Supporting information

General Techniques for chemical synthesis

All reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Anhydrous solvents were obtained by passing through commercially available alumina columns (Innovative Technology, Inc.). NovaPEG Rink Amide Resin was purchased from Novabiochem®, and was swollen in DCM before each reaction. Solid phase reactions were carried in SPE tubes fitted with a frit and a tap. Automated solid phase synthesis was carried out on an Intavis AG Multipep RS instrument. LC-MS were recorded using an HP 1100 series or Thermo Electron Corporation HPLC with a Thermo Finnigan Surveyor MSQ Mass Spectrometer System coupled with a Thermo Scientific column (50 x 2.1 mm), or a DIONEX Ultimate 3000 UHPLC with a Thermo LCQ Fleet Mass Spectrometer System using PINNACLE DB C18 column (1.9 mm, 50 x 2.1 mm) operated by positive mode or a HP 1100 series or Thermo Electron Corporation HPLC with a Thermo Finnigan Surveyor MSQ Mass Spectrometer System using a Thermo Scientific column (50 x 2.1 mm). MALDI spectra were measured using a Brucker Daltonics Autoflex TOF spectrometer or using a Bruker Daltonics Autoflex spectrometer. Microwave reactions were carried out in a CEM discovery instrument. Polymer-bound intermediates were characterized by LC-MS and/or MALDI (2,5-Dihydroxybenzoic acid or α-Cyano-4-hydroxycinnamic acid matrix and desorbed with laser between 15-55%) following a cleavage from the resin. Cleavages were carried out on 0.1-0.3 mg of dry resin with 20 μL of TFA for 20 min at room temperature. The TFA solution was either evaporated or added to 200 μL of Et_2O and centrifuged at 15000g for 5 min to pellet the precipitated compound. The resulting white pellet was then washed with Et_2O (200 μL) and re-dissolved in 1:1 $MeCN/H_2O$ (40μL) for analysis. The mix and split synthesis was performed according to previously established protocols.

General procedures for the supported synthesis of PNA-encoded libraries and building blocks preparation.

Procedure 1. Capping. To 10 mg of NovaPEG Rink amide resin were added 200 μL of capping mixture (9.2 mL of acetic anhydride and 13 mL of 2,6-lutidine in 188 mL of

DMF) and the resin was shaken for 15 min. Subsequently, the resin was washed with 6 x 200 µL of DMF and 6 x 200 µL of CH_2Cl_2.

Procedure 2. Capping in Intavis AG Multipep RS Synthesizer. To 10 mg of NovaPEG Rink amide resin were added 100 µL of capping mixture (9.2 mL of acetic anhydride and 13 mL of 2,6 lutidine in 188 mL of DMF). After 5 min, the resin was washed with 2 x 250 µL of DMF.

Procedure 3. Fmoc deprotection. To 100 mg of NovaPEG Rink amide resin were added 2.0 mL of 20% piperidine solution in DMF, and the resin was shaken for 5 min. Subsequently, the resin was washed with 6 x 2 mL of DMF and 6 x 2 mL of CH_2Cl_2, and the deprotection sequence was repeated a second time.

Procedure 4. Fmoc deprotection in Intavis AG Multipep RS Synthesizer. To 10 mg of NovaPEG Rink amide resin were added 100 µL of 20% piperidine solution in DMF. After 2 min, the resin was washed with 250 µL DMF the reaction was repeated twice with a final wash with 5 x 250 µL of DMF and 3 x 250 µL of CH_2Cl_2.

Procedure 5. First amino acid coupling on resin, loading reduction. To a solution of 0.09 mmol (1.0 equiv, 0.2 mmol/g loading) of amino acid in 7.0 mL of NMP were added 68.9 mg (0.45 mmol, 5.0 equiv) of HOBt followed by 210 µL (1.35 mmol, 15.0 equiv) of DIC. The mixture was activated for 5 min at room temperature, and then added to 450 mg of NovaPEG Rink amide resin. The reaction mixture was shaken for 16 hours and subsequently the resin was washed with 6 x 10 mL of DMF and 6 x 10 mL of CH_2Cl_2. The remaining free amino groups were capped as described in ***procedure 1*** (30 min.)

Procedure 6. Carboxylic acid coupling (including amino acids, PEG-spacer and Cy3) also in Intavis AG Multipep RS Synthesizer

using HCTU or HATU activation: To a solution of 0.08 mmol (4.0 equiv) of amino acid in NMP (1 mL) were added 140 µL (0.07 mmol, 3.5 equiv) of HCTU or HATU (0.5 M) in NMP followed by 67 µL of base solution [DIPEA 1.2 M (0.25 mmol, 4.0 equiv) and 2,6 lutidine 1.8M (0.38 mmol, 6.0 equiv) in NMP]. The mixture was activated for 5 min at room temperature, and then added to 100 mg of resin (0.02 mmol, 1.0 equiv). The reaction mixture was shaken for 2 hours and subsequently the resin was washed with 6 x DMF and 6 x CH_2Cl_2.

***using HOBt/DIC activation*:**

The corresponding carboxylic acid (0.01 mmol, 5.0 equiv) was dissolved in 200µL of NMP and HOBt (1.5 mg, 0.01 mmol, 5.0 equiv) followed by DIC (4.7 µL, 0.03 mmol, 15.0 equiv) were added. The mixture was activated for 15 min, then added to the corresponding resin (10 mg, 0.002 mmol, 1.0 equiv) and the reaction was shaken for 12 hours. Finally, the resin was washed with 6 x 250 µL of DMF and 6 x 250 µL CH_2Cl_2.

Procedure 7a. Fmoc-protected amino acids introduction (first point of diversity). To a solution of the corresponding Fmoc-protected amino acid (10.0 µmol, 10.0 equiv) in 50 µL of dry NMP, 30 µL of HOBt 0.33 M in NMP (10.0 µmol, 10.0 equiv) was added subsequently followed by 20 µL of DIC 1.5 M in NMP (10.0 µmol, 10.0 equiv). The mixture was activated for 15 minutes, added over the corresponding resin 5mg (1.0 µmol, 1.0 equiv) and shaken overnight. The 96 well plates used to perform the 100 reactions in parallel were and washed with 6 x 250 µL of DMF and 6 x 250 µL of CH_2Cl_2.

Procedure 7b. Carboxylic acid coupling of small molecules (second point of diversity).

For the introduction of the second point of diversity, small molecule containing carboxylic acid were coupled using the following procedure: a solution of the small molecule (10.0 µmol, 10.0 equiv) in 30 µL of NMP were added 18 µL (9.0 µmol, 9.0 equiv) of HATU 0.5 M in NMP, followed by 20 µL of base solution [DIPEA 1.2 M (0.008 mmol, 4.0 equiv) and 2,6 lutidine 1.8 M (0.012 mmol, 6.0 equiv) in NMP]. The mixture was then added to 5 mg (2.0 µmol, 1.0 equiv) of the corresponding resin. After 12 hours, the resin was then filtered and washed with 6 x 250 µL of DMF and 6 x 250 µL of CH_2Cl_2.

Procedure 8. On-resin activation of carboxylic acid handle and introduction of second point of diversity (heterocyclic amines-library I). To 5 mg of resin (1.0 µmol, 1 equiv) were added 40 µL (20.0 µmol, 20.0 equiv) of DIC 0.5 M in NMP, followed by 50 µL (25.0 µmol, 25.0 equiv) of HOBt 0.5 M in NMP. After 30 minutes of activation, 22 µL (11.0 µmol, 11.0 equiv) of a 0.5 M solution in NMP of heteroaromatic amine was added over the corresponding resin. After 12 hours, the resin was then filtered and washed with 6 x 250 µL of DMF and 6 x 250 µL of CH_2Cl_2.

Procedure 9. Alloc deprotection (Alloc-aspartate). 10 mg of resin (0.002 mmol, 1.0 equiv) were treated with 200 µL of 2 mL stock solution composed of: 6.9 mg (0.006 mmol, 30 mol %) of $Pd(PPh_3)_4$, 30 µL (0.02 mmol, 10 equiv) of diethyl malonate, 52 mg (0.02 mmol, 10 equiv) of PPh3 all dissolved in 2 mL of degassed $CHCl_3$. The reaction was shaken for 2 hours, and finally washed with 6 x 250 µL DMF and 6 x 250 µL CH_2Cl_2.

Procedure 10. Mtt deprotection (also in Intavis AG Multipep RS Synthesizer). 10 mg of resin were treated with 200 µL of a HOBt solution (122 mg in 10 mL of 50% hexafluoroisopropanol in 1,2 dichoroethane) for 3 min. The reaction was repeated 4 times with a CH_2Cl_2wash after the second cycle.

Procedure 11. PNA synthesis in Intavis AG Multipep RS Synthesizer. Fmoc or Mtt were removed as described in procedure 3 and 9 respectively. Then, to a solution of 8.0 µmol (4.0 equiv) of the corresponding PNA monomer (the nucleobases are Boc protected) in 40 µL of NMP were added 14 µL (7.0 µmol, 3.5 equiv) of HCTU or HATU 0.5 M in NMP (for Mtt monomers, use 21.3 µL (7.0 µmol, 3.5 equiv) of TNTU 0.33 M in NMP), followed by 6.7 µL of base solution [DIPEA 1.2 M (0.008 mmol, 4.0 equiv) and 2,6-lutidine 1.8M (0.012 mmol, 6.0 equiv) in NMP]. The mixture was then added to 10 mg of the corresponding resin. After 20 min the resin was filtered and washed with DMF and the sequence was repeated, then, the resin was washed with 6 x 100 µL of DMF and 6 x 100 µL of CH_2Cl_2. Finally, the resin was capped (procedure 2).

Procedure 12. Cleavage from the resin. 10 mg of resin were treated with 200 µL TFA for 4 hours. Next, the resulting solution was precipitated in 2 mL of Et_2O and centrifuged to recover the product as a pellet. The precipitate was re-dissolved in 500 µL of H_2O, the resulting was solution was filtered and then freeze-dried.

Procedure 13. Copper catalysed cycloaddition (CuAAC). To the corresponding resin (11.5 mg) were added successively 173 µL (0.0173 mmol, 7.5 equiv) of alkyne 0.1M in NMP, 17.3 µL (17.2 µmol, 7.5 equiv) of sodium ascorbate 198 mg/mL in H_2O, 4.4 µL

(0.57 μmol, 0.25 equiv) of copper sulphate 21.4 mg/mL in H_2O and 44 μL (1.1 mmol, 0.5 equiv of TBTA). After 16 hours, the resin was washed with 6 x 250 μL of sodium diethyl dithiocarbamate 0.02 M in DMF, 6 x 250 μL of DMF, 6 x MeOH and 6 x CH_2Cl_2.

Procedures 14 to 18*: *Small molecules synthesis (second point of diversity library I)

***Procedure 14. Phenol alkylation with methy bromoacetate*: a)** A microwave vial was charged with a suspension of the phenol (1.0 equiv), K_2CO_3 (1.5 equiv) and methyl bromoacetate (1.0 equiv.) in anhydrous DMF (1.5 M). The vial was sealed under a stem of N_2 gas and heated in a μW reactor at 100 °C for 15 minutes. The mixture was then diluted in EtOAc (15 mL) and washed with brine (5 mL 3X). The combined organic layers were dried over Na_2SO_4 and filtered, the solvent was evaporated and column chromatography (PE/EtOAc 8:2) was performed, to give the alkylated derivatives in yield ranging from 85 to 50%.

b) NaH method: A solution of the starting phenol or chalcone derivatives (1.0 equiv), in anhydrous THF (0.5 M), was brought to 0 °C by means of an ice bath; to this solution was added NaH (1.1 equiv) in two portions, after 15 minutes stirring, neat methyl or ethyl bromoacetate (1.0 equiv) was added dropwise. The reaction was stirred at 0 °C until completion as judged from TLC analysis; the mixture was then diluted with EtOAc (15 mL) and washed with brine (5 mL 3X). The combined organic layers were dried over Na_2SO_4 and filtered; the solvent was evaporated to yield the desired compound, which was used without any further purification.

***Procedure 15. Methyl ester hydrolysis:* a)** LiOH (1.5 equiv.) was added to a THF/H_2O 1:1 solution of the starting methyl ester (1.0 equiv.) and the mixture was stirred until completion of the reaction as judged by TLC analysis. The mixture was brought to 0 °C by means of an ice bath and HCl (aq) 2M was added until pH=1, then extracted with EtOAc (10 mL 3X) and washed with brine (5 mL 3X). The combined organic layers were dried over Na_2SO_4 and filtered; the solvent was evaporated to yield the corresponding carboxylic acid, which was used without any further purification.

b) KOH/EtOH method: Ground KOH (2.0 equiv.) was added to an EtOH/H_2O 3:1 solution of the starting ester (1.0 equiv.); the mixture was sonicated for about 5 minutes and then stirred at RT for 30 minutes. The mixture was then washed with Et_2O (5 mL 2X) and the organic phases discarded, HCl (aq) 2M was added until pH=1, acidic aqueous phase was then extracted with EtOAc (10 mL 3X) and washed with brine (5 mL 3X). The combined organic layers were dried over Na_2SO_4 and filtered; the solvent was evaporated to yield the corresponding carboxylic acid, which was used without any further purification.

Procedure 16. Microwave-assisted Suzuki coupling: A microwave vial was charged with methyl α-bromoacrylate (1.0 equiv.), boronic acid (2.0 equiv.), Na_2CO_3 (aq) 2M (3.0 equiv.), in-house prepared $Pd(PPh_3)_4$ (0.1 equiv.) and Toluene/EtOH 2:1 (0.02 M). The vial was sealed under a stream of N_2 gas, the mixture was deoxygenated three times by freeze-thaw cycles, and then heated in a μW reactor at 120 °C for 15 minutes. The mixture was then diluted in EtOAc (15 mL) and washed with brine (5 mL 3X). The combined organic layers were dried over Na_2SO_4 and filtered, the solvent was evaporated and column chromatography (PE/EtOAc 95:5) was performed, to give the Suzuki product in yield ranging from 60 to 15%.

Procedure 17. Baylis-Hillman reaction: The Baylis-Hillman addiction products were synthesized according to a known literature procedure.[1] A 1.5 mL Eppendorf vial was charged with the corresponding aldehyde (1.0 equiv), quinuclidine (0.75 equiv), MeOH (0.75 equiv), and methyl acrylate (1.2 equiv). The vial was briefly sonicated to dissolve the solids or alternatively a minimum amount of DMF was added, the mixture was sparked with a stream of N_2 and the vial sealed. After 24 to 48 hours the reaction was diluted in EtOAc (15 mL) and washed with saturated NH_4Cl (aq) (5 mL). The combined organic layers were dried over Na_2SO_4 and filtered, the solvent was evaporated and column chromatography (PE/EtOAc 9:1) was performed, to give the Bailys-Hilmann adducts in yield ranging from 30 to 85%.

Procedure 18. Azide generation. To a solution of 294 mg (1.4 mmol, 11 equiv) of imidazole-1-sulfonyl azide hydrochloride in 12.6 mL of MeOH were added successively 305 mg (2.2 mmol, 18 equiv) of finely ground K_2CO_3 and 8.0 mg (0.05 mmol, 0.35 equiv) of anhydrous $CuSO_4$ and the resulting suspension was sonicated for few minutes (solution has to be light blue). 250 μL of the previously prepared solution were added to

[1] V. Aggarwal, V. K. Aggarwal, I. Emme, S. Y. Fulford, *J. Org. Chem.* 2003, **68**, 692-700.

10 mg (2.0 μmol, 1.0 equiv) of the corresponding resin. After 16 hours, the resin was washed with 6 x 250 μL of sodium diethyl dithiocarbamate 0.02 M in DMF, 6 x 250 μL of DMF, 6 x MeOH and 6 x CH_2Cl_2, and the sequence was repeated.

Procedure 19. Azide reduction. 10 mg of resin were treated with solution of TCEP- tris 2-carboxyethyl phosphine (200μL, 0.35 M in 9-1 DMF- H_2O) for 2 hours. Finally, the resin was washed with 6 x 250 μL H_2O, 6 x 250 μL DMF and 6 x 250 μL CH_2Cl_2.

Procedure 20. Nucleophilic aromatic substitution on chloroquinazoline. 10 mg of resin (0.002 mmol, 1.0 equiv) were treated with a solution of 5.8 mg (0.02 mmol, 10.0 equiv) of 4-Chloro-6-Iodoquinazoline, or 6.5 mg (0.02 mmol, 10.0 equiv) of 4,6-dichloro-8-iodoquinazoline, in 200 μL of DMF/DCE 1:1 containing 7 μL of Hunig's base (0.04 mmol, 20.0 equiv). The reaction was shaken overnight and then washed 6 x 250 μL of DMF and 6 x 250 μL of CH_2Cl_2.

Procedure 21. General procedure for Suzuki coupling (second point of diversity): 10 mg of resin (0.002 mmol, 1.0 equiv) in a 1.5 mL Eppendorf tube were treated with 50 μL solution of 0.05 mg (0.002 mmol, 0.01 equiv, 10%) $Pd(OAc)_2$ (alternatively, in-house prepared $Pd(PPh_3)_4$ 20% can be used) and 1.9 mg (0.004 mmol, 0.02 equiv, 20%) of XPHOS or 1.6 mg (0.004 mmol, 0.02 equiv, 20%) of SPHOS in dry dioxane depending on the boronic acid.

To the resin was then added a solution of boronic acid derivatives (pinacolate ester, tetrafluoroborate salt or boronic acid) (0.016 mmol, 8.0 equiv) dissolved in 100 μL of a 1:1 mixture absolute Ethanol and dry dioxane. Finally, 9 μL of Na_2CO_3 2M solution (0.018 mmol, 9.0 equiv) were added and the reaction was heated either in an oil bath or Eppendorf shaker for 2 hours at 80°C. The reaction was then transferred back to a small fritted column and washed 6 x 250 μL of DMF, 6 x 250 μL of H_2O and 6 x 250 μL of CH_2Cl_2. Procedure was repeated if necessary.

Procedure 22. Alloc deprotection (second point of diversity and Alloc-aspartate): 10 mg of resin (0.002 mmol, 1.0 equiv) were treated with 200 μL of 2 mL stock solution composed of: 6.9 mg (0.006 mmol, 30 mol %) of $Pd(PPh_3)_4$, 30 μL (0.02 mmol, 10 equiv) of diethyl malonate, 52 mg (0.02 mmol, 10 equiv) of PPh3 all dissolved in 2 mL of degassed $CHCl_3$. The reaction was shaken for 2 hours, and finally washed with 6 x 250 μL DMF and 6 x 250 μL CH_2Cl_2.

Procedure 23. Michael acceptors coupling: To a solution of 0.02 mmol (10 equiv) of Michael acceptor carboxylic acid in NMP (100 μL) were added 6.8 mg (0.018 mmol, 9 equiv) of HATU in NMP (25 μL) followed by 4 μL (0.02 mmol, 10 equiv) of DIPEA and 4.6 μL (0.04 mmol, 20 equiv) of 2,6 lutidine. The mixture was activated for 5 min at room temperature, and then added to 10 mg of resin (0.002 mmol, 1.0 equiv). The reaction mixture was shaken for 1 hour and subsequently the resin was washed with 6 x 250 μL DMF and 6 x 250 μL CH_2Cl_2.

<u>Procedures 24 to 39</u>*: *<u>Small molecules synthesis (second point of diversity library II)</u>

***Procedure 24. Methyl ester formation*:** Staring material (1.0 equiv) was dissolved in anhydrous CH_2Cl_2 (0.2 M) and the solution was brought to 0 °C by means of an ice bath. CO_2Cl_2 (1.5 equiv) was added to the solution in a dropwise manner followed by a catalytic amount of dry DMF. Upon gas evolution, the mixture was stirred at RT for 1 hour, brought back to 0 °C and absolute methanol (6.5 equiv) was added dropwise. The mixture was left stirring at RT until completion of the reaction as judged by TLC analysis.

$NaHCO_3$ (sat. aq) was added to the reaction mixture after which an extraction with CH_2Cl_2 (3x) was carried out. The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated. The crude mixture was used in the next step without any purification. Yields were ranging from 50 to 85 %.

Procedure 25. β-TMS ethanol ester formation: Starting material (1.0 equiv) was dissolved in anhydrous DMF (0.9 M) and DIC (1.0 equiv) was added to the solution. Upon stirring at RT for 10 minutes TMS-ethanol (1.2 equiv) and Et_3N (1.1 equiv) were added to the mixture and the reaction was then heated to 85 °C overnight. NH_4Cl (sat. aq) was added to the reaction mixture after which an extraction with EtOAc (3x) was carried out. The combined organic layers were dried over Na_2SO_4, after which the solvent was evaporated and column chromatography (PE/EtOAc 90:10) was performed to give the protected derivative in 25 to 45% yield.

Procedure 26. EOM protection: To a stirred solution of the alcohol (1.0 equiv) in CH_2Cl_2 (0.16 M) was added at 0 °C DIPEA (2.0 equiv) and dropwise EOMCl (1.5 equiv) after which stirring was continued for 2 hours. $NaHCO_3$ (sat. aq) was added to the reaction mixture after which an extraction with CH_2Cl_2 (3x) was carried out. The combined organic layers were dried over Na_2SO_4, after which the solvent was evaporated and column chromatography (PE/EtOAc 80:20) was performed to give the protected derivative in 75 to 84% yield.

Procedure 27. Zinc dust reduction. Following a literature procedure, to a solution of the appropriate nitro compound (1.0 equiv) in dry CH_2Cl_2 (0.036 M) were added at 0 °C zinc dust (14.0 equiv) and acetic acid (150 equiv). Upon completion of the reaction as judged by TLC analysis, the mixture was filtered over Celite©, quenched with sat. $NaHCO_3$ (aq.) and extracted with CH_2Cl_2 (3x). The organic layers were dried over Na_2SO_4 and concentrated to give the crude amine, which was used in the subsequent reaction without further purification. Yields were ranging from 84 to 90 %.

Procedure 28. Bestmann-Ohira homologation: To a stirred suspension at 0 °C of finely powdered K2CO3 (2.0 equiv) in dry MeOH (0.25 M) was added, in two portions, Bestmann-Ohira reagent (1.2 equiv). After 10 minutes of stirring, aldehyde starting material was delivered drop wise as a solution in dry MeOH (0.6 M), and the reaction was stirred at room temperature until completion (max 2 hours). Water and Et_2O were then added, water phase was extracted 3 times with Et_2O and the combined organic layers were dried over Na_2SO_4, filtrated and evaporated under vacuum. The resulting residue was then purified by column chromatography (silica gel, PE/EtOAc 90:10 to 70:30). Yields were ranging from 21 to 45 %.

Procedure 29. Alloc protection: To a stirred solution of starting aniline (1 equiv), in dry CH_2Cl_2 (0.2 M) cooled to 0 °C, was added DIPEA (1.5 equiv) and subsequently allyl chloroformate (1.2 equiv). The mixture was then brought to room temperature and stirred until completion of the reaction, as judged by TLC analysis. $NaHCO_3$ (sat. aq) was added to the reaction mixture after which an extraction with CH_2Cl_2 (3x) was carried out. The combined organic layers were dried over Na_2SO_4, after which the solvent was evaporated and column chromatography (silica gel, PE/EtOAc 80:20) was performed to give the protected derivative. Yields were ranging from 45 to 75 %.

Procedure 30. Deprotection of β-TMS ethanol ester and TMS-acetylene derivative: To a solution of the corresponding TMSE ester or protected alkyne (1.0 equiv) in THF (0.2 M) was added TBAF (2.0 equiv) and stirred at RT for 3 hours. The reaction mixture was quenched with aq. NH_4Cl and extracted with EtOAc (3X). The organic layer was washed with NH_4Cl (sat. aq) (X 3), dried over Na_2SO_4 and evaporated. The crude acid was used as such in next step. The alkyne was subjected to column chromatography (PE/EtOAc 80:20) obtaining the desired compound in 71% yield.

Procedure 31. Methyl ester hydrolysis: Starting material (1.0 equiv) was dissolved in a 5:1 mixture of dioxane/H_2O (0.4 M), LiOH (4.0 equiv) was then added and the reaction was then stirred at RT for 12 hours. Upon completion of the reaction as judged by TLC analysis, the mixture was neutralized with NH_4Cl (sat. aq) and $KHSO_4$ 10% (aq) and extracted with EtOAc (3X). The combined organic layers were dried over Na_2SO_4, after which the solvent was evaporated and column chromatography (silica gel, CH_2Cl_2 /MeOH/H_2CO_2 95:4:1) was performed to give the free carboxylic acid. Yields were ranging from 69 to 75 %.

Procedure 32. Michael acceptor introduction. ***Procedure for propiolic acid introduction:*** Propiolic acid (46 mg, 0.636 mmol, 2.0 equiv) and DCC (131 mg, 0.636 mmol, 2.0 equiv) were dissolved in dry CH_2Cl_2 and the mixture was brought to 0 °C. Upon stirring for 10 minutes, DMAP (cat.) was added, followed by a solution of starting aniline (100 mg, 0.318 mmol, 1.0 equiv) in dry CH2Cl2 (2 mL). Upon completion of the reaction as judged by TLC analysis, the mixture was diluted with NH_4Cl (sat. aq) and extracted with EtOAc (3X). The combined organic layers were dried over Na_2SO_4, after which the solvent was evaporated and column chromatography (silica gel, PE/EtOAc 80:20) was performed to give the desired compound (89 mg, 76% Yield). ***Procedure for vinyl sulphonyl chloride introduction:*** Starting aniline (100 mg, 0.318 mmol, 1.0 equiv) was dissolved in dry CH_2Cl_2 (5 mL) and the mixture was brought to 0 °C. Et_3N (136 μl, 0.318 mmol, 1.0 equiv) was added to the stirred mixture of aniline followed by 2-chloeoethansulfonyl chloride. Upon stirring at 0 °C for 30 minutes, the reaction was brought to RT and stirred overnight. The mixture was diluted with NH_4Cl (sat. aq) and extracted with CH_2Cl_2 (3X). The combined organic layers were dried over Na_2SO_4, after which the solvent was evaporated and column chromatography (silica gel, PE/EtOAc 80:20) was performed to give the desired compound (98 mg, 77% Yield).

Procedure 33. LAH reduction of β-TMS ethanol or methyl esters: Starting material (1.0 equiv) was dissolved in dry THF (0.8 M) and the solution was brought at 0 °C. Lithium aluminium hydride 1.0 M in THF was slowly added to the cooled solution (2.0 equiv). Subsequently, the mixture was brought to RT and stirred for 30 minutes. After completion of the reaction, as judged by TLC analysis, the reaction was quenched with 1 M NaOH and extracted with EtOAc (3X). The combined organic layers were dried over Na_2SO_4, after which the solvent was evaporated and column chromatography (silica gel, PE/EtOAc 80:20 to 70:30) was performed to give the desired compounds in 58% yield (TMSE ester), and 78% yield for methyl ester substrate.

Procedure 34. Dess-Martin oxidation: To a solution in dry CH_2Cl_2 (10 ml, 0.05 M) of starting material (130 mg, 0.43 mmol, 1.0 equiv) was added Dess Martin periodinane (230 mg, 0.516 mmol, 1.2 equiv) in two portions. After completion of the reaction, as judged by TLC analysis, the mixture was diluted with $NaHCO_3$ (sat. aq) and extracted with CH_2Cl_2 (3X). The combined organic layers were dried over Na_2SO_4, after which the solvent was evaporated and column chromatography (silica gel, PE/EtOAc 90:10) was performed to give the desired compound (75 mg, 57% Yield).

Procedure 35. Appel chlorination: Starting alcohol (365 mg, 1.3 mmol, 1.0 equiv) and PPh_3 (341 mg, 1.3 mmol, 1.0 equiv) were dissolved in CCl_4 (15 ml) and refluxed for 24 hours. The solvent was evaporated in vacuo and the residue was purified by column chromatography (silica gel, PE to PE/EA 95:5) to give the desired compound (125 mg, 33% yield).

Procedure 36. Silver trifluoroacetate Iodination: To a solution in CH_2Cl_2 (10 ml, 0.4 M) of starting material 1 g, 3.9 mmol, 1.0 equiv) were added sequentially I2 (0.99 mg, 3.9 mmol, 1.0 equiv) and Silver trifluoroacetate (0.86 mg, 3.9 mmol, 1.0 equiv). After completion of the reaction, as judged by TLC analysis, the reaction was diluted with 30 ml of NH_4Cl (sat. aq) and extracted with CH_2Cl_2 (3 x 30 ml). The combined organic layers were dried over Na_2SO_4, after which the solvent was evaporated and column chromatography (silica gel, PE/EtOAc 90:10 to 80:20) was performed to give the desired compound (930 mg, 62.5% yield).

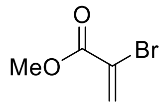
Procedure 37. Sonogashira coupling with TMS acetylene: Starting Iodo arene (480 mg, 1.25 mmol, 1.0 equiv), $PdCl_2(PPh_3)_2$ (43 mg, 5 mol%), CuI (12 mg, 5 mol%) and Et_3N (498 µL, 3.76 mmol, 3 equiv) were dissolved in CH_3CN (5 ml) and degassed via freeze-pump cycles (3X). Upon degassing, TMS acetylene (530 µL, 3.76 mmol, 3.0 equiv) was added and the mixture heated at 60 °C for 2 hours. The reaction was diluted with 30 ml of NH_4Cl (sat. aq) and extracted with EtOAc (3 x 30 ml). The combined organic layers were dried over Na_2SO_4, after which the solvent was evaporated and column chromatography (silica gel, PE/EtOAc 90:10 to 80:20) was performed to give the desired compound (240 mg, 54% yield).

Procedure 38. 4-Hydroxyquinazoline synthesis: The substituted antranilic acid was dissolved in neat formamide and refluxed until a solid precipitate appeared (generally after 12-20 hours). The reaction mixture was then cooled down to room temperature with an ice bath, the precipitate was then filtered on a frit, washed with cold water and dried under high vacuum overnight. The resulting solid was used in the next step without further purification.

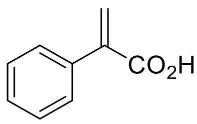
Procedure 39. 4-Chloroquinazoline synthesis: (4,6-dichloro-8-iodoquinazoline **5** and 4-chloro-6-iodoquinazoline **6**): Substituted hydroxyquinazoline obtained from the previous protocols were dissolved in neat thionyl chloride and to this solution was added a catalytic amount of dry DMF (gas evolution). After DMF addition, the mixture was refluxed under a continuous steam of N_2 until completion of the reaction, as judged by LC-MS (ESI) analysis. The reaction mixture was then cooled down to room temperature, thionyl chloride was evaporated under vacuum and the residue was taken up in CH_2Cl_2 and washed with $NaHCO_3$ (sat.aq) 3X. Organic extracts were then dried over Na_2SO_4 filtered and evaporated to yield a residue that was then purified by column chromatography (silica gel, CH_2Cl_2/MeOH 98:2 to 95:5) to afford 4-chloroquinazoline as a fluffy yellowish solid.

Synthesis of building blocks for the 10K PNA encoded library of covalent binders. Library I.

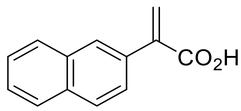
Bromo acrylate **SM-1** needed for Suzuki-Miyaura coupling was synthesized following a known literature procedure.[149]



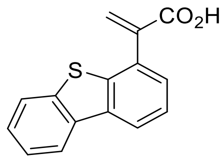
^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 7.01 (d, *J* = 4 Hz, 1H); 6.30 (d, *J* = 4 Hz, 1H); 3.87(s, 3H) ppm.



Compound **SM-2** was synthesized using ***procedure 16*** between bromoacrylate **SM-1** and the corresponding boronic acid, followed by ***procedure 15a*** to obtain the free carboxylic acid.
^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 7.48-7.39 (m, 5H); 6.57 (s, 1H), 6.06 (s, 1H) ppm.

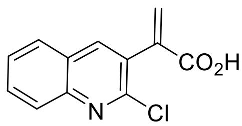


Compound **SM-3** was synthesized using ***procedure 16*** between bromoacrylate **SM-1** and the corresponding boronic acid, followed by ***procedure 15a*** to obtain the free carboxylic acid.
^{1}H NMR (MeOD, 400 MHz, 23 °C) δ = 7.88-7.74 (m, 3H); 7.47-7.44 (m, 4H); 6.67 (s, 1H), 5.89 (s, 1H) ppm. LC-MS (ESI): m/z [M + H] calc. for $C_{13}H_{10}O_2$: 197.08, found: 198.5.



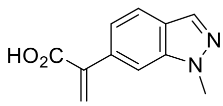
Compound **SM-4** was synthesized using ***procedure 16*** between bromoacrylate **SM-1** and the corresponding boronic acid, followed by ***procedure 15a*** to obtain the free carboxylic acid.

^{1}H NMR (MeOD, 400 MHz, 23 °C) δ = 8.17-8.16 (m, 2H); 7.84-7.82 (m, 1H); 7.49-7.35 (m, 4H); 6.68 (s, 1H), 6.13 (s, 1H) ppm.



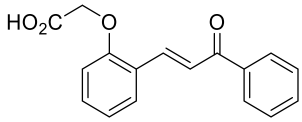
Compound **SM-5** was synthesized using ***procedure 16*** between bromoacrylate **SM-1** and the corresponding boronic acid, followed by ***procedure 15a*** to obtain the free carboxylic acid.

^{1}H NMR (d^{6}-DMSO, 400 MHz, 23 °C) δ = 8.44 (s, 1H); 8.07 (d, *J* = 8 Hz, 1H); 8.00 (d, *J* = 8 Hz, 1H); 7.86 (m, 1H); 7.71 (m, 1H); 6.52 (s, 1H), 6.07 (s, 1H) ppm. LC-MS (ESI): m/z [M + H] calc. for $C_{12}H_8ClNO_2$: 233.02, found: 233.65.



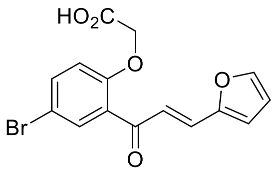
Compound **SM-6** was synthesized using ***procedure 16*** between bromoacrylate **SM-1** and the corresponding boronic acid, followed by ***procedure 15a*** to obtain the free carboxylic acid.

^{1}H NMR (MeOD, 400 MHz, 23 °C) δ = 7.99 (s, 1H); 7.72 (d, *J* = 8 Hz, 1H); 7.63 (s, 1H); 7.23-7.21 (m, 1H); 6.43 (s, 1H), 6.05 (s, 1H); 4.07 (s, 3H) ppm. LC-MS (ESI): m/z [M + H] calc. for $C_{11}H_{10}N_2O_2$: 202.07, found: 202.75.

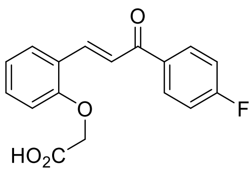


Compound **SM-7** was synthesized using ***procedure 14*** between methyl bromoacetate and the corresponding phenol or chalcone, followed by ***procedure 15a*** to obtain the free carboxylic acid.

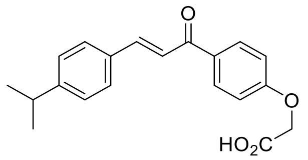
1H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 8.12 (m, 3H); 7.96 (d, *J* = 12 Hz, 1H); 7.69-7.39 (m, 5H); 7.14 (m, 1H); 6.90 (d, *J* = 8 Hz, 1H); 4.86 (s, 2H) ppm. LC-MS (ESI): m/z [M + H] calc. for $C_{17}H_{14}O_4$: 282.09, found: 282.74.



Compound **SM-8** was synthesized using ***procedure 14*** between methyl bromoacetate and the corresponding phenol or chalcone, followed by ***procedure 15a*** to obtain the free carboxylic acid. 1H NMR (CDCl3, 400 MHz, 23 °C) δ = 7.86 (bs, 1H); 7.67-7.59 (m, 3H); 7.24 (d, *J* = 16 Hz, 1H); 6.95 (d, *J* = 8 Hz, 1H); 6.84 (bd, *J* = 4 Hz, 1H); 6.58-6.56 (m, 1H); 4.81 (s, 2H) ppm. LC-MS (ESI): m/z [M + H] calc. for $C_{15}H_{11}BrO_5$: 349.98, found: 352.67

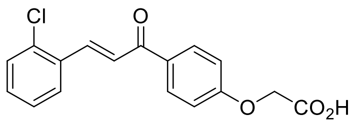


Compound **SM-9** was synthesized using ***procedure 14*** between methyl bromoacetate and the corresponding phenol or chalcone, followed by ***procedure 15a*** to obtain the free carboxylic acid. 1H NMR (d6-DMSO, 400 MHz, 23 °C) δ = 8.28 (m, 2H); 8.17 (d, *J* = 16 Hz, 1H); 8.04 (d, *J* = 16 Hz, 1H); 7.93 (d, *J* = 8 Hz, 1H); 7.46-7.38 (m, 3H); 7.09-7.05 (m, 2H); 4.86 (s, 2H) ppm. LC-MS (ESI): m/z [M + H] calc. for $C_{17}H_{13}FO_4$: 300.08, found: 300.72.



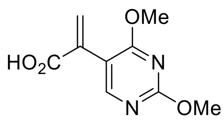
Compound **SM-10** was synthesized using ***procedure 14*** between methyl bromoacetate and the corresponding phenol or chalcone, followed by ***procedure 15a*** to obtain the free carboxylic acid.

^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 8.08 (d, *J* = 8 Hz, 1H); 7.84 (d, *J* = 16 Hz, 1H); 7.61 (d, *J* = 8 Hz, 1H); 7.52 (d, *J* = 16 Hz, 1H) 7.32-7.25 (m, 2H); 7.04 (d, *J* = 8 Hz, 1H); 4.80 (s, 2H); 2.98 (spt, *J* = 8 Hz, 1H); 1.31 (s, 3H); 1.29 (s, 3H) ppm. LC-MS (ESI): m/z [M + H] calc. for $C_{20}H_{20}O_4$: 324.14, found: 324.77.



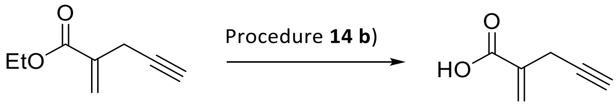
Compound **SM-11** was synthesized using ***procedure 14*** between methyl bromoacetate and the corresponding phenol or chalcone, followed by ***procedure 15a*** to obtain the free carboxylic acid.

^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 8.20 (d, *J* = 16 Hz, 1H); 8.08 (d, *J* = 8 Hz, 1H); 7.79-7.76 (m, 1H); 7.53-7.47 (m, 2H); 7.38-7.35 (m, 2H); 7.06 (d, *J* = 8 Hz, 1H); 4.82 (s, 2H) ppm. LC-MS (ESI): m/z [M + H] calc. for $C_{17}H_{13}ClO_4$: 316.05, found: 316.69.



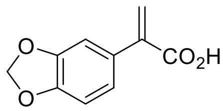
Compound **SM-12** was synthesized using ***procedure 16*** between bromoacrylate **SM-1** and the corresponding boronic acid, followed by ***procedure 15a*** to obtain the free carboxylic acid.

^{1}H NMR (MeOD, 400 MHz, 23 °C) δ = 8.12 (s, 1H); 6.40 (s, 1H); 5.89 (s, 1H); 4.01 (s, 3H); 3.99 (s, 3H) ppm. LC-MS (ESI): m/z [M + H] calc. for $C_9H_{10}N_2O_4$: 210.07, found: 210.76



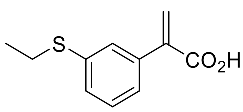
Compound **SM-13** was synthesized according to a known protocol (WO 2006092495), followed by ***procedure 15b*** to obtain the free carboxylic acid.

^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 6.52 (d, *J* = 4 Hz, 1H); 6.21 (d, *J* = 4 Hz, 1H); 3.27 (bs, 2H); 2.26 (t, *J* = 4 Hz, 1H) ppm.



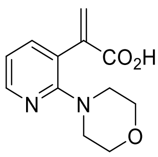
Compound **SM-14** was synthesized using ***procedure 16*** between bromoacrylate **SM-1** and the corresponding boronic acid, followed by ***procedure 15a*** to obtain the free carboxylic acid.

^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 6.92 (m, 2H); 6.82 (m, 1H); 6.30 (s, 1H); 6.00 (s, 2H); 5.85 (s, 1H) ppm.



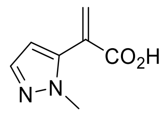
Compound **SM-15** was synthesized using ***procedure 16*** between bromoacrylate **SM-1** and the corresponding boronic acid, followed by ***procedure 15a*** to obtain the free carboxylic acid.

^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 7.40-7.31 (m, 3H); 7.26-7.23 (m, 1H); 6.40 (s, 1H); 5.93 (s, 1H); 2.93 (q, *J* = 8 Hz, 2H); 1.36 (t, *J* = 8 Hz, 3H) ppm.

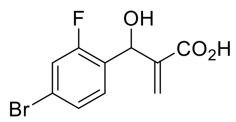


Compound **SM-16** was synthesized using ***procedure 16*** between bromoacrylate **SM-1** and the corresponding boronic acid, followed by ***procedure 15a*** to obtain the free carboxylic acid.

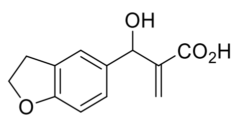
^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 8.35 (dd, *J* = 8, *J* = 4 Hz, 3H); 7.55 (m, 1H); 7.02 (dd, *J* = 8, *J* = 4 Hz, 3H); 6.22 (d, *J* = 4 Hz, 1H); 5.84 (d, *J* = 4 Hz, 1H); 3.77 (m, 4H); 3.16 (m, 4H) ppm. LC-MS (ESI): m/z [M + H] calc. for $C_{12}H_{14}N_2O_3$: 234.10, found: 234.77.



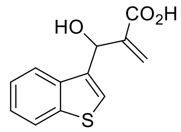
Compound **SM-17** was synthesized using ***procedure 16*** between bromoacrylate **SM-1** and the corresponding boronic acid, followed by ***procedure 15a*** to obtain the free carboxylic acid.
^{1}H NMR (MeOD, 400 MHz, 23 °C) δ = 7.45 (bs, 1H); 6.68 (bd, 1H); 6.31 (m, 1H); 6.06 (bd, 1H); 3.78 (s, 3H) ppm.



Compound **SM-18** was synthesized using ***procedure 17*** between methyl acrylate and the corresponding aldehyde, followed by ***procedure 15a*** to obtain the free carboxylic acid.
^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 7.40-7.33 (m, 2H); 7.27-7.24 (m, 1H); 6.25 (s, 1H); 5.91 (s, 1H); 5.84 (s, 1H) ppm. LC-MS (ESI): m/z [M + H] calc. for $C_{10}H_8BrFO_3$: 273.96, found: 276.02.

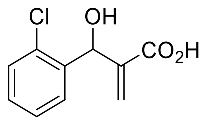


Compound **SM-19** was synthesized using ***procedure 17*** between methyl acrylate and the corresponding aldehyde, followed by ***procedure 15a*** to obtain the free carboxylic acid.
^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 7.21 (s, 1H); 7.10 (d, *J* = 8 Hz, 1H); 6.74 (d, *J* = 8 Hz, 1H); 6.48 (s, 1H); 6.01 (s, 1H); 5.52 (s, 1H); 4.57 (t, *J* = 8 Hz, 2H); 3.10 (t, *J* = 8 Hz, 2H) ppm.



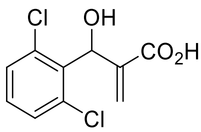
Compound **SM-20** was synthesized using ***procedure 17*** between methyl acrylate and the corresponding aldehyde, followed by ***procedure 15a*** to obtain the free carboxylic acid.

^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 7.82 (m, 2H); 7.42 (s, 1H); 7.38-7.36 (m, 2H); 6.51 (s, 1H); 5.97 (s, 1H); 5.90 (s, 1H) ppm.



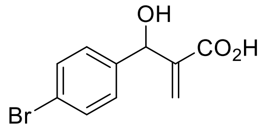
Compound **SM-21** was synthesized using ***procedure 17*** between methyl acrylate and the corresponding aldehyde, followed by ***procedure 15a*** to obtain the free carboxylic acid.

1H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 7.59 (d, *J* = 8 Hz, 1H); 7.41-7.27 (m, 3H); 6.56 (s, 1H); 6.02 (s, 1H); 5.72 (s, 1H) ppm.



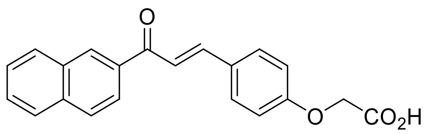
Compound **SM-22** was synthesized using ***procedure 17*** between methyl acrylate and the corresponding aldehyde, followed by ***procedure 15a*** to obtain the free carboxylic acid.

^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 7.33 (d, *J* = 8 Hz, 2H); 7.19 (t, *J* = 8 Hz, 1H); 6.50 (s, 1H); 6.35 (s, 1H); 5.77 (s, 1H) ppm.



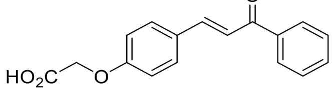
Compound **SM-23** was synthesized using ***procedure 17*** between methyl acrylate and the corresponding aldehyde, followed by ***procedure 15a*** to obtain the free carboxylic acid.

^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 7.50 (dd, *J* = 8; 2 Hz, 2H); 7.26 (dd, *J* = 8; 2 Hz, 2H); 6.50 (s, 1H); 5.97 (s, 1H); 5.53 (s, 1H) ppm. LC-MS (ESI): m/z [M + H] calc. for $C_{10}H_9BrO_3$: 255.97, found: 257.71.

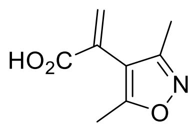


Compound **SM-24** was synthesized using ***procedure 14*** between methyl bromoacetate and the corresponding phenol or chalcone, followed by ***procedure 15a*** to obtain the free carboxylic acid.

^{1}H NMR (MeOD, 400 MHz, 23 °C) δ = 8.11-8.09 (m, 2H); 8.01-7.94 (m, 2H); 7.85-7.78 (m, 4H); 7.67-7.58 (m, 2H); 7.04 (bd, *J* = 8 Hz, 2H); 4.75 (s, 2H) ppm. LC-MS (ESI): m/z [M + H] calc. for $C_{21}H_{16}O_4$: 332.10, found: 332.74

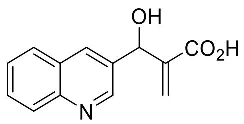


Compound **SM-25** was synthesized using ***procedure 14*** between methyl bromoacetate and the corresponding phenol or chalcone, followed by ***procedure 15a*** to obtain the free carboxylic acid. 1H NMR ($CDCl_3$, 300 MHz, 23 °C) δ = 8.03 (d, *J* = 9 Hz, 2H); 7.81(d, *J* = 18 Hz, 1H); 7.57 (m, 6H); 6.99 (m, 2H); 4.77 (s, 2H) ppm. LC-MS (ESI): m/z [M + H] calc. for $C_{17}H_{14}O_4$: 282.0892, found: 283.11



Compound **SM-26** was synthesized using ***procedure 16*** between bromoacrylate **SM-1** and the corresponding boronic acid, followed by ***procedure 15a*** to obtain the free carboxylic acid.

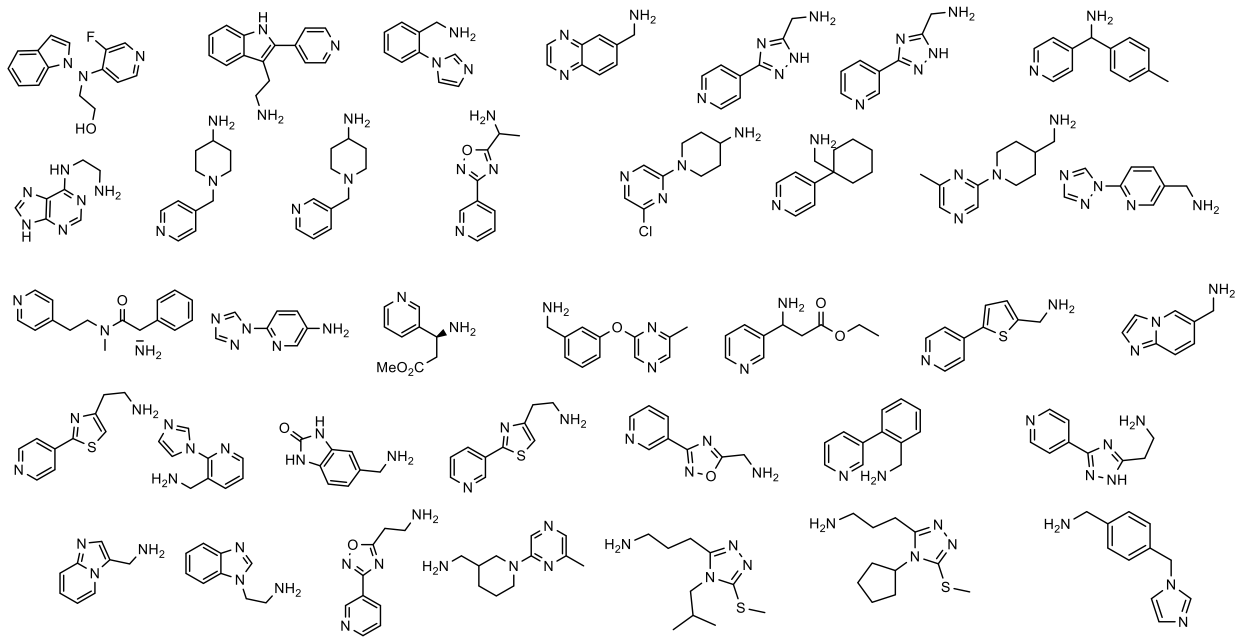
[1]H NMR ($CDCl_3$, 300 MHz, 23 °C) δ = 6.63 (d, *J* = 3 Hz, 1H); 5.77 (d, *J* = 3 Hz, 1H); 2.34 (s, 3H); 2.19 (s, 3H) ppm. LC-MS (ESI): m/z [M + H] calcd. for $C_8H_9NO_3$ 167.06, found: 167.96

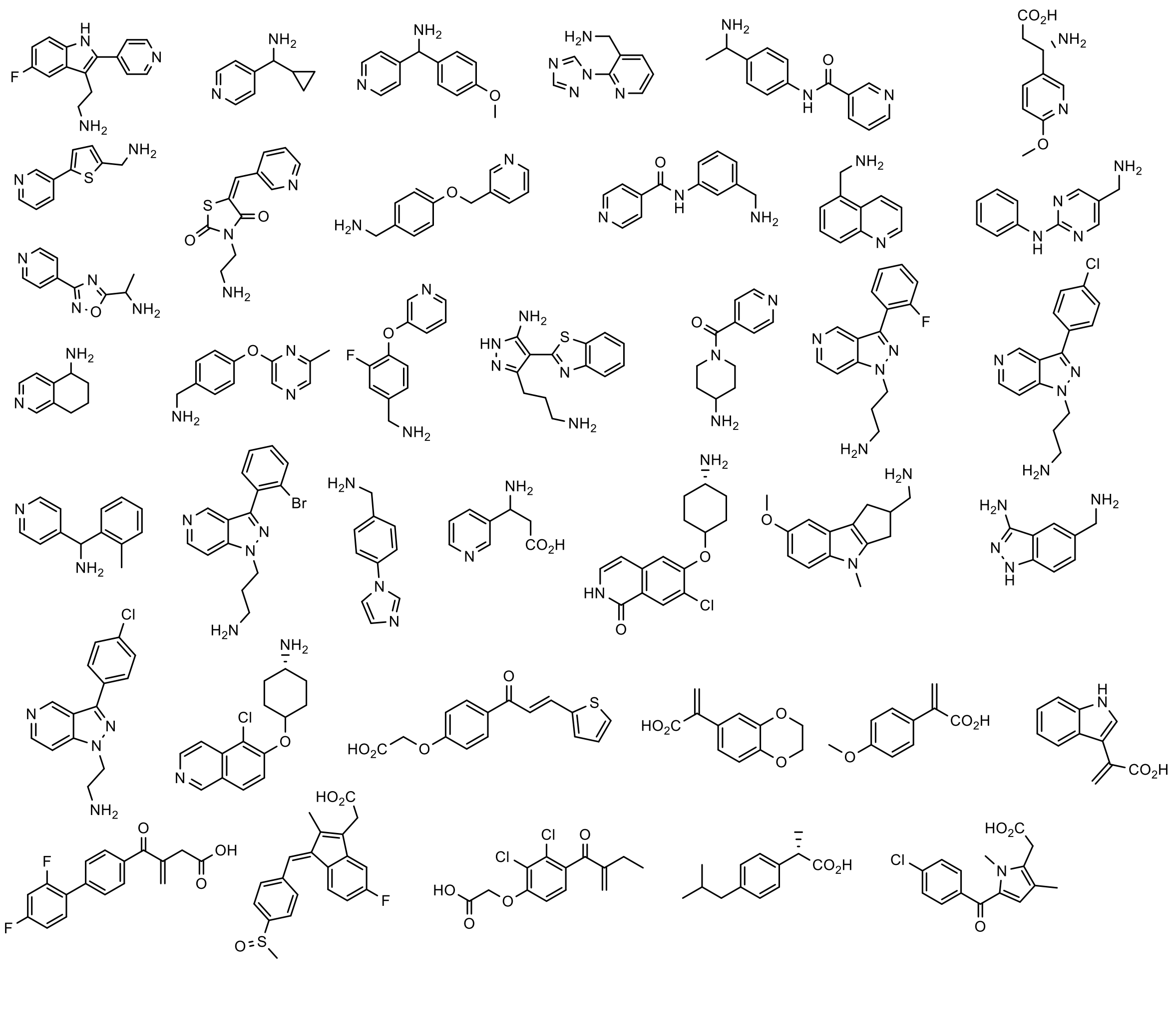


Compound **SM-27** was synthesized using ***procedure 17*** between methyl acrylate and the corresponding aldehyde, followed by ***procedure 15a*** to obtain the free carboxylic acid.

1H NMR (MeOD, 400 MHz, 23 °C) δ = 8.87 (bs, 1H); 8.28 (bs, 1H); 8.01 (d, *J* = 8.5 Hz, 1H); 7.95 (d, *J* = 8.5 Hz, 1H); 7.76 (t, *J* = 7.5 Hz, 1H); 7.61 (t, *J* = 7.5 Hz, 1H); 6.44 (s, 1H); 6.2 (s, 1H); 5.79 (s, 1H) ppm. LC-MS (ESI): m/z [M + H] calc. for $C_{13}H_{11}NO_3$: 229.07, found: 229.71

Collection of amines and carboxylic acid used as a second point of diversity in the 10K PNA encoded library of covalent binders. Library I.

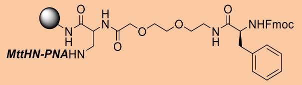
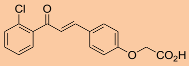
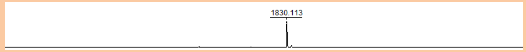
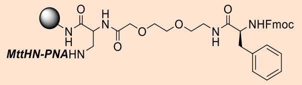
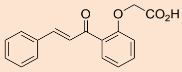
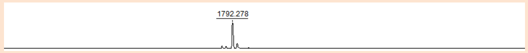
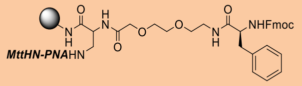
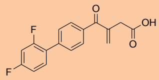
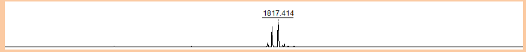
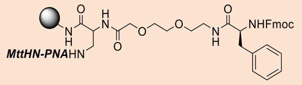
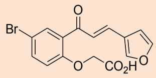
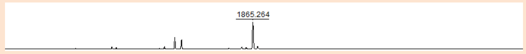
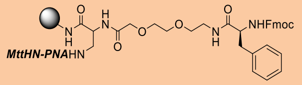
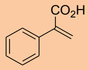
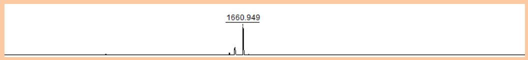
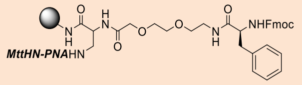
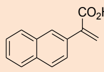
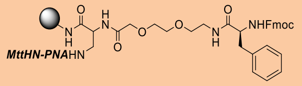
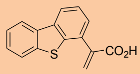
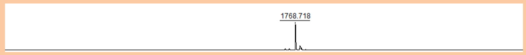
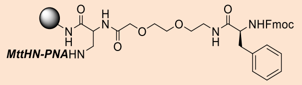
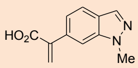
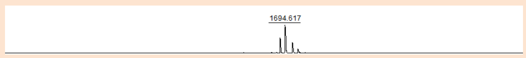
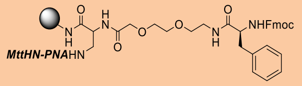
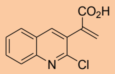
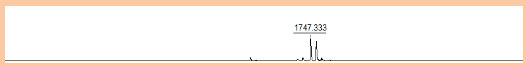
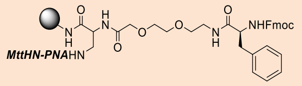
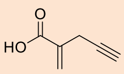
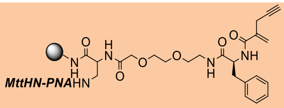
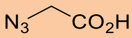
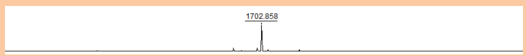
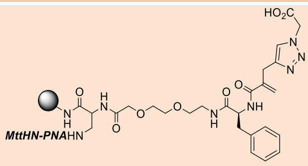
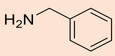
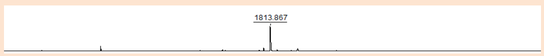
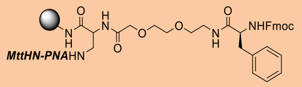
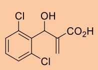
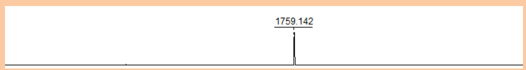




Solid phase validation of building blocks for the synthesis of the 10K PNA encoded library of covalent binders. Library I.

One or more building blocks from each class (chalcone, acrylates, Baylis-Hillman) were conjugated to a PNA tag composed of four nucleobases (4mer PNA), one of them being Thymine with a serine modification on the side-chain [A; C; G; TS; (Boc-Mtt) protection] and tested under a series of conditions. The best reaction conditions are shown in the following table (Table **1-SI**). All reactions were tested on 5 mg (0.001 mmol) of Rink amide NovaPEG resin.

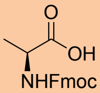
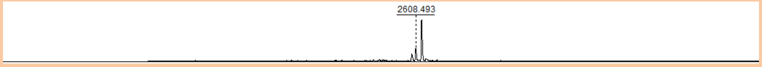
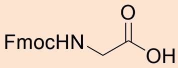
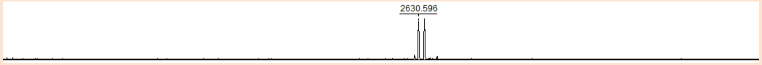
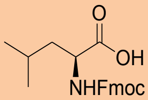
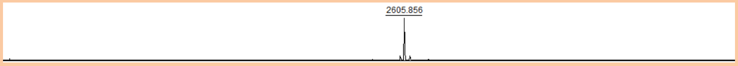
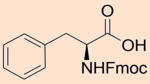
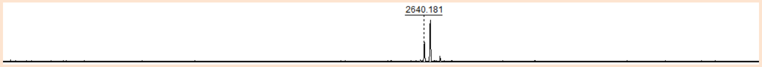
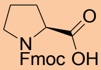
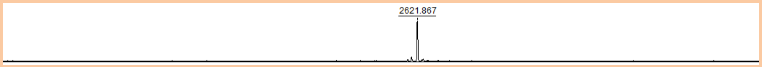
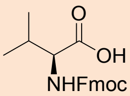
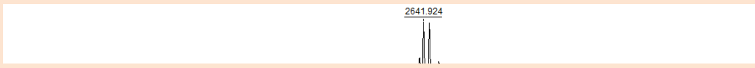
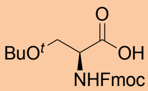
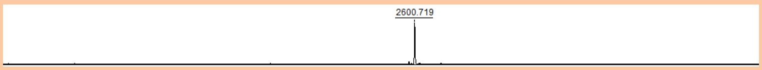
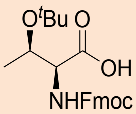
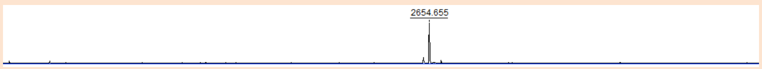
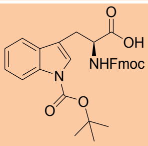
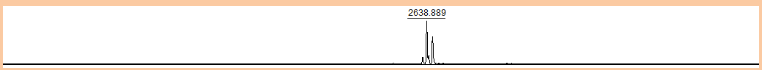
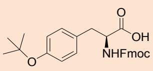
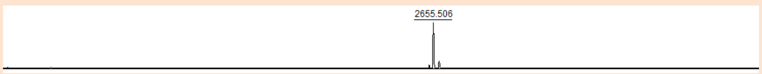
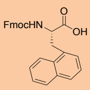
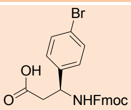
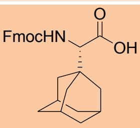
Table 1-SI: Building blocks validation on model system

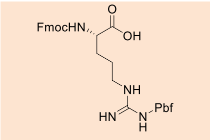
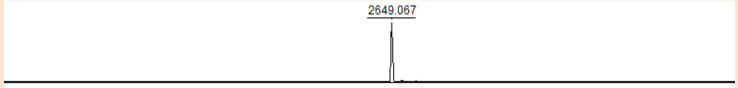
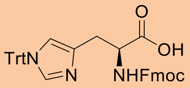
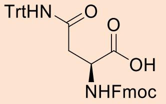
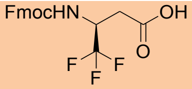
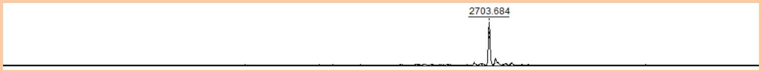
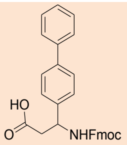
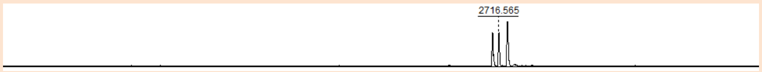
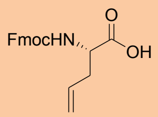
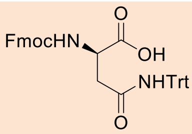
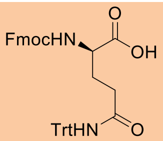
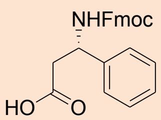
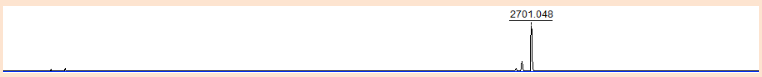
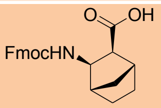
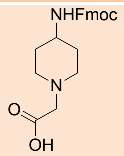
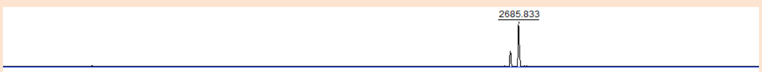
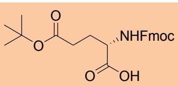
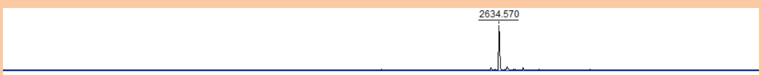
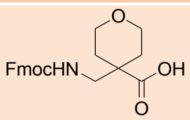
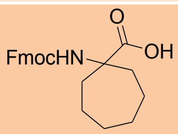
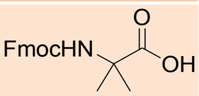
	Starting resin	*Molecule*	*Proc*	*Calc. m/z ($M+Na^+$)*	*Found m/z ($M+Na^+$) (MALDI: 750-2500 Da)*
1			***7b***	1829.68	
2			***7b***	1795.72	
3			***7b***	1817.71	
4			***7b***	1863.61	
5			***7b***	1661.68	
6			***7b***	1711.70	
7			***7b***	1767.67	
8			***7b***	1692.72	
9			***7b***	1746.66	
10			***7b***	1623.55	
11			***12***	1702.85	
12			***8***	1813.94	
13			***7b***	1759.65	

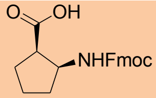
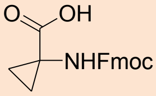
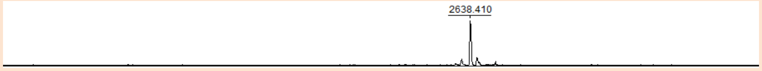
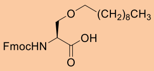
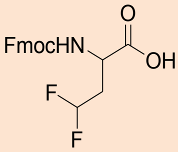
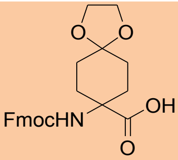
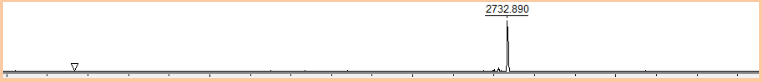
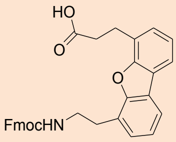
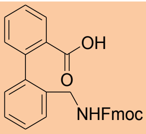
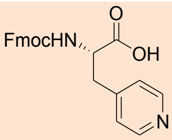
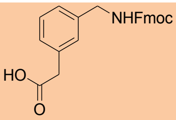
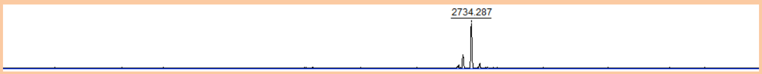
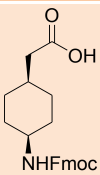
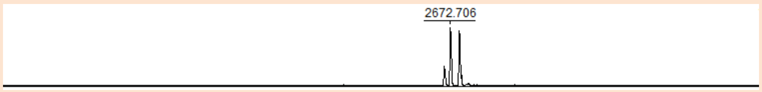
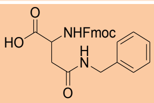
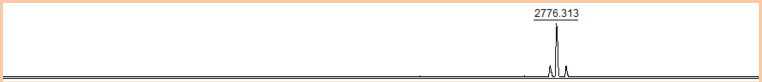
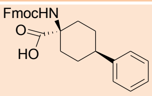
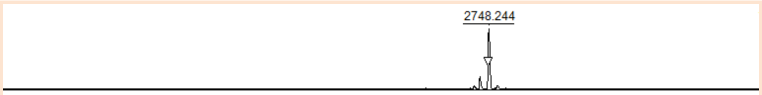
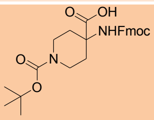
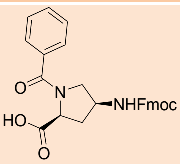
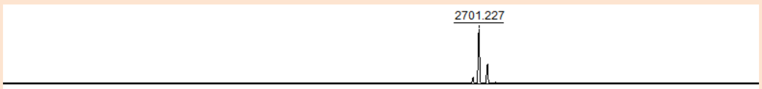
Introduction and PNA-encoding of the first point of diversity in the 10K PNA encoded library of covalent binders. Library I.

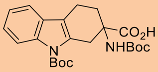
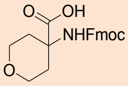
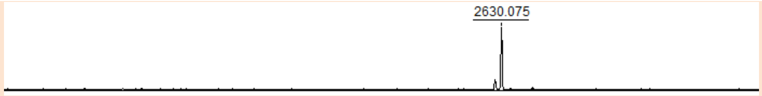
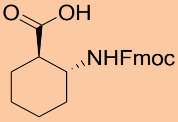
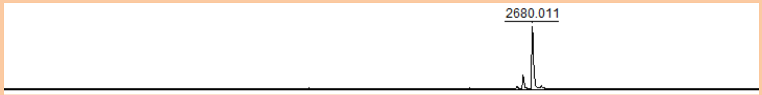
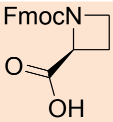
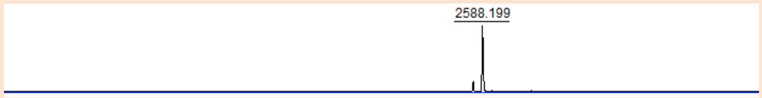
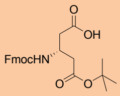
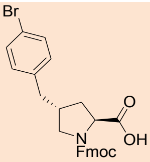
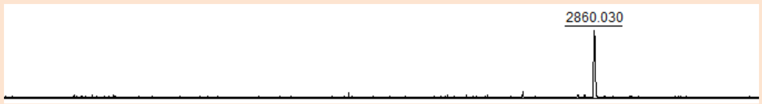
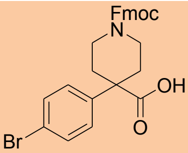
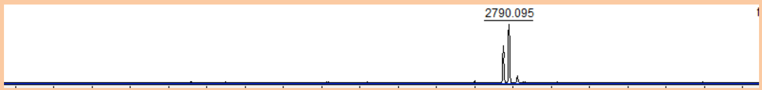
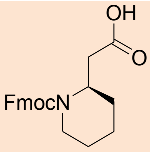
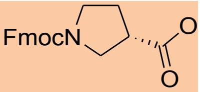
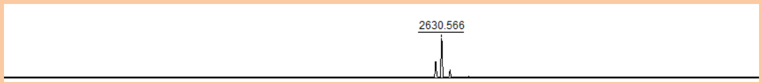
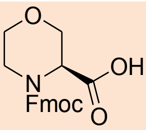
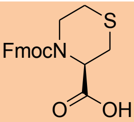
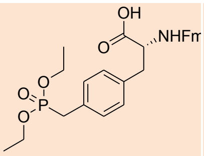
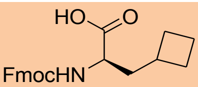
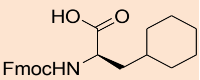
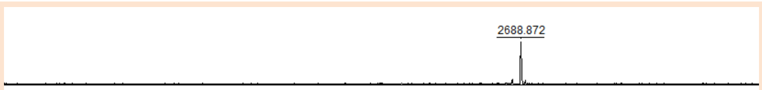
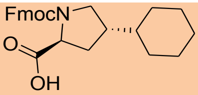
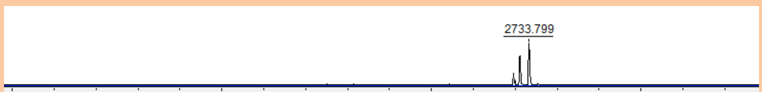
Nova Peg Rink amide resin was charged with an orthogonally protected FmocDap-(Mtt)-OH using ***procedure 5*** with loading reduction to 0.2 mmol/g followed by capping of the remaining free functionalities (***procedure 1***); Fmoc was subsequently deprotected following ***procedure 3*** and PEG spacer was introduced using ***procedure 6*** (both HATU or DIC/HOBt procedure could be used). The obtained spacer was split in 100 pools and each well (5 mg, 1.0 μM) was acylated, upon Fmoc deprotection of the PEG linker (***procedure 3***), with a unique Fmoc-protected amino acid following ***procedure 7a***. Each of the first point of diversity introduced was then encoded with a unique 7mer (Boc-Mtt) protected PNA oligomer. Upon Mtt deprotection of the Dap linker (***procedure 10***), cycles of TNTU mediated PNA syntheses (***procedure 11***) were carried out on 5 mg (0.001 mmol) of resin for each reaction. A small amount of resin for every single reaction was cleaved by TFA 98%, precipitated in Et_2O, and analysed by MALDI-TOF. The complete list of 7mers used to code each first point of diversity in given in table 2-SI.

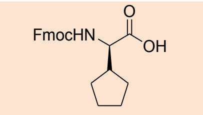
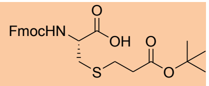
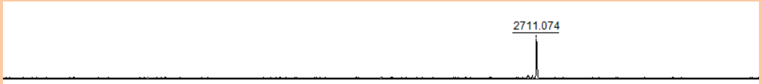
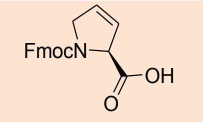
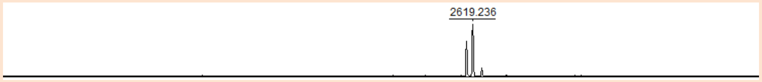
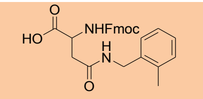
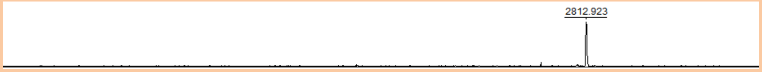
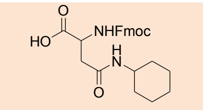
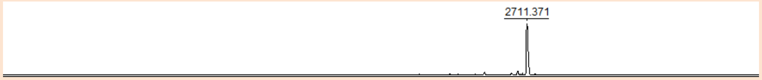
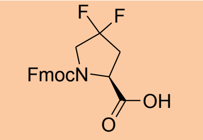
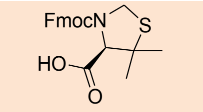
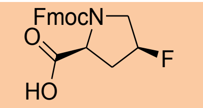
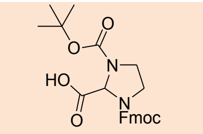
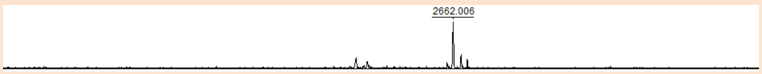
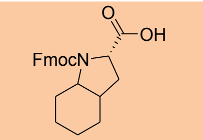
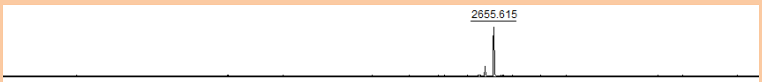
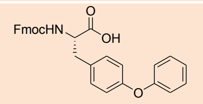
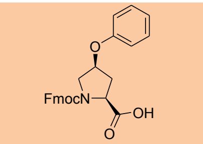
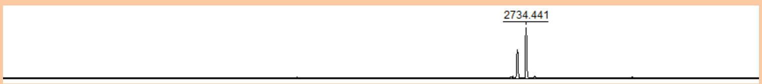
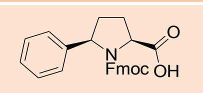
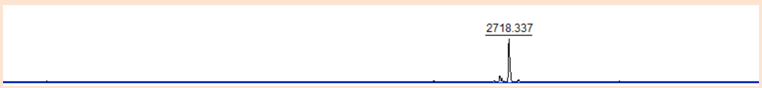
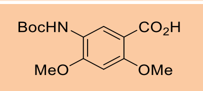
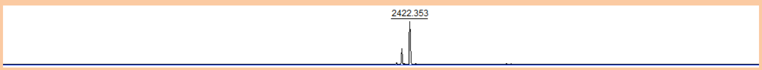
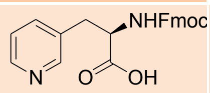
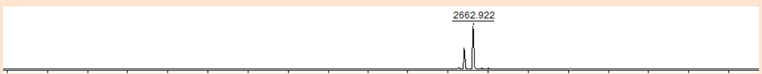
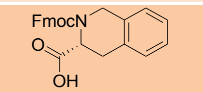
Table 2-SI: Maldis of the 100 7mer conjugates with the first point of diversity

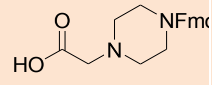
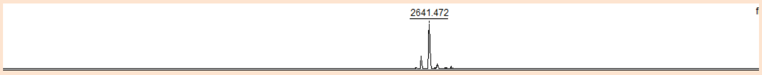
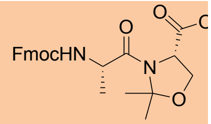
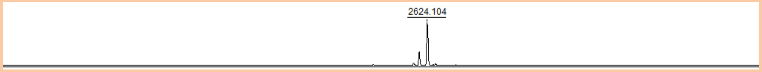
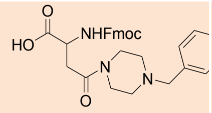
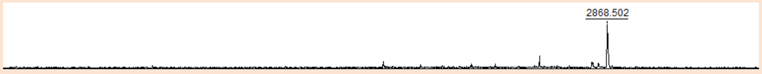
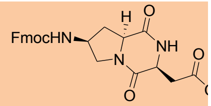
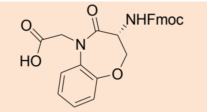
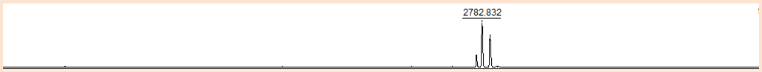
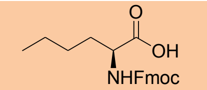
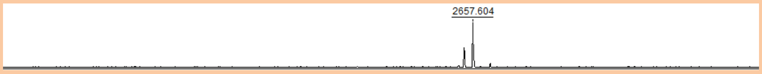
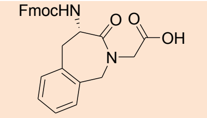
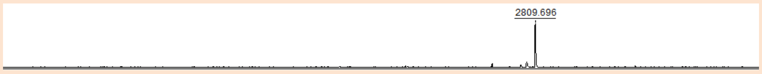
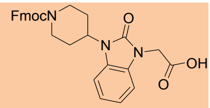
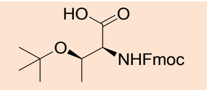
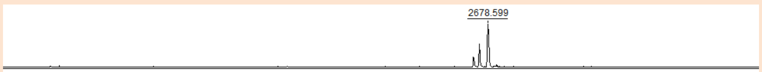
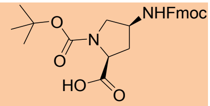
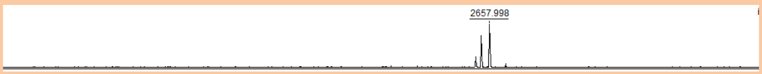
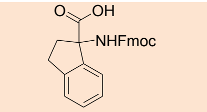
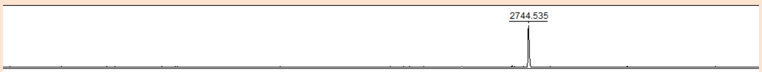
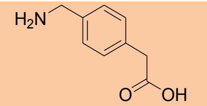
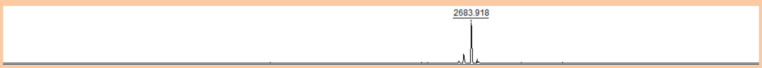
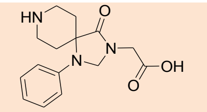
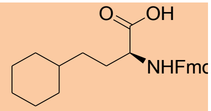
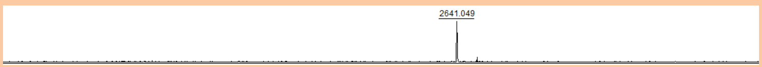
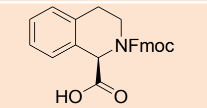
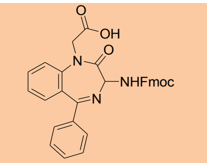
Cod 1	Structure	PNA seq*	MW	Maldi (1000-3800 Da)
1		GGAAGCT	2603.26	
2		GTAAGGG	2629.26	
3		GTAACCG	2605.32	
4		GTAAGCC	2639.34	
5		GCAACGA	2621.87 (M+Na)$^+$	
6		GCAAGAG	2640.33	
7		GCAACTG	2578.35	
8		GCAAGGT	2632.39	
9		GCAACCT	2638.88	
10		GCAAGTC	2654.44	
11		GAGAGCA	2738.45	
12		GGGAGTA	2819.87 (M+Na)$^+$	
13		GTGAGGA	2763.46	

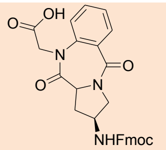
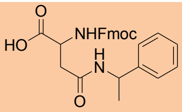
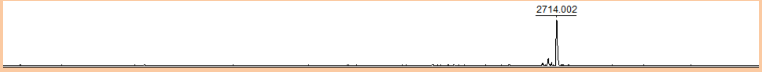
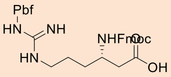
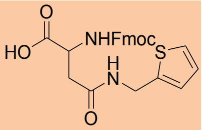
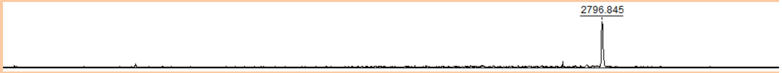
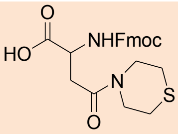
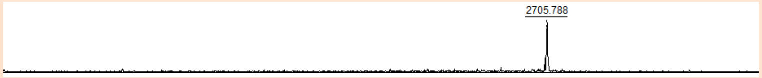
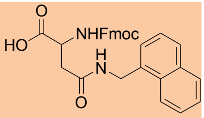
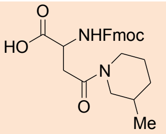
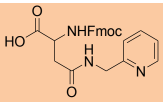
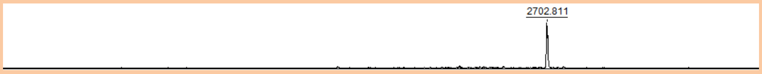
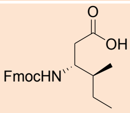
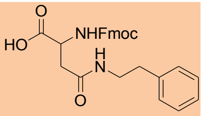
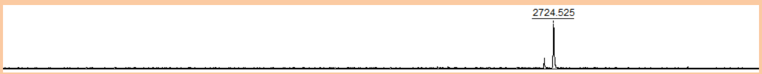
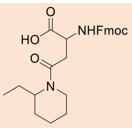
14		GTGACCA	2647.68	
15		GTGAGTG	2699.65	
16		GTGAGCT	2636.59	
17		GCGAGAA	2703.68 (M+Na)+	
18		GCGACTA	2715.41	
19		GCGAGTT	2620.29	
20		GGTAGCA	2667.24 (M+Na)+	
21		GTTAGCG	2672.07 (M+Na)+	
22		GCTAGGA	2701.04 (M+Na)+	
23		GCTACCA	2612.15 (M+Na)+	
24		GCTAGTG	2685.83 (M+Na)+	
25		GCTAGCT	2634.57 (M+Na)+	
26		GACACAC	2624.96 (M+Na)+	
27		GTCAGCA	2654.23 (M+Na)+	
28		GCCAGTA	2601.08 (M+Na)+	

29		GGAAAGG	2692.37	
30		GGAATCG	2638.41 (M+Na)$^+$	
31		GAAGGGA	2830.57 (M+Na)$^+$	
32		GAAGCCA	2644.02 (M+Na)$^+$	
33		GAAGGTG	2732.89 (M+Na)$^+$	
34		GAAGGCT	2819.79 (M+Na)$^+$	
35		GGAGGAA	2790.47	
36		GGAGCTA	2680.35	
37		GGAGGTT	2734.28 (M+Na)$^+$	
38		GTAGCGA	2671.38	
39		GTAGGAG	2776.34	
40		GTAGCTG	2748.24 (M+Na)$^+$	
41		GTAGGGT	2689.47	
42		GTAGCCT	2699.38	

43		GTAGGTC	2735.53	
44		GCAGCAA	2628.32	
45		GCAGGAT	2680.01 (M+Na)$^{+}$	
46		GCAGCTT	2588.20 (M+Na)$^{+}$	
47		GAGGGTA	2722.28 (M+Na)$^{+}$	
48		GTGGGAA	2860.03 (M+Na)$^{+}$	
49		GTGGCTA	2789.31	
50		GTGGGTT	2679.33	
51		GATGGCA	2629.31	
52		GGTGGTA	2676.31	
53		GTTGGGA	2692.37	
54		GTTGCCA	2780.43	
55		GTTGGTG	2679.35	
56		GTTGGCT	2688.87 (M+Na)$^{+}$	
57		GCTGGAA	2733.79 (M+Na)$^{+}$	

58		GCTGCTA	2608.31	
59		GCTGGTT	2711.07 $(M+Na)^+$	
60		GTCGGTA	2618.27	
61		GAATGGG	2812.92 $(M+Na)^+$	
62		GAATCCG	2711.37 $(M+Na)^+$	
63		G ATCGA	2688.75 $(M+Na)^+$	
64		GGATGAG	2737.86 $(M+Na)^+$	
65		GGATCTG	2638.28	
66		GGATGGT	2662.01 $(M+Na)^+$	
67		GGATCCT	2655.61 $(M+Na)^+$	
68		GGATGTC	2784.66 $(M+Na)^+$	
69		GTATCGG	2734.44 $(M+Na)^+$	
70		GTATGGC	2718.33 $(M+Na)^+$	
71		GTATCCC	2422.35 $(M+Na)^+$	
72		GCATCAG	2662.92 $(M+Na)^+$	
73		GCATCGT	2664.64 $(M+Na)^+$	

74		GCATGAC	2641.47 $(M+Na)^+$	
75		GCATCTC	2624.10 $(M+Na)^+$	
76		GAGTGGA	2868.50 $(M+Na)^+$	
77		GAGTCCA	2724.47 $(M+Na)^+$	
78		GAGTGTG	2781.41	
79		GAGTGCT	2757.60 $(M+Na)^+$	
80		GGGTGAA	2809.69 $(M+Na)^+$	
81		GGGTCTA	2802.82 $(M+Na)^+$	
82		GGGTGTT	2678.59 $(M+Na)^+$	
83		GTGTCGA	2657.99 $(M+Na)^+$	
84		GTGTGAG	2744.53 $(M+Na)^+$	
85		GTGTCTG	2683.91 $(M+Na)^+$	
86		GTGTGGT	2825.50	
87		GTGTCCT	2641.38	
88		GTGTGTC	2673.34	
89		GCGTCAA	2805.78 $(M+Na)^+$	

90		GCGTGAT	2816.94 $(M+Na)^+$	
91		GCGTCTT	2714.002 $(M+Na)^+$	
92		GATTGCG	2692.74	
93		GGTTGGA	2796.84 $(M+Na)^+$	
94		GGTTCCA	2705.78 $(M+Na)^+$	
95		GGTTGTG	2830.09 $(M+Na)^+$	
96		GGTTGCT	2732.86 $(M+Na)^+$	
97		GTTTGGG	2702.81 $(M+Na)^+$	
98		GTTTCCG	2702.98 $(M+Na)^+$	
99		GCTTCGA	2724.52 $(M+Na)^+$	
100		GCTTGAG	2756.54 $(M+Na)^+$	

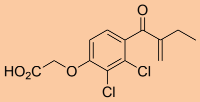
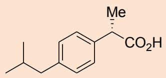
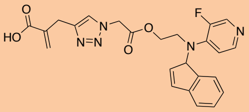
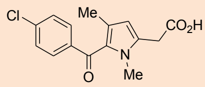
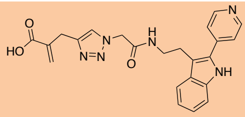
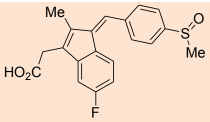
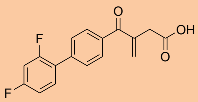
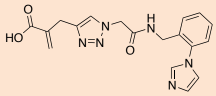
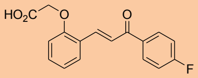
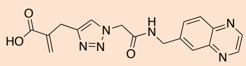
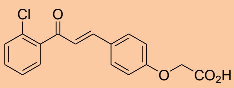
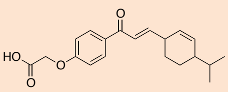
**The PNA were synthesized using alternately serine-modified monomers starting with Gs*

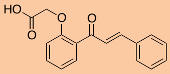
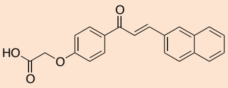
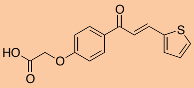
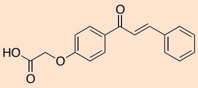
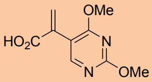
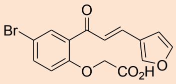
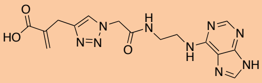
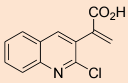
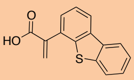
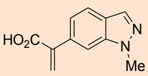
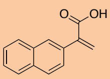
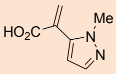
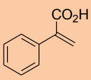
Encoding of the second point of diversity: 14mer PNA synthesis on pooled mix and introduction of the second point of diversity in the 10K PNA encoded library of covalent binders. Library I.

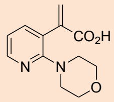
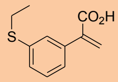
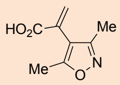
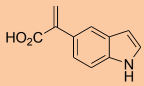
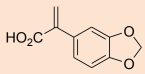
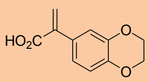
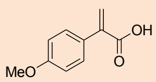
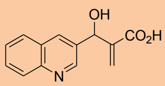
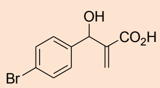
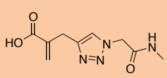
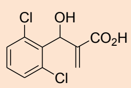
The 100 unique 7mer PNA coding the first point of diversity, were mixed and split In 100 wells using a technique previously reported, obtaining a statistic mixture of all the 100 PNA 7mers in each well. At this stage another unique 7mer PNA tag is synthetized for each well obtaining, by doing so, a permutation of 10'000 PNA 14mers. This second TAG will allow us to code for the second point of diversity. The PNA synthesis was carried out on 2.5 mg (0.5 μmol) of resin for each reaction following cycles of Mtt deprotection (***procedure 10***) and TNTU mediated PNA coupling (***procedure 11***). A small amount of resin for every single reaction was cleaved by TFA 99%, precipitated in Et_2O, and analysed by MALDI-TOF.

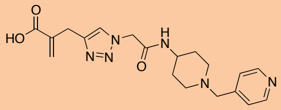
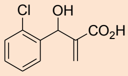
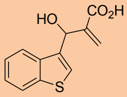
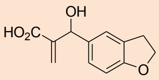
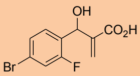
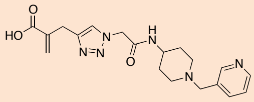
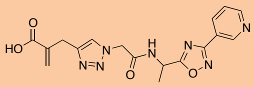
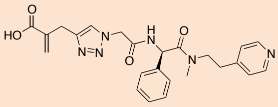
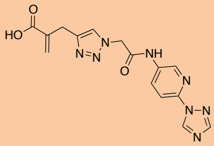
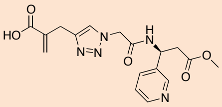
Fmoc amino acids previously introduced were deprotected using ***procedure 4***, followed by ***procedure 7b*** both for the small molecules containing carboxylic acid as well as for the ene-yne acid. Wells containing the ene-yne were then subjected to copper catalysed alkyne-azide cycloaddition (***procedure 13***) with 2-azidoacetic acid, and then the carboxylic acid handle was activated and reacted with 66 different heteroaromatic amines following ***procedure 8***.

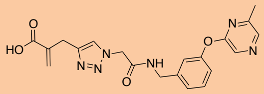
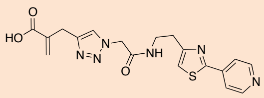
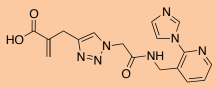
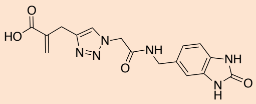
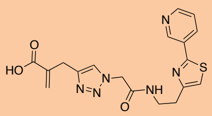
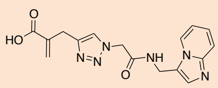
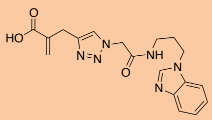
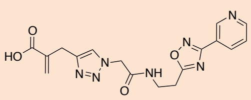
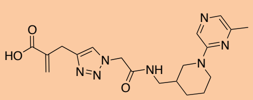
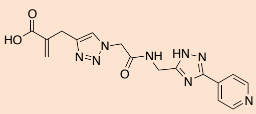
Table 3-SI: MALDI-TOF data of encoded 100 pooled mix: second point of diversity

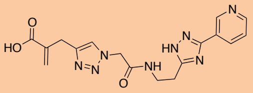
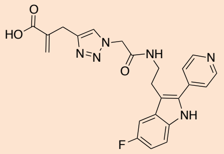
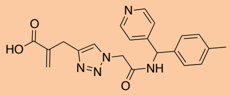
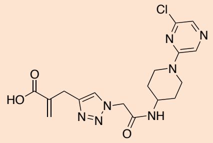
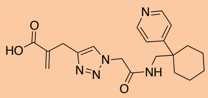
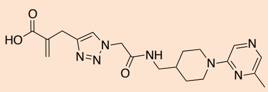
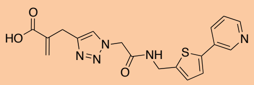
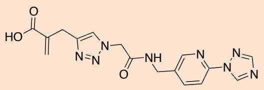
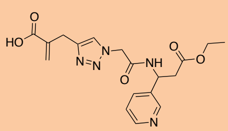
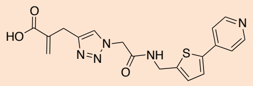
Cod. 2	Small molecule	PNA seq	Calc. m/z [M+H]	Experimental m/z [M+H]
1		CTTCTGG	4373.85-4818.85	4373.45-4819.35
2		CTTGGTG	4413.88-4858.88	4413.55-4856.94
3		CTTCCTG	4333.83-4778.83	4524.67-4692.22
4		CTTGTCG	4373.85-4818.85	4379.75482.77
5		ACTGCAG	4391.88-4836.88	4400.66-4817.41
6		GCTGTAG	4422.89-4868.89	4425.18-4866.77
7		TCTGGAG	4422.89-4867.89	4417.70-4870.06
8		TCTCCAG	4342.84-4787.84	4457.69-4774.56
9		TCTGTGG	4413.87-4858.87	4413.64
10		TCTGCTG	4373.85-4818.85	4366.44-4807.98
11		CCTGAAG	4391.88-4836.88	4389.50-4840.44
12		CCTCTAG	4342.84-4777.84	4343.89-4777.73

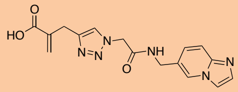
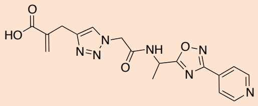
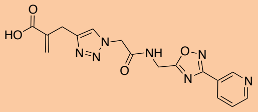
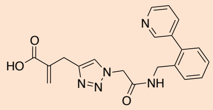
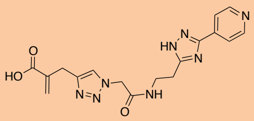
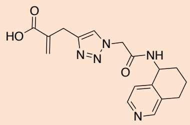
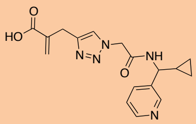
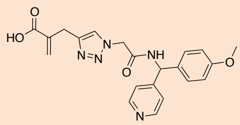
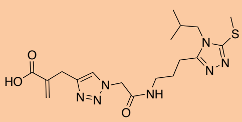
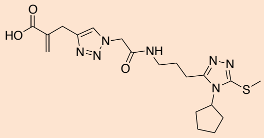
13		CCTGTTG	4373.85–4818.85	4372.62–4818.00
14		AACCTGG	4391.88–4836.88	4391.98–4840.44
15		AACGGTG	4431.90–4876.90	4432.17–4877.17
16		AACGAGG	4440.92–4885.92	4446.22–4880.06
17		AACGTCG	4391.88–4836.88	4390.18–4838.79
18		AACCGAG	4400.89–4845.90	4400.99–4841.63
19		AATCTAG	4390.89–4835.89	4391.08–4835.24
20		GACCAAG	4400.89–4845.89	4403.25–4849.20
21		GACGATG	4431.90–4876.90	4432.18–4879.08
22		GACCTTG	4382.87–4827.89	4388.38–4830.99
23		TACCAGG	4391.88–4836.88	4390.44–4853.94
24		TACCGTG	4382.87–4827.87	4383.20–4826.26
25		TACGACG	4391.88–4836.88	4390.63–4839.03

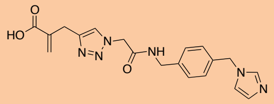
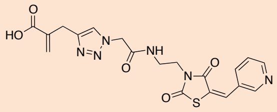
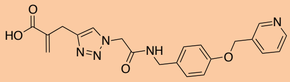
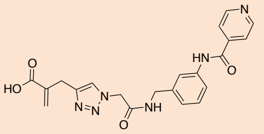
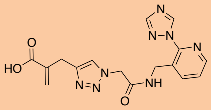
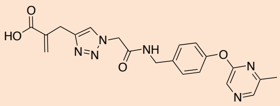
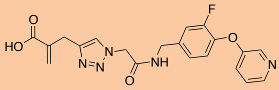
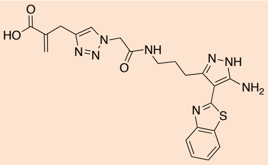
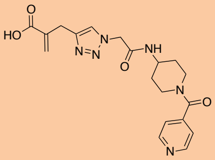
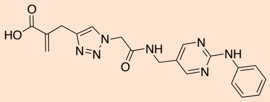
26		TACCTCG	4342.84–4787.84	4341.87–4787.37
27		CACCATG	4351.86–4796.86	4349.27–4801.02
28		AGCGAAG	4440.92–4885.92	4407.09–4889.06
29		AGCCTAG	4391.88–4836.88	4396.94–4832.17
30		AGCGTTG	4422.89–4867.89	4422.23–4869.35
31		TGCCAAG	4391.88–4836.88	4398.29–4835.72
32		TGCGATG	4422.89–4867.89	4425.62–4863.65
33		TGCCTTG	4373.85–4818.85	4375.77–4818.24
34		ATCGGAG	4431.91–4876.90	4431.50–4879.31
35		ATCCCAG	4351.86–4814.86	4355.89–4817.44
36		ATCGTGG	4422.89–4867.89	4422.23–4869.12

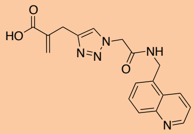
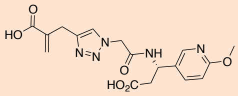
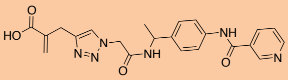
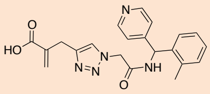
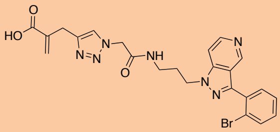
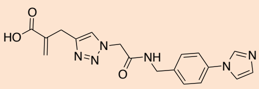
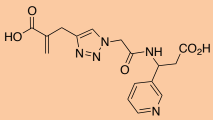
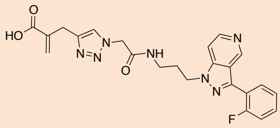
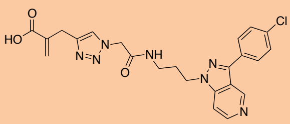
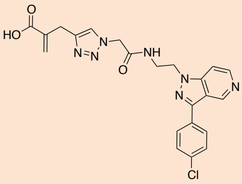
37		ATCGCTG	4382.86–4827.87	4388.04–4799.49
38		GTCGAAG	4431.90–4876.91	4436.03–4875.52
39		GTCCTAG	4382.87–4827.87	4387.47–4817.06
40		GTCGTTG	4413.88–4858.88	4413.64–4858.44
41		TTCCGAG	4382.86–4827.86	4382.52–4826.50
42		TTCGAGG	4422.89–4867.89	4421.09–4866.02
43		TTCCTGG	4373.85–4818.85	4377.79–4815.17
44		TTCGGTG	4413.87–4858.87	4414.54–4851.81
45		TTCCCTG	4333.83–4778.83	4340.42–4734.71
46		TTCGTCG	4373.85–4818.85	4371.50–4815.40

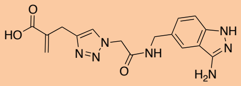
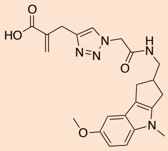
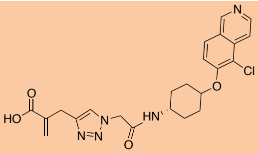
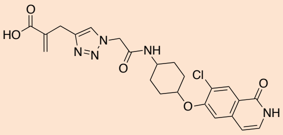
47		CTCCAAG	4351.86–4796.86	4351.29–4797.73
48		CTCGATG	4382.87–4827.87	4391.98–4829.33
49		CTCCTTG	4333.86–4778.83	4333.93–4777.85
50		ACCGTAG	4391.88–4836.88	4389.84–4838.43
51		TCCGAAG	4391.88–4836.88	4391.19–4833.47
52		TCCCTAG	4342.84–4787.84	4345.80–4785.14
53		TCCGTTG	4373.85–4818.85	4377.23–4816.23
54		GCGTAAG	4431.90–4876.91	4433.19–4879.67
55		GCGATAG	4431.90–4894.91	4447.02–4893.22
56		GCGAATG	4431.90–4876.90	4424.37–4871.36

57		GCGTATG	4422.891-866.89	4423.47-4868.04
58		GCGATTG	4422.89-4867.89	4420.75-4864.96
59		GCGTTTG	4413.88-4858.88	4413.98-4859.74
60		CGCATAG	4391.88-4836.88	4390.96-4837.25
61		CGCTTAG	4382.87-4827.87	4383.08-4826.85
62		CGCATTG	4382.87-4827.88	4389.61-4828.98
63		CGCTTTG	4373.85-4818.85	4386.92-4810.81
64		GGGAAAG	4373.85-4818.85	4386.92-4810.81
65		GGGTTAG	4462.91-4907.91	4462.22-4907.26
66		GGGTATG	4462.92-4907.91	4461.77-4909.64

67		GGGATTG	4462.91-4907.91	4466.31-4907.97
68		GGGTTTG	4453.90-4898.90	4453.14-4899.88
69		CCGAAAG	4400.89-4854.90	4404.34 -4846.95
70		CCGTTAG	4382.87-4827.87	4382.18-4828.98
71		CCGTATG	4382.87-4827.87	4385.11-4826.38
72		CCGATTG	4382.87-4827.87	4385.11-4830.40
73		CCGTTTG	4373.85-4818.85	4375.43-4818.83
74		TTGCCAG	4382.87-4827.87	4399.76-4815.84
75		TTGAGGG	4462.91-4907.91	4464.72-4909.88
76		TTGGCTG	4413.91-4858.88	4415.59-4853.50

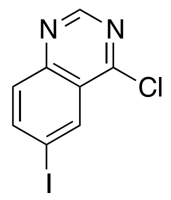
77		TTGCACG	4382.87-4827.87	4380.16-4824.49
78		TTGTGCG	4413.88-4858.88	4414.20-4858.56
79		TTGACCG	4328.87-4827.87	4472.54-4825.67
80		TTACCCG	4342.84-4787.84	4378.86-4772.92
81		TTCGCAG	4382.87-4827.87	4386.46-4816.94
82		TTCTGGG	4413.88-4858.88	4412.62-4855.00
83		TTCGTGG	4413.88-4876.88	4415.78-4875.63
84		TTCACGG	4382.86-4827.87	4394.12-4819.06
85		TTCAGCG	4382.87-4827.87	4388.94-4823.31
86		TTCTCCG	4333.83-4778.83	4368.43-4772.91

87		AACCCTG	4351.86-4796.86	4394.57-4791.48
88		AAAGCCG	4400.90-4863.89	4478.36-4807.97
89		AAAGGGG	4480.94-4925.94	4482.12-4922.87
90		GGCTATG	4422.89-4866.89	4420.19-4864.62
91		AATGCCG	4391.88-4836.88	4389.16-4832.29
92		TTTGCCG	4373.85-4818.853	4394.12-4799.26
93		AAACCGG	4440.92-4885.92	4469.49-4858.79
94		TAACGCG	4391.88-4854.88	4399.19-4860.81
95		AGAGGTG	4471.93-4916.93	4478.25-4915.24
96		AGAGACG	4440.92-4885.92	4436.93-4882.17

97		AGACCTG	4391.88-4836.88	4388.26-4835.49
98		AGAAGCG	4400.92-4885.92	4442.37-4889.30
99		AGACTGG	4431.90-4876.90	4437.16-4874.33
100		AGATGAG	4455.93-4900.93	4453.25-4906.57

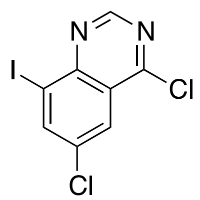
Synthesis of building blocks for the kinase targeted PNA-encoded library.

Library II.



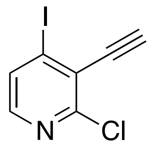
Starting from the corresponding antranilic acid (commercially available, Sigma-Aldrich), compound **SM-28** was synthesized using ***procedure 38*** followed by ***procedure 39*** obtaining the desired compound.

^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 9.08 (s, 1H); 8.67 (s, 1H); 8.21 (d, *J* = 8 Hz, 1H); 7.80 (d, *J* = 8 Hz, 1H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C) δ = 154.0, 150.1, 143.8, 134.5, 130.3, 125.3, 94.6 ppm. LC-MS (ESI): m/z [M + H] calcd. for $C_8H_4ClIN_2$ 289.91, found : 287.08.



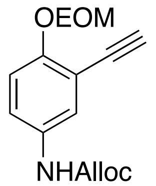
Starting from Methyl 2-amino-5-chloro-3-iodobenzoate (commercially available, Sigma-Aldrich), compound **SM-29** was synthesized as follows: Methyl 2-amino-5-chloro-3-iodobenzoate (1 g, 3.2 mmol) was dissolved in absolute ethanol (3 mL) and the solution, brought at 0 °C, was treated with a solution of 1 M NaOH (6 mL) over 15 minutes. After completion of the reaction, as judged by TLC analysis, ethanol was evaporated and the resulting solution was treated with 1 M HCl until pH= 5. Aqueous phase was then extracted with EtOAc (50 mL X 3), organic extracts were then dried over Na_2SO_4 filtrated and evaporated to afford pure carboxylic acid (850 mg) Yield: 93% then ***procedure 38*** was followed by ***procedure 39***.

^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 9.16 (s, 1H); 8.52 (s, 1H); 8.30 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C) δ = 162.0, 154.4, 149.3, 145.5, 135.5, 125.2, 125.5, 102.7 ppm. LC-MS (ESI): m/z [M + H] calcd. for $C_8H_3Cl_2IN_2$ 323.87, found: 323.13/325.05



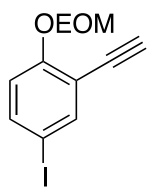
Starting from 2-Chloro-4-iodonicotinaldehyde (commercially available, Sigma-Aldrich), compound **SM-30** was synthesized using ***procedure 28***.

^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 7.96 (d, *J* = 4 Hz, 1H); 7.76 (d, *J* = 4 Hz, 1H), 3.82 (s, 1H) ppm.^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C) δ = 152.3, 147.7, 132.5, 126.0, 113.5, 88.6, 80.1 ppm. LC-MS (ESI): m/z [M + H] calcd. for C_7H_3ClIN 262.90, found : 264.07/266.02



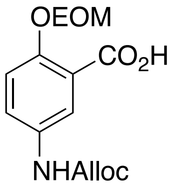
Starting from 2-hydroxy-5-nitrobenzaldehyde (commercially available, Sigma-Aldrich), compound **SM-31** was synthesized using ***procedure 26*** followed by ***procedures 21, 29*** and ***28***.

^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 7.49 (bs, 1H); 7.37 (bs, 1H); 7.14 (d, *J* = 12 Hz, 1H); 6.65 (bs, 1H); 5.98 (m, 1H); 5.39 (bd, 1H); 5.35 (bd, 1H); 5.28 (s, 2H); 4.67 (dt, *J* = 8, 4 Hz, 2H); 3.80 (q, *J* = 8 Hz, 2H); 3.28 (s, 1H); 1.25 (t, *J* = 8 Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C) δ = 154.8, 153.4, 132.4, 131.8, 124.5, 121.2, 118.3, 116.0, 112.9, 93.9, 81.3, 79.6, 65.9, 64.5, 15.1 ppm. LC-MS (ESI): m/z [M + H] calcd. for $C_{15}H_{17}NO_4$ 275.12, found : 279.03.



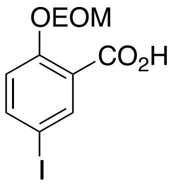
Starting from 2-hydroxy-5-iodobenzaldehyde (commercially available, Sigma-Aldrich), compound **SM-32** was synthesized using ***procedure 26*** followed by ***procedure 28***.

^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 7.78 (s, 1H); 7.60 (d, *J* = 12.0 Hz, 1H); 6.98 (d, *J* = 12.0, 1H); 5.31 (s, 1H); 3.77 (q, *J* = 8 Hz, 2H); 3.33 (s, 1H); 1.25 (t, *J* = 8 Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C) δ = 158.4, 145.7, 142.2, 138.9, 116.9, 114.8, 93.5, 83.2, 82.2, 78.4, 64.7, 15.1 ppm.



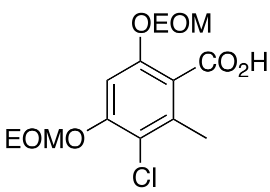
Starting from 5-nitrosalicylic acid (commercially available, Sigma-Aldrich), compound **SM-33** was synthesized using ***procedure 24*** or ***procedure 25*** alternatively followed by ***procedure 26***, ***procedure 27***, ***procedure 29*** and finally ***procedure 31***, *or* ***procedure 30*** to deprotect TMS ester.

^{1}H NMR (CDCl$_3$, 400 MHz, 23 °C) δ = 7.93 (bs, 2H); 7.30 (d, *J* = 8.0, Hz, 1H); 5.98 (m, 1H); 5.44 (s, 2H); 5.39 (bd, 1H); 5.28 (bd, 1H); (4.69 d, *J* = 8 Hz, 2H); 3.80 (q, *J* = 8 Hz, 2H); 1.25 (t, *J* = 8 Hz, 3H) ppm. ^{13}C NMR (CDCl$_3$, 100 MHz, 25 °C) δ = 165.3, 153.5, 152.2, 133.26, 133.25, 125.7, 123.3, 118.6, 118.3, 116.2, 94.9, 66.5, 66.1, 15.1 ppm.



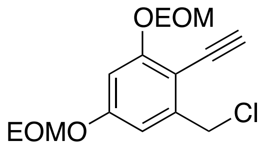
Starting from 5-iodosalicylic acid (commercially available, Sigma-Aldrich), compound **SM-34** was synthesized using ***procedure 24*** or ***procedure 25*** alternatively, followed by ***procedure 26***, and ***procedure 31***, or ***procedure 30*** to deprotect the TMS ester.

^{1}H NMR (MeOD, 400 MHz, 23 °C) δ = 8.07 (d, *J* = 4.0 Hz, 1H); 7.79 (dd, *J* = 8.0, 4 Hz, 1H); 7.12 (d, *J* = 8.0 Hz, 1H); 5.33 (s, 2H); 3.77 (q, *J* = 8.0 Hz, 2H); 1.21 (t, *J* = 8.0 Hz, 3H) ppm.



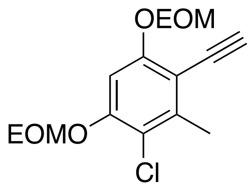
Starting from 3-chloro-4,6-dihydroxy-2-methylbenzoic acid, compound **SM-35** was synthesized using ***procedure 24*** followed by ***procedure 26***, and ***procedure 31***.

^{1}H NMR (CDCl$_3$, 400 MHz, 23 °C) δ = 7.05 (s, 1H); 5.34 (s, 2H); 5.28 (s, 2H); 3.78 (dq, *J* = 12, *J* = 8 Hz, 4H); 2.45 (s, 3H); 1.25 (td, *J* = 8, 4 Hz) ppm. ^{13}C NMR (CDCl$_3$, 100 MHz, 25 °C) δ = 171.3, 154.9, 153.6, 136.3, 118.3, 117.7, 101.8, 94.2, 93.8, 64.7 (2C), 17.8, 15.03 (2C) ppm. LC-MS (ESI): m/z [M + H] calcd. for $C_{14}H_{19}ClO_6$ 318.09, found : 318.72.



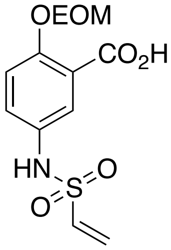
Starting from 3,5-dihydroxybenzoic acid, compound **SM-36** was synthesized using ***procedure 24*** followed by ***procedure 26, procedure 33, procedure 36, procedure 37, procedure 30*** and finally ***procedure 35***.

^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 6.88-6.86 (m, 2H); 5.31 (s, 2H); 5.25 (s, 2H); 4.74 (s, 2H); 3.78 (dq, *J* = 12, 8 Hz, 4H); 3.53 (s, 1H); 2.55 (s, 3H); 1.25 (td, *J* = 8, 4 Hz) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C) δ = 160.1, 158.6, 142.1, 110.1 (2C), 105.5, 103.7, 93.7, 93.1, 85.2, 64.5 (2C), 44.5, 15.1 (2C) ppm. LC-MS (ESI): m/z [M + H] calcd. for $C_{15}H_{19}ClO_4$ 298.10, found : 299.21.



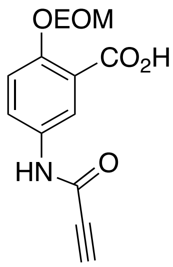
Starting from 3-chloro-4,6-dihydroxy-2-methylbenzoic acid, compound **SM-37** was synthesized using ***procedure 25, procedure 26, procedure 33, procedure 34*** and finally ***procedure 28***.

^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 7.00 (s, 1H); 5.31 (s, 2H); 5.30 (s, 2H); 3.78 (dq, *J* = 12, *J* = 8 Hz, 4H); 3.47 (s, 1H); 2.55 (s, 3H); 1.25 (td, *J* = 8, 4 Hz) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C) δ = 158.1, 153.1, 141.1, 117.03, 107.4, 101.7, 93.9, 93.5, 84.6, 78.4, 64.7, 64.5, 18.7 (2C), 15.1 ppm. LC-MS (ESI): m/z [M + H] calcd. for $C_{15}H_{19}ClO_4$ 298.10, found : 299.18.



Starting from 5-nitrosalicylic acid (commercially available, Sigma-Aldrich), compound **SM-38** was synthesized using ***procedure 25*** followed by ***procedure 26, procedure 27, procedure 32*** (vinylsulphonyl chloride introduction) and finally ***procedure 30*** to deprotect the TMS ester.

^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 7.60 (d, *J* = 4.0 Hz, 1H); 7.35 (dd, *J* = 8.0, 4.0 Hz, 1H); 7.20 (d, *J* = 8.0 Hz, 1H); 6.58 (dd, *J* = 16.0, 8.0 Hz, 1H); 6.22 (d, *J* = 16.0 Hz, 1H); 5.95 (d, *J* = 12.0 Hz, 1H); 5.27 (s, 2H); 3.81 (q, *J* = 8.0 Hz, 2H); 1.28 (t, *J* = 8.0 Hz, 3H) ppm.



Starting from 5-nitrosalicylic acid (commercially available, Sigma-Aldrich), compound **SM-39** was synthesized using ***procedure 25*** followed by ***procedure 26, procedure 27, procedure 32*** (propiolic acid introduction) and finally ***procedure 30*** to deprotect the TMS ester.

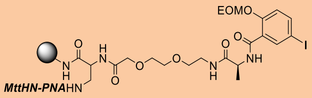
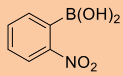
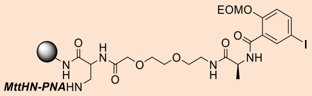
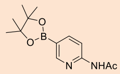
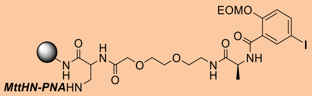
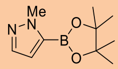
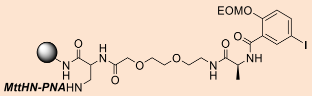
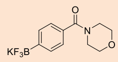
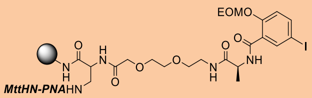
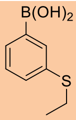
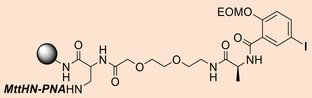
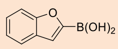
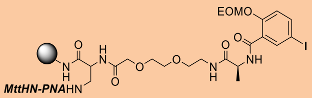
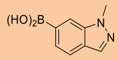
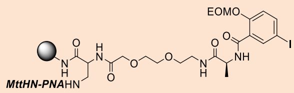
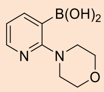
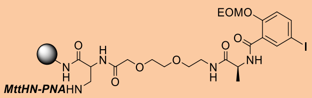
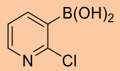
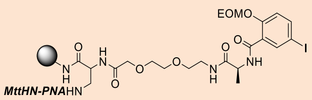
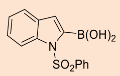
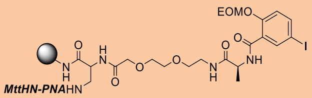
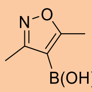
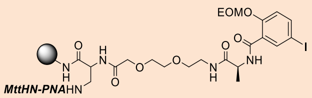
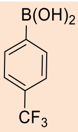
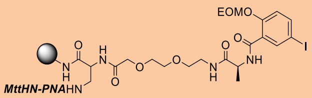
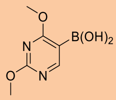
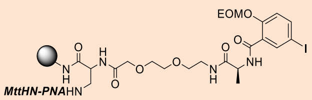
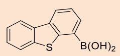
^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C) δ = 8.15 (dd, *J* = 8.0, 4.0 Hz, 1H); 8.00 (d, *J* = 4.0, 1H); 7.35 (d, *J* = 4.0 Hz, 1H); 5.30 (s, 2H); 3.82 (q, *J* = 8.0 Hz, 2H); 3.00 (s, 1H); 1.28 (t, *J* = 8.0 Hz, 3H) ppm.

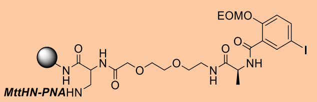
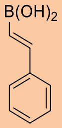
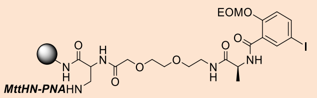
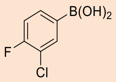
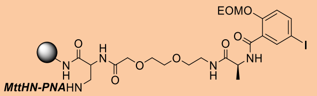
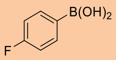
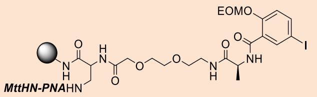
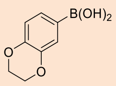
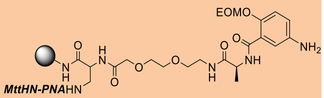
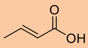
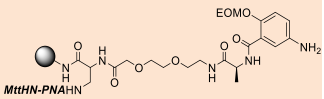
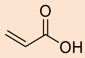
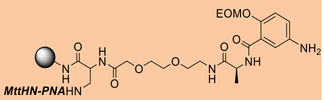
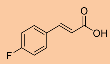
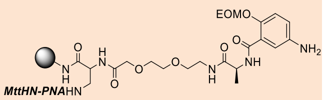
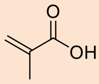
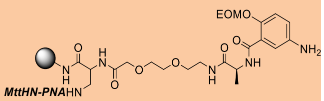
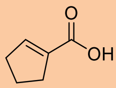
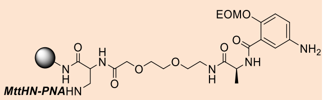
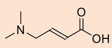
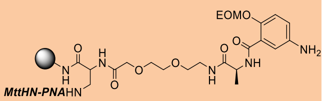
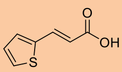
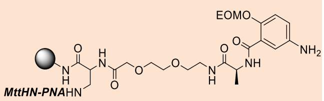
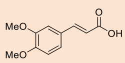
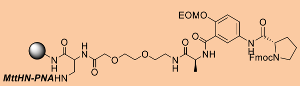
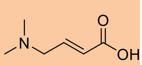
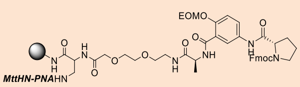
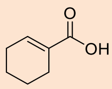
Reaction conditions optimization for the introduction of building blocks to build the second point of diversity for the kinase targeted PNA-encoded library.

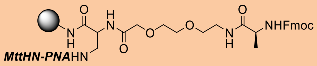
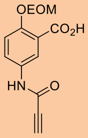
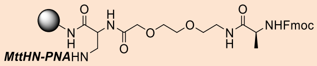
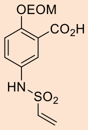
Library II.

All the building blocks needed for the diversification steps were conjugated to a PNA tag composed of four nucleobases (4mer PNA), one of them being Thymine with a serine modification on the side-chain [A; C; G; TS; (Boc-Mtt) protection] and tested under a series of conditions. The best reaction conditions are shown in the following table (Table 4-SI). All reactions were tested on 5 mg (0.001 mmol) of Rink amide NovaPEG resin.

Table 4-SI: Building blocks validation on model system

	Starting resin	BA-MA	Conditions	MW (SM/DC)* m/z [M + H]	Yield
1			Pd(OAc)$_2$ 10% SPHOS 20% 2M Na$_2$CO$_3$, Dioxane 80 °C	1721.55/1717.58	>95%
2			Pd(OAc)$_2$ 10% SPHOS 20% 2M Na$_2$CO$_3$, Dioxane 80 °C	1721.55/1717.58	70% DC
3			Pd(OAc)$_2$ 10% SPHOS 20% 2M Na$_2$CO$_3$, Dioxane 80 °C	1721.55/1676.71	60% DC, 20% deiodination
4			Pd(OAc)$_2$ 10% SPHOS 20% 2M Na$_2$CO$_3$, Dioxane 80 °C	1721.55/1676.71	60% DC 40% SM
5			Pd$_2$(dba)$_3$ 10% SPHOS 20% 2M Na$_2$CO$_3$, Dioxane 80 °C	1721.55/1732.57	80% DC
6			Pd(OAc)$_2$ 10% SPHOS 20% 2M Na$_2$CO$_3$, Dioxane 80 °C	1721.55/1712.89	80% DC
7			Pd(OAc)$_2$ 10% SPHOS 20% 2M Na$_2$CO$_3$, Dioxane 80 °C	1721.55/1725.87	>90% DC
8			Pd(OAc)$_2$ 10% SPHOS 20% 2M Na$_2$CO$_3$, Dioxane 80 °C	1721.55/1758.84	>90% DC
9			Pd(OAc)$_2$ 10% SPHOS 20% 2M Na$_2$CO$_3$, Dioxane 80 °C	1721.55/1706.71	>70% DC
10			Pd(OAc)$_2$ 10% XPHOS 20% 2M Na$_2$CO$_3$, Dioxane 80 °C	1721.55/1851.64	>85% DC
11			Pd(OAc)$_2$ 10% XPHOS 20% 2M Na$_2$CO$_3$, Dioxane 80 °C	1721.55/1690.70	60% DC, 40% deiodination
12			Pd(OAc)$_2$ 10% SPHOS 20% 2M Na$_2$CO$_3$, Dioxane 80 °C	1721.55/1740.68	>90% DC
13			Pd(OAc)$_2$ 10% XPHOS 20% 2M Na$_2$CO$_3$, Dioxane 80 °C	1721.55/1734.64	>95% DC
14			Pd(OAc)$_2$ 10% XPHOS 20% 2M Na$_2$CO$_3$, Dioxane 80 °C	1721.55/1779.20	>80% DC

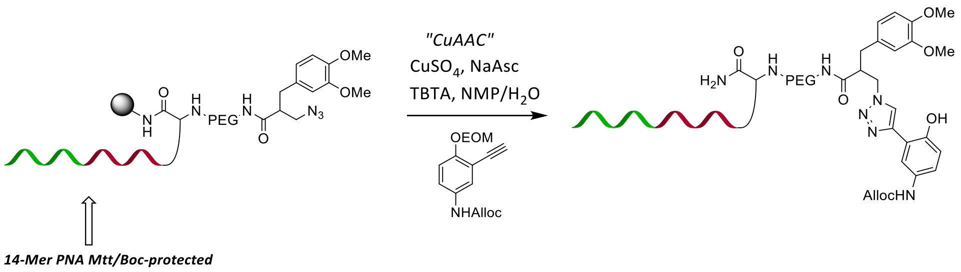
15			$Pd(OAc)_2$ 10% XPHOS 20% 2M Na_2CO_3, Dioxane 80 °C	1721.55/1698.97	>80% DC, 10% SM
16			$Pd(OAc)_2$ 10% XPHOS 20% 2M Na_2CO_3, Dioxane 80 °C	1721.55/1723.76	>90% DC
17			$Pd(OAc)_2$ 10% XPHOS 20% 2M Na_2CO_3, Dioxane 80 °C	1721.55/1689.80	>60% DC
18			$Pd(OAc)_2$ 10% XPHOS 20% 2M Na_2CO_3, Dioxane 80 °C	1721.55/1730.77	>60% DC
19			HATU 9 equiv Hunig's 10 equiv. 2,6-Lutidine 20 equiv. Acid 10 equiv. dry NMP, 1h	1611.64/1678.99	>95% DC
20			HATU 9 equiv Hunig's 10 equiv. 2,6-Lutidine 20 equiv. Acid 10 equiv. dry NMP, 1h	1611.64/1664.91	>90% DC
21			HATU 9 equiv Hunig's 10 equiv. 2,6-Lutidine 20 equiv. Acid 10 equiv. dry NMP, 1h	1611.64/1759.66	>95% DC
22			HATU 9 equiv Hunig's 10 equiv. 2,6-Lutidine 20 equiv. Acid 10 equiv. dry NMP, 1h	1611.64/1679.66	>95% DC
23			HATU 9 equiv Hunig's 10 equiv. 2,6-Lutidine 20 equiv. Acid 10 equiv. dry NMP, 1h	1611.64/1705.88	>95% DC
24			HATU 9 equiv Hunig's 10 equiv. 2,6-Lutidine 20 equiv. Acid 10 equiv. dry NMP, 1h	1611.64/1723.79	>80% DC
25			HATU 9 equiv Hunig's 10 equiv. 2,6-Lutidine 20 equiv. Acid 10 equiv. dry NMP, 1h	1611.64/1747.88	>95% DC
26			HATU 9 equiv Hunig's 10 equiv. 2,6-Lutidine 20 equiv. Acid 10 equiv. dry NMP, 1h	1611.64/1801.94	>85% DC
27			HATU 9 equiv Hunig's 10 equiv. 2,6-Lutidine 20 equiv. Acid 10 equiv. dry NMP, 1h	1930.76/1818.82	>95% DC
28			HATU 9 equiv Hunig's 10 equiv. 2,6-Lutidine 20 equiv. Acid 10 equiv. dry NMP, 1h	1930.76/1815.90	>80% DC

29			1. Piperidine 20% in DMF 2. DIC 10 equiv HOBt 5 equiv small molecule 5 equiv	1698.67/1663.63	>95% DC
30			1. Piperidine 20% in DMF 2. DIC 10 equiv HOBt 5 equiv small molecule 5 equiv	1698.67/1701.79	>90% DC

*SM = Starting material, DC = Desired compound

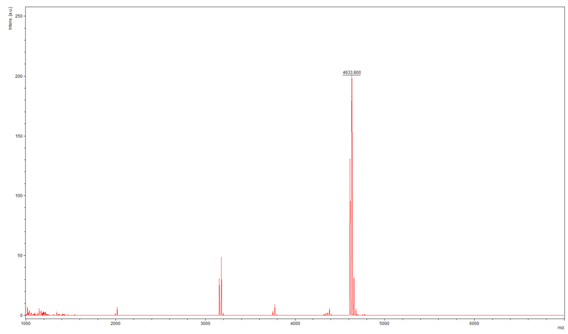
Validation of whole synthetic sequence for selected building blocks on 14*mer* PNA for the kinase targeted PNA-encoded library. Library II.

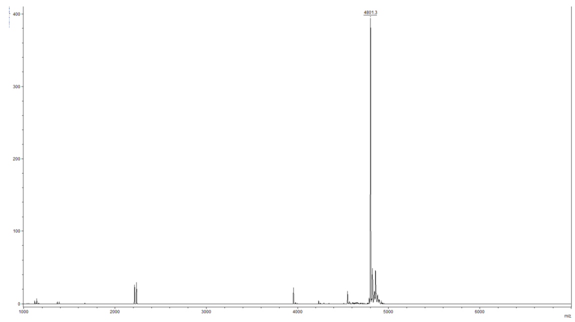
Different 14mer sequences of oligonucleotides were used for each synthetic sequence in order to avoid any bias. Each reaction was carried out on 5 mg (0.001 mmol) scale on NovaPEG Rink amide resin. All the recorded MALDI-TOF spectral data are given for cleaved reaction mixtures (TFA 99%, 1 hour, RT) followed by precipitation in Et_2O.

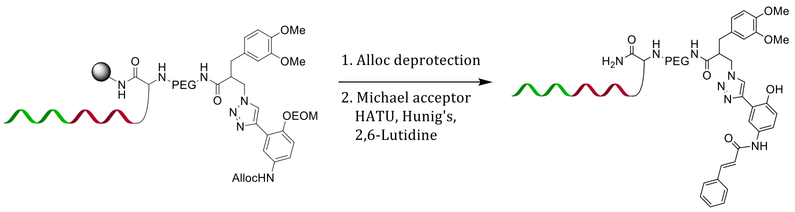


MALDI-TOF SM: m/z [M + H] 4633.800

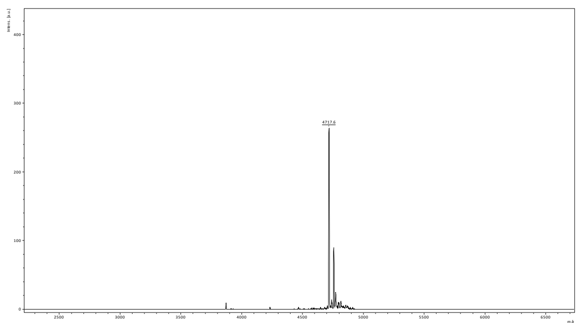
MALDI-TOF DC: m/z [M + H] 4801.300

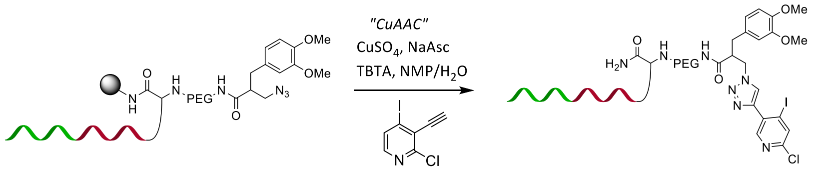






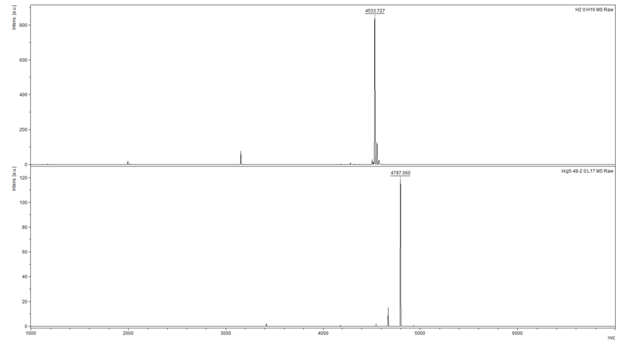
MALDI-TOF DC: m/z [M + H] 4717.600

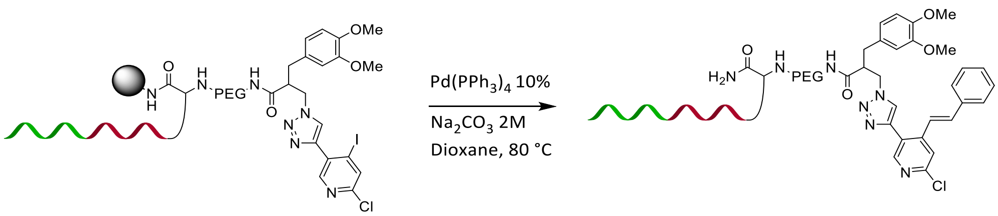




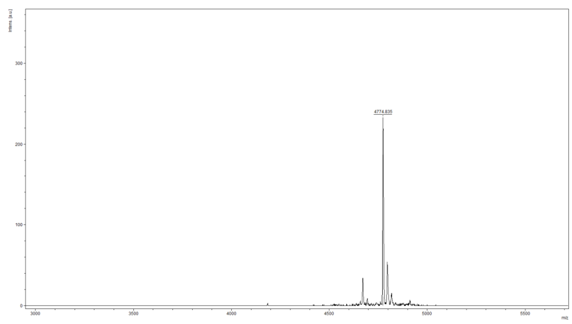
MALDI-TOF SM: m/z [M + H] 4533.727

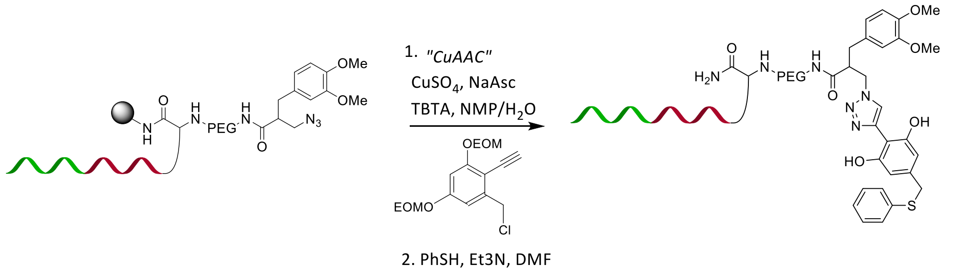
MALDI-TOF DC: m/z [M + H] 4797.060

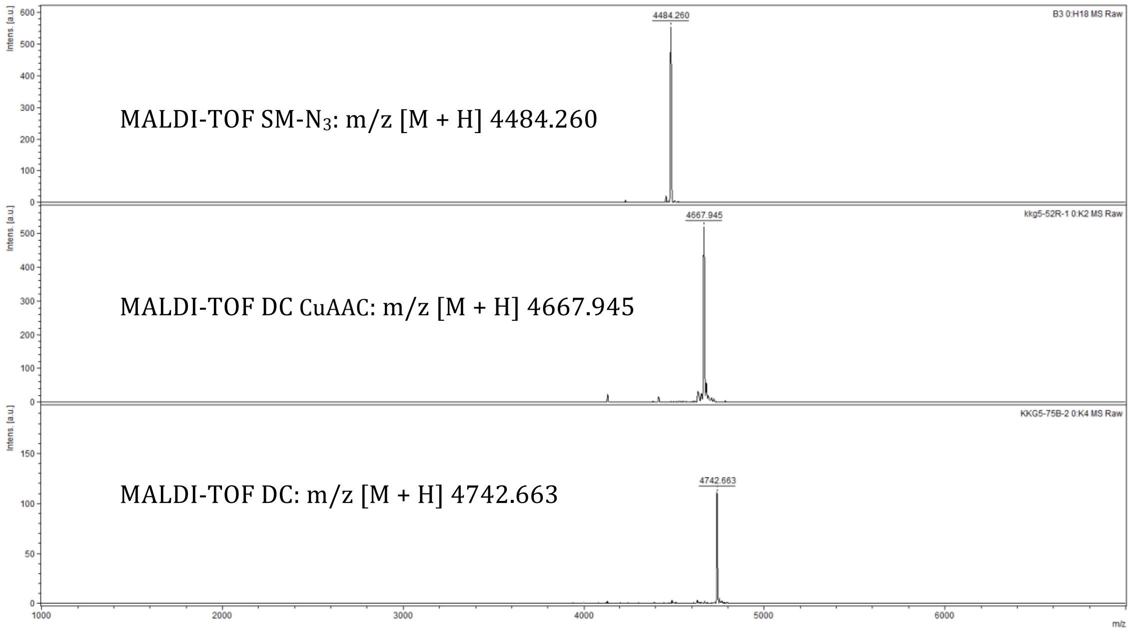


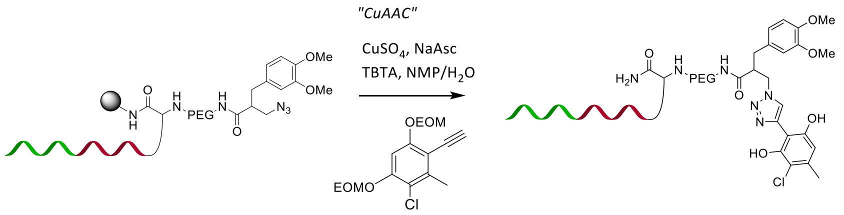


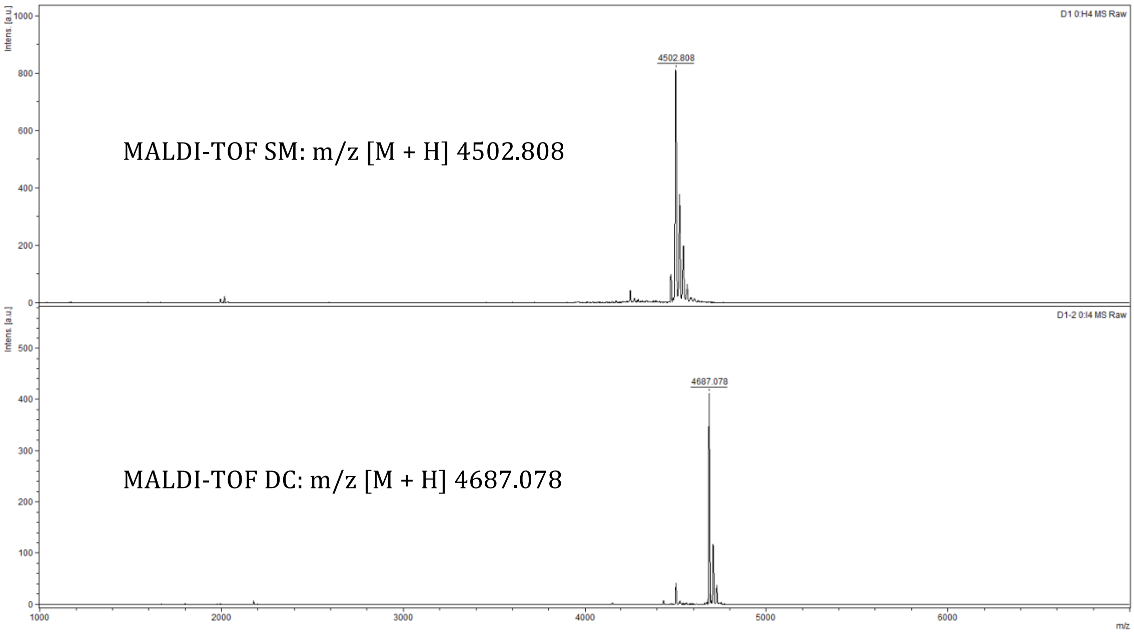
MALDI-TOF DC: m/z [M + H] 4774.853

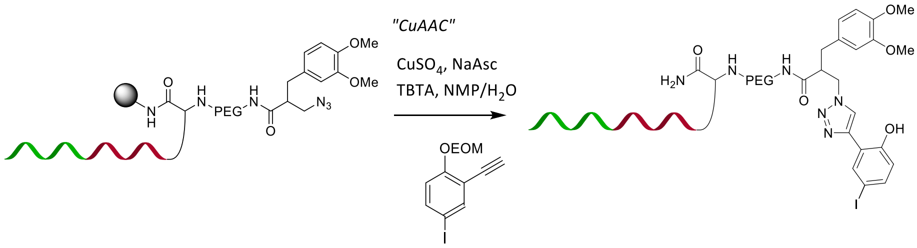



OMe
OMe
N
H
O
H
N
PEG
H
N
O
N3
1. "CuAAC"
CuSO4, NaAsc
TBTA, NMP/H2O
OEOM
EOMO
Cl
2. PhSH, Et3N, DMF
H2N
OH
HO
S

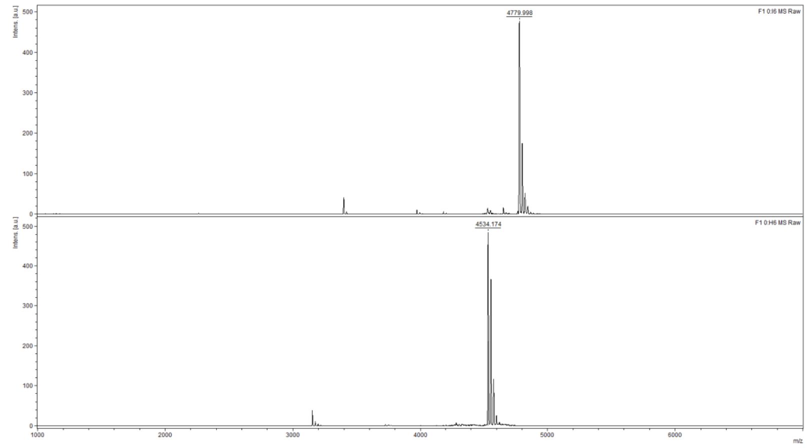

Intens. [a.u.]
4484.260
B3 0:H18 MS Raw
MALDI-TOF SM-N3: m/z [M + H] 4484.260
4667.945
kkg5-52R-1 0:K2 MS Raw
MALDI-TOF DC CuAAC: m/z [M + H] 4667.945
KKG5-75B-2 0:K4 MS Raw
MALDI-TOF DC: m/z [M + H] 4742.663
4742.663
1000
2000
3000
4000
5000
6000
m/z


"CuAAC"
CuSO4, NaAsc
TBTA, NMP/H2O
OEOM
EOMO
Cl
OMe
OMe
PEG
N3
H2N
OH
HO

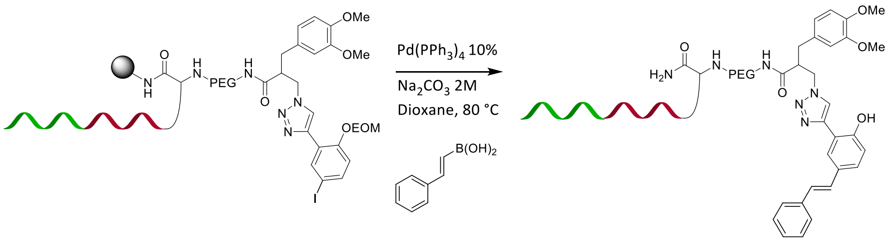

MALDI-TOF SM: m/z [M + H] 4502.808
MALDI-TOF DC: m/z [M + H] 4687.078
4502.808
4687.078
D1 0:H4 MS Raw
D1-2 0:I4 MS Raw
Intens. [a.u.]
m/z



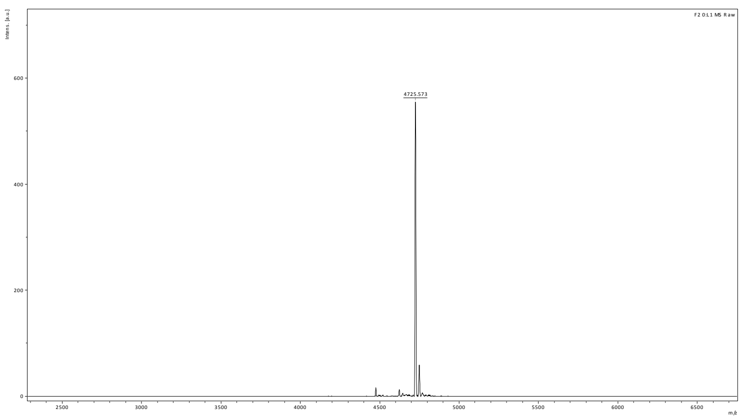
MALDI-TOF SM: m/z [M + H] 4534.174

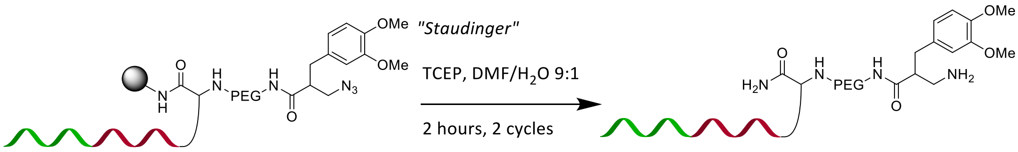


MALDI-TOF DC: m/z [M + H] 4779.998



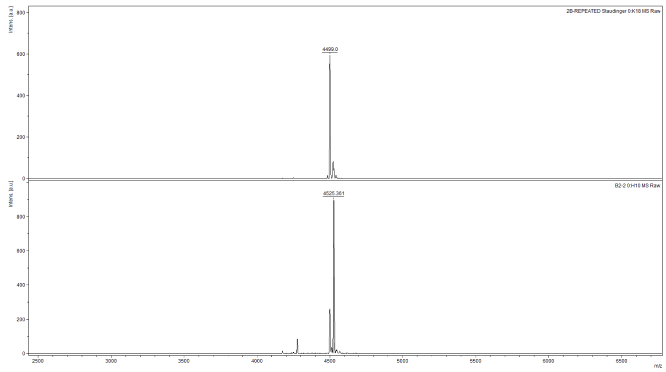
MALDI-TOF DC: m/z [M + H] 4725.573

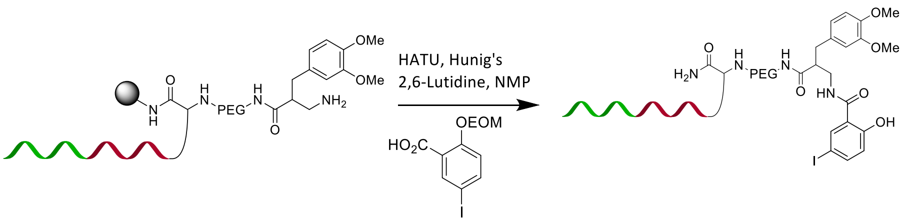




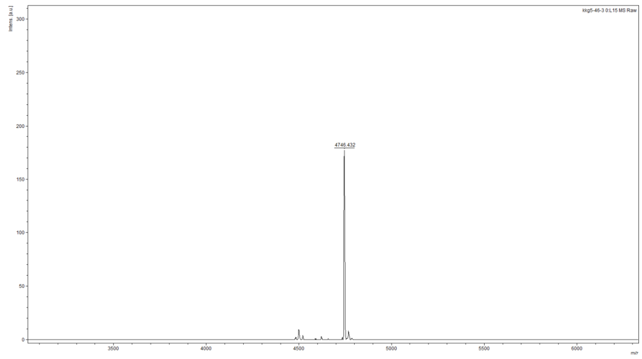
MALDI-TOF SM: m/z [M + H] 4525.351

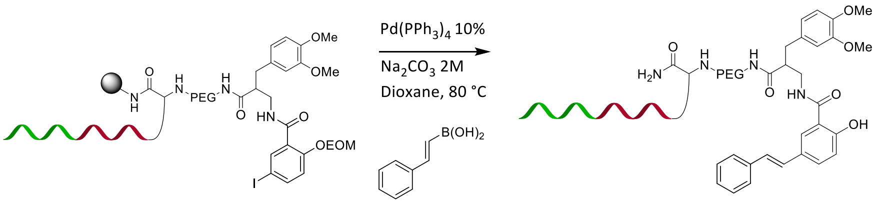
MALDI-TOF DC: m/z [M + H] 4499.0



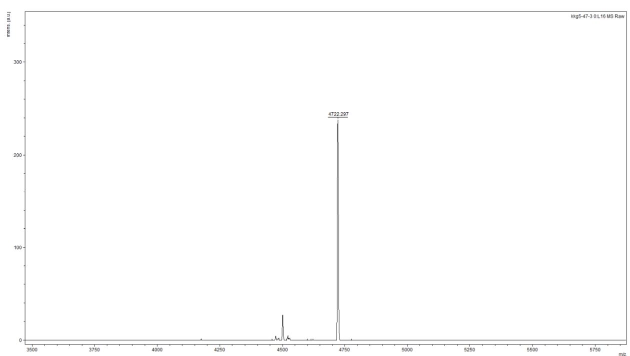


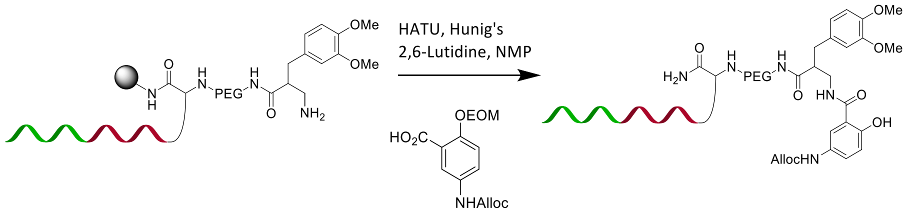
MALDI-TOF DC: m/z [M + H] 4746.432

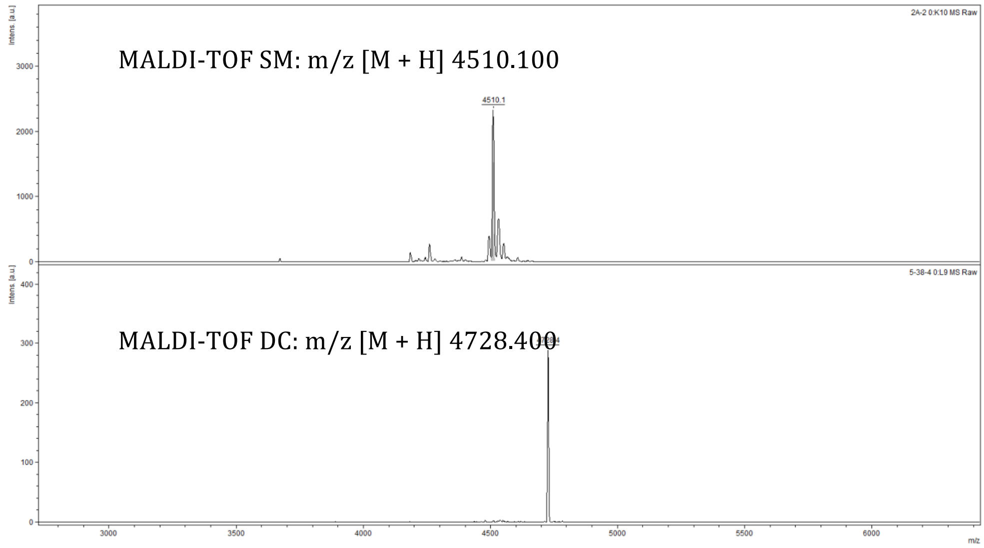


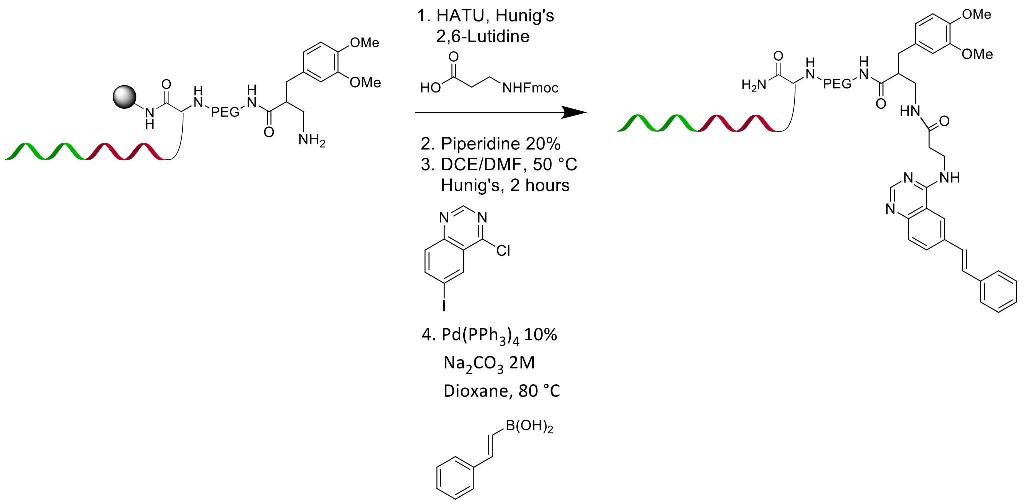


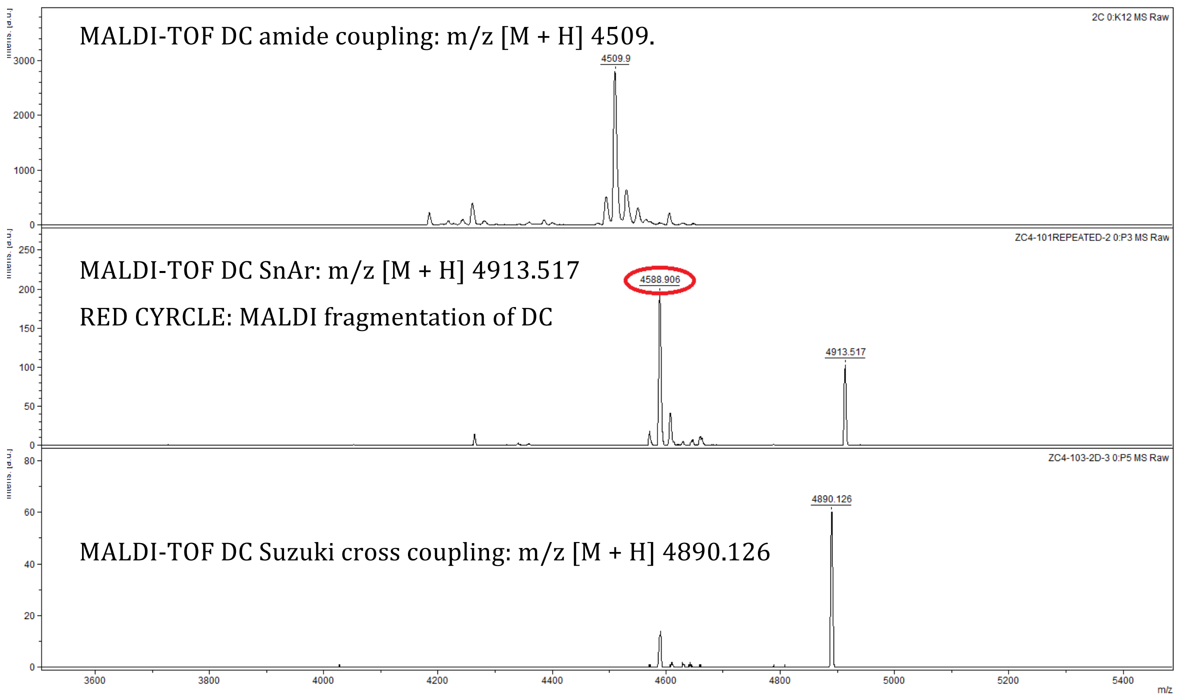
MALDI-TOF DC: m/z [M + H] 4722.297




HATU, Hunig's
2,6-Lutidine, NMP
OMe
OMe
PEG
NH2
OEOM
HO2C
NHAlloc
H2N
HN
OH
AllocHN


MALDI-TOF SM: m/z [M + H] 4510.100
4510.1
MALDI-TOF DC: m/z [M + H] 4728.400
Intens. [a.u.]
m/z


1. HATU, Hunig's
2,6-Lutidine
HO
NHFmoc
2. Piperidine 20%
3. DCE/DMF, 50 °C
Hunig's, 2 hours
Cl
I
4. Pd(PPh3)4 10%
Na2CO3 2M
Dioxane, 80 °C
B(OH)2
OMe
OMe
NH2
PEG
H2N
HN
NH


MALDI-TOF DC amide coupling: m/z [M + H] 4509.
4509.9
2C 0:K12 MS Raw
MALDI-TOF DC SnAr: m/z [M + H] 4913.517
RED CYRCLE: MALDI fragmentation of DC
4588.906
4913.517
ZC4-101REPEATED-2 0:P3 MS Raw
MALDI-TOF DC Suzuki cross coupling: m/z [M + H] 4890.126
4890.126
ZC4-103-2D-3 0:P5 MS Raw
m/z

Kinase targeted PNA-encoded library synthesis. Library II.

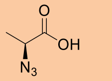
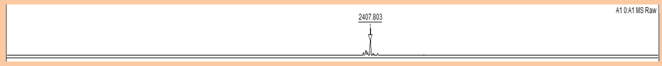
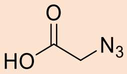
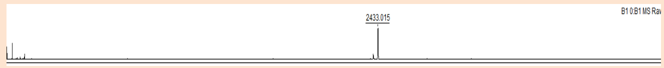
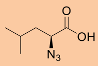
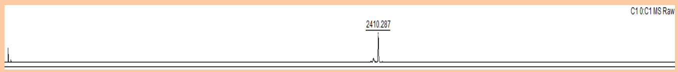
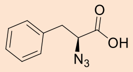
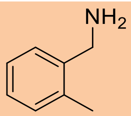
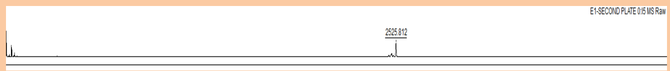
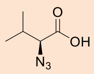
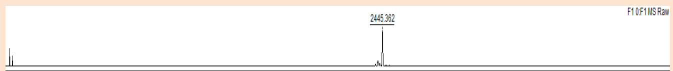
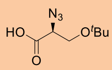
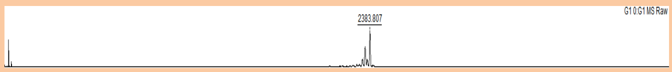
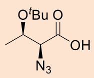
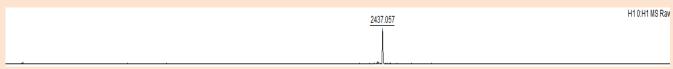
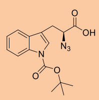
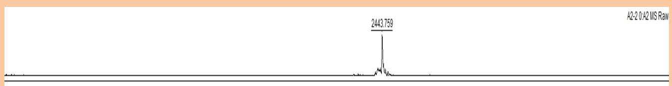
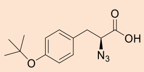
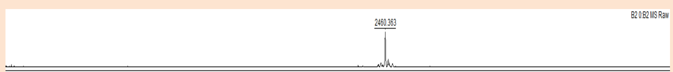
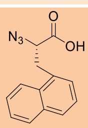
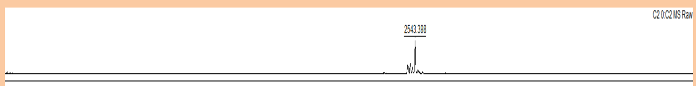
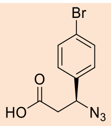
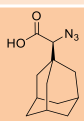
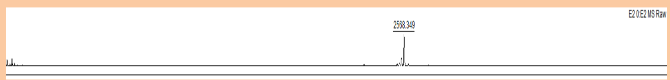
First point of diversity: coupling of 100 different amino acids.

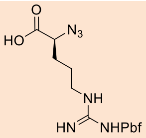
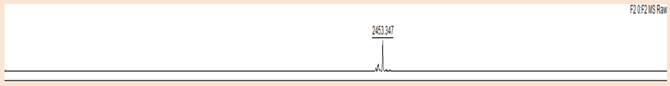
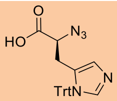
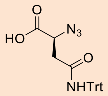
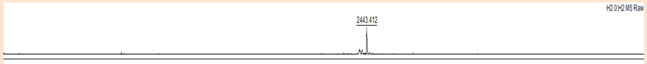
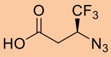
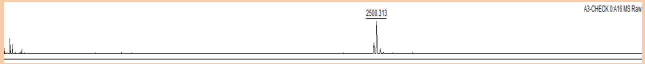
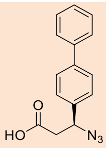
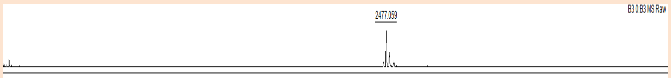
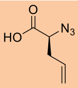
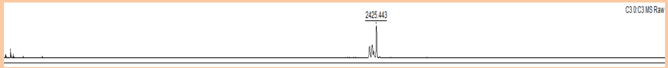
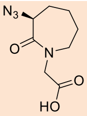
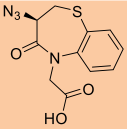
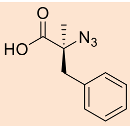
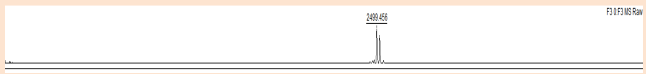
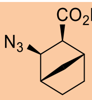
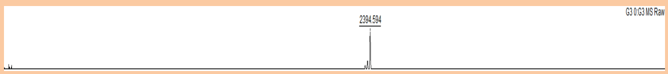
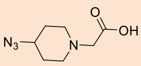
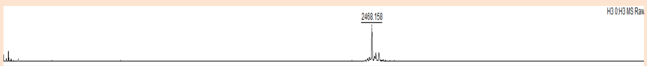
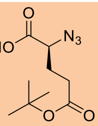
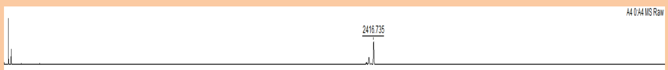
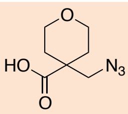
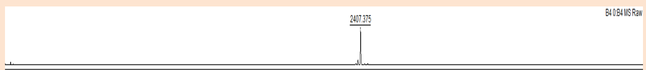
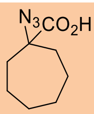
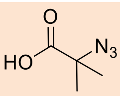
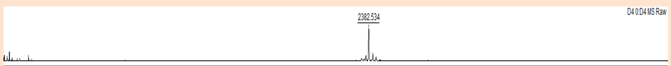
500 mgs of NovaPEG resin were charged with orthogonally protected (Mtt)-Fmoc-DAP with loading reduction (0.2 mmol/g, ***procedure 5***), followed by capping of the unreacted amine functionalities (***procedure 1***). Subsequently, Fmoc was deprotected (***procedure 3***), PEG spacer introduced (***procedure 6***) and final Fmoc cleavage was carried out (***procedure 3***). The obtained material was then divided in 100 wells (5 mgs each) and 100 different amino acid were introduced using carbodiimide chemistry (***procedure 7a***), upon MALDI analysis of the 100 reactions, Fmoc protection was cleaved (***procedure 3***) and the resulting free amine was converted to an azido group (***procedure 18***)

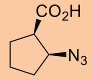
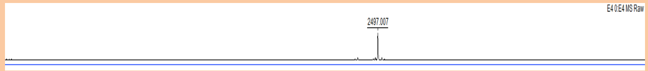
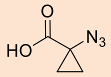
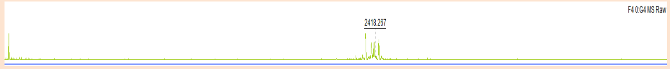
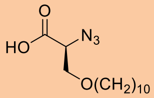
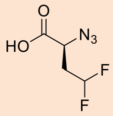
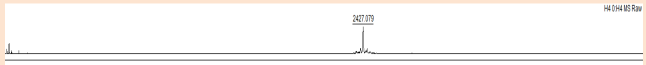
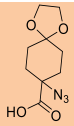
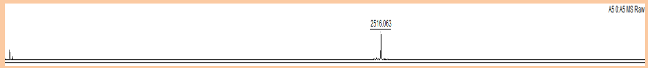
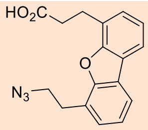
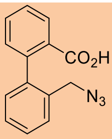
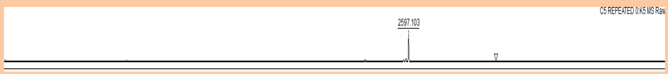
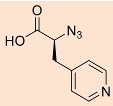
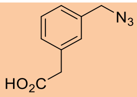
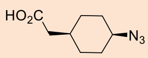
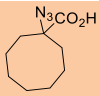
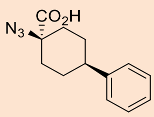
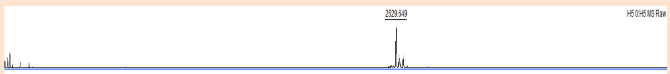
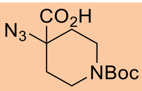
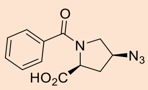
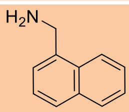
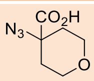
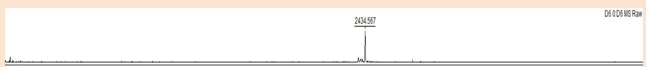
Encoding of the first point of diversity: 7mer PNA synthesis

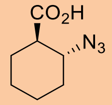
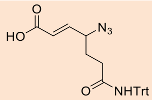
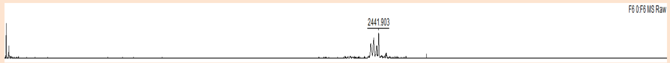
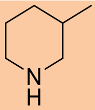
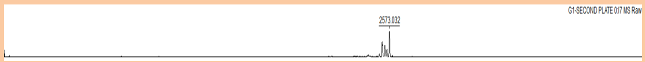
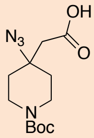
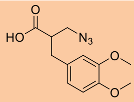
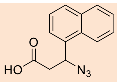
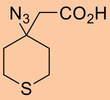
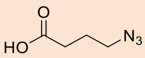
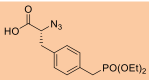
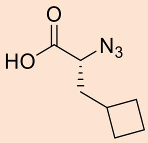
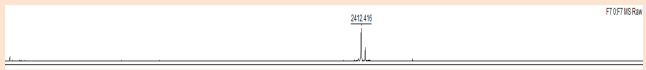
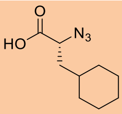
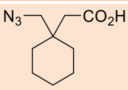
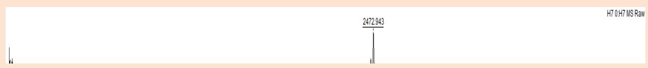
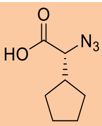
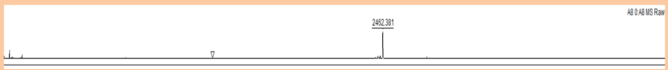
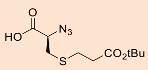
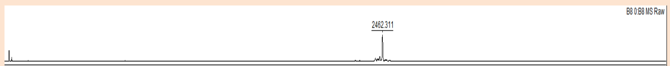
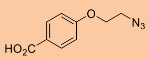
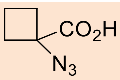
Each of the first point of diversity introduced was then coded with unique 7mer (Boc-Mtt) protected PNA oligomer. Upon Mtt deprotection of the Dap linker **(*procedure 10***), cycles of TNTU mediated PNA synthesis (***procedure 11***), were carried out on 5 mg (0.001 mmol) of resin for each reaction. A small amount of resin for every single reaction was cleaved by TFA 98%, precipitated in Et_2O, and analysed by MALDI-TOF. The complete list of 7mer used to code each first point of diversity in given in table 5-SI.

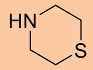
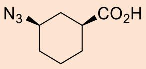
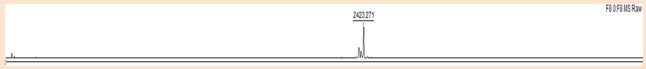
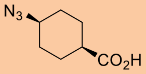
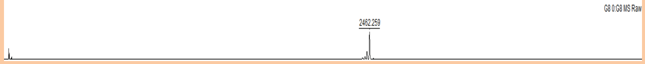
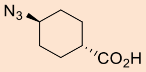
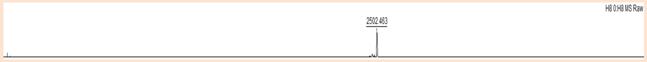
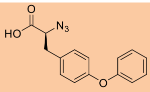
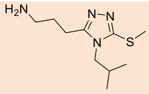
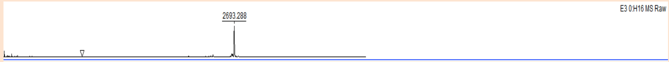
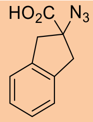
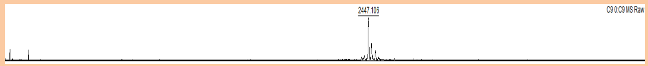
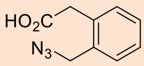
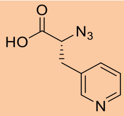
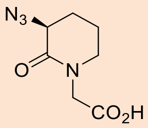
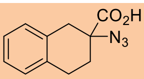
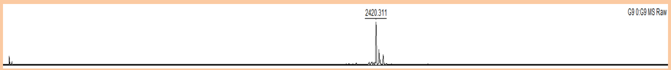
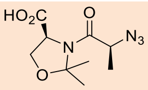
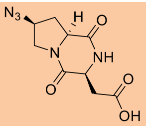
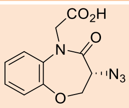
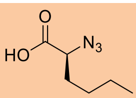
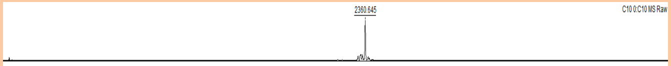
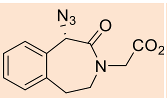
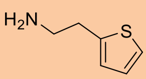
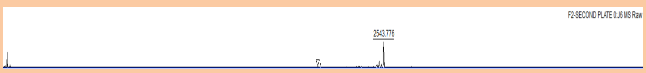
Table 5-SI: Encoding the first point of diversity with 7mer PNA

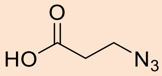
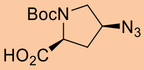
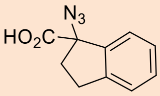
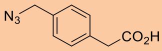
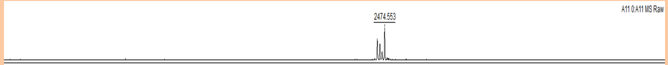
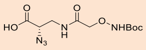
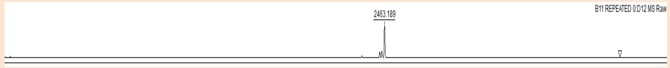
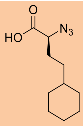
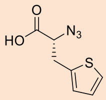
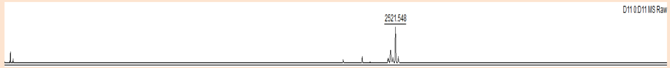
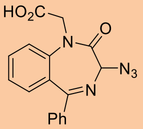
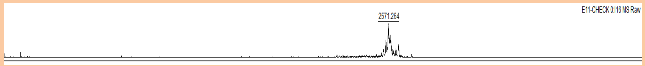
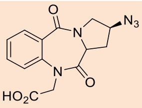
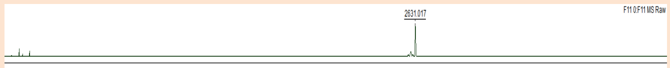
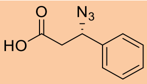
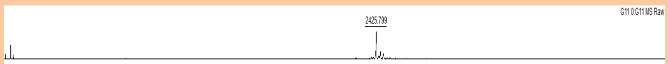
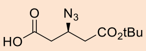
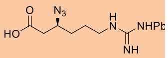
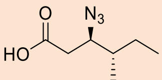
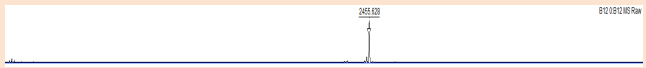
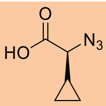
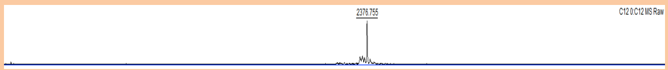
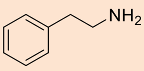
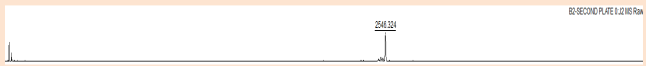
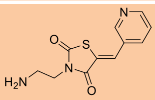
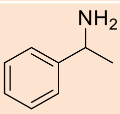
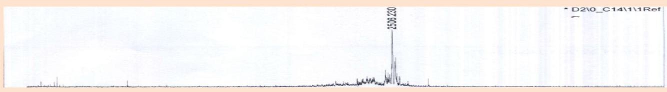
Cod 1	Structure	PNA seq*	Calc. m/z [M+H]	Maldi (1000-3500 Da)
1		GGAAGCT	2406.261	
2		GTAAGGG	2432.265	
3		GTAACCG	2408.317	
4		GTAAGCC	2442.337	
5		GCAACGA	2522.341	
6		GCAAGAG	2443.335	
7		GCAACTG	2380.347	
8		GCAAGGT	2434.391	
9		GCAACCT	2441.413	
10		GCAAGTC	2456.44	
11		GAGAGCA	2541.445	
12		GGGAGTA	2601.285	
13		GTGAGGA	2566.485	

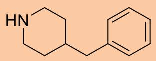
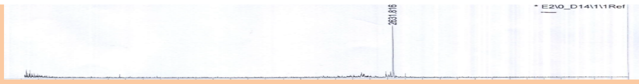
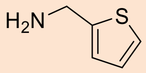
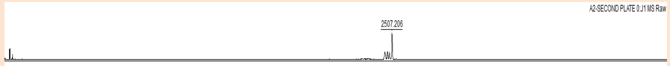
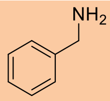
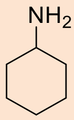
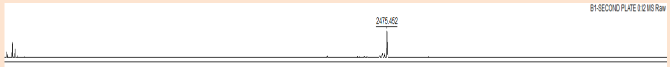
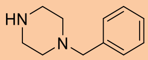
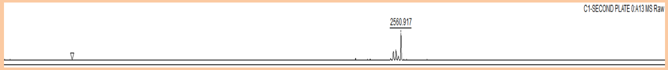
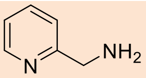
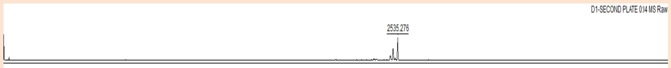
14		GTGACCA	2450.68	
15		GTGAGTG	2501.65	
16		GTGAGCT	2438.59	
17		GCGAGAA	2483.28	
18		GCGACTA	2518.41 (loss of N_3)	
19		GCGAGTT	2423.28	
20		GGTAGCA	2503.38	
21		GTTAGCG	2560.45	
22		GCTAGGA	2496.39	
23		GCTACCA	2392.31	
24		GCTAGTG	2466.96	
25		GCTAGCT	2413.36	
26		GACACAC	2405.32	
27		GTCAGCA	2434.36	
28		GCCAGTA	2380.27	

29		GGAAAGG	2495.37	
30		GGAATCG	2418.23 (loss of N_2 and N_3)	
31		GAAGGGA	2611.569	
32		GAAGCCA	2425.261	
33		GAAGGTG	2515.42	
34		GAAGGCT	2600.501	
35		GGAGGAA	2593.469	
36		GGAGCTA	2483.351 (loss of N_2)	
37		GGAGGTT	2513.371	
38		GTAGCGA	2474.381	
39		GTAGGAG	2527.425	
40		GTAGCTG	2527.437	
41		GTAGGGT	2534.47 (Loss of N_2)	
42		GTAGCCT	2502.383	
43		GTAGGTC	2580.367	
44		GCAGCAA	2431.321	

45		GCAGGAT	2460.351	
46		GCAGCTT	2438.15	
47		GAGGGTA	2571.365	
48		GTGGGAA	2515.52	
49		GTGGCTA	2547.427	
50		GTGGGTT	2554.417 (Loss of N_3)	
51		GATGGCA	2492.421	
52		GGTGGTA	2450.291	
53		GTTGGGA	2663.481	
54		GTTGCCA	2411.313	
55		GTTGGTG	2510.407	
56		GTTGGCT	2470.383	
57		GCTGGAA	2460.351	
58		GCTGCTA	2459.45	
59		GCTGGTT	2480.323	
60		GTCGGTA	2423.28 (Loss of N_2 and N_3)	

61		GAATGGG	2575.385	
62		GAATCCG	2420.327	
63		G ATCGA	2460.351	
64		GGATGAG	2500.375	
65		GGATCTG	2565.447	
66		GGATGGT	2690.00	
67		GGATCCT	2445.337	
68		GGATGTC	2473.347 (Loss of N_3)	
69		GTATCGG	2474.337 (loss of N_2 peak)	
70		GTATGGC	2480.347	
71		GTATCCC	2419.335	
72		GCATCAG	2449.39	
73		GCATCGT	2495.353	
74		GCATGAC	2519.337	
75		GCATCTC	2359.279	
76		GAGTGGA	2591.445	
77		GAGTCCA	2504.313 ($M/z + K^+$)	

78		GAGTGTG	2437.271	
79		GAGTGCT	2438.42	
80		GGGTGAA	2534.395 (main peak loss of N3)	
81		GGGTCTA	2473.347	
82		GGGTGTT	2516.447	
83		GTGTCGA	2493.417	
84		GTGTGAG	2519.391	
85		GTGTCTG	2608.463 (main peak loss of N3)	
86		GTGTGGT	2628.457	
87		GTGTCCT	2524.327	
88		GTGTGTC	2442.373	
89		GCGTCAA	2464.73	
90		GCGTGAT	2453.352	
91		GCGTCTT	2374.005	
92		GATTGCG	2544.337	
93		GGTTGGA	2711.00	
94		GGTTCCA	2544.340 (loss of N3)	

95		GGTTGTG	2628.51	
96		GGTTGCT	2505.327	
97		GTTTGGG	2561.317	
98		GTTTCCG	2573.307	
99		GCTTCGA	2559.393	
100		GCTTGAG	2531.297	

**The PNA were synthesized using alternately serine-modified monomers starting with Gs*

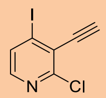
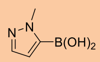
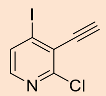
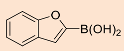
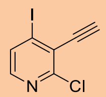
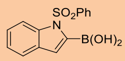
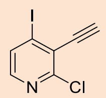
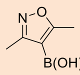
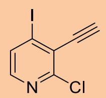
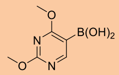
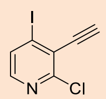
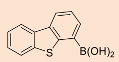
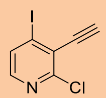
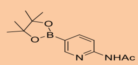
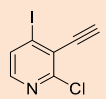
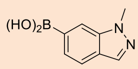
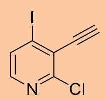
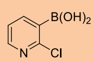
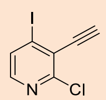
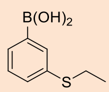
Encoding of the second point of diversity: 14mer PNA synthesis on pooled mix

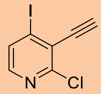
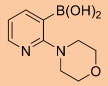
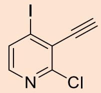
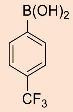
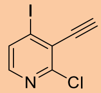
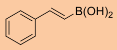
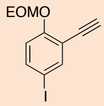
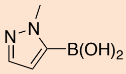
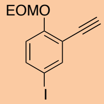
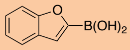
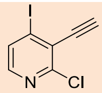
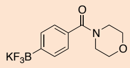
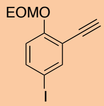
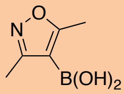
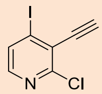
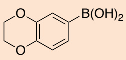
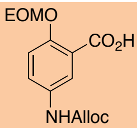
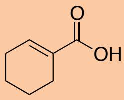
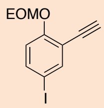
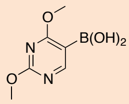
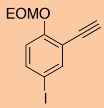
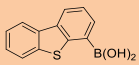
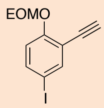
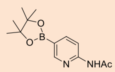
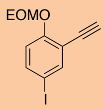
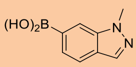
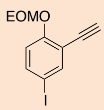
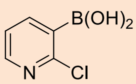
The 100 unique 7mer PNA coding the first point of diversity, were mixed and split In 100 wells using a technique previously reported, obtaining a statistic mixture of all the 100 PNA 7mers in each well. At this stage another unique 7mer PNA tag is synthetized for each well obtaining, by doing so, a permutation of 10'000 PNA 14mers. This second tag will allow the encoding for the second point of diversity. The PNA synthesis was carried out on 2.5 mg (0.5 μmol) of resin for each reaction following cycles of Mtt deprotection (***procedure 10***) and TNTU mediated PNA coupling (***procedure 11***). A small amount of resin for every single reaction was cleaved by TFA 99%, precipitated in Et_2O, and analysed by MALDI-TOF.

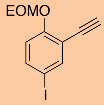
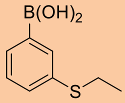
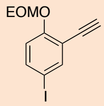
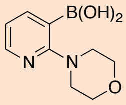
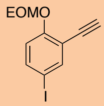
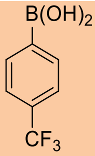
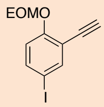
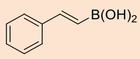
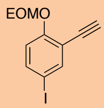
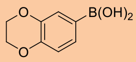
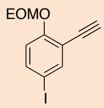
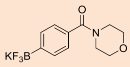
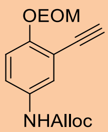
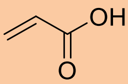
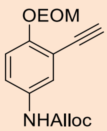
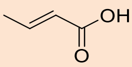
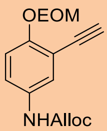
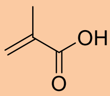
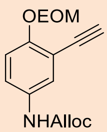
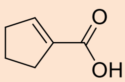
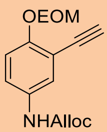
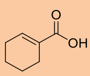
PNA encoded synthesis (PES) on pooled mix, second point of diversity

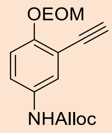
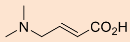
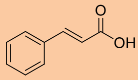
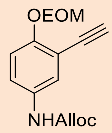
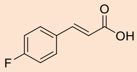
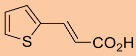
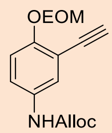
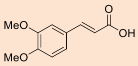
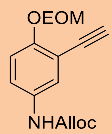
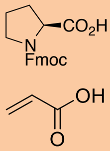
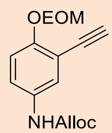
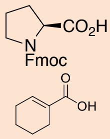
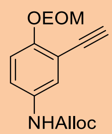
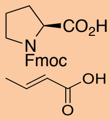
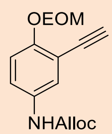
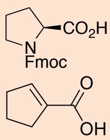
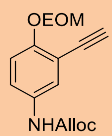
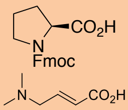
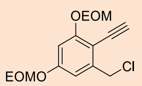
The second point of diversity was introduced accordingly to the procedures reported in the general technique for solid phase synthesis. Alkyne–containing building blocks were reacted with the azide functionality contained in the first point of diversity through CuAAC (***procedure 12***). The obtained triazoles containing iodo functionality were then diversified via Suzuki cross-coupling (***procedure 21***). Alternatively, triazoles containing Alloc-protected aniline were diversified introducing different Michael acceptors (***procedure 22*** for Alloc deprotection and ***procedure 23***). For the introduction of building blocks containing a carboxylic acid the azide on the resin had to be reduced via a Staudinger protocol (***procedure 19***), and the resulting amine acylated with the respective building blocks via HATU mediate coupling (***procedure 7b***). As described above, carboxylic acid containing iodo functionality were further derivatized using Suzuki cross-coupling (***procedure 21***), alternatively, carboxylic acid containing Alloc-protected aniline were diversified introducing different Michael acceptors (***procedure 22*** for Alloc deprotection and ***procedure 23***). For the introduction of quinazoline building blocks, the corresponding chloroquinazoline was coupled to the resin (***procedure 20***) upon Staudinger reduction of the azide of the first diversity point (***procedure 19***). Quinazolines containing an iodo functionality were further derivatized using Suzuki cross coupling (***procedure 21***).

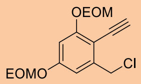
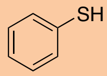
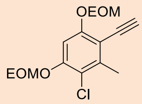
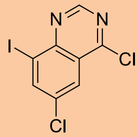
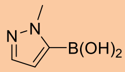
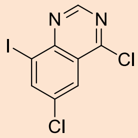
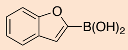
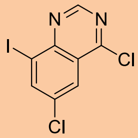
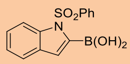
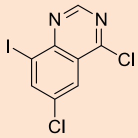
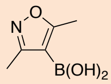
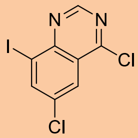
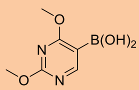
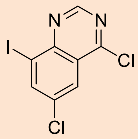
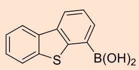
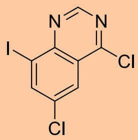
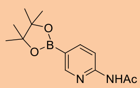
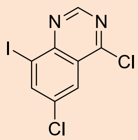
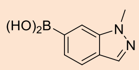
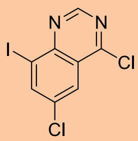
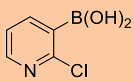
Table 6-SI: MALDI-TOF data of encoded 100 pooled mix: second point of diversity Maldis

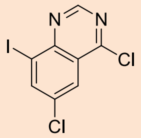
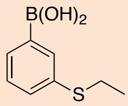
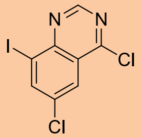
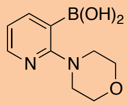
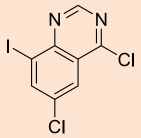
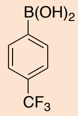
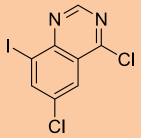
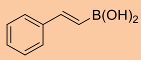
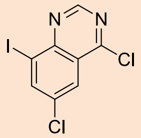
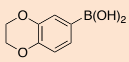
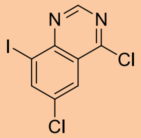
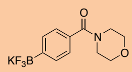
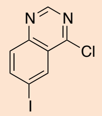
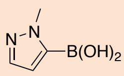
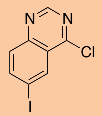
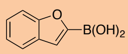
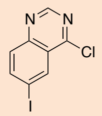
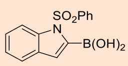
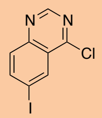
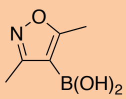
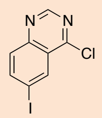
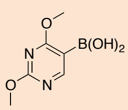
Cod. 2			PNA seq	Calc. m/z [M+H]	Exp. m/z [M+H]
1			CTTCTGG	4347.853-4684.853	4350.3-4688.8
2			CTTGGTG	4387.877-4724.877	4390.9-4726.0
3			CTTCCTG	4307.829-4644.829	4310.3-4647.2
4			CTTGTCG	4347.853-4684.853	4350.8-4687.9
5			ACTGCAG	4365.881-4702.881	4465.4-4703.0
6			GCTGTAG	4396.891-4733.891	4399.6-4736.3
7			TCTGGAG	4396.891-4733.891	4399.5-4738.1
8			TCTCCAG	4316.843-4653.843	4316.5-4656.5
9			TCTGTGG	4387.877-4724.877	4390.6-4729.9
10			TCTGCTG	4347.853-4684.853	4351.5-4688.4

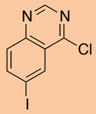
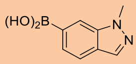
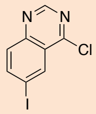
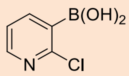
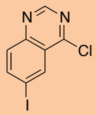
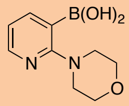
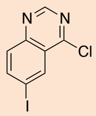
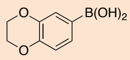
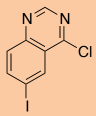
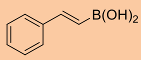
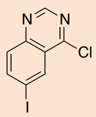
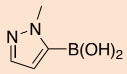
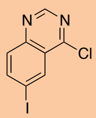
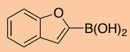
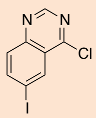
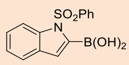
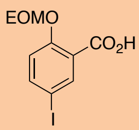
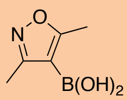
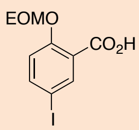
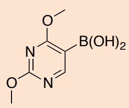
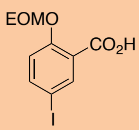
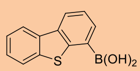
11			CCTGAAG	4365.881-4702.881	4363.6-4707.3
12			CCTCTAG	4316.843-4643.843	4320.4-4647.0
13			CCTGTTG	4347.853-4684.853	4351.9-4687.4
14			AACCTGG	4374.895-4711.895	4375.5-4713.7
15			AACGGTG	4414.919-4751.919	4417.9-4756.0
16			AACGAGG	4365.881-4702.881	4365.8-4703.8
17			AACGTCG	4405.905-4742.905	4428.0-4765.2
18			AACCGAG	4325.857-4662.857	4326.3-4664.9
19			AATCTAG	4365.881-4702.881	4367.8-4704.9
20			GACCAAG	4374.895-4711.895	4376.8-4711.1
21			GACGATG	4405.905-4742.905	4406.5-4748.4
22			GACCTTG	4356.867-4693.8867	4357.7-4697.7
23			TACCAGG	4365.881-4702.881	4366.1-4703.1
24			TACCGTG	4356.867-4693.867	4358.5-4700.2

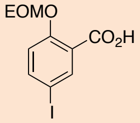
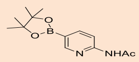
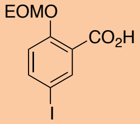
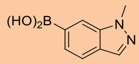
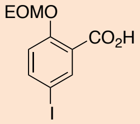
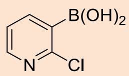
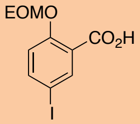
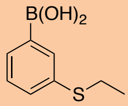
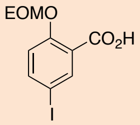
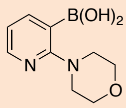
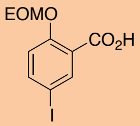
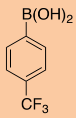
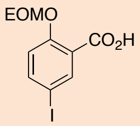
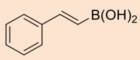
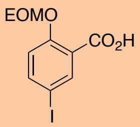
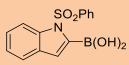
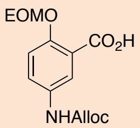
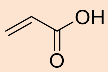
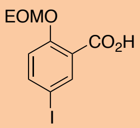
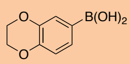
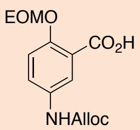
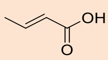
25			TACGACG	4365.881-4702.881	4381.2-4772.3
26			TACCTCG	4316.843-4653.843	4318.3-4656.4
27			CACCATG	4325.857-4662.857	4325.7-4663.1
28			AGCGAAG	4414.919-4751.919	4416.1-4753.9
29			AGCCTAG	4365.881-4702.881	4367.1-4705.4
30			AGCGTTG	4696.891-4733.891	4697.3-4735.5
31			TGCCAAG	4365.881-4702.881	4367.5-4707.8
32			TGCGATG	4396.891-4733.891	4392.4-4738.6
33			TGCCTTG	4347.853-4684.853	4348.7-4686.9
34			ATCGGAG	4405.905-4742.905	4407.5-4746.9
35			ATCCCAG	4325.857-4680.857	4326.7-4683.7

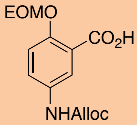
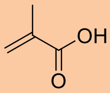
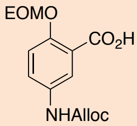
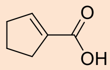
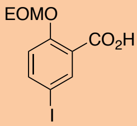
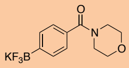
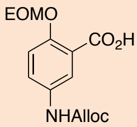
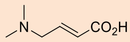
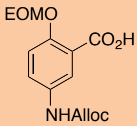
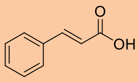
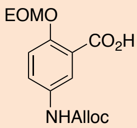
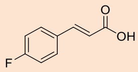
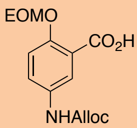
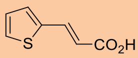
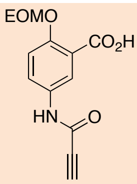
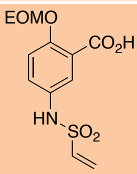
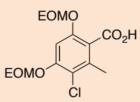
36			ATCGTGG	4396.891-4733.891	4393.5-4735.7
37			ATCGCTG	4356.867-4693.867	4358.6-4695.7
38			GTCGAAG	4405.905-4742.905	4406.8-4744.0
39			GTCCTAG	4356.867-4693.867	4356.3-4794.1
40			GTCGTTG	4387.877-4724.877	4389.2-4724.5
41			TTCCGAG	4356.867-4693.867	4358.3-4796.2
42			TTCGAGG	4396.891-4733.891	4493.7-4731.4
43			TTCCTGG	4347.853-4684.853	4348.9-4785.2
44			TTCGGTG	4387.877-4724.877	4388.3-4725.6
45			TTCCCTG	4307.829-4644.829	4308.1-4646.5
46			TTCGTCG	4347.853-4684.853	4348.2-4787.6

47			CTCCAAG	4325.857-4662.857	4326.7-4667.4
48			CTCGATG	4356.867-4693.867	4357.6-4697.3
49			CTCCTTG	4307.867-4644.829	4309.5-4645.8
50			ACCGTAG	4365.881-4702.881	4368.8-4705.3
51			TCCGAAG	4365.881-4702.881	4367.3-4705.9
52			TCCCTAG	4316.843-4653.843	4318.4-4657.7
53			TCCGTTG	4347.853-4684.853	4348.2-4788.5
54			GCGTAAG	4405.905-4742.905	4400.3-4745.4
55			GCGATAG	4405.905-4760.905	4406.7-4765.8
56			GCGAATG	4405.905-4742.905	4406.1-4743.4
57			GCGTATG	4396.891-4732.891	4497.0-4733.5

58			GCGATTG	4396.891-4733.891	4398.9-4734.5
59			GCGTTTG	4387.877-4724.877	4388.7-4726.8
60			CGCATAG	4365.881-4702.881	4366.3-4705.4
61			CGCTTAG	4356.867-4693.867	4357.8-4695.4
62			CGCATTG	4356.867-4693.876	4357.4-4694.6
63			CGCTTTG	4347.853-4684.853	4348.4-4785.8
64			GGGAAAG	4454.943-4791.943	4456.3-4794.8
65			GGGTTAG	4436.915-4773.915	4437.8-4775.4
66			GGGTATG	4436.915-4773.915	4436.6-4774.8
67			GGGATTG	4436.915-4773.915	4437.5-4774.2
68			GGGTTTG	4427.901-4764.901	4429.8-4765.4

69			CCGAAAG	4374.895-4711.895	4375.8-4713.3
70			CCGTTAG	4356.867-4693.867	4356.1-4694.4
71			CCGTATG	4356.867-4693.867	4357.7-4796.0
72			CCGATTG	4356.867-4693.867	4357.8-4794.0
73			CCGTTTG	4347.853-4684.853	4348.3-4685.2
74			TTGCCAG	4356.867-4693.867	4357.5-4696.6
75			TTGAGGG	4436.915-4773.915	4437.2-4774.7
76			TTGGCTG	4387.877-4724.877	4388.4-4727.57
77			TTGCACG	4356.867-4693.867	4357.3-4694.4
78			TTGTGCG	4387.877-4724.877	4387.0-4726.5
79			TTGACCG	4356.867-4693.867	4357.4-4697.2

80			TTACCCG	4316.843-4653.843	4415.8-4658.6
81			TTCGCAG	4356.867-4693.867	4357.3-4695.1
82			TTCTGGG	4387.877-4724.877	4388.3 -4727.5
83			TTCGTGG	4387.877-4742.877	4388.5-4743.6
84			TTCACGG	4356.867-4693.867	4357.4-4698.9
85			TTCAGCG	4356.867-4693.867	4357.1-4699.2
86			TTCTCCG	4307.829-4644.829	4305.5-4646.6
87			AACCCTG	4454.943-4791.943	4456.6-4796.8
88			AAAGCCG	4414.919-4751.919	4417.5-4757.3
89			AAAGGGG	4374.895-4729.895	4375.4-4731.3
90			GGCTATG	4396.891-4732.891	4392.8-4734.0

91			AATGCCG	4365.881-4702.881	4366.6-4706.5
92			TTTGCCG	4347.853-4684.853	4349.3-4687.5
93			AAACCGG	4364.893-4701.893	4365.1-47078.4
94			TAACGCG	4365.881-4720.881	4366.2-4725.5
95			AGAGGTG	4445.929-4782.929	4446.9-4784.4
96			AGAGACG	4414.919-4751.919	4416.9-4756.5
97			AGACCTG	4365.881-4702.881	4366.4-4706.3
98			AGAAGCG	4414.919-4751.919	4419.4-4757.2
99			AGACTGG	4405.905-4742.905	4408.6-4748.2
100			AGATGAG	4429.93-4766.93	4431.8-4766.9

Synthesis of PNA-encoded known kinases binders as controls

For validation purposes a series of PNA tagged positive control kinase binders were included: BIM (bisindolylmaleimide-based general ATP competitor inhibitor), Dasatinib[2] (a potent tyrosine kinase inhibitor), PD-0325901[3] (an allosteric Mek2 inhibitor), as well as Canertinib[4] (an irreversible ERBB2 inhibitor). Each of these well characterized kinase inhibitors was linked to a unique 14mer PNA tag and mixed to the 10.000 member library previous to microarray hybridization. ERBB2-Canertinib covalent interaction allowed us to establish the washing conditions needed to discriminate between covalent binders on the library and non-covalent binders.

Nova Peg Rink amide resin (10 mg, 0.44 mmol/g full loading) was charged with an orthogonally protected FmocDap-(Mtt)-OH using ***procedure 5*** with loading reduction to 0.2 mmol/g followed by capping of the remaining free functionalities (***procedure 1***), Fmoc was subsequently deprotected following ***procedure 3*** and PEG spacer was introduced using ***procedure 6*** (both HATU or DIC/HOBt procedure could be used). Upon Mtt deprotection of the Dap linker (***procedure 10***), cycles of TNTU mediated PNA synthesis (***procedure 11***) were carried out on 10 mg (0.002 mmol) of resin for each reactions (four positive controls) in parallel, sequences used: sequence **1-2** for canertinib, sequence **5-70** for dasatinib, **79-75** for BMI and **99-99** for PD-0325901.

Upon 14mer PNA synthesis, Mtt was cleaved using ***procedure 10*** and the N-terminus was acylated with cyanine dye (Cy3-CO_2H) using ***procedure 6*** (HATU). Fmoc group was then cleaved (***procedure 3***) followed by coupling with pentynoic acid using **procedure 6** (HATU).

Coupling of alkyne-containing resins to azide-containing positive controls **Dasatinib-azide, BIM-azide** and **PD-0325901-azide** was carried out using CuAAC following

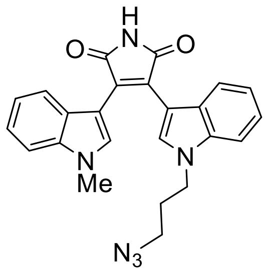
[2] Das, J.; Chen, P.; Norris, D.; Padmanabha, R.; Lin, J.; Moquin, R. V.; Shen, Z.; Cook, L. S.; Doweyko, A. M.; Pitt, S.; Pang, S.; Shen, D. R.; Fang, Q.; de Fex, H. F.; McIntyre, K. W.; Shuster, D. J.; Gillooly, K. M.; Behnia, K.; Schieven, G. L.; Wityak, J.; Barrish, J. C., 2-aminothiazole as a novel kinase inhibitor template. Structure-activity relationship studies toward the discovery of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1- piperazinyl)]-2-methyl-4-pyrimidinyl]amino)]-1,3-thiazole-5-carboxamide (dasatinib, BMS-354825) as a potent pan-Src kinase inhibitor. *J Med Chem* **2006,** *49* (23), 6819-32.

[3] Barrett, S. D.; Bridges, A. J.; Dudley, D. T.; Saltiel, A. R.; Fergus, J. H.; Flamme, C. M.; Delaney, A. M.; Kaufman, M.; LePage, S.; Leopold, W. R.; Przybranowski, S. A.; Sebolt-Leopold, J.; Van Becelaere, K.; Doherty, A. M.; Kennedy, R. M.; Marston, D.; Howard, W. A., Jr.; Smith, Y.; Warmus, J. S.; Tecle, H., The discovery of the benzhydroxamate MEK inhibitors CI-1040 and PD 0325901. *Bioorg Med Chem Lett* **2008,** *18* (24), 6501-4.

[4] Smaill, J. B.; Rewcastle, G. W.; Loo, J. A.; Greis, K. D.; Chan, O. H.; Reyner, E. L.; Lipka, E.; Showalter, H. D.; Vincent, P. W.; Elliott, W. L.; Denny, W. A., Tyrosine kinase inhibitors. 17. Irreversible inhibitors of the epidermal growth factor receptor: 4-(phenylamino)quinazoline- and 4-(phenylamino)pyrido[3,2-d]pyrimidine-6-acrylamides bearing additional solubilizing functions. *J Med Chem* **2000,** *43* (7), 1380-97.

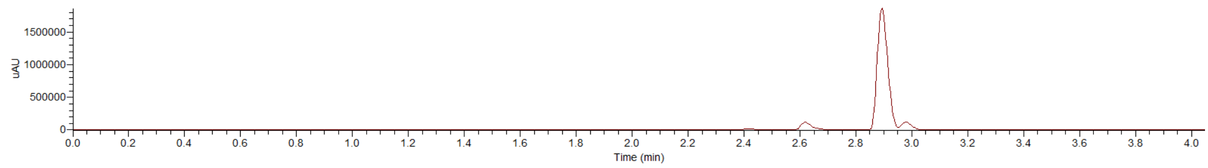
procedure 13; subsequent cleavage from the resin (***procedure 12***) and HPLC purification afforded the desired compounds.

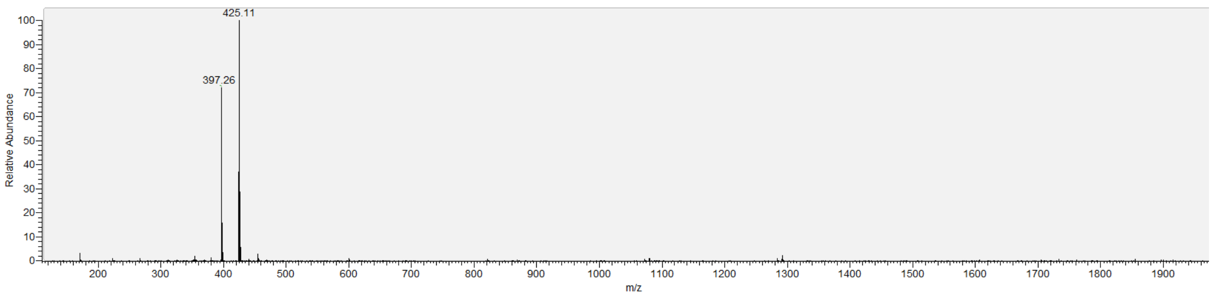
For the synthesis of canertinib conjugate After the Fmoc deprotection of the peg spacer, diglycolic anhydride was reacted under basic conditions obtaining a carboxylic acid, which was activated with DIC/HOBt (***procedure 6***) and reacted with a canertinib derivative **40** bearing a primary amine functionality instead of the morpholino present in the parent compound.

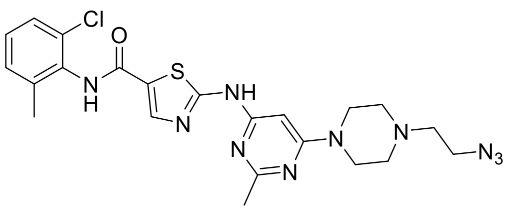


BIM-azide

Bis-indole maleimide azide, **BIM-azide**. LC-MS (ESI): m/z [M + H]+ calcd. for $C_{24}H_{20}N_6O_2$ 424.4640, found: 425.11.

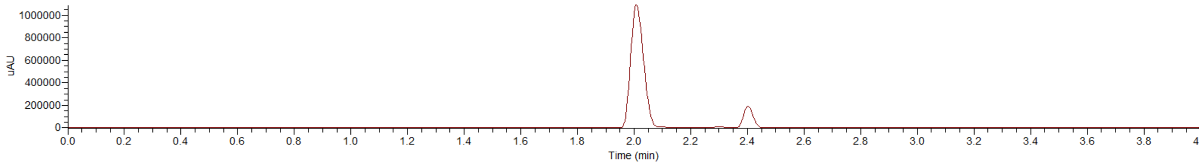


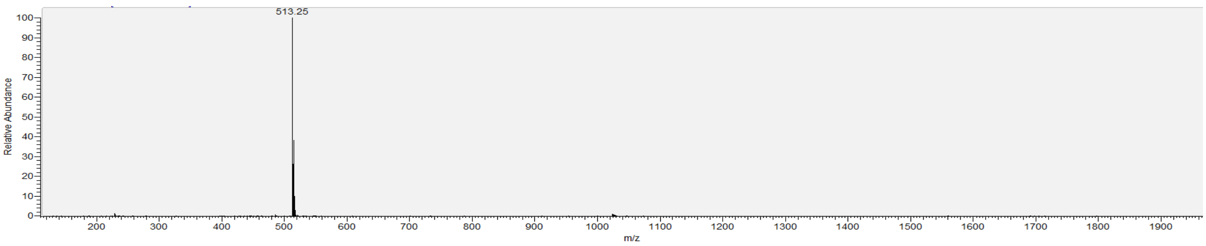


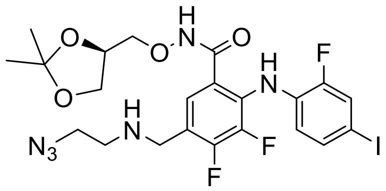


Dasatinib-azide

Dasatinib-azide. LC-MS (ESI): m/z [M + H]+ calcd. for $C_{22}H_{25}ClN_{10}OS$ 513.0210, found: 513.25.

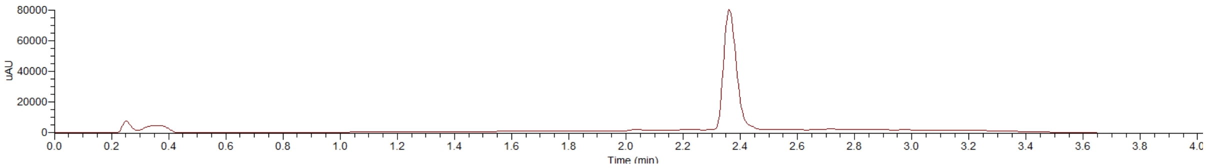


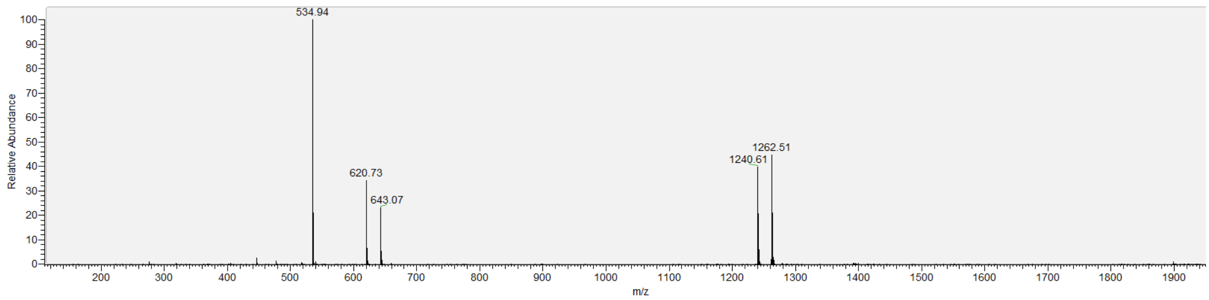


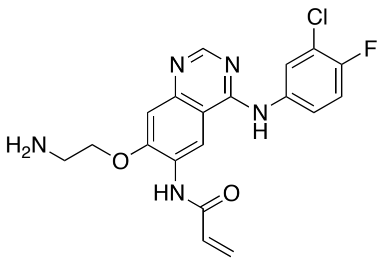


PD-032590-azide

PD-032590-azide. LC-MS (ESI): m/z [M + H]+ calcd. for $C_{22}H_{24}F_3IN_6O_4$ 620.3717, found: 620.73, [M + Na]+ 643.07.



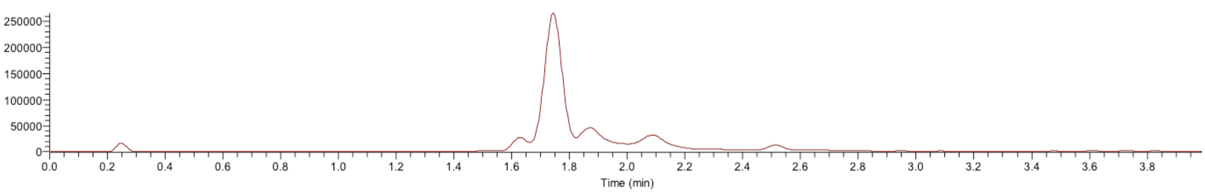


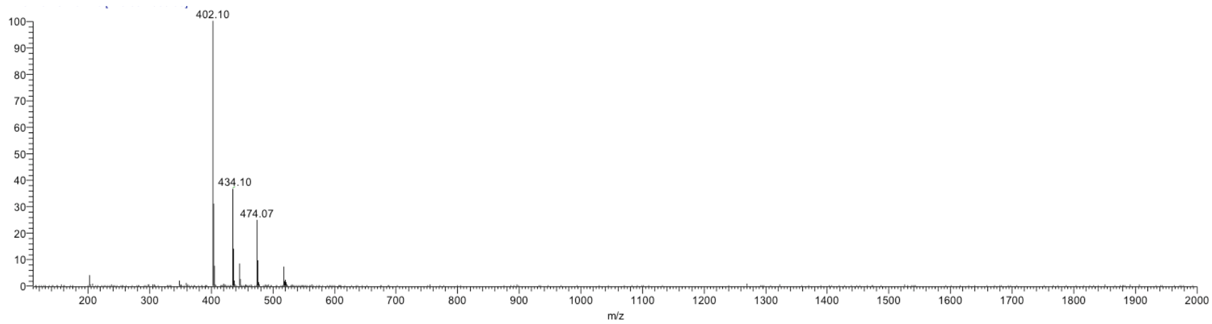


Amino carnetinib 40

Amino carnetinib 40 LC-MS (ESI): m/z [M + H]+ calcd. for $C_{19}H_{18}ClFN_5O_2$ 402.82, found:

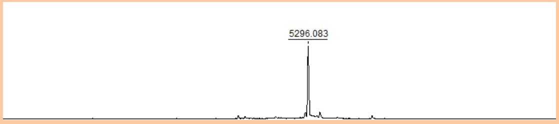
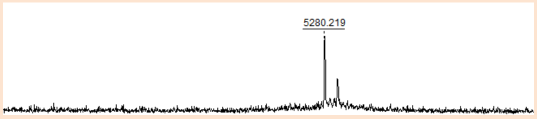
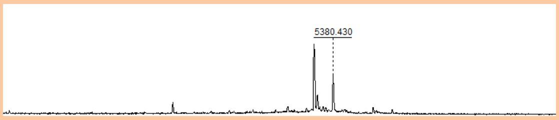
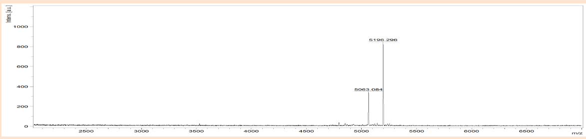
402.10





402.10
434.10
474.07
m/z

Table 7-SI: MALDI of positive control-PNA-Cy3 conjugates

PNA-seq	Control	MW calc.	m/z (M+H$^+$) found (MALDI: 4000-6000 Da)
79-75 GAGTGCTTTGAGGG	**BMI**	5294.670	
5-70 GCAACGACCGTTAG	**Dasatinib**	5279.230	
99-99 GCTTCGAAGACTGG	**PD-0325901**	5380.501 (5290.416 fragmented in Maldi)	
1-2 GGAAGCTCTTGGTG	**Canertinib**	5196.39 (5062.79 fragmented in Maldi	

PNA/DNA display library screening

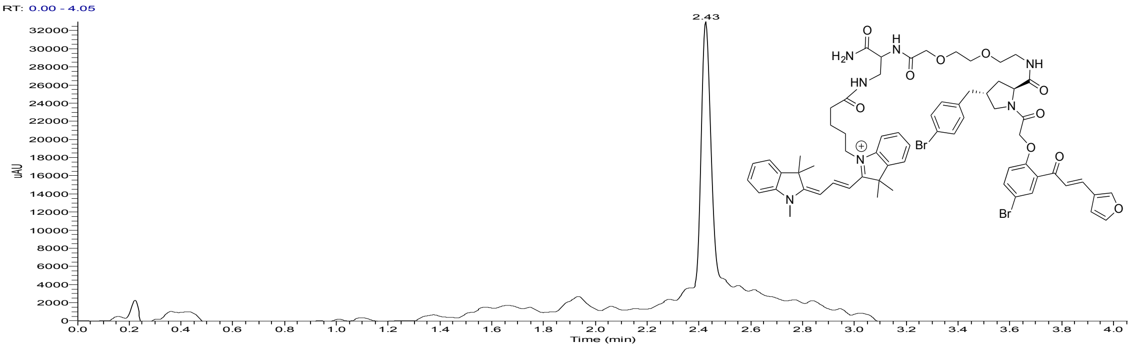
The PNA library was hybridized on a custom DNA microarray slide Agilent (design 048196) on which we spotted the complementary DNA sequences to the 14 mers PNA tags encoding the 10.000 members of our library. Each of the 10.000 members of the library is represented 4 times, allowing us to evaluate the reproducibility of the interaction observed. The PNA tagged library was diluted at 5 μM on PBS buffer complemented with 40% formamide and 10 μg/ml salmon sperm DNA. The hybridization was carried out for 18 h at 50 ^{0}C. The hybridized slide was washed twice on PBS buffer, briefly rinsed with mQ water and dried. The known 14mer PNA tagged inhibitors were mixed to the 10.000 member library previous to microarray hybridization at 5 nM each (10x molar excess respect the native library members). ERBB2-Canertinib covalent interaction allowed us to establish the washing conditions needed to discriminate between covalent binders on the library and non-covalent binders.

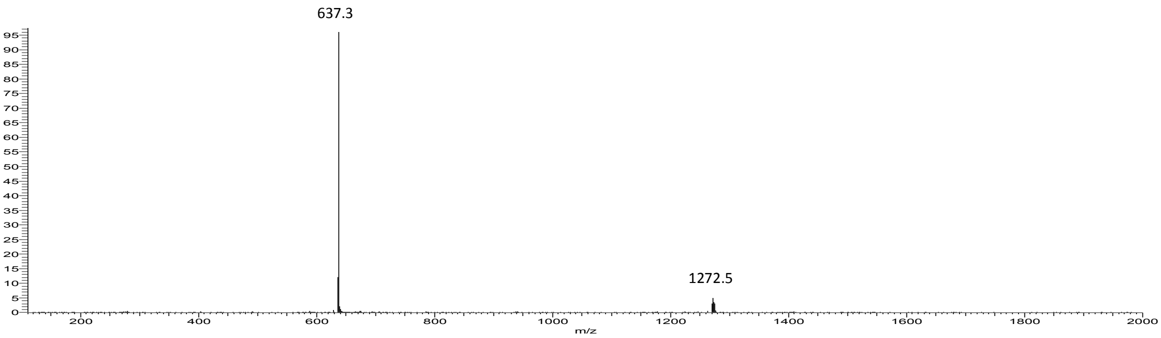
Recombinant protein (ERBB2 Life Technologies Cat# PV3366 or Human MEK2 protein Cat# ab124543 his tagged purified) was diluted to 100 nM on kinase buffer (50 mM Hepes pH 7.4, 5mM $MgCl_2$, 5mM $MnCl_2$, 0.05% Tween 20). The protein was incubated for one hour at room temperature on the hybridized slide and then the non-bound protein was washed by immersion of the slide on PBS (*standard wash*), or a *stringency wash*, for the discovery of covalent binders, using PBS, 1% SDS for 15 min with gentle rotation. The slide was then washed twice 5 min on PBS-T, again briefly rinsed with mQ water and dried. The presence of the kinase on the microarray features was detected by immuno staining the slide. Anti His antibody DyLigth ^{Tm} 649 conjugated (Rockland Immunogenics Cat# 200 343 382) was diluted 1/5000 in PBS-T, 0.5% BSA. The slide was incubated with the diluted antibody for 20 min at RT, PBS-T washed, dried and scanned on Genepix 4100A Personal Scanner. The scanned array was quantified using GenepixPro7 Software (Molecular Devices).

Synthesis of selected compounds from the 10K PNA encoded library of *covalent binders (Library I)*, as Cy3 conjugates.

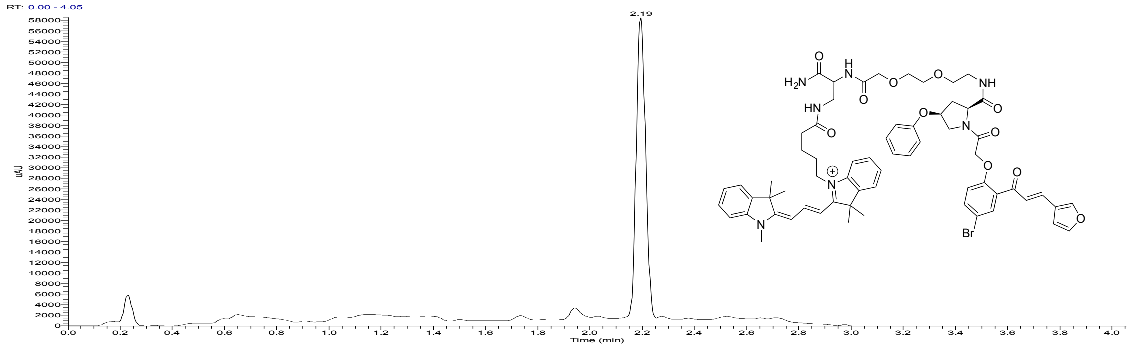
Nova Peg Rink amide resin (10 mg, 0.44 mmol/g full loading) was charged with an orthogonally protected FmocDap-(Mtt)-OH using ***procedure 5*** with loading reduction to 0.2 mmol/g followed by capping of the remaining free functionalities (***procedure 1***), Fmoc was subsequently deprotected following ***procedure 3*** and PEG spacer was introduced using ***procedure 6*** (both HATU or DIC/HOBt procedure could be used). Upon Mtt deprotection of the Dap linker (***procedure 10***), the N-terminus was acylated with cyanine dye (Cy3-CO_2H) using ***procedure 6*** (HATU). Fmoc group was then cleaved (***procedure 3***) followed by coupling with corresponding amino acid using **procedure 6** (DIC/HOBt). For codon 97 the resin was instead charge with an orthogonally protected Fmoc-Asp-(OAll)-OH using ***procedure 6*** DIC/HOBT without loading reduction. Alloc was then deprotected using ***procedure 9***, and the resulting carboxylic acid was derivatized with 2-picoyl amine using ***procedure 8***. Final Fmoc deprotection (***procedure 3***) was followed by introduction of the corresponding small molecule using ***procedure 6*** (both HATU or DIC/HOBt procedure could be used). Compound was then released from the solid support (***procedure 12***) and purified via HPLC.

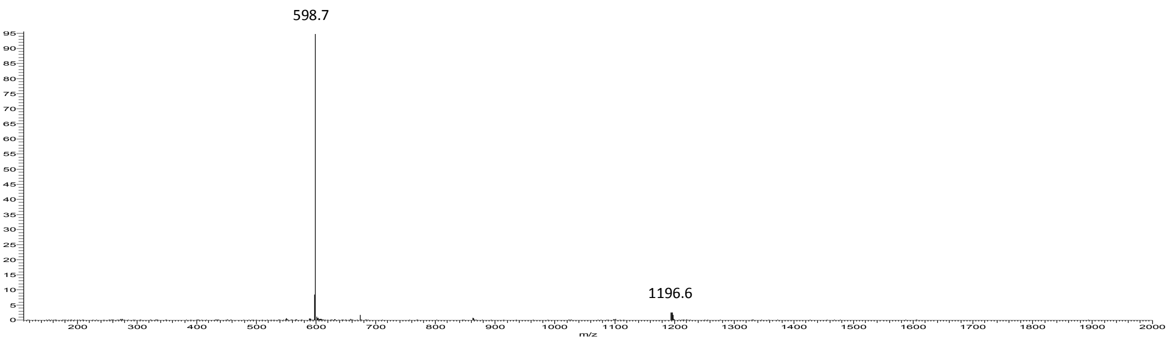
48-18- Cy3 $C_{65}H_{74}Br_2N_7O_{10}^+$ 1273.15



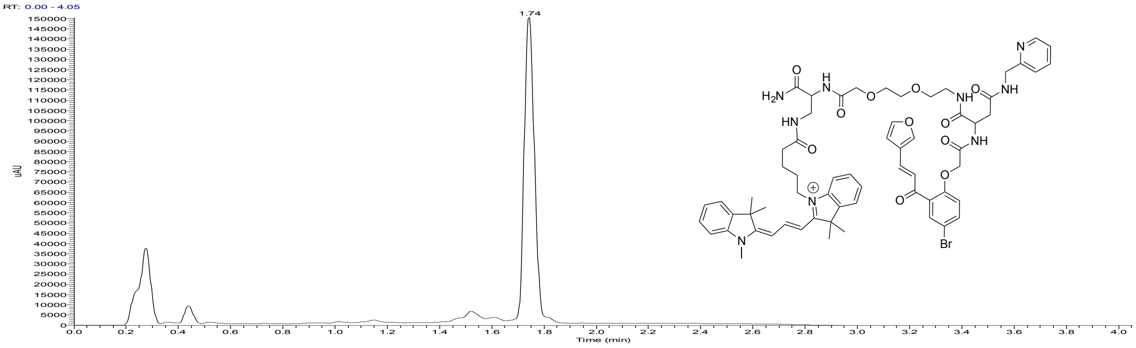


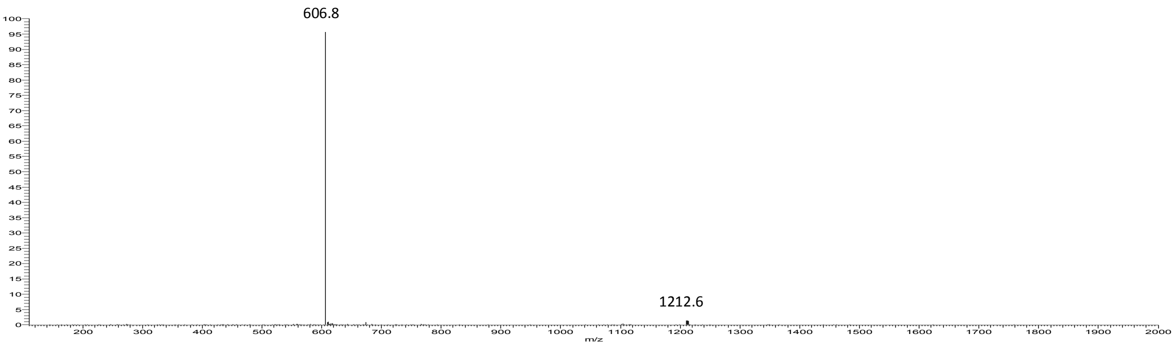
69-18- Cy3 $C_{64}H_{73}BrN_7O_{11}^+$ 1196.23



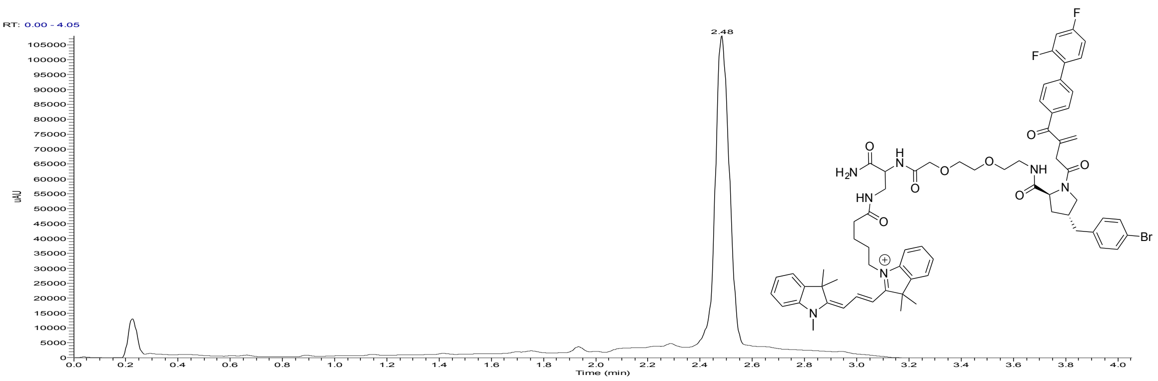


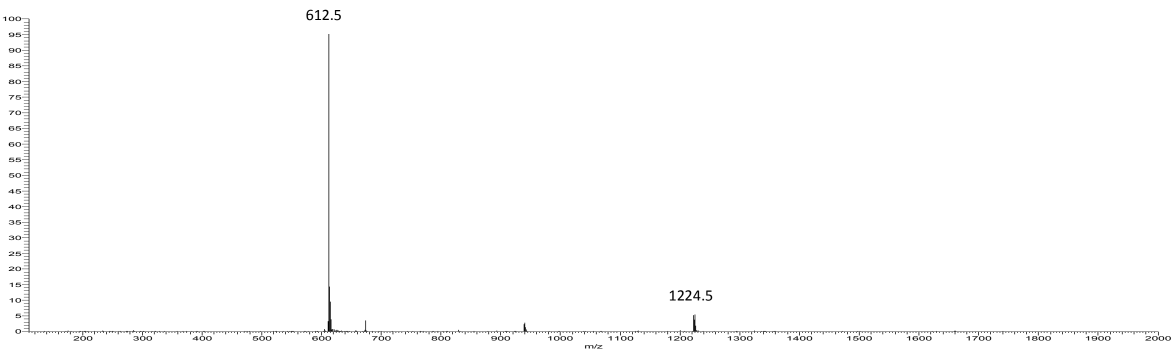
97-18- Cy3 $C_{63}H_{73}BrN_9O_{11}^+$ 1212.23



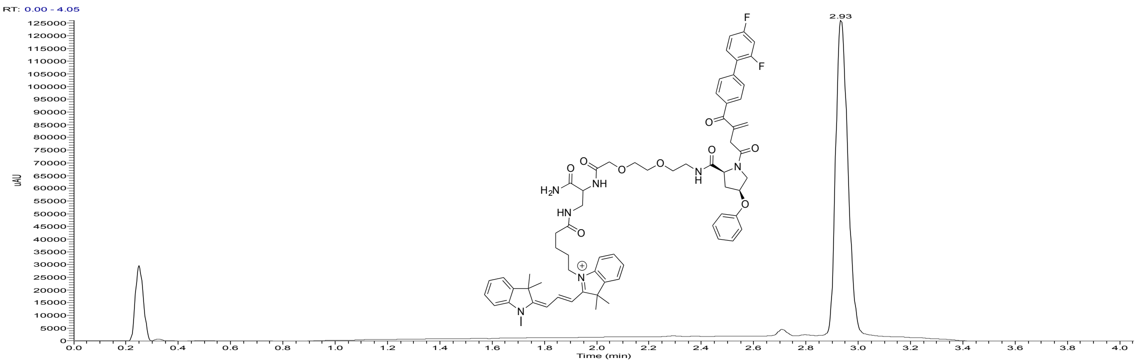


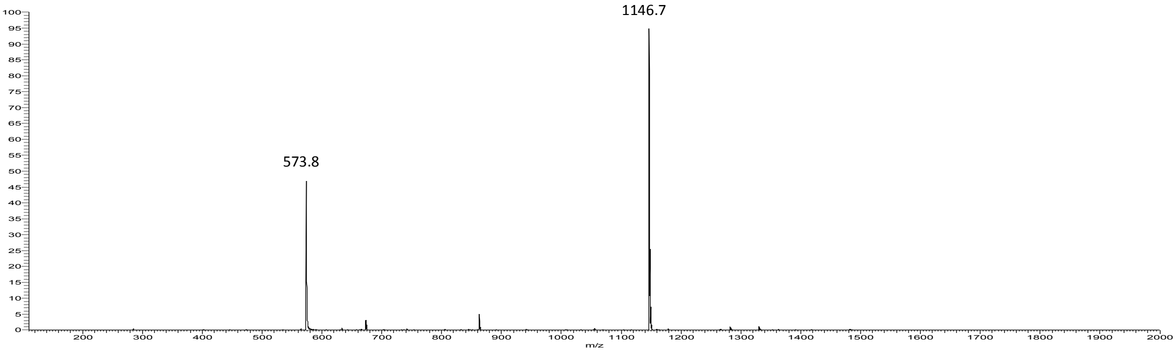
48-7-Cy3 $C_{67}H_{75}BrF_2N_7O_8^+$ 1224.28



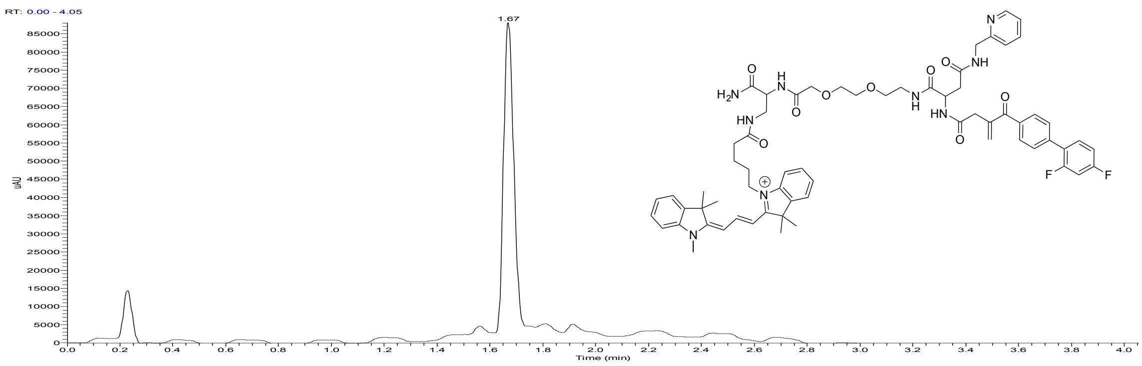


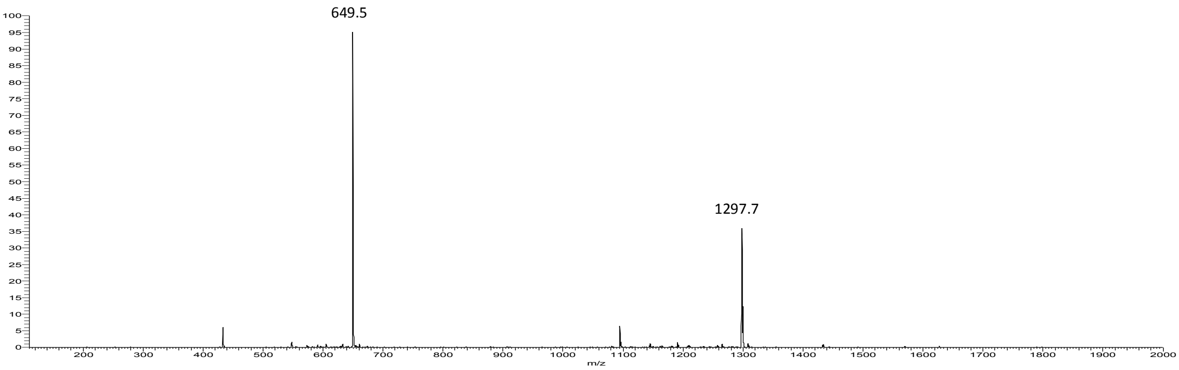
69-7-Cy3 $C_{66}H_{74}F_2N_7O_9^+$ 1147.35



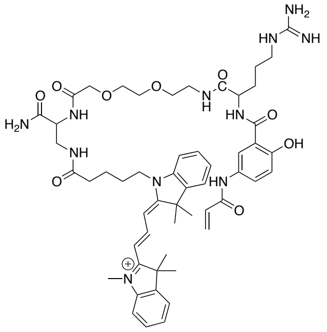


97-7-Cy3 $C_{65}H_{74}F_2N_9O_9^+$ 1163.36

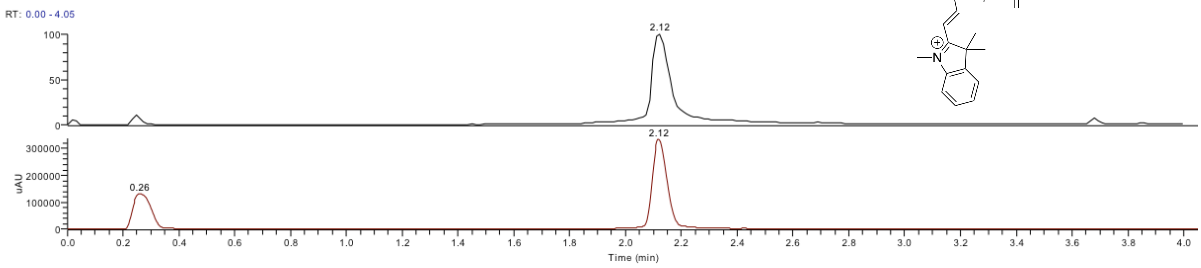


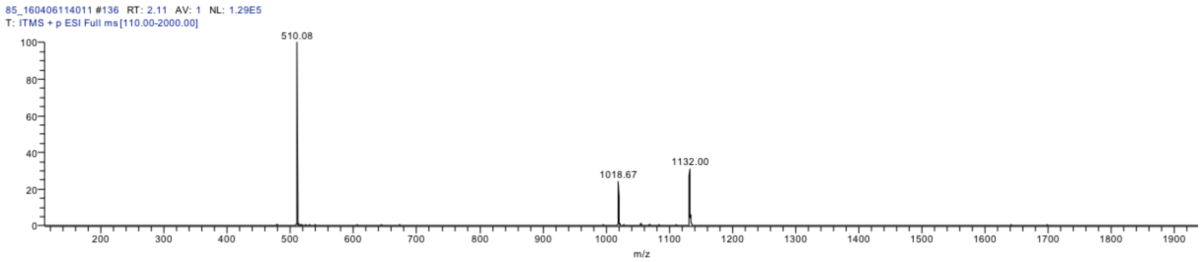


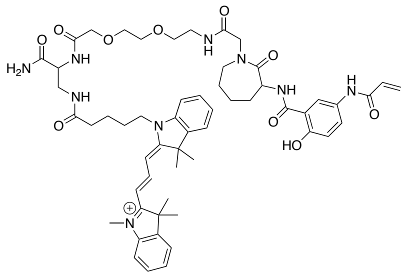
Synthesis of selected compounds from the kinase targeted 10K PNA encoded chemical library (Library II), as Cy3 conjugates.



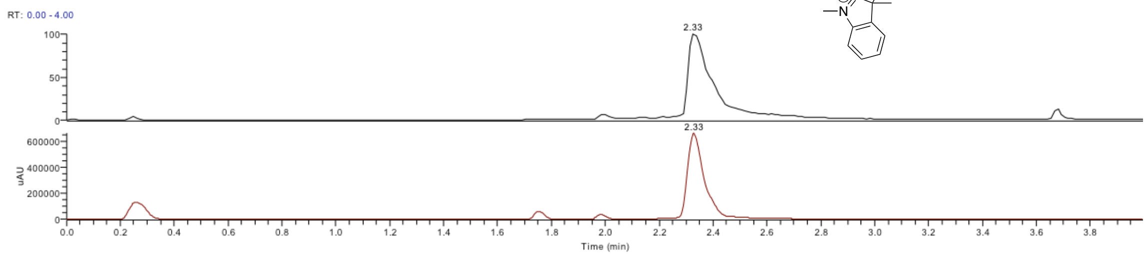
14-88b $C_{54}H_{72}N_{11}O_9^+$ 1019.22

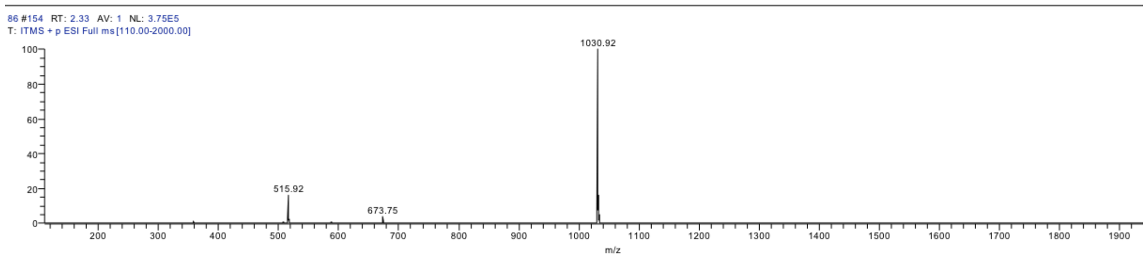




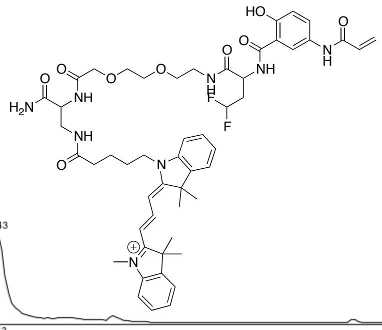


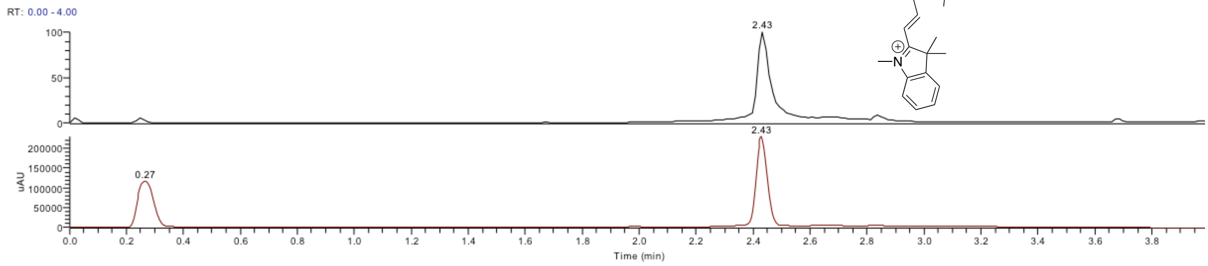
20-88b $C_{56}H_{72}N_9O_{10}^+$ 1031.22

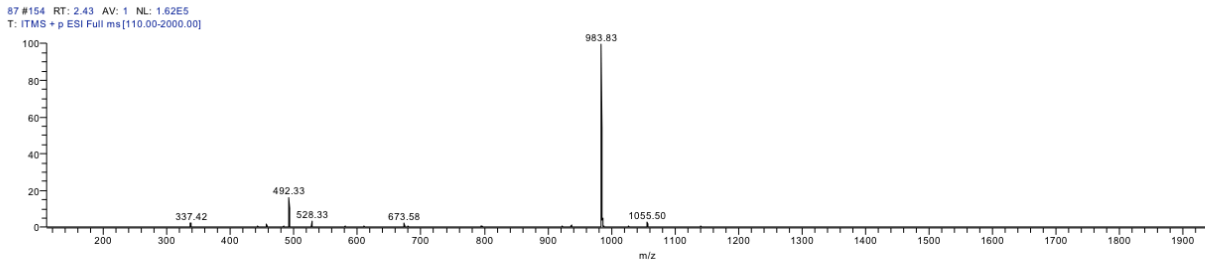




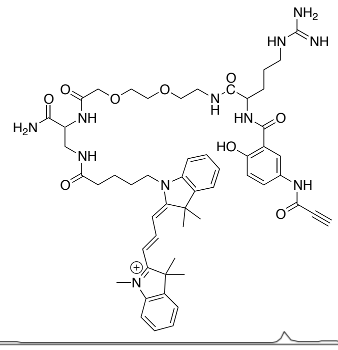
32-88b $C_{52}H_{65}F_2N_8O_9^+$ 984.12

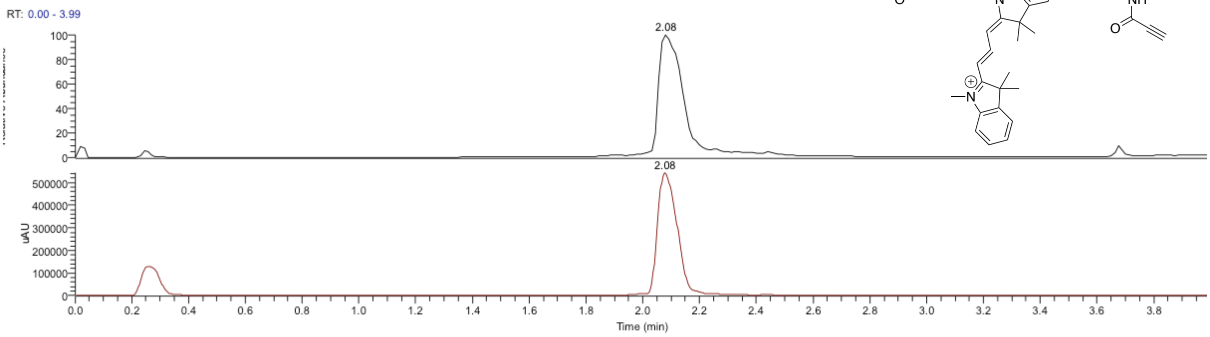


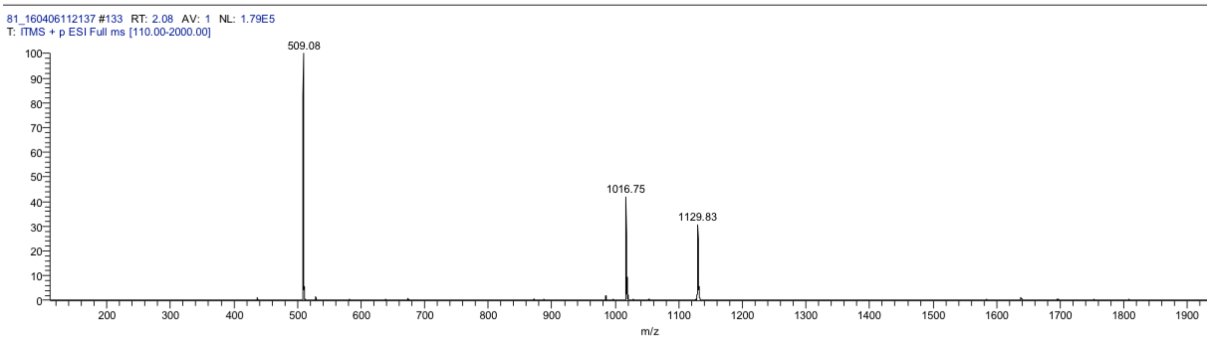




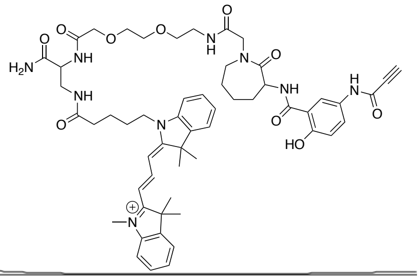
14-98b $C_{54}H_{70}N_{11}O_9^+$ 1017.20

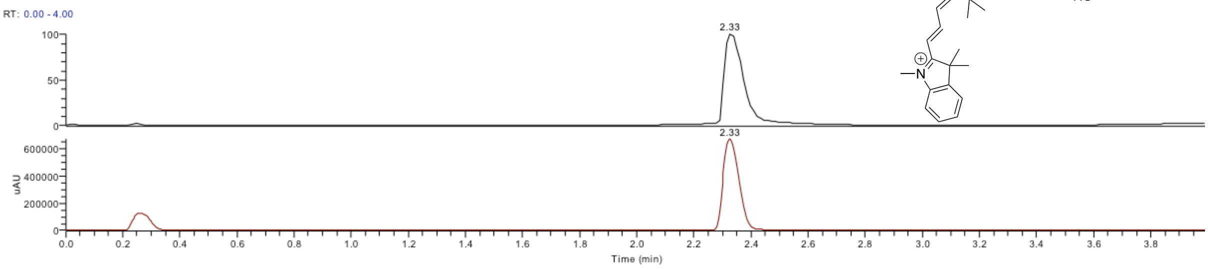


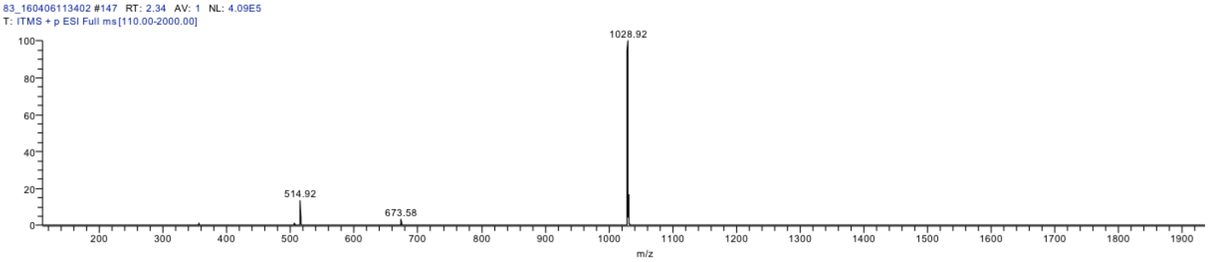




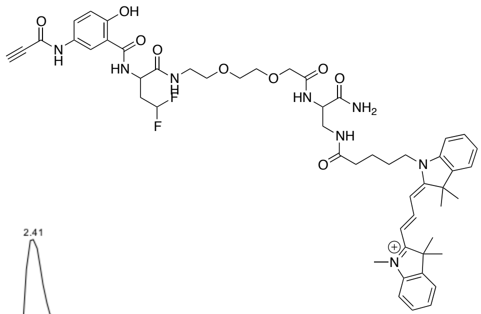
20-98b $C_{56}H_{70}N_9O_{10}^+$ 1029.21

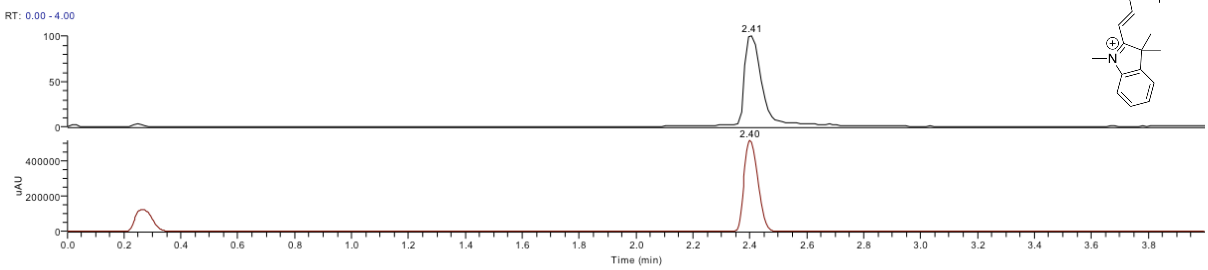


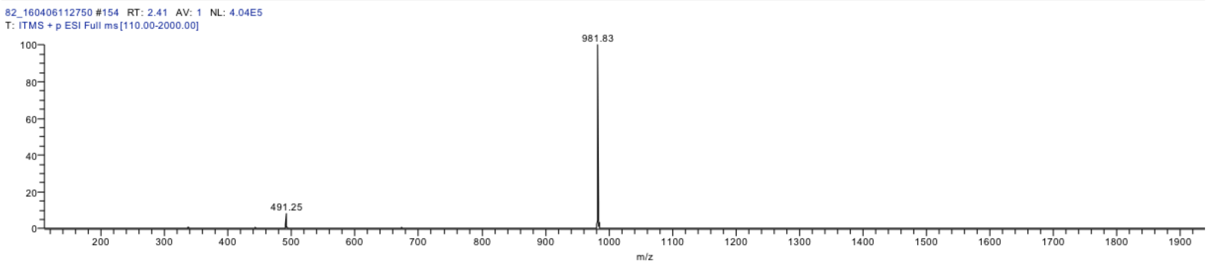




32-98b $C_{52}H_{63}F_2N_8O_9^+$ 982.10







Covalent Protein small molecule interaction

Labelling experiments

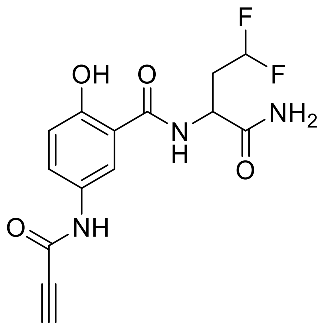
Recombinant protein kinases his tagged were incubated at 400 nM in kinase buffer (50 mM Hepes pH 7.4, 5 mM $MgCl_2$, 5mM $MnCl_2$, 0.05% Tween 20), with Cy3 conjugated small molecule. The reactions were incubated at RT for 1 hour and then the labelling was stopped by addition of 1 mM DTT, SDS sample buffer followed by heating denaturation at 95° C. The samples were loaded on SDS PAGE and the gels were scanned to detect Cy3 fluorescence.

MEK2 labelling in presence of competitors

Recombinant purified MEK2 –his at 500 nM in buffer kinase complemented with 10 μg of HEK 293T cell extract was incubated with compounds **69-7** and **97-7** at 2 μM final concentration, in the presence of 2 μM **PD053901** (Mek2 allosteric inhibitor) or 2 μM of **NW-466** (a Hypothemycin analog) as ATP competitors, to evaluate the capacity of these compounds to compete the labelling of MEK2.

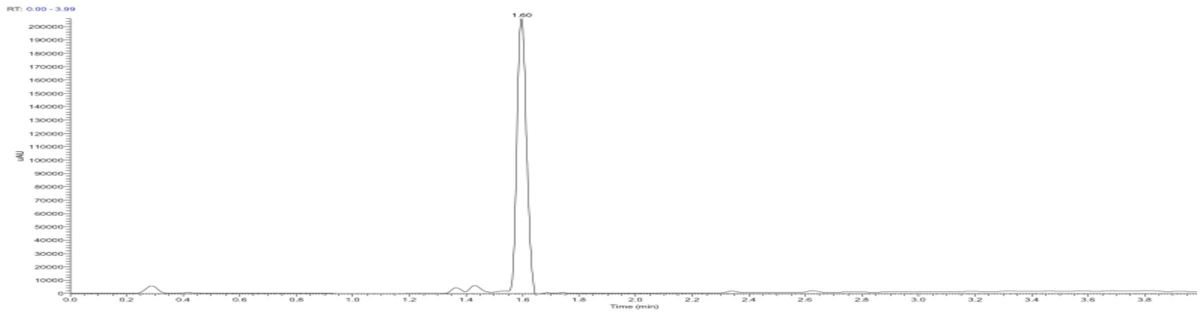
Kinetics of MEK2 covalent labelling

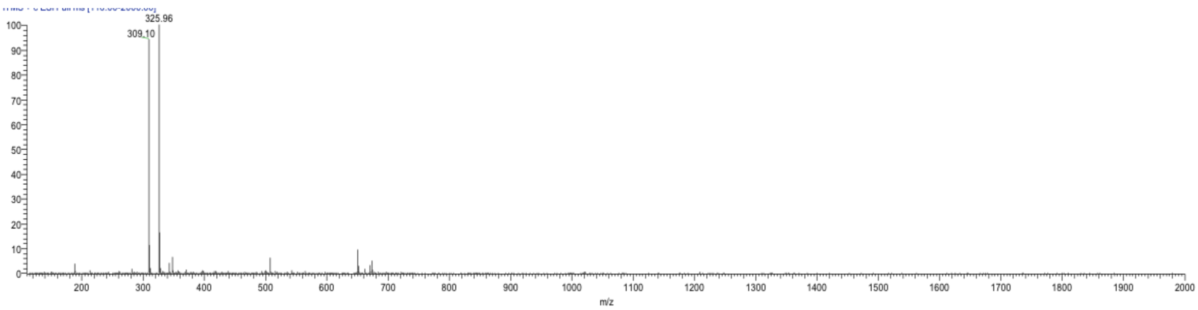
MEK2-his tagged (in house purified) 500nM was incubated with compounds **69-7** and **97-7** Cy3 conjugate at 2μM in buffer (50 mM Hepes, pH 7.4, 150 mM NaCl, 3mM $MgCl_2$, 3mM $MnCl_2$, 0.01% Tween 20, 10 μg of HEK cell extract) at RT followed by 5 min denaturation at 95 ^{0}C/SDS/10 mM DTT, at different time points to study the kinetics of the covalent binding. The kinetics of labelling was quantified using ImageJ software and the Cy3 fluorescence plotted against incubation time.



^{1}H NMR (500 MHz, DMSO, 25°C) δ = 11.51 (s, 1H), 10.59 (s, 1H), 8.05 (d, *J* = 5 Hz, 1H), 7.53 (dd, *J* = 10 Hz, *J* = 5 Hz, 1H), 6.90 (d, *J* = 10 Hz, 1H), 6.09 (tt, J_{H-F} = 55Hz, *J* = 5 Hz, 1H), 4.65 (m, 1H), 4.36 (s, 1H), 3.52 (m, 2H).

LC-MS (ESI): m/z $[M + H]^+$ for $C_{14}H_{13}F_2N_3O_4$ calculated 325,087, found: 325.96





KinomeScan™ kinase selectivity profiles for 32-98.

Unconjugated **32-98** was profiled at a concentration of 1 μM against a diverse panel of more than 400 kinases by DiscoveRx Corporation. Scores for primary screen hits are reported as a percent of the DMSO control (% control). For kinases where no score is shown, no measurable binding was detected. The lower the score, the lower the Kd is likely to be, such that scores of zero represent strong hits. Scores are related to the probability of a hit but are not strictly an affinity measurement. At a screening concentration of 1.0 μM, a score of less than 10% implies that the false positive probability is less than 20% and the Kd is likely less than 100 nM.