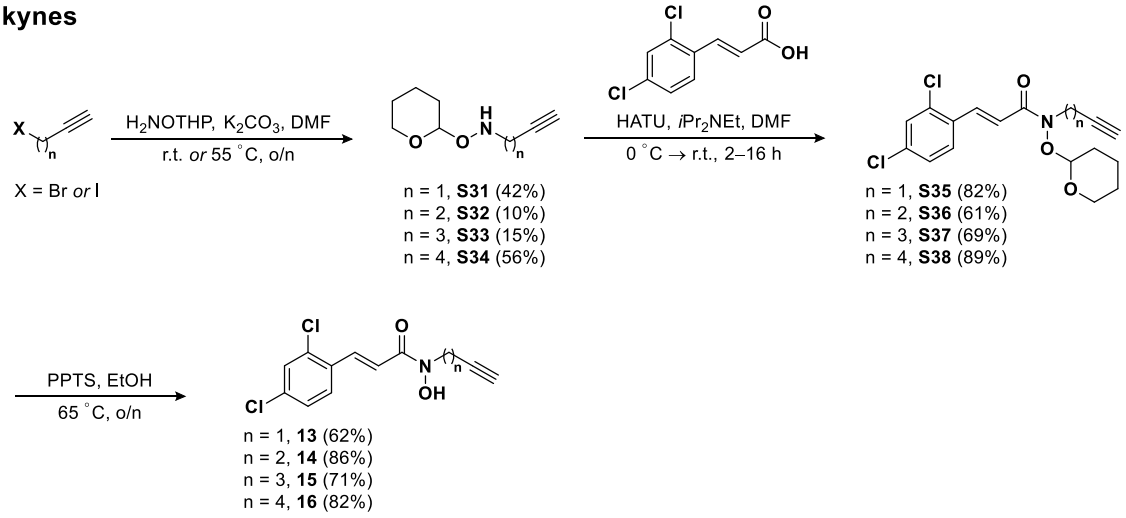


4,4-Difluoro-2-butenamides

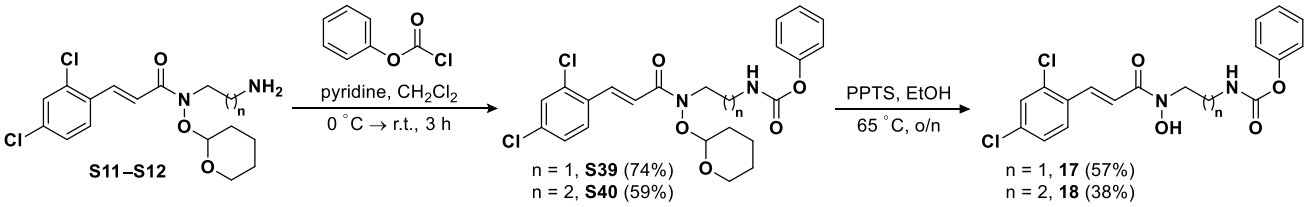


4.1.3 Scheme S3. Synthesis of alkyne, *O*-phenyl carbamate and cyanocyclopropyl compounds

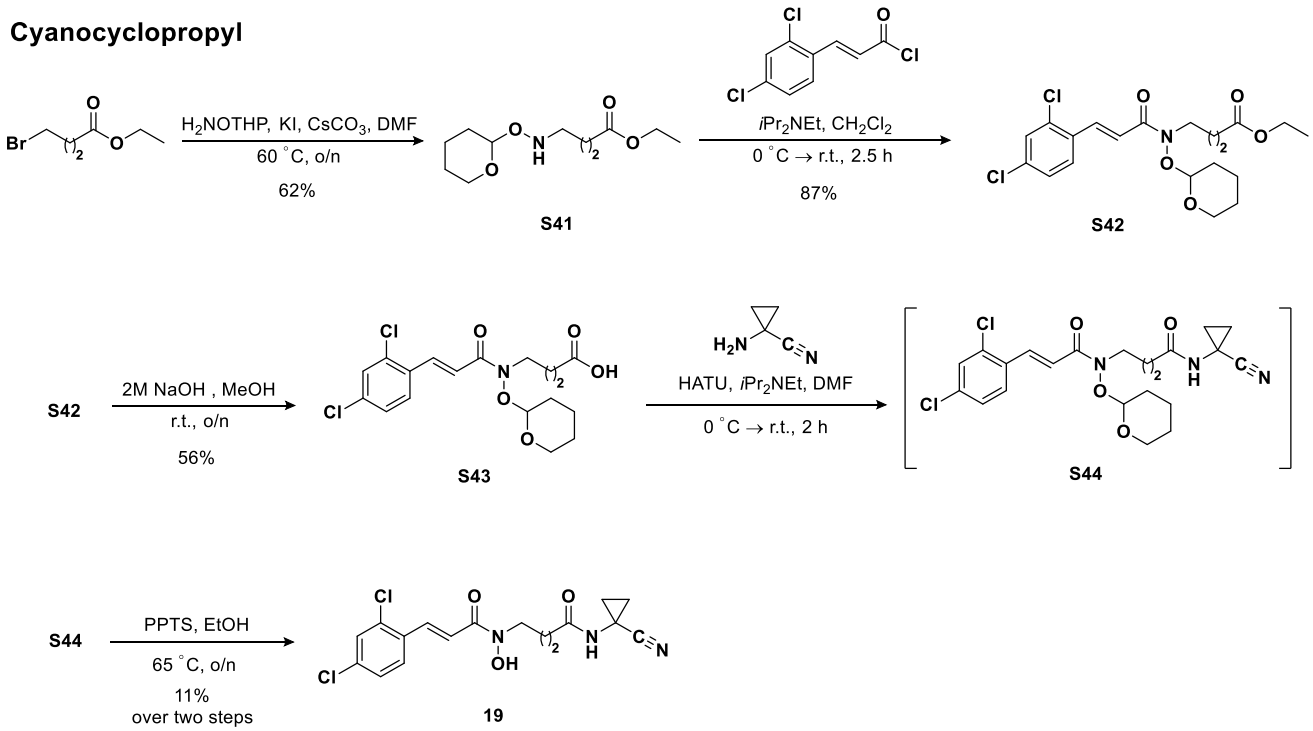
Alkynes



***O*-phenyl carbamates**

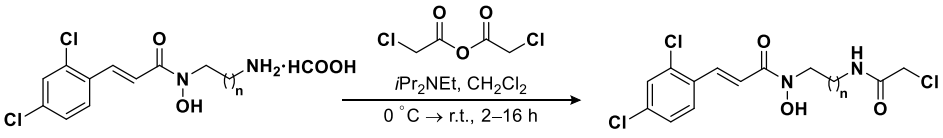


Cyanocyclopropyl



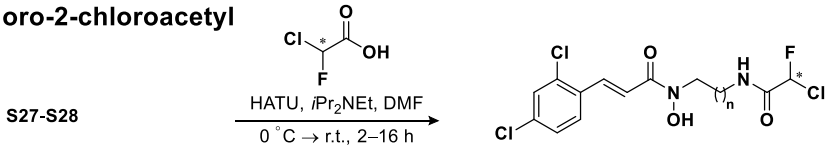
4.1.4 Scheme S4. Synthesis of alkyl halide compounds

Chloroacetyl



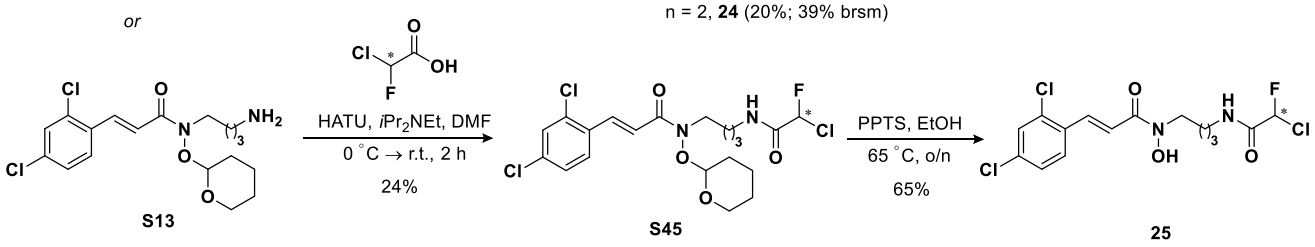
n = 1, **20** (60%)
n = 2, **21** (48%)
n = 2, **22** (38%)

2-Fluoro-2-chloroacetyl

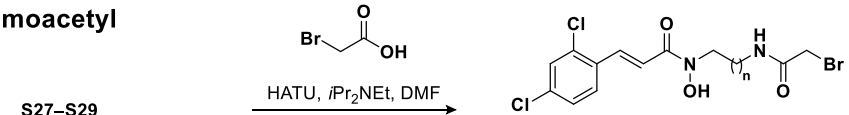


n = 1, **23** (24%)
n = 2, **24** (20%; 39% brsm)

or

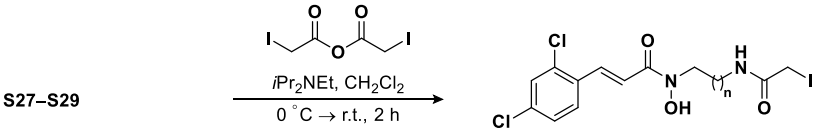


Bromoacetyl



n = 1, **26** (7%)
n = 2, **27** (4%)
n = 2, **28** (19%)

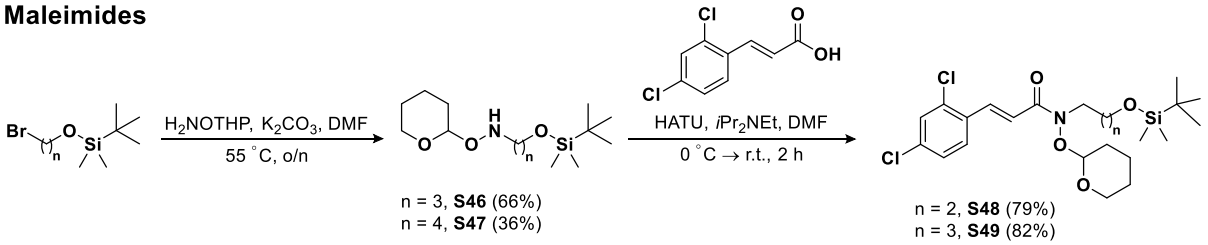
Iodoacetyl



n = 1, **29** (37%)
n = 2, **30** (36%)
n = 2, **31** (36%)

4.1.5 Scheme S5. Synthesis of maleimide, epoxide and aldehyde compounds

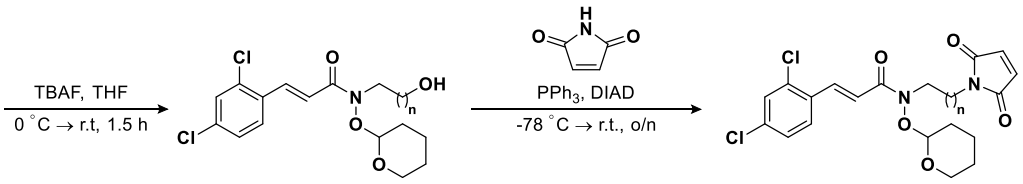
Maleimides



n = 3, **S46** (66%)
n = 4, **S47** (36%)

n = 2, **S48** (79%)
n = 3, **S49** (82%)

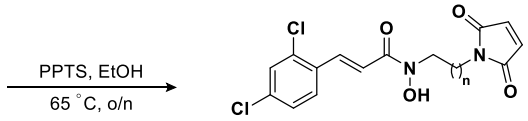
S48–S49



n = 2, **S50** (77%)
n = 3, **S51** (90%)

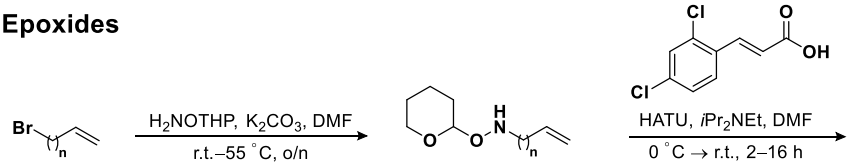
n = 2, **S52** (28%; 37% brsm)
n = 3, **S53** (23%; 38% brsm)

S52–S53



n = 2, **32** (40%)
n = 3, **33** (56%)

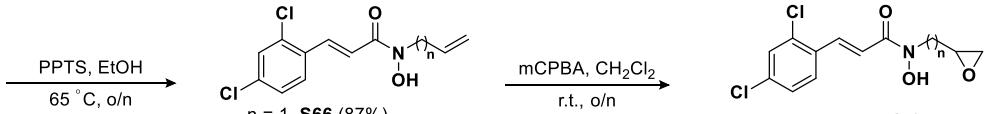
Epoxides



n = 1, **S54** (67%)
n = 2, **S55** (24%)
n = 3, **S56** (34%)
n = 4, **S57** (56%)
n = 5, **S58** (40%)
n = 6, **S59** (56%)

n = 1, **S60** (95%)
n = 2, **S61** (85%)
n = 3, **S62** (94%)
n = 4, **S63** (77%)
n = 5, **S64** (95%)
n = 6, **S65** (95%)

S60–S65



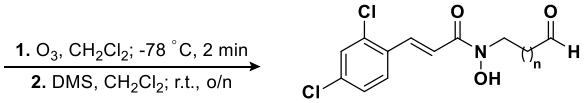
n = 1, **S66** (87%)
n = 2, **S67** (74%)
n = 3, **S68** (84%)
n = 4, **S69** (85%)
n = 5, **S70** (87%)
n = 6, **S71** (86%)

n = 1, unsuccessful*
n = 2, unsuccessful*
n = 3, **34** (17%)
n = 4, **35** (28%)
n = 5, **36** (56%)
n = 6, **37** (44%)

*Readily form elimination/diol products

Aldehydes

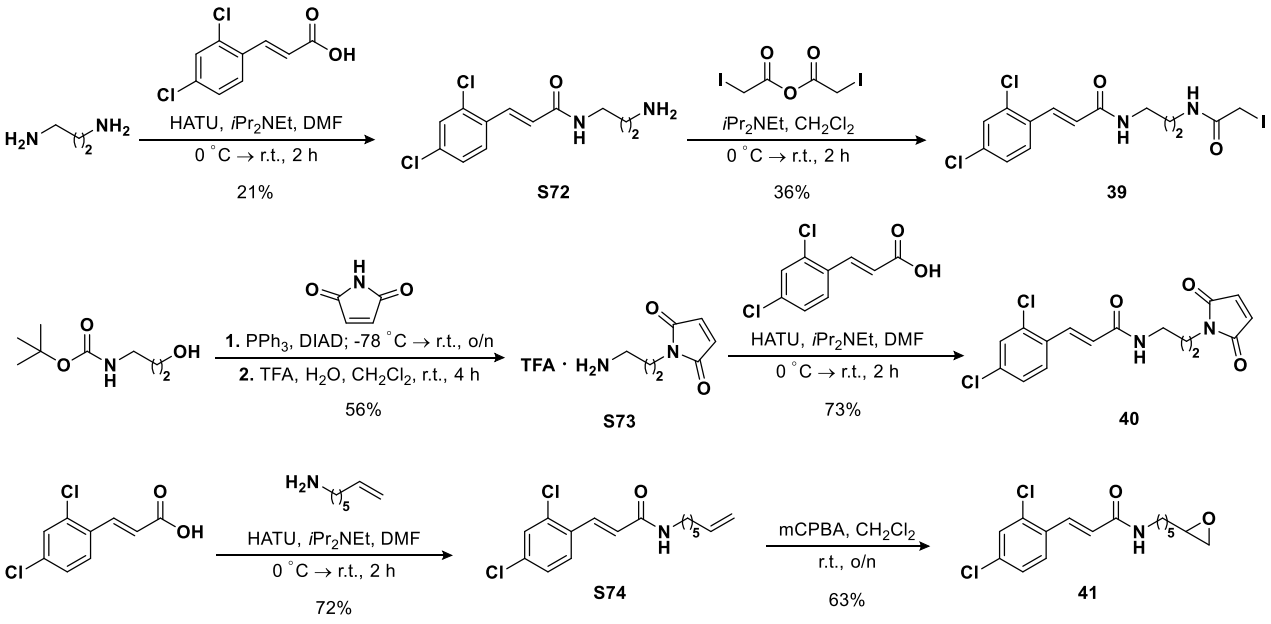
S68–S69



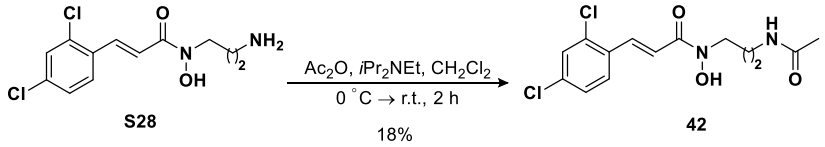
n = 4, **38** (12%) *Readily decomposes
n = 5, Unsuccessful* Reaction optimization was not further pursued

4.1.6 Scheme S6. Synthesis control compounds

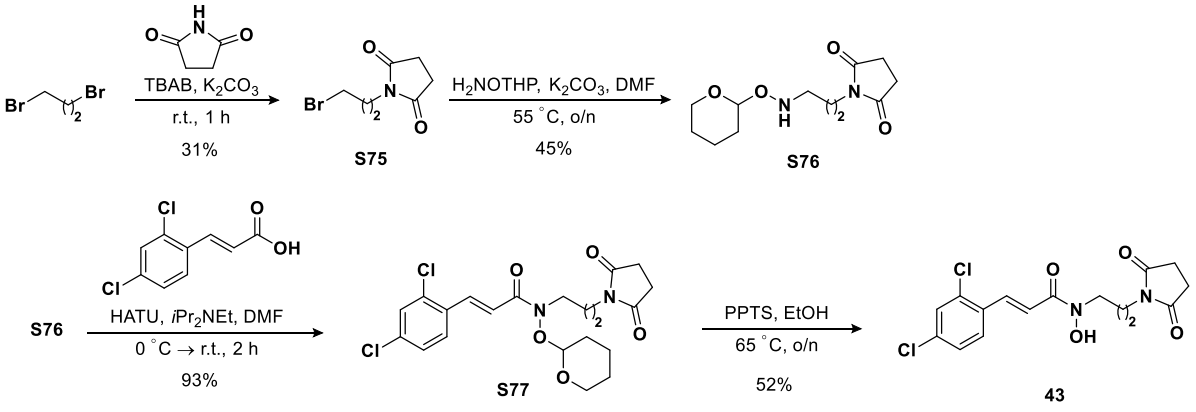
Amide controls



Acetyl



Succinimide



4.2 Chemical experimental procedures and instrumentation

All reagents and solvents were of analytical grade and used without further purification as obtained from commercial suppliers unless otherwise stated. Reactions were conducted under argon atmosphere whenever anhydrous solvents were used. Reactions were monitored by thin-layer chromatography (TLC) using silica gel coated glass plates (analytical SiO_2-60, F-254) and/or by high performance liquid chromatography-mass spectrometry (HPLC-MS). TLC plates were visualized under UV light and/or by dipping in either: potassium permanganate stain, ninhydrin stain or bromocresol green stain, visualizing with heating by heat gun. HPLC-MS analysis was performed on an Agilent 1260 Infinity II instrument coupled to a single quadrupole InfinityLab LC/MSD instrument running a gradient of eluent I (0.1% formic acid in H_2O) and eluent II (0.1% formic acid in MeCN) rising linearly from 0% to 95% of II during t = 0.00−6.00 min and then isocratic with eluent II from t = 6.00–10.0 min, at a flow rate of 0.5 mL/min on a Zorbax 300SB-C8 column. Flash liquid automated column chromatography (ACC) was performed on a CombiFlash Rf+ Lumen using RediSep Rf Gold® silica-gel columns (normal-phase (NP)) or C_{18} HP 4–125 g cartridges (reverse-phase (RP)) using various gradients of MeCN–H_2O containing 0.1% formic acid, at flow rates between 18–85 mL/min. Crude products were dry-loaded on celite unless stated otherwise. Nuclear magnetic resonance (NMR) spectra were recorded on either (a) a Bruker AVIII HD 600 equipped with a cryogenically cooled 5 mm CPDCH probe (^{1}H NMR and ^{13}C NMR recorded at 600 and 151 MHz, respectively) or (b) a Bruker NEO 399 (^{1}H NMR and ^{13}C NMR recorded at 400 and 101 MHz, respectively) or (c) a Bruker NEO 500 (^{1}H NMR and ^{13}C NMR recorded at 500 and 126 MHz, respectively). Chemical shifts are reported in ppm with reference to the residual solvent peak (δ_H $CDCl_3$ 7.26 ppm; δ_C $CDCl_3$ 77.16 ppm; δ_H DMSO-d_6 2.50 ppm; δ_C DMSO-d_6 39.52 ppm; δ_H MeOD-d_4 3.31 ppm; δ_C MeOD-d_4 49.00 ppm). Multiplicities are reported with coupling constants and are given to the nearest 0.1 Hz. When needed, heteronuclear single quantum coherence (HSQC) was used to determine overlapping peaks. High resolution-mass spectrometry (HRMS) was carried out using an Agilent 1260 Infinity II instrument coupled to an Agilent 6230 TOF-MS spectrometer using electrospray ionization ($ES^{+/-}$), giving masses correct to four decimal places.

4.3 General synthetic methods

4.3.1 Method A1: Nucleophilic substitution

O-(tetrahydro-2H-pyran-2-yl)hydroxylamine (OTX; 1.0–1.5 equiv.) and K_2CO_3 (1.0–1.5 equiv.) were dissolved in DMF and the chosen aliphatic bromo-compound (1.0 equiv.) added and the reaction stirred overnight at 55–60 °C. See individual compounds for work-ups and purifications.

4.3.2 Method A2: Nucleophilic substitution

N-substituted bromoalkyl isoindolinone/bromoalkyl ester (1.0 equiv.) and OTX (1.0 equiv.) were added to a suspension of Cs_2CO_3 (1 equiv.) and KI (1 equiv.) in DMF and stirred overnight at 60 °C. The reaction mixture was diluted with EtOAc, and washed with water (3×) and brine, dried ($MgSO_4$), concentrated in vacuo and purified by NP ACC.

4.3.3 Method A3: Nucleophilic substitution

OTX (1.5 equiv.) and K_2CO_3 (1.5–2.0 equiv.) were dissolved in DMF and the chosen aliphatic bromoalkene/bromoalkyne (1.0 equiv.) added and the reaction stirred overnight at room temperature (r.t). Water was added to the reaction mixture and extracted 2–3 times with Et_2O or EtOAc:hexanes (10:1, v/v). The combined organic layers were washed with water (3×), dried (Na_2SO_4), concentrated in vacuo to give an oil, which was purified by NP (wet load in hexane) or RP ACC. See individual compounds for purifications.

4.3.4 Method B: Acylation reactions

The desired acid chloride/anhydride (1.1–2.0 equiv.) was dissolved in anh. CH_2Cl_2 at 0 °C and a solution of the chosen amine (1 equiv.) and *i*Pr_2NEt (1.1–3.0 equiv.), or pyridine (2.0 equiv.), in anh. CH_2Cl_2 was added dropwise and the reaction mixture stirred for 10 min at 0 °C and then for 2 h at r.t. See individual compounds for work-ups and purifications.

4.3.5 Method C1: HATU acylation reactions

A mixture of the chosen acid (1.0 equiv.), amine/hydroxylamine (1.0–1.5 equiv.) and *i*Pr_2NEt (1.1–3.0 equiv.) were dissolved in anh. DMF and stirred at 0 °C. HATU (1.1–1.5 equiv.) was added and the reaction mixture stirred for 5 min at 0 °C and then at r.t until completion (1–16 h). DMF was removed *in vacuo* to give a crude yellow oil, which was purified by NP or RP ACC, or, water was added to the reaction mixture until no more precipitate formation was observed, and the precipitated solution extracted 2–3 times with CH_2Cl_2 or EtOAc–Hexane (9:1). The combined organic layers were washed with water (3×) and brine, dried (Na_2SO_4), concentrated *in vacuo* and purified by NP or RP ACC. See individual compounds for work-ups and purifications.

4.3.6 Method C2: HATU acylation reactions

A mixture of the chosen amine (1.0 equiv.) and *i*Pr_2Net (1.3 equiv.) were dissolved in anh. DMF (1.0 mL) and stirred for 5 min. A mixture containing bromoacetic acid (0.9 equiv.), HATU (1.2 equiv,) and *i*Pr_2Net (1.0 equiv.) dissolved in DMF (1.0 mL) was stirred for 3 min and added dropwise to the other mixture and the reaction mixture stirred for 1 h. DMF was removed *in vacuo* and the crude residue purified by NP

ACC. The product was further purified by RP ACC (10–45% MeCN–H_2O in 0.1% formic acid) to afford the product after lyophilization.

4.3.7 Method D: Phthalimide deprotection

Intermediates S7–S10 (1 equiv.) were dissolved in *n*-butanol and ethylenediamine (5 equiv.) was added slowly. The reaction mixture was heated to 80 °C and stirred overnight. The reaction mixture was cooled to r.t, diluted with water and extracted with CH_2Cl_2 (×2). The combined organic layers were dried ($MgSO_4$), concentrated *in vacuo* and purified by NP ACC. See individual compounds for work-ups and purifications.

4.3.8 Method E: TFA deprotection.

Intermediates S24–S26 were dissolved in CH_2Cl_2:TFA:H_2O (75:20:5, v/v) and stirred overnight at r.t. MeOH was added and the reaction mixture reduced *in vacuo* and the crude semi-solid purified by RP ACC (0–15% hold MeCN–H_2O in 0.1% formic acid) to afford the product after lyophilization.

4.3.9 Method F: *O*-THP deprotection

The chosen protected hydroxamate and PPTS (0.2–0.5 equiv.) were dissolved in abs. EtOH and the reaction stirred at 65 °C overnight. See individual compounds for work-ups and purifications.

4.3.10 Method G: Epoxidation

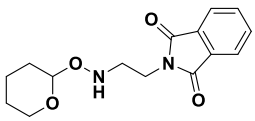
The chosen alkene (1.0 equiv.) was dissolved in anh. CH_2Cl_2 and purged with argon* followed by addition of mCPBA (max. 77%; 1.0–1.5 equiv.) and stirred overnight at r.t. MeOH was added and the reaction mixture reduced *in vacuo* and the crude product purified by RP ACC. See individual compounds for work-ups and purifications individual deviations.

*Procedures carried out in atmospheric conditions resulted in poorer yields and formation of unwanted side products – oxidative cleavage of N–C bond was observed.

4.4 Compound synthesis

4.4.1 Acrylamides

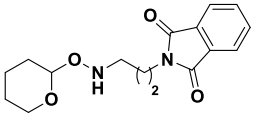
2-(2-(((tetrahydro-2*H*-pyran-2-yl)oxy)amino)ethyl)isoindoline-1,3-dione (S1). Synthesized by



method A1 using 2-(2-bromoethyl)isoindoline-1,3-dione (2.16 g, 8.53 mmol, 1.0 equiv.), OTX (1.00 g, 8.53 mmol, 1.0 equiv.), K_2CO_3 (1.18 g, 8.53 mmol, 1.0 equiv.) and DMF (20 mL). The reaction mixture was poured into water and extracted with EtOAc (2×) and the combined organic layers washed with water (3×), dried ($MgSO_4$) and concentrated *in vacuo*. The crude residue was purified by NP ACC to afford the titled compound (360 mg, 1.24 mmol, 14%) as a colorless oil. **TLC** (66% EtOAc–hexanes): R_f = 0.5. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.80–7.74 (m, 2H), 7.67–7.62 (m, 2H), 5.88 (s, 1H), 4.75 (s, 1H), 4.01–3.90 (m, 1H), 3.85–3.78 (m, 1H),

3.78–3.70 (m, 1H), 3.50–3.42 (m, 1H), 3.22 (br.s, 2H), 1.70–1.59 (m, 2H), 1.52–1.37 (m, 4H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 168.4, 133.9, 132.2, 123.2, 101.4, 62.8, 49.9, 36.3, 29.0, 25.3, 19.9. **MS** (ES^+) *m/z* calcd for $C_{15}H_{19}N_2O_4^+$ $[M+H]^+$, 291.1; found: 291.1.

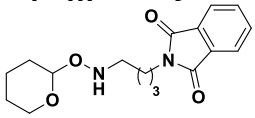
2-(3-(((tetrahydro-2*H*-pyran-2-yl)oxy)amino)propyl)isoindoline-1,3-dione (S2). Synthesized by



method A2 using 2-(3-bromopropyl)isoindoline-1,3-dione (1.15 g, 4.27 mmol, 1.0 equiv.), OTX (500 mg, 4.27 mmol, 1.0 equiv.), Cs_2CO_3 (1.39 g, 4.27 mmol, 1.0 equiv.), KI (708 mg, 4.27 mmol, 1.0 equiv.) and DMF (10 mL). The tilled compound (928 mg, 3.05 mmol, 71%) was collected as a colorless oil*. **TLC** (50% EtOAc–hexanes): R_f = 0.3. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.81 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.69 (dd, *J* = 5.5, 3.0 Hz, 2H), 5.89 (br.s, 1H), 4.79–4.73 (m, 1H), 3.92–3.84 (m, 1H), 3.82–3.71 (m, 2H), 3.55–3.48 (m, 1H), 3.09–2.90 (m, 2H), 1.96–1.87 (m, 2H), 1.76–1.66 (m, 2H), 1.55–1.45 (m, 4H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 168.5, 134.0, 132.2, 123.3, 101.7, 63.2, 49.4, 35.9, 29.3, 26.6, 25.4, 20.3. **MS** (ES^+) *m/z* calcd for $C_{16}H_{21}N_2O_4^+$ $[M+H]^+$, 305.1; found: 305.1.

*Impurity arising from indoline at ~20%, but compound taken forward without further purification.

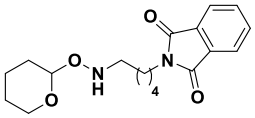
2-(4-(((tetrahydro-2*H*-pyran-2-yl)oxy)amino)butyl)isoindoline-1,3-dione (S3). Synthesized by



method A2 using 2-(4-bromobutyl)isoindoline-1,3-dione (1.20 g, 4.27 mmol, 1.0 equiv.), OTX (500 mg, 4.27 mmol, 1.0 equiv.), Cs_2CO_3 (1.39 g, 4.27 mmol, 1.0 equiv.) and KI (708 mg, 4.27 mmol, 1.0 equiv.) and DMF (10 mL). The titled compound (845 mg, 2.65 mmol, 62%) was collected as a colorless oil*. **TLC** (50% EtOAc–hexanes): R_f = 0.3. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 7.88–7.85 (m, 2H), 7.84–7.82 (m, 2H), 6.58 (br.s, 1H), 4.58 (dd, *J* = 5.3, 3.0 Hz, 1H), 3.77–3.71 (m, 1H), 3.57 (t, *J* = 7.0 Hz, 2H), 3.42–3.34 (m, 1H), 2.85–2.72 (m, 2H), 1.69–1.54 (m, 4H), 1.54–1.46 (m, 1H), 1.46–1.30 (m, 5H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 168.0, 134.4, 131.6, 123.0, 101.0, 61.8, 51.0, 37.4, 29.0, 25.7, 25.0, 24.1, 19.8. **MS** (ES^+) *m/z* calcd for $C_{17}H_{23}N_2O_4^+$ $[M+H]^+$, 319.2; found: 319.1.

*Impurity arising from THP ~20%, but compound taken forward without further purification.

2-(5-(((tetrahydro-2*H*-pyran-2-yl)oxy)amino)pentyl)isoindoline-1,3-dione (S4). Synthesized by

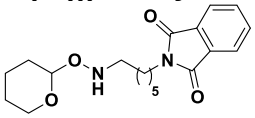


method A2 using 2-(5-bromopentyl)isoindoline-1,3-dione (1.26 g, 4.27 mmol, 1.0 equiv.), OTX (500 mg, 4.27 mmol, 1.0 equiv.), Cs_2CO_3 (1.39 g, 4.27 mmol, 1.0 equiv.), KI (708 mg, 4.27 mmol, 1.0 equiv.) and DMF (10 mL). The titled compound (624 mg, 1.80 mmol, 42%) was collected as a colorless oil*. **TLC** (50% EtOAc–hexanes): R_f = 0.4. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.82 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.69 (dd, *J* = 5.5, 3.0 Hz, 2H), 4.78–4.73 (m, 1H), 3.93–3.86 (m, 1H), 3.70–3.63 (m, 2H), 3.58–3.51 (m, 1H), 3.00–2.88 (m, 2H), 1.73–1.62 (m, 3H), 1.58–1.45 (m, 6H), 1.43–1.30 (m, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 168.5, 134.0, 132.3, 123.3,

101.5, 63.2, 52.2, 38.1, 29.3, 28.6, 27.3, 26.9, 25.5, 20.4. **MS** (ES^+) *m/z* calcd for $C_{18}H_{25}N_2O_4^+$ $[M+H]^+$, 333.2; found: 333.1.

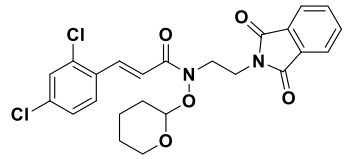
*Trace impurity arising from indoline, but compound taken forward without further purification.

2-(6-(((tetrahydro-2*H*-pyran-2-yl)oxy)amino)hexyl)isoindoline-1,3-dione (S5). Synthesized by



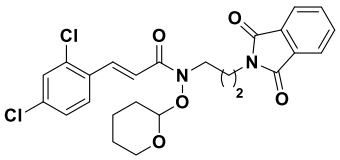
method A2 using 2-(6-bromohexyl)isoindoline-1,3-dione (1.32 g, 4.27 mmol, 1.0 equiv.), OTX (500 mg, 4.27 mmol, 1.0 equiv.), Cs_2CO_3 (1.39 g, 4.27 mmol, 1.0 equiv.), KI (708 mg, 4.27 mmol, 1.0 equiv.) and DMF (10 mL). The titled compound (896 mg, 2.70 mmol, 63%) was collected as a colorless oil. **TLC** (50% EtOAc–hexanes): R_f = 0.3. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.84–7.80 (m, 2H), 7.72–7.67 (m, 2H), 5.71 (br.s, 1H), 4.79–4.73 (m, 1H), 3.93–3.86 (m, 1H), 3.67 (t, *J* = 7.3 Hz, 2H), 3.58–3.50 (m, 1H), 3.02–2.89 (m, 2H), 1.82–1.64 (m, 5H), 1.66–1.43 (m, 6H), 1.43–1.35 (m, 2H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 168.5, 134.0, 132.3, 123.3, 101.6, 63.3, 52.1, 38.0, 29.3, 28.6, 27.1, 25.5, 24.6, 20.3, one aliphatic C overlapped. **MS** (ES^+) *m/z* calcd for $C_{19}H_{27}N_2O_4^+$ $[M+H]^+$, 347.2; found: 347.2.

(*E*)-3-(2,4-dichlorophenyl)-*N*-(2-(1,3-dioxoisoindolin-2-yl)ethyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S6). Synthesized by method B using (*E*)-3-(2,4-dichlorophenyl)acryloyl chloride



(333 mg, 1.39 mmol, 1.1 equiv.), **S1** (360 mg, 1.24 mmol, 1.0 equiv.) and *i*Pr_2NEt (476 µL, 2.77 mmol, 2.2 equiv.) and anh. CH_2Cl_2 (8.0 mL). The reaction mixture was washed with sat. $NaHCO_3$ (3×), dried ($MgSO_4$), concentrated *in vacuo* and purified by NP ACC to afford the titled compound (489 mg, 1.00 mmol, 81%) as a colorless oil. **TLC** (33% EtOAc–hexanes): R_f = 0.4. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.85 (d, *J* = 15.9 Hz, 1H), 7.81 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.68 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.49 (d, *J* = 8.4 Hz, 1H), 7.39 (d, *J* = 2.1 Hz, 1H), 7.26–7.20 (m, 1H), 6.91 (d, *J* = 15.8 Hz, 1H), 4.96–4.92 (m, 1H), 4.20–4.06 (m, 2H), 4.09–4.03 (m, 2H), 3.98–3.90 (m, 1H), 3.62–3.54 (m, 1H), 1.80–1.47 (m, 6H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 168.3, 167.5, 138.2, 135.8, 135.6, 134.0, 132.3, 132.2, 130.1, 128.6, 127.5, 123.3, 119.7, 105.2, 63.8, 47.8, 35.7, 29.1, 25.0, 19.6. **MS** (ES^+) *m/z* calcd for $C_{24}H_{22}Cl_2N_2NaO_5^+$ $[M+Na]^+$, 511.1; found: 511.0.

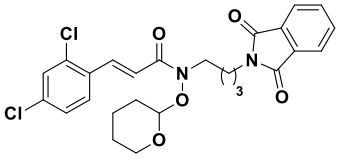
(*E*)-3-(2,4-dichlorophenyl)-*N*-(3-(1,3-dioxoisoindolin-2-yl)propyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S7). Synthesized by method B using (*E*)-3-(2,4-dichlorophenyl)acryloyl chloride



(942 mg, 4.00 mmol, 1.5 equiv.), **S2** (830 mg, 2.72 mmol, 1.0 equiv.), *i*Pr_2NEt (850 µL, 4.88 mmol, 1.8 equiv.) and anh. CH_2Cl_2 (20 mL). The reaction mixture was treated in the same manner as outlined in the synthesis of **S6**. The titled compound (912 mg, 1.81 mmol, 67%) was collected as a pale-yellow foam. **TLC** (50% EtOAc–hexanes): R_f = 0.5. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.95 (d, *J* = 15.8 Hz, 1H), 7.83 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.70 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.50 (d, *J* = 8.5 Hz, 1H), 7.42 (d, *J* = 2.1 Hz, 1H), 7.24 (dd, *J* =

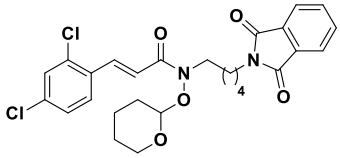
8.4, 2.1 Hz, 1H), 7.00 (d, *J* = 15.8 Hz, 1H), 4.92 (dd, *J* = 3.0, 2.5 Hz, 1H), 4.04–3.97 (m, 1H), 3.95–3.89 (m, 1H), 3.87–3.80 (m, 1H), 3.77 (t, *J* = 7.2 Hz, 2H), 3.59–3.51 (m, 1H), 2.22–2.05 (m, 2H), 1.90–1.77 (m, 2H), 1.77–1.66 (m, 1H), 1.66–1.52 (m, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 168.4, 166.9, 138.1, 135.8, 135.6, 134.0, 132.4, 132.3, 130.1, 128.6, 127.5, 123.3, 120.1, 105.3, 64.1, 46.6, 36.0, 29.3, 26.4, 25.0, 19.9. **MS** (ES^+) *m/z* calcd for $C_{25}H_{24}Cl_2N_2NaO_5^+$ $[M+Na]^+$, 525.1; found: 525.1.

(*E*)-3-(2,4-dichlorophenyl)-*N*-(4-(1,3-dioxoisoindolin-2-yl)butyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S8). Synthesized by method B using (*E*)-3-(2,4-dichlorophenyl)acryloyl chloride



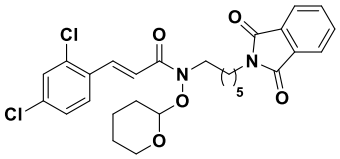
(744 mg, 3.16 mmol, 1.2 equiv.), **S3** (877 mg, 2.63 mmol, 1.0 equiv.), *i*Pr_2NEt (510 µL, 2.92 mmol, 1.1 equiv.) and anh. CH_2Cl_2 (15 mL). The reaction mixture was treated in the same manner as outlined in the synthesis of **S6**. The titled compound (1.13 g, 2.17 mmol, 82%) was collected as a colorless oil. **TLC** (33% EtOAc–hexanes): R_f = 0.4. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.96 (d, *J* = 15.8 Hz, 1H), 7.83 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.70 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.42 (d, *J* = 2.1 Hz, 1H), 7.25 (ddd, *J* = 8.4, 2.1, 0.6 Hz, 1H), 7.01 (d, *J* = 15.8 Hz, 1H), 4.91 (dd, *J* = 5.5, 2.8 Hz, 1H), 4.02–3.92 (m, 2H), 3.83–3.74 (m, 1H), 3.72 (t, *J* = 6.8 Hz, 2H), 3.63–3.55 (m, 1H), 1.89–1.55 (m, 10H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 168.5, 166.8, 138.0, 135.8, 135.6, 134.0, 132.4, 132.3, 130.1, 128.7, 127.5, 123.3, 120.2, 105.0, 64.1, 48.0, 37.7, 29.3, 26.1, 25.0, 24.6, 19.9. **MS** (ES^+) *m/z* calcd for $C_{26}H_{26}Cl_2N_2NaO_5^+$ $[M+Na]^+$, 539.1; found: 539.1.

(*E*)-3-(2,4-dichlorophenyl)-*N*-(5-(1,3-dioxoisoindolin-2-yl)pentyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S9). Synthesized by method B using (*E*)-3-(2,4-dichlorophenyl)acryloyl chloride



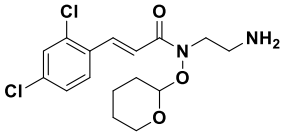
(942 mg, 3.16 mmol, 1.5 equiv.), **S4** (830 mg, 2.72 mmol, 1.0 equiv.), *i*Pr_2NEt (850 µL, 4.88 mmol, 1.8 equiv.) and anh. CH_2Cl_2 (20 mL). The reaction mixture was treated in the same manner as outlined in the synthesis of **S6**. The titled compound (1.13 g, 2.13 mmol, 78%) was collected a colorless oil. **TLC** (50% EtOAc–hexanes): R_f = 0.5. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.93 (d, *J* = 15.8 Hz, 1H), 7.81 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.68 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.51 (d, *J* = 8.4 Hz, 1H), 7.41 (d, *J* = 2.1 Hz, 1H), 7.23 (ddd, *J* = 8.4, 2.1, 0.6 Hz, 1H), 7.00 (d, *J* = 15.8 Hz, 1H), 4.91 (dd, *J* = 5.3, 2.9 Hz, 1H), 4.00–3.88 (m, 2H), 3.76–3.70 (m, 1H), 3.68 (t, *J* = 7.2 Hz, 2H), 3.63–3.56 (m, 1H), 1.90–1.67 (m, 7H), 1.65–1.56 (m, 3H), 1.43–1.33 (m, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 168.5, 166.7, 137.8, 135.7, 135.6, 133.9, 132.4, 132.3, 130.1, 128.6, 127.5, 123.2, 120.3, 104.9, 64.0, 48.4, 37.9, 29.2, 28.4, 26.7, 25.0, 24.2, 19.8. **MS** (ES^+) *m/z* calcd for $C_{27}H_{28}Cl_2N_2NaO_5^+$ $[M+Na]^+$, 553.1; found: 553.1.

(*E*)-3-(2,4-dichlorophenyl)-*N*-(6-(1,3-dioxoisoindolin-2-yl)hexyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S10). Synthesized by method B using (*E*)-3-(2,4-dichlorophenyl)acryloyl chloride



(490 mg, 2.08 mmol, 1.2 equiv.), **S5** (600 mg, 1.73 mmol, 1.0 equiv.) and *i*Pr_2NEt (340 µL, 1.95 mmol, 1.1 equiv.) and anh. CH_2Cl_2 (10 mL). The reaction mixture was treated in the same manner as outlined in the synthesis of **S6**. The titled compound (725 mg, 1.33 mmol, 65%) was collected as a colorless oil. **TLC** (50% EtOAc–hexanes): R_f = 0.6. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.95 (d, *J* = 15.8 Hz, 1H), 7.85–7.78 (m, 2H), 7.69 (dd, *J* = 5.4, 2.8 Hz, 2H), 7.51 (d, *J* = 8.4 Hz, 1H), 7.41 (d, *J* = 2.2 Hz, 1H), 7.23 (d, *J* = 8.6 Hz, 1H), 7.01 (d, *J* = 15.8 Hz, 1H), 4.91 (br.s, 1H), 4.01–3.87 (m, 2H), 3.75–3.69 (m, 1H), 3.66 (t, *J* = 7.4 Hz, 2H), 3.63–3.56 (m, 1H), 1.91–1.59 (m, 10H), 1.40–1.34 (br.s, 4H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 168.5, 166.7, 137.8, 135.7, 135.6, 134.0, 132.4, 132.3, 130.1, 128.6, 127.5, 123.3, 120.3, 104.9, 63.9, 48.6, 38.1, 29.3, 28.6, 27.0, 26.7, 26.5, 25.0, 19.8. **MS** (ES^+) *m/z* calcd for $C_{28}H_{30}Cl_2N_2NaO_5^+$ $[M+Na]^+$, 567.1; found: 567.1.

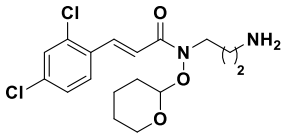
(*E*)-*N*-(2-aminoethyl)-3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S11).



Intermediate **S6** (480 mg, 0.98 mmol, 1.0 equiv.) was dissolved in abs. EtOH (10 mL) and hydrazine (62 µL, 3.92 mmol, 4.0 equiv.) was added and the reaction mixture stirred overnight at r.t. The resulting precipitate was filtered, washed with abs. EtOH, and the filtrate concentrated *in vacuo* and the crude residue purified by NP ACC. The titled compound (281 mg, 0.78 mmol, 80%) was collected as a pale-yellow oil*. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.85 (d, *J* = 15.8 Hz, 1H), 7.46 (d, *J* = 8.6 Hz, 1H), 7.39 (d, *J* = 2.2 Hz, 1H), 7.20 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.80–6.74 (m, 1H), 6.39 (d, *J* = 15.7 Hz, 1H), 4.79–4.74 (m, 1H), 3.97–3.90 (m, 1H), 3.62–3.53 (m, 3H), 3.12 (t, *J* = 5.4 Hz, 2H), 1.81–1.69 (m, 2H), 1.58–1.48 (m, 4H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 165.4, 135.6, 135.5, 135.3, 132.0, 130.0, 128.3, 127.5, 124.5, 102.2, 63.9, 51.0, 37.7, 29.4, 25.3, 20.5. **MS** (ES^+) *m/z* calcd for $C_{16}H_{21}Cl_2N_2O_3^+$ $[M+H]^+$, 359.1; found: 359.0.

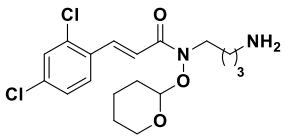
*Impurity arising from THP at ~25%, but compound taken forward without further purification.

(*E*)-*N*-(3-aminopropyl)-3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S12).



Synthesized by method D using **S7** (731 mg, 1.46 mmol, 1.0 equiv.), ethylenediamine (486 µL, 7.30 mmol, 5.0 equiv.) and *n*-butanol (15 mL). The titled compound (311 mg, 0.83 mmol, 57%) was collected as a colorless oil. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.87 (d, *J* = 15.7 Hz, 1H), 7.47 (d, *J* = 8.5 Hz, 1H), 7.40 (d, *J* = 2.1 Hz, 1H), 7.21 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.77 (br.s, 1H), 6.39 (d, *J* = 15.7 Hz, 1H), 5.87 (br.s, 1H), 4.83–4.78 (m, 1H), 3.95–3.87 (m, 1H), 3.59–3.44 (m, 3H), 3.09 (m, 2H), 1.84–1.69 (m, 4H), 1.59–1.48 (m, 4H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 165.3, 135.6, 135.4, 135.3, 132.1, 130.0, 128.3, 127.5, 124.6, 101.5, 63.2, 50.5, 38.8, 29.3, 26.7, 25.4, 20.2. **MS** (ES^+) *m/z* calcd for $C_{17}H_{23}Cl_2N_2O_3^+$ $[M+H]^+$, 373.1; found: 373.1.

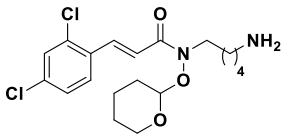
(*E*)-*N*-(4-aminobutyl)-3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S13).



Synthesized by method D using **S8** (946 mg, 1.44 mmol, 1.0 equiv.), ethylenediamine (480 µL, 7.20 mmol, 5.0 equiv.) and *n*-butanol (15 mL). The titled compound (301 mg, 0.78 mmol, 54%) was collected as a colorless oil*. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.97 (d, *J* = 15.8 Hz, 1H), 7.51 (d, *J* = 8.5 Hz, 1H), 7.42 (d, *J* = 2.2 Hz, 1H), 7.24 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.02 (d, *J* = 15.7 Hz, 1H), 4.95–4.90 (m, 1H), 4.01–3.89 (m, 2H), 3.81–3.70 (m, 1H), 3.64–3.56 (m, 1H), 2.71 (t, *J* = 7.0 Hz, 2H), 1.88–1.69 (m, 4H), 1.68–1.56 (m, 3H), 1.51–1.43 (m, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.8, 137.9, 135.7, 135.6, 132.4, 130.1, 128.6, 127.5, 120.2, 105.0, 64.0, 50.7, 42.0, 31.0, 29.3, 25.0, 24.5, 19.9. **MS** (ES^+) *m/z* calcd for $C_{18}H_{25}Cl_2N_2O_3^+$ $[M+H]^+$, 387.1; found: 387.1.

*Ethylenediamine impurity at ~33%, but compound taken forward without further purification.

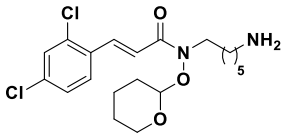
(*E*)-*N*-(5-aminopentyl)-3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S14).



Synthesized by method D using **S9** (1.01 g, 2.07 mmol, 1.0 equiv.), ethylenediamine (669 µL, 10.4 mmol, 5.0 equiv.) and *n*-butanol (20 mL). The title compound (178 mg, 0.44 mmol, 21%) was collected as a pale-yellow oil*.**^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.98 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.43 (d, *J* = 2.5 Hz, 1H), 7.28–7.22 (m, 1H), 7.03 (d, *J* = 16.2 Hz, 1H), 4.95–4.91 (m, 1H), 4.01–3.89 (m, 2H), 3.80–3.71 (m, 1H), 3.64–3.58 (m, 1H), 2.69 (t, *J* = 7.0 Hz, 2H), 1.90–1.78 (m, 1H), 1.78–1.69 (m, 3H), 1.67–1.58 (m, 4H), 1.52–1.44 (m, 4H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 166.8, 137.9, 135.8, 135.6, 132.4, 130.1, 128.6, 127.5, 120.3, 105.0, 64.0, 50.9, 42.2, 33.6, 29.3, 27.0, 25.0, 24.2, 19.9. **MS** (ES^+) *m/z* calcd for $C_{19}H_{27}Cl_2N_2O_3^+$ $[M+H]^+$, 401.1; found: 401.1.

*Impurity arising from DCHA at ~20%, ethylenediamine at ~20%, but compound taken forward without further purification.

(*E*)-*N*-(6-aminohexyl)-3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S15).



Synthesized by method D using **S10** (695 mg, 1.28 mmol, 1.0 equiv.), ethylenediamine (426 µL, 6.40 mmol, 5.0 equiv.) and *n*-butanol (15 mL). The title compound (331 mg, 0.80 mmol, 62%) was collected as a colorless oil*. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.92 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.5 Hz, 1H), 7.40 (d, *J* = 2.1 Hz, 1H), 7.23 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.02 (d, *J* = 15.8 Hz, 1H), 4.92–4.88 (m, 1H), 3.97–3.92 (m, 1H), 3.91–3.83 (m, 1H), 3.75–3.67 (m, 1H), 3.62–3.56 (m, 1H), 2.98 (t, *J* = 7.6 Hz, 2H), 1.89–1.48 (m, 10H), 1.46–1.27 (m, 4H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 166.8, 137.9, 135.8, 135.5, 132.3, 130.0, 128.7, 127.6, 120.2, 104.8, 64.1, 50.7, 39.9, 29.2, 27.5, 26.8, 26.2, 26.1, 25.0, 19.8. **MS** (ES^+) *m/z* calcd for $C_{20}H_{29}Cl_2N_2O_3^+$ $[M+H]^+$, 415.2; found: 415.1.

*Ethylenediamine impurity at ~15%, but compound taken forward without further purification.

(*E*)-*N*-(2-acrylamidoethyl)-3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S16). Synthesized by method B using **S11** (80 mg, 0.22 mmol, 1.0 equiv.), acryloyl chloride (60 µL, 0.26 mmol, 1.2 equiv.), *i*Pr_2NEt (51 µL, 0.29 mmol, 1.3 equiv.) and anh. CH_2Cl_2 (1.6 mL). Upon completion the reaction mixture was diluted with EtOAc and washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. The crude residue was purified by NP ACC to afford the titled compound (51 mg, 0.12 mmol, 56%) as a colorless oil*. **TLC** (66% EtOAc–hexanes): R_f = 0.3. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.85 (d, *J* = 15.7 Hz, 1H), 7.48 (d, *J* = 8.5 Hz, 1H), 7.39 (d, *J* = 2.1 Hz, 1H), 7.20 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.94 (br.s, 1H), 6.67 (dd, *J* = 17.2, 10.5 Hz, 1H), 6.41 (dd, *J* = 17.1, 1.9 Hz, 1H), 6.37 (d, *J* 15.7 Hz, 1H), 5.76 (dd, *J* = 10.4, 1.9 Hz, 1H), 4.94–4.89 (m, 1H), 4.06–3.93 (m, 3H), 3.75–3.57 (m, 3H), 1.86–1.77 (m, 2H), 1.76–1.67 (m, 1H), 1.65–1.56 (m, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 168.6, 165.6, 135.6, 135.4, 135.3, 132.0, 130.0, 129.9, 128.4, 127.5, 126.4, 124.3, 105.2, 64.0, 49.4, 39.3, 28.9, 24.9, 19.6. **MS** (ES^+) *m/z* calcd for $C_{19}H_{22}Cl_2N_2O_4^+$ $[M+Na]^+$, 435.1; found: 435.0.

*Trace impurity, but compound taken forward without further purification.

(*E*)-*N*-(3-acrylamidopropyl)-3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S17). Synthesized by method B using **S12** (135 mg, 0.36 mmol, 1.0 equiv.), acryloyl chloride (53 µL, 0.65 mmol, 1.8 equiv.), *i*Pr_2NEt (120 µL, 0.69 mmol, 2.0 equiv.) and anh. CH_2Cl_2 (1.6 mL). Upon completion the reaction mixture was diluted with CH_2Cl_2 and washed with water, dried ($MgSO_4$) and concentrated *in vacuo*. The crude residue was purified by NP ACC to afford the titled compound (139 mg, 0.33 mmol, 90%) as a pale-brown oil. **TLC** (66% EtOAc–hexanes): R_f = 0.3. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.92 (d, *J* = 15.7Hz, 1H), 7.54 (d, *J* = 8.5 Hz, 1H), 7.42 (d, *J* = 2.1 Hz, 1H), 7.24 (ddd, *J* = 8.4, 2.1, 0.6 Hz, 1H), 6.92–6.88 (m, 1H), 6.74 (dd, *J* = 17.1, 10.4 Hz, 1H), 6.44 (dd, *J* = 17.1, 1.9 Hz, 1H), 6.43 (d, *J* = 15.7 Hz, 1H), 5.80 (dd, *J* = 10.4, 1.9 Hz, 1H), 4.92 (dd, *J* = 5.2, 2.6 Hz, 1H), 4.02–3.94 (m, 2H), 3.88 (dt, *J* = 14.9, 5.6 Hz, 1H), 3.67–3.60 (m, 1H), 3.51–3.41 (m, 1H), 3.29–3.21 (m, 1H), 1.94–1.89 (m, 2H), 1.88–1.79 (m, 2H), 1.76–1.70 (m, 1H), 1.67–1.59 (m, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 168.1, 165.3, 135.5, 135.4, 132.1, 131.5, 129.9, 129.8, 128.4, 127.4, 126.3, 124.7, 105.1, 63.9, 45.5, 35.8, 29.0, 26.8, 24.9, 19.6. **MS** (ES^+) *m/z* calcd for $C_{20}H_{24}Cl_2N_2NaO_4^+$ $[M+Na]^+$, 449.1; found: 449.1.

(*E*)-*N*-(4-acrylamidobutyl)-3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S18). Synthesized by method B using **S13** (135 mg, 0.35 mmol, 1.0 equiv.), acryloyl chloride (43 µL, 0.54 mmol, 1.5 equiv.), *i*Pr_2NEt (102 µL, 0.59 mmol, 1.7 equiv.) and anh. CH_2Cl_2 (3.0 mL). The reaction mixture was worked up

and purified in the same manner as outlined in the synthesis of **S16**. The titled compound (122 mg, 0.28 mmol, 79%) was collected as a colorless oil*. **TLC** (50% EtOAc–hexanes): R_f = 0.3. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.94 (d, *J* = 15.8 Hz, 1H), 7.50 (d, *J* = 8.5 Hz, 1H), 7.39 (d, *J* = 2.1 Hz, 1H), 7.22 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.01 (d, *J* = 15.8 Hz, 1H), 6.54–6.45 (m, 1H), 6.24 (dd, *J* = 17.0, 1.8 Hz, 1H), 6.12 (dd, *J* = 17.0, 10.0 Hz, 1H), 5.57 (dd, *J* = 10.0, 1.8 Hz, 1H), 4.90 (dd, *J* = 5.4, 2.6 Hz, 1H), 3.99–3.84 (m, 2H), 3.82–3.70 (m, 1H), 3.63–3.52 (m, 1H), 3.34 (q, *J* 6.5 Hz, 2H), 1.88–1.66 (m, 5H), 1.64–1.49 (m, 5H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.9, 165.8, 138.0, 135.8, 135.5, 132.1, 131.1, 130.0, 128.5, 127.5, 126.0, 120.0, 104.9, 64.0, 40.4, 39.2, 29.2, 26.3, 24.9, 24.7, 19.8. **MS** (ES^+) *m/z* calcd for $C_{21}H_{26}Cl_2N_2NaO_4^+$ $[M+Na]^+$, 463.1; found: 463.1.

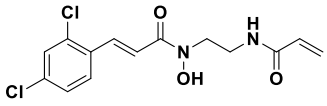
*Trace impurity, but compound taken forward without further purification.

(*E*)-*N*-(5-acrylamidopentyl)-3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S19). Synthesized by method B using **S14** (91 mg, 0.23 mmol, 1.0 equiv.), acryloyl chloride (21 µL, 0.27 mmol, 1.2 equiv.), *i*Pr_2NEt (59 µL, 0.34 mmol, 1.5 equiv.) and anh. CH_2Cl_2 (2.0 mL). Upon completion the reaction mixture was diluted with CH_2Cl_2 and washed with sat. $NaHCO_3$ (2×) and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The crude residue was purified by NP ACC to afford the titled compound (77 mg, 0.17 mmol, 74%) as a colorless oil. **TLC** (50% EtOAc–hexanes): R_f = 0.4. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.99 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.5 Hz, 1H), 7.43 (d, *J* = 2.1 Hz, 1H), 7.25 (dd, *J* = 8.4, 2.5 Hz, 1H, overlapped with solvent peak), 7.04 (d, *J* = 15.8 Hz, 1H), 6.25 (dd, *J* = 17.0, 1.6 Hz, 1H), 6.13 (dd, *J* = 17.0, 10.2 Hz, 1H), 6.00–5.97 (m, 1H), 5.57 (dd, *J* = 10.3, 1.6 Hz, 1H), 4.93 (dd, *J* = 5.2, 2.9 Hz, 1H), 4.01–3.92 (m, 2H), 3.81–3.74 (m, 1H), 3.63–3.59 (m, 1H), 3.32 (q, *J* = 6.7, 2H), 1.91–1.79 (m, 2H), 1.80–1.68 (m, 3H), 1.68–1.54 (m, 5H), 1.43–1.32 (m, 2H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 169.6, 165.8, 138.1, 135.9, 135.6, 132.3, 131.2, 130.1, 128.6, 127.6, 126.1, 120.1, 105.0, 64.0, 40.5, 39.5, 29.3, 28.6, 26.7, 25.0, 23.8, 19.8. **MS** (ES^+) *m/z* calcd for $C_{22}H_{28}Cl_2N_2NaO_4^+$ $[M+Na]^+$, 477.1; found: 477.1.

(*E*)-*N*-(6-acrylamidohexyl)-3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S20). Synthesized by method B using **S15** (150 mg, 0.36 mmol, 1.0 equiv.), acryloyl chloride (34 µL, 0.43 mmol, 1.2 equiv.), *i*Pr_2NEt (92 µL, 0.53 mmol, 1.5 equiv.) and anh. CH_2Cl_2 (3.0 mL). The reaction mixture was worked up and purified in the same manner as outlined in the synthesis of **S16**. The titled compound (96 mg, 0.20 mmol, 56%) was collected as a colorless oil. **TLC** (50% EtOAc–hexanes): R_f = 0.4. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.97 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.5 Hz, 1H), 7.43 (d, *J* = 2.1 Hz, 1H), 7.27–7.23 (m, 1H), 7.04 (d, *J* = 15.8 Hz, 1H), 6.27 (dd, *J* = 16.9, 1.5 Hz, 1H), 6.15 (dd, *J* = 17.0, 10.2 Hz, 1H), 5.99 (br.s, 1H), 5.61 (dd, *J* = 10.2, 1.6 Hz, 1H), 4.93 (dd, *J* = 5.2, 2.9 Hz, 1H), 4.01–3.92 (m, 2H), 3.79–

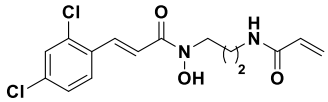
3.73 (m, 1H), 3.65–3.58 (m, 1H), 3.36–3.23 (m, 2H), 1.90–1.79 (m, 2H), 1.77–1.69 (m, 3H), 1.68–1.59 (m, 3H), 1.59–1.49 (m, 2H), 1.44–1.30 (m, 4H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 169.5, 165.7, 138.0, 135.9, 135.6, 132.3, 131.3, 130.1, 128.6, 127.6, 126.1, 120.1, 104.9, 64.1, 40.5, 39.8, 39.1, 29.3, 26.9, 26.0, 25.9, 25.0, 19.9. **MS** (ES^+) *m/z* calcd for $C_{23}H_{30}Cl_2N_2NaO_4^+$ $[M+Na]^+$, 491.1; found: 491.1.

(*E*)-*N*-(2-acrylamidoethyl)-3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamide (2). Synthesized by method



F using **S16** (50 mg, 0.12 mmol, 1.0 equiv.), PPTS (15 mg, 0.06 mmol, 0.5 equiv.) and abs. EtOH (1.0 mL). The reaction mixture was reduced *in vacuo* and the crude oil dissolved in EtOAc and washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by NP ACC to give the product as an oil which was triturated with 50% EtOAc–hexanes to give the titled compound (21 mg, 0.06 mmol, 53%) as a white solid. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 9.98 (s, 1H), 8.37 (br.t, *J* = 6.0 Hz, 1H), 7.75–7.69 (m, 2H), 7.66 (d, *J* = 15.7 Hz, 1H), 7.49 (dd, *J* = 8.4, 2.3 Hz, 1H), 6.93–6.79 (m, 1H), 6.67 (d, *J* = 15.7 Hz, 1H), 6.15 (dd, *J* = 17.1, 2.4 Hz, 1H), 5.71 (d, *J* = 10.5 Hz, 1H), 3.70 (br.t, *J* = 6.6 Hz, 2H), 3.42 (q, *J* = 6.4 Hz, 2H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 165.3, 164.8, 134.5, 134.1, 133.1, 131.7, 129.4, 128.8, 128.0, 127.6, 127.2, 125.6, 47.0, 36.1. **HPLC**, t_R 5.85 min (>98%, UV_{254}). **HRMS** (ES^+) m/z calcd for $C_{14}H_{14}Cl_2N_2NaO_3^+$ $[M+Na]^+$,351.0274; found 351.0282.

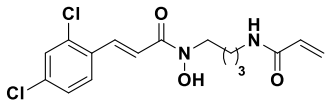
(*E*)-*N*-(3-acrylamidopropyl)-3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamide (3). Synthesized by



method F using **S17** (92 mg, 0.22 mmol, 1.0 equiv.), PPTS (10 mg, 0.04 mmol, 0.2 equiv.) and abs. EtOH (2.0 mL). The crude product was worked up and purified in the same manner as outlined in the synthesis of **2**. The titled compound (34 mg, 0.10 mmol, 46%) was collected as a pale-brown solid. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 9.95 (s, 1H), 8.25 (br.t, *J* = 5.6 Hz, 1H), 7.73 (d, *J* = 8.5 Hz, 1H), 7.71 (d, *J* = 2.2 Hz, 1H), 7.65 (d, *J* = 15.7 Hz, 1H), 7.50 (ddd, *J* = 8.6, 2.2, 0.6 Hz, 1H), 6.89–6.81 (m, 1H), 6.69 (d, *J* = 15.7 Hz, 1H), 6.16 (dd, *J* = 17.2, 2.4 Hz, 1H), 5.71 (dd, *J* = 10.4, 2.5 Hz, 1H), 3.61 (t, *J* = 7.1 Hz, 2H), 3.20 (td, *J* = 7.0, 5.6 Hz, 2H), 1.76 (quint, *J* = 7.1 Hz, 2H). **^{13}C NMR*** (151 MHz, DMSO-d_6) δ 164.9, 164.2, 134.4, 134.0, 132.7, 131.8, 129.4, 128.8, 128.0, 127.4, 127.2, 126.0, 45.6, 36.6, 26.6. **HPLC**, t_R 5.80 min (>96%, UV_{254}). **HRMS** (ES^+) m/z calcd for $C_{15}H_{16}Cl_2N_2O_3^+$ $[M+H]^+$, 343.0611; found 343.0611.

*Trace impurity from NMR tube in ^{13}C spectra.

(*E*)-N-(4-acrylamidobutyl)-3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamide (4). Synthesized by method

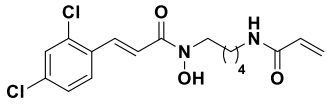


F using **S18** (122 mg, 0.28 mmol, 1.0 equiv.), PPTS (12 mg, 0.05 mmol, 0.2 equiv.) and abs. EtOH (2.5 mL). Water was added to obtain a crude precipitate, which was dissolved in CH_2Cl_2, washed with water (2×), dried ($MgSO_4$) and concentrated *in vacuo*. The residue was triturated CH_2Cl_2–hexanes to give the titled compound (19 mg, 0.05 mmol 19%)

as a white solid*. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 10.05 (s, 1H), 8.08 (br.t, J = 5.7 Hz, 1H), 7.88 (d, J = 8.5 Hz, 1H), 7.77–7.69 (m, 2H), 7.47 (dd, J = 8.5, 2.1 Hz, 1H), 7.31 (d, J = 15.9 Hz, 1H), 6.20 (dd, J = 17.1, 10.1 Hz, 1H), 6.06 (dd, J = 17.1, 2.2 Hz, 1H), 5.56 (dd, J = 10.1, 2.3 Hz, 1H), 3.63 (br.t, J = 6.7 Hz, 2H), 3.15 (q, J 6.7 Hz, 2H), 1.63–1.55 (m, 2H), 1.49–1.38 (m, 2H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 168.7, 164.5, 134.9, 134.6, 134.2, 131.85, 131.79, 129.4, 129.2, 128.0, 124.8, 121.2, 47.3, 38.3, 26.3, 23.9. **HPLC**, t_R 5.87 min (>91%*, UV_{254}). **HRMS** (ES+) m/z calcd for $C_{16}H_{18}Cl_2N_2O_3^+$ $[M+H]^+$, 357.0767; found 357.0768.

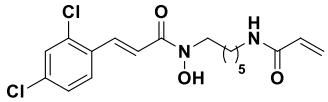
*Small impurity present but disregarded as compound shows no activity against BoNT/A LC.

(*E*)-*N*-(5-acrylamidopentyl)-3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamide (5). Synthesized by



method F using **S19** (77 mg, 0.17 mmol, 1.0 equiv.), PPTS (5 mg, 0.02 mmol, 0.1 equiv.) and abs. EtOH (1.5 mL). Sat. $NaHCO_3$ and CH_2Cl_2 was added to the reaction mixture to form a precipitate, which was filtered and washed with water to obtain the titled compound (16 mg, 0.04 mmol, 25%) as a pale-yellow solid. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 10.04 (s, 1H), 8.06 (br.t, J = 5.6 Hz, 1H), 7.88 (d, J = 8.5 Hz, 1H), 7.76–7.69 (m, 2H), 7.47 (dd, J = 8.5, 2.2 Hz, 1H), 7.31 (d, J = 15.9 Hz, 1H), 6.20 (dd, J = 17.1, 10.2 Hz, 1H), 6.05 (dd, J = 17.1, 2.2 Hz, 1H), 5.54 (dd, J = 10.2, 2.3 Hz, 1H), 3.66–3.57 (m, 2H), 3.11 (q, J = 6.7 Hz, 2H), 1.63–1.55 (m, 2H), 1.49–1.42 (m, 2H), 1.32–1.24 (m, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 168.6, 164.4, 134.8, 134.6, 134.2, 131.9, 131.8, 129.4, 129.2, 128.0, 124.8, 121.3, 47.4, 38.4, 28.7, 26.0, 23.6. **HPLC**, t_R 6.03 min (>95%, UV_{254}). **HMRS** (ES+) m/z calcd for $C_{17}H_{20}Cl_2N_2O_3^+$ $[M+H]^+$, 371.0924; found 371.0928.

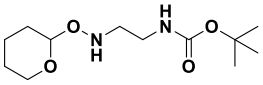
(*E*)-*N*-(6-acrylamidohexyl)-3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamide (6). Synthesized by method



F using **S20** (96 mg, 0.20 mmol, 1.0 equiv.), PPTS (10 mg, 0.04 mmol, 0.2 equiv.) and abs. EtOH (2.0 mL). Sat. $NaHCO_3$ and 1,2-dichloroethane was added to the reaction mixture to form a precipitate, which was filtered and washed with water to obtain the titled compound (37 mg, 0.10 mmol, 48%) as a white solid. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 10.02 (s, 1H), 8.04 (br.t, J = 5.6 Hz, 1H), 7.88 (d, J = 8.6 Hz, 1H), 7.77–7.68 (m, 2H), 7.47 (dd, J = 8.5, 2.2 Hz, 1H), 7.31 (d, J = 15.8 Hz, 1H), 6.20 (dd, J = 17.1, 10.2 Hz, 1H), 6.05 (dd, J = 17.1, 2.2 Hz, 1H), 5.55 (dd, J = 10.2, 2.3 Hz, 1H), 3.61 (br.t, J = 6.7 Hz, 2H), 3.11 (q, J = 6.6 Hz, 2H), 1.62–1.54 (m, 2H), 1.45–1.38 (m, 2H), 1.33–1.24 (m, 4H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 168.6, 164.4, 134.8, 134.6, 134.2, 131.9, 131.8, 129.4, 129.2, 128.0, 124.7, 121.3, 47.5, 41.2, 38.4, 29.0, 26.1, 25.8. **HPLC**, t_R 6.20 min (>96%, UV_{254}). **HMRS** (ES+) m/z calcd for $C_{18}H_{22}Cl_2N_2O_3^+$ $[M+H]^+$, 385.1080; found 385.1081.

4.4.2 2-fluoroacrylamides

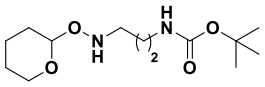
***Tert*-butyl (2-(((tetrahydro-2*H*-pyran-2-yl)oxy)amino)ethyl)carbamate (S21)**. Synthesized by method



A1 using OTX (528 mg, 4.50 mmol, 1.5 equiv.), *tert*-butyl (2-bromoethyl)carbamate (672 mg, 3.00 mmol, 1.0 equiv.), K_2CO_3 (622 mg, 4.50 mmol, 1.5 equiv.) and DMF (4.0 mL). The resulting precipitate was filtered, washed with CH_2Cl_2 and reduced *in vacuo*. The crude residue was purified by NP ACC to afford the titled compound (299 mg, 1.15 mmol, 38%) as a colorless oil*. **TLC** (66% EtOAc–hexanes): R_f = 0.4. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 5.11 (br.s, 1H), 4.84–4.76 (m, 1H), 3.95–3.88 (m, 1H), 3.63–3.52 (m, 1H), 3.38–3.26 (m, 2H), 3.14–3.01 (m, 2H), 1.85–1.66 (m, 2H), 1.64–1.48 (m, 4H), 1.43 (s, 9H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 156.2, 101.8, 79.3, 63.5, 51.5, 38.4, 29.2, 28.5, 25.4, 20.3. MS (ES^+) *m/z* calcd for $C_{12}H_{25}N_2O_4^+$ $[M+H]^+$, 261.2; found: 261.1.

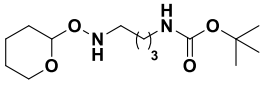
*Trace impurity arising from bromocarbamate, but compound taken forward without further purification.

***Tert*-butyl (3-(((tetrahydro-2*H*-pyran-2-yl)oxy)amino)propyl)carbamate (S22)**. Synthesized by



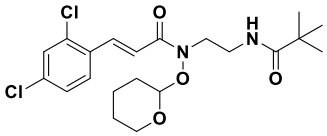
method A1 using OTX (264 mg, 2.25 mmol, 1.5 equiv.), *tert*-butyl (3-bromopropyl)carbamate (357 mg, 1.50 mmol, 1.0 equiv.), K_2CO_3 (311 mg, 2.25 mmol, 1.5 equiv.) and DMF (4.0 mL). The reaction mixture was worked up and purified in the same manner as outlined in the synthesis of compound **S21**. The titled compound (193 mg, 0.70 mmol, 47%) was collected as a colorless oil. **TLC** (50% EtOAc–hexanes): R_f = 0.4. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 4.96–4.78 (m, 2H), 3.96–3.88 (m, 1H), 3.61–3.54 (m, 1H), 3.22 (q, *J* = 6.6 Hz, 2H), 3.11–2.98 (m, 2H), 1.82–1.68 (m, 4H), 1.62–1.49 (m, 4H), 1.43 (s, 9H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 156.3, 101.6, 79.3, 63.3, 49.7, 38.7, 29.2, 28.6, 27.6, 25.4, 20.2. **MS** (ES^+) *m/z* calcd for $C_{13}H_{27}N_2O_4^+$ $[M+H]^+$, 275.2; found: 275.1.

***Tert*-butyl (4-(((tetrahydro-2*H*-pyran-2-yl)oxy)amino)butyl)carbamate (S23)**. Synthesized by method



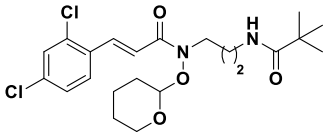
A1 using OTX (528 mg, 4.50 mmol, 1.5 equiv.), *tert*-butyl (4-bromobutyl)carbamate (756 mg, 3.00 mmol, 1.0 equiv.), K_2CO_3 (622 mg, 4.50 mmol, 1.5 equiv.) and DMF (4.0 mL). The reaction mixture was worked and purified in the same manner as outlined for compound **S21**. The titled compound (615 mg, 2.13 mmol, 71%) was collected as a colorless oil. **TLC** (50% EtOAc–hexanes): R_f = 0.4. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 4.85–4.77 (m, 1H), 4.64 (br.s, 1H), 3.95–3.88 (m, 1H), 3.60–3.52 (m, 1H), 3.12 (q, *J* = 6.3 Hz, 2H), 3.04–2.92 (m, 2H), 1.81–1.67 (m, 2H), 1.64–1.47 (m, 8H), 1.42 (s, 9H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 156.1, 101.6, 79.2, 63.3, 51.7, 40.5, 29.3, 28.5, 27.8, 25.4, 24.6, 20.3. **MS** (ES^+) *m/z* calcd for $C_{14}H_{29}N_2O_4^+$ $[M+H]^+$, 289.2; found: 289.2.

***Tert*-butyl (*E*)-(2-(3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamido)ethyl) carbamate (S24)**. Synthesized by method C1 using **S21** (71 mg, 0.33 mmol, 1.0 equiv.), *trans*-2,4-



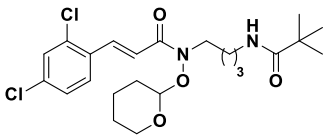
dichlorocinnamic acid (93 mg, 0.38 mmol, 1.2 equiv.), HATU (136 mg, 0.38 mmol, 1.2 equiv.), *i*Pr_2NEt (170 µL, 0.98 mmol, 3.0 equiv.) and anh. DMF (3.0 mL). The reaction mixture was worked up using EtOAc as outlined in method C1 and the crude residue purified by NP ACC to afford the titled compound (122 mg, 0.27 mmol, 82%) as a colorless oil. **TLC** (50% EtOAc–hexanes): R_f = 0.7. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.00 (d, *J* = 15.8 Hz, 1H), 7.53 (d, *J* = 8.5 Hz, 1H), 7.44 (d, *J* = 2.1 Hz, 1H), 7.27–7.24 (m, 1H, overlapped with solvent peak), 7.00 (d, *J* = 15.8 Hz, 1H), 5.07 (s, 1H), 4.95 (d, *J* = 5.3 Hz, 1H), 4.05–3.96 (m, 2H), 3.94–3.87 (m, 1H), 3.66–3.60 (m, 1H), 3.52–3.39 (m, 2H), 1.91–1.82 (m, 2H), 1.69–1.58 (m, 4H), 1.41 (s, 9H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 156.1, 138.4, 136.0, 135.7, 132.2, 130.2, 128.7, 127.6, 119.9, 105.1, 79.5, 64.2, 49.4, 39.1, 29.2, 28.5, 25.0, 19.9, one C=O not observed. **MS** (ES^+) *m/z* calcd for $C_{21}H_{28}Cl_2N_2NaO_5^+$ $[M+Na]^+$, 481.1; found: 481.1.

***Tert*-butyl (*E*)-(3-(3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamido)propyl) carbamate (S25)**. Synthesized by method C1 using **S22** (166 mg, 0.61 mmol, 1.1 equiv.), *trans*-2,4-



dichlorocinnamic acid (119 mg, 0.55 mmol, 1.0 equiv.), HATU (136 mg, 0.38 mmol, 1.2 equiv.), *i*Pr_2NEt (288 µL, 1.65 mmol, 3.0 equiv.) and anh. DMF (3.0 mL). The reaction mixture was worked up using EtOAc as outlined in method C1 and the crude residue purified by NP ACC to afford the titled compound (256 mg, 0.54 mmol, 98%) as a colorless oil. **TLC** (50% EtOAc–hexanes): R_f = 0.6. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.99 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.44 (d, *J* = 2.1 Hz, 1H), 7.25 (dd, *J* = 2.0, 0.6 Hz, 1H, overlapped with solvent peak), 7.03 (d, *J* = 15.8 Hz, 1H), 5.19 (br.s, 1H), 4.97–4.89 (m, 1H), 4.04–3.93 (m, 2H), 3.91–3.80 (m, 1H), 3.68–3.58 (m, 1H), 3.24–3.14 (m, 1H), 3.14–3.05 (m, 1H), 1.92–1.81 (m, 4H), 1.77–1.71 (m, 1H), 1.67–1.59 (m, 3H), 1.44 (s, 9H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 167.4, 156.2, 138.4, 135.9, 135.7, 132.3, 130.2, 128.6, 127.6, 119.8, 105.2, 79.2, 64.1, 45.8, 37.3, 29.3, 28.6, 27.5, 25.9, 19.9. **MS** (ES^+) *m/z* calcd for $C_{22}H_{30}Cl_2N_2NaO_5^+$ $[M+Na]^+$, 495.1; found: 495.1.

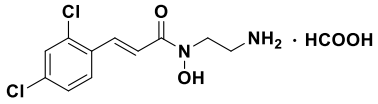
***Tert*-butyl (*E*)-(4-(3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamido)butyl) carbamate (S26)**. Synthesized by method C1 using **S23** (271 mg, 0.94 mmol, 1.1 equiv.), *trans*-2,4-



dichlorocinnamic acid (185 mg, 0.85 mmol, 1.0 equiv.), HATU (358 mg, 0.94 mmol, 1.1 equiv.), *i*Pr_2NEt (444 µL, 2.55 mmol, 3.0 equiv.) and anh. DMF (3.0 mL). The reaction mixture was worked up using EtOAc as outlined in method C1 and the crude residue purified by NP ACC to afford the titled compound (389 mg, 0.80 mmol, 94%) as a colorless oil. **TLC** (50% EtOAc–hexanes): R_f = 0.6. **^{1}H NMR** (600 MHz,

$CDCl_3$) δ 7.98 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.5 Hz, 1H), 7.43 (d, *J* = 2.1 Hz, 1H), 7.25 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.02 (d, *J* = 15.8 Hz, 1H), 4.92 (dd, *J* = 5.3, 2.8 Hz, 1H), 4.59 (br.s, 1H), 4.02–3.90 (m, 2H), 3.80–3.71 (m, 1H), 3.64–3.58 (m, 1H), 3.15 (t, *J* = 7.1 Hz, 2H), 1.91–1.79 (m, 2H), 1.78–1.69 (m, 3H), 1.67–1.58 (m, 3H), 1.52 (quint, *J* = 7.4 Hz, 2H), 1.43 (s, 9H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 166.9, 156.1, 138.0, 135.8, 135.6, 132.4, 130.1, 128.6, 127.6, 120.2, 105.0, 79.3, 64.1, 48.2, 40.4, 29.3, 28.6, 27.4, 25.0, 24.5, 19.9. **MS** (ES^+) *m/z* calcd for $C_{23}H_{32}Cl_2N_2NaO_5^+$ $[M+Na]^+$, 509.2; found: 509.2.

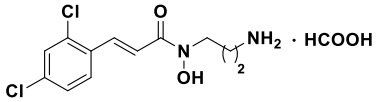
(*E*)-2-(3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamido)ethan-1-aminium formate (S27). Synthesized



by method E using **S24** (325 mg, 0.71 mmol), TFA (1.5 mL), CH_2Cl_2 (8.0 mL) and water (0.5 mL). The titled compound (163 mg, 0.51 mmol, 72%) was collected as a pink fluffy solid*. **^{1}H NMR** (600 MHz, MeOD-d_4) δ 7.99 (d, *J* = 15.9 Hz, 1H), 7.80 (d, *J* = 8.5 Hz, 1H), 7.55 (d, *J* = 2.1 Hz, 1H), 7.39 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.34 (d, *J* = 15.9 Hz, 1H), 4.04 (t, *J* = 5.8 Hz, 2H), 3.26 (t, *J* = 5.8 Hz, 2H). **^{13}C NMR** (151 MHz, MeOD-d_4) δ 168.8, 138.5, 137.3, 136.4, 133.2, 130.8, 130.0, 129.0, 120.7, 47.5, 38.6. **MS** (ES^+) *m/z* calcd for $C_{11}H_{13}Cl_2N_2O_2^+$ $[M+H]^+$, 275.0; found: 275.0.

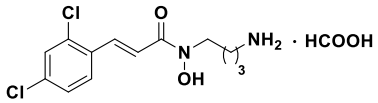
*Impurity arising from **S24** present at ~10%, but compound taken forward without further purification.

(*E*)-3-(3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamido)propan-1-aminium formate (S28). Synthesized

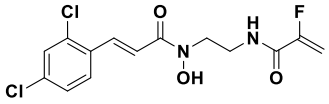


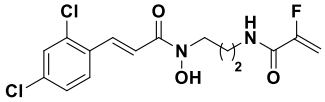
by method E using **S25** (246 mg, 0.47 mmol), TFA (1.5 mL), CH_2Cl_2 (8.0 mL) and water (0.5 mL). The titled compound (156 mg, 0.47 mmol, 89%) was collected as a pale-yellow fluffy hygroscopic solid. **^{1}H NMR** (600 MHz, MeOD-d_4) δ 7.98 (d, *J* = 15.9 Hz, 1H), 7.79 (d, *J* = 8.5 Hz, 1H), 7.55 (d, *J* = 2.1 Hz, 1H), 7.39 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.35 (d, *J* = 15.9 Hz, 1H), 3.87 (t, *J* = 6.4 Hz, 2H), 2.99 (t, *J* = 7.3 Hz, 2H), 2.06 (quint, *J* = 7.0 Hz, 2H). **^{13}C NMR** (151 MHz, MeOD-d_4) δ 168.2, 138.4, 137.2, 136.4, 133.2, 130.8, 130.0, 129.0, 120.6, 46.6, 38.2, 26.1. **MS** (ES^+) *m/z* calcd for $C_{12}H_{15}Cl_2N_2O_2^+$ $[M+H]^+$, 289.1; found: 289.0.

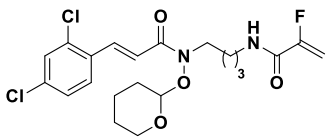
(*E*)-4-(3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamido)butan-1-aminium formate (S29). Synthesized



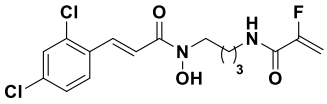
by method E using **S26** (373 mg, 0.77 mmol), TFA (1.5 mL), CH_2Cl_2 (8.0 mL) and water (0.5 mL). The titled compound (183 mg, 0.52 mmol, 68%) was collected as a yellow fluffy hygroscopic solid. **^{1}H NMR** (600 MHz, MeOD-d_4) δ 7.95 (d, *J* = 15.9 Hz, 1H), 7.78 (d, *J* = 8.5 Hz, 1H), 7.55 (d, *J* = 2.1 Hz, 1H), 7.42–7.31 (m, 2H), 3.80 (t, *J* = 6.6 Hz, 2H), 3.06–2.92 (m, 2H), 1.80 (quint, *J* = 6.9 Hz, 2H), 1.73–1.67 (m, 2H). **^{13}C NMR** (151 MHz, MeOD-d_4) δ 167.7, 138.1, 137.1, 136.4, 133.3, 130.8, 129.9, 129.0, 121.0, 48.5 (overlapped with solvent peak), 40.3, 25.6, 24.7. **MS** (ES^+) *m/z* calcd for $C_{13}H_{17}Cl_2N_2O_2^+$ $[M+H]^+$, 303.1; found: 303.0.

(*E*)-3-(2,4-dichlorophenyl)-*N*-(2-(2-fluoroacrylamido)ethyl)-*N*-hydroxyacrylamide (7). Synthesized  by method C1 using 2-fluoroacrylic acid, sodium salt (7 mg, 0.06 mmol, 1.0 equiv.), **S27** (18 mg, 0.06 mmol, 1.0 equiv.), HATU (23 mg, 0.06 mmol, 1.0 equiv.), *i*Pr_2NEt (33 µL, 0.19 mmol, 3.0 equiv.) and anh. DMF (2.0 mL). DMF was removed *in vacuo* and the crude oil purified by RP ACC (gradient: 10→50% hold MeCN–H_2O in 0.1% formic acid). Appropriate fractions were pooled, extracted with CH_2Cl_2, dried (Na_2SO_4) and reduced *in vacuo* to give an off-white solid (6 mg). The product was further purified by NP ACC to afford the titled compound (3 mg, 8.60 µmol, 14%) as an off-white solid. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 10.14 (br.s, 1H), 8.56 (t, *J* = 5.9 Hz, 1H), 7.90 (d, *J* = 8.5 Hz, 1H), 7.76–7.71 (m, 2H), 7.48 (dd, *J* = 8.2, 2.1 Hz, 1H), 7.30 (d, *J* = 15.9 Hz, 1H), 5.51 (d, *J* = 46.9 Hz, 1H), 5.25 (d, *J* = 15.5 Hz, 1H), 3.75 (br.s, 2H), 3.41 (q, *J* = 6.4 Hz, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 165.0, 159.2 (d, *J* 30.2 Hz), 156.3 (d, *J* 267.9 Hz), 135.1, 134.7, 134.3, 131.7, 129.4, 129.2, 128.0, 121.1, 98.6 (d, *J* 14.9 Hz), 47.0, 36.1. **HPLC**, t_R 6.01 min (>98%, UV_{254}). **HMRS** (ES^+) *m/z* calcd for $C_{14}H_{14}Cl_2FN_2O_3^+$ $[M+H]^+$, 347.0360; found: 347.0377.

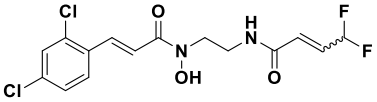
(*E*)-3-(2,4-dichlorophenyl)-*N*-(3-(2-fluoroacrylamido)propyl)-*N*-hydroxyacrylamide (8). Synthesized  by method C1 using 2-fluoroacrylic acid, sodium salt (19 mg, 0.17 mmol, 1.0 equiv.), **S28** (57 mg, 0.17 mmol, 1.0 equiv.), HATU (64 mg, 0.17 mmol, 1.0 equiv.), *i*Pr_2NEt (89 µL, 0.51 mmol, 3.0 equiv.) and DMF (3.0 mL). The reaction mixture was worked up using EtOAc as outlined in method C1 the crude residue purified by RP ACC (gradient: 10→50% MeCN–H_2O in 0.1% formic acid) to afford the titled compound (19 mg, 0.05 mmol, 29%) as a colorless fluffy solid after lyophilization. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 10.14 (br.s, 1H), 8.50 (br.t, *J* = 5.7 Hz, 1H), 7.89 (d, *J* = 8.5 Hz, 1H), 7.74 (d, *J* = 15.8 Hz, 1H), 7.71 (d, *J* = 2.2 Hz, 1H), 7.47 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.30 (d, *J* = 15.9 Hz, 1H), 5.51 (dd, *J* = 48.1, 3.4 Hz, 1H), 5.24 (dd, *J* = 15.7, 3.4 Hz, 1H), 3.69–3.58 (m, 2H), 3.20 (q, *J* = 6.7 Hz, 2H), 1.79 (quint, *J* = 7.1 Hz, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 164.6, 158.8 (d, *J* = 31.7 Hz), 156.5 (d, *J* = 269.3 Hz), 135.0, 134.7, 134.2, 131.8, 129.4, 129.2, 128.0, 121.1, 98.3 (d, *J* = 14.8 Hz), 45.8, 36.5, 26.3. **HPLC**, t_R 6.08 min (>98%, UV_{254}). **HMRS** (ES^+) *m/z* calcd for $[C_{15}H_{16}Cl_2FN_2O_3]^+$: 361.0517; found: 361.0522.

(*E*)-3-(2,4-dichlorophenyl)-*N*-(4-(2-fluoroacrylamido)butyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S30). Synthesized by method C1 using 2-fluoroacrylic acid, sodium salt (14 mg,  0.13 mmol, 1.1 equiv.), **S13** (44 mg, 0.11 mmol, 1.0 equiv.), HATU (48 mg, 0.13 mmol, 1.1 equiv.), *i*Pr_2NEt (60 µL, 0.34 mmol, 3.0 equiv.) and DMF (3.0 mL). Upon completion, the reaction mixture was diluted with EtOAc:hexanes (9:1, v/v) and washed with 5% (aq., w/v) citric acid (2×), sat. $NaHCO_3$ (2×) and brine, dried (Na_2SO_4) and concentrated *in vacuo*. The crude residue was purified by NP to afford the titled

compound (14 mg, 0.03 mmol, 27%) as a colorless semi-solid. **TLC** (50% EtOAc–hexanes): R_f = 0.3. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.98 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.43 (d, *J* = 2.1 Hz, 1H), 7.27–7.24 (m, 1H, overlapped with solvent peak), 7.03 (d, *J* = 15.8 Hz, 1H), 6.47 (s, 1H), 5.66 (dd, *J* = 47.8, 3.2 Hz, 1H), 5.09 (dd, *J* = 15.4, 3.2 Hz, 1H), 4.92 (dd, *J* = 5.4, 2.8 Hz, 1H), 4.00–3.93 (m, 2H), 3.80 (dt, *J* = 14.1, 6.8 Hz, 1H), 3.65–3.58 (m, 1H), 3.40 (q, *J* = 6.8 Hz, 2H), 1.91–1.58 (m, 10H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 167.0, 159.8 (d, *J* = 30.5 Hz), 156.6 (d, *J* = 270.3 Hz), 138.2, 135.9, 135.7, 132.3, 130.2, 128.6, 127.6, 120.0, 105.2, 98.8 (d, *J* = 15.0 Hz), 64.2, 48.0, 39.1, 29.3, 26.6, 25.0, 24.6, 19.9. **MS** (ES^+) *m/z* calcd for $C_{21}H_{25}Cl_2FN_2NaO_4^+$ $[M+Na]^+$, 481.1; found: 481.1.

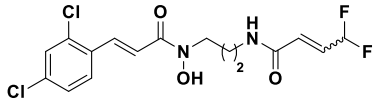
(*E*)-3-(2,4-dichlorophenyl)-*N*-(4-(2-fluoroacrylamido)butyl)-*N*-hydroxyacrylamide (9). Synthesized  by method F using **S30** (14 mg, 0.03 mmol, 1.0 equiv.), PPTS (6 mg, 0.02 mmol, 0.8 equiv.) and abs. EtOH (3.0 mL). The reaction mixture was concentrated *in vacuo* and purified by RP ACC (gradient: 10→55% MeCN–H_2O in 0.1% formic acid) to afford the titled compound (6 mg, 0.02 mmol, 53%) as a colorless fluffy material after lyophilization. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 10.21 (br.s, 1H), 8.52 (t, *J* = 5.8 Hz, 1H), 7.88 (d, *J* = 8.5 Hz, 1H), 7.76–7.68 (m, 2H), 7.47 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.31 (d, *J* = 15.8 Hz, 1H), 5.50 (dd, *J* = 48.1, 3.4 Hz, 1H), 5.22 (dd, *J* = 15.7, 3.3 Hz, 1H), 3.63 (t, *J* = 6.4 Hz, 2H), 3.21–3.12 (m, 2H), 1.62–1.53 (m, 2H), 1.51–1.44 (m, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 164.4, 158.8 (d, *J* = 31.8 Hz), 156.6 (d, *J* = 269.3 Hz), 134.8, 134.6, 134.2, 131.8, 129.4, 129.2, 128.0, 121.3, 98.2 (d, *J* = 14.9 Hz), 47.3, 38.4, 26.1, 23.8. **HPLC**, t_R 6.09 min (>98%, UV_{254}). **HMRS** (ES^+) *m/z* calcd for $C_{16}H_{18}Cl_2FN_2O_3^+$ $[M+H]^+$, 375.0674; found: 375.0692.

4.4.3 4,4-difluoro-2-butenamides

***N*-(2-((*E*)-3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamido)ethyl)-4,4-difluoro-2-butenamide (10)**.  Synthesized by method C1 using 4,4-difluoro-2-butenoic acid (7 mg, 0.06 mmol, 1.0 equiv.), **S27** (18 mg, 0.06 mmol, 1.0 equiv.), HATU (22 mg, 0.06 mmol, 1.0 equiv.), *i*Pr_2NEt (30 µL, 0.17 mmol, 3.0 equiv.) and DMF (2.0 mL). DMF was removed *in vacuo* and the crude product purified by RP ACC (gradient: 10–50% hold MeCN–H_2O in 0.1% formic acid). Appropriate fractions were pooled, extracted with CH_2Cl_2, dried (Na_2SO_4) and reduced *in vacuo* to afford the titled compound (7 mg, 0.02 mmol, 30%) as an off-white solid*. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 9.47 (br.s, 1H), 7.90 (d, *J* = 15.8 Hz, 1H), 7.62 (d, *J* = 8.5 Hz, 1H), 7.41 (d, *J* = 2.2 Hz, 1H), 7.28–7.20 (m, 2H, overlapped with solvent peak), 6.71–6.61 (m, 1H), 6.28 (d, *J* = 16.3 Hz, 1H), 6.08 (td, *J* = 55.1, 3.9 Hz, 2H), 3.90 (br.s, 2H), 3.70 (br.s, 2H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 167.5, 167.0 (d, *J* 13.8 Hz), 139.7, 136.2, 135.6, 134.4 (t, *J* 23.8 Hz), 131.8, 130.0, 128.6, 128.3, 127.7, 119.2, 112.5, (t, *J* 237.2 Hz), 46.7, 36.7. **HPLC**, t_R 6.18 min (>98%, UV_{280}). **HMRS** (ES^+) *m/z* calcd for $C_{15}H_{15}Cl_2F_2N_2O_3^+$ $[M+H]^+$, 379.0422; found: 379.0440.

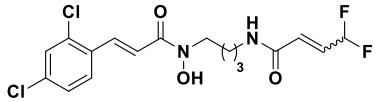
*Residual DMF and grease present from high vacuum line.

***N*-(3-((*E*)-3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamido)propyl)-4,4-difluoro-2-butenamide (11)**.



Synthesized by method C1 using 4,4-difluoro-2-butenoic acid (11 mg, 0.09 mmol, 1.0 equiv.), **S28** (30 mg, 0.09 mmol, 1.0 equiv.), HATU (34 mg, 0.09 mmol, 1.0 equiv.), *i*Pr_2NEt (31 µL, 0.18 mmol, 2.0 equiv.) and DMF (3.0 mL). The reaction mixture was worked up in the same manner as outlined in the synthesis of 8. The crude residue was purified by RP ACC (gradient: 10→55% MeCN–H_2O in 0.1% formic acid) to afford the titled compound (9 mg, 0.02 mmol, 22%) as a colorless fluffy material after lyophilization. **^{1}H NMR** (600 MHz, MeOD-d_4) δ 7.94 (d, *J* = 15.9 Hz, 1H), 7.78 (d, *J* = 8.5 Hz, 1H), 7.54 (d, *J* = 2.2 Hz, 1H), 7.41–7.29 (m, 2H), 6.72–6.61 (m, 1H), 6.47–6.42 (m, 1H), 6.38–6.26 (m, 1H), 3.79 (t, *J* = 6.8 Hz, 2H), 3.34 (t, *J* = 6.9 Hz, 2H), 1.93 (quint, *J* = 6.9 Hz, 2H). **^{13}C NMR** (151 MHz, MeOD-d_4) δ 167.7, 166.2, 138.0, 137.0, 136.4, 134.1 (t, *J* = 24.2 Hz), 133.4, 130.84 (t, *J* = 10.1 Hz), 130.78, 129.9, 128.9, 121.1, 114.8 (t, *J* = 234.4 Hz), 47.2, 38.1, 27.5. **HPLC**, t_R 6.16 min (>98%, UV_{254}). **HMRS** (ES^+) *m/z* calcd for $C_{16}H_{17}Cl_2F_2N_2O_3^+$ $[M+H]^+$, 393.0579; found: 393.0593.

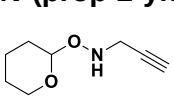
***N*-(4-((*E*)-3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamido)butyl)-4,4-difluoro2-2butenamide (12)**.



Synthesized by method C1 using 4,4-difluoro-2-butenoic acid (13 mg, 0.11 mmol, 1.0 equiv.), **S29** (37 mg, 0.11 mmol, 1.0 equiv.), HATU (41 mg, 0.11 mmol, 1.0 equiv.), *i*Pr_2NEt (111 µL, 0.64 mmol, 6.0 equiv.) and DMF (2.0 mL). The crude product was purified using the same protocol as outlined in the synthesis of 11 and the titled compound (19 mg, 0.05 mmol, 45%) afforded as a colorless fluffy material. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 10.14 (br.s, 1H), 8.41 (br.t, *J* = 5.7 Hz, 1H), 7.88 (d, *J* = 8.5 Hz, 1H), 7.77–7.69 (m, 2H), 7.47 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.31 (d, *J* = 15.8 Hz, 1H), 6.73–6.51 (m, 2H), 6.49–6.43 (m, 1H), 3.63 (t, *J* = 6.9 Hz, 2H), 3.17 (td, *J* = 7.0, 5.6 Hz, 2H), 1.60 (quint, *J* = 7.0 Hz, 2H), 1.51–1.39 (m, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 164.4, 162.7, 134.8, 134.6, 134.2, 131.8, 131.1 (t, *J* = 23.6 Hz), 130.9 (t, *J* = 10.5 Hz), 129.4, 129.2, 128.0, 121.2, 113.9 (t, *J* = 233.5 Hz), 47.2, 38.5, 26.1, 23.8. **HPLC**, t_R 6.22 min (>98%, UV_{280}). **HMRS** (ES^+) *m/z* calcd for $C_{17}H_{19}Cl_2F_2N_2O_3^+$ $[M+H]^+$, 407.0735; found: 407.0754.

4.4.4 Alkynes

***N*-(prop-2-yn-1-yl)-*O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (S31)**. Synthesized by method A3



using OTX (1.97 g, 16.8 mmol, 2.0 equiv.), propargyl bromide (80% solution in toluene; 899 µL, 8.41 mmol, 1.0 equiv.), K_2CO_3 (2.32 g, 16.8 mmol, 2.0 equiv.) and DMF (10 mL). The crude oil was purified using NP ACC to afford the titled compound (549 mg, 3.54 mmol, 42%) as a colorless oil*. **TLC** (20% EtOAc–hexanes): R_f = 0.3. **^{1}H NMR** (500 MHz, $CDCl_3$): δ 5.03 (br.s, 1H), 4.87 (dd, *J* = 5.1, 3.0 Hz, 1H), 3.98–3.89 (m, 1H), 3.76 (dd, *J* = 16.7, 2.4 Hz, 1H), 3.71 (dd, *J* = 16.6, 2.5 Hz,

1H), 3.61–3.51 (m, 1H), 2.24 (t, *J* = 2.5 Hz, 1H), 1.83–1.67 (m, 2H), 1.61–1.47 (m, 4H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 101.8, 80.0, 72.1, 63.2, 41.8, 29.1, 25.4, 20.1. **MS** (ES^+) *m/z* calcd for $C_8H_{14}NO_2^+$ $[M+H]^+$, 156.1; found: 156.1.

*Product is volatile – rotary evaporator cooled to 10 °C and high vacuum avoided.

***N*-(but-3-yn-1-yl)-*O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (S32)**. Synthesized by method A3 using OTX (1.76 g, 15.04 mmol, 2.0 equiv.), 4-bromo-1-butyne (706 µL, 7.52 mmol, 1.0 equiv.), K_2CO_3 (2.08 g, 15.0 mmol, 2.0 equiv.) and DMF (10 mL). The crude oil was purified by RP ACC (gradient: 20→100% MeCN–H_2O in 0.1% formic acid). Appropriate fractions were pooled and extracted with CH_2Cl_2, dried (Na_2SO_4) and reduced *in vacuo* to afford the titled compound (132 mg, 0.78 mmol, 10%) as a colorless oil. **TLC** (20% EtOAc–hexanes): R_f = 0.2. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.23 (br.s, 1H), 4.78–4.70 (m, 1H), 3.90–3.82 (m, 1H), 3.54–3.47 (m, 1H), 3.06 (t, *J* = 6.6 Hz, 2H), 2.49–2.36 (m, 2H), 1.96 (t, *J* = 2.6 Hz, 1H), 1.76–1.68 (m, 1H), 1.68–1.60 (m, 1H), 1.55–1.42 (m, 4H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 101.4, 81.9, 69.8, 63.0, 50.6, 29.1, 25.3, 20.1, 17.3. **MS** (ES^+) *m/z* calcd for $C_9H_{16}NO_2^+$ [M+H], 170.1; found: 170.1.

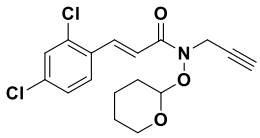
***N*-(pent-4-yn-1-yl)-*O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (S33)**. Synthesized by method A3 using OTX (1.59 g, 13.6 mmol, 2.0 equiv.), 5-bromo-1-pentyne (1.00 g, 6.80 mmol, 1.0 equiv.), K_2CO_3 (1.88 g, 13.6 mmol, 2.0 equiv.) and DMF (10 mL). The crude oil was purified using RP ACC (gradient: 10→100% MeCN–H_2O in 0.1% formic acid). Appropriate fractions were pooled and extracted with CH_2Cl_2, dried (Na_2SO_4) and reduced *in vacuo* to afford the titled compound (190 mg, 1.04 mmol, 15%) as a colorless oil.* **TLC** (20% EtOAc–hexanes): R_f = 0.2. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.73–4.70 (m, 1H), 3.89–3.83 (m, 1H), 3.53–3.48 (m, 1H), 3.08–2.95 (m, 2H), 2.23 (td, *J* = 7.1, 2.7 Hz, 2H), 1.91 (t, *J* = 2.7 Hz, 1H), 1.73–1.69 (m, 2H), 1.68–1.55 (m, 2H), 1.54–1.42 (m, 4H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 101.5, 83.9, 68.7, 63.1, 50.9, 29.2, 26.2, 25.3, 20.2, 16.2; **MS** (ES^+) *m/z* calcd for $C_{10}H_{18}NO_2^+$ $[M+H]^+$, 184.1; found: 184.1.

*Impurity arising from reagent at ~25%, but compound taken forward without further purification.

***N*-(hex-5-yn-1-yl)-*O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (S34)**. Synthesized by method A3* using OTX (330 mg, 2.82 mmol, 1.5 equiv.), 6-iodo-1-heptyne (247 µL, 1.88 mmol, 1.0 equiv.), K_2CO_3 (390 mg, 2.82 mmol, 1.5 equiv.) and DMF (5.0 mL). The crude oil was purified using NP ACC to afford the titled compound (206 mg, 1.04 mmol, 56%) as a colorless oil. **TLC** (50% EtOAc–hexanes): R_f = 0.5. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 4.82–4.78 (m, 1H), 3.95–3.89 (m, 1H), 3.59–3.54 (m, 1H), 3.06–2.94 (m, 2H), 2.21 (td, *J* = 6.9, 2.7 Hz, 2H), 1.94 (t, *J* = 2.7 Hz, 1H), 1.82–1.48 (m, 10H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 101.6, 84.3, 68.6, 63.3, 51.7, 29.3, 26.5, 26.2, 25.5, 20.3, 18.4. **MS** (ES^+) *m/z* calcd for $C_{11}H_{20}NO_2^+$ $[M+H]^+$, 198.1; found: 198.1

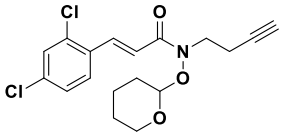
*Stirred at 55 °C overnight and using 6-iodo-1-heptyne as the bromo-alkyne is not commercially available.

(*E*)-3-(2,4-dichlorophenyl)-*N*-(prop-2-yn-1-yl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S35).



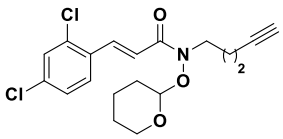
Synthesized by method C1 using *trans*-2,4-dichlorocinnamic acid (50 mg, 0.23 mmol, 1.0 equiv.), **S31** (43 mg, 0.28 mmol, 1.2 equiv.), HATU (106 mg, 0.28 mmol, 1.2 equiv.), *i*Pr_2NEt (48 µL, 0.28 mmol, 1.2 equiv.) and DMF (2.0 mL). DMF was removed *in vacuo* and the crude oil purified by NP ACC to afford the titled compound (67 mg, 0.19 mmol, 82%) as a colorless semi-solid. **TLC** (20% EtOAc–hexanes): R_f = 0.4. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.04 (d, *J* = 15.8 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 1H), 7.44 (d, *J* = 2.1 Hz, 1H), 7.27–7.24 (m, 1H, overlapped with solvent peak), 7.06 (d, *J* = 15.8 Hz, 1H), 5.09–5.05 (m, 1H), 4.87 (dd, *J* = 17.7, 2.5 Hz, 1H), 4.38 (dd, *J* = 17.7, 2.4 Hz, 1H), 4.13–4.05 (m, 1H), 3.69–3.62 (m, 1H), 2.25 (t, *J* = 2.4 Hz, 1H), 1.96–1.77 (m, 3H), 1.70–1.59 (m, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 167.8, 139.1, 136.1, 135.8, 132.2, 130.2, 128.7, 127.6, 119.6, 105.5, 78.2, 71.8, 64.0, 38.6, 29.2, 25.0, 19.7. **MS** (ES^+) *m/z* calcd for $C_{17}H_{17}Cl_2NNaO_3^+$ $[M+Na]^+$, 376.0; found: 376.0.

(*E*)-*N*-(but-3-yn-1-yl)-3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S36).



Synthesized by method C1 using *trans*-2,4-dichlorocinnamic acid (26 mg, 0.12 mmol, 1.0 equiv.), **S32** (22 mg, 0.13 mmol, 1.1 equiv.), HATU (49 mg, 0.13 mmol, 1.1 equiv.), *i*Pr_2NEt (23 µL, 0.13 mmol, 1.1 equiv.) and DMF (2.0 mL). DMF was removed *in vacuo* and the crude oil purified by NP ACC to afford the titled compound (27 mg, 0.07 mmol, 61%) as a colorless oil. **TLC** (20% EtOAc–hexanes): R_f = 0.4. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.00 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.43 (d, *J* = 2.1 Hz, 1H), 7.26–7.24 (m, 1H), 7.02 (d, *J* = 15.8 Hz, 1H), 4.97–4.93 (m, 1H), 4.15–4.08 (m, 1H), 4.04–3.98 (m, 1H), 3.95–3.87 (m, 1H), 3.66–3.60 (m, 1H), 2.64–2.59 (m, 2H), 2.00 (t, *J* = 2.6 Hz, 1H), 1.91–1.80 (m, 2H), 1.79–1.71 (m, 1H), 1.67–1.59 (m, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 167.1, 138.4, 135.9, 135.7, 132.3, 130.2, 128.6, 127.6, 120.0, 105.2, 81.3, 70.0, 64.1, 38.7, 29.2, 25.0, 19.9, 17.1. **MS** (ES^+) *m/z* calcd for $C_{18}H_{20}Cl_2NO_3^+$ $[M+H]^+$, 368.1; found: 368.1.

(*E*)-3-(2,4-dichlorophenyl)-*N*-(pent-4-yn-1-yl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S37).

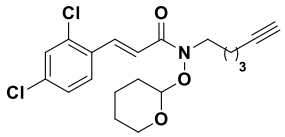


Synthesized by method C1 using *trans*-2,4-dichlorocinnamic acid (50 mg, 0.23 mmol, 1.0 equiv.), **S33** (46 mg, 0.25 mmol, 1.1 equiv.), HATU (96 mg, 0.25 mmol, 1.1 equiv.), *i*Pr_2NEt (44 µL, 0.25 mmol, 1.1 equiv.) and DMF (2.0 mL). DMF was removed *in vacuo* and the crude oil purified by NP ACC to afford the titled compound (61 mg, 0.16 mmol, 69%) as a colorless oil*. **TLC** (20% EtOAc–hexanes): R_f = 0.5. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.98 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.43 (d, *J* = 2.1 Hz, 1H), 7.25 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.03 (d, *J* = 15.8 Hz, 1H), 4.98–4.91 (m, 1H), 4.05–3.95 (m, 2H), 3.86 (ddd, *J* = 14.2, 7.7, 6.2 Hz,

1H), 3.66–3.58 (m, 1H), 2.27 (td, *J* = 7.1, 2.7 Hz, 2H), 2.00–1.91 (m, 3H), 1.89–1.79 (m, 2H), 1.78–1.71 (m, 1H), 1.68–1.59 (m, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 166.9, 138.0, 135.8, 135.6, 132.4, 130.1, 128.6, 127.5, 120.2, 104.9, 83.7, 69.0, 64.0, 47.8, 29.2, 26.1, 25.0, 19.8, 16.2. **MS** (ES^+) *m/z* calcd for $C_{19}H_{22}Cl_2NO_3^+$ $[M+H]^+$, 382.1; found: 382.1.

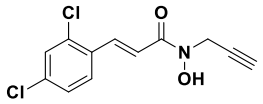
*Trace impurity arising from starting material, but compound taken forward without further purification.

(*E*)-3-(2,4-dichlorophenyl)-*N*-(hex-5-yn-1-yl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S38).



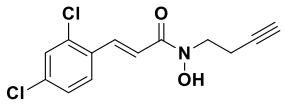
Synthesized by method C1 using *trans*-2,4-dichlorocinnamic acid (70 mg, 0.32 mmol, 1.0 equiv.), **S34** (70 mg, 0.35 mmol, 1.1 equiv.), HATU (135 mg, 0.35 mmol, 1.1 equiv.), *i*Pr_2NEt (112 µL, 0.64 mmol, 2.0 equiv.) and DMF (2.0 mL). The reaction mixture was worked up using EtOAc as outlined in method C1 and the crude residue purified by NP ACC to afford the titled compound (113 mg, 0.29 mmol, 89%) as a colorless oil. **TLC** (50% EtOAc–hexanes): R_f = 0.6. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.98 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.5 Hz, 1H), 7.43 (d, *J* = 2.1 Hz, 1H), 7.25 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.03 (d, *J* = 15.8 Hz, 1H), 4.98–4.91 (m, 1H), 4.02–3.94 (m, 2H), 3.80–3.74 (m, 1H), 3.65–3.59 (m, 1H), 2.24 (td, *J* = 7.0, 2.7 Hz, 2H), 1.94 (t, *J* = 2.7 Hz, 1H), 1.91–1.80 (m, 4H), 1.78–1.71 (m, 1H), 1-68–1.54 (m, 5H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 166.8, 138.0, 135.8, 135.6, 132.4, 130.1, 128.6, 127.5, 120.2, 105.0, 84.2, 68.7, 64.0, 48.1, 29.3, 26.3, 25.8, 25.0, 19.9, 18.3. **MS** (ES^+) *m/z* calcd for $C_{20}H_{23}Cl_2NNaO_3^+$ $[M+Na]^+$, 418.1; found: 418.1.

(*E*)-3-(2,4-dichlorophenyl)-*N*-hydroxy-*N*-(prop-2-yn-1-yl)acrylamide (13). Synthesized by method F



using **S35** (51 mg, 0.14 mmol, 1.0 equiv.), PPTS (7 mg, 0.03 mmol, 0.2 equiv.) and abs. EtOH (2.0 mL). The reaction mixture was concentrated *in vacuo* and purified by NP ACC to afford the titled compound (24 mg, 0.09 mmol, 62%) as an off-white solid. **^{1}H NMR** (600 MHz, MeOD-d_4) δ 7.96 (d, *J* = 15.9 Hz, 1H), 7.78 (d, *J* = 8.5 Hz, 1H), 7.55 (d, *J* = 2.1 Hz, 1H), 7.38 (ddd, *J* = 8.5, 2.2, 0.6 Hz, 1H), 7.30 (d, *J* = 15.8 Hz, 1H), 4.50 (d, *J* = 2.4 Hz, 2H), 2.67 (br.t, *J* = 2.5 Hz, 1H). **^{13}C NMR** (151 MHz, MeOD-d_4) δ 167.7, 138.5, 137.2, 136.5, 133.2, 130.8, 130.0, 128.9, 120.8, 78.5, 73.3, 39.2. **HPLC**, t_R 6.15 min (>98%, UV_{280}). **HMRS** (ES^+) *m/z* calcd for $C_{12}H_{10}Cl_2NO_2^+$ $[M+H]^+$, 270.0083; found: 270.0083.

(*E*)-*N*-(but-3-yn-1-yl)-3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamide (14). Synthesized by method F



using **S36** (27 mg, 0.07 mmol, 1.0 equiv.), PPTS (4 mg, 0.02 mmol, 0.2 equiv.) and abs. EtOH (2.0 mL). The reaction mixture was concentrated *in vacuo* and purified by NP ACC to afford the titled compound (18 mg, 0.06 mmol, 86%) as a colorless solid. **^{1}H NMR** (400 MHz, MeOD-d_4) δ 7.94 (d, *J* = 15.9 Hz, 1H), 7.78 (d, *J* = 8.5 Hz, 1H), 7.54 (d, *J* = 2.1 Hz, 1H), 7.38 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.33 (d, *J* = 16.0 Hz, 1H), 3.88 (br.t, *J* = 7.2 Hz, 2H), 2.61–2.52 (m, 2H), 2.30 (t, *J* = 2.7 Hz, 1H). **^{13}C NMR** (151 MHz, MeOD-d_4) δ 167.7, 138.1, 137.1, 136.4, 133.4, 130.8, 129.9, 128.9,

121.0, 81.5, 71.1, 30.8, 17.3. **HPLC**, t_R 6.21 min (>95%, UV_{254}). **HMRS** (ES^+) *m/z* calcd for $C_{13}H_{12}Cl_2NO_2^+$ $[M+H]^+$, 284.0240; found: 284.0250.

(*E*)-3-(2,4-dichlorophenyl)-*N*-hydroxy-*N*-(pent-4-yn-1-yl)acrylamide (15). Synthesized by method F using **S37** (61 mg, 0.16 mmol, 1.0 equiv.), PPTS (8 mg, 0.03 mmol, 0.2 equiv.) and abs. EtOH (2.0 mL). The reaction mixture was concentrated *in vacuo* and purified by NP ACC to afford the titled compound (34 mg, 0.11 mmol, 71%) as an off-white solid. **^{1}H NMR** (400 MHz, MeOD-d_4) δ 7.92 (d, *J* = 15.9 Hz, 1H), 7.76 (d, *J* = 8.5 Hz, 1H), 7.52 (d, *J* = 2.1 Hz, 1H), 7.36 (ddd, *J* = 8.5, 2.1, 0.6 Hz, 1H), 7.32 (d, *J* = 14.5 Hz, 1H), 3.82 (t, *J* = 6.6 Hz, 2H), 2.31–2.22 (m, 3H), 1.90 (quint, *J* = 7.0 Hz, 2H). **^{13}C NMR** (151 MHz, MeOD-d_4) δ 167.6, 137.9, 137.0, 136.3, 133.4, 130.7, 129.9, 128.9, 121.1, 84.0, 70.1, 48.6 (overlapped with solvent peak), 27.0, 16.6. **HPLC**, t_R 6.42 min (>97%, UV_{254}). **HMRS** (ES^+) *m/z* calcd for $C_{14}H_{14}Cl_2NO_2^+$ $[M+H]^+$, 298.0396; found: 298.0396.

(*E*)-3-(2,4-dichlorophenyl)-*N*-hydroxy-*N*-(hex-5-yn-1-yl)acrylamide (16). Synthesized by method F using **S38** (94 mg, 0.24 mmol, 1.0 equiv.), PPTS (32 mg, 0.13 mmol, 0.5 equiv.) and abs. EtOH (5.0 mL). The reaction mixture was concentrated *in vacuo* and purified by RP ACC (gradient: 10→60% MeCN–H_2O in 0.1% formic acid) to afford the titled compound (61 mg, 0.20 mmol, 82%) as a colorless fluffy material after lyophilization. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 10.08 (s, 1H), 7.88 (d, *J* = 8.5 Hz, 1H), 7.73 (d, *J* = 15.8 Hz, 1H), 7.71 (d, *J* = 2.2 Hz, 1H), 7.47 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.31 (d, *J* = 15.9 Hz, 1H), 3.63 (t, *J* = 6.9 Hz, 2H), 2.76 (t, *J* = 2.7 Hz, 1H), 2.19 (td, *J* = 7.1, 2.7 Hz, 2H), 1.71–1.64 (m, 2H), 1.49–1.42 (m, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 164.5, 134.9, 134.6, 134.2, 131.8, 129.4, 129.2, 128.0, 121.2, 84.3, 71.4, 47.0, 25.4, 25.2, 17.4. **HPLC**, t_R 6.60 min (>98%, UV_{254}). **HMRS** (ES^+) *m/z* calcd for $C_{15}H_{16}Cl_2NO_2^+$ $[M+H]^+$, 312.0553; found: 312.0567.

4.4.5 *O*-phenyl carbamates

Phenyl (*E*)-(2-(3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamido)-ethyl) carbamate (S39). Synthesized by method B using **S11** (100 mg, 0.28 mmol, 1.0 equiv.), phenyl chloroformate (52 µL, 0.42 mmol, 1.5 equiv.), pyridine (45 µL, 0.56 mmol, 2.0 equiv.) and anh. CH_2Cl_2 (2.0 mL). The reaction mixture was worked up and purified in the same manner as outlined in the synthesis of **S16**. The titled compound (99 mg, 0.21 mmol, 74%) was collected as a colorless oil*. **TLC** (50% EtOAc–hexanes): R_f = 0.3. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.87 (d, *J* = 15.7 Hz, 1H), 7.43–7.38 (m, 2H), 7.34–7.28 (m, 2H), 7.20–7.16 (m, 2H), 7.13–7.09 (m, 2H), 6.88 (br.t, *J* = 5.5 Hz, 1H), 6.36 (d, *J* = 15.7 Hz, 1H), 5.15 (br.t, *J* = 3.5 Hz, 1H), 4.07 –3.95 (m, 2H), 3.92–3.85 (m, 1H), 3.80–3.65 (m, 3H), 1.86–1.73 (m, 3H), 1.68–1.55 (m, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 165.6, 156.4, 150.8, 135.7, 135.6, 135.3, 131.9, 123.0, 129.5,

128.4, 127.5, 125.9, 124.2, 121.5, 103.9, 63.6, 51.2, 38.1, 28.7, 25.1, 19.3. **MS** (ES^+) *m/z* calcd for $C_{23}H_{24}Cl_2N_2NaO_5^+$ $[M+Na]^+$, 501.1; found: 501.1.

*Impurity arising from starting material at ~20%, but compound taken forward without further purification.

Phenyl (*E*)-(3-(3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamido)propyl) carbamate (S40). Synthesized by method B using **S12** (50 mg, 0.13 mmol, 1.0 equiv.), phenyl chloroformate (34 µL, 0.27 mmol, 2.0 equiv.), pyridine (22 µL, 0.27 mmol, 2.0 equiv.) and anh. CH_2Cl_2 (1.0 mL). The reaction mixture was worked up and purified in the same manner as outlined in the synthesis of **S16**. The titled compound (39 mg, 0.08 mmol, 59%) was collected as a colorless oil. **TLC** (66% EtOAc–hexanes): R_f = 0.3. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.88 (d, *J* = 15.6 Hz, 1H), 7.43 (d, *J* = 8.4 Hz, 1H), 7.39–7.35 (m, 3H), 7.23–7.21 (m, 1H), 7.20–7.17 (m, 1H), 7.15–7.11 (m, 2H), 6.90 (br.t, *J* = 6.2 Hz, 1H), 6.34 (d, *J* = 15.6 Hz, 1H), 5.16–5.13 (m, 1H), 4.06–3.99 (m, 1H), 3.90–3.76 (m, 2H), 3.70–3.65 (m, 1H), 3.60–3.52 (m, 1H), 3.45–3.37 (m, 1H), 2.00 (quint, *J* = 6.2 Hz, 2H), 1.84–1.75 (m, 3H), 1.70–1.56 (m, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 165.3, 156.3, 150.9, 135.6, 135.5, 135.3, 132.0, 130.0, 129.6, 128.3, 127.4, 126.0, 124.4, 121.6, 103.8, 63.3, 48.4, 36.5, 28.7, 26.9, 25.1, 19.1. **MS** (ES^+) *m/z* calcd for $C_{24}H_{26}Cl_2N_2NaO_5^+$ $[M+Na]^+$, 515.1; found: 515.0.

Phenyl (*E*)-(2-(3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamido)ethyl)carbamate (17). Synthesized by method F using **S39** (99 mg, 0.21 mmol, 1.0 equiv.), PPTS (10 mg, 0.04 mmol, 0.2 equiv.) and abs. EtOH (2.0 mL). The reaction mixture was concentrated *in vacuo* and purified by NP ACC to afford the titled compound (47 mg, 0.12 mmol, 57%) as a white solid*. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.96 (br.s, 1H), 7.97 (d, *J* = 15.6 Hz, 1H), 7.39 (d, *J* = 2.1 Hz, 1H), 7.29–7.18 (m, 3H, overlapped with solvent peak), 7.17–7.09 (m, 2H), 7.08–7.03 (m, 2H), 6.87 (br.s, 1H), 6.21 (d, *J* = 15.6 Hz, 1H), 3.83–3.75 (m, 2H), 3.65–3.58 (m, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 168.0, 156.2, 151.0, 137.3, 136.3, 135.6, 131.2, 130.1, 129.4, 128.2, 127.5, 125.8, 122.4, 121.8, 49.4, 36.6. **HPLC**, t_R 6.37 min (>98%, UV_{254}). **HMRS** (ES+) m/z calcd for $C_{18}H_{17}Cl_2N_2O_4^+$ $[M+H]^+$, 395.0560; found 395.0579.

*Trace impurity from previous step, but compound sufficiently pure for biochemical evaluation.

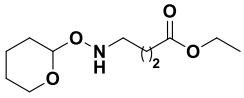
Phenyl (*E*)-(3-(3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamido)propyl)carbamate (18). Synthesized by method F using **S40** (38 mg, 0.08 mmol, 1.0 equiv.), PPTS (2 mg, 0.01 mmol, 0.1 equiv.) and abs. EtOH (1.0 mL). The reaction mixture was concentrated *in vacuo* and purified by NP ACC to afford the titled compound (12 mg, 0.03 mmol, 38%) as a white solid*. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 9.86 (s, 1H), 8.28 (t, *J* = 5.6 Hz, 1H), 7.73–7.68 (m, 2H), 7.65 (d, *J* = 15.7 Hz, 1H), 7.49 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.42–7.36

(m, 2H), 7.25–7.20 (m, 1H), 7.15–7.08 (m, 2H), 6.69 (d, *J* = 15.7 Hz, 1H), 3.59 (t, *J* = 7.0 Hz, 2H), 3.28 (d, *J* = 6.3 Hz, 2H), 1.84 (p, *J* = 7.0 Hz, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 164.3, 154.5, 151.0, 134.4, 134.0, 132.7, 131.8, 129.4, 129.3, 128.8, 128.0, 126.0, 125.3, 121.7, 48.3, 36.4, 26.7. **HPLC**, t_R 6.36 min (>98%, UV_{254}). **HMRS** (ES+) m/z calcd for $C_{19}H_{19}Cl_2N_2O_4^+$ $[M+H]^+$, 409.0716; found 409.0725.

*Trace impurity from starting material, but compound sufficiently pure for biochemical evaluation.

4.4.6 Cyanocyclopropyl

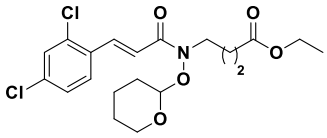
Ethyl 4-(((tetrahydro-2*H*-pyran-2-yl)oxy)amino)butanoate (S41). Synthesized by method A2 using



OTX (500 mg, 4.27 mmol, 1.0 equiv.), ethyl 4-bromobutanoate (832 mg, 4.27 mmol, 1.0 equiv.), Cs_2CO_3 (1.39 g, 4.27 mmol, 1.0 equiv.), KI (708 mg, 4.27 mmol, 1.0 equiv.) and DMF (10 mL). The titled compound (610 mg, 2.64 mmol, 62%) was collected as a colorless oil*. **TLC** (50% EtOAc–hexanes): R_f = 0.3. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 5.76 (br.s, 1H), 4.79–4.71 (m, 1H), 4.10 (q, *J* = 7.2 Hz, 2H), 3.93–3.85 (m, 1H), 3.59–3.50 (m, 1H), 3.05–2.90 (m, 2H), 2.37 (t, *J* = 7.4 Hz, 2H), 1.90–1.64 (m, 4H), 1.59–1.46 (m, 4H), 1.23 (t, *J* = 7.2 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 173.5, 101.6, 63.2, 60.4, 51.3, 32.0, 29.3, 25.4, 23.0, 20.3, 14.3. MS (ES^+) *m/z* calcd for $C_{11}H_{22}NO_4^+$ $[M+H]^+$, 232.2; found: 232.2.

*Trace impurity arising from ethyl 4-bromobutanoate, but compound taken forward without further purification.

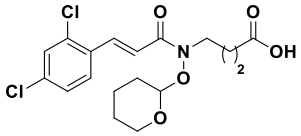
Ethyl (*E*)-4-(3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamido)butanoate (S42).



Synthesized by method B using (*E*)-3-(2,4-dichlorophenyl)acryloyl chloride (768 mg, 3.26 mmol, 1.2 equiv.), **S41** (605 mg, 2.62 mmol, 1.0 equiv.), *i*Pr_2NEt (595 µL, 3.41 mmol, 1.3 equiv.) and anh. CH_2Cl_2 (15 mL). The reaction mixture was worked up and purified in the same manner as outlined in the synthesis of **S6**. The titled compound (984 mg, 2.28 mmol, 87%) was collected as a colorless oil*. **TLC** (50% EtOAc–hexanes): R_f = 0.6. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.97 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.5 Hz, 1H), 7.42 (d, *J* = 2.1 Hz, 1H), 7.24 (ddd, *J* = 8.5, 2.2, 0.7 Hz, 1H), 7.02 (d, *J* = 15.8 Hz, 1H), 4.97–4.88 (m, 1H), 4.12 (q, *J* = 7.2 Hz, 2H), 4.02–3.93 (m, 2H), 3.85–3.78 (m, 1H), 3.64–3.58 (m, 1H), 2.37 (t, *J* = 7.4 Hz, 2H), 2.07–2.00 (m, 2H), 1.88–1.70 (m, 3H), 1.66–1.56 (m, 3H), 1.24 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 173.2, 166.9, 138.0, 135.8, 135.6, 132.3, 130.1, 128.6, 127.5, 120.1, 104.9, 64.0, 60.5, 47.9, 31.6, 29.2, 25.0, 22.6, 19.8, 14.3. MS (ES^+) *m/z* calcd for $C_{20}H_{25}Cl_2NNaO_5^+$ $[M+Na]^+$, 452.1; found: 452.1.

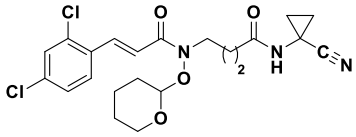
*Impurities present at ~10%, but taken forward without further purification.

(*E*)-4-(3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamido)butanoic acid (S43).



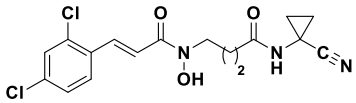
Intermediate **S42** (954 mg, 2.21 mmol) was dissolved in MeOH (8.0 mL) and 2 M $NaOH_{(aq)}$ (2.0 mL) was added and the solution was stirred at r.t overnight. The reaction mixture was acidified with 2 M $HCl_{(aq)}$ and extracted with EtOAc (2×). The combined organic layers were washed with water (2×), brine, dried ($MgSO_4$), and concentrated *in vacuo*. The crude residue was purified by NP ACC to afford the titled compound (498 mg, 1.24 mmol, 56%) as a pale-yellow oil. **TLC** (10% MeOH–CH_2Cl_2): R_f = 0.3. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 7.91 (br.s, 1H), 7.78 (d, *J* = 15.7 Hz, 1H), 7.74 (d, *J* = 2.0 Hz, 1H), 7.53–7.47 (m, 1H), 7.17 (d, *J* = 15.7 Hz, 1H), 5.05–4.99 (m, 1H), 3.96–3.82 (m, 2H), 3.74–3.67 (m, 1H), 3.63–3.55 (m, 1H), 2.23 (br.s, 2H), 1.91–1.67 (m, 5H), 1.63–1.48 (m, 3H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 164.8, 135.9, 134.9, 134.4, 132.9, 131.5, 129.4, 128.1, 121.0, 112.5, 103.6, 75.6, 63.1, 28.4, 24.5, 19.1, 18.8, one C=O not observed. **MS** (ES^-) *m/z* calcd for $C_{18}H_{20}Cl_2NO_5^-$ $[M-H]^-$, 400.1; found: 400.0.

(*E*)-*N*-(1-cyanocyclopropyl)-4-(3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamido)butanamide (S44). Synthesized by method C1 using **S43** (160 mg, 0.41 mmol, 1.0 equiv.), 1-aminocyclopropane-1-carbonitrile, hydrochloride salt (59 mg, 0.50 mmol, 1.2 equiv.), HATU (184 mg, 0.48 mmol, 1.2 equiv.), *i*Pr_2NEt (204 µL, 1.17 mmol, 2.9 equiv.) and DMF (3.0 mL). The reaction mixture was worked up using EtOAc as outlined in method C1 and the crude residue purified by NP ACC to afford the titled compound (122 mg) as a colorless oil.* **TLC** (75% EtOAc–hexanes): R_f = 0.2. **MS** (ES^+) *m/z* calcd for $C_{22}H_{25}Cl_2N_3NaO_4^+$ $[M+Na]^+$, 488.1; found: 488.1.



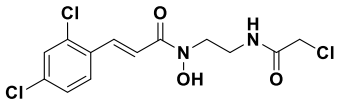
*~35% impurity present – NMR assignment troublesome, taken forward without characterization.

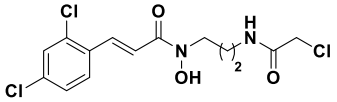
(*E*)-*N*-(1-cyanocyclopropyl)-4-(3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamido)butanamide (19).

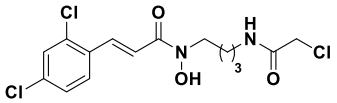


Synthesized by method F using the oil obtained from the synthesis of **S44** (122 mg), PPTS (30 mg, 0.12 mmol) and abs. EtOH (2.0 mL). The reaction mixture was concentrated *in vacuo* and purified by NP ACC to afford the titled compound (16 mg, 0.04 mmol, 11%-over two steps) as a white solid. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 10.05 (br.s, 1H), 8.74 (s, 1H), 7.88 (d, *J* = 8.6 Hz, 1H), 7.76–7.69 (m, 2H), 7.48 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.30 (d, *J* = 15.9 Hz, 1H), 3.67–3.56 (m, 2H), 2.12 (t, *J* = 7.5 Hz, 2H), 1.81 (quint, *J* = 7.2 Hz, 2H), 1.47–1.41 (m, 2H), 1.13–1.08 (m, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 173.0, 164.5, 134.9, 134.7, 134.2, 131.8, 129.4, 129.2, 128.0, 121.2, 120.9, 47.0, e31.9, 22.0, 19.7, 15.6. **HPLC**, t_R 5.99 min (>98%, UV_{254}). **HMRS** (ES^+) *m/z* calcd for $C_{17}H_{18}Cl_2N_3O_3^+$ $[M+H]^+$, 382.0720; found: 382.0724.

4.4.7 Chloroacetyl

(*E*)-*N*-(2-(2-chloroacetamido)ethyl)-3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamide (20). Synthesized  by method B using **S27** (26 mg, 0.08 mmol, 1.0 equiv.), chloroacetic anhydride (14 mg, 0.08 mmol, 1.0 equiv.), *i*Pr_2NEt (42 µL, 0.24 mmol, 3.0 equiv.) and anh. CH_2Cl_2 (2.0 mL). The reaction mixture was reduced *in vacuo* and the crude product purified by RP ACC (gradient: 10→60% MeCN–H_2O in 0.1% formic acid) to afford the titled compound (17 mg, 0.05 mmol, 60%) as a colorless fluffy material after lyophilization. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 10.17 (br.s, 1H), 8.32 (t, *J* = 5.7 Hz, 1H), 7.89 (d, *J* = 8.5 Hz, 1H), 7.74 (d, *J* = 15.9 Hz, 1H), 7.72 (d, *J* = 2.2 Hz, 1H), 7.48 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.31 (d, *J* = 15.9 Hz, 1H), 4.06 (br.s, 2H), 3.71 (t, *J* = 6.8 Hz, 2H), 3.35 (q, *J* = 6.4 Hz, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 166.3, 165.0, 135.1, 134.7, 134.3, 131.7, 129.4, 129.2, 128.0, 121.1, 47.0, 42.5, 36.3. **HPLC**, t_R 5.88 min (>98%, UV_{280}). **HMRS** (ES^+) *m/z* calcd for $C_{13}H_{14}Cl_3N_2O_3^+$ $[M+H]^+$, 351.0065; found: 351.0082.

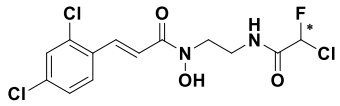
(*E*)-*N*-(3-(2-chloroacetamido)propyl)-3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamide (21).  Synthesized by method B using **S28** (34 mg, 0.10 mmol, 1.0 equiv.), chloroacetic anhydride (17 mg, 0.10 mmol, 1.0 equiv.), *i*Pr_2NEt (51 µL, 0.29 mmol, 3.0 equiv.) and anh. CH_2Cl_2 (2.0 mL). The reaction mixture was reduced *in vacuo* and the crude product purified by RP ACC (gradient: 10→60% MeCN–H_2O in 0.1% formic acid) to afford the titled compound (17 mg, 0.05 mmol, 48%) as a colorless fluffy material after lyophilization. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 10.17 (br.s, 1H), 8.23 (br.t, *J* = 5.7 Hz, 1H), 7.89 (d, *J* = 8.5 Hz, 1H), 7.74 (d, *J* = 15.8 Hz, 1H), 7.72 (d, *J* = 2.2 Hz, 1H), 7.48 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.31 (d, *J* = 15.9 Hz, 1H), 4.05 (s, 2H), 3.64 (t, *J* = 7.0 Hz, 2H), 3.13 (q, *J* = 6.6 Hz, 2H), 1.75 (quint, *J* = 7.1 Hz, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 165.9, 164.6, 135.0, 134.7, 134.2, 131.8, 129.4, 129.2, 128.0, 121.2, 45.7, 42.7, 36.7, 26.4. **HPLC**, t_R 5.95 min (>98%, UV_{280}). **HMRS** (ES^+) *m/z* calcd for $C_{14}H_{16}Cl_3N_2O_3^+$ $[M+H]^+$, 365.0221; found: 365.0238.

(*E*)-*N*-(4-(2-chloroacetamido)butyl)-3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamide (22). Synthesized  by method B using **S29** (38 mg, 0.11 mmol, 1.0 equiv.), chloroacetic anhydride (18 mg, 0.11 mmol, 1.0 equiv.), *i*Pr_2NEt (58 µL, 0.33 mmol, 3.0 equiv.) and anh. CH_2Cl_2 (2.0 mL). The reaction mixture was reduced *in vacuo* and the crude product purified by RP ACC (gradient: 10→60% MeCN–H_2O in 0.1% formic acid) to afford the titled compound (16 mg, 0.04 mmol, 38%) as a colorless fluffy material after lyophilization. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 10.22 (br.s, 1H), 8.23 (t, *J* = 5.7 Hz, 1H), 7.88 (d, *J* = 8.5 Hz, 1H), 7.78–7.69 (m, 2H), 7.48 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.32 (d, *J* = 15.8 Hz, 1H), 4.03 (s, 2H), 3.62 (br.t, *J* = 6.9 Hz, 2H), 3.11 (q, *J* = 7.0 Hz, 2H), 1.58 (quint, *J* = 7.0 Hz, 2H), 1.43 (quint, *J* = 7.0 Hz, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 165.8,

164.4, 134.8, 134.6, 134.2, 131.8, 129.4, 129.2, 128.0, 121.3, 47.2, 42.7, 38.7, 26.1, 23.8. **HPLC**, t_R 5.98 min (>95%, UV_{280}). **HMRS** (ES^+) *m/z* calcd for $[C_{15}H_{28}Cl_3N_2O_3]^+$ $[M+H]^+$, 379.0378; found: 379.0369.

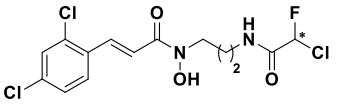
4.4.8 2-chloro-2-fluoroacetyl

(*E*)-*N*-(2-(2-chloro-2-fluoroacetamido)ethyl)-3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamide (23).



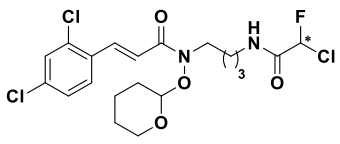
Synthesized by method C1 using 2-chloro-2-fluoroacetic acid (9 mg, 0.07 mmol, 1.0 equiv.), **S27** (22 mg, 0.07 mmol, 1.0 equiv.), HATU (28 mg, 0.07 mmol, 1.0 equiv.), *i*Pr_2NEt (37 µL, 0.21 mmol, 3.0 equiv.) and DMF (2.0 mL). DMF was removed *in vacuo* and the crude residue purified by RP ACC (10–45% hold MeCN–H_2O in 0.1% formic acid). Appropriate fractions were pooled, extracted with CH_2Cl_2, dried (Na_2SO_4) and reduced *in vacuo* to afford the titled compound (6 mg, 0.02 mmol, 24%) as an off-white solid. **^{1}H NMR** (600 MHz, MeOD-d_4) δ 7.94 (d, *J* = 15.9 Hz, 1H), 7.78 (d, *J* = 8.5 Hz, 1H), 7.54 (d, *J* = 2.2 Hz, 1H), 7.38 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.33 (d, *J* = 16.2 Hz, 1H), 6.51 (d, *J* = 49.8 Hz, 1H), 3.98–3.93 (m, 1H), 3.91–3.82 (m, 1H), 3.59 (t, *J* = 6.1 Hz, 2H). **^{13}C NMR** (151 MHz, MeOD-d_4) δ 168.3, 167.2 (d, *J* 24.1), 138.1, 137.1, 136.4, 133.3, 130.8, 130.0, 128.9, 121.0, 95.1 (d, *J* 235.2 Hz), 37.8, 30.8. **HPLC**, t_R 6.06 min (>95%, UV_{254}). **HMRS** (ES^+) *m/z* calcd for $C_{13}H_{13}Cl_{3F}N_2O_3^+$ $[M+H]^+$, 368.9970; found: 368.9984.

(*E*)-*N*-(3-(2-chloro-2-fluoroacetamido)propyl)-3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamide (24).



Synthesized by method C1 using 2-chloro-2-fluoroacetic acid (19 mg, 0.17 mmol, 1.0 equiv.), **S28** (57 mg, 0.17 mmol, 1.0 equiv.), HATU (64 mg, 0.17 mmol, 1.0 equiv.), *i*Pr_2NEt (88 µL, 0.50 mmol, 3.0 equiv.) and DMF (2.0 mL). DMF was removed *in vacuo* and the crude residue purified by RP ACC (gradient: 10→60% MeCN–H_2O in 0.1% formic acid) to afford the titled compound (13 mg, 0.03 mmol, 20%) as a colorless fluffy material after lyophilization. Unreacted starting material could be recovered (24 mg, 39% yield brsm). **^{1}H NMR** (600 MHz, DMSO-d_6) δ 10.24 (br.s, 1H), 8.75–8.61 (m, 1H), 7.89 (d, *J* = 8.5 Hz, 1H), 7.74 (d, *J* = 15.8 Hz, 1H), 7.72 (d, *J* = 2.2 Hz, 1H), 7.48 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.31 (d, *J* = 15.9 Hz, 1H), 6.74 (d, *J* = 49.4 Hz, 1H), 3.73– 3.59 (m, 2H), 3.18 (q, *J* = 6.6 Hz, 2H), 1.79 (quint, *J* = 7.1 Hz, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 164.6, 163.5 (d, *J* = 22.9 Hz), 135.0, 134.7, 134.2, 131.8, 129.4, 129.2, 128.0, 121.2, 94.0 (d, *J* = 252.0 Hz), 45.6, 36.6, 26.2. **HPLC**, t_R 6.11 min (>98%, UV_{254}). **HMRS** (ES^+) *m/z* calcd for $[C_{14}H_{15}Cl_3FN_2O_3]^+$: 383.0127; found: 383.0144.

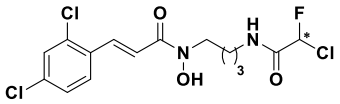
(*E*)-*N*-(4-(2-chloro-2-fluoroacetamido)butyl)-3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S45). Synthesized by method C1 using 2-chloro-2-fluoroacetic acid (16 mg, 0.14 mmol, 1.0 equiv.), **S13** (49 mg, 0.14 mmol, 1.0 equiv.), HATU (53 mg, 0.14 mmol, 1.0 equiv.), *i*Pr_2NEt (73 µL, 0.42 mmol, 3.0 equiv.) and DMF (3.0 mL). The crude product was purified using the same protocol as outlined



in the synthesis of **S30** and the titled compound (13 mg, 0.03 mmol, 24%) collected as a colorless semi-solid*. **TLC** (50% EtOAc–hexanes): R_f = 0.3. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.99 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.43 (d, *J* = 2.1 Hz, 1H), 7.28–7.24 (m, 1H, overlapped with solvent peak), 7.03 (d, *J* = 15.7 Hz, 1H), 6.66 (br.s, 1H), 6.29 (d, *J* = 50.9 Hz, 1H), 4.93 (dd, *J* = 5.4, 2.7 Hz, 1H), 4.01–3.92 (m, 2H), 3.86–3.77 (m, 1H), 3.65–3.58 (m, 1H), 3.45–3.35 (m, 2H), 1.91–1.71 (m, 5H), 1.66–1.59 (m, 5H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 167.1, 164.3 (d, *J* = 21.9 Hz), 138.3, 135.9, 135.7, 132.3, 130.1, 128.6, 127.6, 119.9, 105.2, 94.2 (d, *J* = 256.1 Hz), 64.2, 47.9, 39.4, 29.3, 26.2, 25.0, 24.5, 19.9. **MS** (ES^+) *m/z* calcd for $C_{20}H_{24}Cl_3FN_2NaO_4^+$ $[M+Na]^+$, 503.1; found: 503.0.

*Impurity at 2.80 and 38.8 ppm (^{1}H/^{13}C), but compound taken forward without further purification.

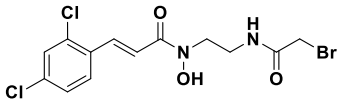
(*E*)-*N*-(4-(2-chloro-2-fluoroacetamido)butyl)-3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamide (25).



Synthesized by method F using **S45** (13 mg, 0.03 mmol, 1.0 equiv.), PPTS (9 mg, 0.03 mmol, 1.3 equiv.) and abs. EtOH (3.0 mL). The reaction mixture was concentrated *in vacuo* and purified by RP (10→55% MeCN–H_2O in 0.1% formic acid) to afford the titled compound (7 mg, 0.02 mmol, 65%) as a colorless fluffy material after lyophilization. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 10.16 (s, 1H), 8.69 (t, *J* = 5.7 Hz, 1H), 7.95–7.83 (m, 1H), 7.78–7.68 (m, 2H), 7.47 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.31 (d, *J* = 15.8 Hz, 1H), 6.71 (d, *J* = 49.4 Hz, 1H), 3.63 (t, *J* = 6.4 Hz, 2H), 3.16 (td, *J* = 7.0, 5.7 Hz, 2H), 1.63–1.54 (m, 2H), 1.52–1.42 (m, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 164.5, 163.5 (d, *J* = 23.0 Hz), 134.9, 134.6, 134.2, 131.8, 129.4, 129.2, 128.0, 121.3, 94.0 (d, *J* = 251.8 Hz), 47.2, 38.5, 25.9, 23.7. **HPLC**, t_R 6.14 min (>98%, UV_{254}). **HMRS** (ES^+) *m/z* calcd for $C_{15}H_{17}Cl_3FN_2O_3^+$ $[M+H]^+$, 397.0283; found: 397.0291.

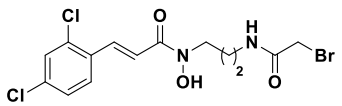
4.4.9 Bromoacetyl

(*E*)-*N*-(2-(2-bromoacetamido)ethyl)-3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamide (26). Synthesized



by method C2 using bromoacetic acid (12 mg, 0.09 mmol, 0.9 equiv.), **S27** (32 mg, 0.10 mmol, 1.0 equiv.), HATU (44 mg, 0.12 mmol, 1.2 equiv.), *i*Pr_2Net (39 µL, 0.23 mmol, 2.3 equiv.) and DMF (2.0 mL). The titled compound (3 mg, 7.57 µmol, 7%) was collected as a colorless fluffy solid. **^{1}H NMR** (600 MHz, MeOD-d_4) δ 7.93 (d, *J* = 15.8 Hz, 1H), 7.78 (d, *J* = 8.5 Hz, 1H), 7.54 (d, *J* = 2.1 Hz, 1H), 7.38 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.34 (d, *J* = 15.9 Hz, 1H), 3.87 (br.t, *J* = 6.1 Hz, 2H), 3.83 (br.s, 2H), 3.53 (t, *J* = 6.0 Hz, 2H). **^{13}C NMR** (151 MHz, MeOD-d_4) δ 170.2, 168.3, 138.1, 137.1, 136.4, 133.4, 130.8, 130.0, 128.9, 121.1, 48.4, 38.1, 28.6. **HPLC**, t_R 5.91 min (>98%, UV_{254}). **HMRS** (ES^+) *m/z* calcd for $C_{13}H_{14}BrCl_2N_2O_3^+$ $[M+H]^+$, 394.9559; found: 394.9578.

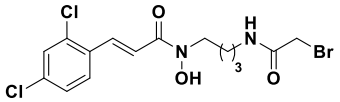
(*E*)-*N*-(3-(2-bromoacetamido)propyl)-3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamide (27).



Synthesized by method C2 using bromoacetic acid (11 mg, 0.08 mmol, 0.9 equiv.), **S28** (30 mg, 0.09 mmol, 1.0 equiv.), HATU (40 mg, 0.11 mmol, 1.2

equiv.), *i*Pr$_2$Net (34 µL, 0.20 mmol, 2.3 equiv.) and DMF (2.0 mL). The titled compound (2 mg, 5.16 µmol, 4%) was collected as an off-white fluffy solid. **^{1}H NMR** (600 MHz, MeOD-d_4) δ 7.94 (d, J = 15.9 Hz, 1H), 7.79 (d, J = 8.4 Hz, 1H), 7.55 (d, J = 2.1 Hz, 1H), 7.38 (dd, J = 8.5, 2.1 Hz, 1H), 7.35 (d, J = 15.9 Hz, 1H), 3.85 (s, 2H), 3.79 (br.t, J = 6.8 Hz, 2H), 3.28 (t, J = 6.8 Hz, 2H), 1.95–1.88 (m, 2H).**^{13}C NMR** (151 MHz, MeOD-d_4) δ 169.6, 167.7, 138.0, 137.0, 136.4, 133.4, 130.8, 130.0, 128.9, 121.1, 47.1, 38.4, 28.8, 27.4. **HPLC**, t_R 6.01 min (>98%, UV$_{254}$). **HMRS** (ES$^+$) *m/z* calcd for $C_{14}H_{16}BrCl_2N_2O_3^+$ [M+H]$^+$, 408.9716; found: 408.9736.

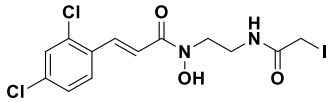
(*E*)-*N*-(4-(2-bromoacetamido)butyl)-3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamide (28). Synthesized



by method C2 using bromoacetic acid (15 mg, 0.11 mmol, 0.9 equiv.), **S24** (42 mg, 0.12 mmol, 1.0 equiv.), HATU (55 mg, 0.14 mmol, 1.2 equiv.), *i*Pr$_2$Net (48 µL, 0.28 mmol, 2.3 equiv.) and DMF (2.0 mL). The titled compound (7 mg, 0.20 mmol, 19%) was collected as an off-white solid. **^{1}H NMR** (600 MHz, MeOD-d_4) δ 7.93 (d, J = 15.9 Hz, 1H), 7.78 (d, J = 8.4 Hz, 1H), 7.54 (d, J = 2.2 Hz, 1H), 7.38 (dd, J = 8.5, 2.1 Hz, 1H), 7.35 (d, J = 15.9 Hz, 1H), 3.82 (s, 2H), 3.76 (br.t, J = 6.9 Hz, 2H), 3.26 (t, J = 7.0 Hz, 2H), 1.74 (quint, J = 7.3 Hz, 2H), 1.62–1.54 (m, 2H). **^{13}C NMR** (151 MHz, MeOD-d_4) δ 169.5, 167.6, 137.8, 137.0, 136.3, 133.4, 130.8, 129.9, 128.9, 121.2, 48.9 (overlapped with solvent peak), 40.5, 28.8, 27.3, 25.1. **HPLC**, t_R 5.98 min (>98%, UV$_{254}$). **HMRS** (ES$^+$) *m/z* calcd for $C_{15}H_{18}BrCl_2N_2O_3^+$ [M+H]$^+$, 422.9872; found: 422.9868.

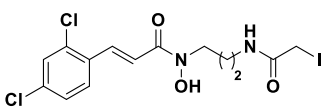
4.4.10 Iodoacetyl

(*E*)-3-(2,4-dichlorophenyl)-*N*-hydroxy-*N*-(2-(2-iodoacetamido)ethyl)acrylamide (29). Synthesized by



method B using **S27** (26 mg, 0.08 mmol, 1.0 equiv.), iodoacetic anhydride (28 mg, 0.08 mmol, 1.0 equiv.), *i*Pr$_2$NEt (42 µL, 0.24 mmol, 3.0 equiv.) and anh. CH_2Cl_2 (3.0 mL). The reaction mixture was reduced *in vacuo* and the crude product purified by RP ACC (gradient: 10→55% MeCN–H_2O in 0.1% formic acid) to afford the titled compound (7 mg, 0.02 mmol, 37%) as a colorless fluffy material after lyophilization. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 10.09 (s, 1H), 8.37 (br.t, J = 5.9 Hz, 1H), 7.89 (d, J = 8.5 Hz, 1H), 7.77–7.71 (m, 2H), 7.48 (dd, J = 8.6, 2.2 Hz, 1H), 7.30 (d, J = 15.8 Hz, 1H), 3.67 (br.t, J = 6.9 Hz, 2H), 3.63 (s, 2H), 3.32–3.28 (m, 2H, overlapped with residual water). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 168.2, 164.9, 135.1, 134.7, 134.3, 131.7, 129.4, 129.2, 128.0, 121.1, 46.9, 36.3, 0.5. **HPLC**, t_R 6.09 min (>96%, UV$_{280}$). **HMRS** (ES$^+$) *m/z* calcd for $C_{13}H_{14}Cl_2IN_2O_3^+$ [M+H]$^+$, 442.9421; found: 442.9430.

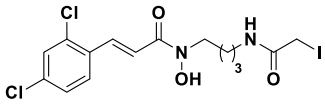
(*E*)-3-(2,4-dichlorophenyl)-*N*-hydroxy-*N*-(3-(2-iodoacetamido)propyl)acrylamide (30). Synthesized



by method B using **S28** (32 mg, 0.10 mmol, 1.0 equiv.), iodoacetic anhydride (35 mg, 0.10 mmol, 1.0 equiv.), *i*Pr$_2$NEt (51 µL, 0.29 mmol, 3.0 equiv.) and anh. CH_2Cl_2 (3.0 mL). The reaction mixture was reduced *in vacuo* and the

crude product purified by RP ACC (gradient: 10→60% MeCN–H_2O in 0.1% formic acid) to afford the titled compound (16 mg, 0.04 mmol, 36%) as a colorless fluffy material after lyophilization. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 10.10 (br.s, 1H), 8.25 (br.t, *J* = 5.6 Hz, 1H), 7.89 (br.s, 1H), 7.76–7.71 (m, 2H), 7.48 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.30 (d, *J* = 15.9 Hz, 1H), 3.68–3.61 (m, 4H), 3.09 (q, *J* = 6.7 Hz, 2H), 1.73 (quint, *J* = 7.1 Hz, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 167.6, 164.6, 135.0, 134.7, 134.2, 131.8, 129.4, 129.2, 128.0, 121.2, 45.7, 36.9, 26.4, 0.8. **HPLC**, t_R 6.01 min (>98%, UV_{280}). **HMRS** (ES^+) *m/z* calcd for $C_{14}H_{16}Cl_2IN_2O_3^+$ $[M+H]^+$, 456.9577; found: 456.9597.

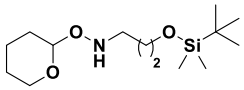
(*E*)-3-(2,4-dichlorophenyl)-*N*-hydroxy-*N*-(4-(2-iodoacetamido)butyl)acrylamide (31). Synthesized by



method B using **S29** (35 mg, 0.10 mmol, 1.0 equiv.), iodoacetic anhydride (35 mg, 0.10 mmol, 1.0 equiv.), *i*Pr_2NEt (52 µL, 0.30 mmol, 3.0 equiv.) and anh. CH_2Cl_2 (3.0 mL). The reaction mixture was reduced *in vacuo* and the crude product purified by RP ACC (gradient: 10→60% MeCN–H_2O in 0.1% formic acid) to afford the titled compound (17 mg, 0.04 mmol, 36%) as a colorless fluffy material after lyophilization. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 10.08 (s, 1H), 8.24 (br.t, *J* = 5.6 Hz, 1H), 7.88 (d, *J* = 8.5 Hz, 1H), 7.78–7.69 (m, 2H), 7.48 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.31 (d, *J* = 15.9 Hz, 1H), 3.67–3.56 (m, 4H), 3.06 (q, *J* = 7.0 Hz, 2H), 1.59 (quint, *J* = 7.1 Hz, 2H), 1.45–1.36 (m, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 167.5, 164.5, 134.9, 134.6, 134.2, 131.8, 129.4, 129.2, 128.0, 121.2, 47.2, 38.7, 26.0, 23.7, 0.9. **HPLC**, t_R 6.07 min (>98%, UV_{280}). **HMRS** (ES^+) *m/z* calcd for $C_{15}H_{28}Cl_2IN_2O_3^+$ $[M+H]^+$, 470.9734; found: 470.9754.

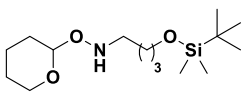
4.4.11 Maleimides

***N*-(3-((*tert*-butyldimethylsilyl)oxy)propyl)-*O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (S46)**.



Synthesized by method A1 using OTX (663 mg, 5.65 mmol, 1.5 equiv.), (3-bromopropoxy)-*tert*-butyldimethylsilane (955 mg, 3.77 mmol, 1.0 equiv.), K_2CO_3 (780 mg, 5.65 mmol, 1.5 equiv.) and DMF (2.0 mL). The reaction mixture was worked up and purified in the same manner as outlined in the synthesis of **S1**. The titled compound (720 mg, 2.49 mmol, 66%) was collected as a colorless oil. **TLC** (33% EtOAc–hexanes): R_f = 0.5. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 4.81–4.77 (m, 1H), 3.95–3.89 (m, 1H), 3.70 (t, *J* = 6.1 Hz, 2H), 3.60–3.53 (m, 1H), 3.13–3.02 (m, 2H), 1.82–1.68 (m, 4H), 1.61–1.48 (m, 4H), 0.89 (s, 9H), 0.04 (s, 6H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 101.5, 63.2, 61.7, 49.8, 30.4, 29.4, 26.1, 25.5, 20.3, 18.4, -5.22, -5.23. **MS** (ES^+) *m/z* calcd for $C_{14}H_{32}NO_3Si^+$ $[M+H]^+$, 290.2; found: 290.2.

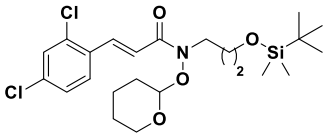
***N*-(4-((*tert*-butyldimethylsilyl)oxy)butyl)-*O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (S47)**.



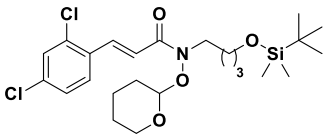
Synthesized by method A1 using OTX (582 mg, 4.97 mmol, 1.5 equiv.), (4-bromobutoxy)-*tert*-butyldimethylsilane (885 mg, 3.31 mmol, 1.0 equiv.), K_2CO_3 (687 mg, 4.97 mmol, 1.5 equiv.) and DMF (2.0 mL). The reaction mixture was worked up and purified in

the same manner as outlined in the synthesis of **S1**. The titled compound (358 mg, 1.18 mmol, 36%) was collected as a colorless oil. **TLC** (50% EtOAc–hexanes): R_f = 0.6. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 4.84–4.75 (m, 1H), 3.96–3.89 (m, 1H), 3.64–3.60 (m, 2H), 3.59–3.54 (m, 1H), 3.05–2.94 (m, 2H), 1.82–1.67 (m, 2H), 1.61–1.47 (m, 8H), 0.88 (s, 9H), 0.03 (s, 6H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 101.6, 63.2, 63.1, 52.2, 30.5, 29.3, 26.1, 25.5, 23.9, 20.3, 18.5, -5.2 (2 × overlapped C). **MS** (ES^+) *m/z* calcd for $C_{15}H_{34}NO_3Si^+$ $[M+H]^+$, 304.2; found: 304.1.

((*E*)-*N*-(3-((*tert*-butyldimethylsilyl)oxy)propyl)-3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S48). Synthesized by method C1 using *trans*-2,4-dichlorocinnamic acid (165 mg, 0.76 mmol, 1.0 equiv.), **S46** (220 mg, 0.76 mmol, 1.0 equiv.), HATU (318 mg, 0.84 mmol, 1.1 equiv.) and *i*Pr_2NEt (265 µL, 1.52 mmol, 2.0 equiv.) and DMF (2.0 mL). The reaction mixture was worked up using EtOAc as outlined in method C1 and the crude residue purified by NP ACC to afford the titled compound (293 mg, 0.60 mmol, 79%) as a colorless oil. **TLC** (33% EtOAc–hexanes): R_f = 0.7. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.98 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.42 (d, *J* = 2.1 Hz, 1H), 7.24 (ddd, *J* = 8.5, 2.2, 0.6 Hz, 1H), 7.02 (d, *J* = 15.8 Hz, 1H), 5.01–4.93 (m, 1H), 4.05–3.96 (m, 2H), 3.88–3.79 (m, 1H), 3.69 (td, *J* = 6.1, 1.8 Hz, 2H), 3.66–3.58 (m, 1H), 2.00–1.72 (m, 5H), 1.68–1.57 (m, 3H), 0.89 (s, 9H), 0.05 (s, 6H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 166.8, 137.7, 135.7, 135.6, 132.5, 130.1, 128.6, 127.5, 120.5, 104.6, 63.9, 60.8, 46.4, 30.4, 29.2, 26.1, 25.1, 19.7, 18.4, -5.19, -5.21. **MS** (ES^+) *m/z* calcd for $C_{23}H_{35}Cl_2NO_4Na^+$ $[M+Na]^+$, 510.2; found: 510.1.



(*E*)-*N*-(4-((*tert*-butyldimethylsilyl)oxy)butyl)-3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S49). Synthesized by method C1 using *trans*-2,4-dichlorocinnamic acid (237 mg, 1.09 mmol, 1.0 equiv.), **S47** (331 mg, 1.09 mmol, 1.0 equiv.), HATU (415 mg, 1.09 mmol, 1.0 equiv.), *i*Pr_2NEt (380 µL, 2.18 mmol, 2.0 equiv.) and DMF (5.0 mL). The reaction mixture was worked up using EtOAc as outlined in method C1 and the crude residue purified by NP ACC to afford the titled compound (449 mg, 0.89 mmol, 82%) as a colorless oil. **TLC** (50% EtOAc–hexanes): R_f = 0.7. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.98 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.5 Hz, 1H), 7.43 (d, *J* = 2.1 Hz, 1H), 7.25 (dd, *J* = 8.4, 2.1, 0.6 Hz, 1H), 7.04 (d, *J* = 15.8 Hz, 1H), 4.96–4.92 (m, 1H), 4.02–3.94 (m, 2H), 3.79–3.72 (m, 1H), 3.66–3.59 (m, 3H), 1.92–1.71 (m, 5H), 1.68–1.52 (m, 5H), 0.89 (s, 9H), 0.05 (s, 6H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 166.8, 137.8, 135.7, 135.6, 132.5, 130.1, 128.6, 127.5, 120.4, 104.8, 63.9, 62.8, 48.5, 30.2, 29.3, 26.1, 25.1, 23.7, 19.8, 18.5, -5.1. **MS** (ES^+) *m/z* calcd for $C_{24}H_{37}Cl_2NO_4Na^+$ $[M+Na]^+$, 524.2; found: 524.1.



(*E*)-3-(2,4-dichlorophenyl)-*N*-(3-hydroxypropyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S50). Intermediate **S48** (270 mg, 0.66 mmol, 1.0 equiv.) was dissolved in anh. THF (5.0 mL) and cooled to 0 °C. TBAF (1 M solution in THF; 0.73 mL, 0.73 mmol, 1.2 equiv.) was added and the reaction warmed to r.t over a period of 1 h. The reaction mixture was concentrated *in vacuo* and purified by RP ACC (gradient: 10→60% MeCN–H_2O in 0.1% formic acid). Appropriate fractions were pooled and extracted with EtOAc, dried (Na_2SO_4), and reduced *in vacuo* to reveal the titled compound (191 mg, 0.51 mmol, 77%) as a colorless semi-solid. **TLC** (66% EtOAc–hexanes): R_f = 0.3. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.01 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.44 (d, *J* = 2.1 Hz, 1H), 7.27–7.24 (m, 1H, overlapped with solvent peak), 7.04 (d, *J* = 15.8 Hz, 1H), 4.97 (dd, *J* = 5.3, 2.6 Hz, 1H), 4.07–3.96 (m, 3H), 3.69–3.52 (m, 3H), 1.95–1.82 (m, 4H), 1.80–1.73 (m, 1H), 1.69–1.59 (m, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 168.1, 138.8, 136.1, 135.7, 132.1, 130.2, 128.7, 127.6, 119.4, 105.2, 64.1, 58.6, 45.2, 30.1, 29.3, 25.0, 19.8. **MS** (ES^+) *m/z* calcd for $C_{17}H_{22}Cl_2NO_4^+$ $[M+H]^+$, 374.1; found: 374.0.

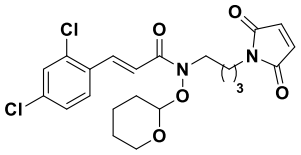
(*E*)-3-(2,4-dichlorophenyl)-*N*-(4-hydroxybutyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S51). The reaction procedure was carried out in the same manner as outlined in the synthesis of **S50** using **S49** (442 mg, 0.88 mmol, 1.0 equiv.), TBAF (1 M solution in THF; 0.97 mL, 0.97 mmol, 1.2 equiv.) and anh. THF (5.0 mL). The reaction was warmed to r.t over a period of 90 min. The reaction mixture was concentrated *in vacuo* and purified by NP ACC to afford the titled compound (305 mg, 0.79 mmol, 90%) as a colorless oil. **TLC** (66% EtOAc–hexanes): R_f = 0.3. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.97 (d, *J* = 15.8 Hz, 1H), 7.51 (d, *J* = 8.4 Hz, 1H), 7.42 (d, *J* = 2.1 Hz, 1H), 7.24 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.03 (d, *J* = 15.8 Hz, 1H), 4.97–4.90 (m, 1H), 4.02–3.92 (m, 2H), 3.86–3.75 (m, 1H), 3.68 (t, *J* = 6.4 Hz, 2H), 3.65–3.58 (m, 1H), 1.91–1.71 (m, 5H), 1.67–1.56 (m, 5H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 166.9, 138.1, 135.8, 135.6, 132.3, 130.1, 128.6, 127.5, 120.1, 105.0, 64.1, 62.6, 48.4, 29.7, 29.3, 25.0, 23.7, 19.8. **MS** (ES^+) *m/z* calcd for $C_{18}H_{24}Cl_2NO_4^+$ $[M+H]^+$, 388.1; found: 388.0.

(*E*)-3-(2,4-dichlorophenyl)-*N*-(3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)propyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S52). The synthesis of **S52** was carried out using a protocol that was adapted from a synthesis outlined by Michael A. Walker.[15] PPh_3 (70 mg, 0.27 mmol, 1.0 equiv.) was dissolved in anh. THF (3.0 mL) and stirred at -78 °C. DIAD (55 µL, 0.27 mmol, 1.0 equiv.) in anh. THF (0.5 mL) was added dropwise to the solution and stirred for 5 min. The reaction flask was removed from the dry-ice/acetone bath and a solution of **S50** (100 mg, 0.27 mmol, 1.0 equiv.) in anh. THF (0.5 mL) was added dropwise with the reaction turning colorless (~5 min). The reaction mixture was re-cooled to -78 °C and maleimide (26 mg,

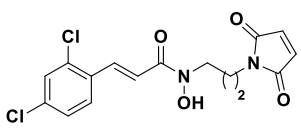
0.27 mmol, 1.0 equiv.) was added and the reaction stirred for 10 min at -78 °C before warming to r.t and stirred overnight. The reaction mixture was concentrated *in vacuo* and the crude residue purified by RP ACC (gradient: 10→70% MeCN–H_2O in 0.1% formic acid) to afford the titled compound (34 mg, 0.08 mmol, 28%) as a colorless semi-solid* after lyophilization. Unreacted starting material could be recovered (37% brsm). **TLC** (50% EtOAc–hexanes): R_f = 0.4. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.96 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.5 Hz, 1H), 7.42 (d, *J* = 2.1 Hz, 1H), 7.25 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.01 (d, *J* = 15.8 Hz, 1H), 6.69 (s, 2H), 4.95–4.88 (m, 1H), 4.00–3.88 (m, 2H), 3.84–3.74 (m, 1H), 3.64–3.54 (m, 3H), 2.12–1.97 (m, 2H), 1.90–1.79 (m, 2H), 1.76–1.69 (m, 1H), 1.66–1.56 (m, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 170.8, 166.9, 138.1, 135.8, 135.6, 134.2, 132.2, 130.1, 128.6, 127.5, 120.0, 105.4, 64.2, 46.4, 35.8, 29.3, 26.3, 25.0, 20.0. **MS** (ES^+) *m/z* calcd for $C_{21}H_{23}Cl_2N_2O_5^+$ $[M+H]^+$, 453.1; found: 453.1.

*Impurity arising from starting material at 10%, but compound taken forward without further purification.

(*E*)-3-(2,4-dichlorophenyl)-*N*-(4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S53). The reaction procedure was carried out in the same manner as outlined in the synthesis of **S52** using PPh_3 (65 mg, 0.25 mmol, 1.0 equiv.), DIAD (51 µL, 0.25 mmol, 1.0 equiv.), **S51** (95 mg, 0.25 mmol, 1.0 equiv.), maleimide (24 mg, 0.25 mmol, 1.0 equiv.) and anh. THF (3.0 mL). The titled compound (27 mg, 0.06 mmol, 23%) was collected as a colorless oil. Unreacted starting material could be recovered (38% brsm). **TLC** (50% EtOAc–hexanes): R_f = 0.5. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.96 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.42 (d, *J* = 2.1 Hz, 1H), 7.25 (ddd, *J* = 8.5, 2.2, 0.6 Hz, 1H), 7.01 (d, *J* = 15.8 Hz, 1H), 6.67 (s, 2H), 4.94–4.87 (m, 1H), 4.00–3.89 (m, 2H), 3.81–3.72 (m, 1H), 3.63–3.58 (m, 1H), 3.55 (t, *J* = 7.0 Hz, 2H), 1.90–1.79 (m, 2H), 1.75–1.67 (m, 3H), 1.66–1.58 (m, 5H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 170.9, 166.8, 138.0, 135.8, 135.6, 134.2, 132.4, 130.1, 128.6, 127.5, 120.2, 105.1, 64.1, 48.0, 37.6, 29.3, 26.0, 25.0, 24.4, 19.9. **MS** (ES^+) *m/z* calcd for $C_{22}H_{25}Cl_2N_2O_5^+$ $[M+H]^+$, 467.1; found: 467.1.

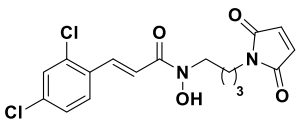


(*E*)-3-(2,4-dichlorophenyl)-*N*-(3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)propyl)-*N*-hydroxyacrylamide (32). Synthesized by method F using **S52** (18 mg, 0.04 mmol, 1.0 equiv.), PPTS (7 mg, 0.03 mmol, 0.7 equiv.) and abs. EtOH (3.0 mL). The reaction mixture was concentrated *in vacuo* and purified by RP ACC (gradient: 10→50% MeCN–H_2O in 0.1% formic acid) to afford the titled compound (6 mg, 0.02 mmol, 40%) as a colorless fluffy material after lyophilization. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 7.89 (d, *J* = 8.5 Hz, 1H), 7.75–7.69 (m, 2H), 7.47 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.29 (d, *J* = 15.8 Hz, 1H), 7.02 (s, 2H), 3.60 (br.s, 2H), 3.45 (t, *J* = 7.2 Hz, 2H, overlapped with residual water), 1.84 (quint, *J* = 7.2 Hz, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 171.0, 164.6, 134.9, 134.7, 134.5, 134.2, 131.8, 129.4, 129.2, 128.0, 121.2, 45.5, 35.1, 25.6.



HPLC, t_R 6.15 min (>96%, UV_{254}). **HMRS** (ES^+) *m/z* calcd for $C_{16}H_{15}Cl_2N_2O_4^+$ $[M+H]^+$, 369.0403; found: 369.0412.

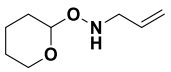
(*E*)-3-(2,4-dichlorophenyl)-*N*-(4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butyl)-*N*-hydroxyacrylamide (33). Synthesized by method F using **S53** (27 mg, 0.06 mmol, 1.0 equiv.), PPTS



(25 mg, 0.10 mmol, 1.7 equiv.) and abs. EtOH (3.0 mL). The reaction mixture was purified using the same protocol as outlined in the synthesis of **36**. The titled compound (13 mg, 0.03 mmol, 56%) was collected as a colorless fluffy material after lyophilization. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 10.18 (s, 1H), 7.88 (d, J = 8.6 Hz, 1H), 7.76–7.68 (m, 2H), 7.47 (dd, J = 8.5, 2.2 Hz, 1H), 7.30 (d, J = 15.8 Hz, 1H), 7.01 (s, 2H), 3.62 (br.s, 2H), 3.43 (t, J = 6.4 Hz, 2H), 1.59–1.46 (m, 4H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 171.1, 164.5, 134.8, 134.6, 134.5, 134.2, 131.8, 129.4, 129.2, 128.0, 121.2, 47.0, 36.8, 25.3, 23.6. **HPLC**, t_R 6.28 min (>98%, UV_{254}). **HMRS** (ES^+) *m/z* calcd for $C_{17}H_{17}Cl_2N_2O_4^+$ $[M+H]^+$, 383.0560; found: 383.0568.

4.4.12 Epoxides

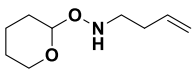
***N*-allyl-*O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (S54)**. Synthesized by method A3 using OTX



(1.45 g, 12.4 mmol, 1.5 equiv.), 3-bromo-propene (715 µL, 8.27 mmol, 1.0 equiv.), K_2CO_3 (1.71 g, 12.40 mmol, 1.5 equiv.) and DMF (5.0 mL). The crude oil was purified using NP ACC to afford the titled compound (867 mg, 5.51 mmol, 67%) as a pale-yellow oil*. **TLC** (20% EtOAc–hexanes): R_f = 0.3. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.87 (ddt, J = 16.8, 10.3, 6.3 Hz, 1H), 5.54 (br.s, 1H), 5.17 (dq, J = 17.3, 1.6 Hz, 1H), 5.12–5.05 (m, 1H), 4.74 (dd, J 5.2 and 3.3 Hz, 1H), 3.89–3.82 (m, 1H), 3.57–3.46 (m, 3H), 1.76–1.67 (m, 1H), 1.67–1.59 (m, 1H), 1.55–1.40 (m, 4H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 134.1, 117.9, 101.4, 63.0, 55.1, 29.1, 25.3, 20.1. **MS** (ES^+) *m/z* calcd for $C_8H_{16}NO_2^+$ $[M+H]^+$, 158.1; found: 158.1.

*Product is volatile – rotary evaporator cooled to 10 °C and high vacuum avoided.

***N*-(but-3-en-1-yl)-*O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (S55)**. Synthesized by method A3



using OTX (1.30 g, 11.10 mmol, 1.5 equiv.), 4-bromo-1-butene (752 µL, 7.41 mmol, 1.0 equiv.), K_2CO_3 (1.54 g, 11.10 mmol, 1.5 equiv.) and DMF (5.0 mL). The crude oil was purified using NP ACC to afford the titled compound (310 mg, 1.81 mmol, 24%) as a colorless oil*. **TLC** (20% EtOAc–hexanes): R_f = 0.2. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.79 (ddt, J = 17.1, 10.2, 6.8 Hz, 1H), 5.09 (dq, J = 17.2, 1.7 Hz, 1H), 5.06–5.01 (m, 1H), 4.78 (dd, J = 5.4, 3.3 Hz, 1H), 3.94–3.88 (m, 1H), 3.59–3.52 (m, 1H), 3.09–2.98 (m, 2H), 2.36–2.23 (m, 2H), 1.82–1.74 (m, 1H), 1.74–1.66 (m, 1H), 1.60–1.46 (m, 4H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 135.9, 116.8, 101.6, 63.1, 51.4, 31.7, 29.4, 25.5, 20.3. **MS** (ES^+) *m/z* calcd for $C_9H_{18}NO_2^+$ $[M+H]^+$, 172.1; found: 172.1.

*Product is volatile – rotary evaporator cooled to 10 °C and high vacuum avoided.

***N*-(pent-4-en-1-yl)-*O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (S56)**. Synthesized by method A3 using OTX (1.18 g, 10.07 mmol, 1.5 equiv.), 5-bromo-1-pentene (795 µL, 6.71 mmol, 1.0 equiv.), K_2CO_3 (1.39 g, 10.07 mmol, 1.5 equiv.) and DMF (5.0 mL). The crude oil was purified using NP ACC to afford the titled compound (425 mg, 2.29 mmol, 34%) as a colorless oil*. **TLC** (20% EtOAc–hexanes): R_f = 0.3. **^{1}H NMR** (500 MHz, $CDCl_3$)**: δ 5.80 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H), 5.02 (dq, *J* = 17.2, 1.7 Hz, 1H), 4.98–4.93 (m, 1H), 4.80–4.75 (m, 1H), 3.95–3.88 (m, 1H), 3.59–3.52 (m, 1H), 3.04–2.92 (m, 2H), 2.15–2.08 (m, 2H), 1.82–1.68 (m, 3H), 1.63 (q, *J* = 7.4 Hz, 2H), 1.58–1.48 (m, 4H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 138.4, 115.0, 101.6, 63.3, 51.8, 31.4, 29.4, 26.6, 25.5, 20.4. **MS** (ES^+) *m/z* calcd for $C_{10}H_{20}NO_2^+$ $[M+H]^+$, 186.1; found: 186.1.

*Product is volatile – rotary evaporator cooled to 10 °C and high vacuum avoided.

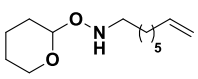
**THP-based impurity (0.8–1.3 ppm), but compound taken forward without further purification.

***N*-(hex-5-en-1-yl)-*O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (S57)**. Synthesized by method A3 using OTX (1.08 g, 9.20 mmol, 1.5 equiv.), 6-bromo-1-hexene (820 µL, 6.13 mmol, 1.0 equiv.), K_2CO_3 (1.27 g, 9.20 mmol, 1.5 equiv.) and DMF (5.0 mL). The crude oil was purified using NP ACC to afford the titled compound (682 mg, 3.42 mmol, 56%) as a pale-yellow oil*. **TLC** (20% EtOAc–hexanes): R_f = 0.3. **^{1}H NMR** (500 MHz, $CDCl_3$): δ 5.78 (ddt, *J* = 17.0, 10.2, 6.7 Hz, 1H), 4.98 (dq, *J* = 17.1, 1.7 Hz, 1H), 4.92 (ddt, *J* = 10.2, 2.3, 1.2 Hz, 1H), 4.78–4.74 (m, 1H), 3.95–3.84 (m, 1H), 3.59–3.48 (m, 1H), 3.03–2.89 (m, 2H), 2.09–2.02 (m, 2H), 1.81–1.73 (m, 1H), 1.73–1.65 (m, 1H), 1.58–1.48 (m, 6H), 1.46–1.39 (m, 2H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 138.7, 114.7, 101.6, 63.3, 52.2, 33.7, 29.3, 26.9, 26.5, 25.5, 20.4. **MS** (ES^+) *m/z* calcd for $C_{11}H_{22}NO_2^+$ $[M+H]^+$, 200.2; found: 200.2.

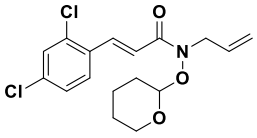
*Product is volatile – rotary evaporator cooled to 10 °C and high vacuum avoided.

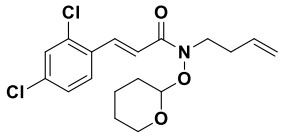
***N*-(hept-6-en-1-yl)-*O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (S58)**. Synthesized by method A3* using OTX (1.08 g, 9.20 mmol, 1.5 equiv.), 6-bromo-1-hexene (820 µL, 6.13 mmol, 1.0 equiv.), K_2CO_3 (1.27 g, 9.20 mmol, 1.5 equiv.) and DMF (5.0 mL). The crude product was purified by NP ACC to afford the titled compound (239 mg, 1.12 mmol, 40%) as a colorless oil. **TLC** (20% EtOAc–hexanes): R_f = 0.3. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.80 (ddt, *J* = 16.9, 10.1, 6.7 Hz, 1H), 5.03–4.96 (m, 1H), 4.93 (ddt, *J* = 10.2, 2.3, 1.2 Hz, 1H), 4.85–4.79 (m, 1H), 3.99–3.87 (m, 1H), 3.62–3.51 (m, 1H), 3.06–2.90 (m, 2H), 2.10–1.99 (m, 2H), 1.85–1.66 (m, 2H), 1.63–1.46 (m, 6H), 1.46–1.29 (m, 4H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 139.0, 114.5, 101.5, 63.3, 52.2, 33.8, 29.3, 28.9, 27.1, 26.8, 25.5, 20.3. **MS** (ES^+) *m/z* calcd for $C_{12}H_{24}NO_2^+$ $[M+H]^+$, 214.2; found: 214.2.

*Reaction stirred at 40 °C overnight.

***N*-(oct-7-en-1-yl)-*O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (S59)**. Synthesized by method A3*  using OTX (461 mg, 3.93 mmol, 1.5 equiv.), 8-bromo-1-octene (500 mg, 2.62 mmol, 1.5 equiv.), K_2CO_3 (543 mg, 3.93 mmol, 1.5 equiv.) and DMF (4.0 mL). The crude oil was purified using NP ACC to afford the titled compound (335 mg, 1.47 mmol, 56%) as a colorless oil. **TLC** (20% EtOAc–hexanes): R_f = 0.4. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.80 (ddt, *J* = 16.9, 10.1, 6.7 Hz, 1H), 5.02–4.95 (m, 1H), 4.95–4.90 (m, 1H), 4.83–4.78 (m, 1H), 3.99–3.86 (m, 1H), 3.62–3.51 (m, 1H), 3.03–2.89 (m, 2H), 2.09–1.98 (m, 2H), 1.86–1.64 (m, 2H), 1.63–1.45 (m, 6H), 1.43–1.22 (m, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 139.2, 114.4, 101.5, 63.3, 52.3, 33.8, 29.3, 29.1, 28.9, 27.2, 27.1, 25.5, 20.3. **MS** (ES^+) *m/z* calcd for $C_{13}H_{26}NO_2^+$ $[M+H]^+$, 228.2; found: 228.2.

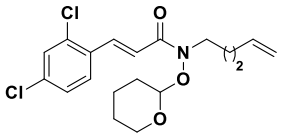
*Reaction stirred at 55 °C overnight.

(*E*)-*N*-allyl-3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S60). Synthesized  by method C1 using *trans*-2,4-dichlorocinnamic acid (150 mg, 0.69 mmol, 1.0 equiv.), **S54** (130 mg, 0.83 mmol, 1.2 equiv.), HATU (315 mg, 0.83 mmol, 1.2 equiv.) and *i*Pr_2NEt (144 µL, 0.83 mmol, 1.2 equiv.) and DMF (3.0 mL). DMF was removed *in vacuo* and the crude residue was purified by RP ACC (gradient: 20→100% MeCN–H_2O in 0.1% formic acid). Appropriate fractions were pooled and extracted with CH_2Cl_2, dried (Na_2SO_4) and reduced *in vacuo* to reveal the titled compound (235 mg, 0.66 mmol, 95%) as a pale-yellow viscous oil. **TLC** (20% EtOAc–hexanes): R_f = 0.4. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.00 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.42 (d, *J* = 2.1 Hz, 1H), 7.24 (ddd, *J* = 8.4, 2.1, 0.6 Hz, 1H), 7.06 (d, *J* = 15.9 Hz, 1H), 5.92 (dddd, *J* = 16.9, 10.2, 6.4, 5.4 Hz, 1H), 5.30–5.25 (m, 1H), 5.22 (dq, *J* = 10.2, 1.4 Hz, 1H), 5.00–4.96 (m, 1H), 4.61 (ddt, *J* = 16.0, 5.5, 1.6 Hz, 1H), 4.30 (ddt, *J* = 16.0, 6.3, 1.3 Hz, 1H), 4.03–3.97 (m, 1H), 3.64–3.57 (m, 1H), 1.90–1.80 (m, 2H), 1.80–1.71 (m, 1H), 1.67–1.56 (m, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 167.0, 138.2, 135.8, 135.6, 132.4, 132.3, 130.1, 128.6, 127.5, 120.2, 118.0, 104.9, 63.9, 51.5, 29.2, 25.0, 19.7. **MS** (ES^+) *m/z* calcd for $C_{17}H_{20}Cl_2NO_3^+$ $[M+H]^+$, 356.1; found: 356.1.

(*E*)-*N*-(but-3-en-1-yl)-3-(2,4-dichlorophenyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S61).  Synthesized by method C using *trans*-2,4-dichlorocinnamic acid (150 mg, 0.69 mmol, 1.0 equiv.), **S55** (142 mg, 0.83 mmol, 1.2 equiv.), HATU (315 mg, 0.83 mmol, 1.2 equiv.), *i*Pr_2NEt (144 µL, 0.83 mmol, 1.2 equiv.) and DMF (3.0 mL). The crude product was purified using the same protocol as outlined in the synthesis of S46 and the titled compound (217 mg, 0.58 mmol, 85%) collected as a pale-yellow viscous oil. **TLC** (20% EtOAc–hexanes): R_f = 0.4. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.97 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.42 (d, *J* = 2.0 Hz, 1H), 7.24 (ddd, *J* = 8.5, 2.2, 0.6 Hz, 1H), 7.02 (d, *J* = 15.9 Hz, 1H), 5.81 (ddt, *J* = 17.1, 10.2, 6.9 Hz, 1H), 5.11 (dq, *J* = 17.1, 1.6 Hz, 1H), 5.07–5.02 (m, 1H), 4.97–4.91 (m, 1H), 4.05–3.96 (m,

2H), 3.80 (ddd, *J* = 14.2, 8.0, 6.2 Hz, 1H), 3.65–3.59 (m, 1H), 2.53–2.41 (m, 2H), 1.92–1.80 (m, 2H), 1.79–1.71 (m, 1H), 1.68–1.58 (m, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 166.9, 137.9, 135.8, 135.6, 135.2, 132.4, 130.1, 128.6, 127.5, 120.3, 117.1, 105.0, 64.0, 48.3, 31.6, 29.3, 25.0, 19.8. **MS** (ES^+) *m/z* calcd for $C_{18}H_{22}Cl_2NO_3^+$ $[M+H]^+$, 370.1; found: 370.1.

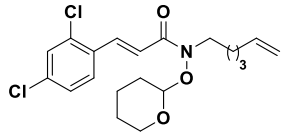
(*E*)-3-(2,4-dichlorophenyl)-*N*-(pent-4-en-1-yl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S62).



Synthesized by method C1 using *trans*-2,4-dichlorocinnamic acid (150 mg, 0.69 mmol, 1.0 equiv.), **S56** (141 mg, 0.76 mmol, 1.1 equiv.), HATU (289 mg, 0.76 mmol, 1.1 equiv.), *i*Pr_2NEt (132 µL, 0.76 mmol, 1.1 equiv.) and DMF (3.0 mL). The DMF was removed *in vacuo* and the crude residue was purified by NP ACC to afford the titled compound (250 mg, 0.65 mmol, 94%) as a colorless oil*. **TLC** (20% EtOAc–hexanes): R_f = 0.4. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.98 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.43 (d, *J* = 2.1 Hz, 1H), 7.25 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.03 (d, *J* = 15.8 Hz, 1H), 5.88–5.76 (m, 1H), 5.08–5.02 (m, 1H), 5.00–4.96 (m, 1H), 4.95–4.92 (m, 1H), 4.01–3.89 (m, 2H), 3.79–3.72 (m, 1H), 3.64–3.59 (m, 1H), 2.14–2.07 (m, 2H), 1.90–1.80 (m, 2H), 1.79–1.69 (m, 2H), 1.67–1.59 (m, 3H), 1.56–1.49 (m, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.8, 137.89, 137.87, 135.7, 135.6, 132.4, 130.1, 128.6, 127.5, 120.3, 115.2, 104.9, 64.0, 51.7, 31.3, 29.3, 26.3, 25.0, 19.9. **MS** (ES^+) *m/z* calcd for $C_{19}H_{24}Cl_2NO_3^+$ $[M+H]^+$, 384.1; found: 384.1.

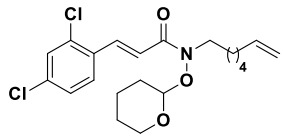
*~35% impurity arising from **S48**, but compound taken forward without further purification.

(*E*)-3-(2,4-dichlorophenyl)-*N*-(hex-5-en-1-yl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S63).



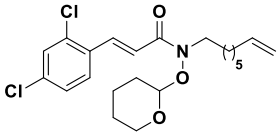
Synthesized by method C1 using *trans*-2,4-dichlorocinnamic acid (150 mg, 0.69 mmol, 1.0 equiv.), **S57** (165 mg, 0.83 mmol, 1.2 equiv.), HATU (315 mg, 0.83 mmol, 1.2 equiv.), *i*Pr_2NEt (144 µL, 0.83 mmol, 1.2 equiv.) and DMF (3.0 mL). The crude product was purified in the samee manner as outlined in the synthesis of **S52** and the titled compound (211 mg, 0.53 mmol, 77%) collected as a colorless oil. **TLC** (20% EtOAc–hexanes): R_f = 0.5. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.97 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.42 (d, *J* = 2.1 Hz, 1H), 7.24 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.03 (d, *J* = 15.8 Hz, 1H), 5.79 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H), 5.00 (dq, *J* = 17.1, 1.7 Hz, 1H), 4.96–4.91 (m, 2H), 4.01–3.90 (m, 2H), 3.74 (ddd, *J* = 14.3, 8.1, 6.1 Hz, 1H), 3.64–3.58 (m, 1H), 2.12–2.05 (m, 2H), 1.91–1.79 (m, 2H), 1.78–1.68 (m, 3H), 1.67–1.58 (m, 3H), 1.43 (quint, *J* = 7.7 Hz, 2H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 166.7, 138.6, 137.8, 135.7, 135.6, 132.4, 130.1, 128.6, 127.5, 120.3, 114.8, 104.9, 64.0, 48.5, 33.5, 29.3, 26.6, 26.2, 25.0, 19.9. **MS** (ES^+) *m/z* calcd for $C_{20}H_{26}Cl_2NO_3^+$ $[M+H]^+$, 398.1; found: 398.1.

(*E*)-3-(2,4-dichlorophenyl)-*N*-(hept-6-en-1-yl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S64).



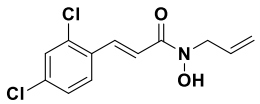
Synthesized by method C1 using *trans*-2,4-dichlorocinnamic acid (430 mg, 1.98 mmol, 1.0 equiv.), **S58** (465 mg, 2.18 mmol, 1.1 equiv.), HATU (829 mg, 2.18 mmol, 1.1 equiv.), *i*Pr_2NEt (1.04 mL, 5.94 mmol, 3.0 equiv.) and DMF (10 mL). The reaction mixture was worked up using EtOAc as outlined in method C1 and the crude product was purified by NP ACC to afford the titled compound (778 mg, 1.89 mmol, 95%) as a colorless oil. **TLC** (20% EtOAc–hexanes): R_f = 0.5. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.97 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.5 Hz, 1H), 7.42 (d, *J* = 2.1 Hz, 1H), 7.24 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.03 (d, *J* = 15.8 Hz, 1H), 5.79 (ddt, *J* = 16.9, 10.1, 6.7 Hz, 1H), 4.98 (dq, *J* = 17.1, 1.7 Hz, 1H), 4.95–4.90 (m, 2H), 4.02–3.95 (m, 1H), 3.95–3.89 (m, 1H), 3.72 (ddd, *J* = 14.3, 8.4, 6.0 Hz, 1H), 3.64–3.58 (m, 1H), 2.08–2.02 (m, 2H), 1.91–1.79 (m, 2H), 1.77–1.68 (m, 3H), 1.67–1.57 (m, 3H), 1.47–1.39 (m, 2H), 1.38–1.30 (m, 2H); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 166.7, 139.0, 137.8, 135.7, 135.6, 132.5, 130.1, 128.6, 127.5, 120.4, 114.5, 104.9, 64.0, 48.6, 33.7, 29.3, 28.7, 27.0, 26.4, 25.0, 19.8. **MS** (ES^+) *m/z* calcd for $C_{21}H_{28}Cl_2NO_3^+$ $[M+H]^+$, 412.1; found: 412.1.

(*E*)-3-(2,4-dichlorophenyl)-*N*-(oct-7-en-1-yl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S65).



Synthesized by method C1 using *trans*-2,4-dichlorocinnamic acid (178 mg, 0.82 mmol, 1.0 equiv.), **S59** (205 mg, 0.90 mmol, 1.1 equiv.), HATU (343 mg, 0.90 mmol, 1.1 equiv.), *i*Pr_2NEt (428 µL, 2.46 mmol, 3.0 equiv.) and DMF (8.0 mL). The reaction mixture was worked up using EtOAc as outlined in method C1 and the crude product was purified by NP ACC to afford the titled compound (330 mg, 0.77 mmol, 95%) collected as a colorless oil. **TLC** (20% EtOAc–hexanes): R_f = 0.5. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.98 (dt, *J* = 15.7, 0.6 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.43 (d, *J* = 2.1 Hz, 1H), 7.25 (ddd, *J* = 8.4, 2.1, 0.6 Hz, 1H), 7.03 (d, *J* = 15.8 Hz, 1H), 5.80 (ddt, *J* = 17.0, 10.2, 6.7 Hz, 1H), 5.02–4.95 (m, 1H), 4.95–4.90 (m, 2H), 4.02–3.96 (m, 1H), 3.96–3.89 (m, 1H), 3.73 (ddd, *J* = 14.3, 8.3, 6.0 Hz, 1H), 3.66–3.58 (m, 1H), 2.07–2.00 (m, 2H), 1.92–1.80 (m, 2H), 1.78–1.67 (m, 3H), 1.67–1.59 (m, 3H), 1.42–1.30 (m, 6H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 166.7, 139.2, 137.8, 135.7, 135.6, 132.5, 130.1, 128.6, 127.5, 120.4, 114.4, 104.9, 64.0, 48.7, 33.8, 29.3, 29.0, 28.9, 27.1, 26.8, 25.1, 19.9. **MS** (ES^+) *m/z* calcd for $C_{22}H_{30}Cl_2NO_3^+$ $[M+H]^+$, 426.2; found: 426.1.

(*E*)-*N*-allyl-3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamide (S66). Synthesized by method F using **S60**



(211 mg, 0.59 mmol, 1.0 equiv.), PPTS (74 mg, 0.30 mmol, 0.5 equiv.) and abs. EtOH (4.0 mL). The reaction mixture was concentrated *in vacuo* and purified by RP ACC (gradient: 10→70% MeCN–H_2O in 0.1% formic acid). Appropriate fractions were pooled and extracted with CH_2Cl_2, dried (Na_2SO_4), and reduced *in vacuo* to reveal the titled compound (140 mg,

0.51 mmol, 87%) as an off-white solid. **^{1}H NMR** (500 MHz, MeOD-d_4) δ 7.95 (d, J = 15.9 Hz, 1H), 7.78 (d, J = 8.5 Hz, 1H), 7.54 (d, J = 2.1 Hz, 1H), 7.40–7.31 (m, 2H), 5.90 (ddt, J = 16.4, 11.1, 5.9 Hz, 1H), 5.30 (dq, J = 17.2, 1.6 Hz, 1H), 5.27–5.21 (m, 1H), 4.33 (d, J = 6.0 Hz, 2H). **^{13}C NMR** (151 MHz, MeOD-d_4) δ 167.4, 138.0, 137.0, 136.3, 133.3, 132.9, 130.7, 129.9, 128.9, 121.1, 118.8, 52.3, **MS** (ES^+) *m/z* calcd for $C_{12}H_{12}Cl_2NO_2^+$ $[M+H]^+$, 272.0; found: 272.0.

(*E*)-*N*-(but-3-en-1-yl)-3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamide (S67). Synthesized by method F using **S61** (212 mg, 0.57 mmol, 1.0 equiv.) and PPTS (72 mg, 0.29 mmol, 0.5 equiv.) and abs. EtOH (4.0 mL). The reaction mixture was concentrated *in vacuo* and purified by RP ACC (gradient: 10→80% MeCN–H_2O in 0.1% formic acid). Appropriate fractions were pooled and extracted with CH_2Cl_2, dried (Na_2SO_4), and reduced *in vacuo* to reveal the titled compound (122 mg, 0.43 mmol, 74%) as a colorless semi-solid that solidified over time to a pearlescent solid. **^{1}H NMR** (600 MHz, MeOD-d_4) δ 7.90 (d, J = 15.9 Hz, 1H), 7.73 (d, J = 8.5 Hz, 1H), 7.49 (d, J = 2.1 Hz, 1H), 7.36–7.29 (m, 2H), 5.83 (ddt, J = 17.1, 10.3, 6.8 Hz, 1H), 5.17–5.10 (m, 1H), 5.04 (dd, J = 10.3, 1.8 Hz, 1H), 3.78 (br.t, J = 7.3 Hz, 2H), 2.44 (br.q, J = 7.1 Hz, 2H). **^{13}C NMR** (151 MHz, MeOD-d_4) δ 167.5, 137.8, 136.9, 136.3, 136.1, 133.3, 130.7, 129.8, 128.8, 121.2, 117.3, 48.9 (overlapped with solvent peak), 32.1. **MS** (ES^+) *m/z* calcd for $C_{13}H_{14}Cl_2NO_2^+$ $[M+H]^+$, 286.0; found: 286.0.

(*E*)-3-(2,4-dichlorophenyl)-*N*-hydroxy-*N*-(pent-4-en-1-yl)acrylamide (S68). Synthesized by method F using **S62** (47 mg, 0.12 mmol, 1.0 equiv.), PPTS (15 mg, 0.06 mmol, 0.5 equiv.) and abs. EtOH (3.0 mL). The reaction mixture was concentrated *in vacuo* and purified by NP ACC to afford the titled compound (27 mg, 0.09 mmol, 84%) as an off-white semi-solid. **^{1}H NMR** (500 MHz, MeOD-d_4). δ 7.93 (d, J = 15.9 Hz, 1H), 7.77 (d, J = 8.5 Hz, 1H), 7.53 (d, J = 2.1 Hz, 1H), 7.40–7.31 (m, 2H), 5.86 (ddt, J = 17.0, 10.2, 6.7 Hz, 1H), 5.06 (dq, J = 17.1, 1.7 Hz, 1H), 5.01–4.95 (m, 1H), 3.74 (t, J = 7.1 Hz, 2H), 2.16–2.08 (m, 2H), 1.79 (quint, J = 7.3 Hz, 2H). **^{13}C NMR** (151 MHz, MeOD-d_4) δ 167.5, 139.0, 137.8, 137.0, 136.3, 133.4, 130.8, 129.9, 128.9, 121.3, 115.6, 48.9 (overlapped with solvent peak), 31.9, 27.1. **MS** (ES^+) *m/z* calcd for $C_{14}H_{16}Cl_2NO_2^+$ $[M+H]^+$, 300.1; found: 300.1.

(*E*)-3-(2,4-dichlorophenyl)-*N*-(hex-5-en-1-yl)-*N*-hydroxyacrylamide (S69). Synthesized by method F using **S63** (176 mg, 0.44 mmol, 1.0 equiv.), PPTS (55 mg, 0.22 mmol, 0.5 equiv.) and abs. EtOH (4.0 mL). The crude product was purified using the same protocol as outlined in the synthesis of **S59** and the titled compound (118 mg, 0.38 mmol, 85%) was collected as an orange semi-solid. **^{1}H NMR** (600 MHz, MeOD-d_4) δ 7.92 (d, J = 15.9 Hz, 1H), 7.75 (d, J = 8.5 Hz, 1H), 7.51 (d, J = 2.1 Hz, 1H), 7.38–7.31 (m, 2H), 5.82 (ddt, J = 17.0, 10.2, 6.7 Hz, 1H), 5.02 (dq, J = 17.2, 1.7 Hz, 1H), 4.97–4.92 (m, 1H), 3.73 (t, J = 7.1 Hz, 2H), 2.14–2.07 (m, 2H), 1.71 (quint, J = 7.3 Hz, 2H),

1.49–1.41 (m, 2H). **^{13}C NMR** (151 MHz, MeOD-d_4) δ 167.4, 139.6, 137.7, 136.9, 136.3, 133.4, 130.7, 129.9, 128.9, 121.2, 115.2, 48.9 (overlapped with solvent peak), 34.4, 27.2, 27.1. **MS** (ES^+) *m/z* calcd for $C_{15}H_{18}Cl_2NO_2^+$ $[M+H]^+$, 314.1; found: 314.1.

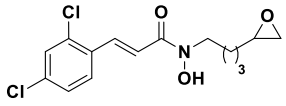
(*E*)-3-(2,4-dichlorophenyl)-*N*-(hept-6-en-1-yl)-*N*-hydroxyacrylamide (S70). Synthesized by method F using **S64** (737 mg, 1.79 mmol, 1.0 equiv.), PPTS (112 mg, 0.45 mmol, 0.25 equiv.) and abs. EtOH (12 mL). The crude product was purified using the same protocol as outlined in the synthesis of **S59** and the titled compound (511 mg, 1.56 mmol, 87%) collected as an off-white solid. **^{1}H NMR** (600 MHz, MeOD-d_4) δ 7.92 (d, *J* = 15.8 Hz, 1H), 7.76 (d, *J* = 8.5 Hz, 1H), 7.53 (d, *J* = 2.1 Hz, 1H), 7.39–7.29 (m, 2H), 5.81 (ddt, *J* = 17.0, 10.1, 6.7 Hz, 1H), 4.99 (br.d, *J* = 17.2 Hz, 1H), 4.92 (br.d, *J* = 10.2 Hz, 1H), 3.72 (br.t, *J* = 7.1 Hz, 2H), 2.11–2.04 (m, 2H), 1.70 (quint, *J* = 7.4 Hz, 2H), 1.50–1.42 (m, 2H), 1.41–1.34 (m, 2H). **^{13}C NMR** (151 MHz, MeOD-d_4) δ 167.4, 139.9, 137.7, 136.9, 136.3, 133.5, 130.8, 129.9, 128.9, 121.3, 114.9, 48.9 (overlapped with solvent peak), 34.8, 29.7, 27.6, 27.2. **MS** (ES^+) *m/z* calcd for $C_{16}H_{20}Cl_2NO_2^+$ $[M+H]^+$, 328.1; found: 328.1.

(*E*)-3-(2,4-dichlorophenyl)-*N*-hydroxy-*N*-(oct-7-en-1-yl)acrylamide (S71). Synthesized by method F using **S65** (294 mg, 0.69 mmol, 1.0 equiv.), PPTS (87 mg, 0.34 mmol, 0.5 equiv.) and abs. EtOH (10 mL). The crude product was purified using the SAME protocol as outlined in the synthesis of **S59** and the titled compound (203 mg, 0.59 mmol, 86%) collected as an off-white solid. **^{1}H NMR** (600 MHz, MeOD-d_4) δ 7.92 (d, *J* = 15.9 Hz, 1H), 7.76 (d, *J* = 8.5 Hz, 1H), 7.52 (d, *J* = 2.1 Hz, 1H), 7.40–7.31 (m, 2H), 5.80 (ddt, *J* = 17.0, 10.2, 6.7 Hz, 1H), 4.98 (d, *J* = 17.2 Hz, 1H), 4.91 (d, *J* = 10.3 Hz, 1H), 3.72 (br.t, *J* = 7.1 Hz, 2H), 2.09–2.02 (m, 2H), 1.73–1.65 (m, 2H), 1.45–1.32 (m, 6H). **^{13}C NMR** (151 MHz, MeOD-d_4) δ 167.4, 140.0, 137.7, 136.9, 136.3, 133.4, 130.8, 129.9, 128.9, 121.3, 114.8, 48.9 (overlapped with solvent peak), 34.8, 30.0, 29.9, 27.7, 27.6. **MS** (ES^+) *m/z* calcd for $C_{17}H_{22}Cl_2NO_2^+$ $[M+H]^+$, 342.1; found: 342.1.

(*E*)-3-(2,4-dichlorophenyl)-*N*-hydroxy-*N*-(3-(oxiran-2-yl)propyl)acrylamide (34). Compound **S68** (68 mg, 0.23 mmol, 1.0 equiv.) was dissolved in CH_2Cl_2 (5.0 mL) and cooled to 0 °C. mCPBA (max 77%; 47 mg, 0.9 equiv.) was added and the reaction stirred at 0 °C for 1 h. HPLC-MS showed no conversion therefore the reaction was warmed to r.t and stirred overnight. Water (10 mL) was added and the organic layer separated. The aqueous layer was extracted with CH_2Cl_2 (2×) and the combined organic layers washed with brine, reduced *in vacuo* and purified by RP ACC (gradient: 10→50% MeCN–H_2O in 0.1% formic acid). Appropriate fractions were pooled, extracted with CH_2Cl_2, and reduced *in vacuo* to reveal the titled compound (12 mg, 0.04 mmol, 17%) as a colorless semi-solid. **^{1}H NMR** (500 MHz, MeOD-d_4) δ 7.93 (d, *J* = 15.9 Hz, 1H), 7.77 (d, *J* = 8.5 Hz, 1H), 7.53 (d, *J* = 2.1 Hz, 1H), 7.40–7.30 (m, 2H), 3.87–3.70 (m, 2H), 3.03–2.93 (m, 1H), 2.76 (dd, *J* = 5.0,

4.0 Hz, 1H), 2.52 (dd, *J* = 5.0, 2.7 Hz, 1H), 1.95–1.76 (m, 2H), 1.71–1.59 (m, 1H), 1.56–1.45 (m, 1H). **^{13}C NMR** (151 MHz, MeOD-d_4) δ 167.6, 137.9, 137.0, 136.3, 133.4, 130.8, 129.9, 128.9, 121.2, 52.9, 48.9 (overlapped with solvent peak), 47.7, 30.7, 24.3. **HPLC**, t_R 6.07 min (>98%, UV_{254}). **HMRS** (ES^+) *m/z* calcd for $[C_{14}H_{16}Cl_2NO_3]^+$: 316.0502; found: 316.0517.

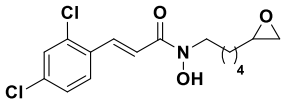
(*E*)-3-(2,4-dichlorophenyl)-*N*-hydroxy-*N*-(4-(oxiran-2-yl)butyl)acrylamide (35). Synthesized by



method G* using **S69** (62 mg, 0.20 mmol, 1.0 equiv.), mCPBA (max 77%; 44 mg, 0.20 mmol, 1.0 equiv.) and CH_2Cl_2 (5.0 mL). The crude product was purified by RP ACC (gradient: 10→50% MeCN–H_2O in 0.1% formic acid). Appropriate fractions were pooled and extracted with CH_2Cl_2, dried (Na_2SO_4) and reduced *in vacuo* to reveal the titled compound (18 mg, 0.06 mmol, 28%) as a colorless semi-solid. **^{1}H NMR** (500 MHz, MeOD-d_4) δ 7.93 (d, *J* = 15.9 Hz, 1H), 7.77 (d, *J* = 8.5 Hz, 1H), 7.53 (d, *J* = 2.1 Hz, 1H), 7.39–7.31 (m, 2H), 3.83–3.67 (m, 2H), 2.96–2.91 (m, 1H), 2.74 (dd, *J* = 5.0, 4.0 Hz, 1H), 2.48 (dd, *J* = 5.0, 2.8 Hz, 1H), 1.76 (quint, *J* = 7.1 Hz, 2H), 1.67–1.58 (m, 1H), 1.57–1.46 (m, 3H). **^{13}C NMR** (151 MHz, MeOD-d_4) δ 167.5, 137.8, 137.0, 136.3, 133.4, 130.8, 129.9, 128.9, 121.2, 53.3, 48.9 (overlapped with solvent peak), 47.7, 33.2, 27.5, 24.2. **HPLC**, t_R 6.30 min (>98%, UV_{254}). **HMRS** (ES^+) *m/z* calcd for $[C_{15}H_{18}Cl_2NO_3]^+$: 330.0658; found: 330.0667.

*Reaction carried out under atmospheric conditions.

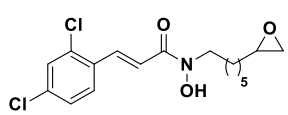
(*E*)-3-(2,4-dichlorophenyl)-*N*-hydroxy-*N*-(5-(oxiran-2-yl)pentyl)acrylamide (36). Synthesized by



method G using **S70** (76 mg, 0.23 mmol, 1.0 equiv.), mCPBA (max 77%; 76 mg, 0.44 mmol, 1.5 equiv.) and CH_2Cl_2 (8.0 mL). The crude product was purified by RP ACC (gradient: 10→60% MeCN–H_2O in 0.1% formic acid). Appropriate fractions* were pooled and extracted with CH_2Cl_2, dried (Na_2SO_4) and reduced *in vacuo* to reveal the titled compound (46 mg, 0.13 mmol, 56%) as a colorless semi-solid. **^{1}H NMR** (600 MHz, MeOD-d_4) δ 7.92 (d, *J* = 15.9 Hz, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.53 (d, *J* = 2.0 Hz, 1H), 7.40–7.30 (m, 2H), 3.73 (t, *J* = 7.1 Hz, 2H), 2.96–2.89 (m, 1H), 2.73 (dd, *J* = 5.0, 4.0 Hz, 1H), 2.47 (dd, *J* = 5.0, 2.7 Hz, 1H), 1.72 (p, *J* = 7.2 Hz, 2H), 1.62–1.47 (m, 4H), 1.47–1.38 (m, 2H).**^{13}C NMR** (151 MHz, MeOD-d_4) δ 167.4, 137.7, 136.9, 136.3, 133.4, 130.7, 129.9, 128.9, 121.2, 53.3, 47.7, 33.4, 27.6, 27.5, 26.7. **HPLC**, t_R 6.44 min (>98%, UV_{280}). **HMRS** (ES^+) *m/z* calcd for $[C_{16}H_{20}Cl_2NO_3]^+$: 344.0815; found: 344.0826.

*Close-running impurity towards end of desired product peak.

(*E*)-3-(2,4-dichlorophenyl)-*N*-hydroxy-*N*-(6-(oxiran-2-yl)hexyl)acrylamide (37). Synthesized by



method G using **S71** (80 mg, 0.23 mmol, 1.0 equiv.), mCPBA (max 77%; 73 mg, 0.42 mmol, 1.4 equiv.) and CH_2Cl_2 (8.0 mL). The crude product was purified using the same protocol as outlined in the synthesis of **34** and the titled compound (37 mg, 0.10 mmol, 44%) collected as a pale-yellow semi-solid. **^{1}H NMR** (600 MHz, DMSO-d_6)

δ 10.01 (s, 1H), 7.92–7.85 (m, 1H), 7.75–7.70 (m, 2H), 7.48 (d, *J* = 8.6 Hz, 1H), 7.31 (d, *J* = 15.8 Hz, 1H), 3.61 (br.s, 2H), 2.86 (br.s, 1H), 2.65 (t, *J* = 4.5 Hz, 1H), 2.41 (dd, *J* = 5.2, 2.6 Hz, 1H), 1.58 (quint, *J* = 7.1 Hz, 2H), 1.49–1.21 (m, 8H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 164.4, 134.8, 134.6, 134.2, 131.8, 129.4, 129.2, 128.0, 121.3, 51.5, 47.5, 46.1, 31.8, 28.5, 26.2, 26.1, 25.4. **HPLC**, t_R 6.69 min (>98%, UV_{280}). **HMRS** (ES^+) *m/z* calcd for $[C_{17}H_{22}Cl_2NO_3]^+$: 358.0971; found: 358.0980.

4.4.13 Aldehyde

(*E*)-3-(2,4-dichlorophenyl)-*N*-hydroxy-*N*-(6-oxohexyl)acrylamide (38). Intermediate **S68** (65 mg, 0.20 mmol) was dissolved in anh. CH_2Cl_2 (10 mL) and transferred to an ozone bubbler system (equipped with an aq. KI ozone trap) under a positive pressure of nitrogen. The reaction mixture was bubbled with nitrogen for 10 min upon cooling to -78 °C and then bubbled with oxygen for 5 min. Ozone was then generated using an ozonator (115 V) and the reaction mixture bubbled with ozone for 2 min*. Upon the KI trap turning brown, the ozonator was immediately turned off and excess ozone purged with nitrogen for 10 min. Upon confirmation of the trioxolane (HPLC-MS), DMS (1.0 mL) was added to the reaction mixture which was stirred overnight at r.t. Solvent was removed under a stream of nitrogen and the crude product was purified by RP ACC (gradient: 10→50% MeCN–H_2O in 0.1% formic acid) to afford the titled compound (8 mg, 0.02 mmol, 12%) as an off-white solid after lyophilization. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 10.10 (br.s, 1H), 9.69–9.61 (m, 1H), 7.87 (dd, *J* = 8.4, 2.3 Hz, 1H), 7.75–7.69 (m, 2H), 7.47 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.31 (d, *J* = 15.8 Hz, 1H), 3.61 (br.t, *J* = 7.0 Hz, 2H), 2.42 (td, *J* = 7.2, 1.6 Hz, 2H), 1.62–1.50 (m, 4H), 1.32–1.22 (m, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 203.5, 164.4, 134.8, 134.6, 134.2, 131.8, 129.4, 129.2, 128.0, 121.3, 47.4, 42.9, 26.1, 25.7, 21.2. **HPLC**, t_R 6.22 min (>98%, UV_{280}). **HMRS** (ES^+) *m/z* calcd for $C_{15}H_{18}Cl_2NO_3^+$ $[M+H]^+$, 330.0658; found: 330.0673.

*The amount of ozone is difficult to control. As the compound contained the cinnamic acid double bond, too much ozone led to complete decomposition of the starting material.

4.4.14 Amide control compounds

(*E*)-*N*-(3-aminopropyl)-3-(2,4-dichlorophenyl)acrylamide formate (S72). Synthesized by method C1 using 1,3-diaminopropane (837 µL, 10.0 mmol, 8.5 equiv.), *trans*-2,4-dichlorocinnamic acid (384 mg, 1.77 mmol, 1.2 equiv.), HATU (673 mg, 1.77 mmol, 1.0 equiv.), *i*Pr_2NEt (617 µL, 3.54 mmol, 2.0 equiv.) and anh. DMF (10 mL). DMF was removed *in vacuo* and the crude residue purified by RP ACC (gradient: 10→30% MeCN–H_2O in 0.1% formic acid) to afford the titled compound (103 mg, 0.32 mmol, 18%*) as an off-white sticky solid after lyophilization. **^{1}H NMR** (600 MHz, MeOD-d_4) δ 8.55 (s, 1H), 7.91 (d, *J* = 15.7 Hz, 1H), 7.72 (d, *J* = 8.5 Hz, 1H), 7.54 (s, 1H), 7.38 (d, *J* = 8.6 Hz, 1H), 6.65 (d, *J* = 15.7 Hz, 1H), 3.43 (t, *J* = 6.7 Hz, 2H), 2.97 (t, *J* =

7.3 Hz, 2H), 1.91 (quint, *J* = 6.9 Hz, 2H). **^{13}C NMR** (151 MHz, MeOD-d_4) δ 170.4, 168.5, 137.1, 136.5, 136.3, 133.1, 130.8, 129.8, 128.9, 124.9, 38.3, 37.2, 29.0. **MS** (ES^+) *m/z* calcd for $C_{12}H_{15}Cl_2N_2O^+$ $[M+H]^+$, 273.1; found: 273.0.

*The use of unprotected 1,3-diaminopropane led to large amounts of disubstituted byproduct. We advise using *N*-Boc-1,3-propanediamine instead.

(*E*)-3-(2,4-dichlorophenyl)-*N*-(3-(2-iodoacetamido)propyl)acrylamide (39). Synthesized by method B using **S72** (21 mg, 0.07 mmol, 1.0 equiv.), iodoacetic anhydride (41 mg, 0.12 mmol, 1.7 equiv.), *i*Pr_2NEt (35 µL, 0.20 mmol, 3.0 equiv.) and anh. CH_2Cl_2:DMF (6.0 mL, 1:1, v/v).* The reaction mixture was poured into 5% (aq., w/v) citric acid and extracted (2×) with EtOAc:hexanes (10:1, v/v). The combined organic layers were washed with brine, dried (Na_2SO_4), concentrated *in vacuo* and purified by RP ACC (gradient: 15→45% MeCN–H_2O in 0.1% formic acid) to afford the titled compound (11 mg, 0.04 mmol, 36%) as a colorless fluffy material after lyophilization. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 8.33–8.14 (m, 2H), 7.75–7.69 (m, 2H), 7.65 (d, *J* = 15.7 Hz, 1H), 7.50 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.69 (d, *J* = 15.7 Hz, 1H), 3.63 (s, 2H), 3.20 (q, *J* = 6.7 Hz, 2H), 3.09 (q, *J* = 6.7 Hz, 2H), 1.60 (quint, *J* = 7.0 Hz, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 167.6, 164.2, 134.4, 134.0, 132.7, 131.8, 129.4, 128.8, 128.0, 126.0, 37.0, 36.6, 28.9, 0.8. **HPLC**, t_R 6.01 min (>98%, UV_{254}). **HMRS** (ES^+) *m/z* calcd for $C_{14}H_{16}Cl_2IN_2O_2^+$ $[M+H]^+$, 440.9628; found: 440.9648.

*DMF added to aid dissolution.

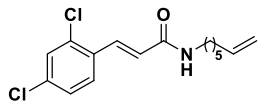
3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propan-1-aminium 2,2,2-trifluoroacetate (S73). The compound was synthesized as previously described[16]* to afford the titled compound (320 mg, 1.19 mmol, 56%), as an off-white solid. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 7.78 (br.s, 3H), 7.04 (s, 2H), 3.46 (t, *J* = 6.9 Hz, 2H), 2.83–2.73 (m, 2H), 1.77 (quint, *J* 6.9 Hz, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 171.1, 158.1 (d, *J* = 32.2 Hz), 134.6, 36.7, 34.4, 26.4. MS (ES^+) *m/z* calcd for $C_7H_{11}N_2O_2^+$ $[M+H]^+$, 155.1; found: 155.0. CAS RN: 886209-47-8. Data in agreement with literature.[16]

*The order of compound addition for the Mitsunobu reaction was adapted to reflect the order of addition for the synthesis of **S70**.

(*E*)-3-(2,4-dichlorophenyl)-*N*-(3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)propyl)acrylamide (40). Synthesized by method C1 using **S73** (66 mg, 0.26 mmol, 1.1 equiv.), *trans*-2,4-dichlorocinnamic acid (52 mg, 0.24 mmol, 1.0 equiv.), HATU (100 mg, 0.26 mmol, 1.1 equiv.), *i*Pr_2NEt (166 µL, 0.96 mmol, 4.0 equiv.) and anh. DMF (2.0 mL). The reaction mixture was worked up using EtOAc as outlined in method C1 and the crude residue purified by NP ACC to afford the titled compound (62 mg, 0.18 mmol, 73%) as a white solid. **TLC** (5% MeOH–CH_2Cl_2): R_f = 0.3. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 8.26 (t, *J* = 5.7 Hz, 1H), 7.72 (d, *J* = 8.5

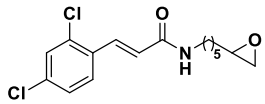
Hz, 1H), 7.69 (d, *J* = 2.2 Hz, 1H), 7.64 (d, *J* = 15.7 Hz, 1H), 7.49 (ddd, *J* = 8.4, 2.2, 0.6 Hz, 1H), 7.01 (s, 2H), 6.68 (d, *J* = 15.7 Hz, 1H), 3.44 (t, *J* = 7.2 Hz, 2H), 3.16 (q, *J* = 6.9 Hz, 2H), 1.70 (quint, *J* = 7.1 Hz, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 171.0, 164.2, 134.5, 134.4, 134.0, 132.8, 131.8, 129.4, 128.8, 128.0, 125.9, 36.5, 35.2, 28.2. **HPLC**, t_R 6.17 min (>98%, UV_{254}). **HMRS** (ES^+) *m/z* calcd for $C_{16}H_{15}Cl_2N_2O_3^+$ $[M+H]^+$, 353.0454; found: 353.0470.

(*E*)-3-(2,4-dichlorophenyl)-*N*-(hept-6-en-1-yl)acrylamide (S74) Synthesized by method C1 using



trans-2,4-dichlorocinnamic acid (100 mg, 0.46 mmol, 1.0 equiv.), hept-6-en-1-amine (62 mg, 0.55 mmol, 1.2 equiv.), HATU (209 mg, 0.55 mmol, 1.2 equiv.), *i*Pr_2NEt (27 µL, 0.69 mmol, 1.5 equiv.) and anh. DMF (2.0 mL). The reaction mixture was worked up using EtOAc as outlined in method C1 and the crude residue purified by RP ACC (gradient: 10–100% MeCN–H_2O in 0.1% formic acid). Appropriate fractions were pooled and extracted with CH_2Cl_2, dried (Na_2SO_4) and reduced *in vacuo* to reveal the titled compound (103 mg, 0.33 mmol, 72%) as a colorless semi-solid that solidified upon standing. **TLC** (50% EtOAc–hexanes): R_f = 0.5. **^{1}H NMR** (600 MHz, MeOD-d_4) δ 7.85 (d, *J* = 15.8 Hz, 1H), 7.67 (d, *J* = 8.6 Hz, 1H), 7.50 (d, *J* = 2.2 Hz, 1H), 7.34 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.61 (d, *J* = 15.7 Hz, 1H), 5.80 (m, 1H), 4.99 (dd, *J* = 17.1, 2.2 Hz, 1H), 4.92 (dd, *J* = 10.3, 2.1 Hz, 1H), 3.30–3.27 (m, 2H, overlapped with solvent peak), 2.06 (q, *J* = 7.0 Hz, 2H), 1.57 (p, *J* = 7.2 Hz, 2H), 1.47–1.34 (m, 4H). **^{13}C NMR** (151 MHz, MeOD-d_4) δ 167.6, 139.9, 136.8, 136.2, 135.8, 133.2, 130.7, 129.6, 128.8, 125.5, 114.9, 40.6, 34.8, 30.2, 29.7, 27.5. **MS** (ES^+) *m/z* calcd for $C_{16}H_{20}Cl_2NO^+$ $[M+Na]^+$, 312.1; found: 312.0.

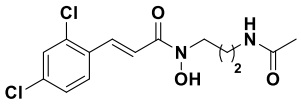
(*E*)-3-(2,4-dichlorophenyl)-*N*-(5-(oxiran-2-yl)pentyl)acrylamide (41) Synthesized by method G using



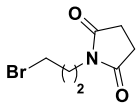
S74 (30 mg, 0.10 mmol, 1.0 equiv.), mCPBA (max 77%; 26 mg, 0.12 mmol, 1.2 equiv.) and CH_2Cl_2 (3.0 mL). The crude product was purified using RP ACC (gradient: 10–70% MeCN–H_2O in 0.1% formic acid). Appropriate fractions were pooled and extracted with CH_2Cl_2, dried (Na_2SO_4) and reduced *in vacuo* to reveal the titled compound (20 mg, 0.06 mmol, 63%) as a colorless semi-solid. **TLC** (50% EtOAc–hexanes): R_f = 0.1. **^{1}H NMR** (600 MHz, MeOD-d_4) δ 8.28 (br.t, *J* = 5.7 Hz, 1H), 7.86 (d, *J* = 15.7 Hz, 1H), 7.69 (d, *J* = 8.5 Hz, 1H), 7.52 (d, *J* = 2.1 Hz, 1H), 7.36 (ddd, *J* = 8.5, 2.2, 0.6 Hz, 1H), 6.62 (d, *J* = 15.7 Hz, 1H), 3.35–3.31 (m, 2H, overlapped with solvent peak), 2.95–2.90 (m, 1H), 2.73 (dd, *J* = 5.0, 4.0 Hz, 1H), 2.47 (dd, *J* = 5.0, 2.8 Hz, 1H), 1.64–1.55 (m, 3H), 1.55–1.47 (m, 3H), 1.47–1.40 (m, 2H). ^{13}C NMR (151 MHz, MeOD-d_4) δ 167.8*, 136.8, 136.2, 135.9, 133.3, 130.8, 129.7, 128.8, 125.5*, 53.3, 47.7*, 40.7*, 33.5*, 30.3*, 27.8, 26.8. **HPLC**, t_R 6.50 min (>98%, UV_{254}). **HMRS** (ES^+) *m/z* calcd for $C_{16}H_{20}Cl_2NO_2^+$ $[M+H]^+$, 328.0866; found: 328.0873.

*Peaks appear as double due to amide rotamers.

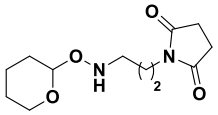
4.4.15 Acetyl control

(*E*)-*N*-(3-acetamidopropyl)-3-(2,4-dichlorophenyl)-*N*-hydroxyacrylamide (42). Synthesized by  method B using **S28** (30 mg, 0.07 mmol, 1.0 equiv.), acetic anhydride (7 µL, 0.07 mmol, 1.0 equiv.), *i*Pr_2NEt (54 µL, 0.31 mmol, 4.2 equiv.) and anh. CH_2Cl_2 (3 mL). The reaction mixture was reduced *in vacuo* and the crude product purified by RP ACC (gradient: 10→50% MeCN–H_2O in 0.1% formic acid) to afford the titled compound (4 mg, 0.01 mmol, 16%) as a colorless fluffy material after lyophilization. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 7.88 (d, *J* = 8.5 Hz, 1H), 7.85 (t, *J* = 5.3 Hz, 1H), 7.75–7.70 (m, 2H), 7.47 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.32 (d, *J* = 15.9 Hz, 1H), 3.62 (br.t, *J* = 6.9 Hz, 2H), 3.06 (q, *J* = 6.6 Hz, 2H), 1.79 (s, 3H), 1.71 (quint, *J* = 7.2 Hz, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 169.1, 164.5, 134.8, 134.6, 134.2, 131.8, 129.4, 129.2, 128.0, 121.4, 45.8, 36.3, 26.7, 22.6. **HPLC**, t_R 5.64 min (>98%, UV_{254}). **HMRS** (ES^+) *m/z* calcd for $C_{14}H_{17}Cl_2N_2O_3^+$ $[M+H]^+$, 331.0611; found: 331.0625.

4.4.16 Succinimide control

1-(3-bromopropyl)pyrrolidine-2,5-dione (S75). The compound was synthesized as previously  described[17] using succinimide (990 mg, 10.0 mmol, 1.0 equiv.), 1,3-dibromopropane (3.04 mL, 30.0 mmol, 3.0 equiv.), K_2CO_3 (4.15 g, 30.0 mmol, 3.0 equiv.) and TBAB (322 mg, 1.00 mmol, 0.1 equiv.). The crude product was purified by NP ACC to afford the titled compound (674 mg, 3.06 mmol, 31%) as a white solid*. **TLC** (50% EtOAc–hexanes): R_f = 0.3. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.66 (t, *J* = 7.0 Hz, 2H), 3.37 (t, *J* = 6.7 Hz, 2H), 2.72 (s, 4H), 2.16 (quint, *J* 6.7 Hz, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 177.2, 37.8, 30.8, 29.9, 28.3. MS (ES^+) *m/z* calcd for $C_7H_{11}BrNO_2^+$ $[M+H]^+$, 220.0; found: 219.9. CAS RN: 88661-56-7. Data in agreement with literature.[18]

*In our hands, the applied method with absence of solvent did not lead to satisfactory yields, but further reaction optimization was not pursued.

1-(3-(((tetrahydro-2*H*-pyran-2-yl)oxy)amino)propyl)pyrrolidine-2,5-dione (S76). Synthesized by  method A1 using OTX (139 mg, 1.19 mmol, 1.5 equiv.), **S75** (174 mg, 0.79 mmol, 1.0 equiv.), K_2CO_3 (164 mg, 1.19 mmol, 1.5 equiv.) and DMF (4.0 mL). The reaction mixture was worked up and purified in the same manner as outlined in the synthesis of **S1**. The titled compound (92 mg, 0.36 mmol, 45%) as a colorless oil. **TLC** (5% MeOH–CH_2Cl_2): R_f = 0.2. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 4.79–4.74 (m, 1H), 3.91–3.85 (m, 1H), 3.64–3.49 (m, 3H), 3.00–2.94 (m, 1H), 2.94–2.86 (m, 1H), 2.67 (s, 4H), 1.85–1.76 (m, 2H), 1.76–1.64 (m, 2H), 1.55–1.44 (m, 4H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 177.4, 101.6, 63.1, 49.4, 36.8, 29.2, 28.2, 25.6, 25.4, 20.2. **MS** (ES^+) *m/z* calcd for $C_{12}H_{21}N_2O_4^+$ $[M+H]^+$, 257.1; found: 257.1.

(*E*)-3-(2,4-dichlorophenyl)-*N*-(3-(2,5-dioxopyrrolidin-1-yl)propyl)-*N*-((tetrahydro-2*H*-pyran-2-yl)oxy)acrylamide (S77). Synthesized by method C1 using *trans*-2,4-dichlorocinnamic acid (71 mg, 0.33 mmol, 1.0 equiv.), **S76** (92 mg, 0.36 mmol, 1.1 equiv.), HATU (136 mg, 0.36 mmol, 1.1 equiv.) and *i*Pr_2NEt (114 µL, 0.65 mmol, 2.0 equiv.) and DMF (3.0 mL). The reaction mixture was worked up using EtOAc as outlined in method C1 and the crude residue purified by NP ACC to afford the titled compound (139 mg, 0.31 mmol, 93%) as a colorless oil.* **TLC** (50% EtOAc–hexanes): R_f = 0.2. **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.95 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.42 (d, *J* = 2.1 Hz, 1H), 7.25 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.01 (d, *J* = 15.8 Hz, 1H), 4.91 (dd, *J* = 5.5, 2.8 Hz, 1H), 4.01–3.88 (m, 2H), 3.84–3.75 (m, 1H), 3.64–3.56 (m, 3H), 2.70 (s, 4H), 2.08–1.99 (m, 2H, overlapped with residual EtOAc), 1.91–1.79 (m, 2H), 1.76–1.69 (m, 1H), 1.67–1.56 (m, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 177.3, 166.9, 138.1, 135.8, 135.6, 132.3, 130.1, 128.6, 127.6, 120.0, 105.4, 64.2, 46.2, 36.7, 29.3, 28.3, 25.5, 25.0, 20.0. **MS** (ES^+) *m/z* calcd for $C_{21}H_{24}Cl_2N_2NaO_5^+$ $[M+Na]^+$, 477.1; found: 477.1.

*Impurity present at ~10% – removed in next step.

(*E*)-3-(2,4-dichlorophenyl)-*N*-(3-(2,5-dioxopyrrolidin-1-yl)propyl)-*N*-hydroxyacrylamide (43). Synthesized by method F using **S77** (117 mg, 0.26 mmol, 1.0 equiv.), PPTS (51 mg, 0.20 mmol, 0.8 equiv.) and abs. EtOH (10 mL). The reaction mixture was concentrated *in vacuo* and purified by RP ACC (gradient: 10→50% MeCN–H_2O in 0.1% formic acid) to afford the titled compound (50 mg, 0.13 mmol, 52%) as a colorless fluffy material after lyophilization. **^{1}H NMR** (600 MHz, DMSO-d_6) δ 10.26 (br.s, 1H), 7.89 (d, *J* = 8.5 Hz, 1H), 7.75–7.70 (m, 2H), 7.47 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.30 (d, *J* = 15.9 Hz, 1H), 3.61 (br.t, *J* = 7.3 Hz, 2H), 3.40 (t, *J* = 7.4 Hz, 2H), 2.61 (s, 4H), 1.81 (quint, *J* = 7.4 Hz, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 177.7, 164.6, 134.9, 134.7, 134.2, 131.8, 129.4, 129.2, 128.0, 121.2, 45.7, 35.9, 28.0, 24.9. **HPLC**, t_R 5.89 min (>98%, UV_{254}). **HMRS** (ES^+) *m/z* calcd for $C_{16}H_{17}Cl_2N_2O_4^+$ $[M+H]^+$, 371.0560; found: 371.0577.

5.0 Supplementary References

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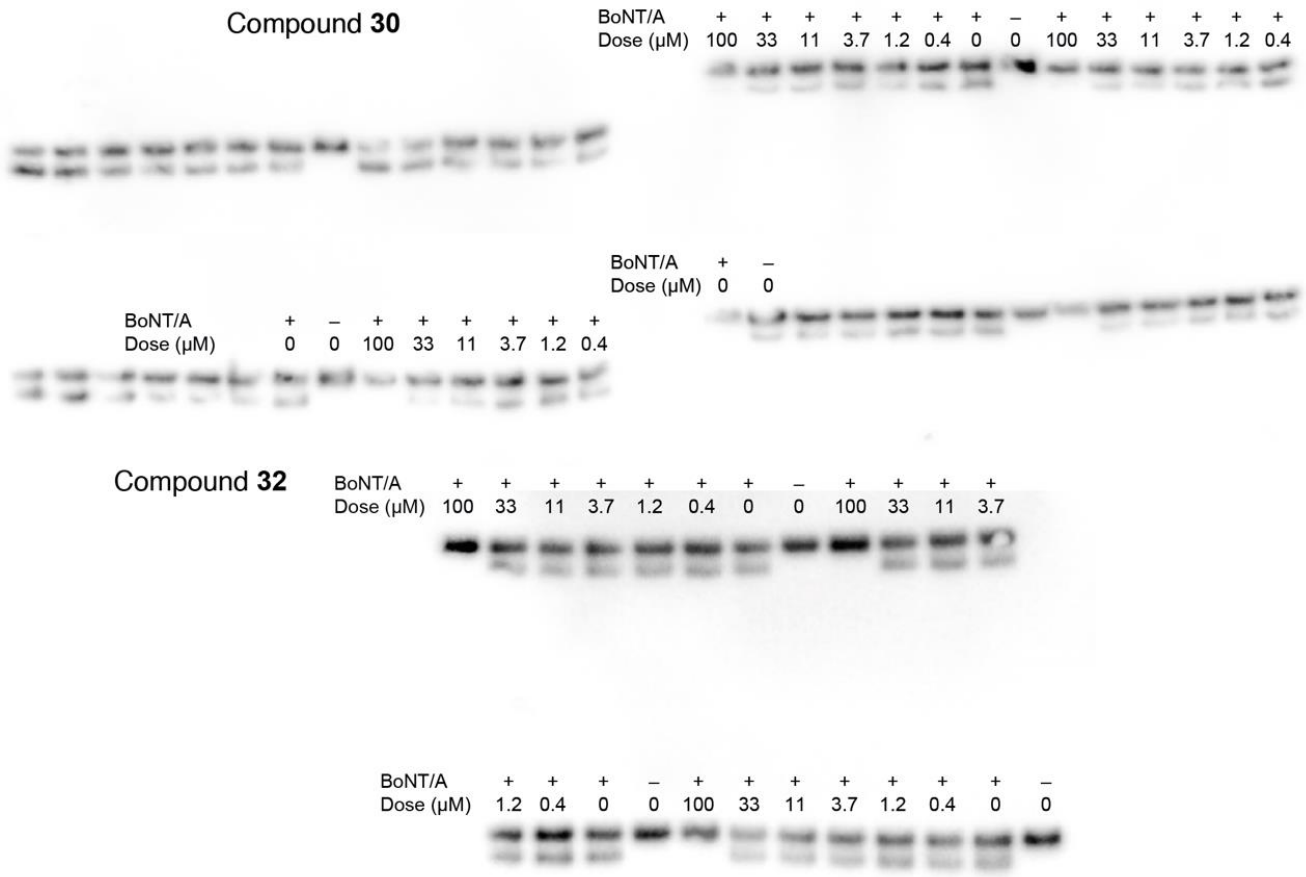
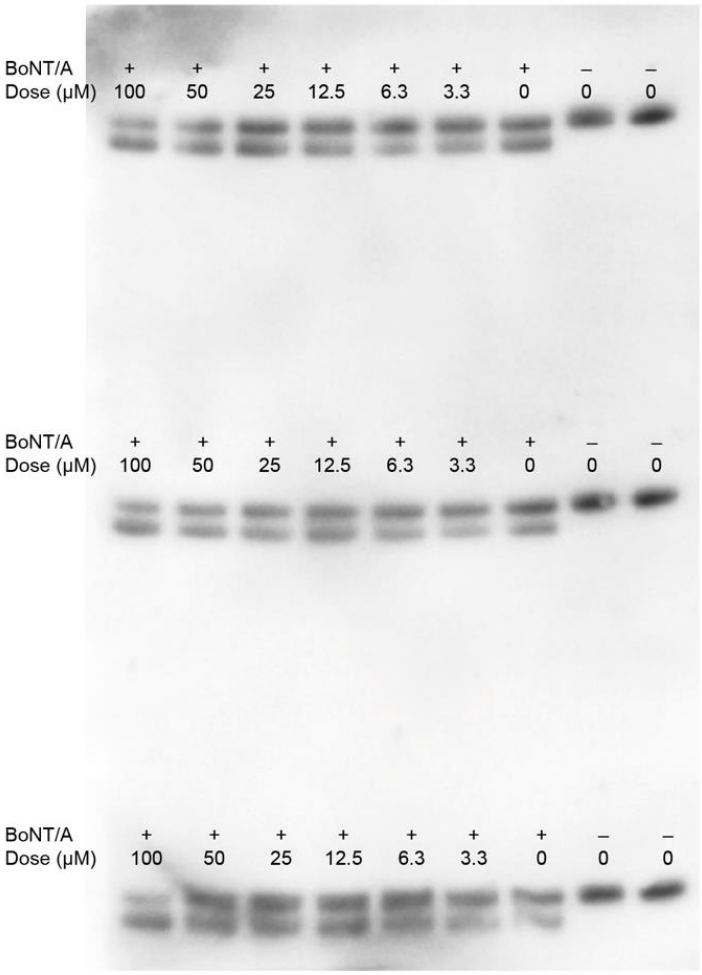
6.0 Appendix

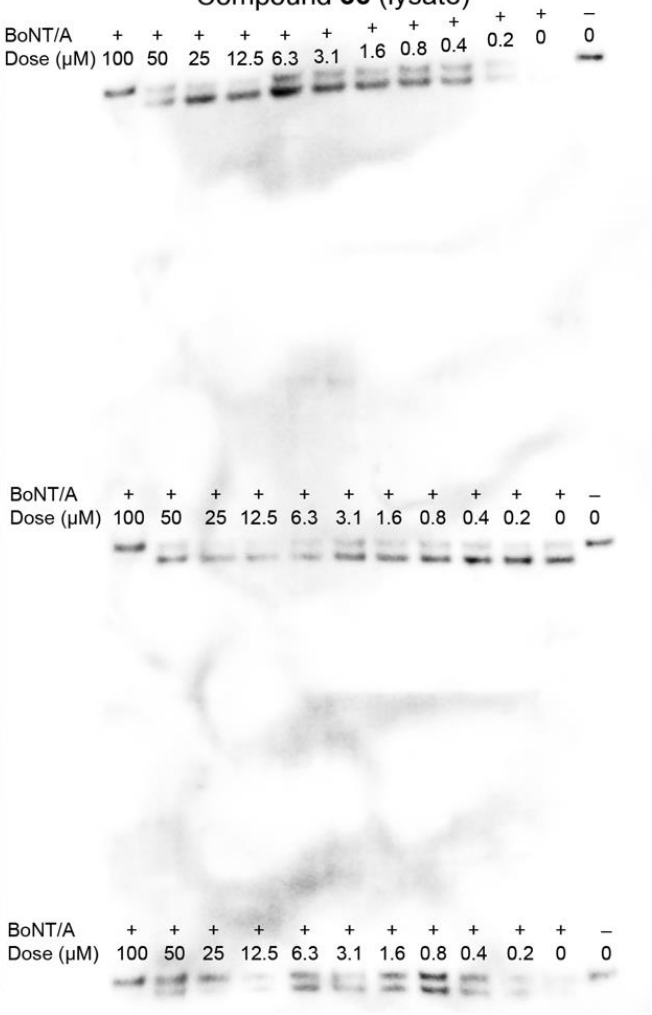
6.1 Full immunoblots of BoNT/A cellular assays



Compound **36** (whole cells)

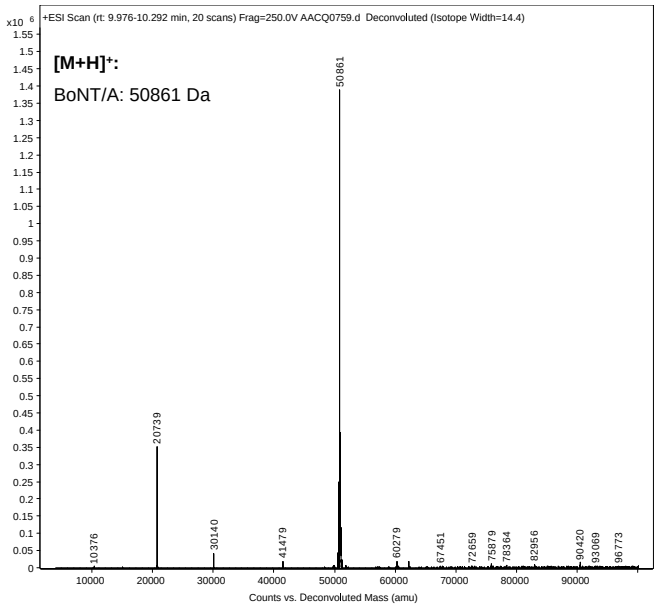


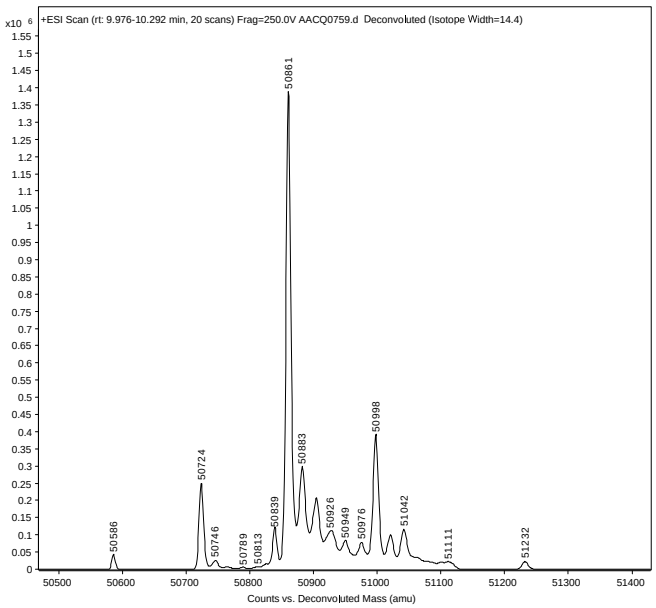
Compound **36** (lysate)



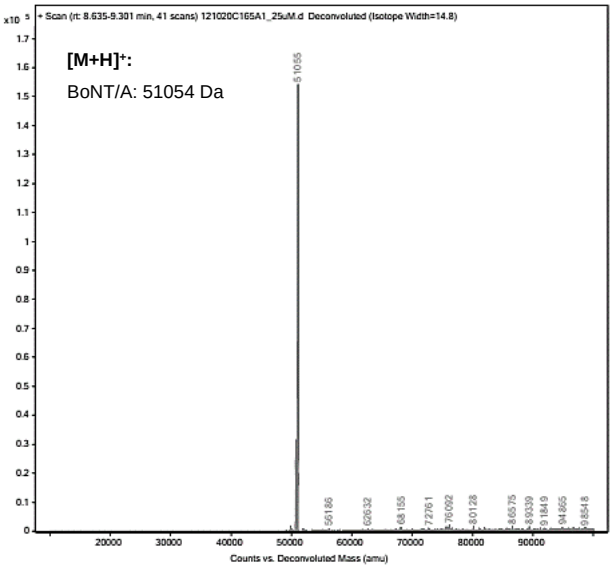
6.2 Raw deconvoluted BoNT/A LC HRMS traces

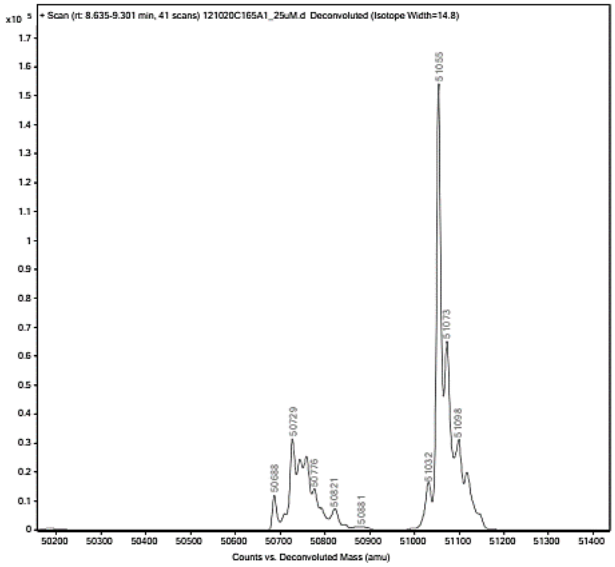
BoNT/A (WT)





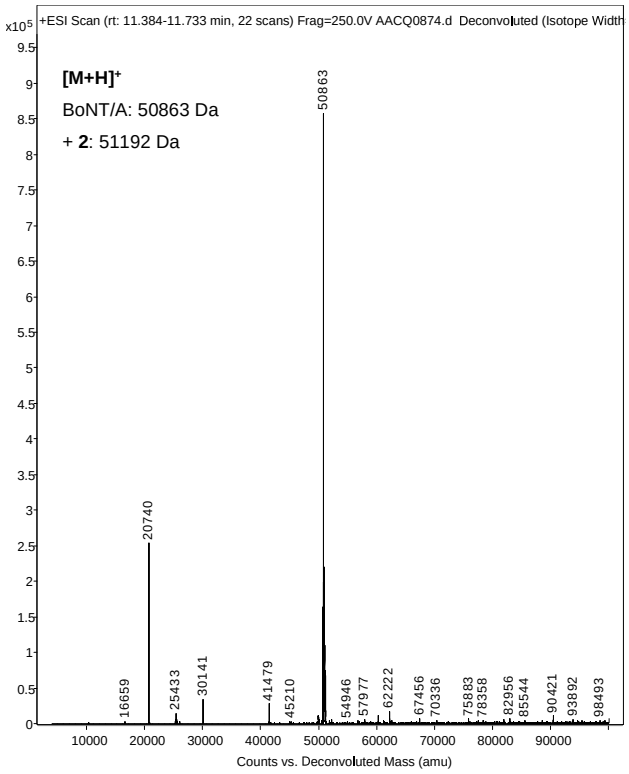
BoNT/A (C165A)

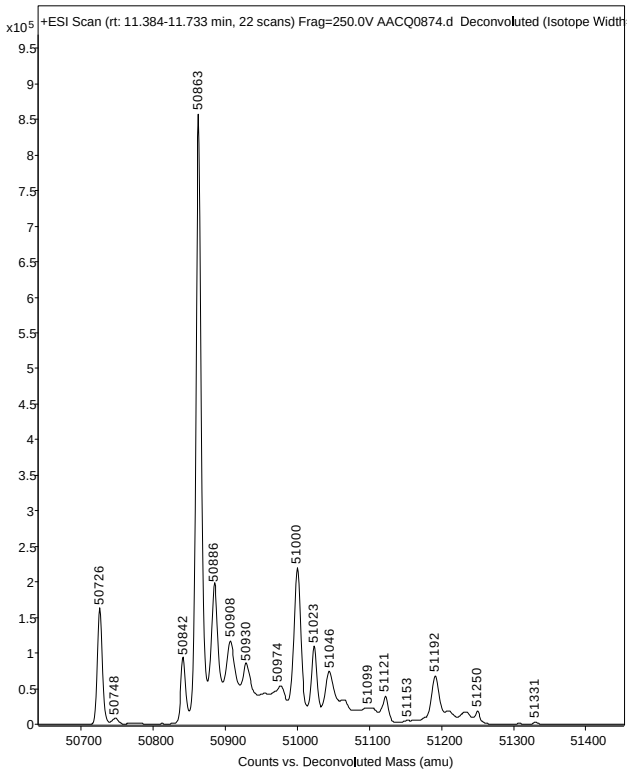




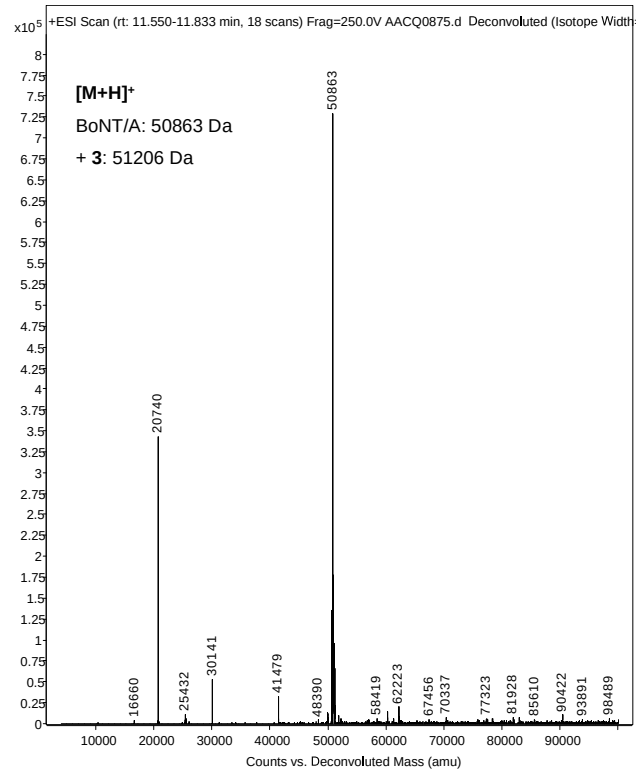
Note: Mass difference in WT and C165A is due to differing amounts of cleavage of terminal amino acids upon expression – C165A is correct construct mass (-minus initial methionine).

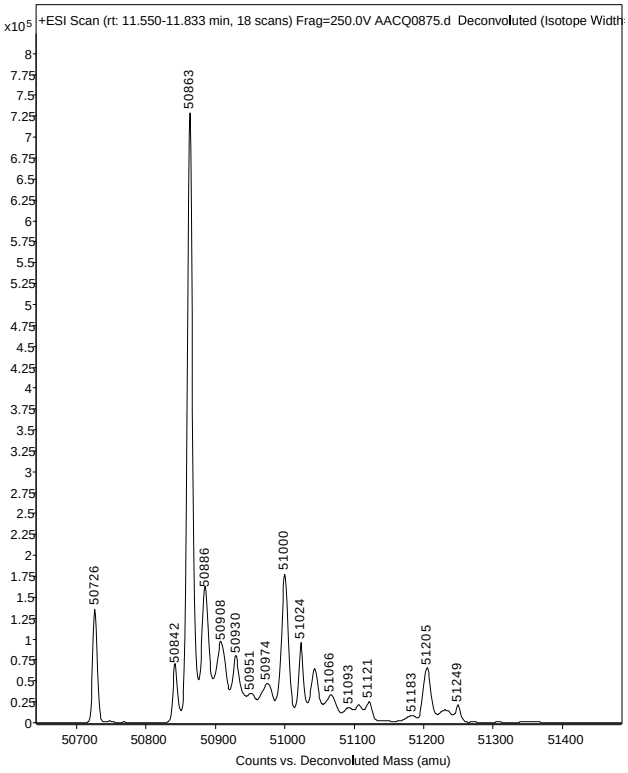
BoNT/A + **2**



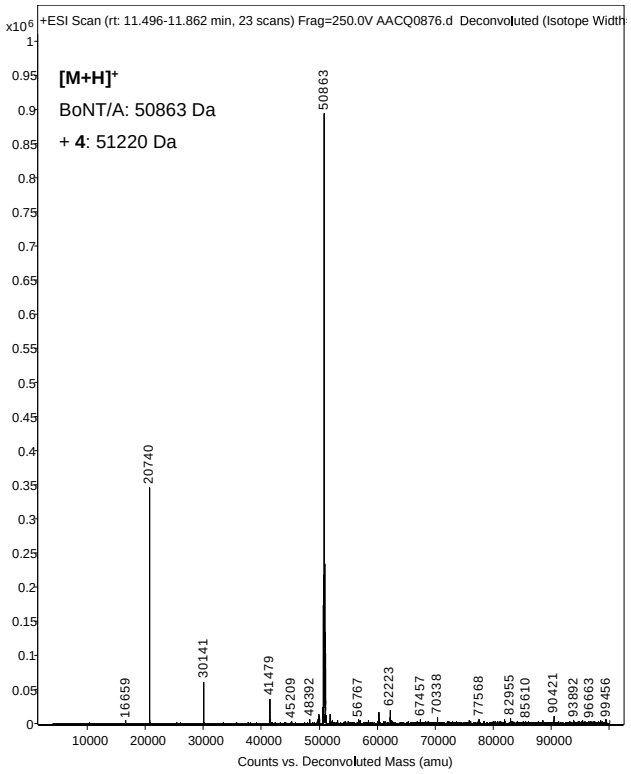


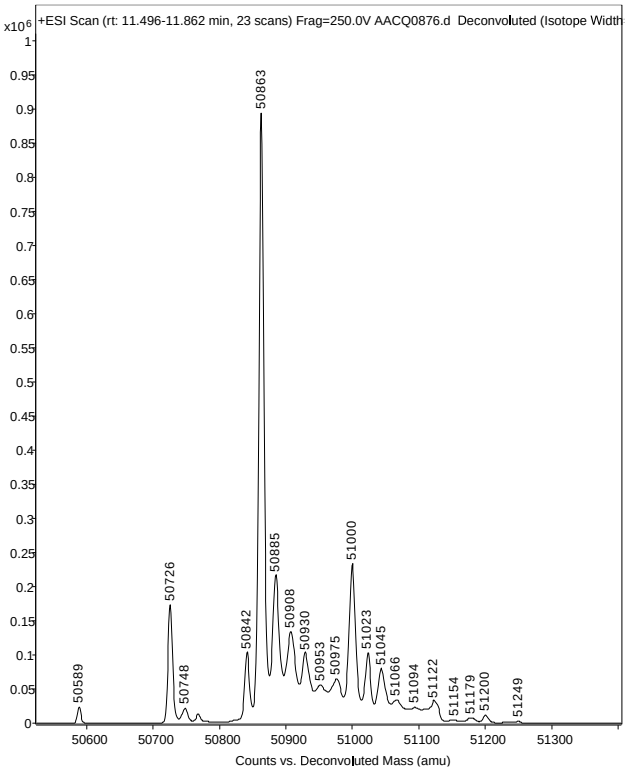
BoNT/A + **3**



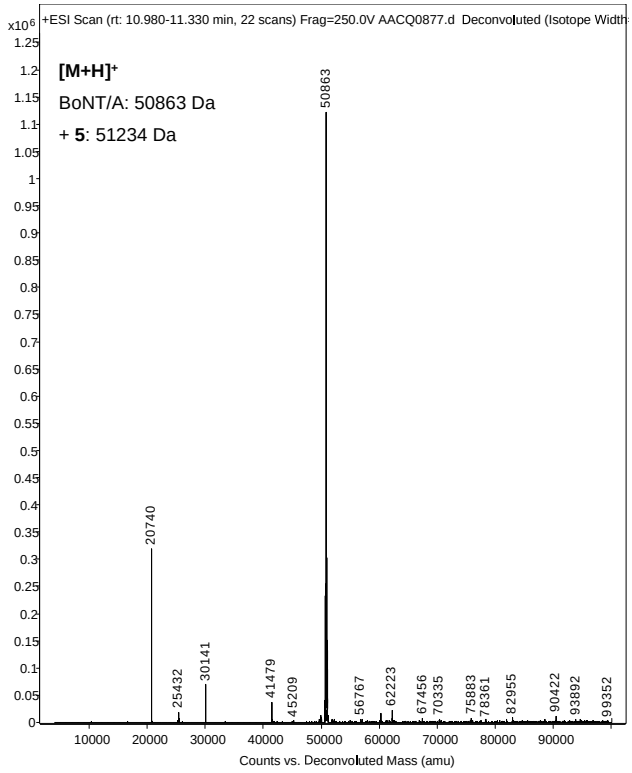


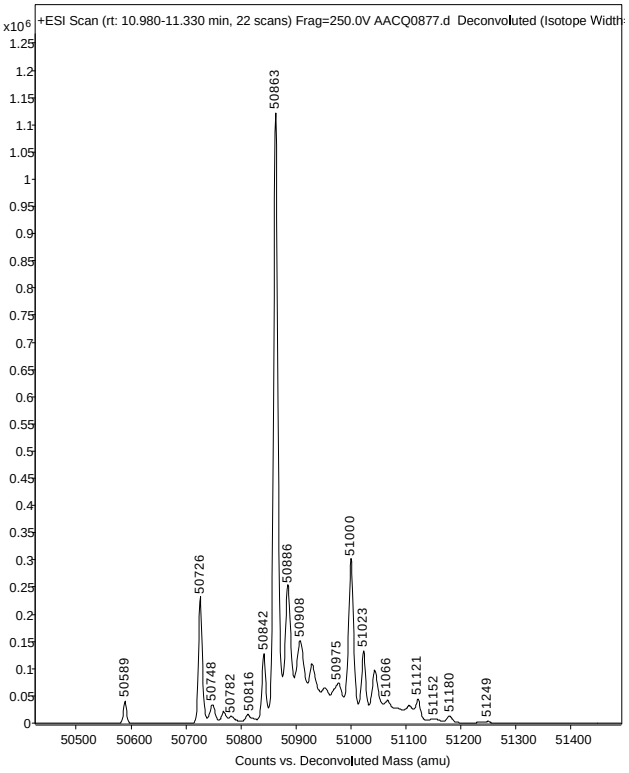
BoNT/A + **4**



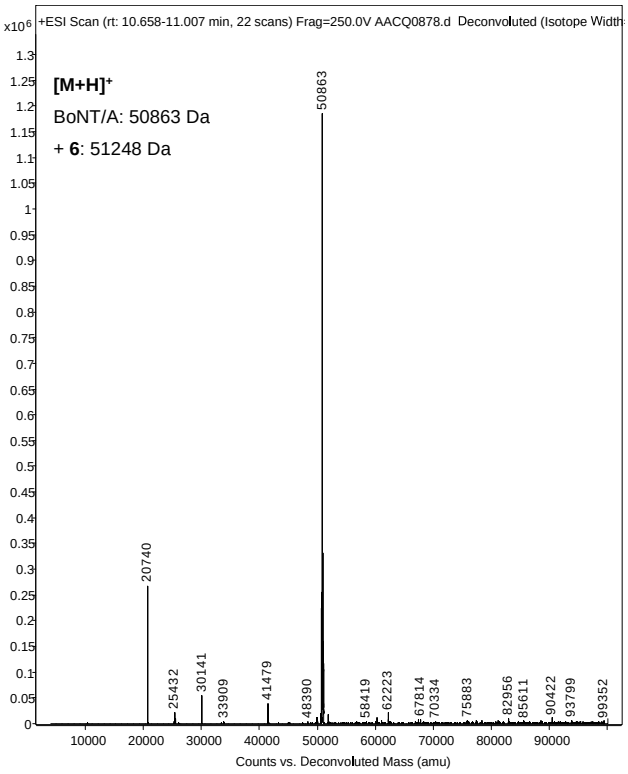


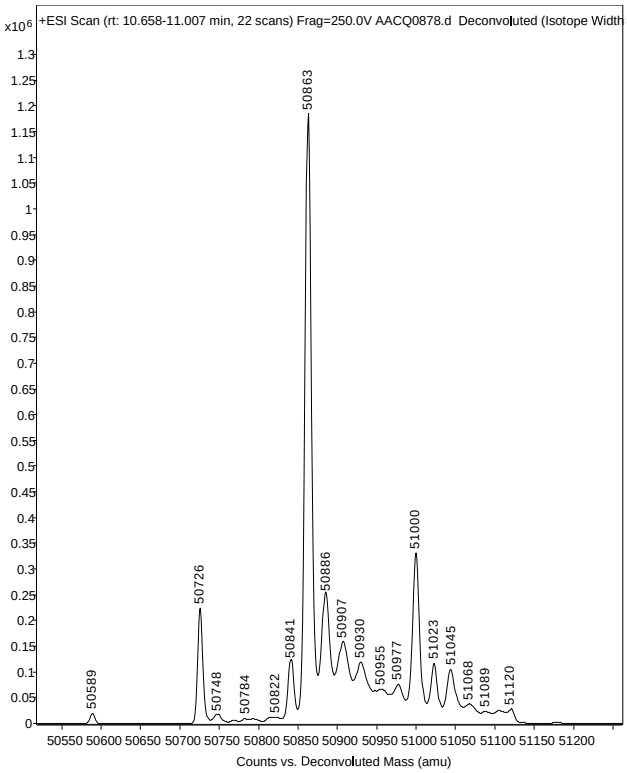
BoNT/A + **5**



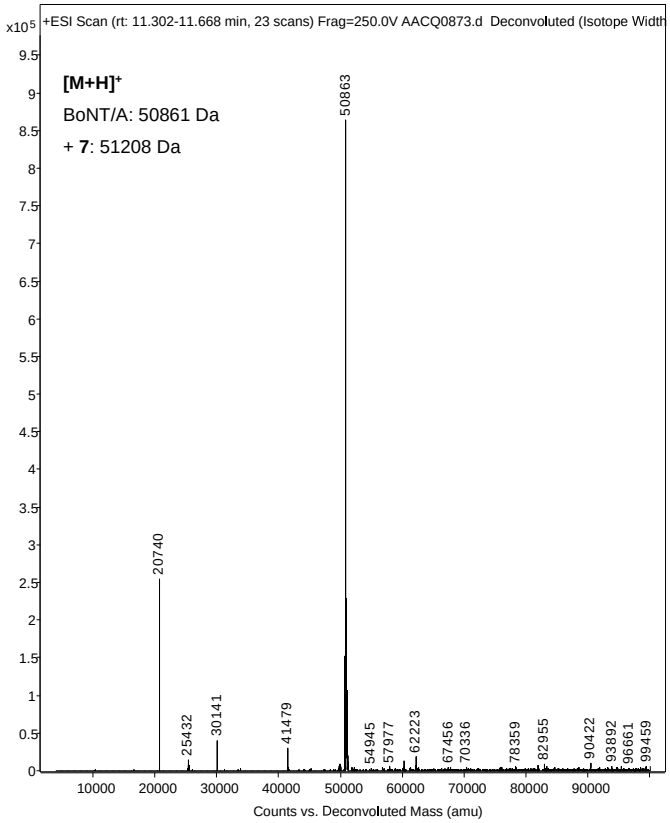


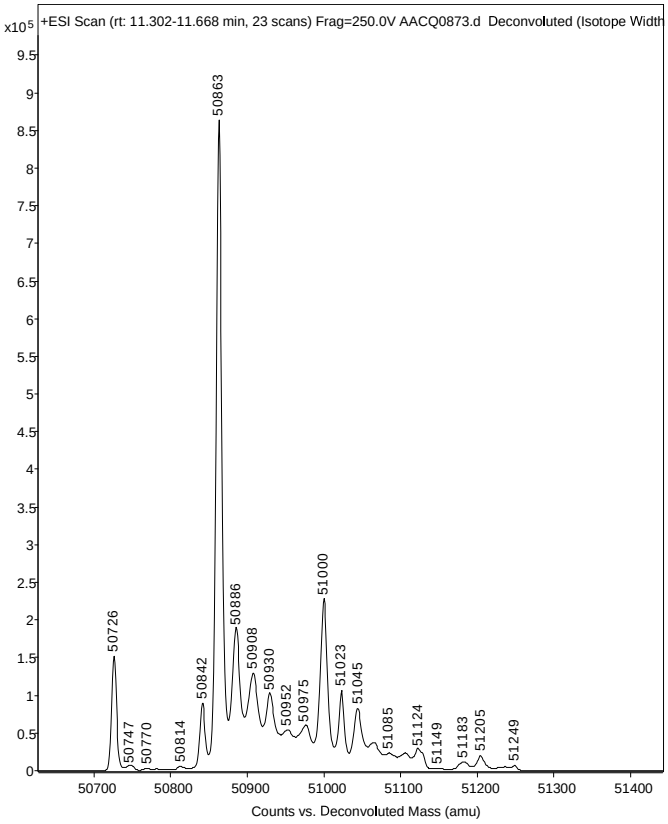
BoNT/A + **6**



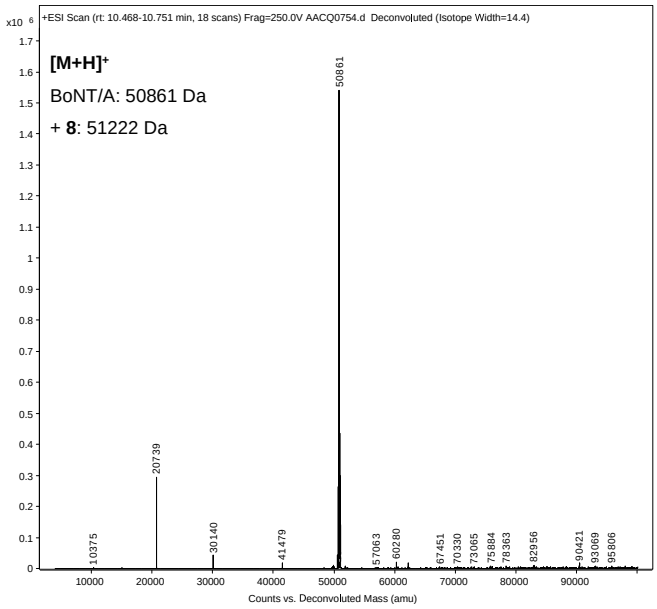


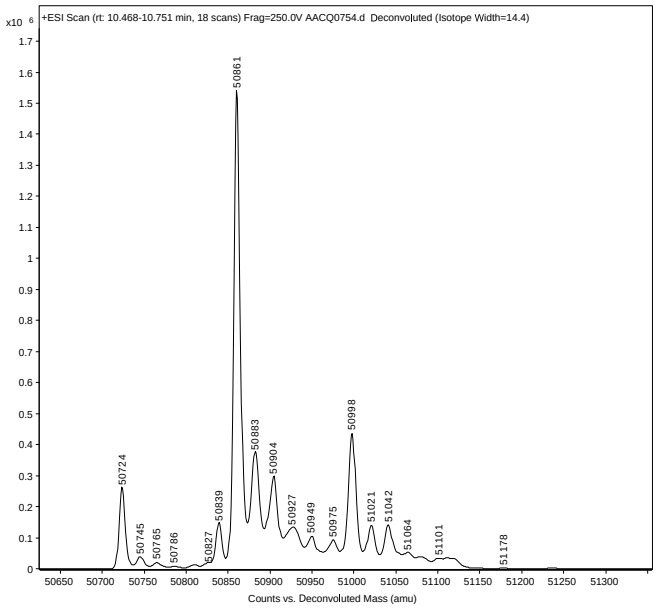
BoNT/A + **7**



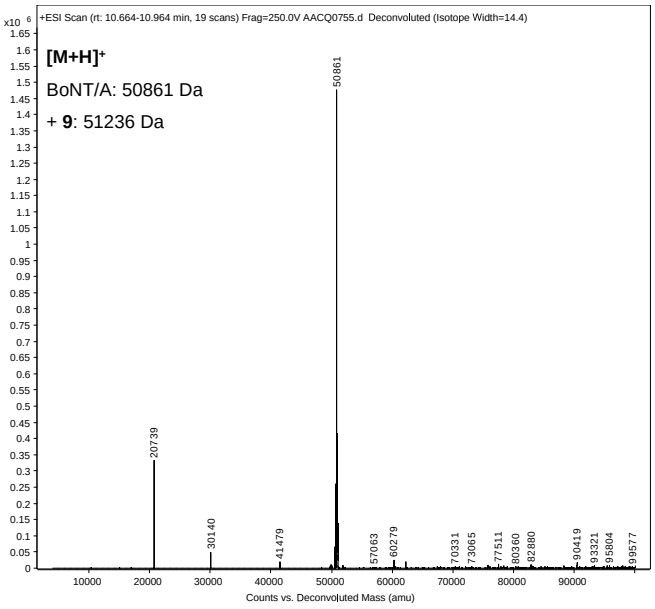


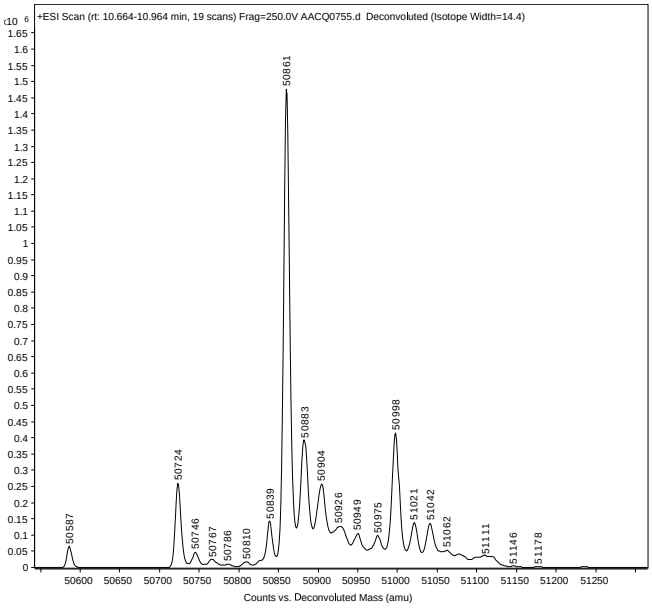
BoNT/A + **8**



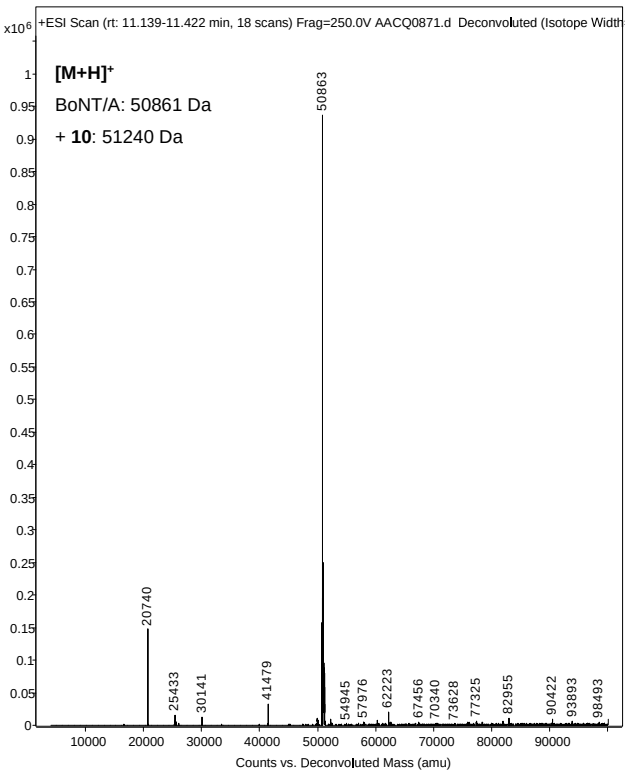


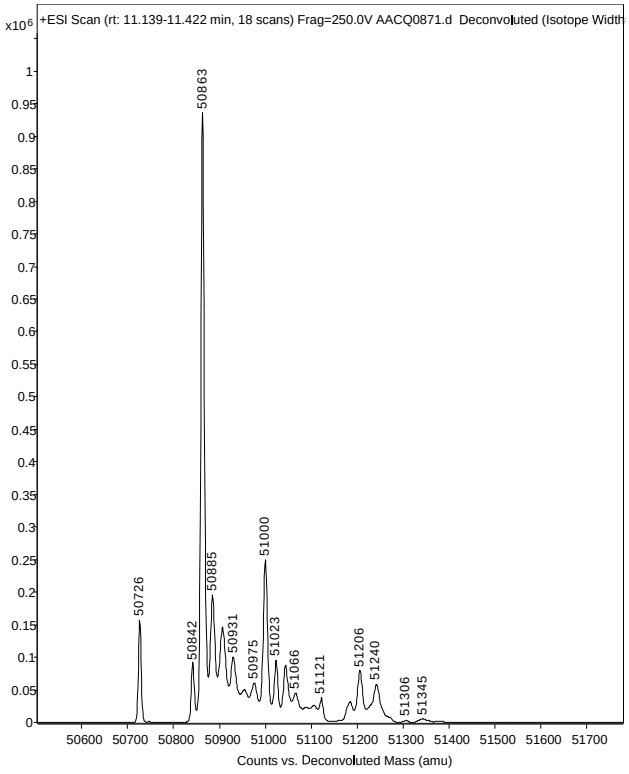
BoNT/A + **9**



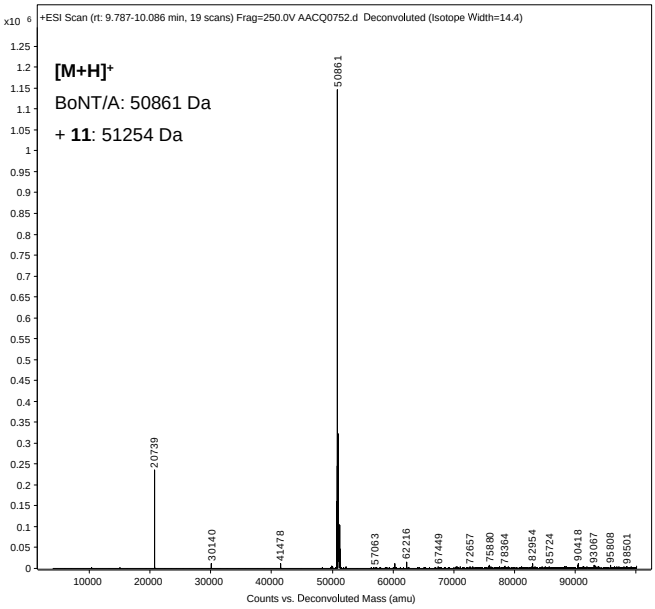


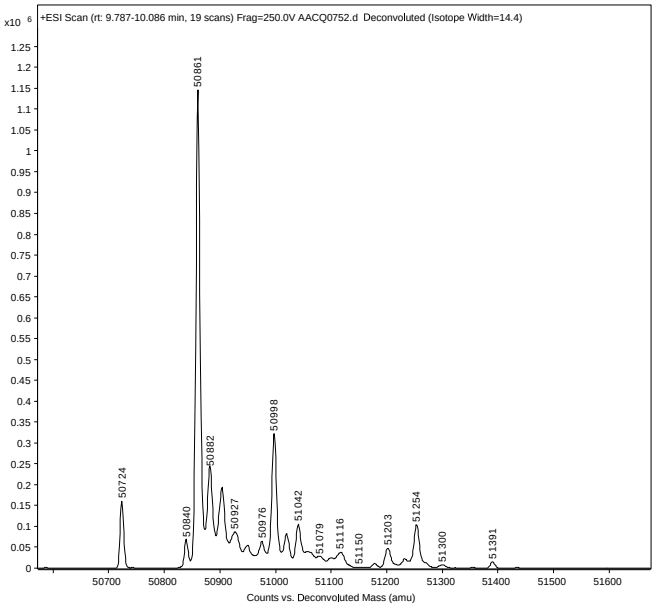
BoNT/A + **10**



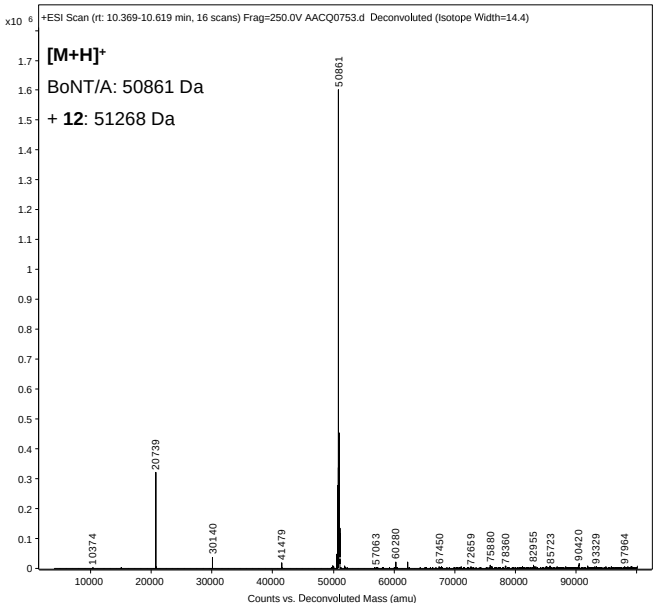


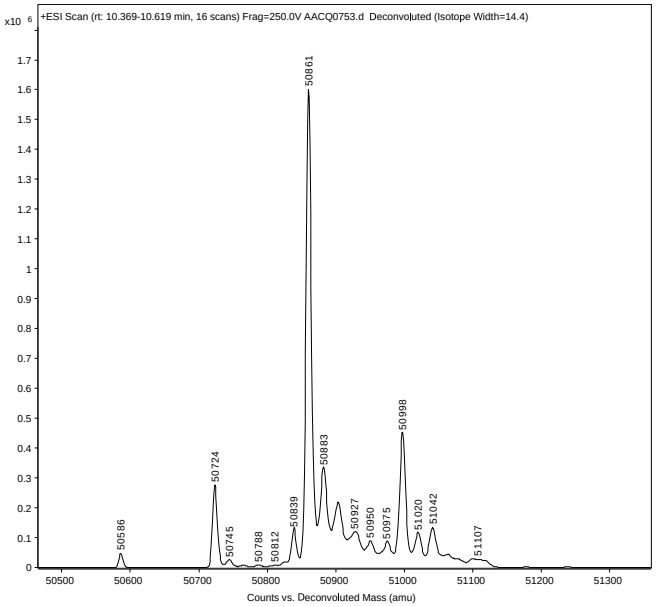
BoNT/A + **11**





BoNT/A + **12**





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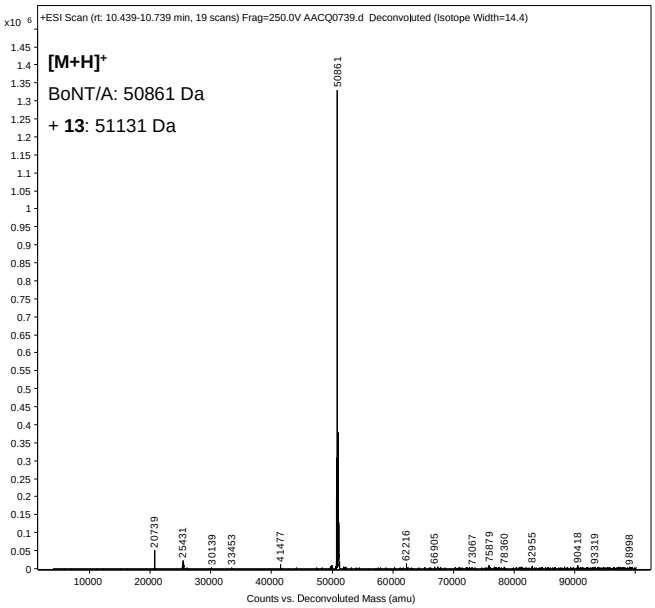
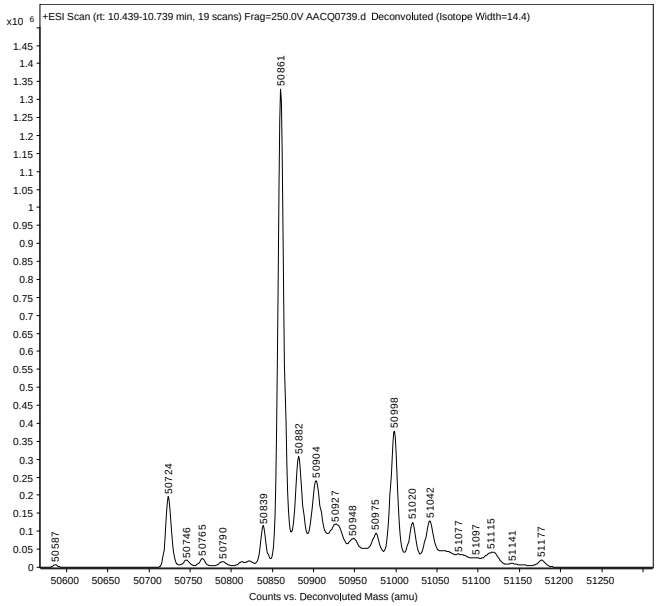
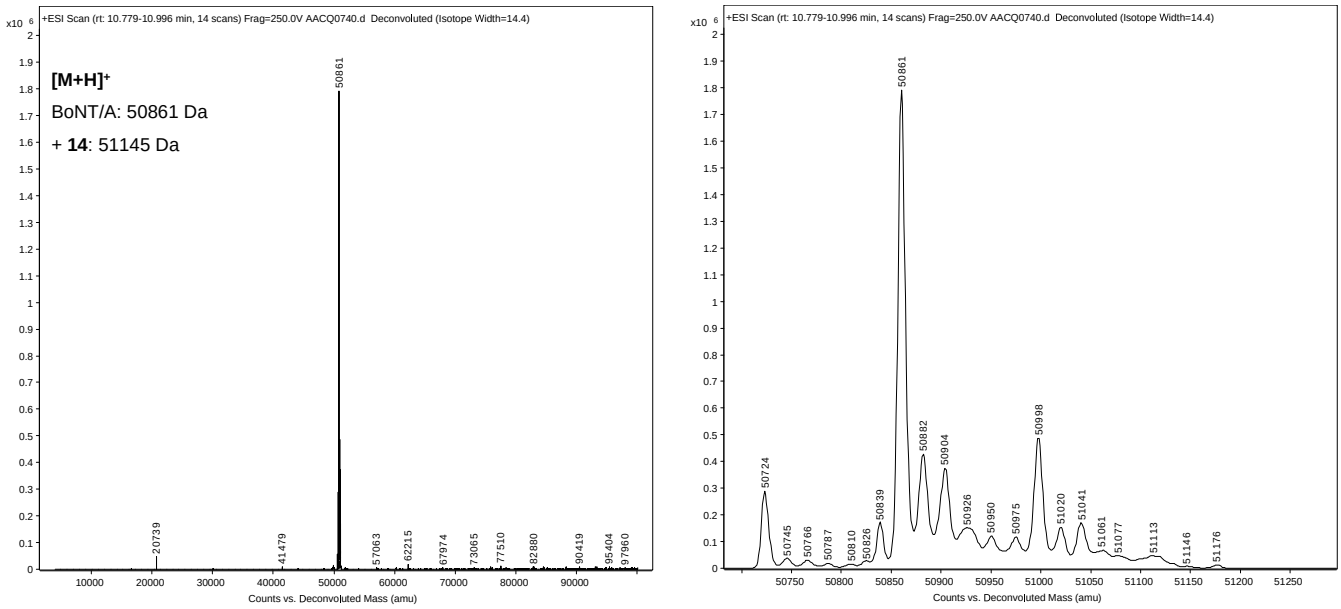
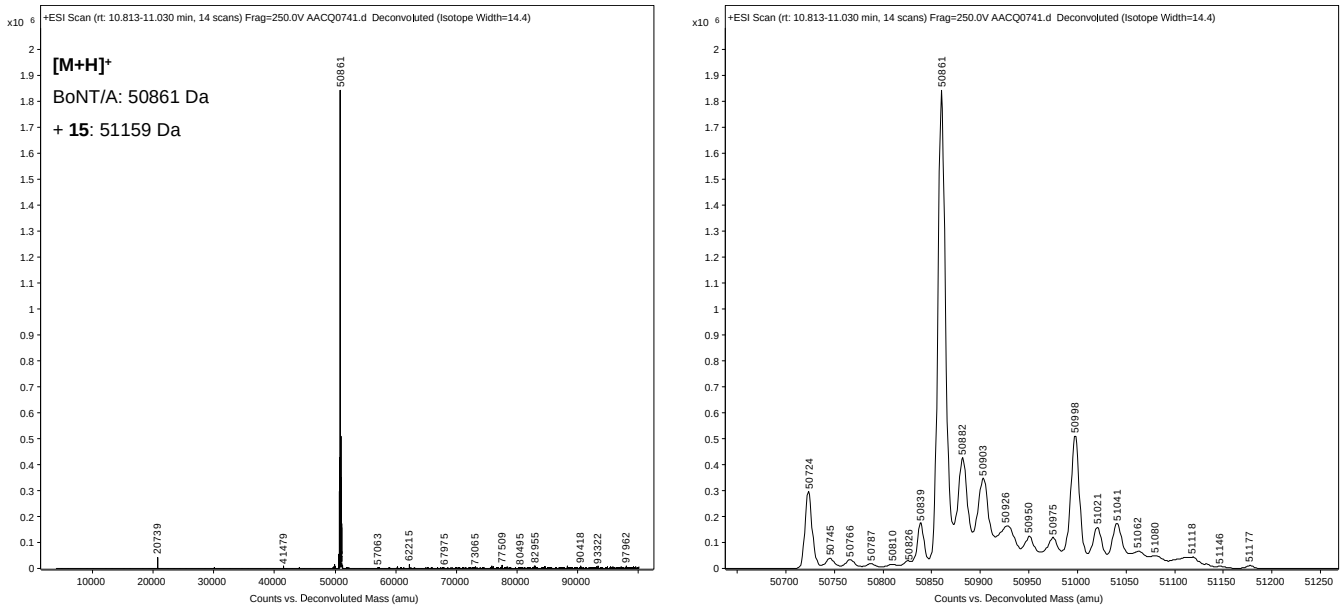




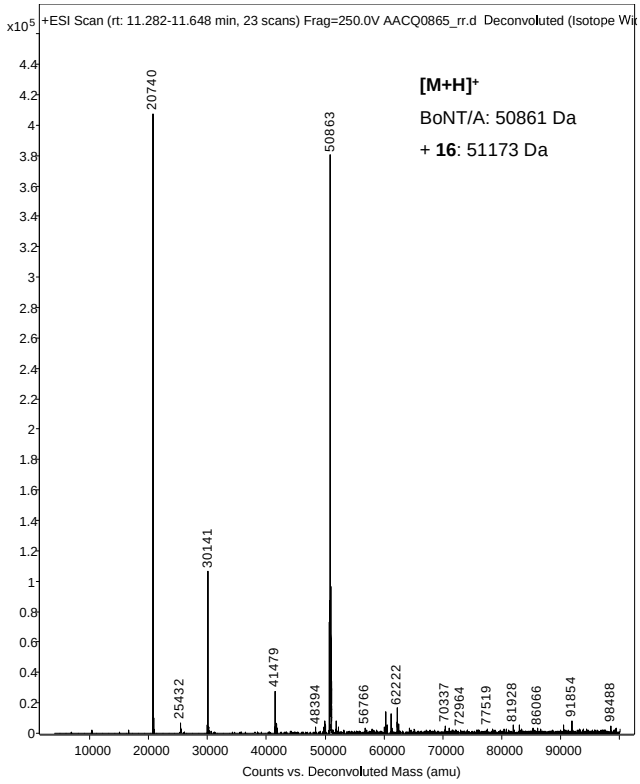
BoNT/A + **14**

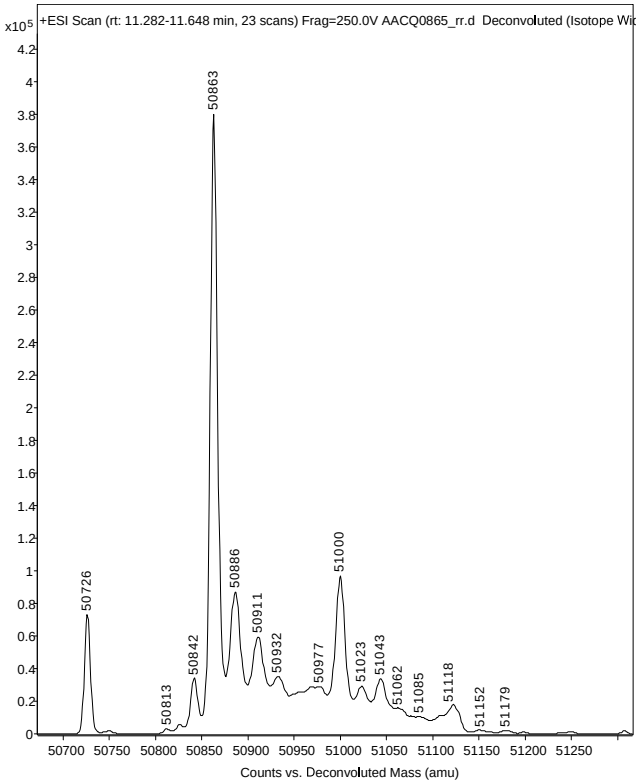


BoNT/A + **15**

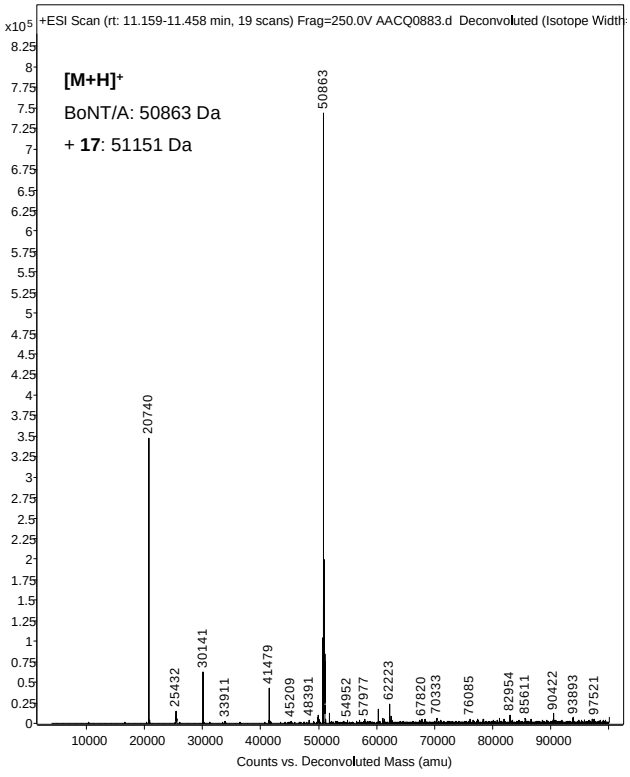


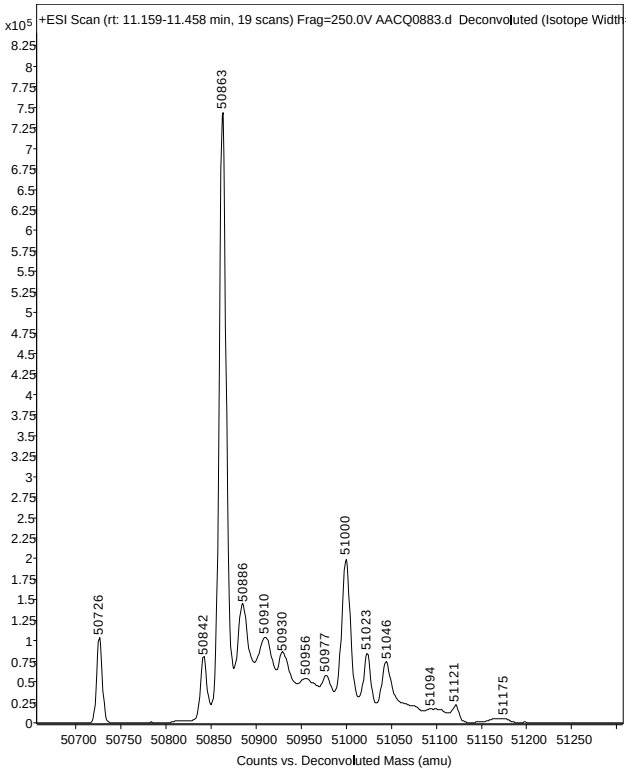
BoNT/A + **16**



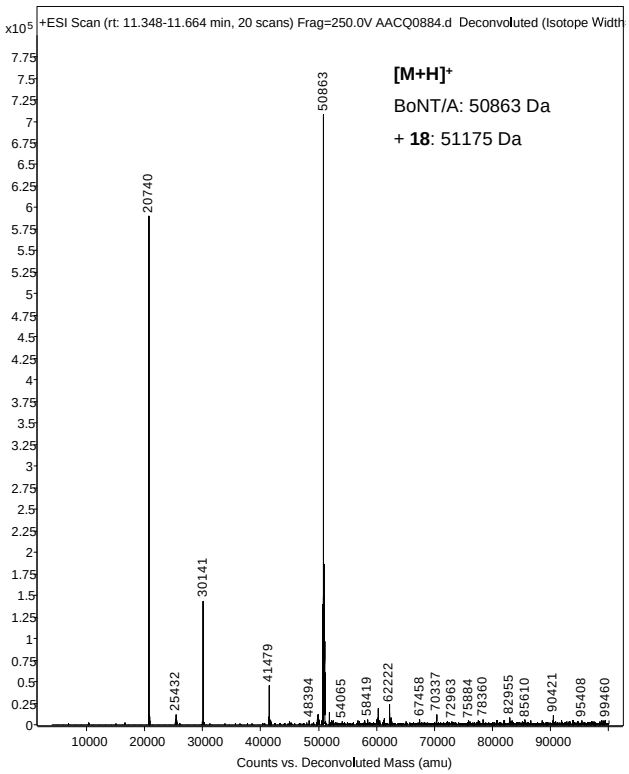


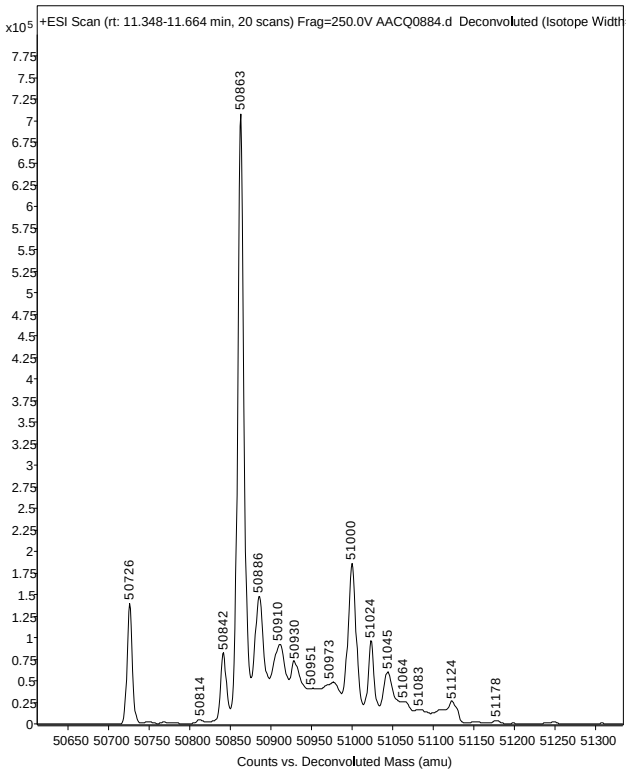
BoNT/A + **17**



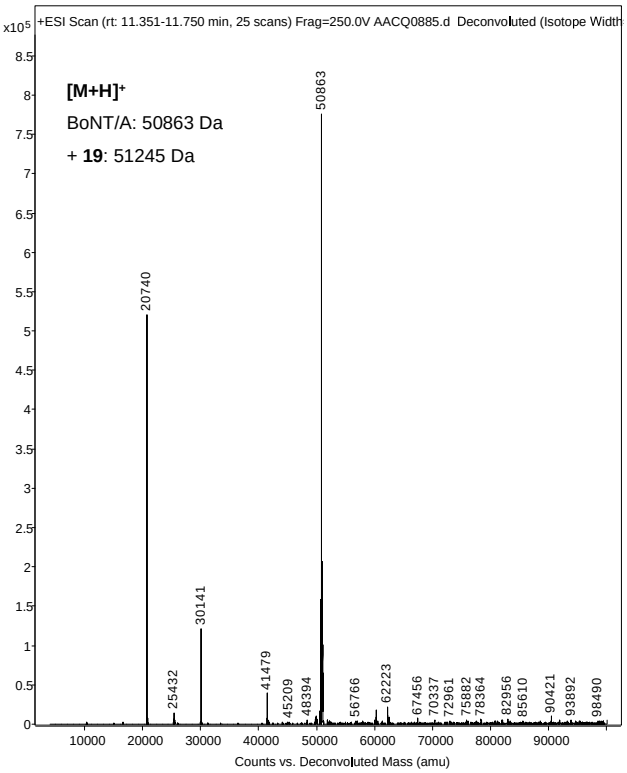


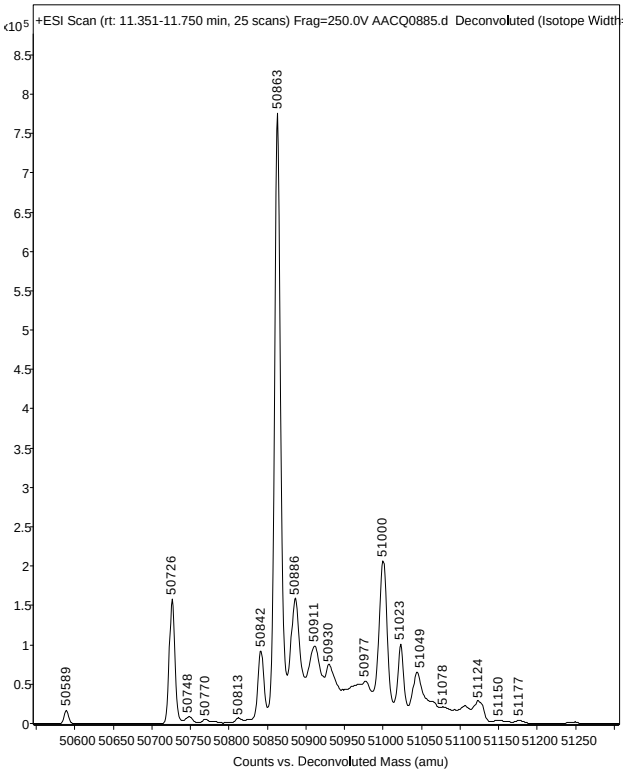
BoNT/A + **18**



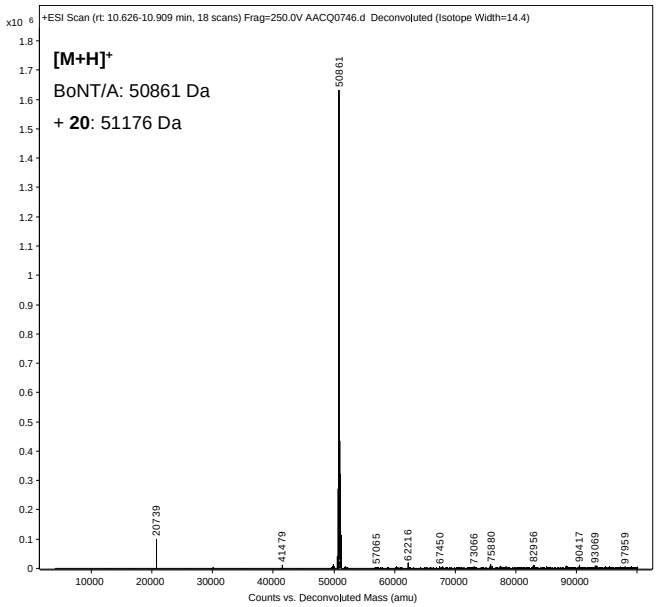


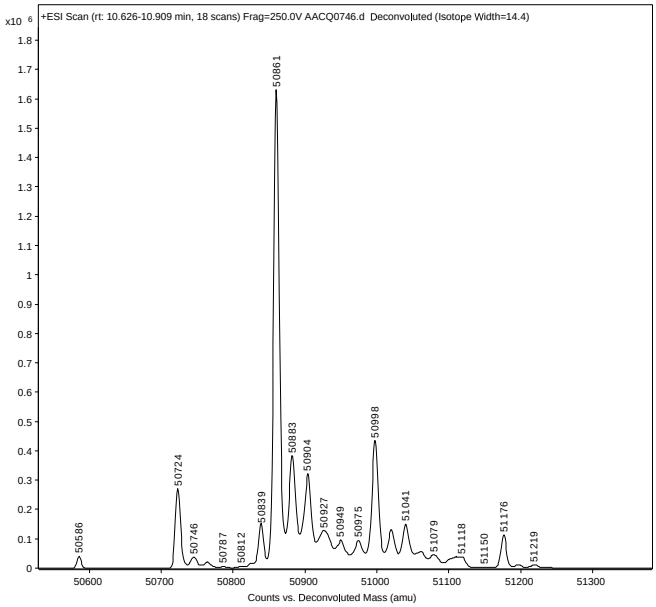
BoNT/A + **19**



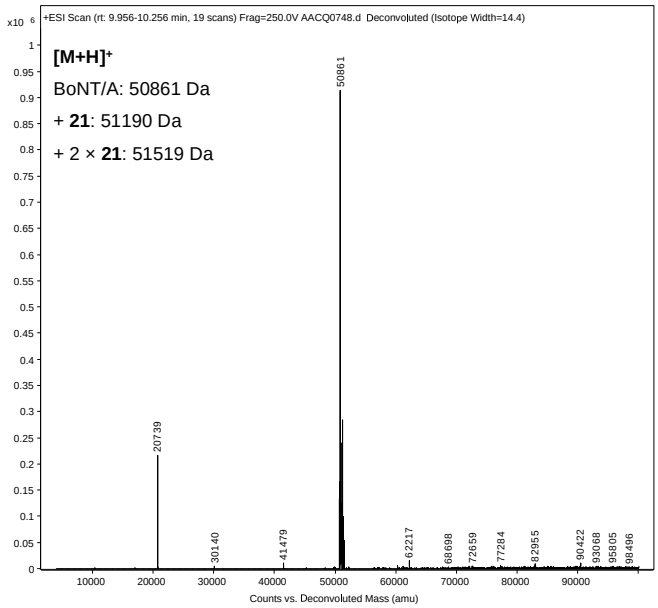


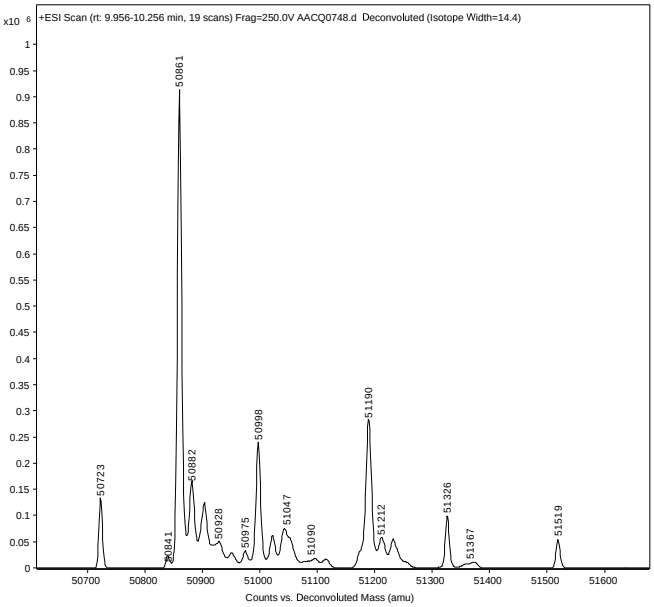
BoNT/A + **20**



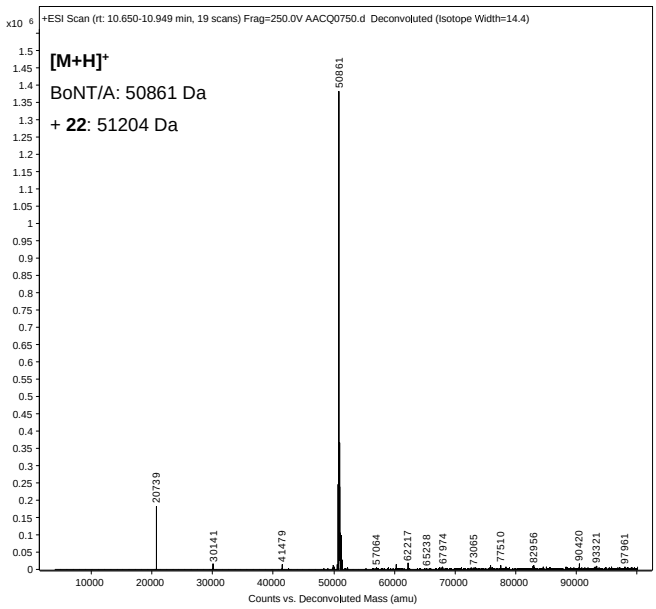


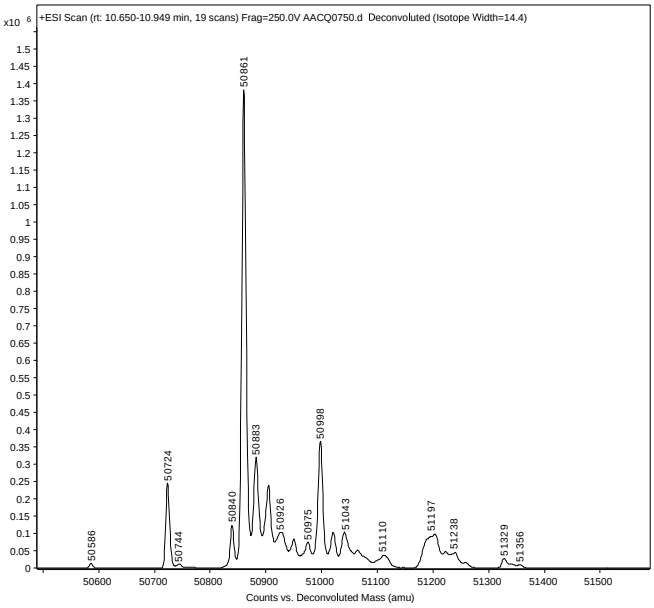
BoNT/A + **21**



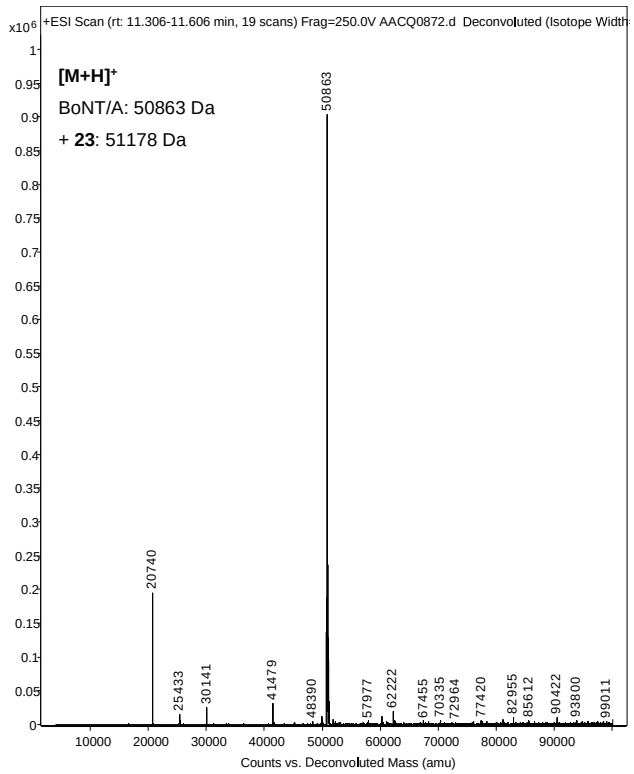


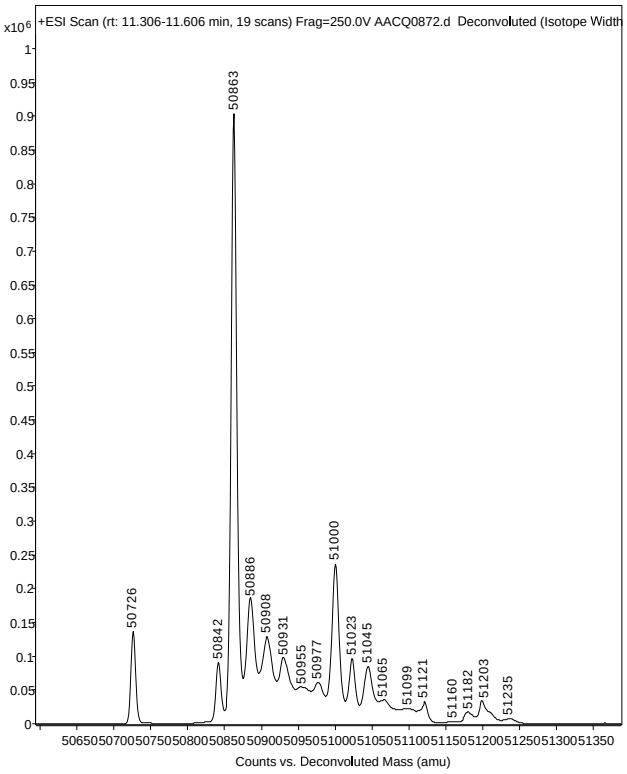
BoNT/A + **22**



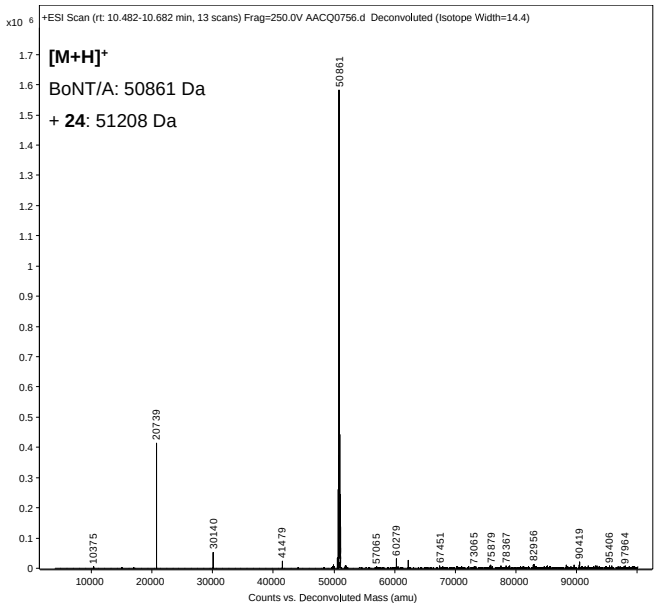


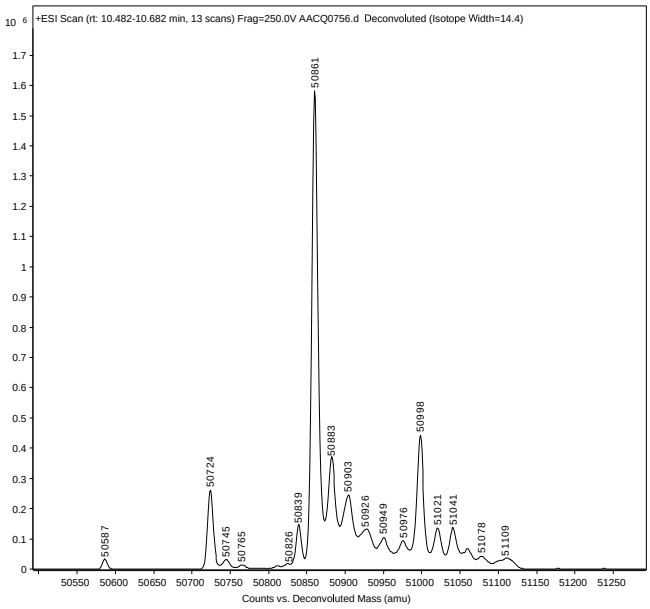
BoNT/A + **23**



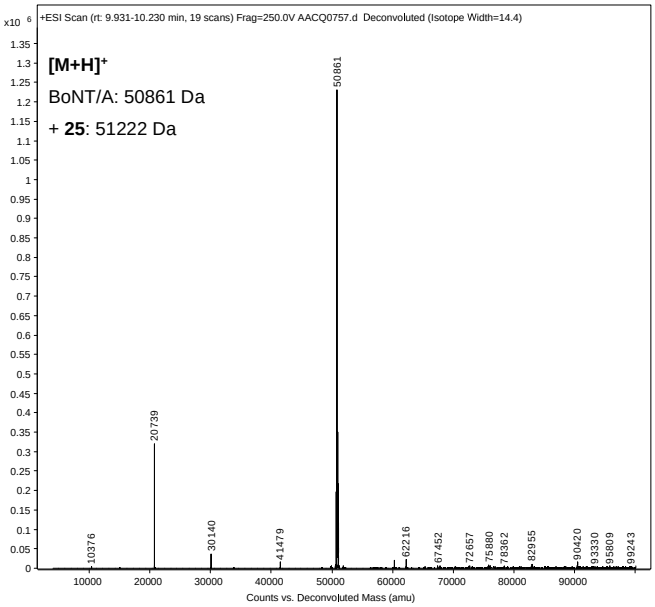


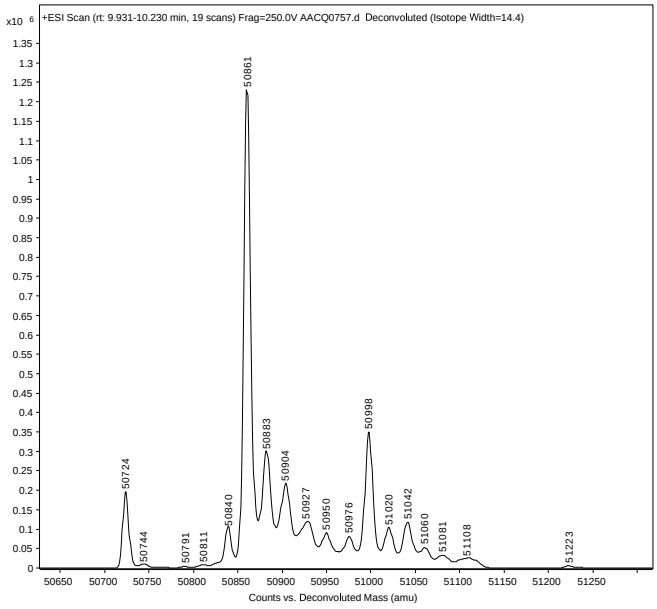
BoNT/A + **24**



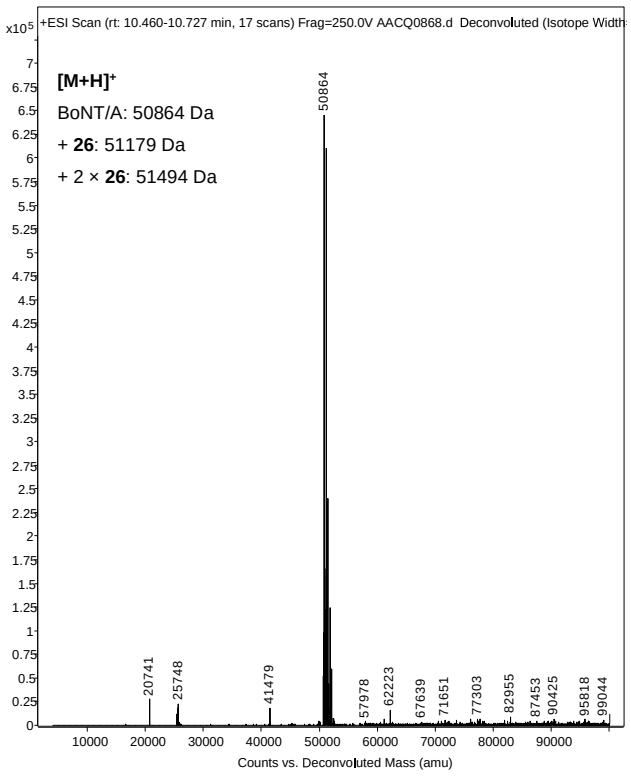


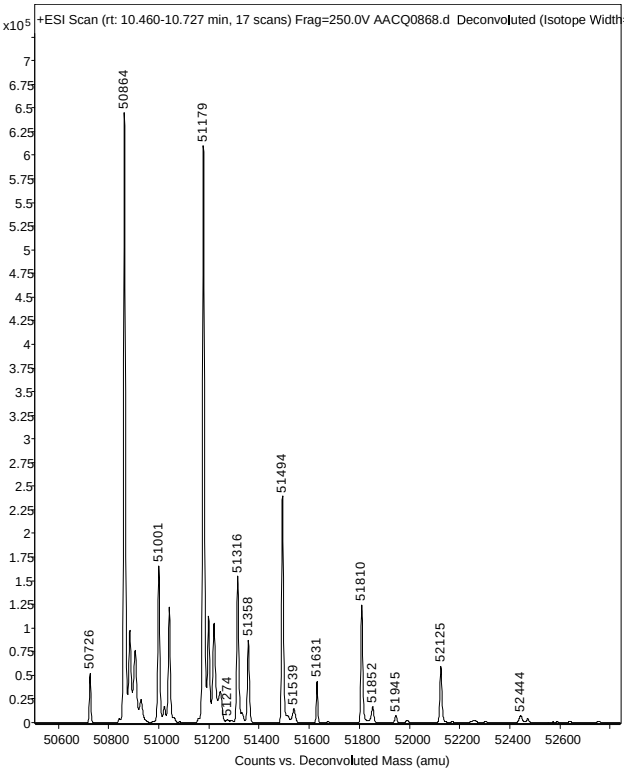
BoNT/A + **25**



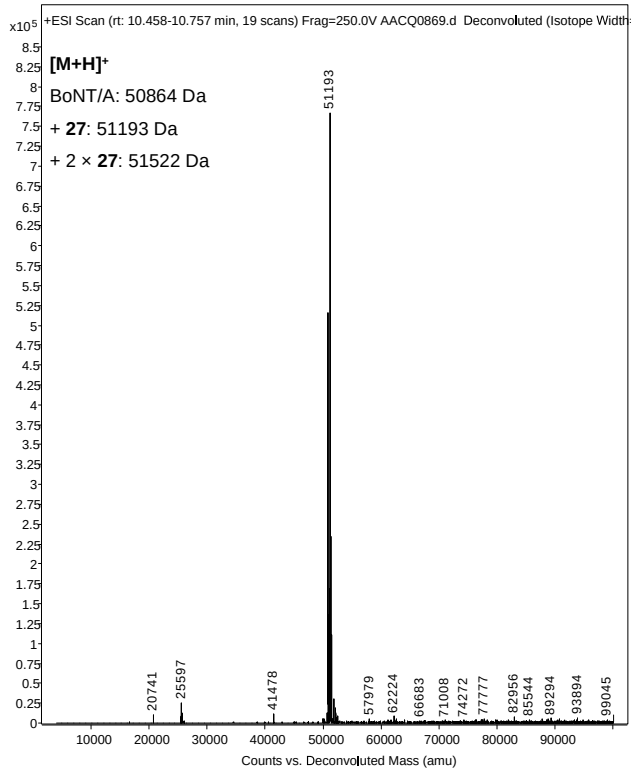


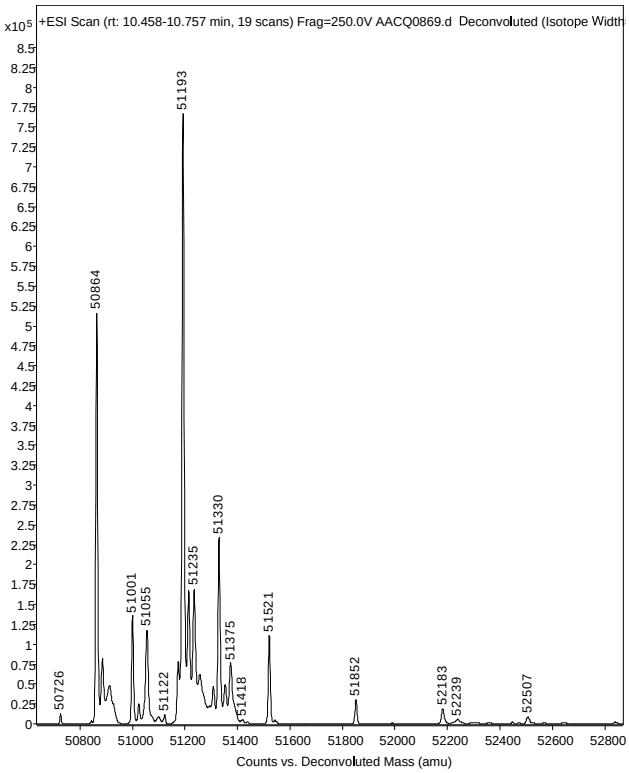
BoNT/A + **26**



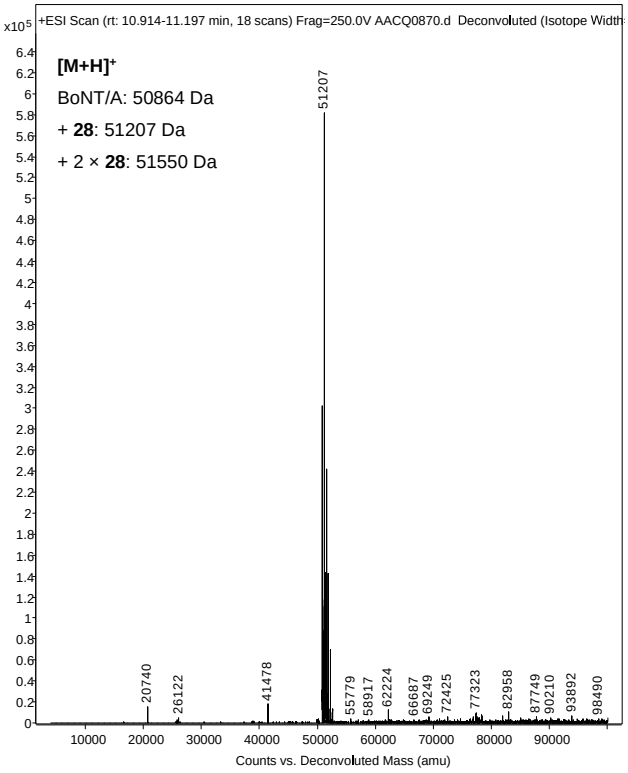


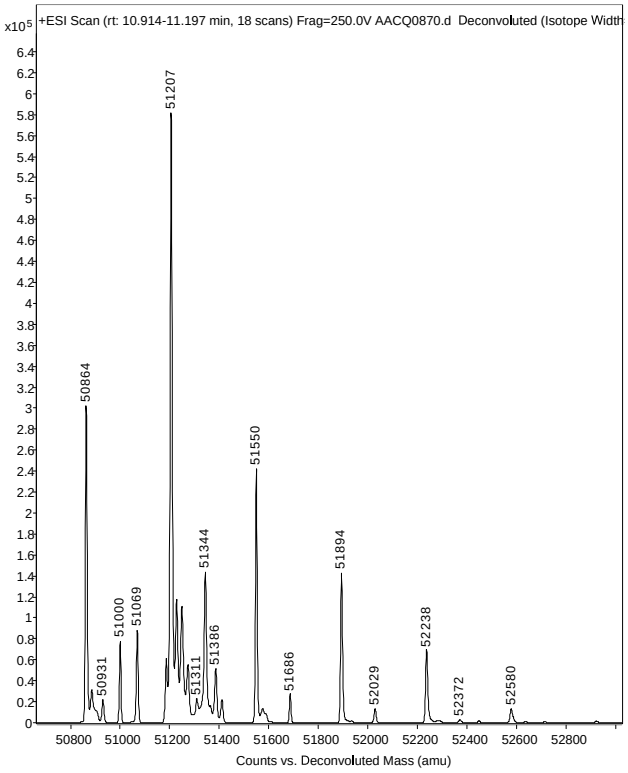
BoNT/A + **27**



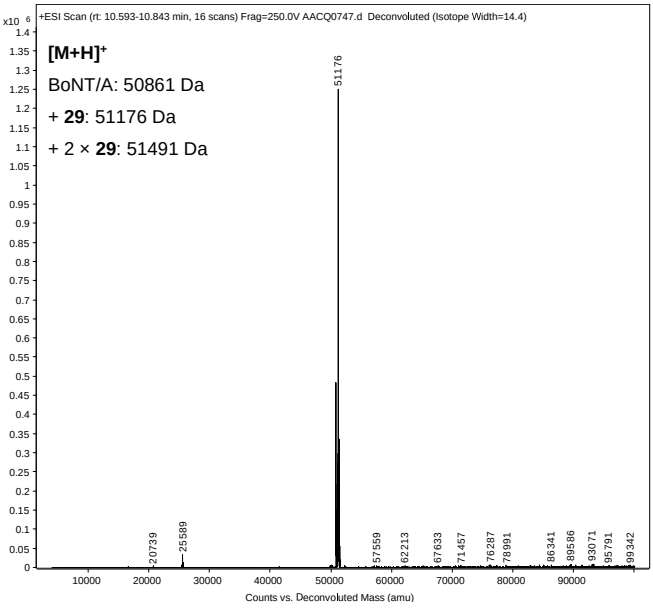


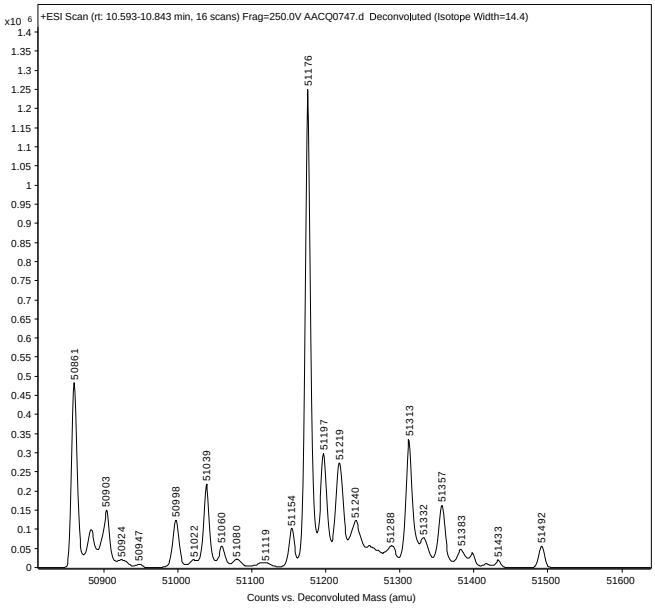
BoNT/A + **28**



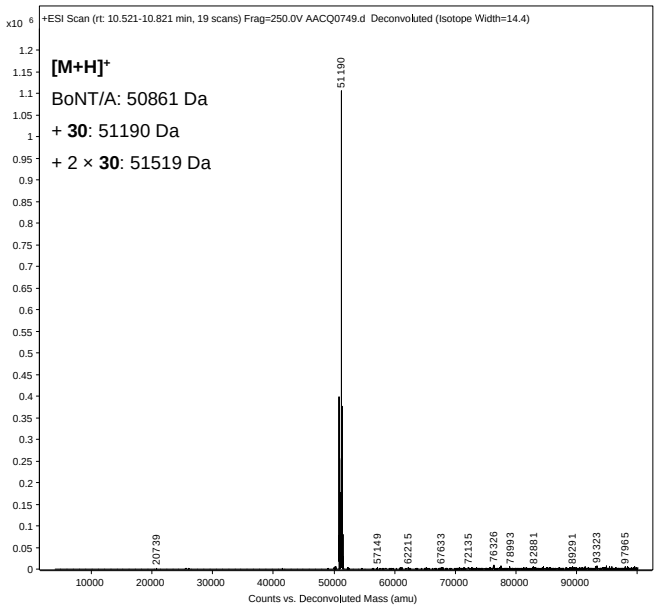


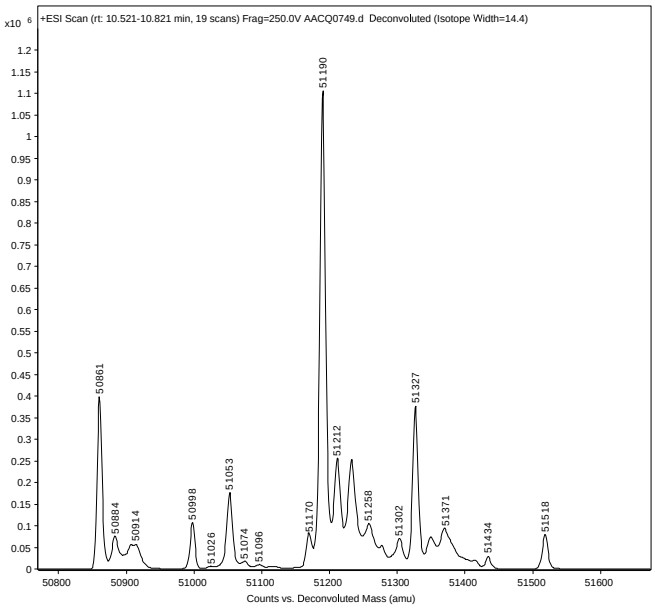
BoNT/A + **29**



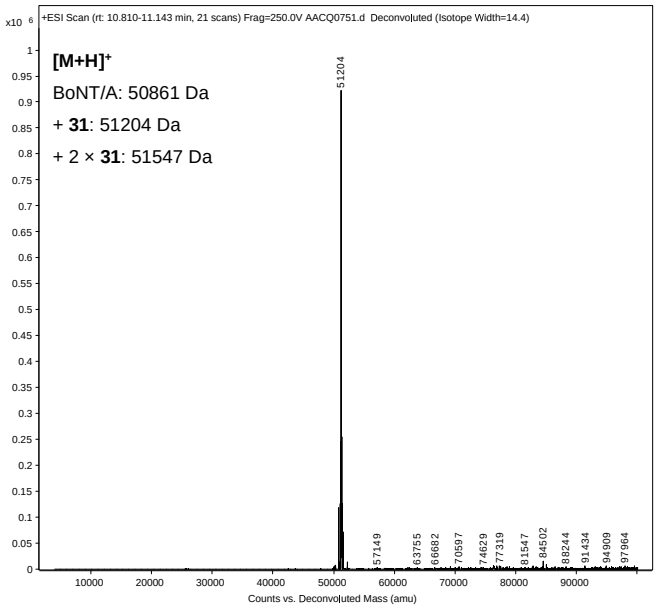


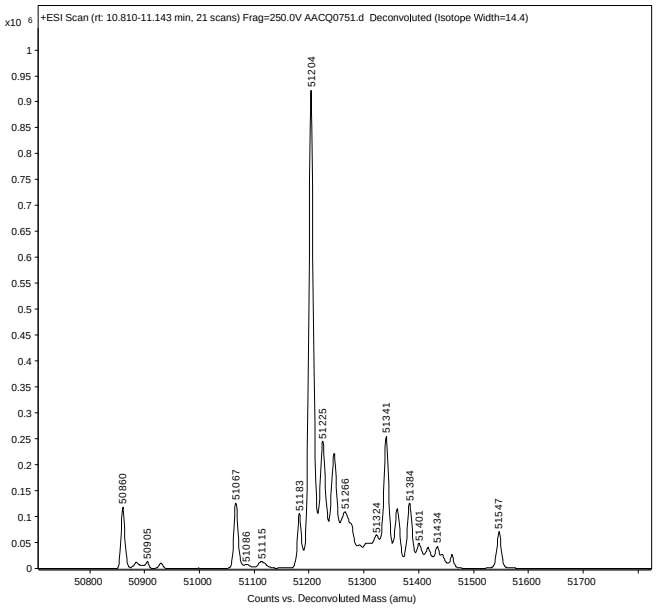
BoNT/A + **30**



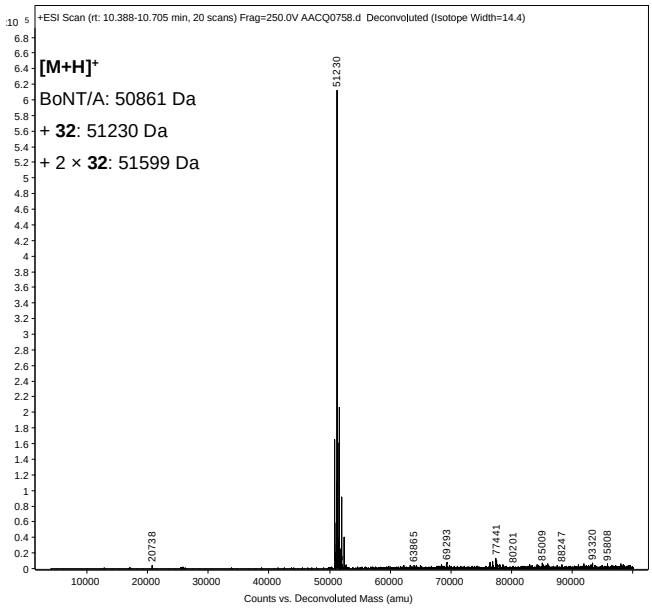


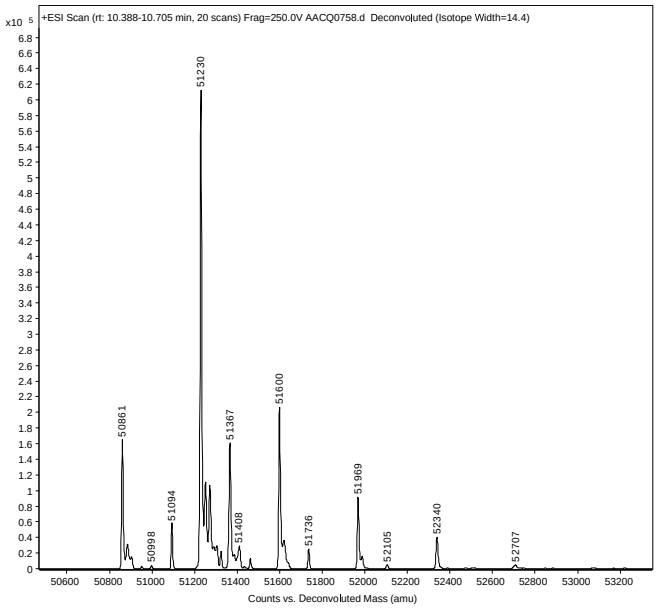
BoNT/A + **31**



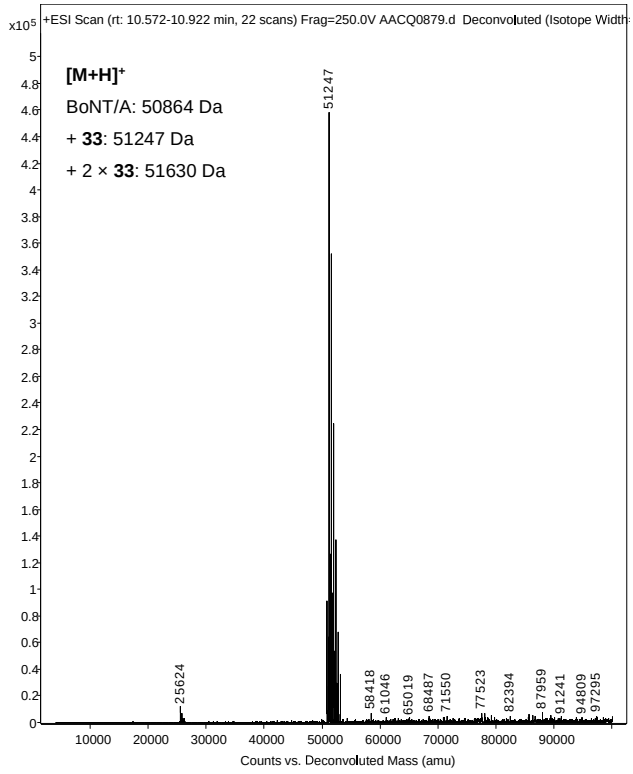


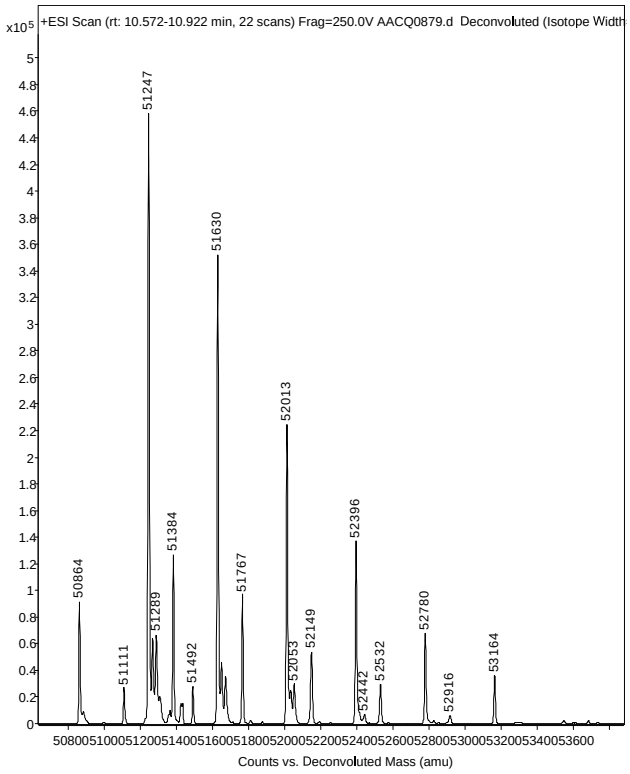
BoNT/A + **32**



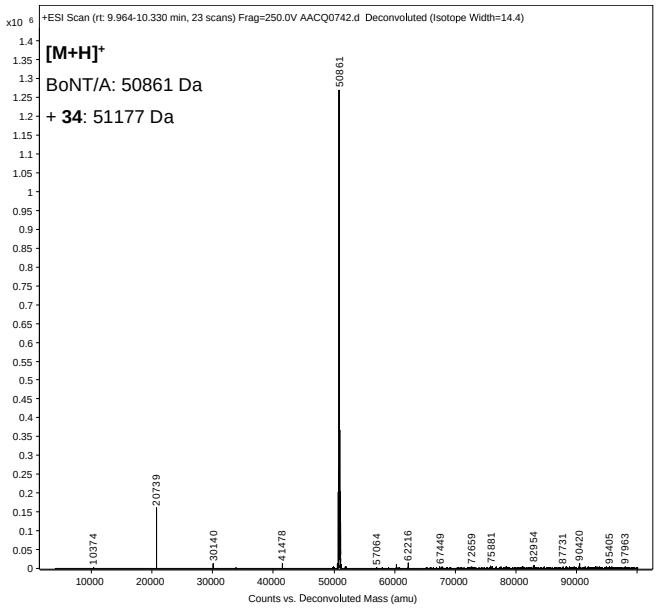


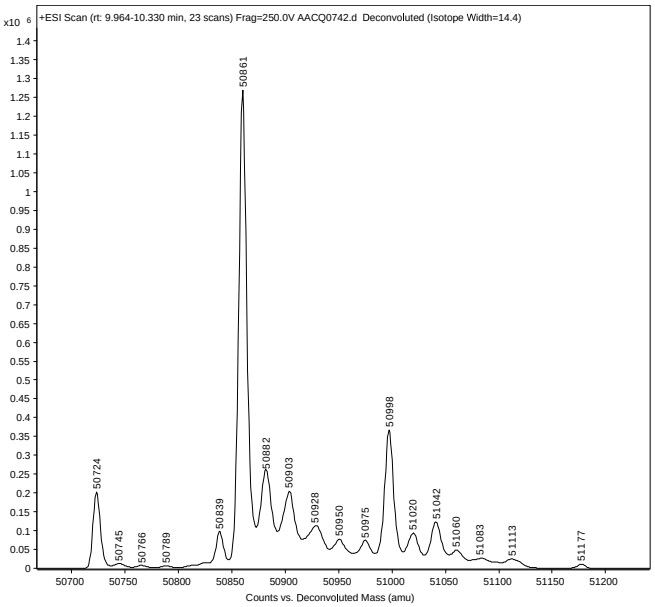
BoNT/A + **33**



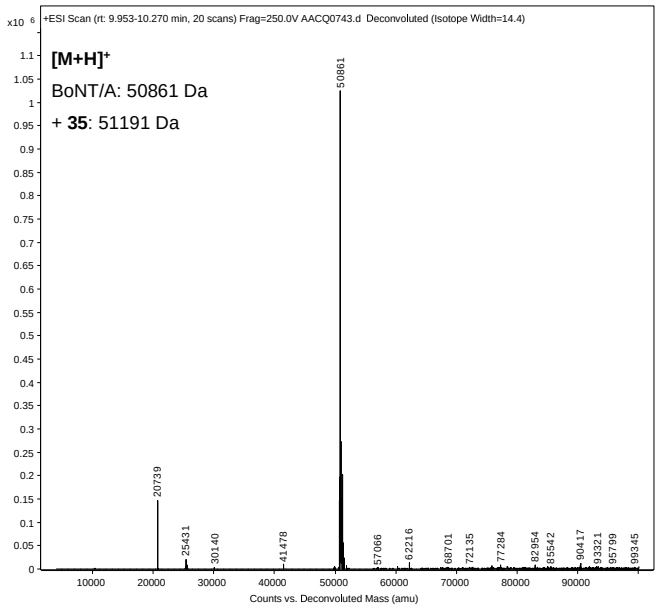


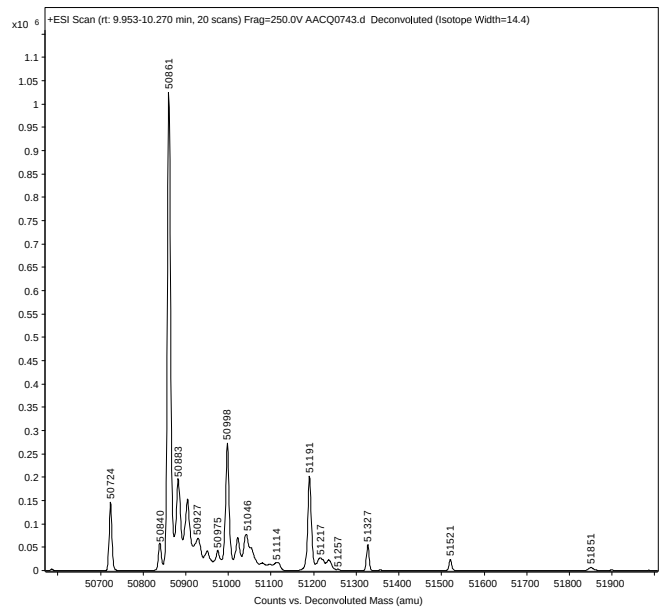
BoNT/A + **34**



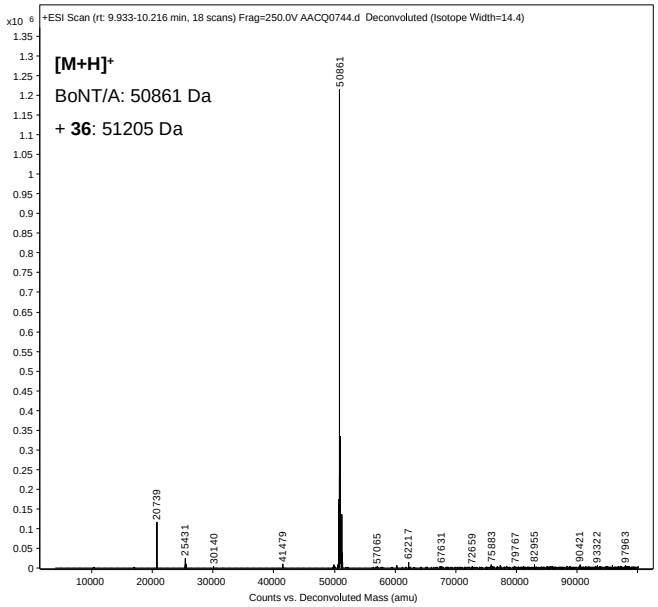


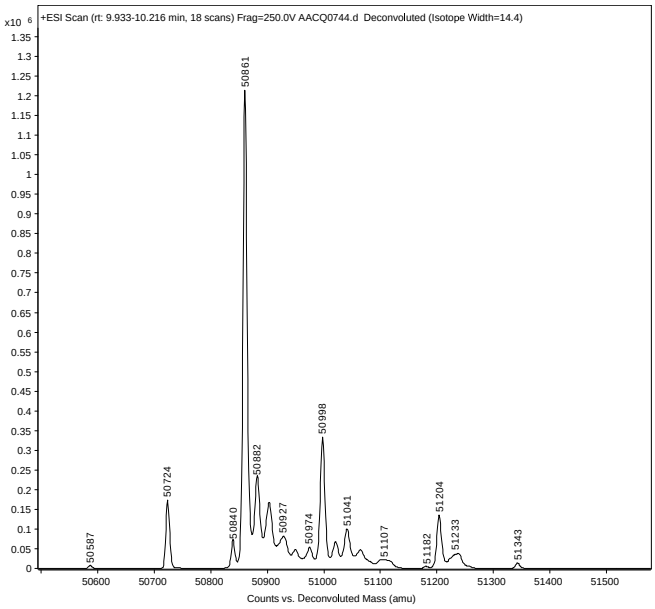
BoNT/A + **35**



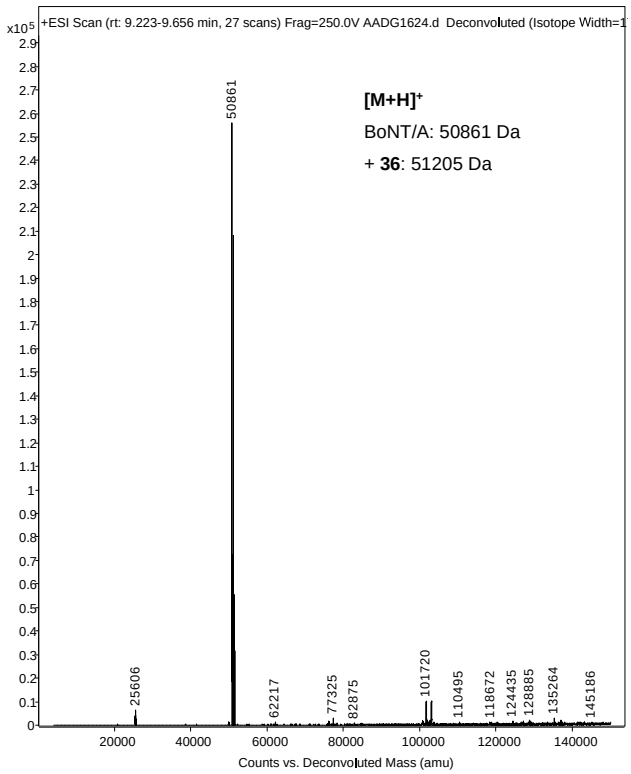


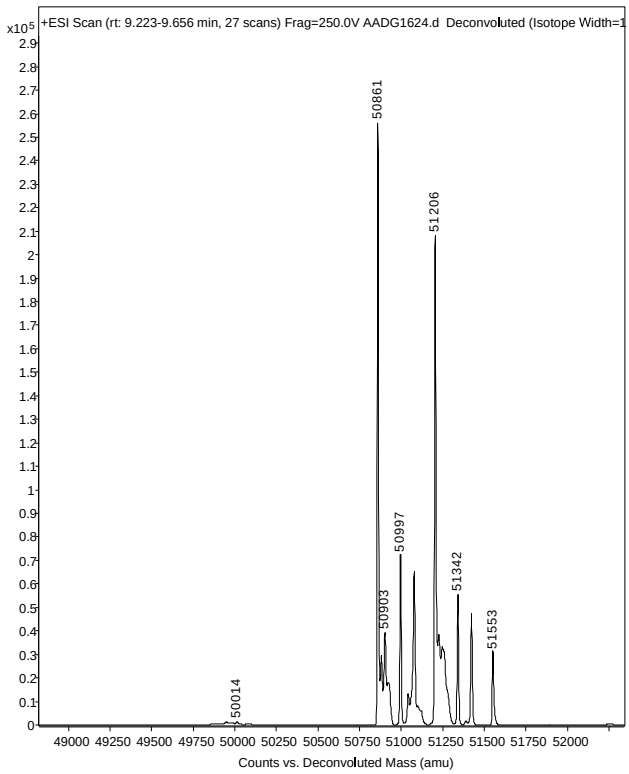
BoNT/A + **36** – 1 h labeling



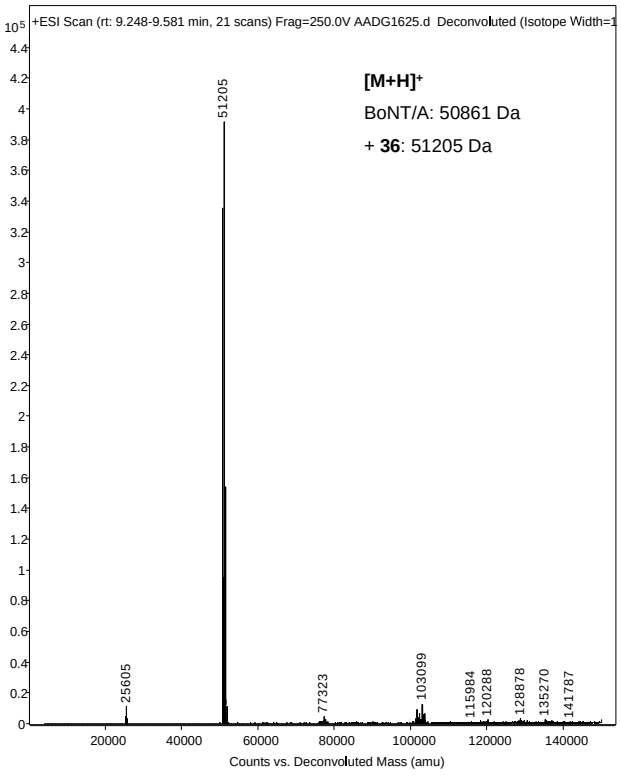


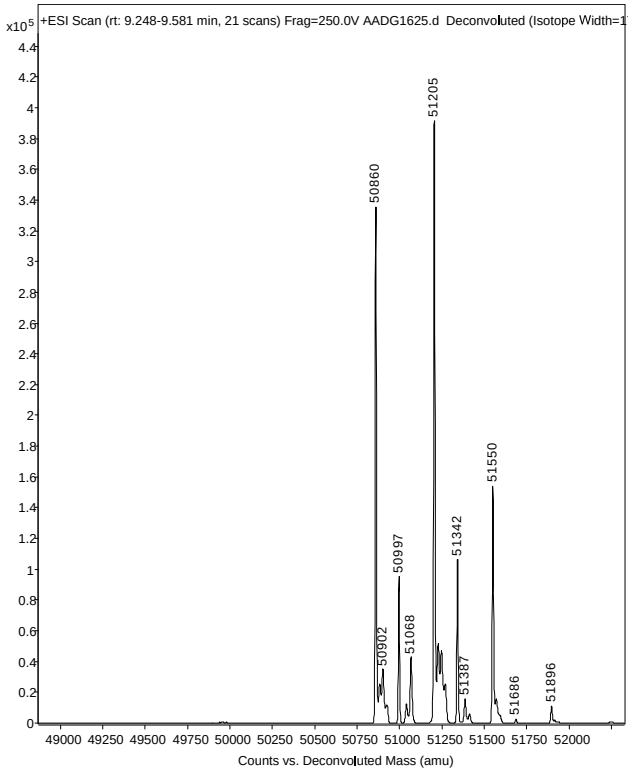
BoNT/A + **36** – 3 h labeling



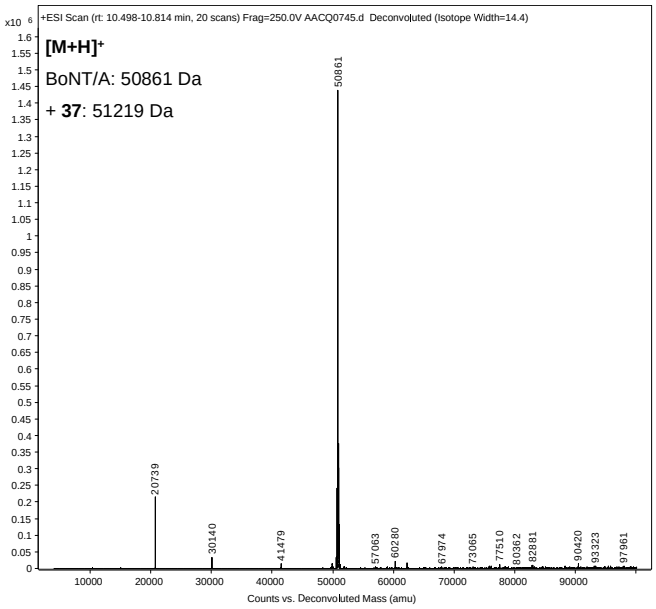


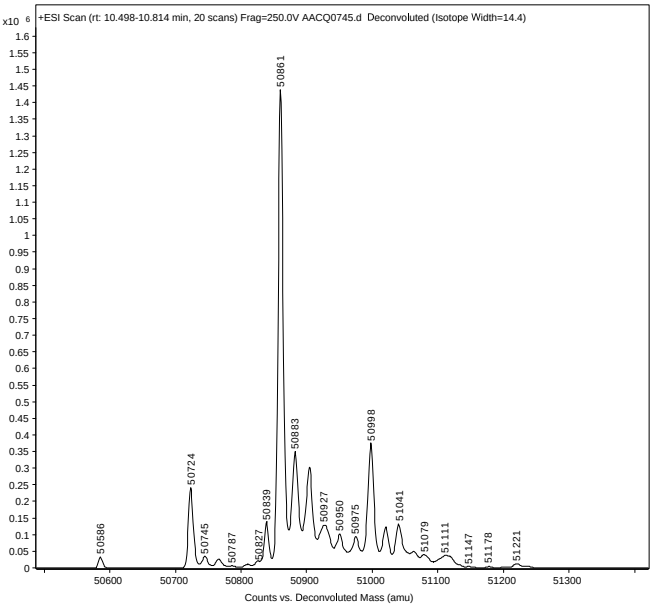
BoNT/A + **36** – 5 h labeling



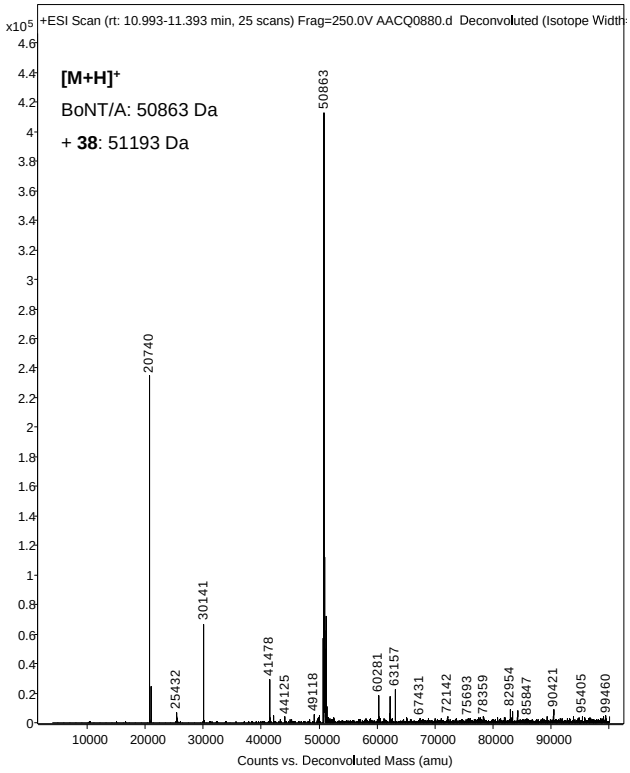


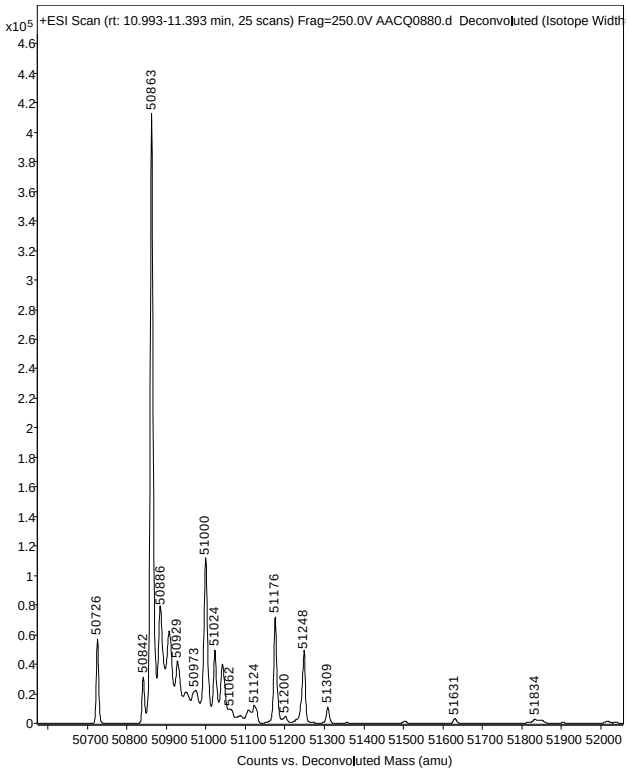
BoNT/A + **37**



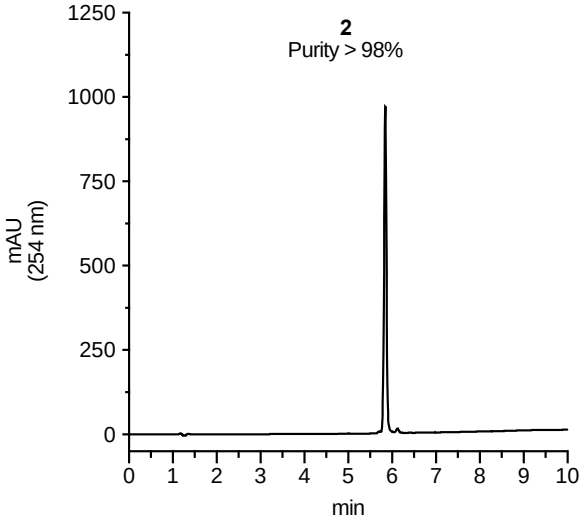


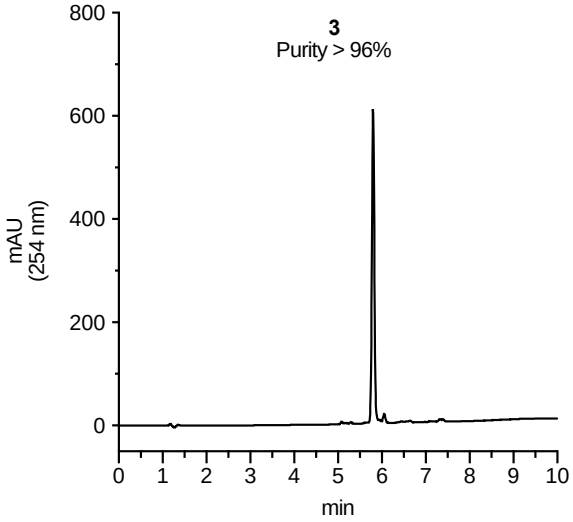
BoNT/A + **38**

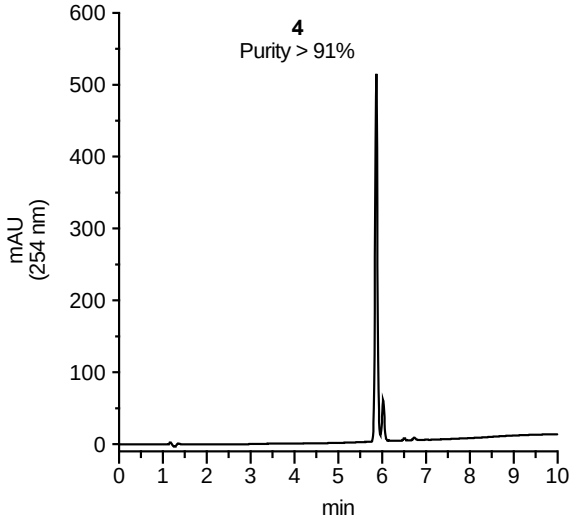

+ESI Scan (rt: 10.993-11.393 min, 25 scans) Frag=250.0V AACQ0880.d Deconvoluted (Isotope Width
x10^5
[M+H]+
BoNT/A: 50863 Da
+ 38: 51193 Da
50863
20740
30141
25432
41478
44125
49118
60281
63157
67431
72142
75693
78359
82954
85847
90421
95405
99460
Counts vs. Deconvoluted Mass (amu)

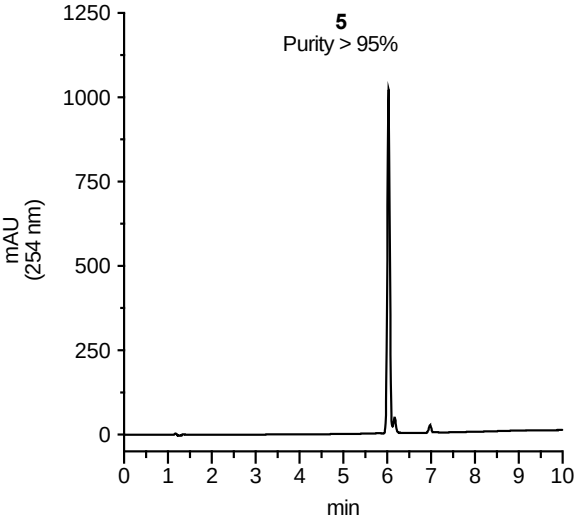

+ESI Scan (rt: 10.993-11.393 min, 25 scans) Frag=250.0V AACQ0880.d Deconvoluted (Isotope Width
x10^5
50863
50726
50842
50886
50929
50973
51000
51024
51062
51124
51176
51200
51248
51309
51631
51834
Counts vs. Deconvoluted Mass (amu)

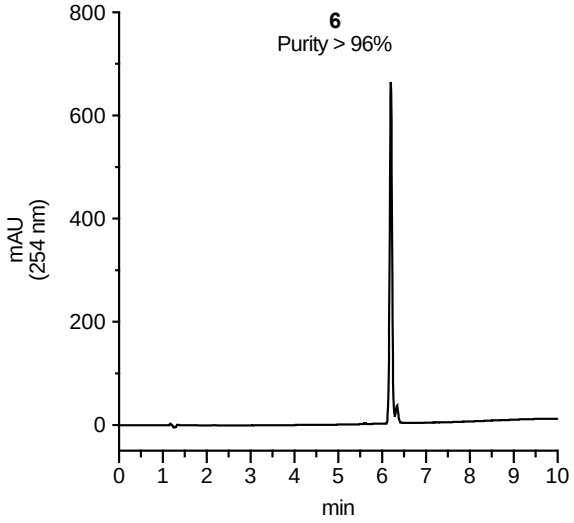
6.3 HPLC Chromatograms

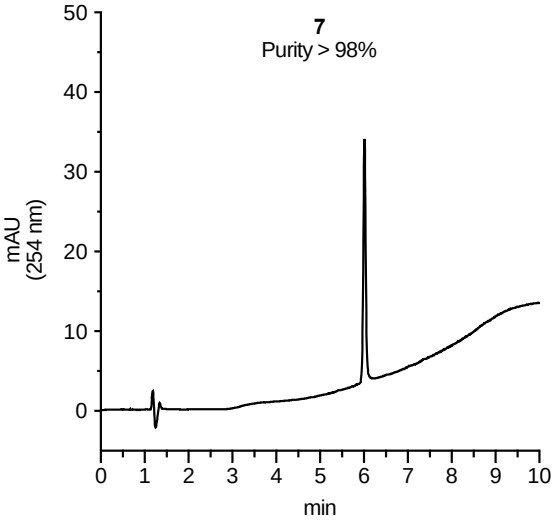


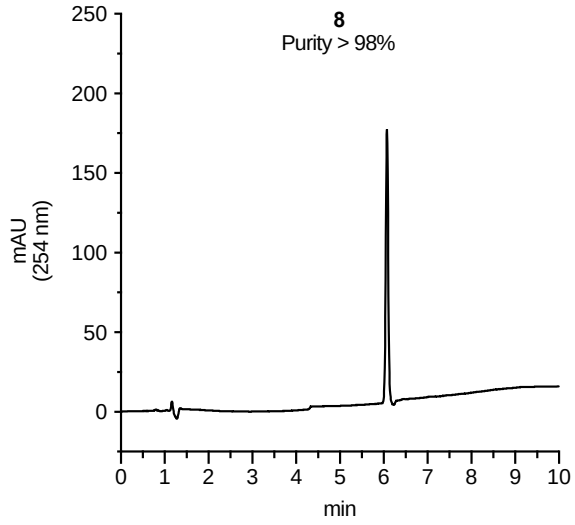


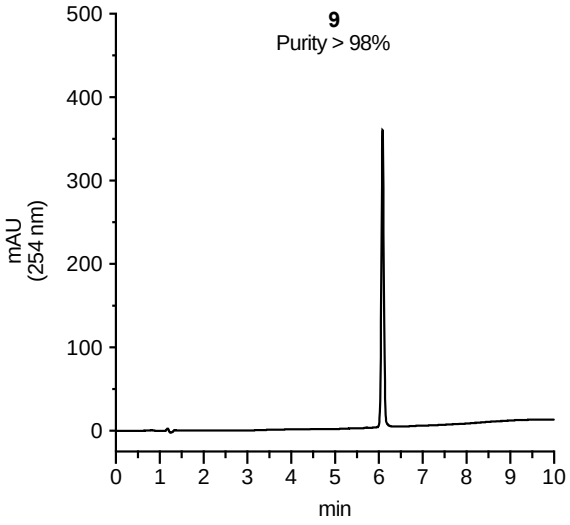


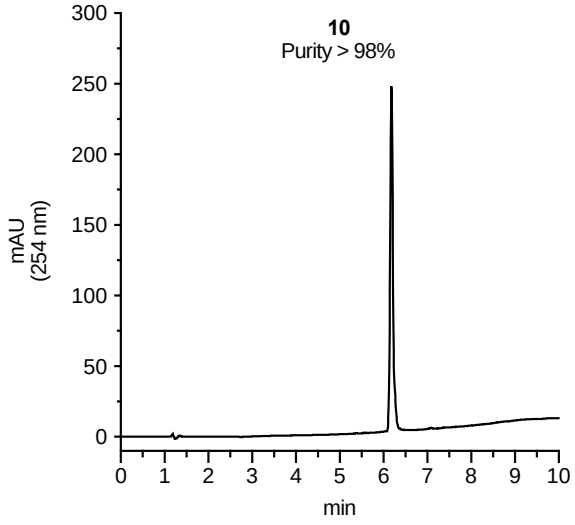


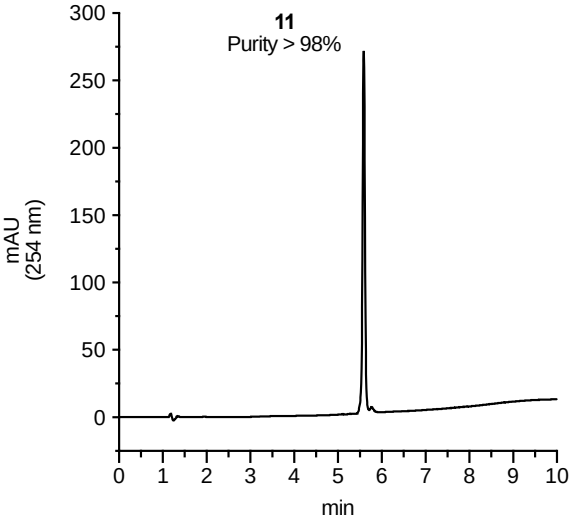


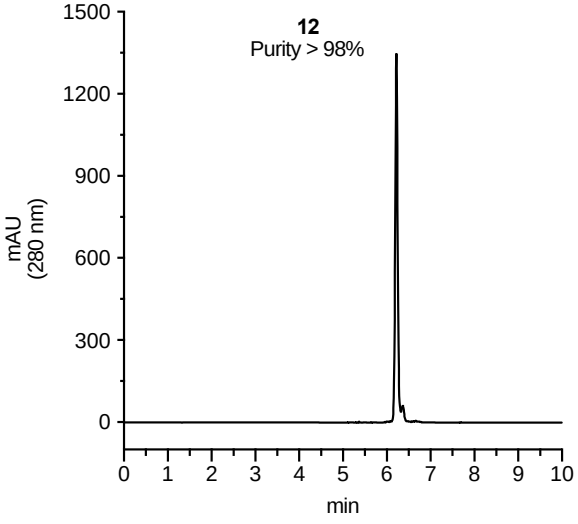


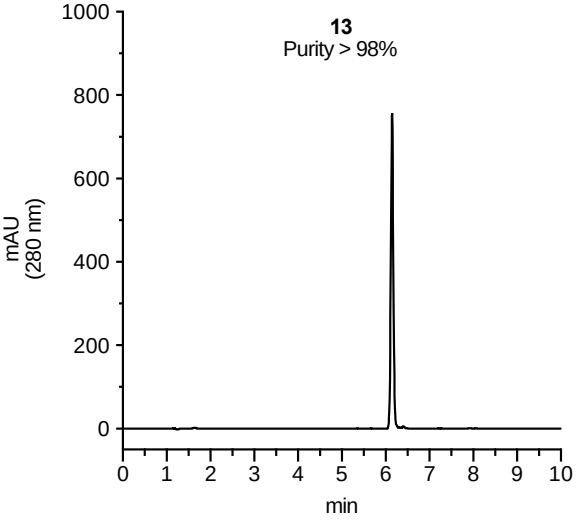

8
Purity > 98%
mAU
(254 nm)
min

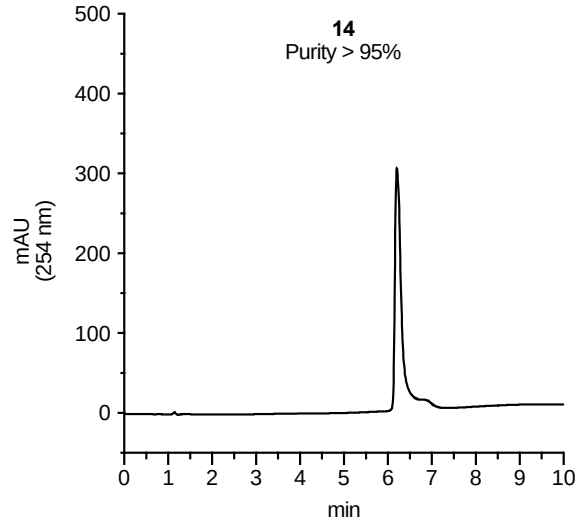

9
Purity > 98%
mAU
(254 nm)
min

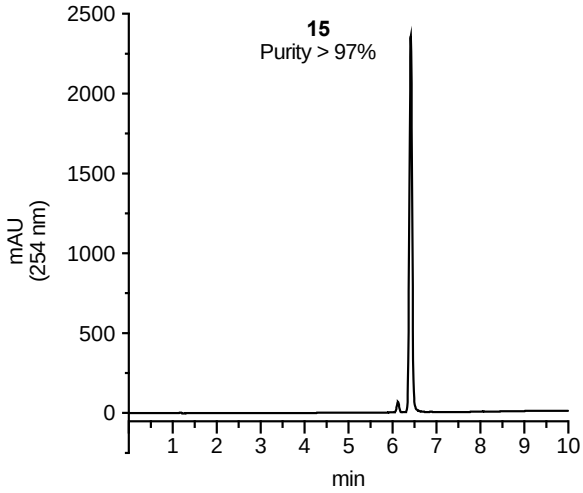

10
Purity > 98%
mAU
(254 nm)
min

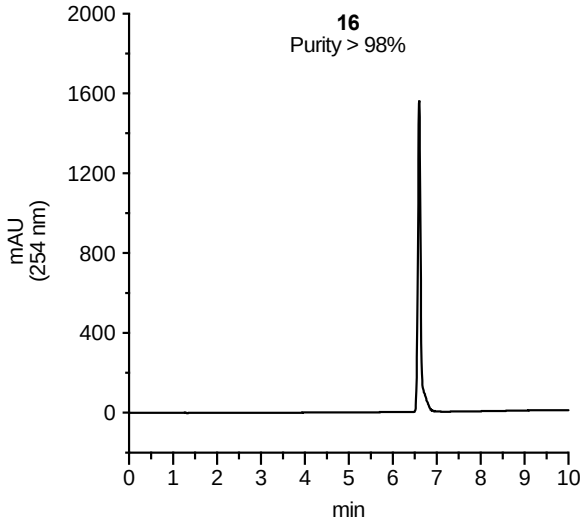

11
Purity > 98%
mAU
(254 nm)
min

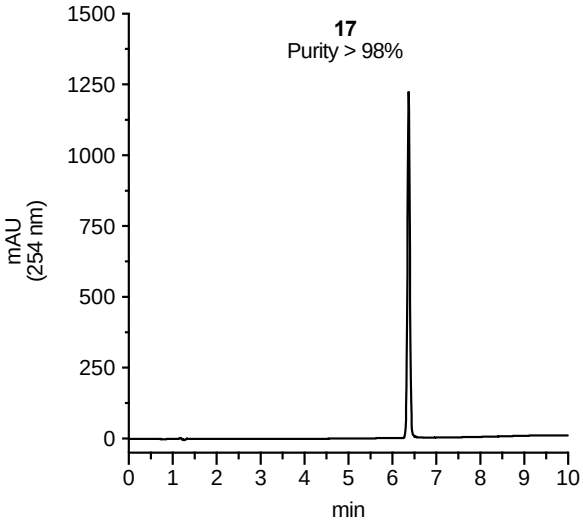

12
Purity > 98%
mAU
(280 nm)
min

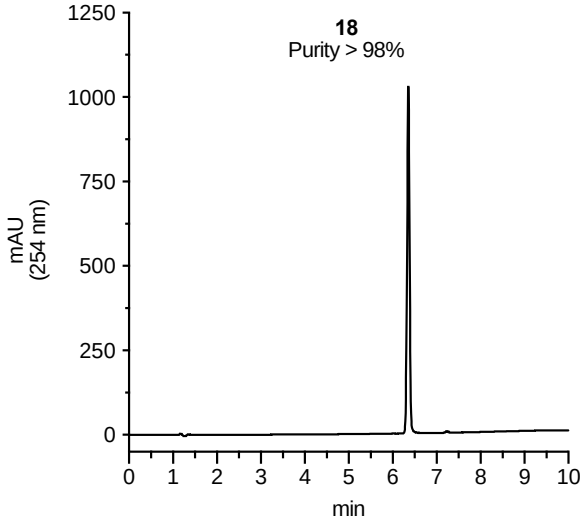

13
Purity > 98%
mAU
(280 nm)
min

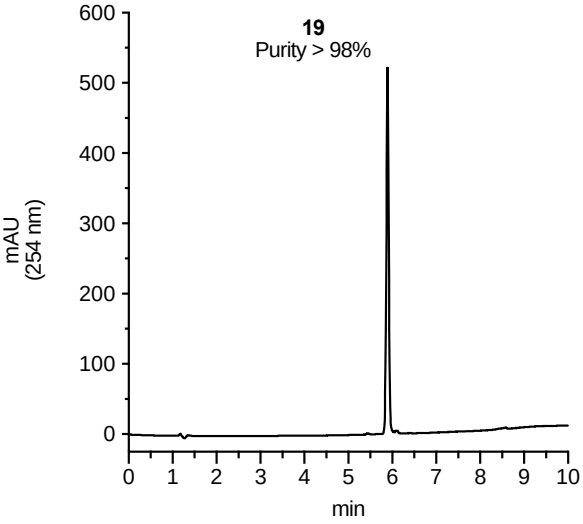

14
Purity > 95%
mAU
(254 nm)
min

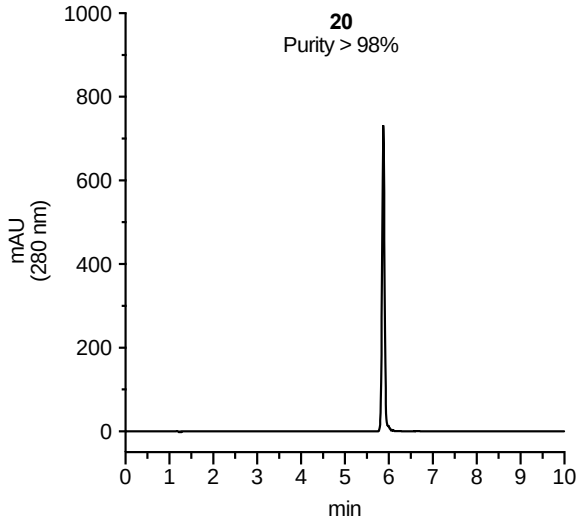

15
Purity > 97%
mAU
(254 nm)
min

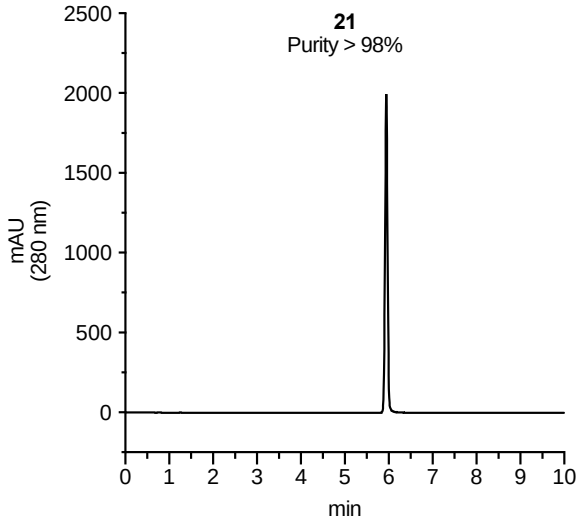

16
Purity > 98%
mAU
(254 nm)
min

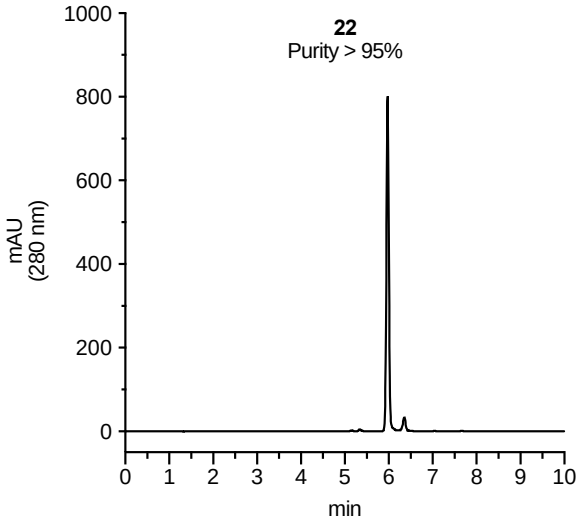

17
Purity > 98%
mAU
(254 nm)
min

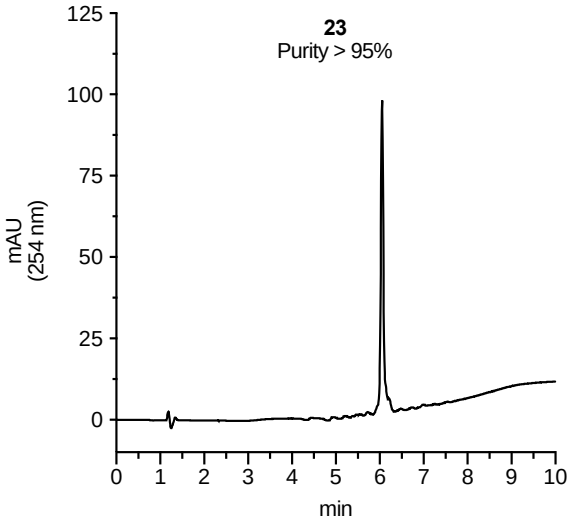

18
Purity > 98%
mAU
(254 nm)
min

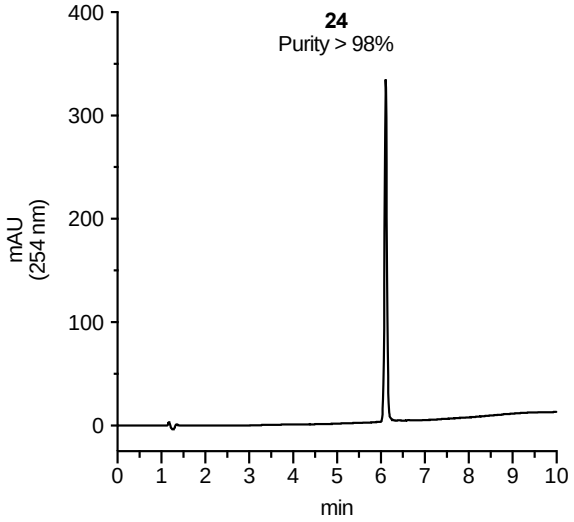

19
Purity > 98%
mAU
(254 nm)
min

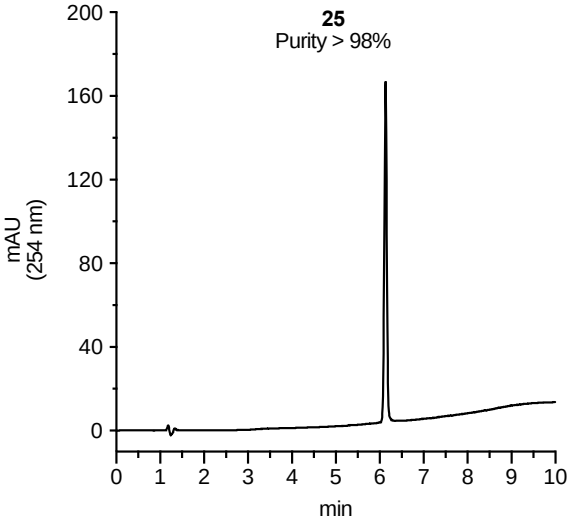

20
Purity > 98%
mAU
(280 nm)
min

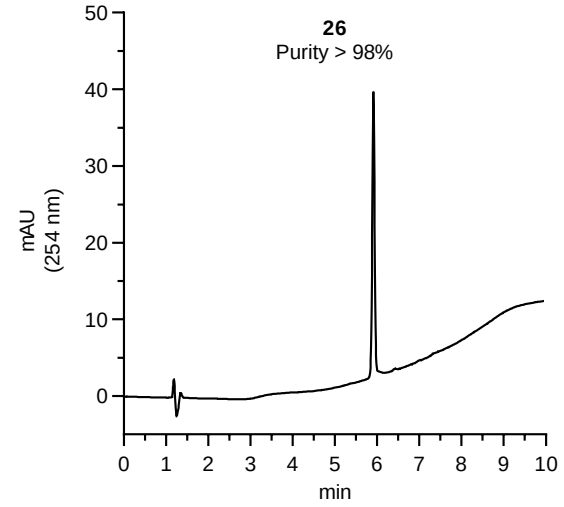

21
Purity > 98%
mAU
(280 nm)
min

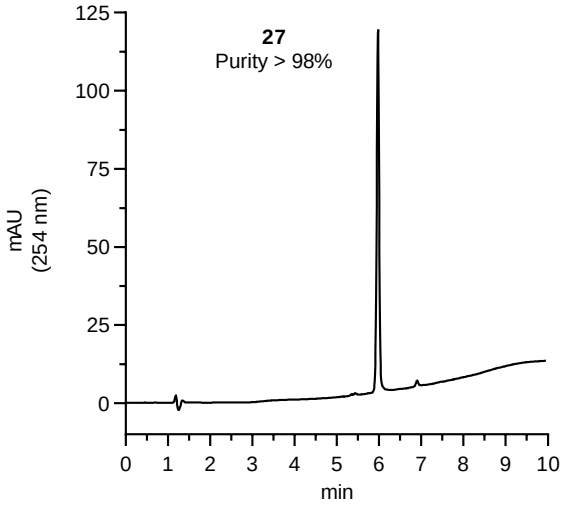

22
Purity > 95%
mAU
(280 nm)
min

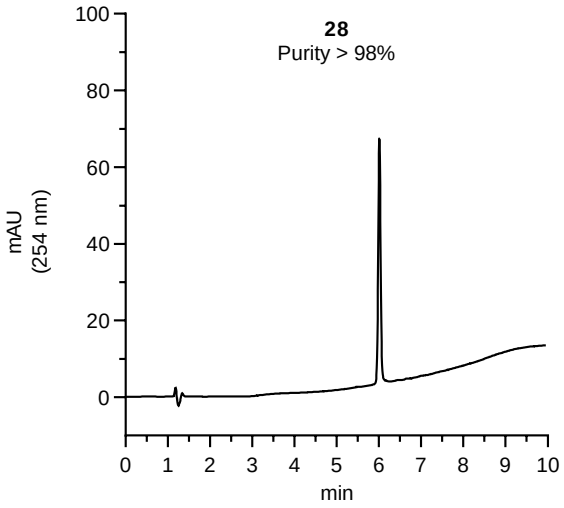

23
Purity > 95%
mAU
(254 nm)
min

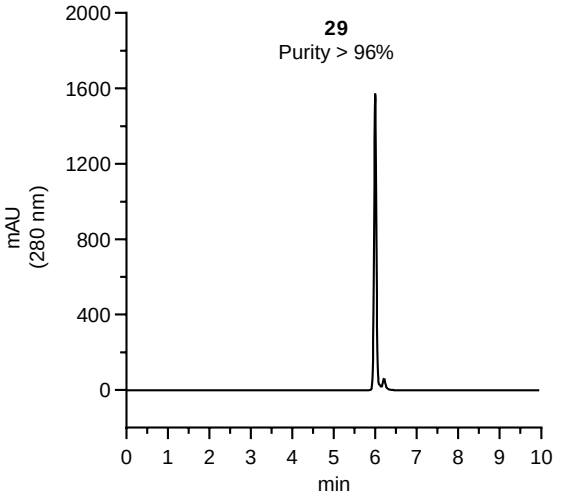

24
Purity > 98%
mAU
(254 nm)
min

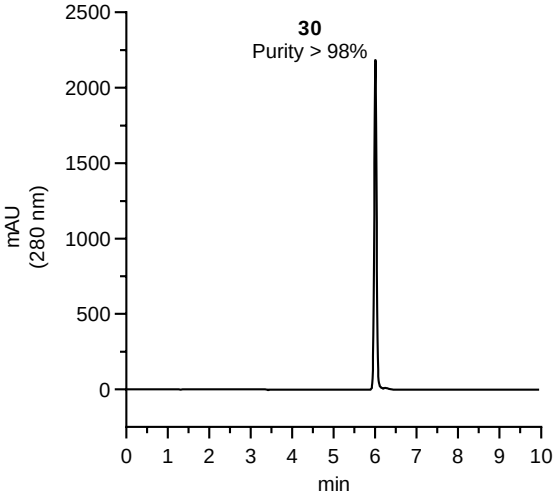

25
Purity > 98%
mAU
(254 nm)
min

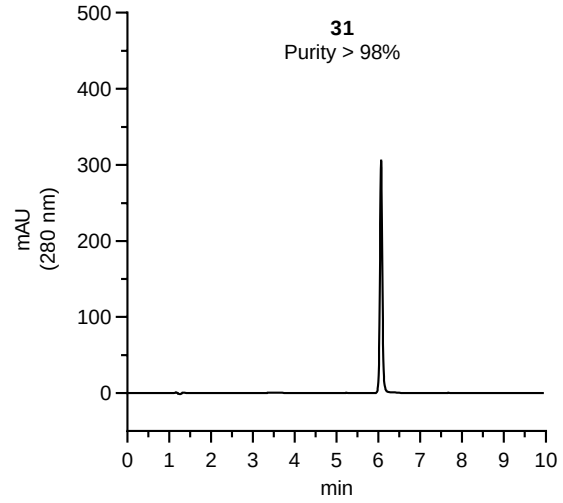

26
Purity > 98%
mAU
(254 nm)
min

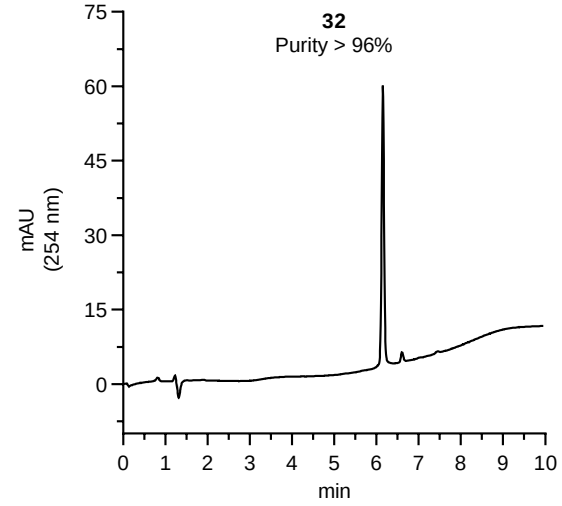

27
Purity > 98%
mAU
(254 nm)
min

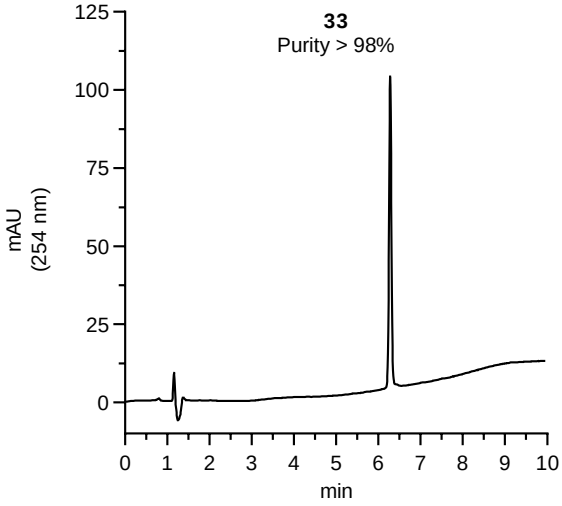

28
Purity > 98%
mAU
(254 nm)
min

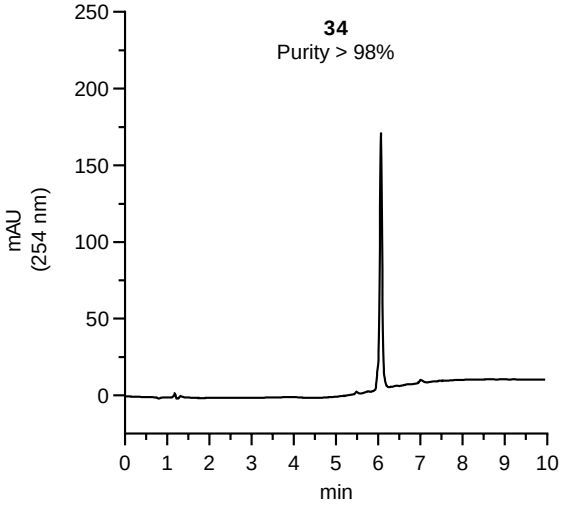

29
Purity > 96%
mAU
(280 nm)
min

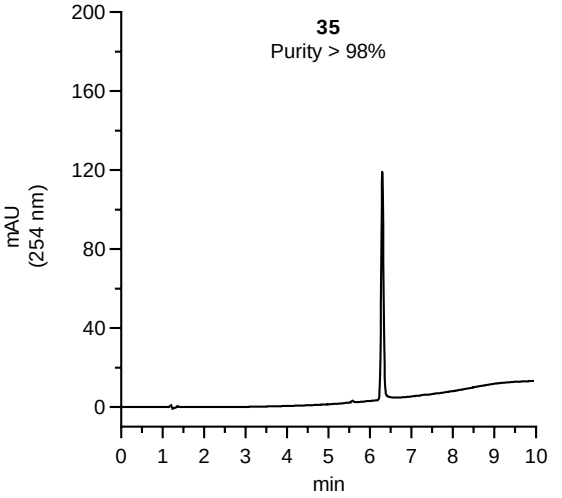

30
Purity > 98%
mAU
(280 nm)
min

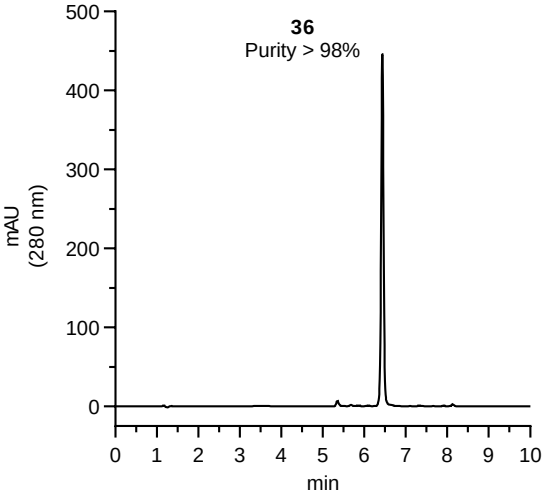

31
Purity > 98%
mAU
(280 nm)
min

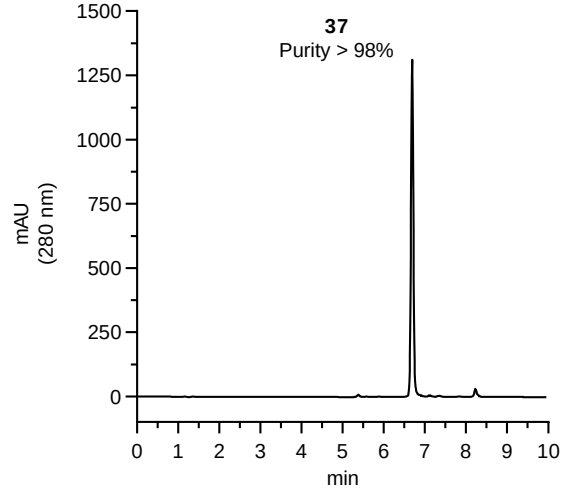

32
Purity > 96%
mAU
(254 nm)
75
60
45
30
15
0
0 1 2 3 4 5 6 7 8 9 10
min

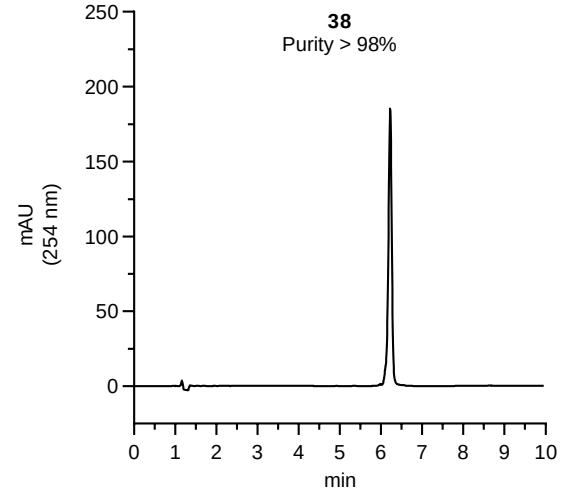

33
Purity > 98%
mAU
(254 nm)
125
100
75
50
25
0
0 1 2 3 4 5 6 7 8 9 10
min

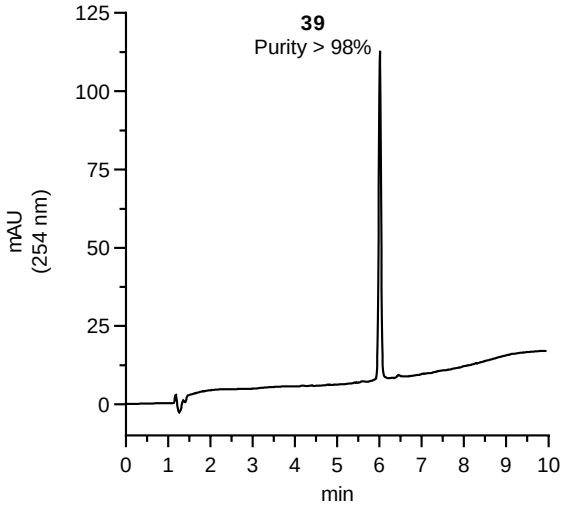

34
Purity > 98%
mAU
(254 nm)
250
200
150
100
50
0
0 1 2 3 4 5 6 7 8 9 10
min

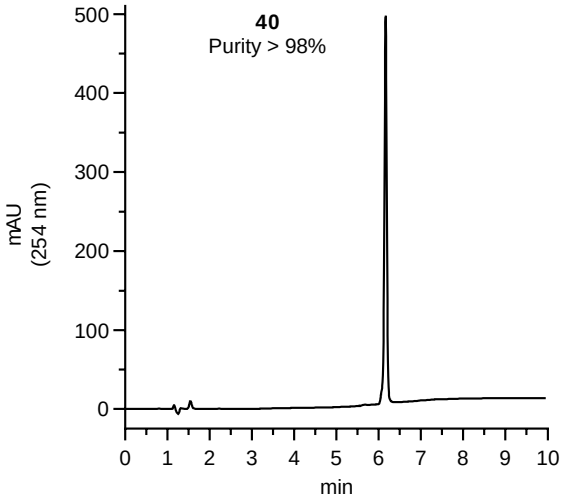

35
Purity > 98%
mAU
(254 nm)
200
160
120
80
40
0
0 1 2 3 4 5 6 7 8 9 10
min

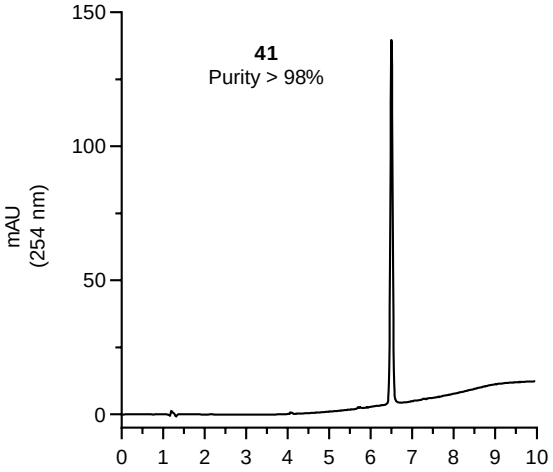

36
Purity > 98%
mAU
(280 nm)
500
400
300
200
100
0
0 1 2 3 4 5 6 7 8 9 10
min

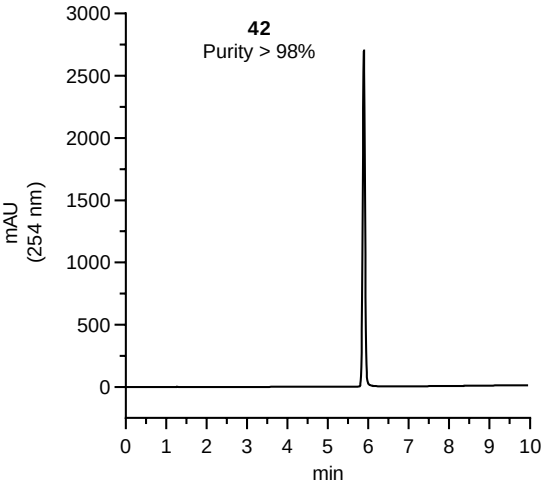

37
Purity > 98%
mAU
(280 nm)
1500
1250
1000
750
500
250
0
0 1 2 3 4 5 6 7 8 9 10
min

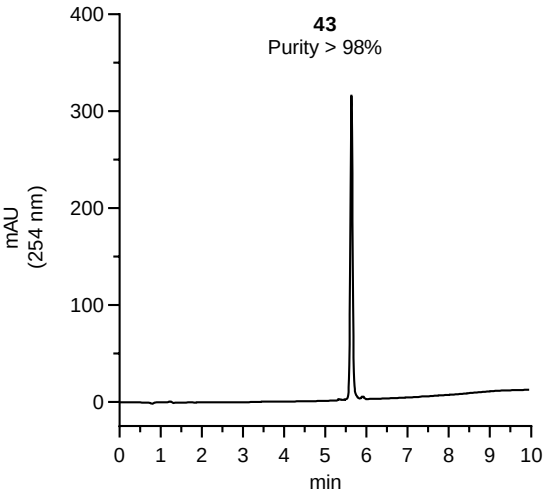

38
Purity > 98%
mAU
(254 nm)
min


39
Purity > 98%
mAU
(254 nm)
min


40
Purity > 98%
mAU
(254 nm)
min


41
Purity > 98%
mAU
(254 nm)


42
Purity > 98%
mAU
(254 nm)
min


43
Purity > 98%
mAU
(254 nm)
min