SUPPLEMENTARY INFORMATION


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General Experimental Procedure: Melting points of samples were determined in open capillary tubes using Buchi Melting point B-540 apparatus and are uncorrected. IR spectra were recorded on an infrared Fourier Transform spectrophotometer using KBr pellets. Column chromatographic separations were performed using silica gel 60-120 mesh, solvent systems gradient EtOAc/Pet ether and pure DCM to 3% MeOH/DCM. \(^1\)H and \(^{13}\)C spectra were obtained using Bruker AC-200 (200 MHz) and 500 MHz NMR spectrometers. The chemical shifts are reported in delta (\(\delta\)) values. The optical rotation values were measured on Bellingham-Stanley Ltd, ADP220 polarimeter. CD spectra were recorded on JASCO-715 Spectropolarimeter. Mass spectra were obtained either by LCMS techniques. Oligomers were characterized by RP HPLC, C18 column and LC-TOF-MS mass spectrometry.

Experimental procedure

\((2S,4R)\)-N1-(benzyloxy carbonyl)-4-hydroxy-2-(hydroxymethyl)-pyrrolidine (1):

To an ice cooled solvent mixture dry THF (175 ml) and absolute Ethanol (250 ml) containing NaBH\(_4\) (3.192 g, 84.3 mmol) in a 3-necked flask, LiCl (3.58 g, 84.3 mmol) was added slowly from the solid addition funnel for 30 mins. The above solution was stirred for 1.0 h and appearance of milky solution indicates the formation of LiBH\(_4\) \textit{in situ}. To the above ice cooled milky solution, (\(2S,4R\))-N1-(benzyloxy carbonyl)-4-hydroxyproline methyl ester (9.45 g, 33.75 mmol) dissolved in absolute ethanol (50 ml) was added from dropping funnel over a period of 30 mins under nitrogen atmosphere and the reaction mixture was stirred over night at rt. Then the pH of the reaction mixture was adjusted to 7.0 by adding saturated solution of NH\(_4\)Cl. The solvent mixture was removed under vacuum and the residue was extracted into ethyl acetate (25 ml x 3). The organic layer was washed with water, brine solution, dried over anhydrous Na\(_2\)SO\(_4\) and concentrated to afford oily product diol 1 (7.5 g, yield 88%, Rf= 0.3, Ethyl acetate: petroleum ether-5:5). \([\alpha]_D^{20} +16.8^\circ\) (c 3.26, CH\(_2\)Cl\(_2\)); \(^1\)H-NMR (CHCl\(_3\)-d, 200 MHz); \(\delta\)H 1.65-1.9 (m, 1H, C3H'), 1.9-2.15 (m, 1H, C3H), 3.4-3.9 (m, 5H, CH\(_2\)OH, C2H, C5H), 4.2 (m, 1H, C4H), 4.4 (bd, 1H, CH\(_2\)OH, exchangeable), 5.2-5.75 (bd, 1H, C4-OH, exchangeable), 7.4 (s, 5H, C\(_6\)H\(_5\)). Anal Calcd (%) for C\(_{13}\)H\(_{17}\)NO\(_4\): C, 62.15; H, 10.75; N, 5.57; Found C, 61.83; H, 10.91; N, 5.53: LCMS; 252.07 [M+1]+.

\((2S,4R)-N1-(benzyloxy carbonyl)-4-hydroxy-2-(p-toluenesulfonyloxymethyl)-pyrrolidine (2):

The diol 1 (7.12 g, 28.36 mmol) dissolved in dry pyridine (200 ml) and cooled to 0\(^\circ\)C. To the this ice cooled solution, freshly crystallized p-toluene sulfonyl chloride (5.95 g, 31.2 mmol) in pyridine was
added from the dropping funnel over a period of 1.5 h under nitrogen atmosphere. The reaction mixture was stirred for 8 h at rt. Pyridine was removed under reduced pressure and co-evaporated with toluene (twice). The residue was extracted in to ethyl acetate (50 ml x 2), washed with water, dried over Na$_2$SO$_4$ and concentrated to yield crude oily residue. The residue was purified by column chromatography to get monotosylate 2. (8.6 g, yield 75%, Rf = 0.36, Ethyl acetate: petroleum ether-5:5). [$\alpha$]$_D^{20}$ +27.7° (c 4.43, CH$_2$Cl$_2$); $^1$H-NMR (CHCl$_3$-d, 200 MHz); δ$_H$ 1.7-2.15 (2H, C$_2$H, C$_3$H), 2.2-2.5 (s, 3H, OCH$_3$), 3.1-3.6 (m, 2H, C$_5$H$_2$), 3.7-4.2 (m, 3H, CH$_3$), 4.25-4.65 (m, 2H, CH$_2$-OTs), 4.7-5.5 (bd, 4H, C$_4$H, COOCH$_2$, OH), 7.26-7.4 (s, 7H, C$_6$H$_5$, two CH-Ts), 7.5-7.8 (m, 2H, two CH-Ts). $^{13}$C-NMR (CHCl$_3$-d, 200 MHz); δ$_C$ 21.0, 35.9, 54.5, 54.8, 66.4, 68.7, 69.8, 127.3, 128.0, 129.5, 132.3, 136.0, 144.5, 154.6. Anal Calcd (%) for C$_{20}$H$_{23}$NO$_6$S: C, 59.25; H, 5.67; N, 3.45; S, 7.90, Found C, 58.92; H, 5.77; N, 3.37; S, 7.67; MS LCMS; 405.00 [M$^+$].

(2$S$,4$R$)-N1-(benzyloxycarbonyl)-4-hydroxy-2-(azidomethyl)-pyrrolidine:
To the solution of monotosylate 2 (6.0 g, 14.8 mmol) in DMF (50 ml), NaN$_3$ (7.7 g, 118.4 mmol) was added. The reaction mixture was stirred at 55°C for 8 h. The solvent was removed under reduced pressure and the residue was extracted into ethyl acetate (25 ml x 3). The combined organic layer was washed with water, brine, dried over anhydrous Na$_2$SO$_4$ and then concentrated to yield (2$S$,4$R$)-N1-(benzyloxycarbonyl)-4-hydroxy-2-(azidomethyl)-pyrrolidine. (3.9 g, yield 96%, Rf = Ethyl acetate:petroleum ether-5:5). IR (neat) (ν) cm$^{-1}$. 3016, 2360, 2106, 1697, 1419. $^1$H-NMR (CHCl$_3$-d, 200 MHz); δ$_H$ 1.95-2.02 (m, 2H, C$_3$H), 3.07-3.85 (m, 4H, C$_5$H, CH$_2$N$_3$, OH), 3.97-4.45 (m, 2H, C$_2$H, C$_4$H), 5.09 (s, 2H, OCH$_2$), 7.31 (s, 5H, C$_6$H$_5$). $^{13}$C-NMR (CHCl$_3$-d, 200 MHz); δ$_C$ 36.9, 37.5, 51.9, 53.2, 55.2, 54.8, 55.6, 66.6, 67.0, 68.4, 68.8, 127.2, 127.5, 128.1, 136.2, 162.5: LCMS; 277.00 [M+1$^+$].

(2$S$,4$R$)-N1-(benzyloxycarbonyl)-4-hydroxy-2-(aminomethyl)-pyrrolidine:
To a solution of the (2$S$,4$R$)-N1-(benzyloxycarbonyl)-4-hydroxy-2-(azidomethyl)-pyrrolidine (3.5 g, 12.7 mmol) in Methanol (5 ml) taken in hydrogenation flask was added Raney Nickel (1.5 ml). The reaction mixture was hydrogenated in a Parr apparatus for 3.5 h at rt and H$_2$ of pressure 35-40 psi. The catalyst was filtered off and then solvent was removed under reduced pressure to yield a residue of the (2$S$,4$R$)-N1-(benzyloxycarbonyl)-4-hydroxy-2-(aminomethyl)-pyrrolidine as colorless oil. Yield (3.0 g, 95.0%). This compound was used for the further reaction without any purification.

(2$S$,4$R$)-N1-(benzyloxycarbonyl)-2-(tert-butoxycarbonylaminomethyl)-4-hydroxy pyrrolidine (3):
The (2$S$,4$R$)-N1-(benzyloxycarbonyl)-4-hydroxy-2-(aminomethyl)-pyrrolidine (3.0 g, 12.0 mmol) was taken in DMSO (10 ml), triethyl amine (1.58 g, 15.6 mmol) and BocN$_3$ (2.05 g, 14.4 mmol) were added. The reaction mixture was heated to 50°C for 8 h. The reaction mixture was poured into 150 ml of ice-cold water and the product extracted into ether (20 ml x 8). The combined ether layer was washed
with water, brine and then concentrated to give boc-protected amine 3. (3.2 g, yield 76%, Rf = 0.6, Ethyl acetate: petroleum ether). $[\alpha]_D^{20} = -12.74^\circ$ (c 0.7, CH$_2$Cl$_2$); $^1$H-NMR (CHCl$_3$-d, 200 MHz); $\delta_H$ 1.41 (s, 9H, Boc), 1.6-2.3 (m, 3H, C2H, C3H$_2$), 3.15-3.75 (m, 4H, C5H$_2$, CH$_2$NH), 3.9-4.2 (m, 1H, C4-OH), 4.3-4.5 (m, 1H, C4H), 5.11 (s, 2H, OCH$_2$), 5.4-5.6 (bd, 1H, carbamate NH), 7.33 (s, 5H, C$_6$H$_5$). $^1$C-NMR (CHCl$_3$-d, 200 MHz); $\delta_C$ 28.3, 38.04, 44.1, 54.9, 56.6, 67.0, 69.2, 79.2, 128.4, 136.4, 156.3, 159.6. Anal Calcd (%) for C$_{18}$H$_{26}$N$_2$O$_5$: C, 61.71; H, 7.42; N, 8.00; Found C, 61.68; H, 7.64; N, 7.78: LCMS; 351.21 [M+1]$^+$.  

**Ethyl-[(2S,4R)-2-(tert-butoxycarbonylamino)methyl]-4-hydroxy pyrrolidin-1-yl]-propeonate:**

To a solution of the ester 3 (3.2 g, 9.5 mmol) in Methanol (5 ml) taken in hydrogenation flask was added 10% Pd/C (0.32 g). The reaction mixture was hydrogenated in a Parr apparatus for 7 h at room temperature and H$_2$ of pressure 60 psi. The catalyst was filtered off and then solvent was removed under reduced pressure to yield a residue of the amine [(2S,4R)-2-(tert-butoxycarbonylamino)methyl]-4-hydroxy pyrrolidine as colorless oil. Yield (1.9 g, 95%). This compound was used for the further reaction without any purification.

The cyclic amine obtained (1.9 g, 8.8 mmol) taken in methanol (20 ml), added ethyl acrylate (0.97 g, 9.68 mmol) and stirred 3 h at rt. The reaction mixture was evaporated to dryness and was extracted into ethyl acetate (25 ml x 3). The organic layer was dried over Na$_2$SO$_4$ and concentrated to give crude residue, which on column chromatography afford Ethyl-[(2S,4R)-2-(tert-butoxycarbonylamino)methyl]-4-hydroxy pyrrolidin-1-yl]-propeonate. (2.0 g, yield 72%, Rf = 0.6, MeOH:CH$_2$Cl$_2$:1:9). $^1$H-NMR (CHCl$_3$-d, 200 MHz); $\delta_H$ 1.24 (t, 3H, ester CH$_3$), 1.41 (s, 9H, Boc), 1.52-1.9 (m, 2H, C3H$_2$), 1.95-2.6 (m, 5H, -CH$_2$-CH$_2$-, C2H), 2.7-3.5 (m, 5H, C5H$_2$, CH$_2$NH, OH), 3.7-4.2 (m, 2H, ester CH$_2$), 4.25-4.4 (m, 1H, C4H), 4.75-5.45 (bd, 1H, NH). $^1$C-NMR (CHCl$_3$-d, 200 MHz); $\delta_C$ 13.96, 28.19, 33.7, 37.7, 40.7, 49.1, 60.2, 61.3, 61.6, 69.2, 78.7, 156.3, 172.4. Anal Calcd (%) for C$_{15}$H$_{28}$N$_2$O$_5$: C, 56.96; H, 8.86; N, 8.86, Found C, 56.65; H, 8.95; N, 8.71: LCMS; 317.00 [M+1]$^+$.  

**Ethyl-[(2S,4S)-2-(tert-butoxycarbonylamino)methyl]-4-(N$^3$-benzoyl thymin-1-yl)- pyrrolidin-1-yl]-propeonate (4):**

To a solution of Ethyl-[(2S,4R)-2-(tert-butoxycarbonylamino)methyl]-4-hydroxy pyrrolidin-1-yl]-propeonate (1.5 g, 4.74 mmol), N3-benzoyl thymine and triphenyl phosphene in dry benzene cooled to 4°C, added DEAD drop wise with syringe under nitrogen atmosphere. The reaction mixture was stirred for another 5 h at rt. The reaction mixture was evaporated to dryness and the residue was purified by column chromatography to obtain monomer ethyl ester 4. (1.8 g, yield 72%, Rf = 0.76, MeOH:CH$_2$Cl$_2$:1:9). $[\alpha]_D^{20} = -69.16^\circ$ (c 0.50, CH$_2$Cl$_2$); $^1$H-NMR (CHCl$_3$-d, 500 MHz); $\delta_H$ 1.29 (t, 3H, ester CH$_3$), 1.41 (s, 9H, Boc), 1.55-1.8 (m, 1H, C3H$_2$), 1.96 (s, 3H, Thy CH$_3$), 2.15-2.3 (m, 1H, C3H), 2.35-2.7 (m, 5H, -CH$_2$-CH$_2$-, C2H), 3.0-3.2 (m, 3H, C5H$_2$, CH$_2$NH), 3.25-3.4 (m, 1H, C5H), 3.95-4.15 (m, 2H, ester
CH2), 4.95-5.15 (m, 1H, C4H), 5.2-5.3 (bd, 1H, NH), 7.15-7.25 (m, 2H, Ar), 7.25-7.35 (m, 1H, Ar),
7.35-8.00 (m, 3H, thy CH, Ar). 13C-NMR (CHCl3-d, 500 MHz); δC  12.1, 13.9, 28.0, 33.2, 35.5, 39.5,
47.5, 57.3, 58.5, 60.4, 63.0, 79.0, 110.6, 128.8, 130.0, 131.6, 134.5, 135.4, 149.6, 156.0, 162.2, 168.9,
172.2. Anal Calcd (%) for C27H36N4O7: C, 61.36; H, 6.81; N, 10.60, Found C, 61.13; H, 6.97; N, 10.43;
LCMS; 528.01 [M]^+, 428.01 [M-tBoc]⁺.

[(2S,4S)-2-(tert-butoxycarbonylaminomethyl)-4-(thymin-1-yl)-pyrrolidin-1-yl]-propeonic acid (5):
The monomer ethyl ester 4 (1.5 g, 2.8 mmol) was dissolved in methanol (6 ml), 2N NaOH (6 ml) was
added and stirred for 7 h. The reaction mixture was washed with ethyl acetate to remove benzoic acid.
The aqueous layer was then neutralized with cation exchange resin (Dowex H+). After removal of the
resin by filtration the aqueous layer was concentrated to a residue which on co-evaporation with
dichloromethane (10 ml x 2) afford monomer 5. (1.1 g, yield 98%), mp, 119-121°C; [α]D20 –78.0° (c
0.5, CH2Cl2); 1H-NMR (D2O, 500 MHz); δH  1.39 (s, 9H, Boc), 1.82 (s, 3H, thy CH3), 2.1-2.3 (m, 1H,
C3H'), 2.45-2.75 (m, 2H, N-CH2-CH2-CO), 2.8-2.9 (m, 1H, C2H), 2.95-3.15 (m, 1H, C5H'), 3.4-3.8
(m, 5H, COCH2CH2-N, CH2NH), 3.95-4.15 (m, 1H, C4H), 7.44 (s, 1H, thy CH). 13C-NMR (D2O, 500
MHz); δC  11.0, 27.45, 31.9, 32.4, 38.0, 50.3, 56.7, 57.5, 66.8, 81.4, 110.4, 142.6, 157.6, 158.0, 168.5,
177.7. Anal Calcd (%) for C18H28N4O6: C, 54.54; H, 7.07; N, 14.14, Found C, 54.23; H, 7.29; N, 13.97;
LCMS; 397.05 [M+H]^+, 297.05 [M+1-tBoc]^+. 
Chloroform-d

$^{28.19}_{78.76} \quad 61.60 \quad 60.28 \quad 51.04 \quad 49.14 \quad 40.72 \quad 33.74 \quad 13.96$
Chloroform-d

(2S,4S)-4

COOEt

NHBoc

Chloroform-d
(2S,4S)-5

COOH

NHBoc
HPLC profile of aegPNA 6
HPLC profile of *bepPNA 7*

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HPLC profile of bepPNA 8
HPLC profile of *bepPNA 9*
HPLC profile of bepPNA 10

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HPLC profile of bepPNA 11

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LC-TOF-MS of *aegPNA 6*

\[ [M+3H]^+ = 2275.90 \]
LC-TOF-MS of *bep*PNA 7

\[ [M+2H]^+ = 2288.0 \]
LC-TOF-MS of *bepPNA 8

\[ [M+2H]^+ = 2288.0 \]
LC-TOF-MS of bepPNA 9

\[ [M+2H]^+ = 2288.04 \]
LC-TOF-MS of bepPNA 10

\[ [\text{M+3H}]^+ = 2326.06 \]
LC-TOF-MS of bepPNA 11

\[ [M+3H]^+ = 2374.43 \]
A. Melting curves of a) *aeg*PNA 6, b) *bep*PNA 7, c) *bep*PNA 8, d) *bep*PNA 9, e) *bep*PNA 10 and f) *bep*PNA 11 with DNA and B. corresponding first derivative curves.

A. Melting curves of a) *aeg*PNA 6, b) *bep*PNA 7, c) *bep*PNA 8, d) *bep*PNA 9, e) *bep*PNA 10 and f) *bep*PNA 11 with RNA (poly rA) and B. corresponding first derivative curves.
**UV-Tm measurements:** The complementary DNA oligomers were synthesised on an Applied Biosystems 3900 DNA Synthesizer. The poly rA was obtained from Sigma-Aldrich. The concentration was calculated on the basis of absorbance from the molar extinction coefficients of the corresponding nucleobases. The complexes were prepared in 10 mM sodium phosphate buffer, pH 7.0 containing NaCl (100 mM) and EDTA (0.1 mM) and were annealed by keeping the samples at 90°C for 5 minutes followed by slow cooling to room temperature. Absorbance versus temperature profiles were obtained by monitoring at 260 nm with Perkin-Elmer Lambda 35 spectrophotometer scanning from 5 to 90°C at a ramp rate of 0.2°C per minute. The data were processed using Microcal Origin 5.0 and Tm values derived from the derivative curves.

**Circular Dichroism:** CD spectra were recorded on a JASCO J-715 spectropolarimeter. The CD spectra of the PNA:DNA complexes and the relevant single strands were recorded in 10 mM sodium phosphate buffer, 100 mM NaCl, 0.1 mM EDTA, pH 7.0. The temperature of the circulating water was kept below the melting temperature of the PNA:DNA complexes, i.e., at 10°C. The CD spectra of the homothymine T8 single strands were recorded as an accumulation of 10 scans from 320 to 195 nm using a 1 cm cell, a resolution of 0.1 nm, band-width of 1.0 nm, sensitivity of 2 m deg, response 2 sec and a scan speed of 50 nm/min. for the PNA2:DNA/RNA complexes, spectra were recorded as an accumulation of 8 scans, response of 1 sec and a scan speed of 200 nm/min.

**Gel mobility shift assay:** The PNAs 6-11 were individually mixed with DNA in 2:1 ratio (PNA strand, 0.4 mM and DNA 0.2 mM) in water. The samples were lyophilized to dryness and re-suspended in sodium phosphate buffer (10 mM, pH 7.0, 10µl) containing EDTA (0.1 mM). The samples were annealed by heating to 85°C for 5 min followed by slow cooling to RT and refrigeration at 4°C overnight. To this, 10µl of 40% sucrose in TBE buffer pH 8.0 was added and the sample was loaded on the gel. Bromophenol blue (BPB) was used as the tracer dye separately in an adjacent well. Gel electrophoresis was performed on a 15% non-denaturing polyacrylamide gel (acrylamide:bis-acrylamide, 29:1) at constant power supply of 200 V and 10 mA, until the BPB migrated to three-fourth of the gel length. During electrophoresis the temperature was maintained at 10°C. The spots were visualized through UV shadowing by illuminating the gel placed on a fluorescent silica gel plate, GF254 using UV-light.
CD-curves for \textit{bep}PNA 8 and the complementary RNA (poly rA) mixtures in the molar ratios of 0:100, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, 100:0 (Buffer, 10 mM Sodium phosphate pH 7.0, 100 mM NaCl, 0.1 mM EDTA)

CD-Job’s plot for \textit{bep}PNA 8 and the complementary RNA (poly rA) mixtures in the molar ratios of 0:100, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, 100:0 at 248 nm (Buffer, 10 mM Sodium phosphate pH 7.0, 100 mM NaCl, 0.1 mM EDTA)
UV-Job’s plot for *bepPNA 10* and the complementary RNA (poly rA) mixtures in the molar ratios of 0:100, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, 100:0 at 260 nm (Buffer, 10 mM Sodium phosphate pH 7.0, 100 mM NaCl, 0.1 mM EDTA)

UV-Job’s plot for *bepPNA 11* and the complementary RNA (poly rA) mixtures in the molar ratios of 0:100, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, 100:0 at 260 nm (Buffer, 10 mM Sodium phosphate pH 7.0, 100 mM NaCl, 0.1 mM EDTA)
**Figure.** pH titration curve of (2S,4S)-bepPNA monomer 5 (free amine) with NaOH and its derivative.
Single-strand melting curves. (Buffer, 10 mM Sodium phosphate pH 7.0, 100 mM NaCl, 0.1 mM EDTA)