# **Supplemental Information**

# Synthesis of Libraries of Peptidomimetic Compounds Containing A 2-

# **Oxopiperazine Unit In The Main Chain**

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### Materials and Instruments:

S-2-chloro-propionic acid was purchased from Alfa Aeser. 2-Chloro-4-methyl valeric acid and S-2-bromo-propionic acids were obtained from Aldrich. S-2-bromo-3-phenyl-propionic acid was purchased from Toronto Research Chemicals Inc. Tetrakis (triphenyl phosphine) Pd (0) was bought from Frontier Scientific. Fmoc-amino acids were purchased from advanced chemtech. Rest of the other chemicals and reagents were purchased either from Aldrich or Fisher Scientific. Rink amide MBHA resin (0.7 mmol/g) and Tentagel macrobead-NH<sub>2</sub> (160 µg, 0.4 mmol/g) resin were obtained from Chemprep and rapp-polymere, respectively. Disposable fritted columns (5 mL, 50 mL) for peptoid syntheses were bought from Intavis AG. Microwave assisted peptoid syntheses were accomplished utilizing 10% of 1550 W household microwave (GE model JE 1860 BH04). HPLC purification of crude peptoids after acid cleavage from Rink amide resin was carried out in Water 1525 binary HPLC pumps and a 2487 dual absorbance detector, or a 2998 photodiode array detector. Buffer A (H<sub>2</sub>O with 5% CH<sub>3</sub>CN and 0.1% trifluoroacetic acid (TFA) and buffer B (CH<sub>3</sub>CN with 0.1% TFA) were used as a mobile phase in Vydac C-18 analytic column (5m, 250 x 4.6 mm, Alltech) and Apollo C-18 5µ preparative column. Purity of the compounds were assessed by analytical HPLC under UV (214), otherwise mentioned in description of the spectrum. MS and MS/MS were recorded in ESI-MS (Waters) and MALDI -TOF (4800 Proteomics Analyser, Applied Biosystems). In MS/MS, the signal-to-noise ratio threshold maintained to 3, however that for fig 2b and 3b in the main text was 20. Cyano-4hydroxycinnamic acid (HCCA) and 2,5-dihydroxybenzoic acid (super-DHB) were used as MALDI matrices (Aldrich). <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded in Bruker 400 instrument operating at 400 MHz for <sup>1</sup>H and 100 MHz for <sup>13</sup>C NMR using Deuterated-chloroform (CDCl<sub>3</sub>) or Deuterated-Dimethylsulfoxide (DMSO) (Cambridge) as a solvent.

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#### Solid Phase Synthesis of compound 6b and analogs:



Rink amide MBHA resin (1 g, 0.7 mmol) was swelled in DMF and coupled with bromoacetic acid (BAA, 2M) activated with diisopropylcarbodiimide (DIC, 3.2 M) in N,N'dimethylformamide (DMF) after fmoc deprotection. Subsequently, the bromide was displaced incubating the product with N-alloc ethylenediamine (alloc-EDA, 1 M) in DMF. The resultant secondary amine was coupled with S- or R-(-)-2 chloropropionic acid (2M) separately, activated with DIC (3M), 37°C, 2h. N-alloc moiety was deprotected using Pd(PPh<sub>3</sub>)<sub>4</sub> (20 mole%) and phenylsilane (12X) in DCM, R.T., 30 mins to afford a primary amine 4 which was incubated with 10% DIEA in DMF, R.T., O.N. to form oxopiperazine moiety 5. Further chain extension from N-terminal oxopiperazine ring was carried out incubating the BAA/DIC followed by bromide displacement with R-(+)-N-methylbenzyl amine at 37°C, 1 h. A crude peptoid 6b was cleaved off the solid support using 70:25:5::TFA:DCM:TIS @ RT, 2 h and the purified with reverse phase HPLC using preparative column (retention time = 8 min). Product was authenticated using ESI-MS. After each acid coupling and halide displacement steps, the beads were washed with DMF (3x), DCM (2x) and DMF. Compound 6 was subjected to NMR study for conformational analysis. <sup>1</sup>HNMR (400 MHZ, D<sub>6</sub>-DMSO) δ 7.5-7.4 (m, 5H), 5.32 (bs, 1H, NH), 4.6 (q, 1 H), 4.4 (m, 1H), 3.8-4.1 (m, 4 H), 3.6 (m, 1H), 3.5 (m, 2H), 3.2 (m, 1H), 1.62 (d, 3H, Me), 1.32 (d, 3H, Me); <sup>13</sup>CNMR (100 MHz, DMSO) δ 169.9, 168.2, 163.8, 159.1, 158.8, 137.2, 129.4, 129.2, 128.2, 57.7, 51.6, 49.4, 47.4, 45.5, 19.0, 16.8; ESI-MS(Obs. MH+) 333.4 (333.18 expected).

#### Synthesis of 2-Chloro-3-phenyl propanoic acid



A solution of (S)-phenyl alanine (0.25 mol, 41 g) in 6 N HCl (325 mL) was stirred at 0°C for 30 mins. Aqueous solution of sodium nitrite (27.5 g, 0.4 mmol, in 50 mL water) was added dropwise over the period of an hour under the constant flow of argon to remove nitrogen gas evolved during the reaction. The mixture was stirred for 5 h at 0°C and subsequently the

compound was extracted with diethyl ether, washed with water, brine and dehydrated with anhydrous sodium sulfate. Solvent was evaporated *in vacuo* to obtain compound **7** as a light yellowish crude product. <sup>1</sup>HNMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.32-7.233 (m, 5H), 4.48 (t, 1H), 3.39 (dd, 1H), 3.18 (dd, 1H); <sup>13</sup>CNMR (100 MHz, CDCl<sub>3</sub>) 174.8, 135.5, 129.4, 128.7, 127.5, 57.2, 40.8.

# Solid phase synthesis protocol for 2-oxopiperazine ring containing analogs used for purity test:

Scheme Sch1: Solid phase syntheses of oxopiperazinone containing analogs for purity test.



Rink amide MBHA resin (50 mg, 0.035 mmol) was swelled in DMF and was coupled with bromoacetic acid (2M) activated with DIC (3.2 M) in DMF, after fmoc-group deprotection. The bromide was displaced with N-alloc ethylenediamine (1 M) in DMF. The resultant secondary amine was coupled either with a solution of S-(-)-2 chloropropionic acid or 2-(S)-chloro, 3-methyl pentanoic acid (15 equivalent) in DMF. N-alloc moiety was deprotected using Pd(PPh<sub>3</sub>)<sub>4</sub> (20 mole%) and phenylsilane (12X) in DCM, R.T., 30 mins to afford a primary amine which was incubated with 10% DIEA in DMF, 37°C, O.N. to form oxopiperazine moiety **5**. The oxopiperazine ring containing methyl or isobutyl substituents were further coupled with 5 different fmoc-amino acid (10x) activated with HOAt (15x) and DIC (30x). The reaction mixtures were incubated for 30 mins @ 50°C and further kept at 37°C additional 2 h. The fmoc groups were deprotected with 20% piperadine in DMF and resultant primary amines were coupled either with DIC activated (S)-2-bromopropionic acid or (S)-2-chloro-4-methyl pentanoic acid. Finally the halides were displaced with 2-methoxy ethylamine at 60-70°C overnight to generate 15 different oxopiperazine containing PTA analogs. Each trimer peptoids were

separately cleaved off the solid support using 70:25:5::TFA:DIC:TIS. The purity of the compound was tested by HPLC and was characterized using MALDI-TOF MS and MS/MS.

## Protocol for peptoid library construction:

# Library-1:

Tentagel MB NH<sub>2</sub> (0.5 g, initial loading 0.4 mmol/g) was soaked in DMF for 30 min, RT. The beads were coupled with fmoc-methionine (5X) activated with N,N,N',N'-Tetramethyl-O-(1Hbenzotriazol-1-yl)uronium hexafluorophosphate (HBTU) (5X), N-Hydroxybenzotriazole (HOBt) (8X) and Diisopropyl ethylamine (DIEA) (10x) in DMF. Mixture was incubated 3 h at room temperature. Fmoc-group was deprotected using 20% piperidine in DMF. Resultant primary amine was treated with BAA/DIC and the bromide was displaced with furfurylamine under microwave condition. Likewise, methoxyethylamine, propargyl amine and additional methoxyethyl amine were repeatedly added along with BAA/DIC into the growing peptoid chain as a part of linker region. The main oxopiperazine containing peptoid oligomer library (~16 K diversity) was created utilizing 14 different linear or  $\alpha$ -branched primary amines along with five oxopiperazine moieties and commercially available piperazine. As a first unit of peptoid library, BAA/DIC was coupled to the secondary amine from the linker, the solid support was split into 15 different fractions, after washing step. Each portion was treated with different primary amines (2M) to displace the bromide under microwave condition. The beads were washed as explained earlier. Resultant secondary amines were pooled and coupled with BAA/DIC. The beads were washed. The bromide was displaced with N-tboc ethylene diamine and the secondary amine was coupled with  $\alpha$ -chloro acid derivatives. t-boc group was deprotected with 50% TFA in Dichloromethane (DCM) (30 min at RT). Beads were washed several times with DCM and then DMF. Cyclization to afford oxopiperazine rings was accomplished using 10% DIEA in DMF. ON, 37°C. Further, chain extension from the oxopiperazine moiety was carried out by repeated coupling of BAA/DIC and primary amines utilizing split and pool method.



**Figure L1**: Oxopiperazine containing peptoid library-1 showing leader sequence in Red color and the primary amines represented as R. The substituents in oxopiperazine derivatives are depicted as R'. As a part of diversity element a commercially available piperazine moiety is also added along with oxopiperazine ring.

# Library-2:

The final 2-oxopiperazine containing PTA library-2 (~21K diversity) was synthesized utilizing 12 primary amines, 5 oxopiperazine moieties and 6 different fmoc-amino acids. As a linker region, library-2 contains methionine followed by 2-methoxy ethylamine and two methylamines coupled repeated with BAA/DIC. The first peptoid monomer and oxopiperazine moieties were incorporated into peptoid as described in library-1. Further chain extension from oxopiperazine moiety in the library-2 was carried out by splitting the pool of beads into five different fractions where each of the fractions are separately coupled with six different fmoc–protected amino acids (20X) activated with HOAt (20x) and DIC (30X). The reaction mixture was incubated at 50°C, 30 mins and further incubated at 37°C, overnight. The beads were washed as described earlier. The products were pooled, the fmoc-group was deprotected with 20% piperadine in DMF to afford primary amine. The beads containing primary amines were washed and then split into five different portions where the halides were finally displaced with different primary amines incubating at 60°C (2M, ON) to afford oxopiperazine containing PTA library-2.



**Figure L2**: Library-2 showing leader sequence in Red color. R, R' and R" represent commerically available primary amine, side chain of  $\alpha$ -haloacids and amino acid side-chains, respectively.

Spectral data of compound 6b synthesized with S-(-)-2 chloropropionic acid and R-(-)-2 chloropropionic acid, recorded in  $D_6$ -DMSO:



Figure S1 : <sup>1</sup>HNMR of 6b synthesized with S-(-)-2 chloropropionic acid recorded at 25°C



**Figure S1.1** :: <sup>1</sup>HNMR of **6b** synthesized with R-(-)-2 chloropropionic acid recorded at 25°C



Figure S2: <sup>1</sup>HNMR of 6b synthesized with S-(-)-2 chloropropionic acid recorded at 37°C



Figure S2.1: <sup>1</sup>HNMR of 6b synthesized with R-(-)-2 chloropropionic acid recorded at 45°C



Figure S3: <sup>13</sup>CNMR of 6b synthesized with S-(-)-2 chloropropionic acid.



Figure S4: <sup>13</sup>CNMR-DEPT of 6b synthesized with S-(-)-2 chloropropionic acid.



Figure S5: COSY of 6b synthesized with S-(-)-2 chloropropionic acid.



Figure S6: 1D-NOE of 6b synthesized with S-(-)-2 chloropropionic acid irradiated at  $\delta$  4.6.



**Figure S7**: HPLC of **6b** that is synthesized with S or R-(-)-2 chloropropionic acid showing retention time at 7.5 min.



Figure S8: ESI-MS of 6b synthesized with S-(-)-2 chloropropionic acid



Figure S8.1: ESI-MS of 6b synthesized with R-(-)-2 chloropropionic acid



Spectral data of S-(2)-Chloro-3-phenylpropionic acid 7, recorded in D-Chloroform:

**Figure S9** : <sup>1</sup>HNMR of S-(2)-Chloro-3-phenyl-propionic acid.



**Figure S10** : <sup>13</sup>CNMR of S-(2)-Chloro-3-phenyl-propionic acid.

**Table T1**: Table showing purity of different chiral-2-oxopiperazine ring containing peptoids. The purities of the compounds are determined based upon peaks resolved by HPLC.



No.	Compound	Purity (%)			
1	6a	98			
2	6b	90			
3	6c	92			
4	6d	98			
5	6e	90			
6.	6f	85			

HPLC and MALD-TOF data of oxopiperazine-containing peptoids.



Figure S11:HPLC (left, rentention time: 14.8 min) & ESI-MS (right) of 6a shown at the top.



Figure S12: HPLC (left, retention time: 16.5 min) and ESI-MS (right) of 6c shown at the top.



Figure S13: HPLC (left, retention time: 17 min) and ESI-MS (right) of 6d shown at the top.



**Figure S14**: HPLC (left, retention time :14.5 min) and MALDI-MS (right) of **6e** shown at the top.



Figure S15: HPLC (left, retention time: 18 min) and ESI-MS (right) of 6f shown at the top.

Scheme Sch2: Solid phase syntheses of 2-oxopiperazinone containing peptide tertiary amide analogs (PTA) for purity test.



**Table T2:** Table showing purity of different analogs of compound **9**. The purities of the 2-oxopiperazine containing PTA analogs are determined based upon peaks resolved by HPLC.



No.	R' [9a]	Purity	No.	R' [9b]	Purity	No.	R' [9c]	Purity
1	CH <sub>3</sub>	91	6	CH <sub>3</sub>	90	11	CH <sub>3</sub>	82
2	(CH <sub>2</sub> ) <sub>4</sub> NH <sub>2</sub>	88	7	(CH <sub>2</sub> ) <sub>4</sub> NH <sub>2</sub>	87	12	(CH <sub>2</sub> ) <sub>4</sub> NH <sub>2</sub>	91
3	CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	85	8	CH <sub>2</sub> COOH	88	13	CH <sub>2</sub> COOH	80
4	CH <sub>2</sub> -Ph	82	9	CH <sub>2</sub> -Ph	86	14	CH <sub>2</sub> -Ph	80
5	CH <sub>2</sub> (O-Bn)	85	10	CH <sub>2</sub> -indole	86	15	CH <sub>2</sub> -indole	83

HPLC and MALD-TOF data of 2-oxopiperazine containing PTA analogs (In each structure, the numbers indicated in red color are not observed in tandem mass):



**Figure S15**: HPLC (middle-left, retention time: 3.5 min, MALDI-MS (middle-right) and MALDI MS/MS of test compound entry 1 (Table T2).



**Figure S16**: HPLC (middle-left, retention time: 3.5 min), MALDI-MS (middle-right) and MALDI MS/MS of test compound entry 2 (Table T2)



**Figure S17**: HPLC (middle-left, retention time: 16.5 min), MALDI-MS (middle-right) and MALDI MS/MS of test compound entry 2 ((Table T2)



**Figure S18**: HPLC (middle-left, retention time: 15 min), MALDI-MS (middle-right) and MALDI MS/MS of test compound entry 3 (Table T2)



**Figure S19**: HPLC (middle-left, retention time: 15.5 min), MALDI-MS (middle-right) and MALDI MS/MS of test compound entry 5 (Table T2)



**Figure S20**: HPLC (middle-left, retention time: 15 min), MALDI-MS (middle-right) and MALDI MS/MS of test compound entry 6 (Table T2)



**Figure S21**: HPLC (middle-right, retention time: 1.5 min), MALDI-MS (middle-left) and MALDI MS/MS of test compound entry 7 (Table T2)



**Figure S22**: HPLC (middle-left, retention time: 15 min), MALDI-MS (middle-right) and MALDI MS/MS of test compound entry 8 (Table T2)



**Figure S23**: HPLC (middle-left, retention time: 18 min, UV 254 nm), MALDI-MS (middle-right) and MALDI MS/MS of test compound entry 9 (Table T2)



**Figure S24**: HPLC (middle-left, retention time: 17.9 min, UV 281 nm), MALDI-MS (middle-right) and MALDI MS/MS of test compound entry 10 (Table T2),.



**Figure S25:** HPLC (middle-left, retention time: 17 min), MALDI-MS (middle-right) and MALDI MS/MS of test compound entry 11 (Table T2)



**Figure S26**: HPLC (middle-left, retention time: 14.9 min), MALDI-MS (middle-right) and MALDI MS/MS of test compound entry 12 (Table T2)



**Figure S27**: HPLC (middle-left, retention time: 16 min), MALDI-MS (middle-right) and MALDI MS/MS of test compound entry 13 (Table T2)



**Figure S28**: HPLC (middle-left, retention time: 17 min, UV 254 nm), MALDI-MS (middle-right) and MALDI MS/MS of test compound entry 14 (Table T2)



**Figure S29**: HPLC (middle-left, retention time: 18 min, UV 281 nm), MALDI-MS (middle-right) and MALDI MS/MS of test compound entry 15 (Table T2).

Following are the structures of peptoids from library-L1 and library-L2 identifed after CNBr mediated cleavage from the resins and subsequent MALDI – MS and MS/MS analyses. Each figure has a peptoid structure showing the mass of corresponding y and b fragments at particular substituent observed during collision induced dissociation (CID) of the parent peak:





**Figure S30**: Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S31**:Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S32**: Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S33**:Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S34**: Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S35**: Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S36**:Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S37**:Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S38**:Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S39**: Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S40**: Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S41**:Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S42**: Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S43**:Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S44**:Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S45**: Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S46**:Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.





**Figure S47**:Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S48**: Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S49**: Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S50**: Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S51**:Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S52**:Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S53**:Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S54**:Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S55**: Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S56**: Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S57**:Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S58**: Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S59**: Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S60**: Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S61**:Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S62**:Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S63**: Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



**Figure S64**: Top: MALDI-MS data showing parent peak (left) of peptoid (right). Bottom: MALDI-TOF MS/MS of the compound.



Scheme Sch3: Solution phase synthesis of 2-oxopiperazine-amino acid compound 22.

**Figure S65:** <sup>1</sup>HNMR of compound **22** in D-chloroform, showing the peaks a, b and c representing  $\alpha$ -H and  $\alpha$ -CH3 group from alanine and protons at C-3 in 2-oxopiperazine ring.



**Figure S65:** NOE Spectrum of compound **22** irradiated at  $\delta$  4.5 (Ha) confirming the proton facing towards C-3, thus adopting a trans-amide bond geometry.



**Figure S66**: MALDI-MS data of compound **7** showing the parent peak [MH<sup>+</sup>] 617.

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