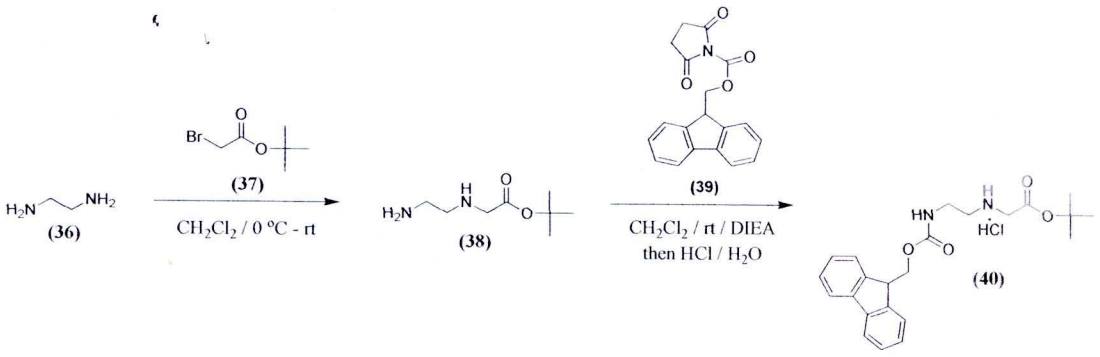
CHAPTER III

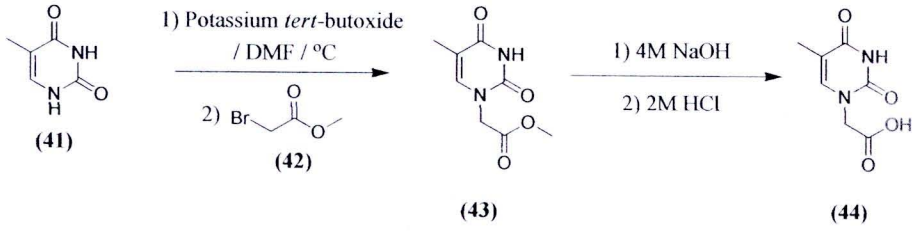
RESEARCH METHOLOGY

Investigation of novel *ss aeg*PNA oligomers-MNPs bimolecular probe was separated into two parts as following: (I) synthesis of *ss aeg*PNA oligomers, (II) immobilization of *ss aeg*PNA oligomers onto functionalized MNPs.

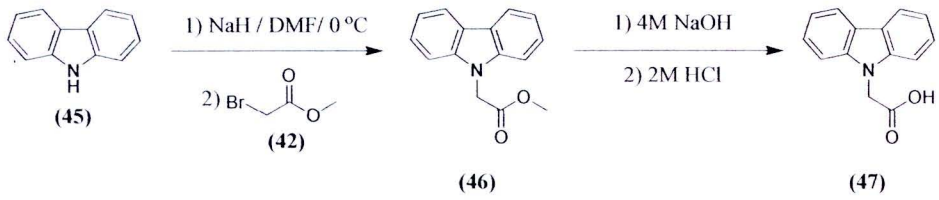
I. Synthesis of *ss aeg*PNA oligomers



Scheme 5 Synthesis of *tert*-Butyl *N*-[2-(*N'*-9-fluorenylmethoxycarbonyl)aminoethyl] glyceinate hydrochloride (40)

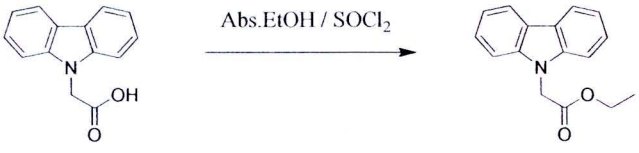


Scheme 6 Synthesis of thymine-1-yl-acetic acid (44)



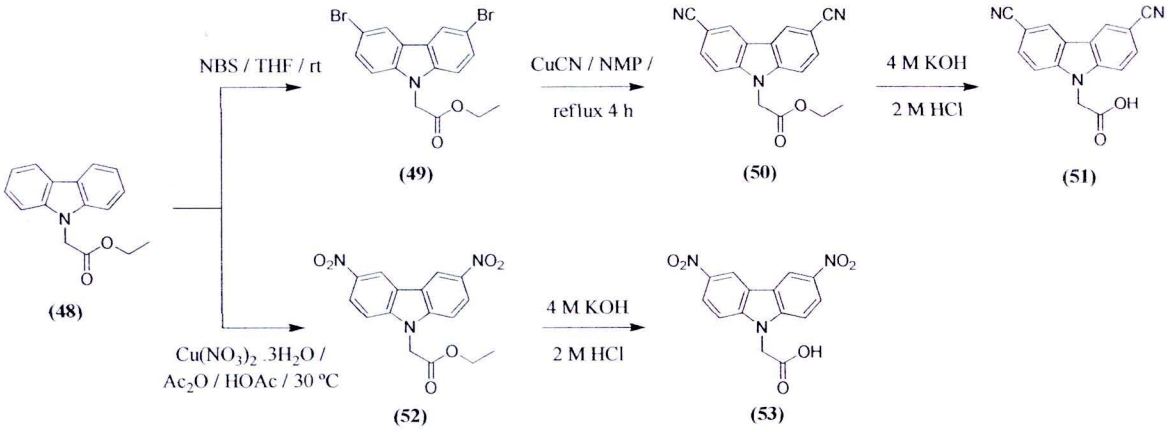
(45) (42) (46) (47)

Scheme 7 Synthesis of carbazole-9-yl-acetic acid (47)



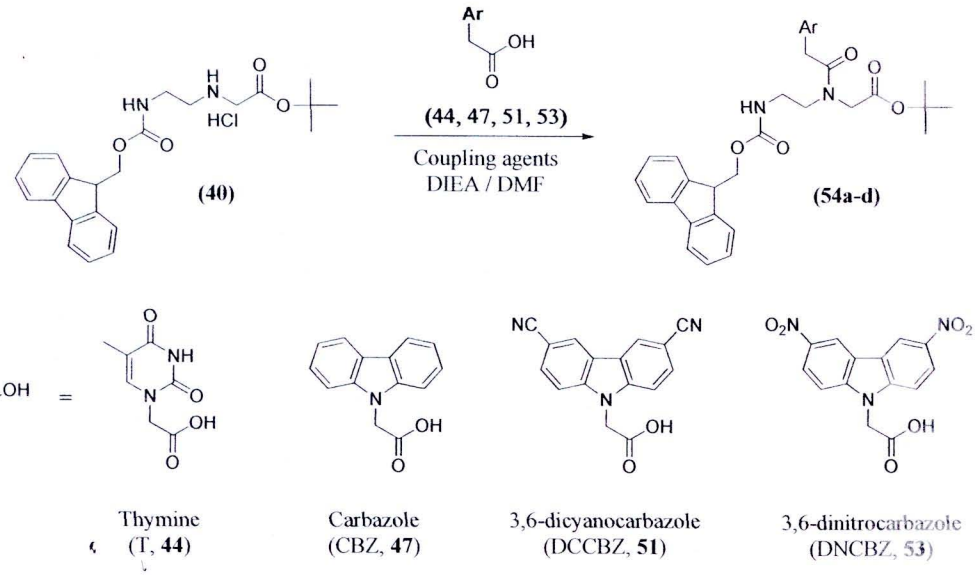
(47) (48)

Scheme 8 Synthesis of carbazole-9-yl-ethyl acetate (48)



(48) (49) (50) (51) (52) (53)

Scheme 9 Synthesis of 3,6-disubstituted carbazole-9-yl-acetic acid (51,53)



Scheme 10 Synthesis of Fmoc *aeg*PNA monomers (54a-d)

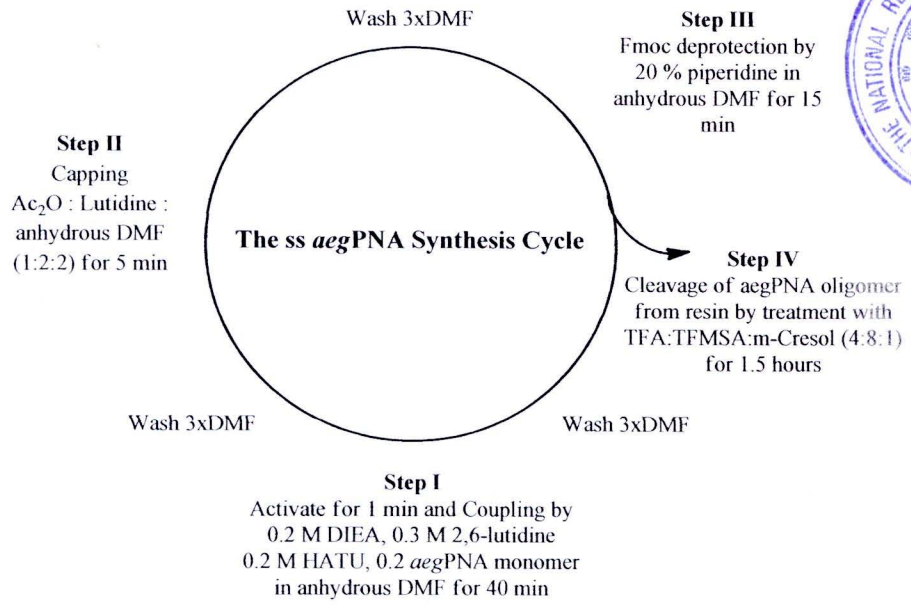
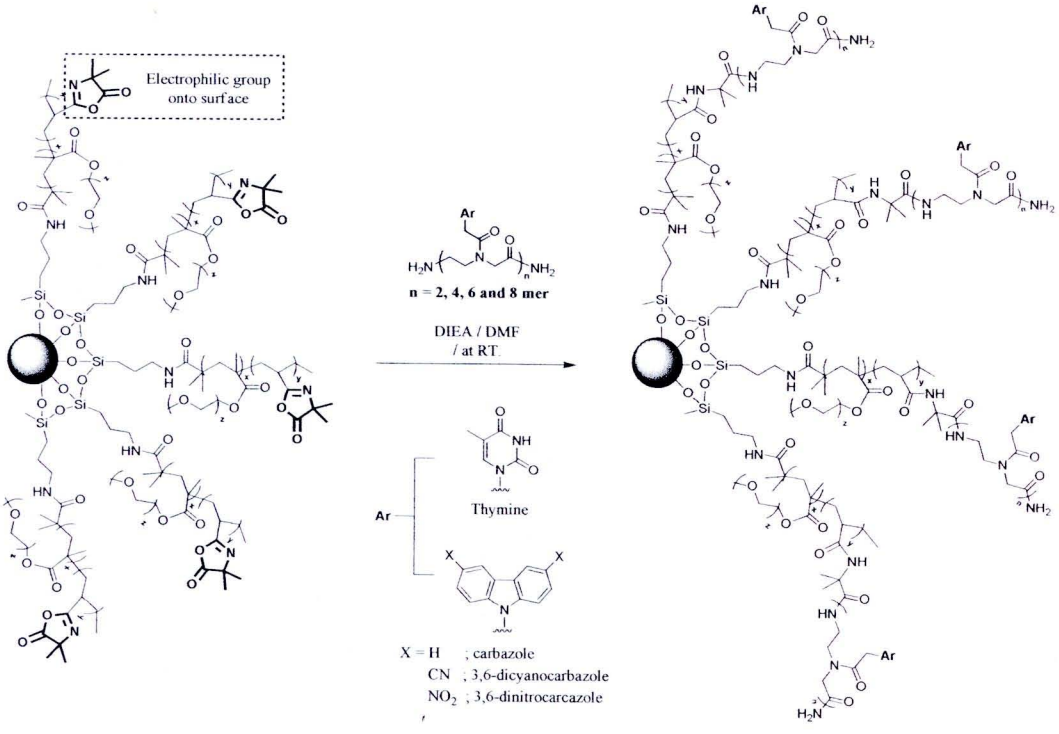


Figure 21 Synthesis of *ss aeg*PNA oligomers (56a-g)

Table 2 Sequence of *ss aeg*PNA oligomers (56a-g)

Compound	Code of *aeg*PNA oligomers	Base sequence (*N*- to *C*- terminus)
Dimer	T_2	NH_2-TT-$CONH_2$
Tetramer	T_4	NH_2-TTTT-$CONH_2$
Hexamer	T_6	NH_2-TTTTTT-$CONH_2$
	TC_6	NH_2-CBZ TTTTT-$CONH_2$
	TCC_6	NH_2-DCCBZ TTTTT-$CONH_2$
	TNC_6	NH_2-DNCBZ TTTTT-$CONH_2$
Octamer	T_8	NH_2-TTTTTTTT-$CONH_2$

II. Immobilization of *ss aeg*PNA oligomers onto functionalized MNPs



Scheme 11 Immobilization of *ss aeg*PNA oligomers onto electrophilic MNPs

General Procedure

1. Equipments

Unless otherwise stated, all glasswares were oven dried. The progresses of reactions were monitored by thin layer chromatography (TLC) (Merck D.C. silica gel 60 F_{254} 0.2 mm-pre-coated aluminium plates). Visualization of TLC plates was accomplished using either UV light (254 nm) or ninhydrin. Evaporation of solvents was performed on Büchi Rotavapor R-114 with a water aspirator model B-480 or a Refco Vacubrand pump. The weight of all chemical substances was determined on sartorius electrical balance. Column chromatography was performed on silica gel having 60-200 μm for column chromatography.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker NMR spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz ^{13}C NMR in appropriate deuterated solvents. Chemical shifts (δ) are reported in part per million (ppm) relative to either tetramethylsiline (TMS) or the residual protonated solvent signal as a reference.

The *ss aeg*PNA oligomers were purified using reverse phase HPLC on a Agilent 1100 series controller system equipped with gradient pump and Agilent 1100 series photodiode array detector. A Vertisep^{TM} C_{18} HPLC column 5 μm particle size 10 x 150 mm was used for the purification of *aeg*PNA oligomers. Peak monitoring and data processing were performed on the base Empower software. The fractions were manually collected and assisted by real-time HPLC chromatogram monitoring. The combined pure *ss aeg*PNA oligomers fractions were concentrated *in vacuo* and Freeze Drying (FD1 0-110, Heto Labequipment). MALDI-TOF mass spectra were measured in positive ion mode with a static accelerating voltage of +20 kV on a Bruker Daltonics Microflex MALDI-TOF mass spectrometer.

The concentration of all *ss aeg*PNA oligomers was performed using a Jasco V-650 Uv-vis spectrophotometer. Immobilization of *ss aeg*PNA oligomers onto MNPs was verified using a Perkin Elmer Model LS 55 Luminescence spectrometer and FT-IR spectra were recorded on a Perkin-Elmer Model 1600 Series FT-IR spectrometer in the

wavenumber range of 4000-400 cm^{-1}. The sample was made by the pressed disc method after mixing dried solid samples with KBr at normal mode and ATR mode.

The active site of electrophilic on MNPs were quantitatively determined using 8603 Mettler Toledo SevenEasy pH conductometer

2. Materials

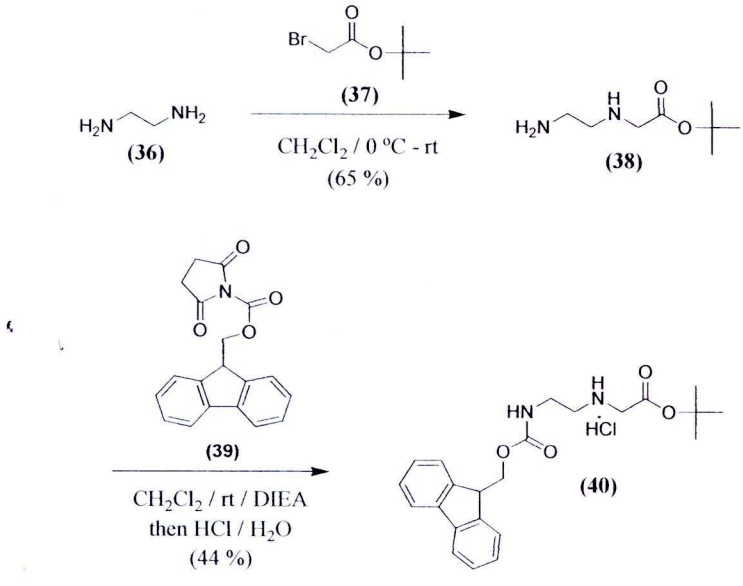
All commercially available chemical were purchased from Fluka Co., Ltd, Merck Co., Ltd, Acros Co., Ltd, Aldrich Co., Ltd, Chempep Co., Ltd and Lab Scan Co., Ltd., and all chemical were used without purification unless otherwise noticed. Commercial grade solvents for column chromatography were distilled prior to use. Solvent for reactions were AR grade and were used without purification. Dichloromethane (DCM) and *N*,*N*-dimethylmethanamide (DMF) were distilled over fresh calcium hydride (CaH_2) under N_2 atmosphere. HPLC grade acetonitrile for HPLC experiments, obtained from Lab Scan, were filtered through Nylon membrane before use. DI water was obtained from ultrapure water system with ELGA (England). For peptide synthesis, anhydrous DMF ($H_2O \leq 0.01$ % dried over activated 3A molecular sieves) using as solvent was purchased from Merck. Methylbenzhylamine resin (MBHA resin loading 0.7-1.4 mmole/g) used as solid support was purchased from Aldrich. Coupling agents were obtained from commercial source and used without purification

IUPAC name 99 % *tert*-Butyl bromoacetate, 96% Carbazole, *N*,*N*-Diisopropylethylamine (DIEA), 98% Ethylenediamine, 98% 1-Hydroxybenzotriazole (HOBt), 99% 2,6-Lutidine, 98% Potassium *tert*-butoxide, 99% Thymine, 99.5% Trifluoroacetic acid (TFA), Acetic anhydride (Ac_2O), 97% Methyl bromoacetate, 98% Trifluoromethanesulfonic acid (TFMSA), 98% *N*-9-fluorenylmethoxycarbonyloxy (succinimide) (Fmoc-OSu), 96% *O*-(Benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate (TBTU), 95% *N*-Bromosuccinimide (NBS), 99% Piperidine, 99.5% Copper (II) nitrate trihydrate ($Cu(NO_3)_2.H_2O$), 99.8% *O*-(7-azabenzotriazol-l-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU)

Deuterated solvent for NMR characterization, Chloroform-*d* ($CDCl_3$) and Dimethyl sulfoxide (DMSO-d_6) were purchased from Aldrich. Electrophilic MNPs were synthesized as previous published by Ruttakornpituk, M et al. [76].

Synthesis of Fmoc *aeg*PNA monomers

1. Synthesis of *tert*-Butyl *N*-[2-(*N'*-9-fluorenylmethoxycarbonyl) aminoethyl] glyceinate hydrochioride (40) [77]



Scheme 12 Synthesis of *tert*-Butyl *N*-[2-(*N'*-9-fluorenylmethoxycarbonyl)aminoethyl] glyceinate hydrochloride (40)

To a solution of ethylenediamine **(36)** (48.9 mL, 0.731 mol) in anhydrous CH_2Cl_2 (300 mL) cooled in an ice bath, was added dropwise over period of 5 h. to a solution of *tert*-Butyl bromoacetate **(37)** (13.5 mL, 0.091 mol) in anhydrous CH_2Cl_2 (150 mL). After stirring overnight, the reaction mixture was washed with water (5 x 120 mL), dried over anhydrous Na_2SO_4, concentrated, and dried *in vacuo* to give crude product of *tert*-butyl *N*-(2-aminoethyl)glycinate **(38)** as an oil. Without further purification, **38** (10.2 g, 0.058 mol) was dissolved in anhydrous CH_2Cl_2 (340 mL), and diisopropylethylamine (DIEA) (9.22 mL, 0.055 mol) was added. Then, a solution of *N*-(9-fluorenylmethoxycarbonyloxy) succinimide **(39)** (18.6 g, 0.055 mol) in anhydrous CH_2Cl_2 (100 mL) was added dropwise over 5 h. The resulting solution was stirred at room temperature approximately 18 h. and was then washed with 1 N aqueous HCl solution

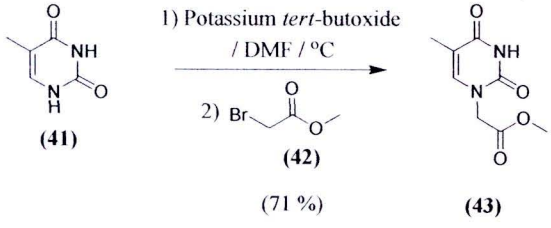
(5 x 100 mL), and brine (1 x 100 mL). The organic layer was dried over anhydrous Na_2SO_4, partially concentrated *in vacuo*, and stored at - 20 °C overnight. The precipitate was collected by filtration and washed with cooled CH_2Cl_2 until the filtrate was colorless. The solids crude product were recrystallized in 40 % acetone:CH_2Cl_2 and dried *in vacuo* to produce the HCl salt of **40** as a white solid in 26 % yield for two steps (10.5 g): R_f = 0.44 (19:1 CH_2Cl_2/MeOH)

^{1}H NMR 400 MHz (DMSO-d_6) δ 9.15 (br, s, 2H, NH), 7.89 (d, 2H, *J*=7.2 Hz, Ar), 7.69 (d, 2H, *J*=7.2 Hz, Ar), 7.52 (t, 1H, *J*=7.3 Hz, NH), 7.42 (t, 2H, *J*=7.2 Hz, Ar), 7.34 (t, 2H, *J*=7.2 Hz, Ar), 4.35 (d, 2H, CH_2, *J*=6.8 Hz), 4.23 (t, 1H, CH, *J*=6.8 Hz), 3.87 (s, 2H, CH_2), 3.31-3.29 (m, 2H, CH_2), 2.99 (t, 2H, CH_2, *J*=6.2 Hz), 1.46 (s, 9H, $C(CH_3)_3$)

^{13}C NMR 100 MHz (DMSO-d_6) δ 165.9, 156.5, 143.9, 141.0, 127.8, 127.6, 125.2, 125.0, 120.1, 83.2, 65.8, 55.2, 47.3, 46.4, 36.5, 27.7

HRMS: Calcd. For $C_{23}H_{29}N_2O_4$: *m/z* 397.21, found *m/z* 396.98 $(M+H)^+$

2. Synthesis of thymine-1-yl-methyl acetate (43) [78]



Scheme 13 Synthesis of thymine-1-yl-methyl acetate (43)

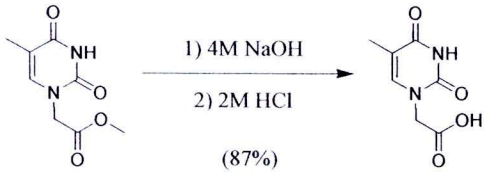
To a suspension of thymine **(41)** (2.00 g, 15.8 mmol) and Potassium *tert*-butoxide (2.13 g, 19.0 mmol) in anhydrous DMF (40 mL) cooled in a water-ice bath, was added dropwise over of 40 min. to methyl bromoacetate **(42)** (1.74 mL, 19.0 mmol). After stirring overnight, the reaction mixture was concentrated *in vacuo*. The residue was brought up in CH_2Cl_2, and the solution was washed with saturated $NaHCO_3$ (5 x 30 mL) and brine (3 x 15 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and

concentrated *in vacuo* to yield desired product **(43)** as a white solid in 71% yield (2.10 g): $R_f = 0.75$ (9:1 CH_2Cl_2/MeOH)

^1H NMR 400 MHz, ($CDCl_3$) δ 8.51 (br, 1H, NH), 6.92 (s, 1H, Ar), 4.43 (s, 2H, CH_2), 3.79 (s, 3H, CH_3), 1.92 (s, 3H, CH_3)

^{13}C NMR 100 MHz ($CDCl_3$) 140.2, 11.5, 53.0, 48.7, 29.8, 12.4, 1.2

3. Synthesis of thymine-1-yl-acetic acid (44) [78]



(43) **(44)**

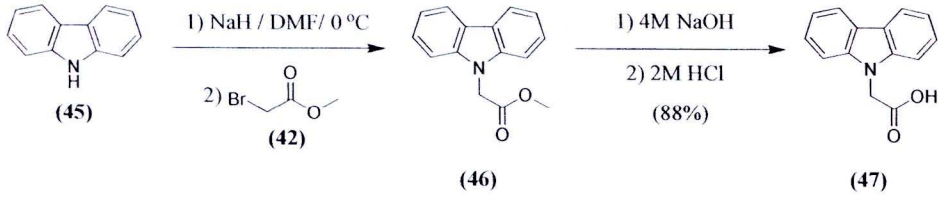
Scheme 14 Synthesis of thymine-1-yl-acetic acid (44)

A solution of **43** in 4M NaOH (5 mL) was stirred at room temperature for 40 min. The mixture was cooled in an ice bath, treated with 2 M HCl (pH = 2), and stirred for 30 min. The percipitate was filtered and dried in *in vacuo* to give **44** as a white solid in 87 % yield. (1.69 g): $R_f = 0.11$ (9:1 CH_2Cl_2/MeOH)

^1H NMR 400 MHz (DMSO-d_6) δ 11.33 (s, lH, NH), 7.50 (s, lH, Ar), 4.40 (s, 2H, CH_2), 1.74 (s, 3H, CH_3)

^{13}C NMR 100 MHz (DMSO-d_6) δ 169.6, 164.4, 151.0, 141.8, 108.3, 48.4, 11.9

4. Synthesis of carbazole-9-yl-acetic acid (47) [79]



(45) (42) (46) (47)

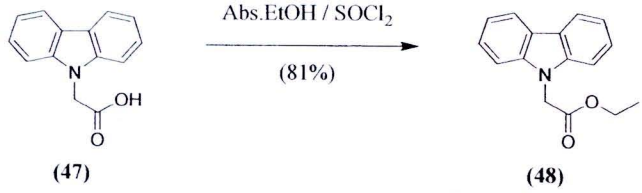
Scheme 15 Synthesis of carbazole-9-yl-acetic acid (47)

To a solution of carbazole **(45)** (3.00 g, 17.9 mmmol) and sodium hydride (1.29 g, 53.8 mmol) in anhydrous DMF (40 mL) cooled in a water-ice bath, was added dropwise over of 30 min. to **42** (2.04 mL, 21.5 mmol). After stirring overnight, the reaction mixture was concentrated *in vacuo* to give crude product of **46**. Then, 4M NaOH (5 mL) was added and then stirred at room temperature for 2 h. The reaction mixture was then cooled in a water-ice bath, treated with 2 M HCl (pH = 2), stirred for 30 min. to give crude product as white solid. Recrystallization of crude product from toluene to yield desired compound **47** as a white crystalline in 88 % yield. (3.55 g): R_f = 0.58 (8:2 CH_2Cl_2/MeOH)

^{1}H NMR 400 MHz (DMSO-d_6) δ 8.15 (d, 2H, Ar, *J*=7.6 Hz), 7.55 (d, 2H, Ar *J*=7.6 Hz), 7.43 (t, 2H, Ar, *J*=7.6 Hz), 7.22 (t, 2H, Ar, *J*=7.6 Hz), 5.22 (s, 2H, CH_2)

^{13}C NMR 100 MHz (DMSO-d_6) δ 170.4, 140.4, 125.9, 122.3, 120.2, 119.2, 109.3, 43.9

5. Synthesis of carbazole-9-yl-ethyl acetate (48) [79]



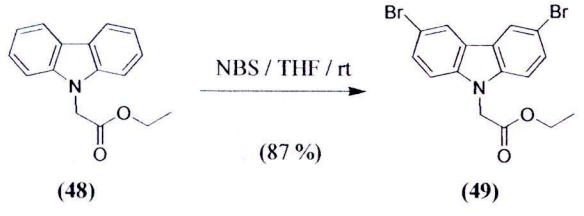
Scheme 16 Synthesis of carbazole-9-yl-ethyl acetate (48)

To a cooled ice bath solution of **47** (2.50 g, 11.1 mmol) in EtOH (20 mL), was added thionyl chloride (0.96 mL, 13.3 mmol). The reaction mixture was stirred at 0 °C for 1.5 h. Then, the reaction mixture was concentrated and triturate with water. After that, precipitate was filtered and dried *in vacuo* to give **48** as a white solid in 81 % yield. (2.28 g): R_f = 0.74 (19:1 CH_2Cl_2/MeOH)

^{1}H NMR 400 MHz ($CDCl_3$) δ 8.11 (d, 2H, Ar, *J*=7.6 Hz), 7.46 (t, 2H, Ar, *J*=7.6, Hz), 7.33 (d, 2H, Ar, *J*=7.6), 7.27 (t, 2H, *J*=7.6 Hz, Ar), 4.99 (s, 2H, CH_2), 4.20 (q, 2H, CH2, *J*=7.2 Hz), 1.22 (t, 3H, CH_3 *J*=7.2 Hz)

^{13}C NMR 100 MHz ($CDCl_3$) δ 168.7, 140.7, 125.9, 123.2, 120.4, 119.8, 108.7, 61.7, 44.5, 14.4

6. Synthesis of 3,6-dibromocarbazole-9-yl-ethyl acetate (49) [80]



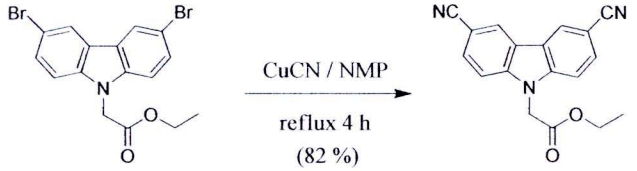
Scheme 17 Synthesis of 3,6-dibromocarbazole-9-yl-ethyl acetate (49)

To a solution *N*-bromosuccinimide (NBS) (1.12 g, 6.32 mmol) in anhydrous THF, was added in a small portion to a solution of **48** (0.80 g, 3.16 mmol). The reaction mixture was stirred at room temperature for 4 h. Then, the reaction mixture was brought up in CH_2Cl_2, washed with water (5 x 50 mL) and brine (3 x 20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to produce **49** as a white solid in 87 % yields. (1.13g): R_f = 0.16 (9:1 Hexane/EtOAc)

^{1}H NMR 400 MHz ($CDCl_3$) δ 8.16 (s, 2H, Ar), 7.58 (d, 2H, Ar, *J*=8.7), 7.22 (d, 2H, Ar, *J*=8.7), 4.95 (s, 2H, CH_2), 4.22 (q, 2H, CH_2, *J*=7.2 Hz), 1.24 (t, 3H, CH_3, *J*=7.2 Hz)

^{13}C NMR 100 MHz ($CDCl_3$) δ 167.7, 139.6, 129.1, 123.7, 123.5, 113.1, 110.3, 61.9, 44.3, 14.3

7. Synthesis of 3,6-cyanocarbazole-9-yl-ethyl acetate (50) [80]



(49) (50)

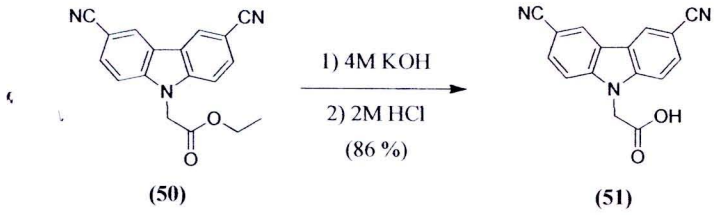
Scheme 18 Synthesis of 3,6-dicyanocarbazole-9-yl-ethyl acetate (50)

To a solution of **49** (0.50 g, 1.22 mmol) in NMP, was added CuCN (0.65 g, 7.29 mmol) and refluxed under nitrogen atmosphere for 5 h. After that, the hot reaction mixture was poured into a solution of $FeCl_3$: HCl : H_2O (2:1:4), and stirred at 50-60 °C for 30 min. The crude product was filtered, brought up in CH_2Cl_2, and washed with water (5 x 50 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* and then recrystallized from methanol to give **50** as a gray powder 82 % yield. (0.30 g): R_f = 0.34 (100 EtOAc)

^{1}H NMR 400 MHz (DMSO-d_6) δ 8.85 (s, 2H, Ar), 7.94 (d, 2H, Ar, J=8.6 Hz), 7.86 (d, 2H, Ar, J=8.6 Hz), 5.54 (s, 2H, CH_2), 4.15 (q, 2H, CH_2, J=6.8 Hz), 1.20 (t, 3H, CH3, J=6.8)

^{13}C NMR 100 MHz (DMSO-d_6) δ 168.0, 143.2, 130.4, 126.3, 121.8, 119.9, 111.6, 102.7, 61.3, 44.4, 14.2

8. Synthesis of 3,6-cyanocarbazole-9-yl-acetic acid (51) [79]



(50) (51)

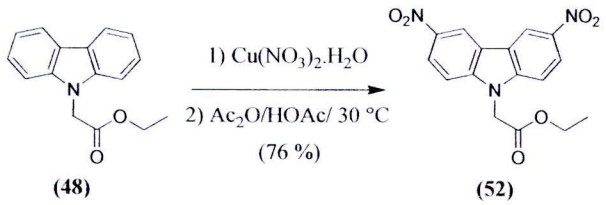
Scheme 19 Synthesis of 3,6-dicyanocarbazole-9-yl-acetic acid (51)

To a solution of **50** in 4M KOH (5 mL), was stirred at room temperature for 1 h. The mixture was cooled in a water-ice bath, treated with 2 M HCl (pH = 2), and stirred for 30 min. The product was filtered and dried in *in vacuo* to give **51** as a gray solid in 86 % yield. (0.23 g): R_f = 0.13 (100 EtOAc)

^{1}H NMR 400 MHz (DMSO-d_6) δ 8.85 (s, 2H, Ar), 7.93 (d, 2H, Ar, J=8.6 Hz), 7.86 (d, 2H, Ar, J=8.6 Hz), 5.43 (s, 2H, CH_2)

^{13}C NMR 100 MHz (DMSO-d_6) δ 169.3, 142.9, 130.0, 126.7, 121.7, 119.9, 111.6, 102.3, 44.6

9. Synthesis of 3,6-dinitrocarbazole-9-yl-ethyl acetate (52) [80]



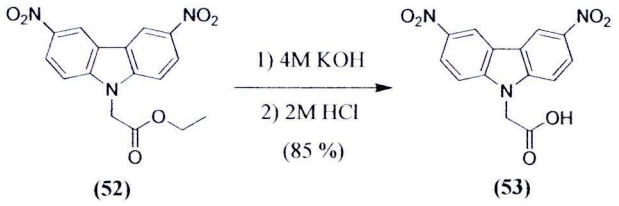
Scheme 20 Synthesis of 3,6-dinitrocarbazole-9-yl-ethyl acetate (52)

To a solution of $Cu(NO_3)_2.3H_2O$ (1.19 g, 4.93 mmol) in mixture of Ac_2O : HOAc (4:1), was added in to a solution of **48** (0.5 g, 1.94 mmol). The reaction mixture was stirred at room temperature for 1 h. and then the reaction mixture was poured into ice water. The precipitate was filtered and dried *in vacuo* and was purified by with flash column chromatography (CH_2Cl_2) to give **52** as a yellow solid in 76 % yield (0.51 g): R_f = 0.61 (100 CH_2Cl_2)

^{1}H NMR 400 MHz (DMSO-d_6) δ 9.50 (s, 2H, Ar), 8.42 (d, 2H, Ar, J=9.2 Hz), 7.88 (d, 2H, Ar, J= 9.2 Hz), 5.61 (s, 2H, CH_2), 4.17 (q, 2H, CH_2, J=6.8 Hz), 1.22 (t, 3H, CH_3, J=6.8 Hz)

^{13}C NMR 100 MHz (DMSO-d_6) δ 167.7, 145.1, 141.5, 122.8, 122.1, 118.3, 111.0, 61.3, 44.6, 13.9

10. Synthesis of 3,6-dinitrocarbazole-9-yl-acetic acid (53) [79]



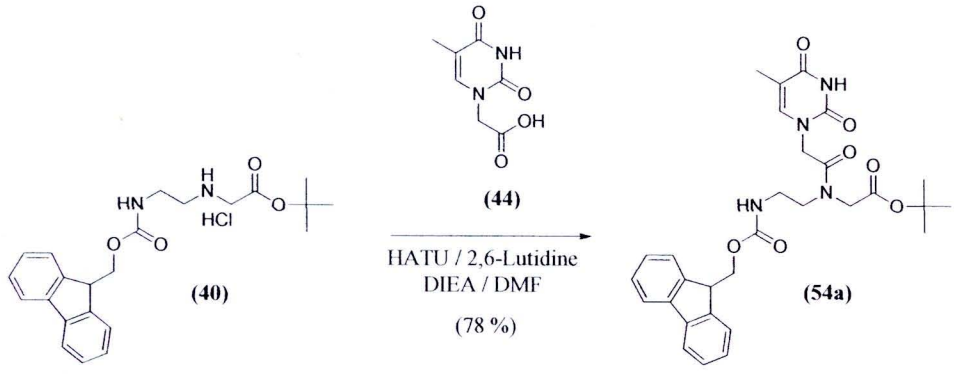
Scheme 21 Synthesis of 3,6-dinitrocarbazole-9-yl-acetic acid (53)

A solution of **52** in 4M KOH (5 mL) was stirred at room temperature for 1 hour. The mixture was cooled in a water-ice bath, treated with 2 M HCl (pH = 2), and stirred for 30 min. The product was filtered and dried in *in vacuo* to give **53** as a yellow solid in 85 % yield (0.31 g): R_f = 0.26 (19:1 CH_2Cl_2/MeOH)

^{1}H NMR 400 MHz (DMSO-d_6) δ 9.47 (s, 2H, Ar), 8.41 (d, 2H, Ar, J=9.2), 7.89 (d, 2H, Ar J=9.2), 5.48 (s, 2H, CH_2)

^{13}C NMR 100 MHz (DMSO-d_6) δ 169.9, 145.7, 142.2, 122.7, 122.1, 119.3, 111.0, 46.0.

11. Synthesis of *tert*-Butyl *N*-[2-(*N'*-9 fluorenylmethoxycarbonyl) aminoethyl]-*N*-[(thymine-1-yl)acetyl]glycinate (54a) [77]



Scheme 22 Synthesis of *tert*-Butyl *N*-[2-(*N'*-9 fluorenylmethoxycarbonyl) aminoethyl]-*N*-[(thymine-1-yl)acetyl]glycinate (54a)

To a solution of **40** (0.50 g, 1.15 mmol) in anhydrous DMF (5 mL), was added DIEA (0.41 mL, 2.31 mmol), thymine-1-ylacetic acid **(44)** (0.25 g, 1.38 mmol), HATU (0.70 g, 1.84 mmol), and 2,6-lutidine (0.40 mL, 3.45 mmol). The reaction mixture was stirred for 5 h. at room temperature and then concentrated *in vacuo*. The residue was brought up in CH_2Cl_2 and washed with saturated $NaHCO_3$ (5 x 50 mL) and brine (5 x 30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in*

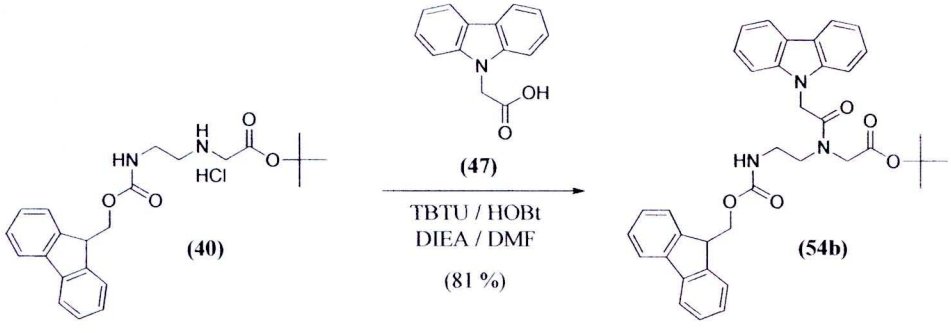
vacuo. The crude product was purified by column chromatography (9:1 CH_2Cl_2/MeOH). The desired product **(54a)** was obtained as a white solid in 78 % yield (0.51 g): R_f = 0.58 (9:1 CH_2Cl_2/MeOH)

^{1}H NMR 400 MHz ($CDCl_3$) δ 8.59/8.56 (rotamer br, lH, NH,), 7.75-7.73 (m, 2H, Ar), 7.61-7.57 (m, 2H, Ar), 7.40-7.36 (m, 2H, Ar), 7.31-7.27 (m, 2H, Ar), 6.96/6.83 (rotamer s, 1H, Ar) 5.98/5.39 (rotamer br, 1H, NH), 4.45-4.36 (rotamer m, 3H, CH_2 and CH), 4.21-4.19 (rotamer m, 2H), 4.06/3.93 (rotamer s, 2H), 3.54-2.94 (m, 4 H), 1.86/1.85 (rotamer s, 3H, CH_3), 1.64/1.46 (rotamer s, 9H, $C(CH_3)$)

^{13}C NMR 100 MHz ($CDCl_3$) δ 168.9, 168.7, 144.1, 143.9, 141.4, 141.2, 141.0, 127.9, 127.7, 127.2, 127.1, 125.2, 125.1, 120.1, 110.9, 110.7, 83.9, 82.8, 66.9, 51.4, 50.0, 49.1, 47.7, 47.4, 39.4, 39.1, 28.2, 12.4

HRMS: Calcd. For $C_{30}H_{34}N_4O_7$: *m/z* 562.61, found *m/z* 563.58 (M+H)

12. Synthesis of *tert*-Butyl *N*-[2-(*N'*-9 fluorenylmethoxycarbonyl) aminoethyl]-*N*-[(carbazole-9-yl)acetyl]glycinate (54b) [79]



Scheme 23 Synthesis of *tert*-Butyl *N*-[2-(*N'*-9 fluorenylmethoxycarbonyl) aminoethyl]-*N*-[(carbazole-9-yl)acetyl]glycinate (54b)

To a solution of **40** (0.50 g, 1.15 mmol) in anhydrous DMF (5 mL) was added DIEA (0.41 mL, 2.31 mmol), carbazole-9-ylacetic acid **(47)** (0.31 g, 1.38 mmol), TBTU (0.44 g, 1.38 mmol), and HOBt (0.19 g, 1.38 mmol). The reaction mixture was stirred for

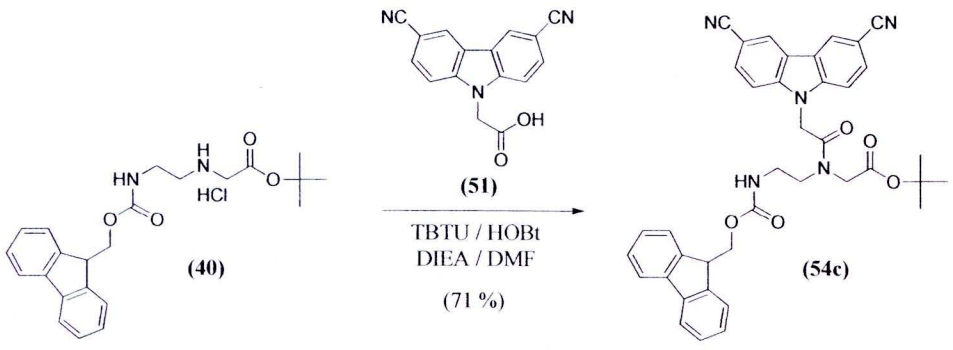
5 h. at room temperature and then concentrated *in vacuo*. The residue was brought up in CH_2Cl_2, and washed with saturated $NaHCO_3$ (5 x 50 mL) and brine (5 x 30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography (19:1 CH_2Cl_2/MeOH). The desired product **(54b)** was obtained as a white solid in 81 % yield (0.56 g): R_f = 0.34 (19:1 CH_2Cl_2/MeOH)

^{1}H NMR 400 MHz ($CDCl_3$) δ 8.08-8.06 (m, 2H, Ar), 7.75-7.73 (m, 2H, Ar), 7.60-7.58 (m, 2H, Ar), 7.55-7.53 (m, 2H, Ar), 7.41-7.19 (m, 8H, Ar) 5.68/5.33 (rotamer br, 1H, NH), 5.07/4.98 (rotamer s, 2H, CH_2), 4.41/4.32 (rotamer d, 2H, CH2, *J*=6.8/6.8 Hz), 4.23/4.16 (rotamer t, 1H, CH, *J*=6.8/6.8 Hz), 4.03/3.90 (rotamer s, 2H, CH_2,), 3.59-3.17 (m, 4H, 2 x CH_2), 1.50/1.44 (rotamer s, 9H, $C(CH_3)$)

^{13}C NMR 100 MHz ($CDCl_3$) δ 169.2, 167.8, 166.7, 166.5, 166.2, 145.5, 143.9, 141.5, 140.7, 139.4, 137.4, 128.9, 127.5, 125.0, 122.7, 122.5, 122.1, 121.3, 121.2, 120.1, 120.0, 118.6, 111.0, 110.8, 110.5, 109.6, 80.9, 65.5, 48.8, 47.4, 46.8, 45.1, 44.6, 27.8, 27.6

HRMS: Calcd. For $C_{37}H_{35}N_3O_5$: *m/z* 626.27, found *m/z* 626.26 (M+Na)

13. Synthesis of *tert*-Butyl *N*-[2-(*N'*-9 fluorenylmethoxycarbonyl) aminoethyl]-*N*-[(3,6-dicyanocarbazole-9-yl)acetyl]glycinate (54c) [79]



Scheme 24 Synthesis of *tert*-Butyl *N*-[2-(*N'*-9 fluorenylmethoxycarbonyl) aminoethyl]-*N*-[(3,6-dicyanocarbazole-9-yl)acetyl]glycinate (54c)

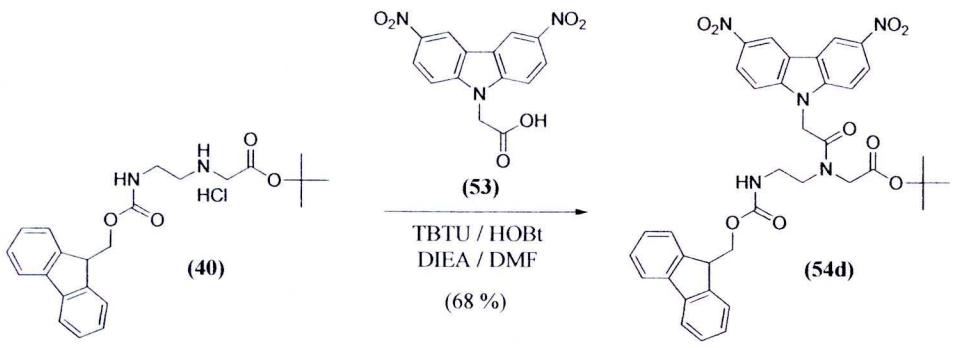
To a solution of **40** (0.25 g, 0.57 mmol) in anhydrous DMF (5 mL) was added DIEA (0.20 mL, 1.42 mmol), 3,6-dicyanocarbazole-9-ylacetic acid **(51)** (0.19 g, 0.68 mmol), TBTU (0.22 g, 0.68 mmol), and HOBt (0.10 g, 0.68 mmol). The reaction mixture was stirred for 7 h. at room temperature and then concentrated *in vacuo*. The residue was brought up in CH_2Cl_2, and washed with saturated $NaHCO_3$ (5 x 50 mL) and brine (5 x 30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography (19:1 CH_2Cl_2/MeOH). The desired product **(54c)** was obtained as a white solid in 71 % yield (0.26 g): R_f = 0.57 (19:1 CH_2Cl_2/MeOH)

^{1}H NMR 400 MHz ($CDCl_3$) δ 8.33 (s, 2H, Ar), 7.78-7.28 (m, 12H, Ar), 6.01/5.70 (rotamer br, 1H, NH), 5.11/5.01 (rotamer s, 2H, CH_2), 4.54/4.40 (rotamer d, 2H, CH_2 *J*=5.9/5.9 Hz), 4.22 (t, 1H, CH, *J*=5.8 Hz), 4.13/3.94 (rotomer s, 2H, CH_2), 3.57-3.37 (m, 4H, 2 x CH_2), 1.43/1.25 (rotamer s, 9H, $C(CH_3)$)

^{13}C NMR 100 MHz ($CDCl_3$) δ 168.9, 166.8, 166.3, 166.1, 156.9, 143.8, 143.4, 143.3, 141.5, 130.7, 130.6, 128.0, 127.8, 127.2, 127.1, 125.8, 125.7, 125.0, 122.5, 122.2, 119.8, 110.3, 104.2, 104.0, 84.3, 83.1, 66.8, 51.2, 49.9, 49.2, 48.9, 47.5, 45.0, 44.5, 39.7, 29.8, 28.3, 28.1

HRMS: Calcd. For $C_{39}H_{35}N_5O_5$: *m/z* 676.26, found *m/z* 676.25 (M+Na)

14. Synthesis of *tert*-Butyl *N*-[2-(*N'*-9 fluorenylmethoxycarbonyl) aminoethyl]-*N*-[(3,6-dinitrocarbazole-9-yl)acetyl]glycinate (54d) [79]



Scheme 25 Synthesis of *tert*-Butyl *N*-[2-(*N'*-9 fluorenylmethoxycarbonyl) aminoethyl]-*N*-[(3,6-dinitrocarbazole-9-yl)acetyl]glycinate (54d)

To a solution of **40** (0.25 g, 0.57 mmol) in anhydrous DMF (5 mL) was added DIEA (0.20 mL, 1.42 mmol), 3,6-dinitrocarbazole-9-ylacetic acid **(53)** (0.21 g, 0.68 mmol), TBTU (0.22 g, 0.68 mmol), and HOBt (0.10 g, 0.68 mmol). The reaction mixture was stirred for 8 h. at room temperature and then concentrated *in vacuo*. The residue was brought up in CH_2Cl_2, and washed with saturated $NaHCO_3$ (5 x 50 mL) and brine (5 x 30 mL).The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography (98:2 CH_2Cl_2/MeOH). The desired product **(54d)** was obtained as a white solid in 68 % yield (0.27 g): R_f= 0.24 (98:2 CH_2Cl_2/MeOH)

^{1}H NMR 400 MHz (DMSO-d_6) δ 9.51 (s, 2H, Ar), 8.42 (d, 2H, Ar, *J*=9.2 Hz), 8.35 (d, 2H, Ar, *J*=9.2 Hz), 7.88-7.26 (m, 10H, Ar), 5.69/5.43 (rotamer s, 1H, NH), 4.41/4.29 (rotamer d, 2H, CH_2 *J*=6.8/6.8 Hz), 4.26/4.22 (rotamer t, 1H, CH), 4.40/3.94 (rotamer s, 2H, CH_2), 3.64-3.10 (m, 4H, 2 x CH_2) 1.54/1.35 (rotamer s, 9H, $C(CH_3)$)

^{13}C NMR 100 MHz (DMSO-d_6) δ 169.2, 167.8, 166.7, 166.6, 166.1, 145.4, 143.8, 141.5, 140.7, 139.4, 137.4, 128.9, 127.6, 125.0, 125.0, 122.7, 122.5, 122.1, 121.4,

121.2, 120.1, 120.0, 118.6, 111.0, 110.8, 110.5, 109.7, 80.9, 65.5, 48.8, 47.5, 46.8, 45.1, 44.6, 27.8, 27.7

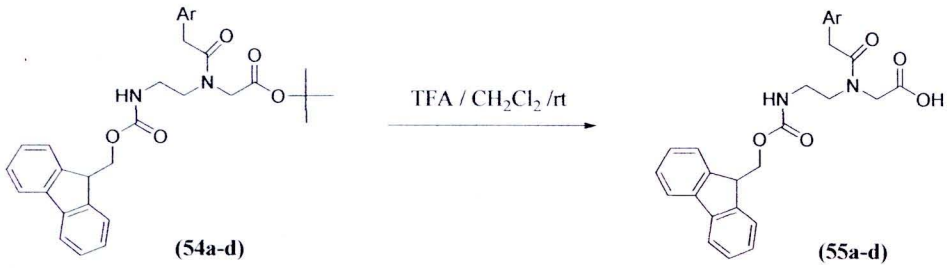
HRMS: Calcd. For $C_{37}H_{35}N_5O_9$: *m/z* 716.70, found *m/z* 716.23 (M+Na)

Synthesis of *ss aeg*PNA oligomers [81]

In this investigation, length and steric on oligomers needs to be explored; therefore, oligomers with different length (dimer/tetramer/hexamer/octamer) and different steric volume were prepared as shown in Table 3.

Table 3 Sequence of *ss aeg*PNA oligomers used in this investigated

Compound	Code of *aeg*PNA oligomers	Base sequence (*N*- to *C*- terminus)	Product
Dimer	T_2	H_2N-TT-$CONH_2$	56a
Tetramer	T_4	H_2N-TTTT-$CONH_2$	56b
Hexamer	T_6	H_2N-TTTTTT-$CONH_2$	56c
	TC_6	H_2N-CBZ TTTTT-$CONH_2$	56d
	TCC_6	H_2N-DCCBZ TTTTT-$CONH_2$	56e
	TNC_6	H_2N-DNCBZ TTTTT-$CONH_2$	56f
Octamer	T_8	H_2N-TTTTTTTT-$CONH_2$	56g



Scheme 26 Preparation of Fmoc-*aeg*PNA acid monomers for solid phase synthesis under acidic condition

1. Preparation of Fmoc-*aeg*PNA-COOH monomers (55a-d)

A portion of desired Fmoc-*aeg*PNA *tert*-butyl ester monomer (150 mg) was treated with a 1:1 (V/V) mixture of TFA:CH_2Cl_2 (2 mL). The reaction mixture was stirred at the room temperature for 2 h. and was co-evaporated with toluene. The resulting crude product was dried *in vacuo* to remove TFA. Finally, the crude product was dissolved with anhydrous DMF and the volume of desired Fmoc-*aeg*PNA-COOH monomer was adjusted to be the 0.2 M stock solution which then ready for manual coupling solid phase synthesis (Scheme 26).

2. Preparation of the reaction pipette and apparatus for solid phase synthesis

All *ss aeg*PNA oligomers were manually synthesized using a custom-built peptide synthesis column which has been was previously developed in this laboratory. The accurately weighed resin was downloaded into the column and first swelled in the required solvent for 2 h. before starting the synthesis cycle.

3. General procedure for *ss aeg*PNA oligomers synthesis

A 1 μmol scale MBHA-resin was downloaded into the custom-built peptide synthesis column and first swelled in anhydrous DMF for 1 h. before starting the synthesis cycle. In general, solid phase synthesis of *ss aeg*PNA oligomers consists of four major steps as follows (Figure 21):

I) Activation of Fmoc-*aeg*PNA-COOH monomer **(55a-d)** by mixing any desired 0.2 M Fmoc-*aeg*PNA COOH solution with 0.2 M HATU solution (10 μL), 0.2 M

DIEA and 0.3 M 2,6-lutidene solution (10 μL) for 1 min. The mixture was agitated every 5 min over a period of 50 min. at the room temperature. Then, the resin was successfully washed with DMF (3 x 200 μL).

II) Next, capping of the unreacted amino group with mixing solution of (1 : 2 : 2) acetic anhydride : 2,6-lutidine : anhydrous DMF (2 x 50 μL) at the room temperature for 5 min. After that, the resin was washed with DMF (3 x 200 μL).

III) After the coupling and capping were completed, the deprotection of the Fmoc group at *N*-terminal from the growing peptide chain was performed by 20 % piperidine in anhydrous DMF at the room temperature for 15 min.

IV) Cleavage of the *ss aeg*PNA oligomers from the resin was successfully achieved by treatment resin with TFA:TFMSA:*m*-cresol (4:8:1) mixture at the room temperature, then agitated every 10 min over a period of 1.5 h. A microcentrifuge tube was then employed to separate the resin from the solution containing the desired *ss aeg*PNA oligomers. The supernatant was removed using a stream of nitrogen gas to give product as brown residue. The residue was then precipitated by addition of cool diethyl ether and the crude *ss aeg*PNA was isolated from supernatant by centrifugation.

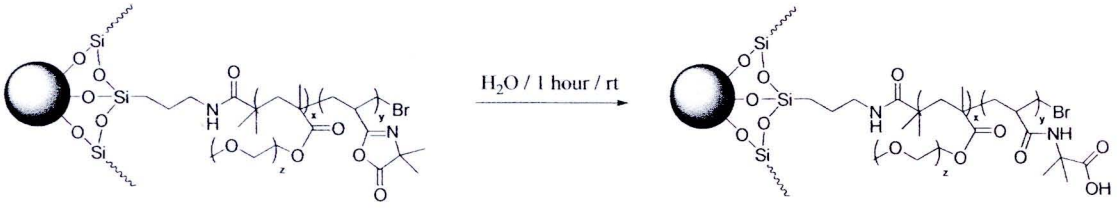
4. Purification and characterization of the *ss aeg*PNA oligomers (56a-g)

The crude of desired *ss aeg*PNA oligomers was purified by reverse-phase HPLC with UV-vis detection at 200, 260 and 300 nm using Vertisep UPS C_{18} semi-prep column (5μm particle size x 15 cm x 10 mm) at 50 °C using 0.1% TFA in H_2O as solvent A and 0.1 % TFA in CH_3CN as solvent B (0 to 5 % over 6 min, 5 to 25 % over 20 min, 25 to 95 % over 4 min, 95 to 100 % over 3 min, 5 mL/min) at flow rate 5.0 mL/min. The desired products were confirmed by MALDI-TOF mass spectrometry using α-cyano-4-hydroxycinnamic acid (CCA) as the matrix.

Loading determination for 2-vinyl-4,4-dimethylazlactone (VDM) as electrophilic group onto MNPs

The electrophilic MNPs were synthesized as previously described (loading ratio PEGMA:VDM = 70:30) [76]. To calculate amount of 2-vinyl-4,4-dimethylazlactone (VDM) on surface of MNPs, the ring-opening reaction of VDM by water (H_2O) to produce carboxylic acid group (Scheme 27) and the corresponding carboxylic groups was qualitatively analyzed by FT-IR spectroscopy and quantitatively determined by conductometric titration [82-83].

First, MNPs containing VDM (2 mg) was dispersed in 5 mM NaOH and then was stirred for 5 h. at room temperature, back-titrated with 5 mM HCl solution until reach the end point. Finally, loading of VDM was determined form the amount of acid used.



Scheme 27 Ring-opening reaction of VDM with water

Immobilization of *ss aeg*PNA oligomers onto the electrophilic MNPs

Electrophilic-coated MNPs was dispersed in anhydrous DMF and was ultrasonicated for 20 min, followed by addition of *ss aeg*PNA oligomers (Table 3) and DIEA in anhydrous DMF solution. The mixture was stirred at room temperature under nitrogen atmosphere. 200 µL of reaction mixtures were withdrawn by fixed time intervals and washed with 1,4-dioxane to remove aggregate, followed by external magnet separation. The attachment of desired *ss aeg*PNA oligomers onto MNPs was reconfirmed by FT-IR spectrometry. The yield of desired *ss aeg*PNA oligomers-MNPs complexes were indirectly calculated from disappearance of *ss aeg*PNA oligomers *via* UV-vis spectroscopy.