Table of Contents:

1. General notes

All reactions using anhydrous conditions were carried out under an argon atmosphere in oven-dried glassware. Anhydrous solvents for reactions were purchased from Acros Organics or Sigma-Aldrich. All other solvents were used as supplied by Fisher Chemicals, Merck or Sigma-Aldrich in HPLC or analytical grade. All other reagents were obtained in the highest commercial grades from Sigma Aldrich, TCI Europe or ABCR, and used without further purification. Reactions were monitored by thin layer chromatography using Merck silica gel 60 F254 TLC aluminium plates and visualized with UV light and/or permanganate stain. Chromatographic purification was performed as flash chromatography on Sigma-Aldrich silica (high-purity grade, pore size 60 Å). Concentration under reduced pressure was performed by rotary evaporation at 40 °C at the appropriate pressure. Preparative reversed-phase high-pressure liquid chromatography (RP-HPLC) were performed on an Agilent 1200 Series HPLC using a Synergi polar-RP 80 Å column (4 µm, 150 × 10 mm, Phenomenex) at a flow rate of 4 mL/min with linear gradients of solvents A and B (A = H_2O, B = ACN).

Proton (^1H) nuclear magnetic resonance (NMR) spectra were recorded at 298 K on a Bruker AV400 (400 MHz) or a Bruker VIII500 (500 MHz) spectrometer. Carbon (^{13}C) NMR spectra were measured at 298 K on a Bruker AV400 (100 MHz) spectrometer or on a Bruker AVIII500 (125 MHz) spectrometer and recorded broadband proton decoupled. Chemical shifts (δ) are given in parts per million (ppm) and are reported relative to residual solvent peaks (internal standards). Coupling constants (J) are reported in hertz (Hz) and multiplicities are classified by the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, sext = sextet, sept = septet, m = multiplet or unresolved, br. = broad signal. Mass spectrometry (LC-ESI-MS) spectra were recorded on an Agilent 6100 Series Single Quadrupole MS system combined with an Agilent 1200 Series LC using a Poroshell 120 EC-C18 threaded column (2.7

μm, 4.6 x 50 mm, Agilent) and applying a gradient of 5 - 95% ACN in H_2O containing 0.1% formic acid (FA). Calculated and measured m/z values are reported as dimensionless quantities.

2. Dual-display DNA-encoded chemical library.

The 148'135-compound dual-display encoded self-assembling chemical (ESAC) library was formed by combinatorial hybridization of two sub-libraries A and B, followed by Klenow-mediated code transfer to one strand (Fig. 1) as previously described. [S1]

3. Sub-library A synthesis.

The synthesis of sub-library A was prepared by using 48-mer oligonucleotides (IBA GmbH) carrying a free amino group at the 5'-end (ω-aminohexyl phosphate diester) which were reacted with activated Fmoc-protected amino acids and carboxylic acids to give the corresponding amide conjugates. Sub-library consists of 265 compounds, of which 42 are capable of forming a covalent bond. Oligonucleotide sequences followed the pattern 5'-GGA GCT TCT GAA TTC TGT GTG CTG *XXX XXX* CGA GTC CCA TGG CGC AGC-3', where *XXX XXX* represents the coding sequence (6 nucleotides) that unambiguously identifies each individual library member.

4. Sub-library B synthesis.

The synthesis of sub-library B of 200 compounds has previously been described and was built using activated Fmoc-protected amino acid, carboxylic acid, carboxylic acid anhydride and sulfonyl chloride building blocks to give the corresponding amide or sulfonamide conjugates.[S1] Sub-library B was further extended with additional 359 compounds including 114 building blocks with can potentially undergo a covalent reaction. For the extended

construction of sub-library B also activated formyl carboxylic acids were used following Ugi multicomponent reaction and reductive amination. All library compounds of total 559 members were initially coupled to a general 41-mer 3′-amino-modified oligonucleotide of the sequence 5′-CAT GGG ACT CG *ddd ddd* CAG CAC ACA GAA TTC AGA AGC TCC-3′ (IBA GmbH and LGC Biosearch), which was designed to be complementary to the sub-library A oligonucleotides. In order to allow for promiscuous duplex formation with the coding region of sub-library A, the sub-library B oligonucleotide contains a six nucleotide abasic spacer region (*d*, deoxyabasic) (Fig. 1).

5. Amide bond formation.

Depending on the chemical properties of the building blocks, different conjugation chemistries were applied. After the coupling reaction, the DNA-compound conjugate was precipitated with EtOH before purifying by HPLC. The HPLC purification was performed on a Agilent 1200 Series HPLC using a XTerra preparative column RP18 (5 μm, 10 x 150 mm, Waters) by applying a compound-dependent gradient of buffer B in A (A: water containing 100 mM TEAA pH 7.0, triethylammoniumacetate; B: 80% ACN, 20% H_2O containing 100 mM TEAA pH 7.0;). The separated and collected oligonucleotide-compound conjugates were vacuum-dried overnight, redissolved in H_2O (100 µL), and analysed by mass spectrometry (LC-ESI-MS). LC-ESI-MS spectra were recorded on an Agilent 6100 Series Single Quadrupole MS system combined with an Agilent 1200 Series LC using a XBridge OST C18 column (2.5 μm, 10 x 50 mm, Waters) and applying a gradient of 5-95% methanol in water containing 400 mM HFIP (hexafluoroisopropanol) and 16.3 mM TEA (triethylamine).

Conjugation chemistry using EDC and S-NHS. Carboxylic acids in dimethyl sulfoxide (DMSO, 12.5 µL, 100 mM), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) in DMSO (12 µL,

100 mM), N-hydroxysulfosuccinimide (S-NHS) in 4:1 DMSO/H_2O, (10 μL, 333 mM) were added to DMSO (190 μL) and allowed to stand at 30 °C for 30 min. Subsequently, a mixture of amino-modified sub-library oligonucleotide in H_2O (5 μL, 5 nmol) and triethylamine hydrochloride in H_2O (500 mM TEA·HCl, pH 10.0, 20 μL) was added and the reaction kept at 30 °C for 12 h. Fmoc amino acids were coupled accordingly. Fmoc-deprotection was carried out as previously described. [S1]

Conjugation chemistry using EDC and HOAt. Carboxylic acids in DMSO (12.5 μL, 100 mM), EDC in DMSO (4 μL, 300 mM), 1-hydroxy-7-azabenzotriazole (HOAt) in DMSO (4 μL, 60 mM) and *N*,*N*-diisopropylethylamine (DIPEA) in DMSO (4 μL, 300 mM) were added to DMSO (16 μL) and allowed to stand at 25 °C for 15 min. Subsequently, a mixture of amino-modified sub-library oligonucleotide in H_2O (5 μL, 5 nmol) and MOPS buffer (100 mM MOPS, 1 M NaCl, pH 8.0, 35 μL) was added and the reaction filled up with H_2O to 125 μL total volume. The reaction was performed at 25 °C for 12 h. Fmoc amino acids were coupled accordingly. Fmoc-deprotection was carried out as previously described. [S1]

Conjugation chemistry using DMTMM. Carboxylic acids in DMSO (12.5 μL, 100 mM) and 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) in H_2O (12 μL, 100 mM) were added to DMSO (40 μL) and allowed to stand at 25 °C for 15 min. Subsequently, a mixture of amino-modified sub-library oligonucleotide in H_2O (5 μL, 5 nmol) and MOPS buffer (100 mM MOPS, 1 M NaCl, pH 8.0, 35 μL) was added and the reaction filled up with H_2O to 125 μL total volume. The reaction was performed at 25 °C for 12 h. Fmoc amino acids were coupled accordingly. Fmoc-deprotection was carried out as previously described. [S1]

Conjugation chemistry using EDC, HOAt and S-NHS. Carboxylic acids in DMSO (20 μL, 150 mM) was added to EDC (20 μL, 150 mM in DMSO), followed by a mixture of HOAt and S-NHS (HOAt 5 mM, S-NHS 200 mM, DMSO:H_2O 3:1, 20 μL). The reaction mixture was incubated at room temperature for 30 min and then cooled to 4 °C. To the cooled solution was added amino-modified sub-library oligonucleotide in H_2O (2 μL, 2 nmol) in water and the resulting mixture was incubated in the fridge overnight (8 °C). The reaction was allowed to warm to room temperature. Ethanol precipitation and HPLC purification was carried out as described above. Fmoc amino acids were coupled accordingly. Fmoc-deprotection and reductive amination was carried out as previously described. [S1, S2],

Ugi reaction. The amino-modified oligonucleotide was coupled to 4-formyl benzoic acid as described above using EDC, HOAt and S-NHS. To the modified DNA (4 nmol) in H_2O (10 μL) was added the neat amine (5-fold excess compared to carboxylic acid and isocyanide). After 10 min incubation 13 μL carboxylic acid (1 M in MeOH, or the equivalent as suspension) and 13 μL isocyanide (1 M in MeOH) were added. The reaction mixture was incubated overnight at room temperature. The reaction progress was monitored by LC-MS. In case of incomplete conversion more equivalents of the reagents were added. Ethanol precipitation and HPLC purification was carried out as described above.

Conjugation of carboxylic acid anhydrides. Carboxylic acid anhydrides in DMSO (25 μl, 100 mM) were mixed with sodium hydrogen phosphate in H_2O (25 μL, 500 mM, pH 7.1), DMSO (195 μL), H_2O (35 μL) and subsequently reacted with amino-modified sub-library oligonucleotide in H_2O (5 μL, 5 nmol) overnight at 30 °C. The reaction was quenched with Tris-Cl (20 μl, 500 mM, pH 8.1) at 30 °C for 1 h.

6. Encoding of sub-library B.

To unambiguously label library members in sub-library B, individual oligonucleotide-compound conjugates were extended with a unique identifier sequence (Fig. 1). For this purpose, 39-mer code oligonucleotides of sequence 5'-CCT GCA TCG AAT GGA TCC GTG *XXX XXX XX* GCA GCT GCG C-3' (IBA GmbH, LGC Biosearch) were used, where *XXX XXX XX* denotes an 8-digit code region. The HPLC-purified oligonucleotide-compound conjugates were ligated to these coding oligonucleotides with the help of a DNA adapter (14 nt) or a chimeric DNA/RNA adapter oligonucleotide (21 nt), which is complementary to both, the sub-library B oligonucleotide-compound conjugates and the sub-library B code oligonucleotides. The adapter oligonucleotide was eventually removed by RNase HII (New England Biolabs) treatment and the ligation products were analyzed by LC-ESI-MS.

Ligation protocol. The HPLC-purified oligonucleotide-compound conjugates were ligated to coding oligonucleotides in a similar fashion as previously described (1). Sub-library B oligonucleotide-compound conjugate in H_2O (12.5 μL, 4 μM), sub-library B code oligonucleotide in H_2O (39 nt, 1.3 eq., 13 μL, 5 μM), sub-library B chimeric DNA/RNA adapter oligonucleotide in H_2O (21 nt, 2 eq., 5 μL, 20 μM), 10x ligation reaction buffer (5 μL, New England Biolabs) and H_2O (8.5 μL) were mixed and heated up to 90 °C for 2 min before the mixture was allowed to cool down to room temperature. T4 DNA ligase (1 μL, New England Biolabs, stock 400'000 units/mL) was added and ligation performed at 16 °C for 10 hours before inactivating the ligase at 65 °C for 15 min. Single ligated oligonucleotide-compound conjugates were pooled to a masterpool which was treated in batches of 50 pmol (1 μM, 50 μL) with RNase HII (New England Biolabs, 1 μL, 5'000 units/mL) and 5.6 μL 10x ThermoPol Reaction Buffer for 12 hour at 37 °C. After adapter degradation the solution was immediately cartridge purified (Eurogentec, SmartPure PCR Kit).

Ligation protocol for building blocks, which can potentially undergo a covalent reaction. To a buffered solution (10 mM phosphate, 50 mM NaCl, 10 mM $MgCl_2$, 1 mM ATP) was added the oligonucleotide-compound conjugate, followed by a DNA adapter (14 nt, 1.33 eq, 4.7 µL, 10 µM) and the coding oligonucleotide (13.5 µL, 3.33 µM). The reaction mixture was heated for 20 min to 53 °C, then allowed to cool to room temperature. To the reaction mixtures was added 10 µL Ligase (New England Biolabs, 1:60 in phosphate buffer, stock: 2'000'000 units/mL). The reaction was kept for 4 hours at 8 °C, then 1 h at room temperature. The reaction mixture was diluted with 120 µL 3 M guanidine hydrochloride, 3.75 M ammonium acetate, 30% isopropanol and applied to a silica spin columns (Macherey Nagel), washed twice with NT3 buffer (Macherey Nagel) and eluted with 30 µL phosphate buffer.

7. Library hybridization and code transfer to sub-library A strand.

To obtain the final library, sub-libraries A and B were hybridized, resulting in a combinatorial collection of duplexes, where each member of sub-library A could pair with each member of sub-library B. For the unambiguous identification of any dual pharmacophore combination by high-throughput sequencing, coding information for A and B needs to be read from the same DNA strand. This was achieved by a Klenow-polymerase assisted sub-library A strand extension of the A/B heteroduplexes, which transferred the coding information from the sub-library B strand onto the sub-library A strand (Fig. 1).

Hybridization and Klenow fill-in protocol. Hybridization was achieved as previously described.[S1] However, the mixture of the two strands was heated to only 72 °C in thiol-free phosphate buffer for 2 min. The Klenow fill-in was achieved by using 40 μL of the hybridization mix (40 pmol), 80 μL phosphate buffer pH 7.0 with 1 mM $MgCl_2$, 1 μL deoxynucleotide solution mix (dNTP, 10 mM) and 0.25 μL Klenow fragment (New England Biolabs, 3'→ 5'exo-, 50

units/μL). After 2 hours the reaction mixture from the Klenow fill-in was aliquoted and diluted for selections as previously described. [S1]

8. Affinity screening of the dual-display ESAC library.

Affinity selections were performed as previously described using a KingFisher magnetic particle processor (Thermo Fisher Scientific). [S1] Streptavidin-coated magnetic beads (0.1 mg, Dynabeads MyOne Streptavidin T1 or C1, Invitrogen, Life Technologies, Thermo Fisher Scientific) were resuspended in PBS (100 µL, 50 mM sodium phosphate, 100 mM NaCl, pH 7.4) and subsequently incubated with biotinylated target protein (100 µL, different concentrations) for 30 min with continuous gentle mixing. Protein-coated beads were washed three times with PBS-Tween (PBST, 200 µL, 50 mM sodium phosphate, 100 mM NaCl, 0.05% vol/vol Tween 20, pH 7.4) supplemented with biotin (100 µM) and subsequently incubated with the ESAC library (100 µL, 5 nM total concentration, in PBST) for 1 h with continuous gentle mixing. After removing unbound library members by washing five times with PBST (200 µL), beads carrying bound library members were resuspended in elution buffer (100 µL, 10 mM Tris-Cl, pH 8.5) and the DNA–compound conjugates separated from the beads by heat denaturation of streptavidin and the target protein (95 °C for 10 min). The DNA portion of eluted library members was amplified by PCR, introducing at the same time additional, selection-specific DNA barcodes and submitted to Illumina highthroughput DNA sequencing (HiSeq 2500, Functional Genomics Center Zurich). Selection results were decoded by an *in-house* developed analytical software.

9. Target proteins.

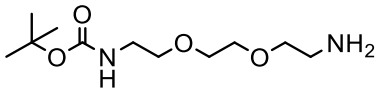
Selections against catalytic domains of c-Jun N-terminal kinases (JNK1), Cyclin G Associated Kinase (GAK) and Bruton tyrosine kinase (BTK). For JNK1 and GAK, the recombinant proteins containing the N-terminal His_6-tag and the C-terminal biotinylated-Avi-tag were prepared as previously described. [S3-S5] For BTK, the kinase domain was expressed in Sf9 cells, and purified using Ni-affinity chromatography and size exclusion chromatography. The purity of all proteins were assessed to be more than 95% by SDS-PAGE. The correct mass and stoichiometric modification with biotin was confirmed using ESI-TOF mass spectrometry.

10. Protein MS analysis.

For each analysis, 40 pmol JNK1 protein and +/− glutathion (GSH) were preincubated for 5 min followed by the treatment of the small molecules (A82, B272-PA, A82-L-B272 stock solutions in DMSO) in a total volume of 100 μL. After 5 minutes the probe was injected onto a reversed phase MassPrep™ on-line desalting cartridge (20 μm, 2.1 x 10 mm, Waters). After desalting, the protein was eluted with an LC gradient (5 - 95% B in 11 minutes, A = 0.1% formic acid in H_2O, B = ACN, flow rate = 1 mL/min) into an Agilent 6100 Series Single Quadrupole MS system combined with an Agilent 1200 Series LC. Mass spectra were deconvoluted over the full width at half peak maximum (FWHM) by using implemented ChemStation software (Agilent). Assay conditions: 0.4 μM JNK1/GAK in 50 mM HEPES, 300 mM NaCl, 10 mM $MgCl_2$, 1 μM TCEP; 0.4 μM BTK in 20 mM Tris, 240 mM NaCl, 30 μM DTT; +/− glutathion (GSH, 0.5 mM final concentration); 0.1 - 4.0 μM final concentration of binder; 1.6% final DMSO concentration.

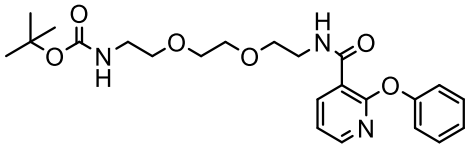
11. Synthetic procedures

Synthesis of Boc-NH protected 2,2'-(Ethylenedioxy)bis(ethylamine) ***1***



Compound **1** was synthesized according to a modified published procedure. [S6] The PEG derivative 1,8-diaminotriethyleneglycol (500 mg, 3.37 mmol) was dissolved in 2 ml methanol and treated with TEA (47 μL, 0.34 mmol) followed by addition of di-tert-butyl dicarbonate (736 mg, 3.37 mmol). The reaction was stirred at 35 °C overnight. The reaction mixture was concentrated to dryness and purified by silica gel chromatography using dichloromethane / MeOH / TEA (9 / 0.9 / 0.1). After combining the product fractions, the solvent was evaporated and the product **1** obtained as viscous golden oil (595 mg, 71% yield).

Synthesis of tert-butyl N-[2-(2-{2-[(2-phenoxypyridin-3-yl) formamido]ethoxy}ethoxy)ethyl] carbamate ***2***



2-Phenoxynicotinic acid (100 mg, 0.46 mmol), EDC-HCl (105 mg, 0.55 mmol) and HOAt (31 mg, 0.23) were dissolved in 2 mL of dry DMF. The mixture was allowed to stir at room temperature for 15 min and was then reacted with compound 1, which was dissolved in 1 ml dry DMF and DIPEA (116 μL, 0.69 mmol). After two hours the reaction solution was diluted with dichloromethane and the organic phase extracted with 100 mM HCl and brine. The combined organic phases were dried with Na_2SO_4 and evaporated to dryness. Silica gel chromatography (0 – 3% MeOH in DCM) gave compound **2** as a white foam (188 mg, 91%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.61 (dd, J = 7.6, 2.0, 1H), 8.29 (br, 1H), 8.21 (dd, J = 4.8, 2.0, 1H), 7.47 – 7.43 (m, 2H), 7.30 – 7.26 (m, 1H), 7.19 – 7.16 (m, 2H), 7.15 (dd, J = 7.6, 4.8,

1H), 3.73 – 3.66 (m, 4H), 3.59 – 3.56 (m, 2H), 3.45 – 3.42 (m, 2H), 3.37 (t, J = 5.2, 2H), 3.23 – 3.19 (m, 2H), 1.43 (s, 9H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 163.40, 160.25, 155.90, 152.66, 149.87, 142.17, 129.73, 125.63, 121.79, 119.29, 116.92, 79.18, 70.32, 70.14, 69.73, 40.19, 39.64, 28.37. LC-ESI-MS: m/z calcd. for $C_{23}H_{31}N_3O_6$: 445.52; found $[M+H]^+$: 446.12.

Synthesis of (2E)-4-(3,4-difluorophenyl)-4-oxo-N-[2-(2-{2-[(2-phenoxypyridin-3-yl)formamido]ethoxy}ethoxy)ethyl]but-2-enamideE)-N-[2-(2-{2-[4-(3,4-difluorophenyl)-4-oxobut-2-enamido]-ethoxy}-ethoxy)-ethyl]-2-phenoxynicotinamide ***A82-L-B272***

Compound **2** (188 mg, 0.42 mmol) was dissolved in 2 mL of dry DCM and 1 mL of 4 M HCl in dioxane. The mixture was allowed to stir at room temperature overnight. Afterwards, the solution was evaporated to dryness and the residue dissolved in 3 mL of a dioxane / H_2O mixture (2/8), divided into three tubes and lyophilized. The content of one tube (35.7 mg, 0.09 mmol) was dissolved in 1 mL of dry DMF and DIPEA (22 μL, 0.13 mmol). In a separate dry flask, 4-(3,4-difluorophenyl)-4-oxobut-2-enoic acid (18 mg, 0.08 mmol), EDC-HCl (20 mg, 0.10 mmol) and HOAt (6 mg, 0.04 mmol) were dissolved in 1.5 mL of dry DMF. The solutions were combined and allowed to stir for 3 hours at room temperature. The reaction solution was evaporated to dryness and directly purified by silica gel chromatography (0 – 3% MeOH in DCM) to obtain compound **A82-L-B272** as a yellowish solid (28.7 mg). The compound was further purified by reversed-phase HPLC (90% H_2O / 10% ACN to 20% H_2O / 80% ACN over 10 min). After lyophilization the title compound was collected as a white solid (16.6 mg, 0.031 mmol, 22% yield).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.61 (dd, J = 7.6, 2.0, 1H), 8.26 (br, 1H), 8.21 (dd, J = 4.8, 2.0, 1H), 7.87 (d, J = 15.2, 1H), 7.87 – 7.80 (m, 2H), 7.48 – 7.44 (m, 2H), 7.33 – 7.28 (m, 2H), 7.20 – 7.18 (m, 2H), 7.15 (dd, J = 7.6, 4.8, 1H), 7.01 (d, J = 15.0, 1H), 6.65 (br, 1H), 3.74 – 3.71 (m, 4H), 3.65 – 3.63 (m, 2H), 3.52 – 3.50 (m, 6H). ^{13}C-NMR (125 MHz, $CDCl_3$, without

^{19}F decoupling): δ = 187.07, 163.73, 163.59, 160.20, 155.01 – 149.47 (2 x dd, F-C), 152.61, 149.92, 142.16, 136.17, 133.95, 131.96, 129.74, 125.93 – 125.84 (1x), 125.67, 121.80, 119.35, 118.07 – 117.62 (2x), 116.78, 70.42, 70.26, 69.70, 69.44, 39.67, 39.65. LC-ESI-MS: m/z calcd. for $C_{28}H_{27}F_2N_3O_6$: 539.54; found $[M+H]^+$: 540.22.

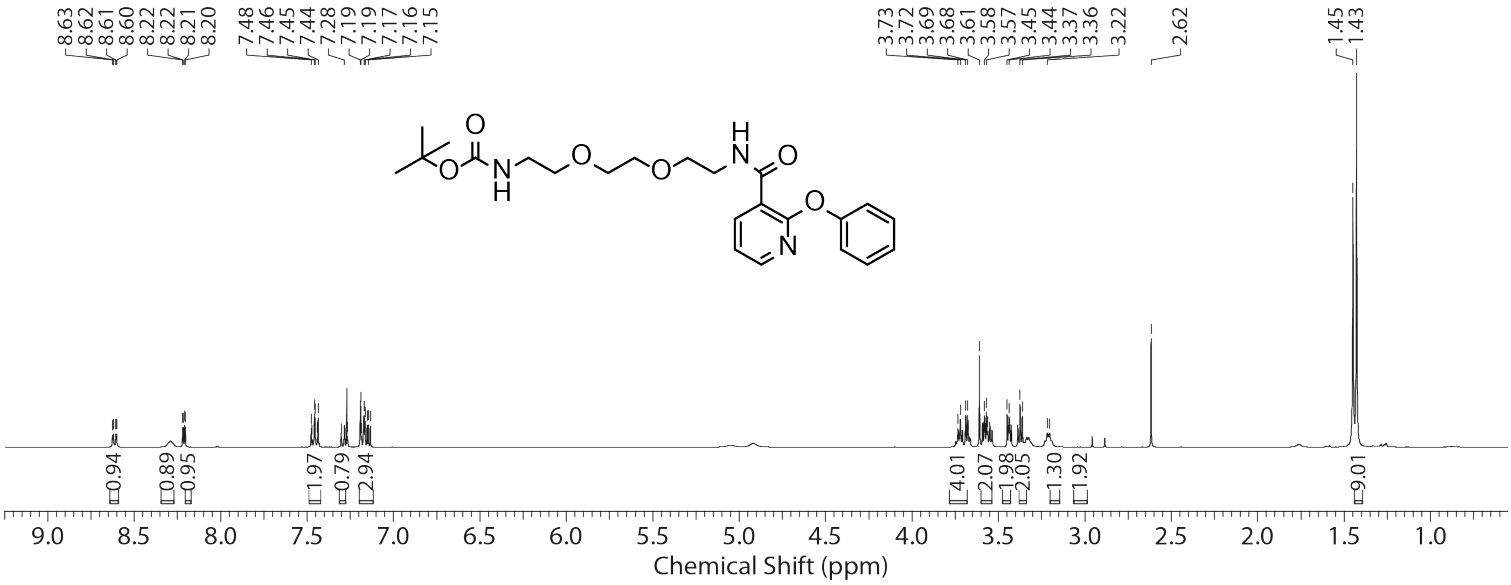
Synthesis of (2E)-4-(3,4-difluorophenyl)-4-oxo-N-propylbut-2-enamide ***B272-PA***

4-(3,4-difluorophenyl)-4-oxobut-2-enoic acid (20 mg, 0.095 mmol), EDC-HCl (22 mg, 0.12 mmol), and HOAt (7 mg, 0.05 mmol) were dissolved in 1 mL of dry DMF. DIPEA (50 μL, 0.29 mmol) was added and the mixture was stirred at room temperature for 10 minutes. Propylamine (9 μL, 0.10 mmol) was added and the solution was allowed to stir at room temperature overnight. The reaction solution was evaporated to dryness and directly purified by silica gel chromatography (Ethyl acetate/Hexane, 1:1) to obtain compound **B272-PA** as a withe solid (10.7 mg). The compound was further purified by reversed-phase HPLC (90% A / 10% B to 20% A / 80% B over 10 min). After lyophilization the title compound was collected as a white solid (4.9 mg, 0.019 mmol, 22% yield).

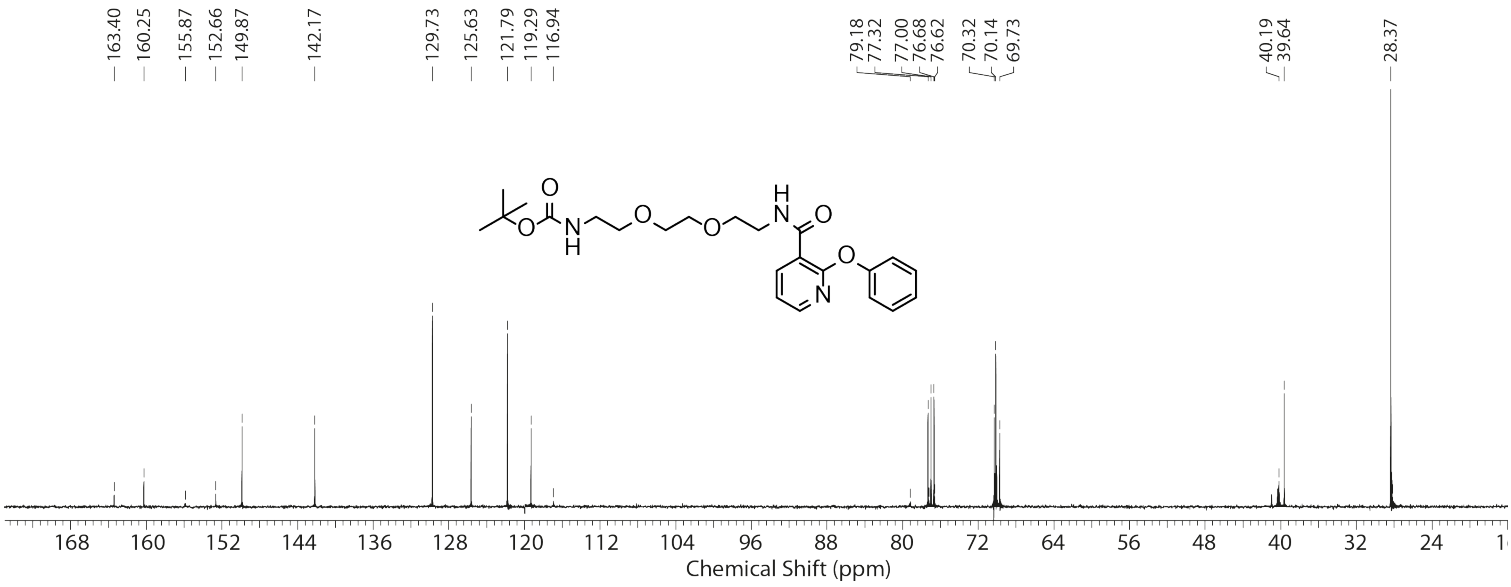
^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.90 (d, J = 14.8, 1H), 7.87 – 7.81 (m, 2H), 7.32 – 7.27 (m, 1H), 7.05 – 7.00 (m, 1H), 6.24 (br, 1H), 3.38 (q, J = 6.2, 2H), 1.62 (sext, J = 7.3, 2H), 0.97 (t, J = 7.4, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$, no ^{19}F decoupling): δ = 187.22, 163.69, 155.13 – 149.57 (2 x dd, F-C), 136.24, 133.96, 132.02, 126.00 – 125.90 (1x), 118.11 – 117.72 (2x), 41.80, 22.68, 11.35. LC-ESI-MS: m/z calcd. for $C_{13}H_{13}F_2NO_2$: 253.25; found $[M+H]^+$: 254.1.

12. Characterization

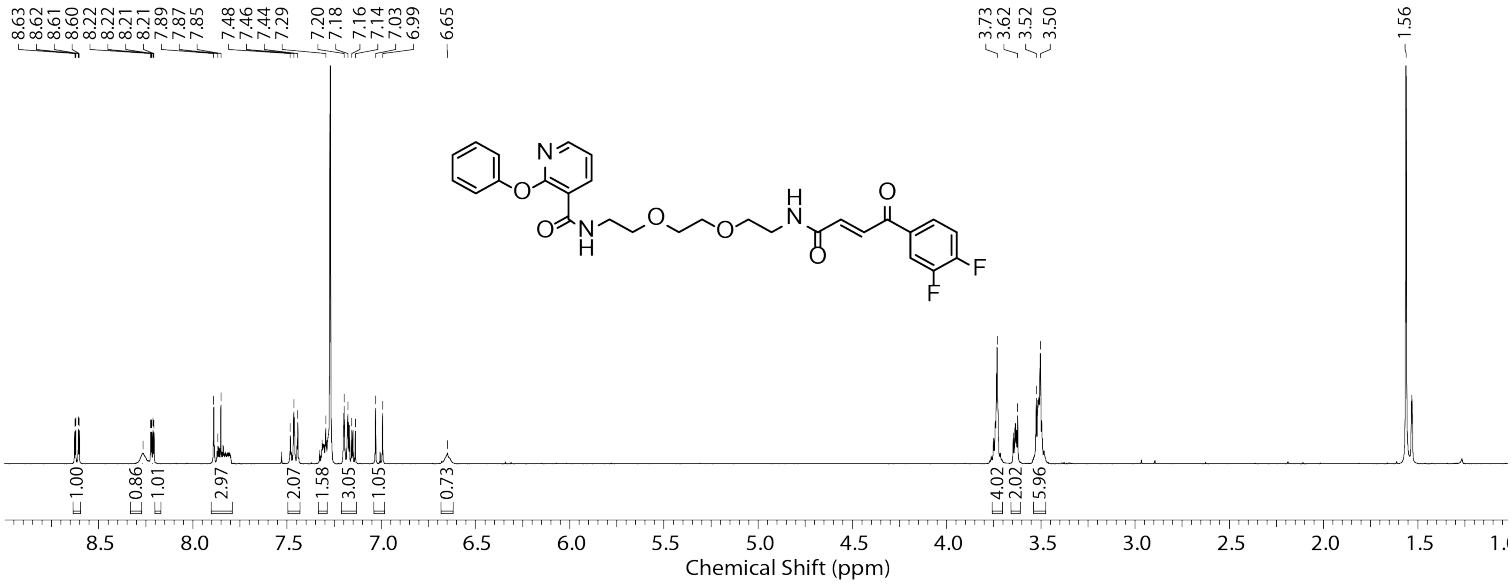
^{1}H NMR of compound **2** in $CDCl_3$ (400 MHz)



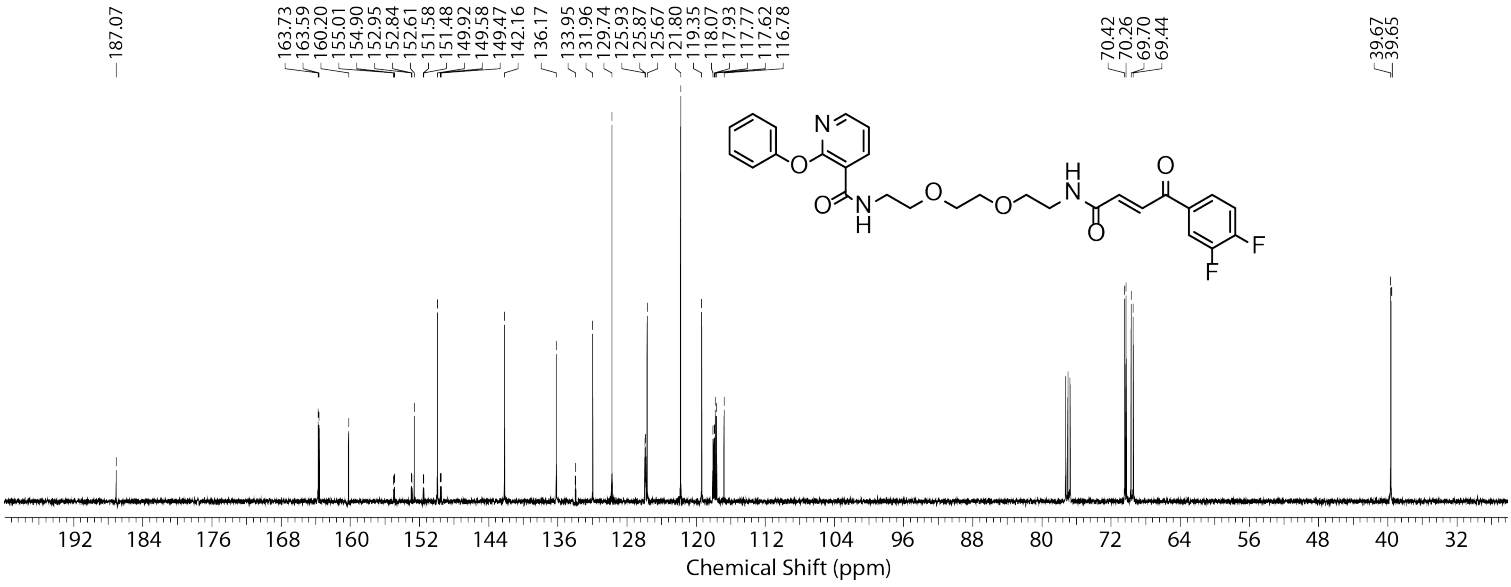
*^{13}C NMR of compound **2** in $CDCl_3$ (100 MHz)*



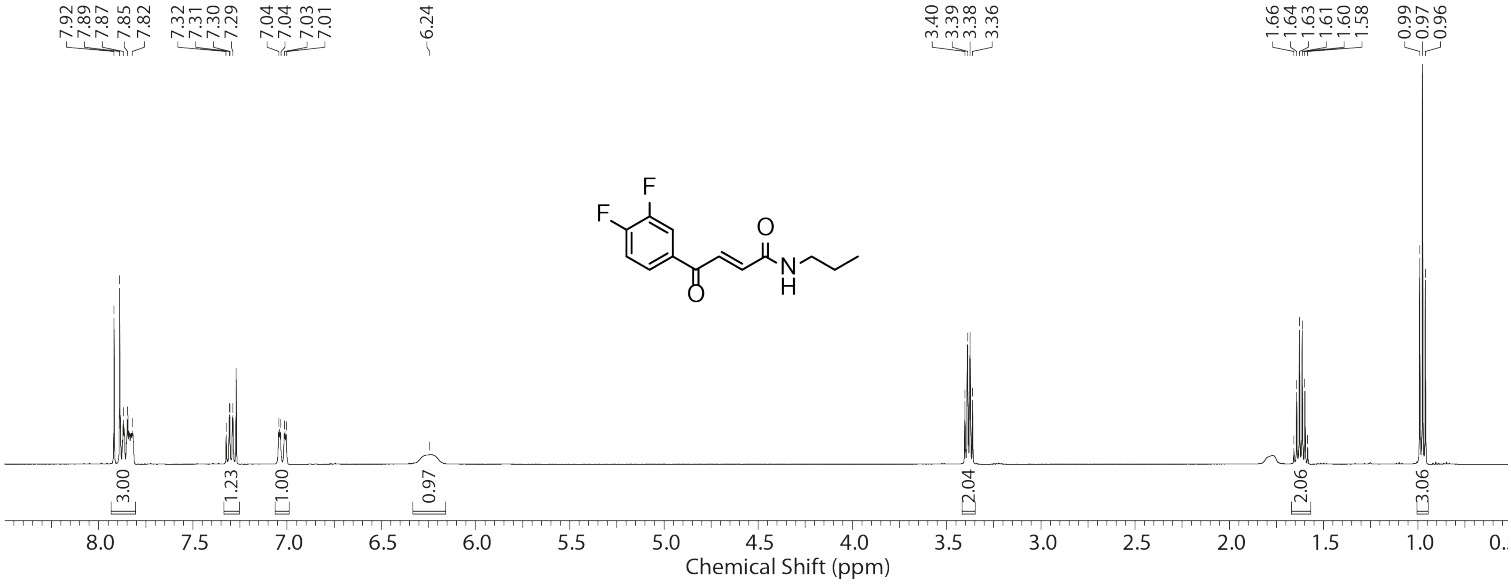
*[1]H NMR of compound **A82-L-B272** in $CDCl_3$ (400 MHz)*



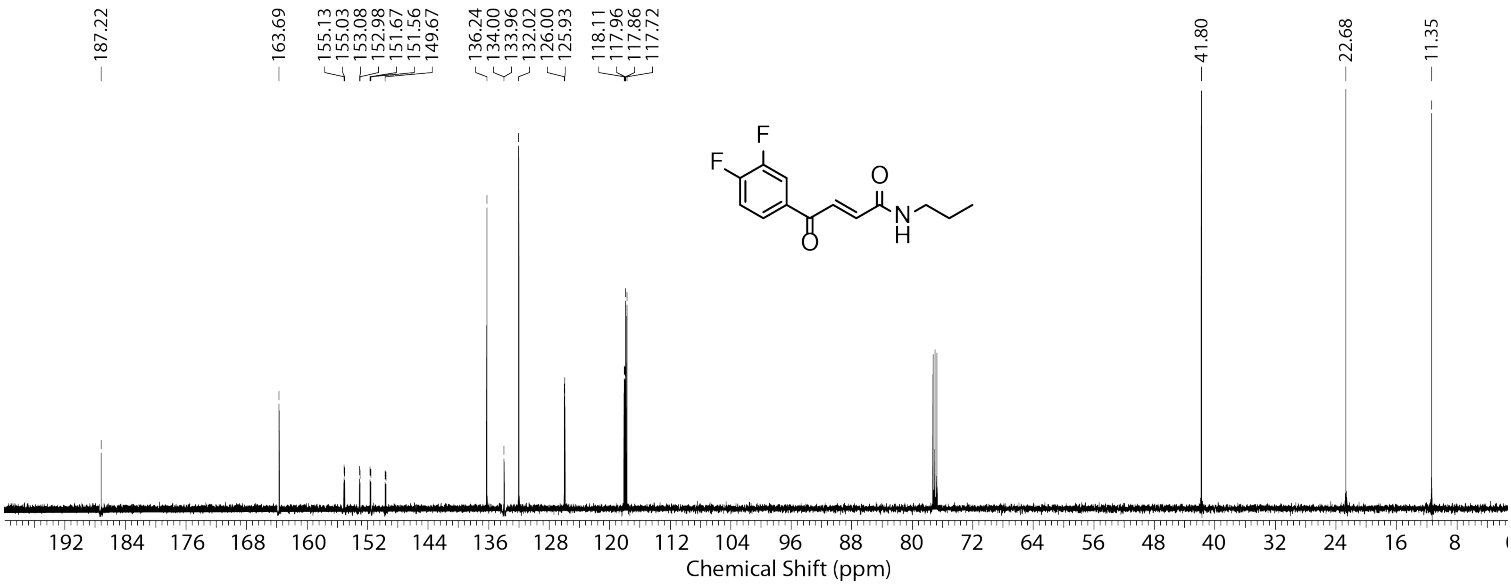
*[13]C NMR of compound **A82-L-B272** in $CDCl_3$ (125 MHz)*



[1]H NMR of compound **B272-PA** *in $CDCl_3$ (400 MHz)*



[13]C NMR of compound **B272-PA** *in $CDCl_3$ (125 MHz)*



13. Selection experiments against positive controls

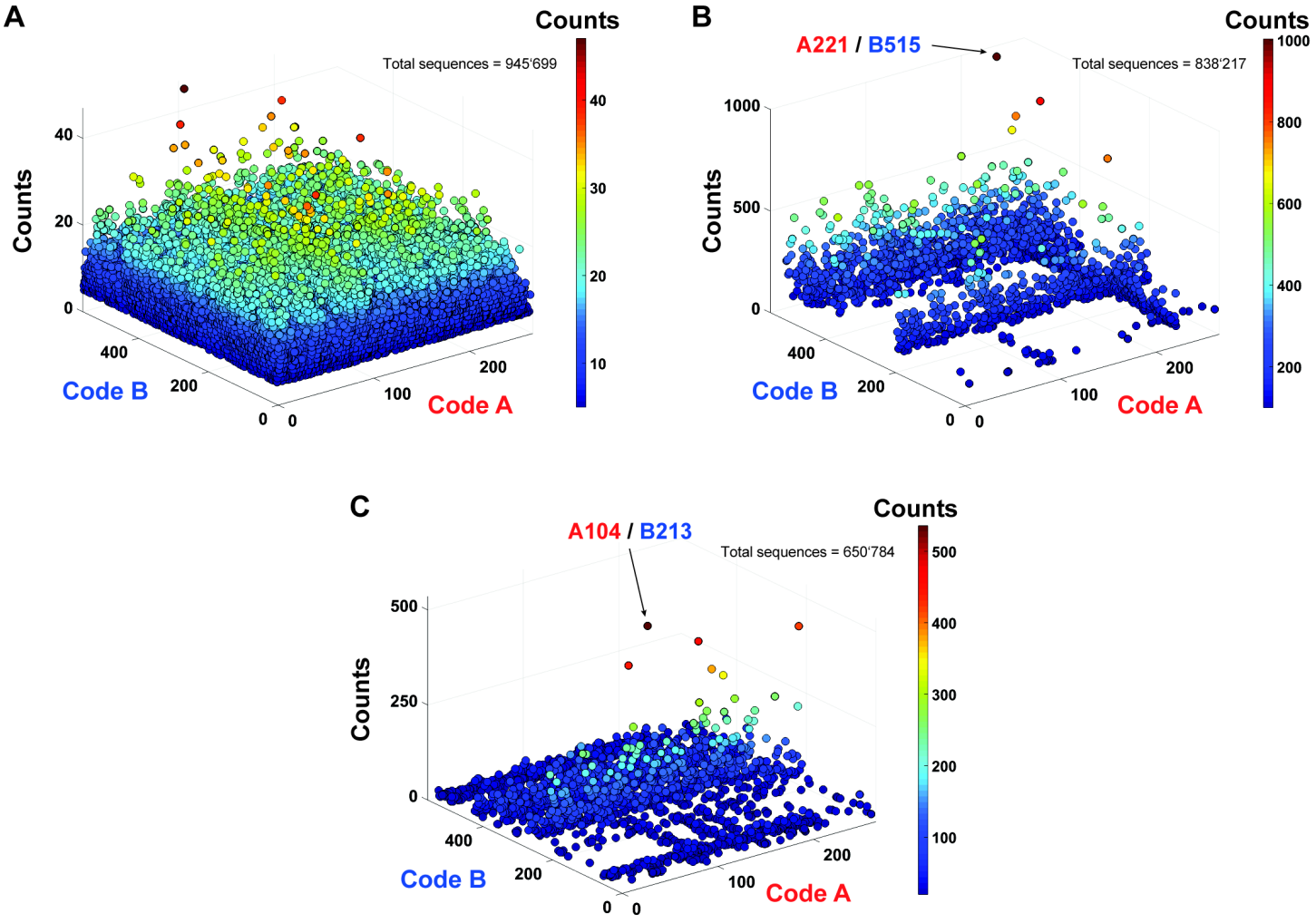


Figure S1. A) Unselected library (cut-off = 5, average count = 6.3). B) Control selection experiment against horseradish peroxidase (positive control = B515, counts hit = 1003, cut-off = 100, average count = 5.6). C) Control selection experiment against carbonic anhydrase IX (positive control = B213, counts hit = 536, cut-off = 20, average count = 4.4).

14. Literature References

[S1] M. Wichert, N. Krall, W. Decurtins, R.M. Franzini, F. Pretto, P. Schneider, D. Neri, J. Scheuermann, *Nat Chem* **2015**, *7*, 241–249.

[S2] R.M. Franzini, F. Samain, M. Abd Elrahman, G. Mikutis, A. Nauer, M. Zimmermann, Scheuermann, J. Hall, D. Neri, *Bioconjug Chem* **2014**, *25*, 1453–1461.

[S3] A. Chaikuad, T. Keates, C. Vincke, M. Kaufholz, M. Zenn, B. Zimmermann, C. Gutiérrez, R. G. Zhang, C. Hatzos-Skintges, A. Joachimiak, S. Muyldermans, F. W. Herberg, S. Knapp, S. Müller, *Biochem. J.* **2014**, *459*, 59–69

[S4] T. Keates, C. D. Cooper, P. Savitsky, C. K. Allerston, C. Phillips, M. Hammarström, N. Daga, G. Berridge, P. Mahajan, N. A. Burgess-Brown, S. Müller, S. Gräslund, O. Gileadi, *N. Biotechnol.* **2012**, *29*, 515–525

[S5] A. Chaikuad, E. M. Tacconi, J. Zimmer, Y. Liang, N. S. Gray, M. Tarsounas, S. Knapp, *Nat. Chem. Biol.* **2014**, *10*, 853–860

[S6] R. Clayton, J. Hardman, C.C. Labranche, K.D. McReynolds, *Bioconjug. Chem.* **2011**, *22*, 2186–2197.