Electronic Supplementary Information

Highly stable triple helix formation by homopyrimidine (L)-acyclic threoninol nucleic acids with single stranded DNA and RNA

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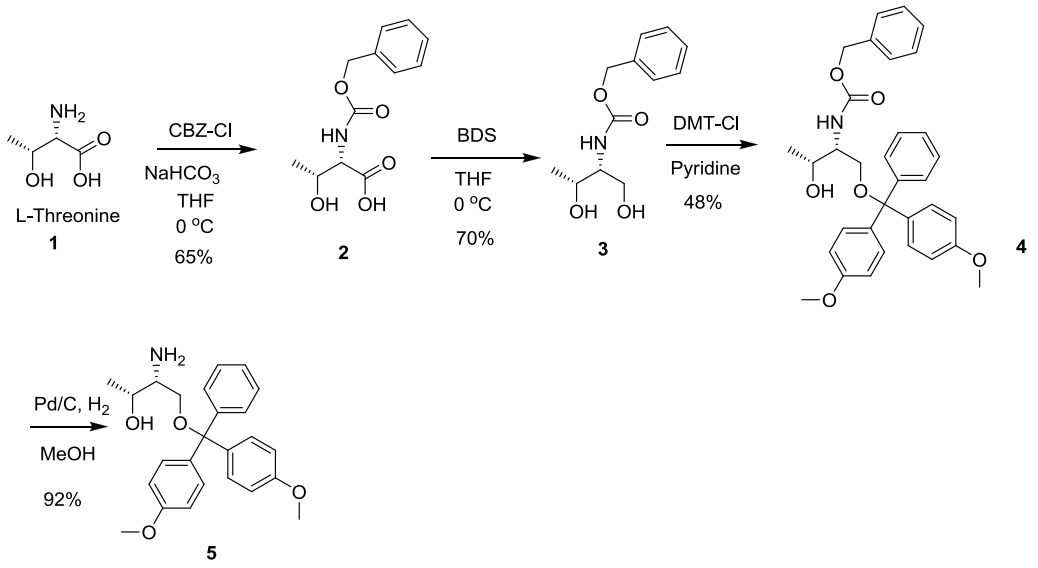
S1. Synthesis of phosphoramidite building blocks of acyclic threoninol nucleic acids (aTNA)

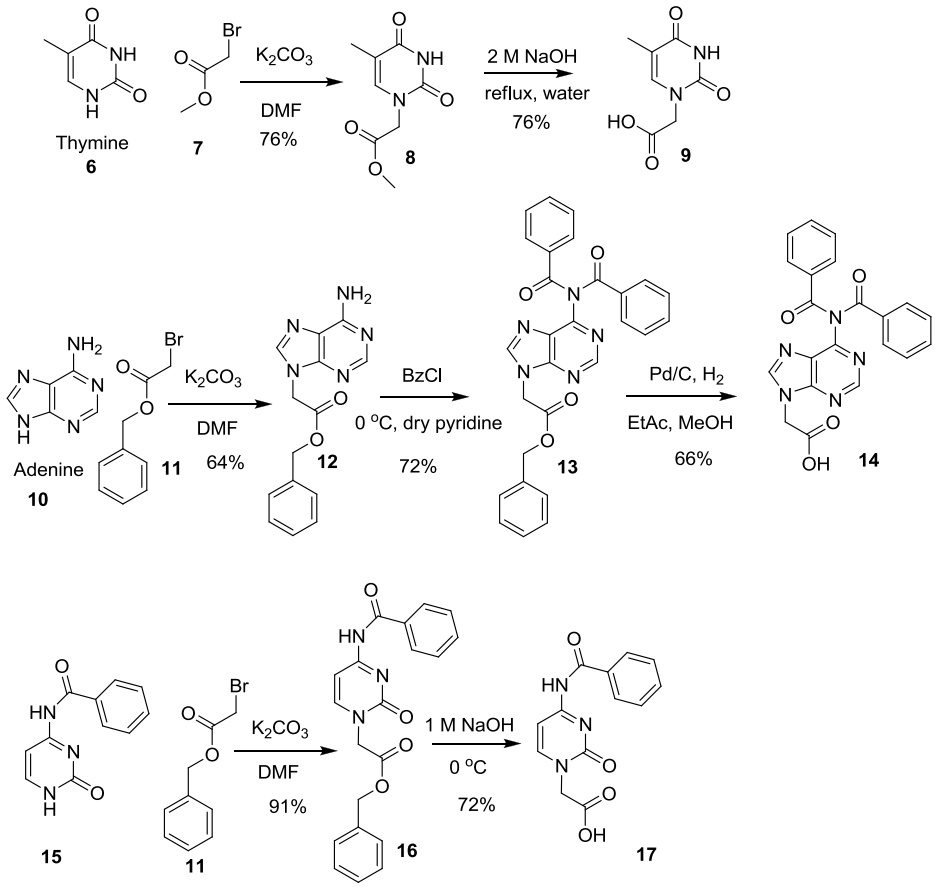
Building blocks of aTNA bearing adenine, cytosine and thymine were prepared from commercially available optically active threonine amino acid. (L)-Threonine and (D)-threonine were used for the synthesis of (L)-aTNA and (D)-aTNA respectively. Although, synthesis of (D)-TNA monomers was reported by using (D)-threoninol as starting material[1], we followed different route in order to improve the synthesis of phosphoramidite monomers.

The primary amino group of (L)-threonine **1** was protected by Cbz chloride in the presence of inorganic base produced Cbz-threonine **2**. Carboxylic acid **2** was reduced by borane dimethyl sulfide complex in anhydrous THF to yield corresponding diol **3**. Subsequently, diol was converted in to DMT alcohol **4** by reacting DMT chloride in anhydrous pyridine. This compound can be used as stock for the generation of reactive amine for further reactions. Next, hydrogenolysis of the Cbz group of compound **4** by Pd/C in the presence of hydrogen afforded DMT amine **5** (Scheme 1).



Scheme 1 Synthesis of DMT amine **5** from (L)-threonine.

Nucleobase acids were synthesized according the reported literature (Scheme 2). Reaction of thymine **6** with methyl bromoacetate **7** in dry DMF gave corresponding thymine ester **8.**

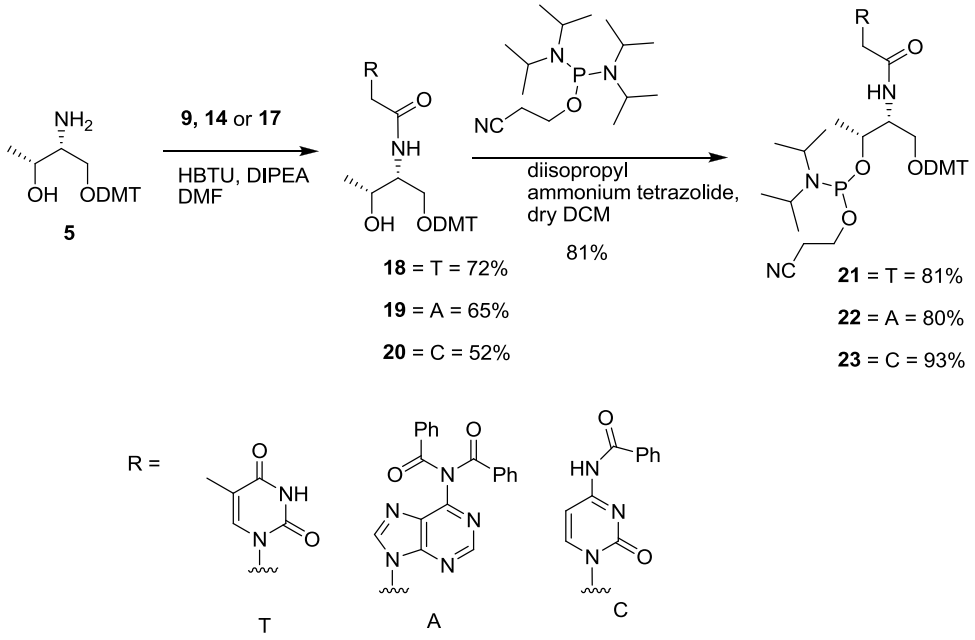


Scheme 2 Synthesis of nucleobase acids.

Subsequently, **8** was refluxed in 2 M sodium hydroxide to afford thymine acid **9**. Alkylation of adenine **10** by benzyl bromoacetate **11** in dry DMF yielded product **12**. The exocyclic amine of compound **12** was protected by benzoyl chloride in anhydrous pyridine gave corresponding product **13**. Benzyl ester of compound **13** was cleaved in combined solvent (ethyl acetate and methanol) by using palladium on carbon in the presence of hydrogen to afford **14**. Similarly, treatment of benzoylated cytosine **15** with benzyl bromoacetate **11** in the presence of potassium carbonate in dry DMF gave product **16**. Compound **16** was treated in 1 N sodium hydroxide at 0 ^{o}C for the deprotection of benzyl ester to give corresponding acid **17**.

The resulting nucleobases acids **9**, **14** and **17** were allowed to react with DMT amine **5** in the presence of HBTU and diisopropyl amine in dry DMF to give corresponding building blocks of DMT alcohols **18**, **19**, **20** respectively (Scheme 3). Regioselectivity of alkylated thymine and adenine were confirmed by Heteronuclear Multiple Bond Correlation (HMBC). Phosphitylation

of DMT-alcohols **18**, **19** and **20** were carried out in the presence of 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphordiamidite and diisopropyl ammonium tetrazolide afforded phosphoramidite building blocks **21**, **22** and **23**. In order to synthesis of thymine (D)-threoninol phosphoramidite similar synthetic route was followed with similar yields.



Scheme 3 Synthesis of phosphoramidite building blocks (L)-aTNA

(2S,3R)-2-(Benzyloxycarbonylamino)-3-hydroxybutanoic acid (**2**)[2]: To a stirred solution of **1** (10 g, 84 mmol) in 1:1 saturated sodium bicarbonate-THF (400 ml) was added benzyl chloroformate (17.14 g, 100.6 mmol) dropwise at 0 °C. The reaction mixture was left stirring for 3 hr. The reaction mixture was washed with diethyl ether and pH (2-3) of the aqueous layer was adjusted by HCl. The product was extracted in ethyl acetate and the organic layer was dried over Na_2SO_4. Ethyl acetate was removed under reduced pressure to give the crude product as a colorless oil (13.8 g, 65 %). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.32 - 7.27 (m, 5H), 5.12 - 5.05 (m, 2H), 4.39 - 4.35 (m, 1H), 4.32 - 4.30 (m, 1H), 1.18 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.4, 157.3, 136.2, 128.7, 128.3, 128.2, 68.0, 67.5, 59.3, 19.5.

Benzyl (2R,3R)-1,3-dihydroxybutan-2-ylcarbamate (**3**)[3]: To a stirred solution of **2** (7 g, 27.6 mmol) in dry THF was added 2 M solution of borane dimethyl sulfide (BDS) complex in THF

(20.7 ml, 41.5 mmol) drop by drop at 0 ^{o}C. The reaction was allowed to stir for next 5 hours at room temperature. The reaction was quenched with methanol and solvent was evaporated under vacuum. Three times co evaporations were carried out with methanol to give pale yellow oil as crude product. Purification was carried out by silica gel chromatography with eluent (gradient 1 to 5 % methanol/DCM) to afford colorless oil (4.6 g, 70 %). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.35 – 7.28 (m, 5H), 5.63 (d, *J* = 8.7 Hz, 1H), 5.09 (s, 2H), 4.13 – 4.08 (m, 1H), 3.75 (d, *J* = 4.6 Hz, 2H), 3.58 – 3.54 (m, 1H), 3.17 – 3.10 (m, 2H), 1.18 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.3, 136.4, 128.6, 128.3, 128.1, 68.1, 67.1, 64.3, 56.5, 20.3.

Benzyl (2R,3R)-1-(bis(4-methoxyphenyl)(phenyl)methoxy)-3-hydroxybutan-2-ylcarbamate (**4**): To a stirred solution of **3** (2.1 g, 8.8 mmol) in anhydrous pyridine, DMT-Cl (3.6 g, 10.6 mmol) was added in one portion under argon atmosphere. The reaction was allowed to stir for 4 hours. Pyridine was removed under high vacuum and solid residues were dissolved in ethyl acetate. The organic layer was washed with water and followed by brine solution and dried over sodium sulphate. The solvent was evaporated and residues were purified by column chromatography over silica gel with eluent (gradient 0 to 100 % dichloromethane/pentane with 1 % triethylamine) afforded 4 as white foam (2.3 g, 48 % yield). $[\alpha]_D^{25}$ = - 2.9 (*c* = 1 in dichloroethane). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.41 - 7.38 (m, 4H), 7.36 - 7.33 (m, 3H), 7.28 - 7.25 (m, 5H), 7.22 - 7.19 (m, 1H), 6.95 (d, *J* = 9.2 Hz, 1H), 6.87 - 6.84 (m, 4H), 5.14 - 5.03 (m, 2H), 3.86 (qt, *J* = 6.1, 3.0 Hz, 1H), 3.73 (s, 6H), 3.64 (ddd, *J* = 14.0, 7.6, 2.8 Hz, 1H), 3.36 (s, 2H), 3.12 (dd, *J* = 9.0, 5.4 Hz, 1H), 2.90 (dd, *J* = 9.0, 6.4 Hz, 1H), 0.98 (d, *J* = 6.3 Hz, 3H).^{13}C NMR (126 MHz, DMSO) δ 158, 156.3, 145.1, 137.3, 135.9, 135.8, 129.7, 129.7, 128.3, 127.7, 127.7, 127.6, 126.5, 113.1, 85.2, 65.3, 65.2, 63.1, 56.6, 55.0, 20.0; HRMS calcd for $C_{33}H_{35}NO_6Na$ $[M + Na]^+$ 564.2357, found $[M + Na]^+$ 564.2363.

(2R,3R)-3-Amino-4-(bis(4-methoxyphenyl)(phenyl)methoxy)butan-2-ol (**5**)[1]: Under an argon atmosphere, palladium on carbon (10 %) (100 mg) was added to a solution of **4** (700 mg, mmol) in methanol. The reaction was carried out in the presence of hydrogen for 3 hr. The product was filtered over filter paper and the filtrate was evaporated, to give **5** as a semi solid paste (484 mg, 92 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.42 - 7.39 (m, 2H), 7.32 - 7.25 (m, 6H), 7.21 (t, *J* = 7.1 Hz, 1H), 6.90 - 6.87 (m, *J* = 8.7 Hz, 4H), 3.73 (s, 6H), 3.64 - 3.61 (m, 1H), 3.01 (dd, *J* = 8.9,

5.3 Hz, 1H), 2.84 (dd, *J* = 8.9, 6.1 Hz, 1H), 2.57 (q, *J* = 5.4 Hz, 1H), 0.96 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 158.0, 145.2, 136.0, 135.9, 129.7, 129.7, 127.8, 127.7, 126.5, 113.1, 85.1, 66.9, 65.5, 56.7, 55.0, 20.2.

Ethyl 2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate (**8**)[4]: The dry potassium carbonate (5.5 g, 39.7 mmol) was added to a suspension of thymine **6** in dry DMF under argon condition. After 30 min. methyl bromoacetate **7** (3.7 ml, 39.7 mmol) was added drop by drop. The reaction was allowed to stir for overnight. The reaction mixture was filtered through sintered funnel and solvent was removed up to dryness. The solid residue was suspended in cold water and 4 M HCl was added and stirred for 30 min. The precipitate was filtered and washed with water. The solid mass was dried under high vacuum to give title compound **8** (6 g, 76 % yield) which was used for the next reaction without any purification. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.40 (s, 1H), 7.50 (d, *J* = 1.3 Hz, 1H), 4.49 (s, 2H), 3.69 (s, 3H), 1.76 (d, *J* = 1.1 Hz, 4H). ^{13}C NMR (126 MHz, DMSO) δ 168.7, 164.3, 150.93, 141.5, 108.6, 52.3, 48.3, 11.9.

2-(5-Methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetic acid (**9**)[4]: 2 M sodium hydroxide (30 ml) was added to a suspension of **8** in 30 ml water and refluxed for 10 min. The reaction mixture was allowed to cool at room temperature. Under the ice cold condition, pH (2-3) was adjusted by conc. HCl. A white precipitate was filtered and washed with cold water. Residues were dried over high vacuum to afford white powder (3.3 g, 76 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.10 (s, 1H), 11.34 (s, 1H), 7.50 (d, *J* = 1.2 Hz, 1H), 4.37 (s, 2H), 1.76 (d, *J* = 1.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 169.7, 164.3, 151.0, 141.8, 108.3, 48.4, 12.0.

Benzyl 2-(6-amino-9H-purin-9-yl)acetate (**12**)[5]: Adenine **10** (10 g, 74 mmol) was suspended in dry DMF. Sodium hydride (3.55 g, 88.8 mmol) was added at ice cold condition under argon atmosphere. The reaction was allowed to stir for 2 hours at room temperature. Additional DMF was added in order to stir the suspension. The solution was cooled in ice bath for 30 min. Subsequently, benzyl 2-bromoacetate **11** (18.6 g, 81.4 mmol) was added drop wise and the reaction mixture was stirred overnight. DMF was evaporated under reduced pressure and water was added to the resulting precipitates and stirred for 1 hr. The solid residues were filtered and washed with water. The product was refluxed in ethanol for 45 min. The product was allowed to precipitate overnight, collected by filtration and washed with cold ethanol. The yellow residues

were dried under high vacuum to give 13.6 g (64.8 %) as title compound. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (d, J = 2.5 Hz, 2H), 7.38 – 7.32 (m, 5H), 7.29 (s, 2H), 5.20 (s, 2H), 5.16 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 167.9, 156.0, 152.7, 149.7, 141.2, 135.4, 128.5, 128.2, 128.0, 118.3, 66.6, 44.0.

Benzyl 2-(6-(N-benzoylbenzamido)-9H-purin-9-yl)acetate (**13**): To a stirred mixture of **12** (5 g, 17.6 mmol) in anhydrous pyridine was added benzoyl chloride (10.42 g, 74.1 mmol). The reaction was allowed to stir for 12 hr. The reaction was stopped by addition of water. The product was dissolve in dichloromethane and washed with water followed by brine solution. The organic layer was concentrated and yellow precipitates were recrystallized in methanol. The product was purified by column chromatography over slica gel column with eluent (gradient 20 to 80 % dichloromethane/pentane) to afford 6.32 g (72.9 %) as solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.65 (s, 1H), 8.16 (s, 1H), 7.86 (d, J = 7.5 Hz, 4H), 7.50 - 7.45 (m, 2H), 7.40 - 7.32 (m, 9H), 5.24 (s, 2H), 5.07 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.4, 166.6, 153.4, 152.5, 152.0, 145.2, 134.2, 133.1, 129.6, 129.0, 128.9, 128.9, 128.7, 128.5, 126.8, 68.3, 44.6; HRMS calcd for $C_{28}H_{21}N_5O_4Na$ $[M + Na]^+$ 514.1486, found $[M + Na]^+$ 514.1494.

2-(6-(N-benzoylbenzamido)-9H-purin-9-yl)acetic acid (**14**): To a suspension of **13** (1.99 g, 4.05 mmol) in methanol (40 ml) and ethyl acetate (60 ml) was added Pd/C (650 mg) under argon atmosphere. The reaction was carried out in the presence of hydrogen pressure for 3 hr. The product was filtered over filter paper. The organic solvent was removed under high vacuum. The product was purified by column chromatography with eluent (gradient, DCM to 5 % MeOH/DCM) to afford 1.09 g (66.8 %) white solid. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.61 (s, 1H), 8.44 (s, 1H), 7.84 - 7.82 (m, 4H), 7.52 (t, J = 7.5 Hz, 2H), 7.38 (t, J = 7.7 Hz, 4H), 5.14 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 173.8, 170.1, 154.9, 153.1, 152.5, 148.5, 135.4, 134.3, 130.4, 129.8, 128.2, 45.4; HRMS calcd for $C_{21}H_{15}N_5O_4Na$ $[M + Na]^+$ 424.1016, found $[M + Na]^+$ 424.1018.

Benzyl 2-(4-benzamido-2-oxopyrimidin-1(2H)-yl)acetate (**16**): To a stirred suspension of **15** (2 g, 9.3 mmol) in dry DMF was added sodium hydride (408 mg, 10.2 mmol) at ice cold condition. The reaction was allowed to stir for 50 minutes. The benzyl bromoacetate **11** (2.6 g, 11.2 mmol) was added drop by drop at room temperature. The reaction was stirred for additional

2 hr. To the reaction mixture ethyl acetate was added and washed with water. The organic solvent was removed under high vacuum. The precipitates were recrystallized in diethyl ether to give title compound **16** (3 g, 91%) as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.27 (s, 1H), 8.19 (d, *J* = 7.3 Hz, 1H), 8.02 (d, *J* = 7.6 Hz, 2H), 7.63 (t, *J* = 7.4 Hz, 1H), 7.52 (t, *J* = 7.6 Hz, 2H), 7.40 – 7.34 (m, 6H), 5.22 (s, 2H), 4.78 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 167.9, 167.3, 163.8, 155.2, 150.6, 135.5, 133.1, 132.7, 128.5, 128.4, 128.2, 127.9, 96.1, 66.4, 50.8.

Benzyl 2-(4-benzamido-2-oxopyrimidin-1(2H)-yl)acetate (**17**)[6]: To a suspension of **16** (5 g, 13.7 mmol) in water (40 ml) was added 1 N sodium hydroxide (40 ml) drop by drop at 0 ^{o}C. Reaction was allowed to stir for 2 hr. After completion of reaction pH (2 to 3) was adjusted by hydrochloric acid. The product was filtered and washed with cold water and dried under high vacuum overnight to give title compound **17** (2.7g, 72 %) as white solid. Next the reaction was carried out without any further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (d, *J* = 7.2 Hz, 1H), 8.02 (d, *J* = 7.6 Hz, 2H), 7.63 (t, *J* = 7.6 Hz, 1H), 7.52 (t, *J* = 7.5 Hz, 2H), 7.36 – 7.29 (m, 1H), 4.60 (s, 2H).

***N*-((2R,3R)-1-(bis(4-methoxyphenyl)(phenyl)methoxy)-3-hydroxybutan-2-yl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamide** (**18**): Under an argon condition to a stirred solution of **5** (712 mg, 1.7 mmol), **9** (316 mg, 1.7 mmol) and diisopropylethylamine (444 mg, 3.4 mmol) in dry DMF was added HBTU (977 mg, 2.58 mmol). The reaction was allowed to stir for 3 hr. The reaction mixture was diluted with ethyl acetate and washed with water. The organic layer was dried over dry sodium sulphate. The solvent was evaporated by vacuum and the resulting residues were purified by column chromatography over silica gel with eluent (gradient 0 to 4 % MeOH/DCM with 1% triethylamine) to give white foam (712 mg, 72 %). $[\alpha]_D^{25}$ = + 2.3 (*c* = 1 in dichloroethane). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.97 (d, *J* = 8.9 Hz, 1H), 7.40 - 7.39 (m, 3H), 7.33 - 7.29 (m, 2H), 7.27 - 7.22 (m, 4H), 6.92 - 6.84 (m, 4H), 4.38 (s, 2H), 3.94 - 3.86 (m, 2H), 3.74 (s, 6H), 3.10 (dd, *J* = 8.9, 6.0 Hz, 1H), 2.88 (dd, *J* = 8.8, 6.1 Hz, 1H), 1.75 (s, 3H), 1.00 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 167.0, 164.5, 158.0, 151.0, 145.1, 142.5, 135.8, 129.7, 129.7, 127.8, 127.7, 126.5, 113.1, 107.7, 85.2, 64.8, 62.7, 55.0, 54.2, 45.7, 20.2, 11.9; HRMS calcd for $C_{32}H_{35}N_3O_7Na$ $[M + Na]^+$ 596.2367, found $[M + Na]^+$ 596.2374.

***N*-benzoyl-N-(9-(2-((2R,3R)-1-(bis(4-methoxyphenyl)(phenyl)methoxy)-3-hydroxybutan-2-ylamino)-2-oxoethyl)-9H-purin-6-yl)benzamide** (**19**): Under an argon condition to a stir solution of **5** (1.2 g, 2.9 mmol), **14** (1.2 g, 2.9 mmol) and diisopropylethylamine (761 mg) in dry DMF was added HBTU (2.2 g, 5.9 mmol). The reaction was allowed to stir for 3 hr. the reaction mixture was diluted with ethyl acetate and washed with water followed by brine solution. The organic layer was dried over dry sodium sulphate. The solvent was evaporated by vacuum and the resulting residues were purified by column chromatography over silica gel with eluent (gradient 0 to 5 % MeOH/DCM with 1 % triethylamine) to give white foam (1.5 g, 65 %). $[\alpha]_D^{25}$ = - 5.8 (*c* = 1 in dichloroethane). ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.51 (s, 1H), 8.46 (s, 1H), 7.85 - 7.83 (m, 4H), 7.55 - 7.51 (m, 2H), 7.42 - 7.36 (m, 6H), 7.31 - 7.27 (m, 4H), 7.25 - 7.21 (m, 2H), 7.14 - 7.18, 6.85 - 6.81 (m, 4H), 5.14 (d, *J* = 11.2 Hz, 2H), 4.03 - 3.97 (m, 2H), 3.75 (s, 6H), 3.30 - 3.29 (m, 1H), 3.16 (dd, *J* = 9.2, 6.1 Hz, 1H), 1.00 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (101 MHz, MeOD) δ 173.8, 168.4, 160.1, 153.1, 152.5, 148.8, 146.4, 137.3, 137.2, 135.4, 134.3, 131.3, 130.5, 129.8, 129.3, 128.7, 128.1, 127.8, 114.1, 87.4, 67.5, 64.5, 57.0, 55.7, 47.1, 20.5; HRMS calcd for $C_{46}H_{43}N_6O_7$ $[M + H]^+$ 791.3188 , found $[M + H]^+$ 791.3189.

N-(1-(2-((2R,3R)-1-(bis(4-methoxyphenyl)(phenyl)methoxy)-3-hydroxybutan-2-ylamino)-2-oxoethyl)-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide (**20**): Under an argon condition to a stirred solution of **5** (1.5 g, 3.7 mmol), **17** (764 mg, 2.8 mmol) and diisopropylethylamine (951 mg) in dry DMF was added HBTU (2.8 g, 7.4 mmol). The reaction was allowed to stir for 24 hr. the reaction mixture was diluted with ethyl acetate and washed with water followed by brine solution. The organic layer was dried over dry sodium sulphate. The solvent was evaporated by vacuum and the resulting residues were purified by column chromatography over silica gel with eluent (gradient 0 to 4% MeOH/DCM with 1% triethylamine) to give white foam (1.3 g, 52%). $[\alpha]_D^{25}$ = - 2.5 (*c* = 1 in dichloroethane). ^{1}H NMR (400 MHz, DMSO) δ 11.20 (s, 1H), 8.08-8.02 (m, 3H), 7.63 (t, *J* = 7.4 Hz, 1H), 7.52 (t, *J* = 7.6 Hz, 2H), 7.40 (d, J = 7.9 Hz, 2H), 7.34 - 7.30 (m, 3H), 7.28 - 7.20 (m, 5H), 6.91 (d, *J* = 8.4 Hz, 4H), 4.63 (d, *J* = 6.2 Hz, 2H), 4.00 - 3.96 (m, 1H), 3.92 - 3.89 (m, 1H), 3.74 (s, 6H), 3.13 (dd, *J* = 8.8, 6.1 Hz, 1H), 2.91 (dd, *J* = 8.7, 6.0 Hz, 1H), 1.00 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 166.7, 163.2, 158.0, 151.3, 145.1, 135.8, 135.8, 133.3, 132.6, 129.7, 128.4, 127.8, 127.8, 126.5, 113.2, 95.4, 85.2, 64.8, 62.7, 55.0, 54.3, 51.3, 20.2; HRMS calcd for $C_{38}H_{39}N_4O_7$ $[M + H]^+$ 663.2813 , found $[M + H]^+$ 663.2814.

(2R,3R)-4-(bis(4-methoxyphenyl)(phenyl)methoxy)-3-(2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido)butan-2-yl 2-cyanoethyl diisopropylphosphoramidite (**21**): Compound **18** (712 mg, 1.24 mmol) was dried under high vacuum and anhydrous dichloromethane was added followed by the addition of diisopropyl ammonium tetrazolide (106 mg, 0.620 mmol). Subsequently, 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphordiamidite (561 mg, 1.86 mmol) was added and allowed to stir at room temperature for 3 hr. The reaction was diluted with dichloromethane and poured in saturated aqueous sodium bicarbonate. The product was extracted in dichloromethane and dried over dry sodium sulphate. The product was purified by column chromatography over slica gel column with eluent (gradient 50 to 100% dichloromethane/pentane with 1% triethylamine) to afford (779 mg, 81 %) as white foam solid. ^{31}P NMR (162 MHz, C_6D6) δ 148.06, 146.64.

(2R,3R)-3-(2-(6-(N-benzoylbenzamido)-9H-purin-9-yl)acetamido)-4-(bis(4-methoxyphenyl)(phenyl)methoxy)butan-2-yl 2-cyanoethyl diisopropylphosphoramidite (**22**): Compound **19** (300 mg, 0.379 mmol) was dried under high vacuum and anhydrous dichloromethane was added followed by the addition of diisopropyl ammonium tetrazolide (32 mg, 0.189 mmol). 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphordiamidite (171 mg, 0.569 mmol) was added and allowed to stir at room temperature for 3 hr. The reaction was diluted with dichloromethane and poured in saturated aqueous sodium bicarbonate. The product was extracted in dichloromethane and dried over dry sodium sulphate. The product was purified by column chromatography over slica gel column with eluent (50 to 100% dichloromethane/pentane with 1% triethylamine) to afford (300 mg, 80 %) as white foam solid. ^{31}P NMR (162 MHz, $CDCl_3$) δ 148.38, 147.12.

(2R,3R)-3-(2-(4-benzamido-2-oxopyrimidin-1(2H)-yl)acetamido)-4-(bis(4-methoxyphenyl)(phenyl)methoxy)butan-2-yl 2-cyanoethyl diisopropylphosphoramidite (**23**): Compound **20** (472 mg, 0.712 mmol) was dried under high vacuum and anhydrous dichloromethane (7 ml) was added followed by the addition of diisopropyl ammonium tetrazolide (61 mg, 0.356 mmol). Subsequently, 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphordiamidite (322 mg, 1.07 mmol) was added and allowed to stir at room temperature for 3 hr. The reaction was diluted with dichloromethane and poured in saturated

aqueous sodium bicarbonate. The product was extracted in dichloromethane and dried over dry sodium sulphate. The product was purified by column chromatography over slica gel column with eluent (gradient 50 to 100% dichloromethane/pentane with 1% triethylamine) to afford (573 mg, 93 %) as white foam solid. ^{31}P NMR (162 MHz, C_6D6) δ 148.34, 146.70.

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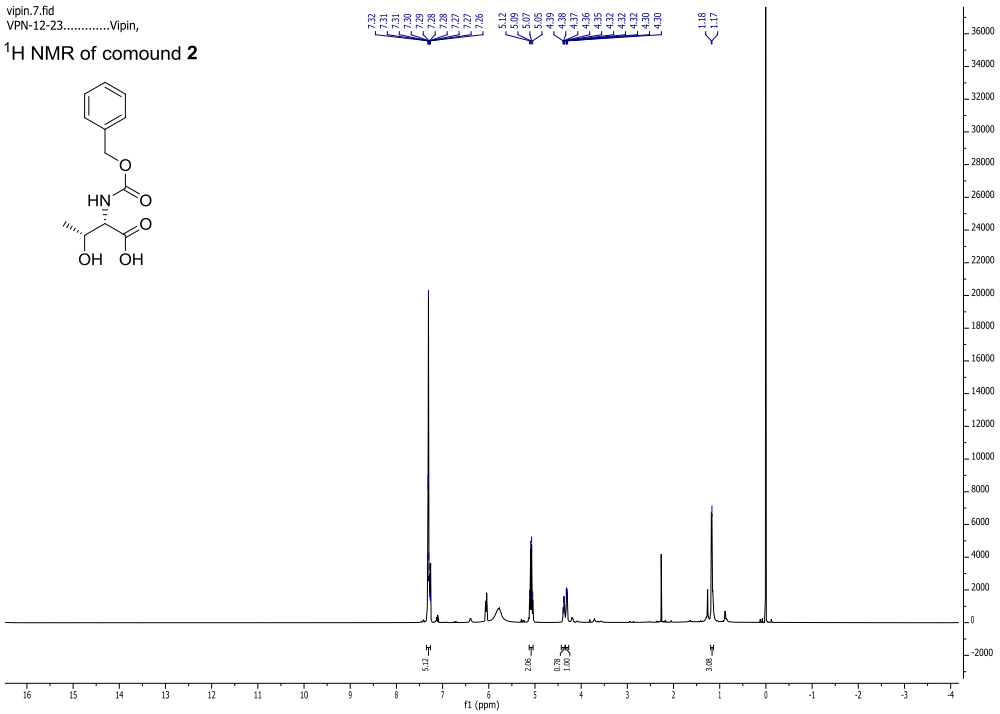
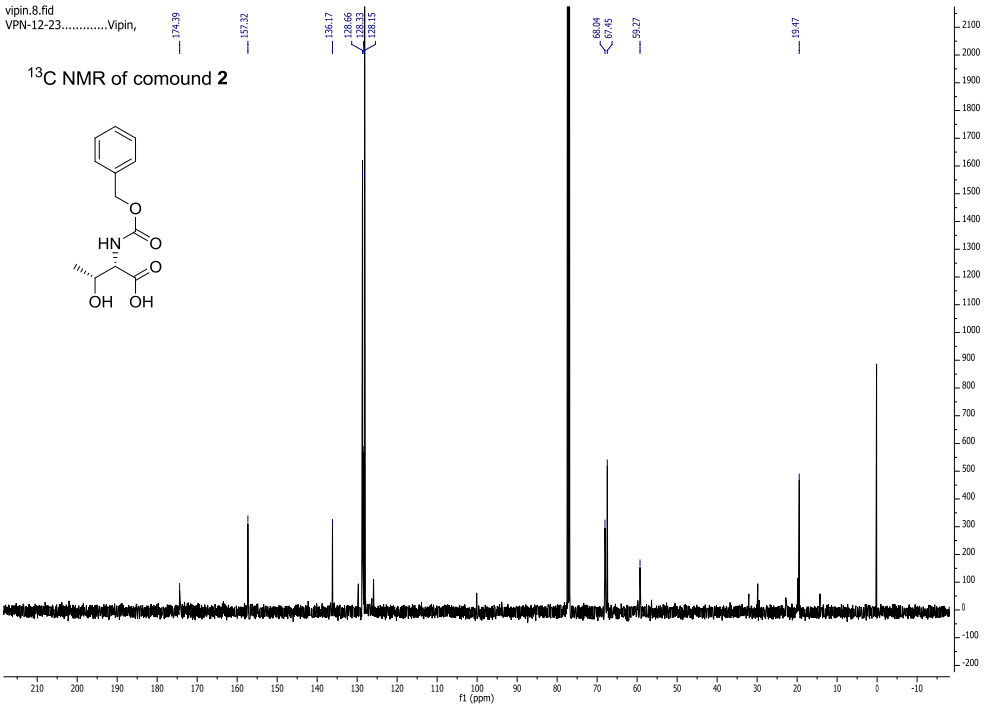
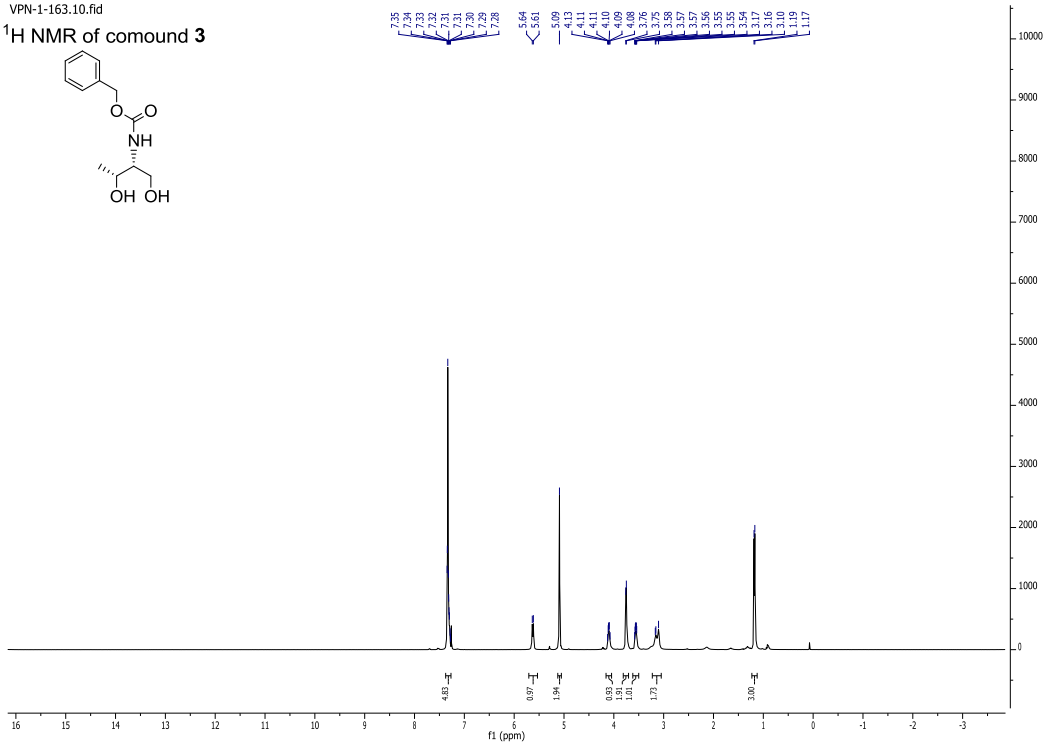
S2. NMR Spectra





VPN-1-163.10.fid

^{1}H NMR of comound **3**



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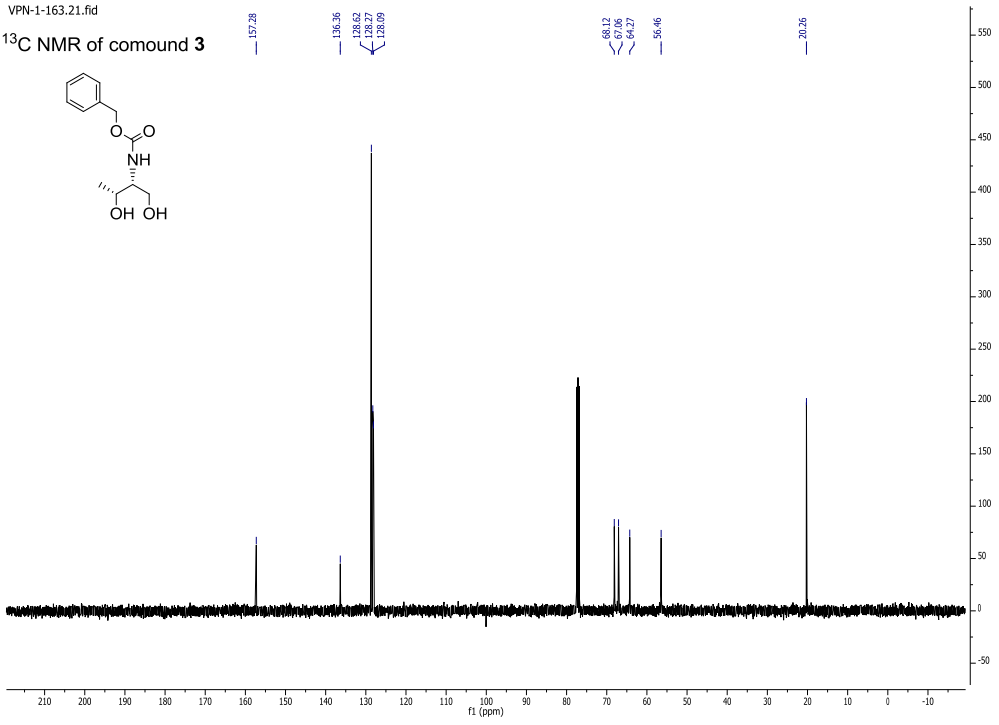
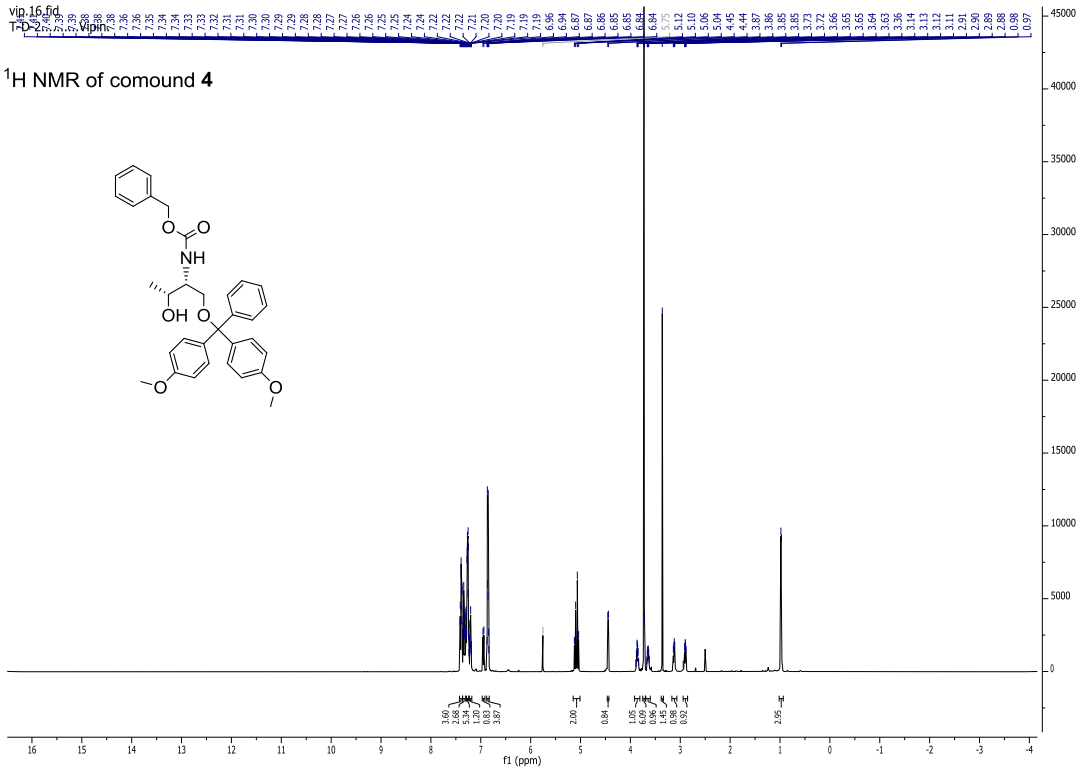
^{13}C NMR of comound **3**

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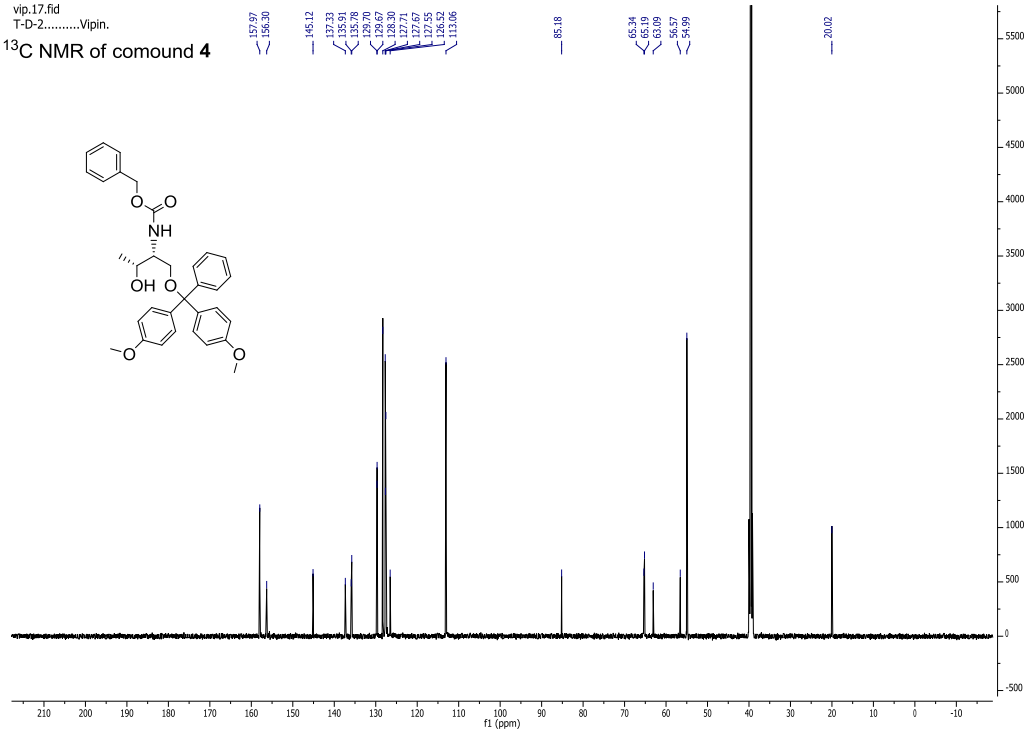
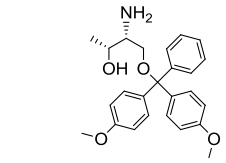
T-D-2.....Vipin.
^{1}H NMR of comound **4**
f1 (ppm)


vip.17.fid
T-D-2.....Vipin.
^{13}C NMR of comound **4**
f1 (ppm)

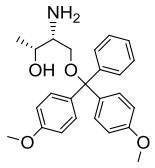
VPN-1-63.10.fid

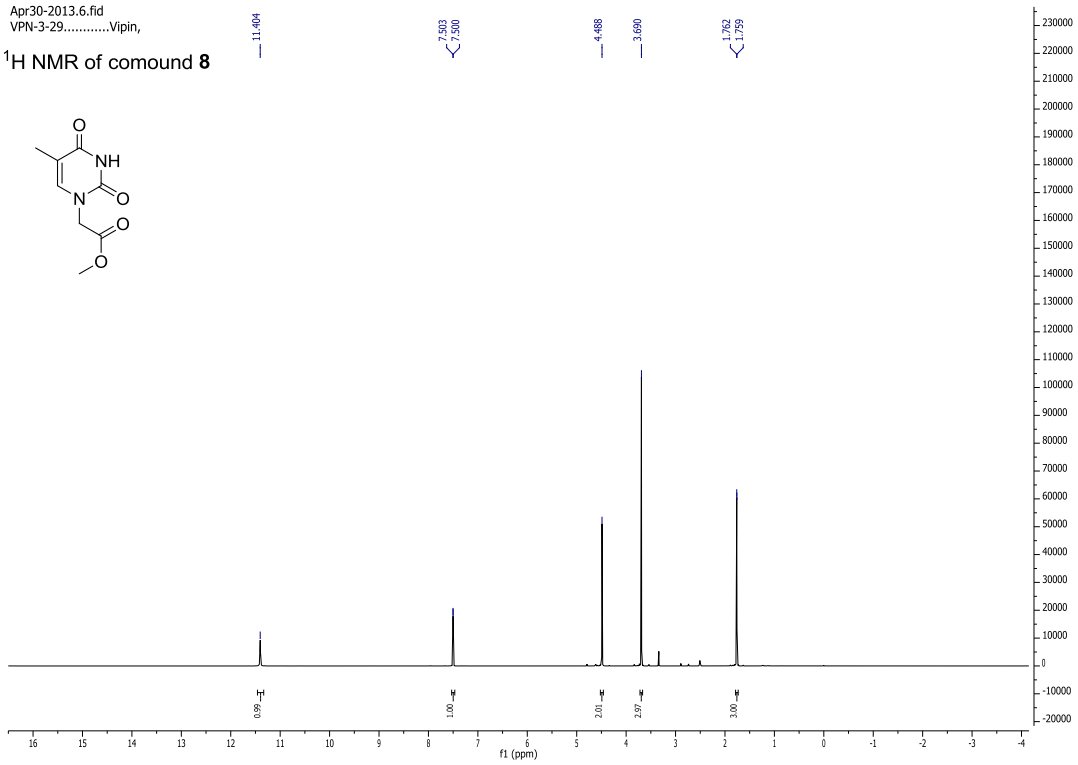
^{1}H NMR of comound **5**

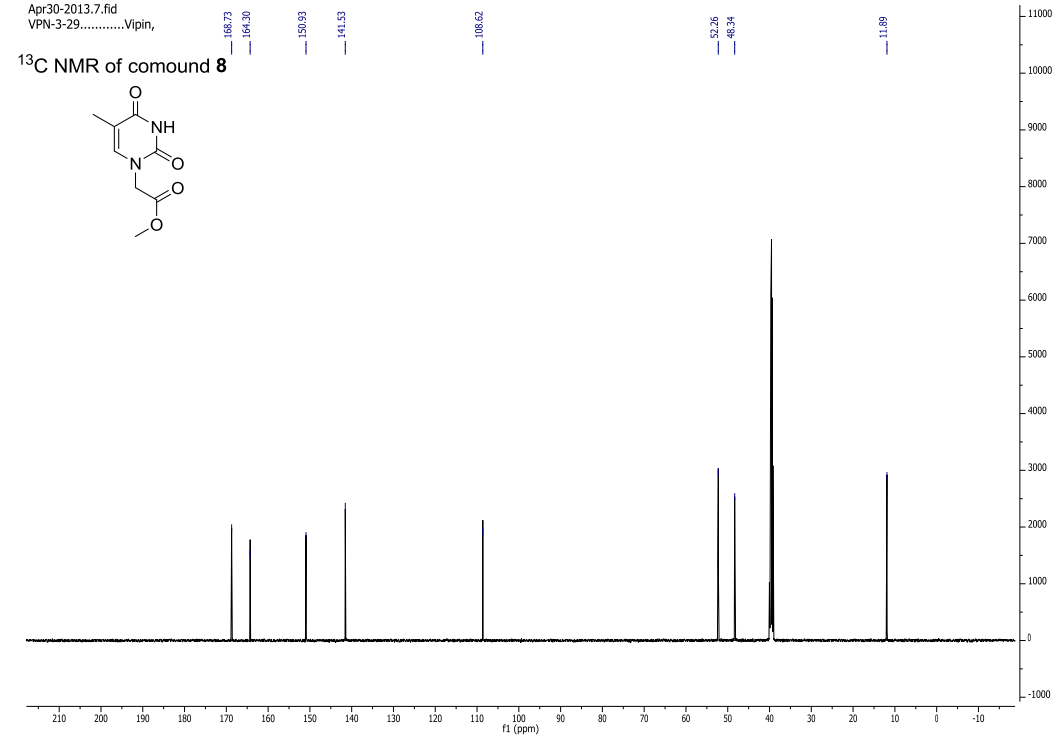


VPN-1-63.11.fid

^{13}C NMR of comound **5**




Apr30-2013.6.fid
VPN-3-29.....Vipin,
^{1}H NMR of comound **8**
11.404
7.503
7.500
4.488
3.690
1.762
1.759
0.99
1.00
2.01
2.97
3.00
f1 (ppm)


Apr30-2013.7.fid
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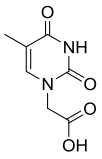
^{13}C NMR of comound **8**
168.73
164.30
150.93
141.53
108.62
52.26
48.34
11.89
f1 (ppm)

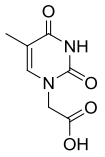
VPN-T acetate.21.fid

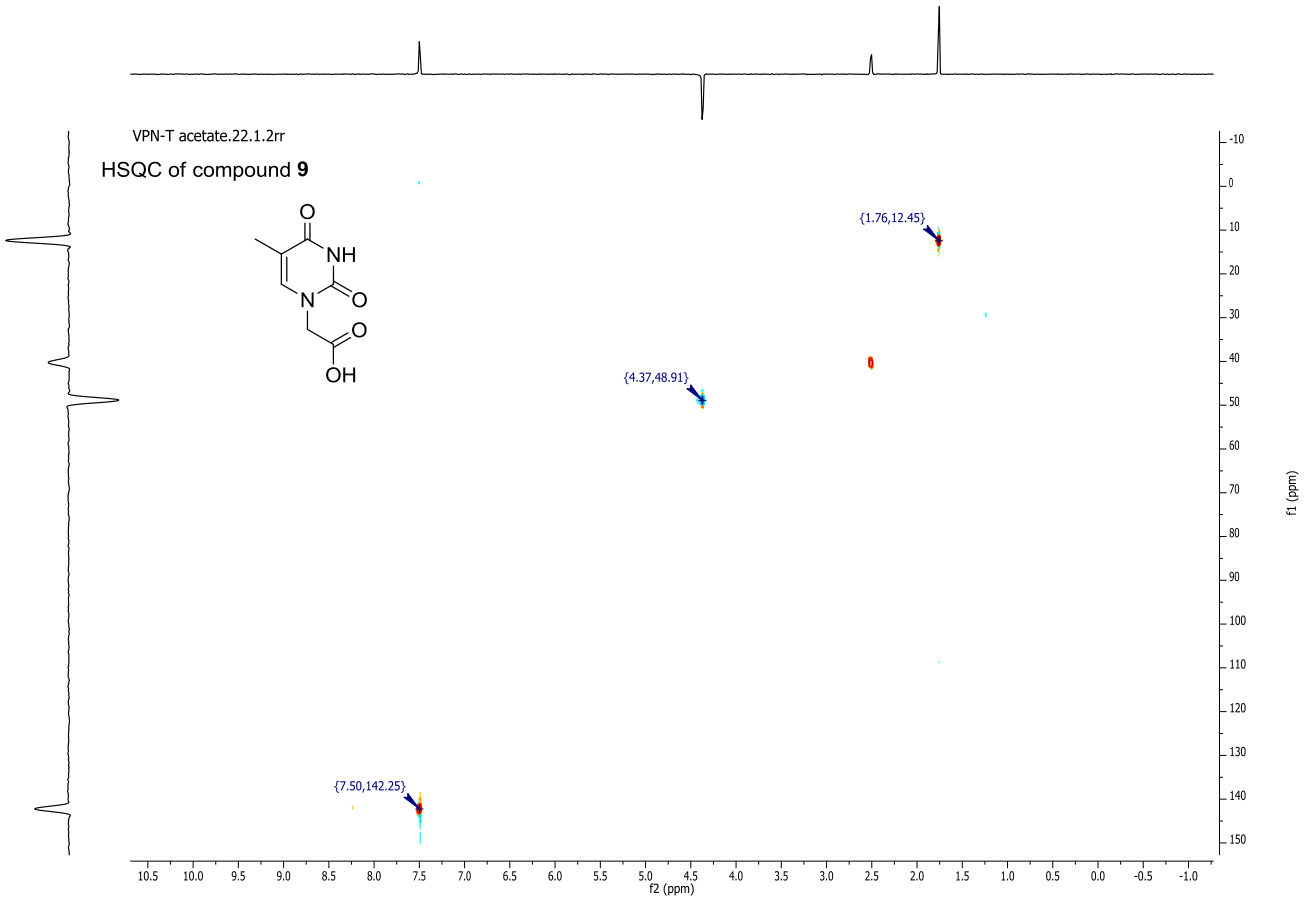
^{1}H NMR of comound **9**

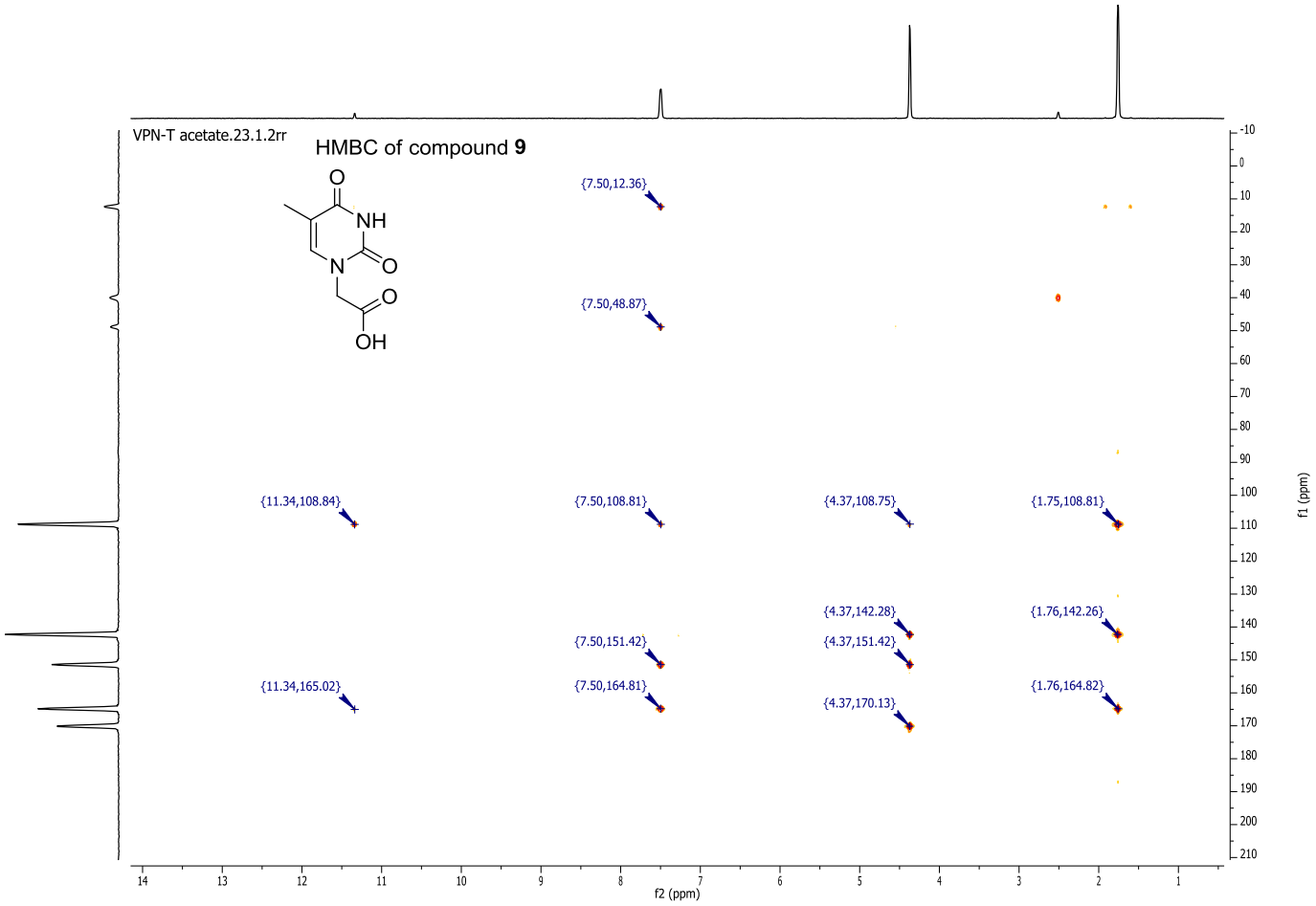


VPN-T acetate.11.fid

^{13}C NMR of comound **9**

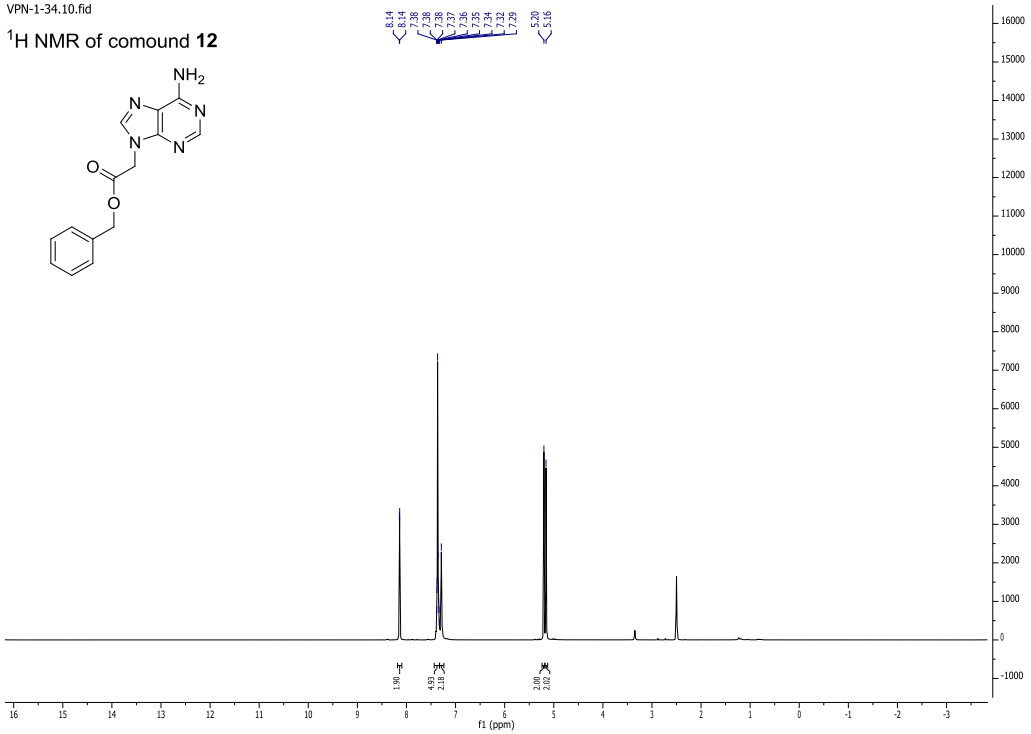



VPN-T acetate.22.1.2rr
HSQC of compound 9
O
NH
N
O
O
OH
{1.76,12.45}
{4.37,48.91}
{7.50,142.25}
f2 (ppm)
f1 (ppm)

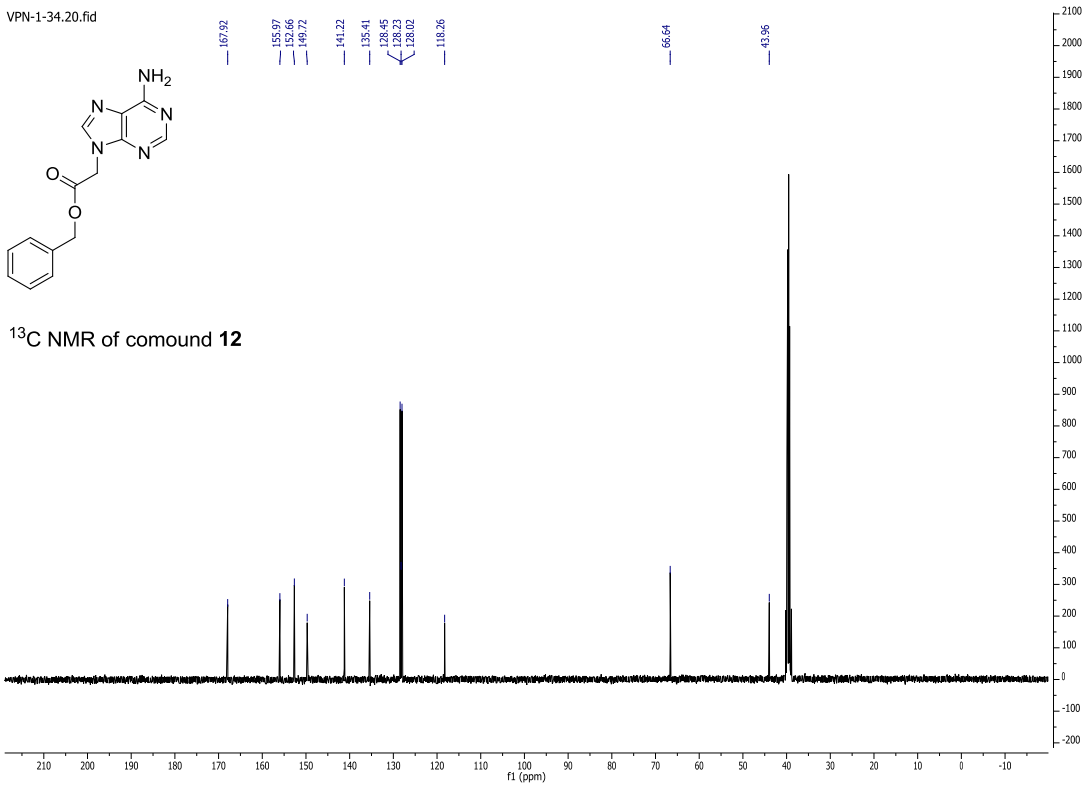

VPN-T acetate.23.1.2rr
HMBC of compound 9
O
NH
N
O
O
OH
{7.50,12.36}
{7.50,48.87}
{11.34,108.84}
{7.50,108.81}
{4.37,108.75}
{1.75,108.81}
{4.37,142.28}
{1.76,142.26}
{7.50,151.42}
{4.37,151.42}
{11.34,165.02}
{7.50,164.81}
{4.37,170.13}
{1.76,164.82}
f2 (ppm)
f1 (ppm)

VPN-1-34.10.fid

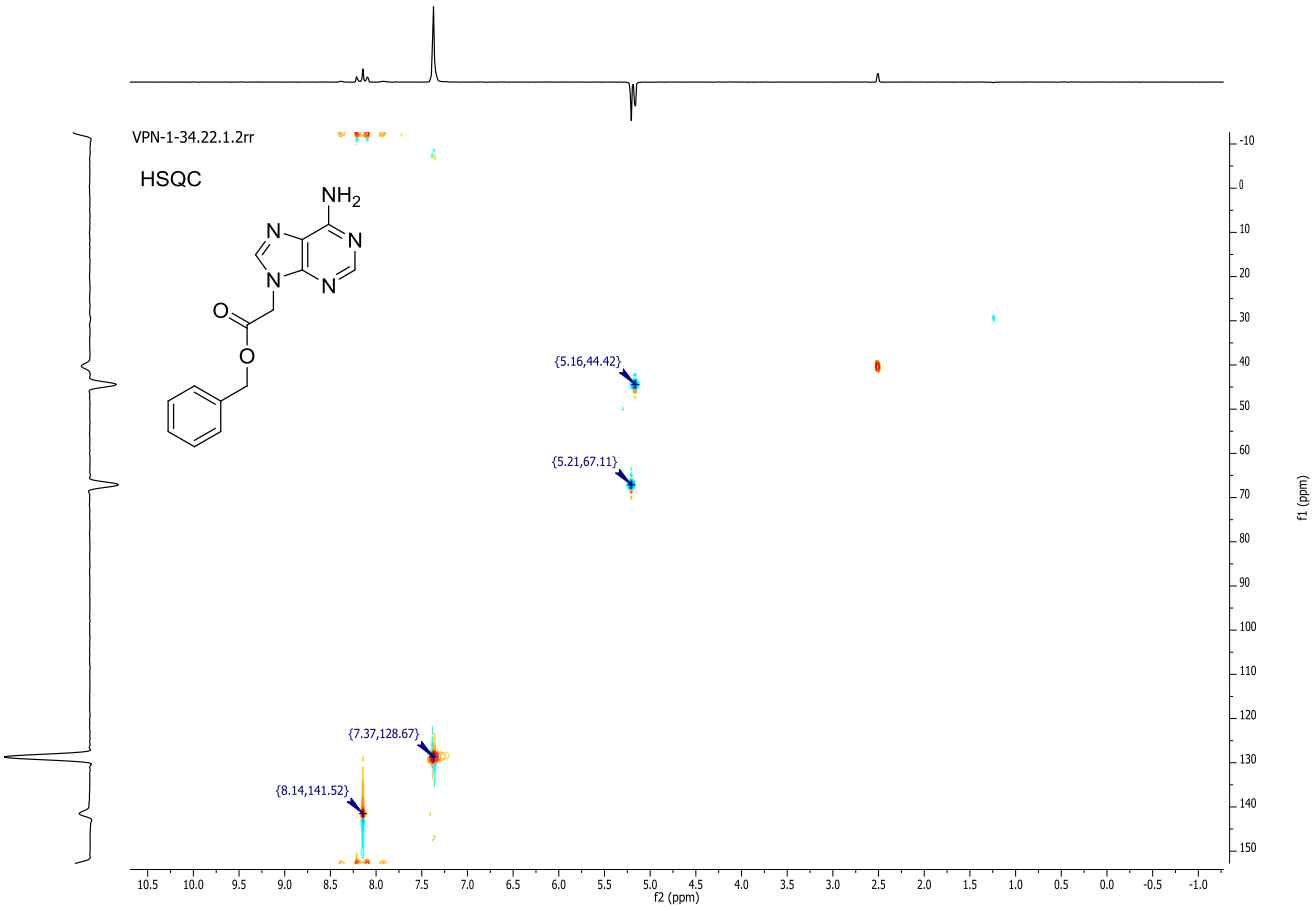
^{1}H NMR of comound **12**



VPN-1-34.20.fid



^{13}C NMR of comound **12**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HSQC
NH2
N
N
N
N
O
O
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{5.21,67.11}
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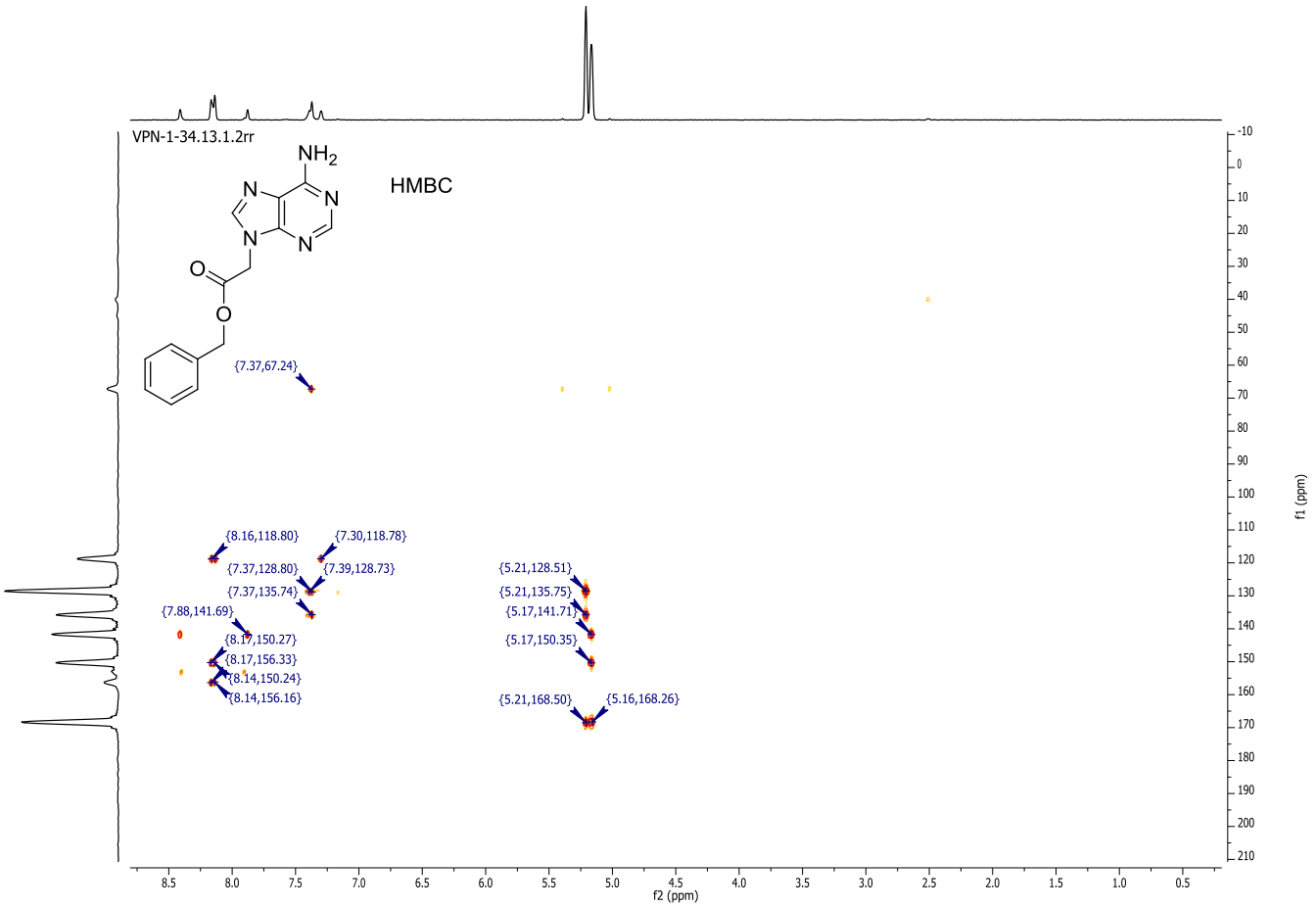
{8.14,141.52}
f2 (ppm)
f1 (ppm)


VPN-1-34.13.1.2rr
HMBC
NH2
N
N
N
N
O
O
{7.37,67.24}
{8.16,118.80}
{7.30,118.78}
{7.37,128.80}
{7.39,128.73}
{7.37,135.74}
{7.88,141.69}
{8.17,150.27}
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{8.14,150.24}
{8.14,156.16}
{5.21,128.51}
{5.21,135.75}
{5.17,141.71}
{5.17,150.35}
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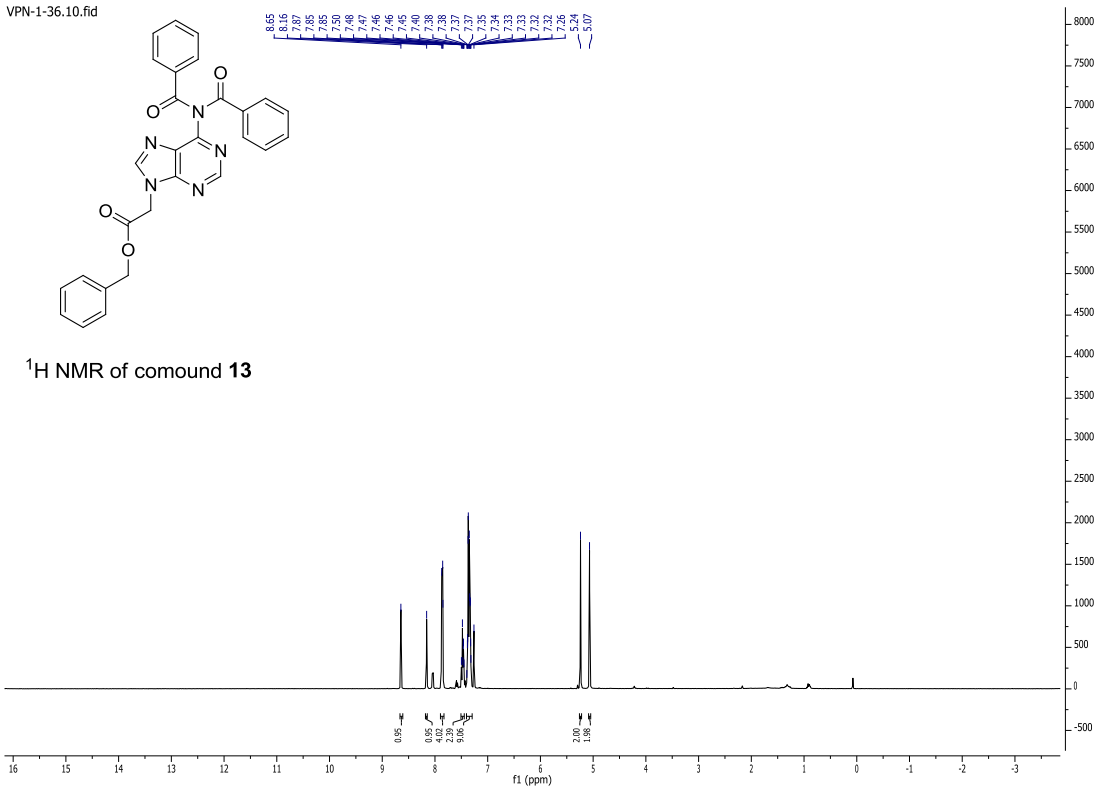
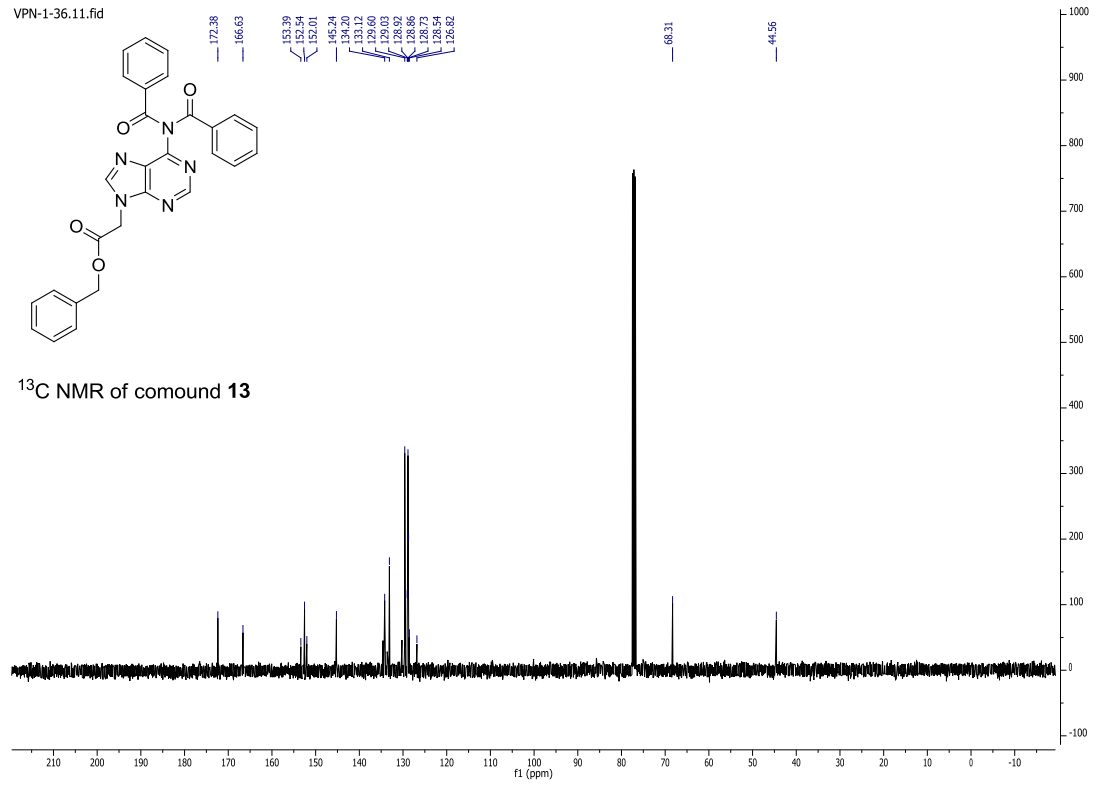
{5.16,168.26}
f2 (ppm)
f1 (ppm)

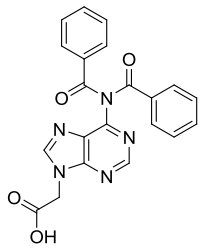


^{1}H NMR of comound **13**



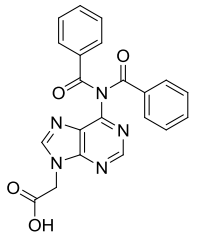
^{13}C NMR of comound **13**

VPN-1-39T.10.fid

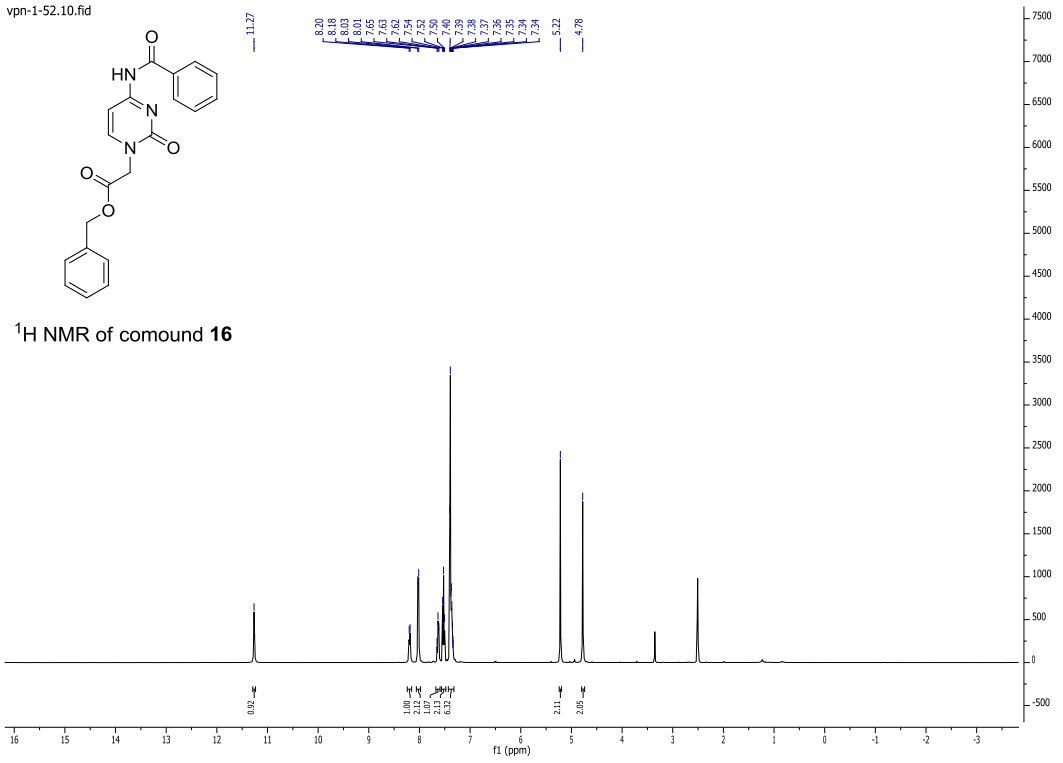


^{1}H NMR of comound **14**

VPN-1-39T.11.fid



^{13}C NMR of comound **14**



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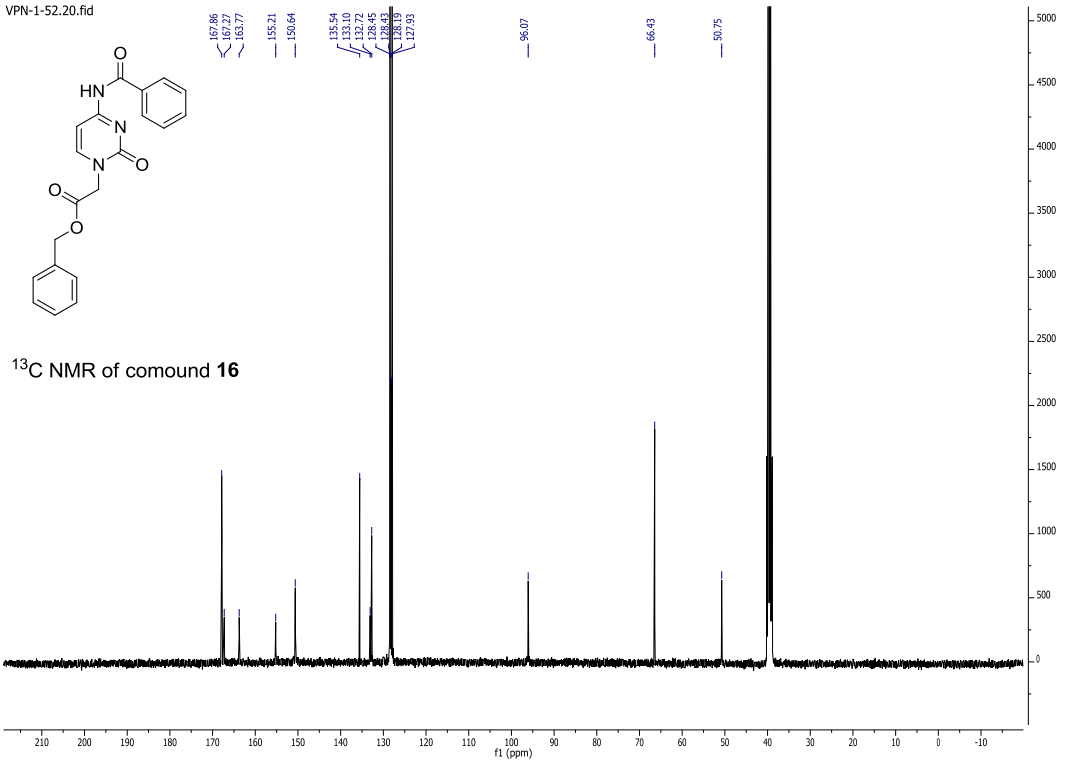
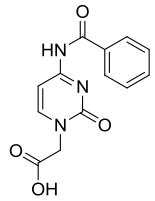


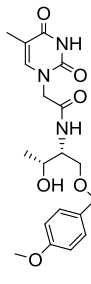
^{13}C NMR of comound **16**

VPN-1-60.10.fid

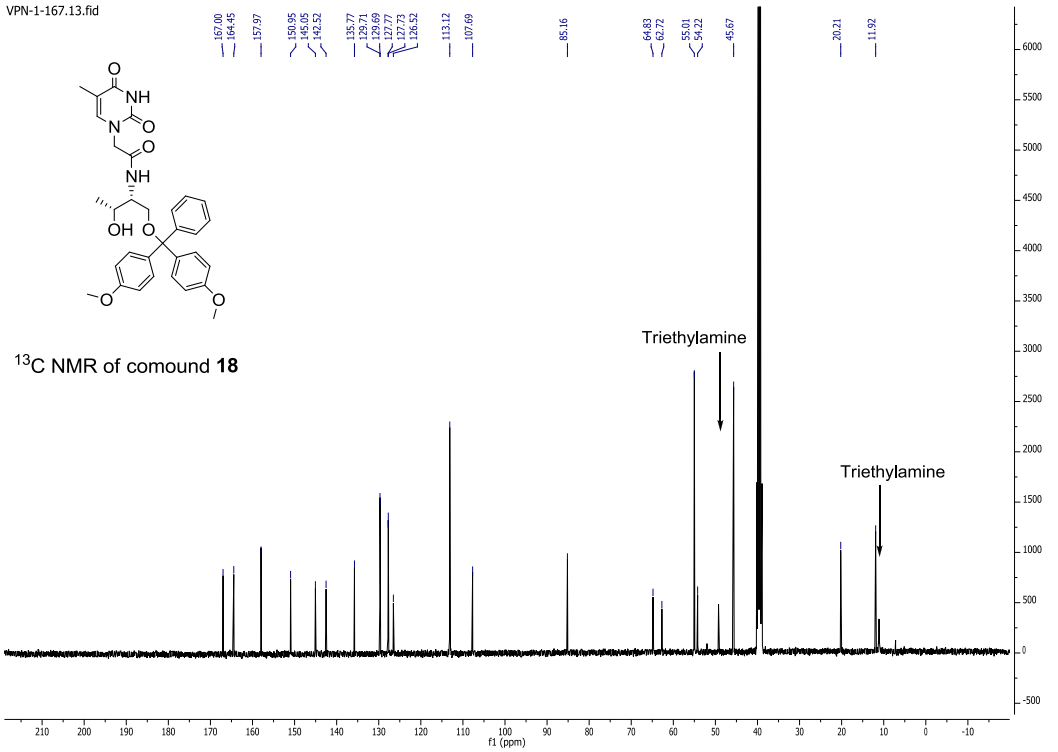


^{1}H NMR of comound **17**

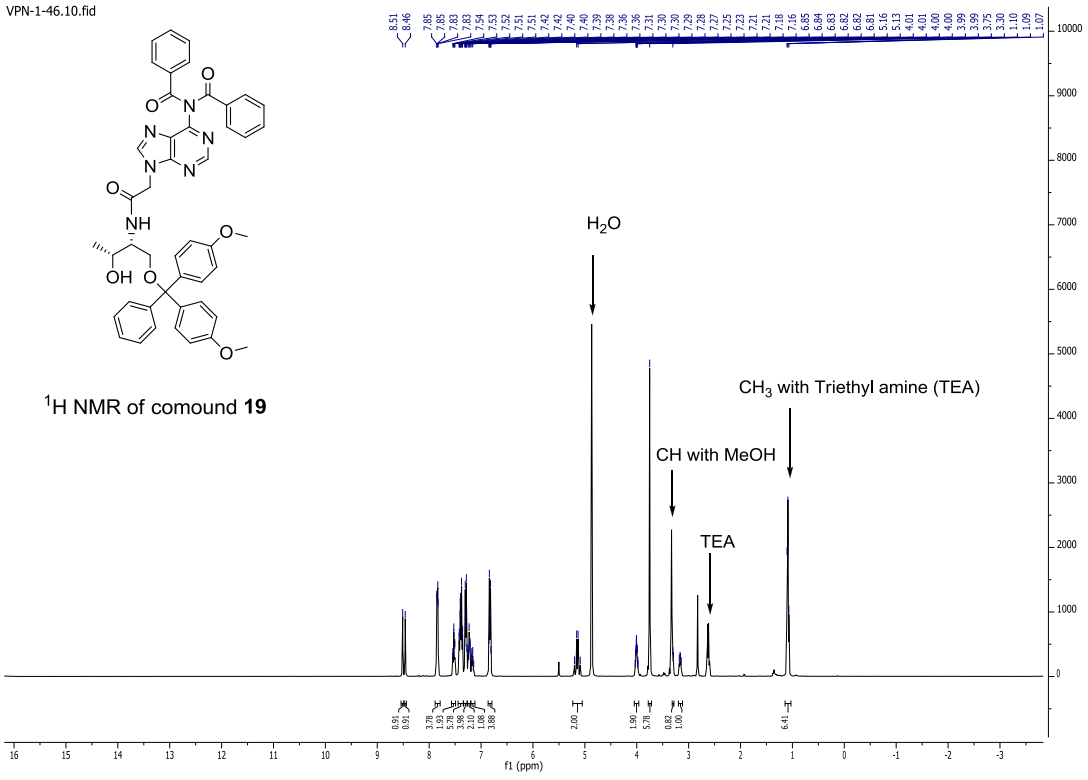
VPN-1-167.10.fid



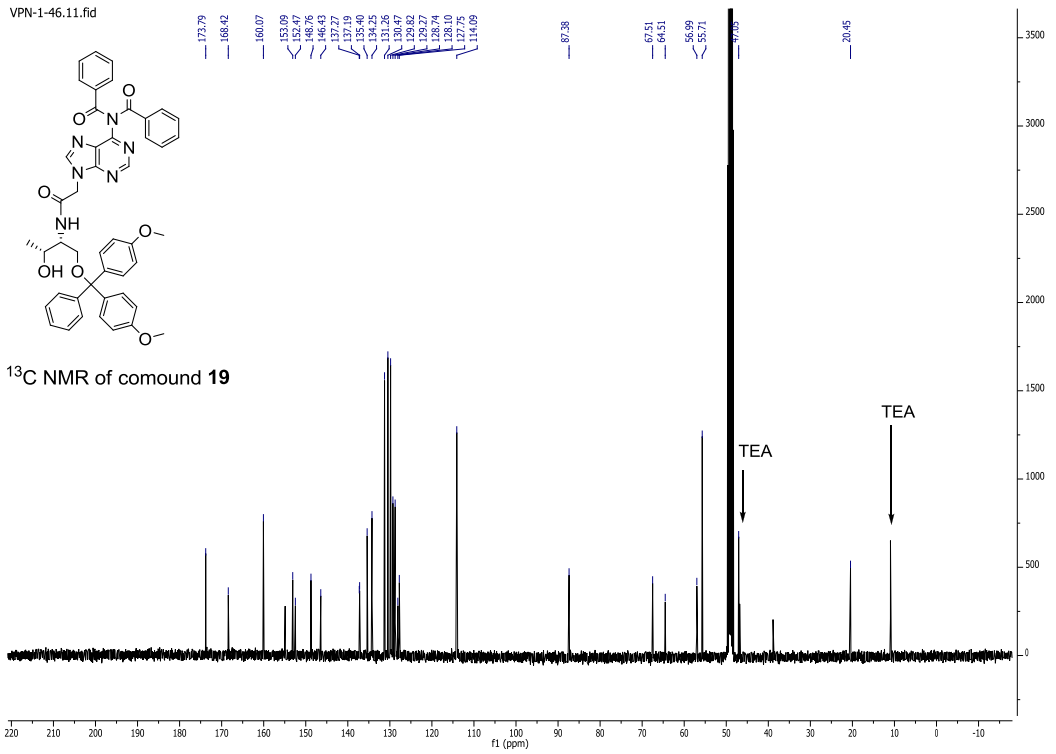
^{1}H NMR of comound **18**



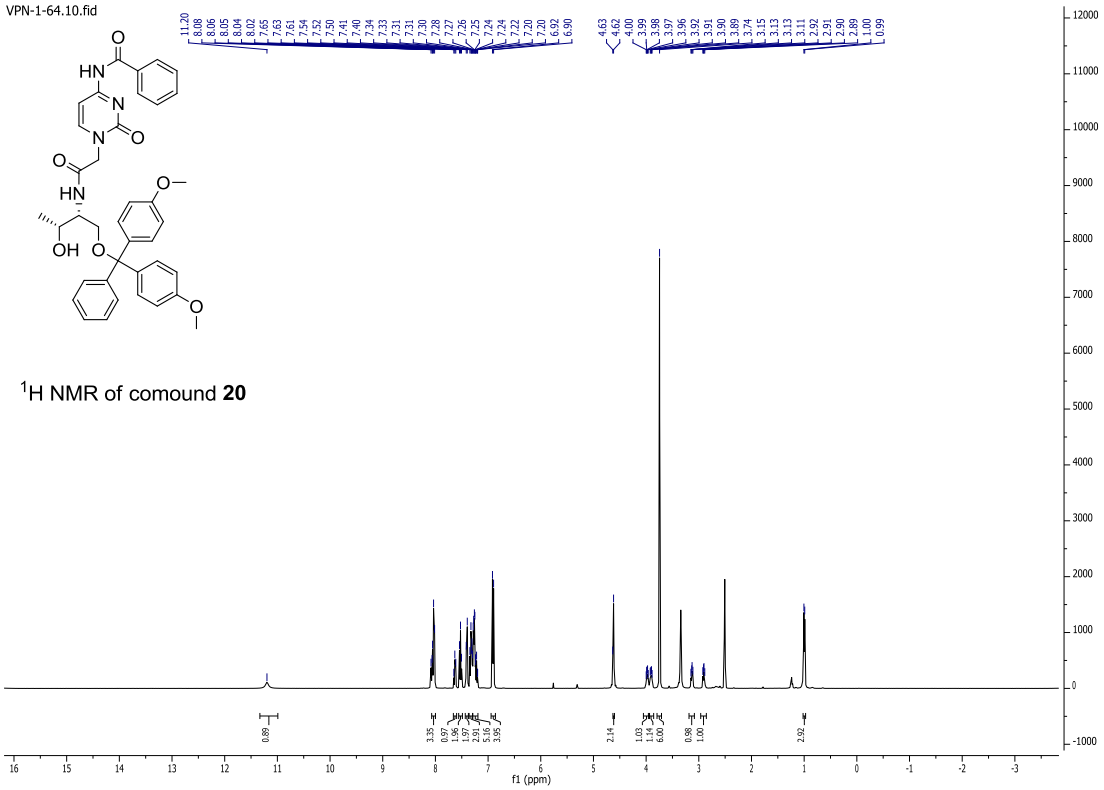
^{13}C NMR of comound **18**



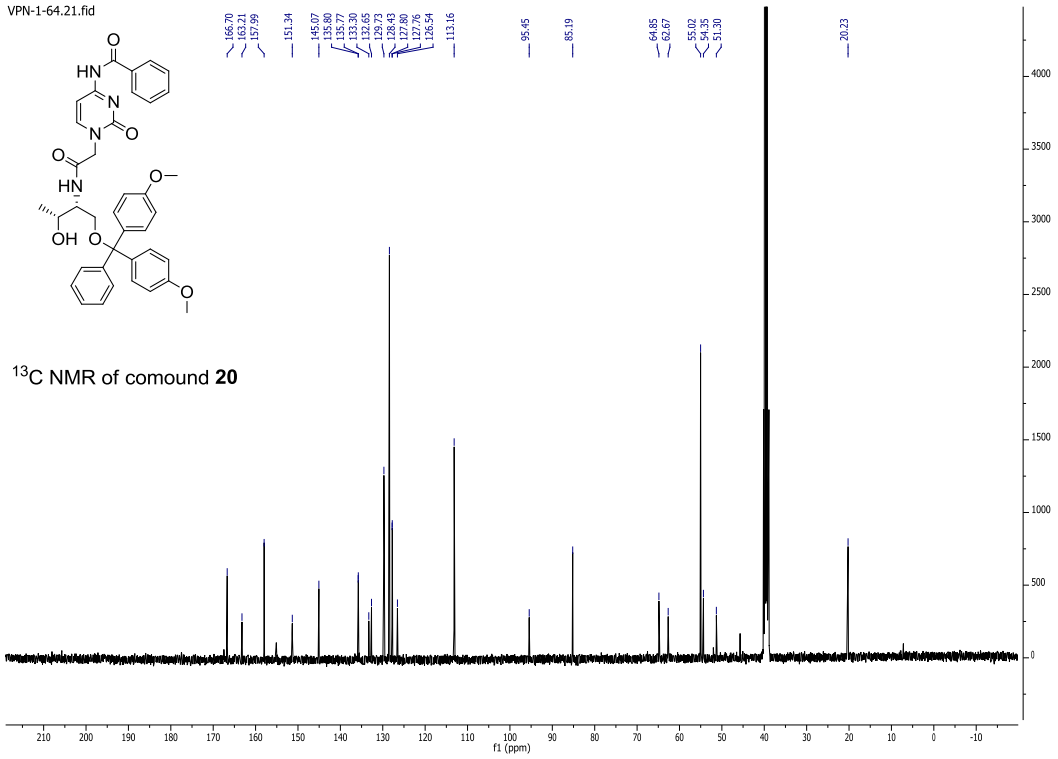
^{1}H NMR of comound **19**



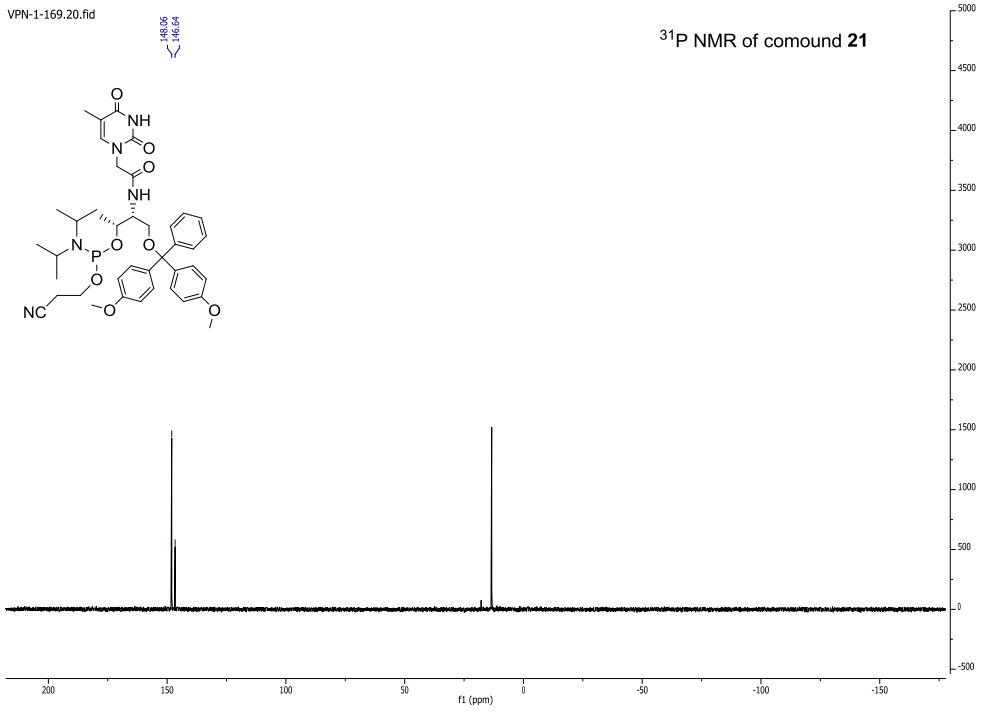
^{13}C NMR of comound **19**



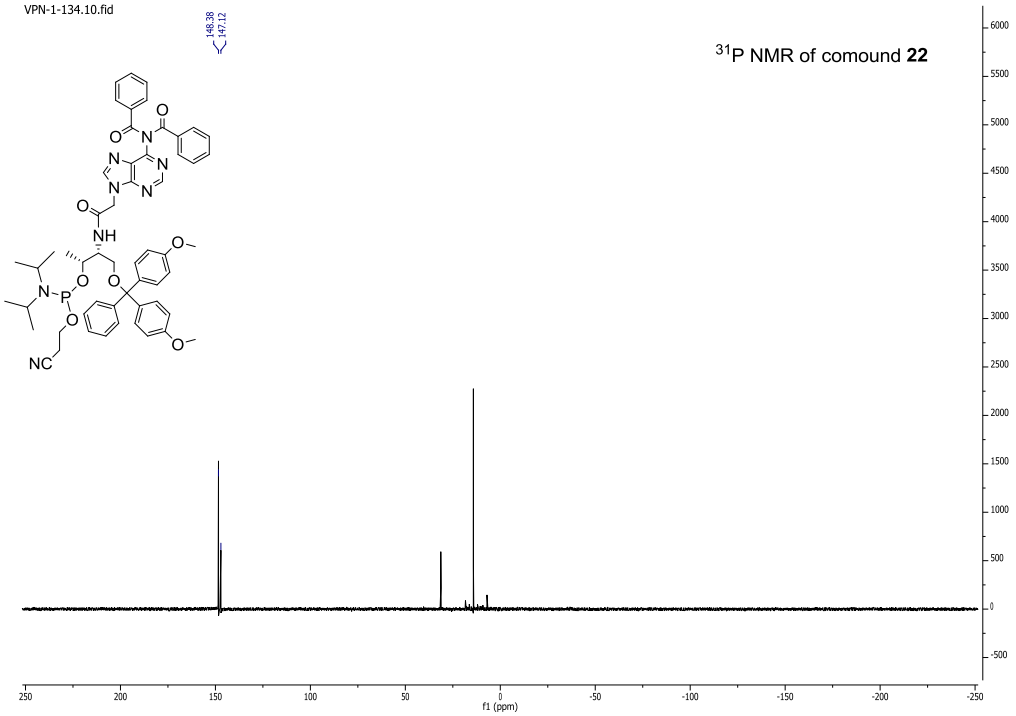
^{1}H NMR of comound **20**



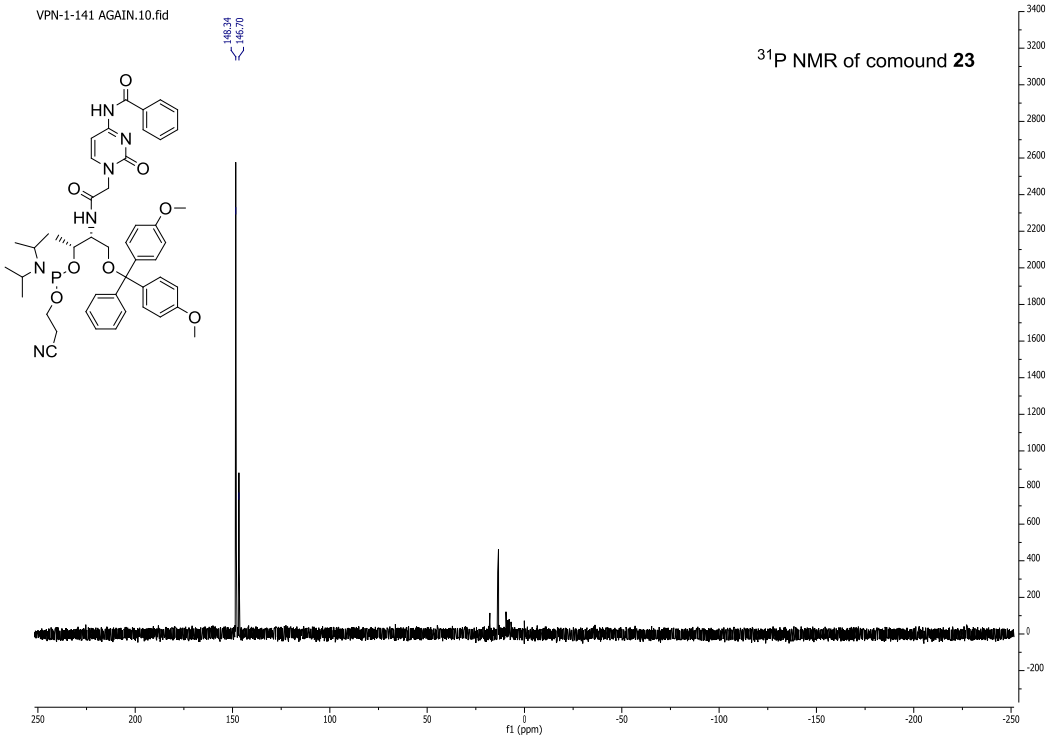
^{13}C NMR of comound **20**



^{31}P NMR of comound **21**



^{31}P NMR of comound **22**



^{31}P NMR of comound **23**

S3. Synthesis of aTNA oligonucleotides

For the aTNA oligonucleotide synthesis, we followed the standard protocol of phosphoramidite coupling in which monomers were dissolved (100 mM solution) in anhydrous acetonitrile. Coupling was performed for 6 min extended time. For this protocol we observed very low yield. Therefore, we dissolved the (L)-aTNA monomers (100 mM) in dichloromethane and couplings were carried out for standard time. By this approach, we observed very efficient synthesis similar to the normal DNA synthesis. Synthesis of (L)-aTNA was carried out on standard controlled pore glass (CPG) containing standard DNA nucleotides. We also synthesized (L)-aTNA on universal CPG, which was cleaved by using described protocol. For the synthesis of (D)-aTNA oligonucleotides similar protocol was followed.

S4. MALDI-TOF-MS

Acyclic aTNA oligonucleotides were analyzed by MALDI-TOF on HPA matrix. A saturated solution of 3-HPA in 50% acetonitrile was prepared. 100 µl of diammonium hydrogen citrate (100 g/ 1L) was added to 900 µl of the saturated 3-HPA solution. 0.5 µl of this matrix was loaded on steel target Maldi plate. Matrix was dried and 0.5 µl of oligo was added and further dried. Next, sample was analyzed on positive mode by Bruker instrument.

Entry	Oligonucleotide sequences [a]	Observed Mass	Expected Mass
ON-1	*5'*-aaaaaaaaaaaaaa-*4'*-dA-3'	5042.473	5042.62
ON-2	*5'*-ttttttttttttt-*4'*-dT-3'	4908.277	4907.52
ON-7	*5'*-tttttctctctctc-*4'*-dT-3'	4832.761	4832.32
ON-8	*5'*-tctctctctctttt-*4'*-dT-3'	4832.127	4832.32
ON-10	*5'*- ttt ttt tt-*4'*	2605.591	2603.84
ON-11	*5'*-ccc ccc cc-*4'*	2485.363	2483.74
ON-12	*5'*-aaaatttatattatt-*4'*-dA-3'	5314.101	5312.75
ON-13	*5'*-taataatataaatt-*4'*-dT-3'	5313.803	5312.75

[a] Small letters dictate acyclic nucleotides. The dA and dT indicate for standard DNA nucleotides. The a-TNA oligonucleotides are shown from *5'* to *4'* direction.

S5. Melting-profiles and CD studies

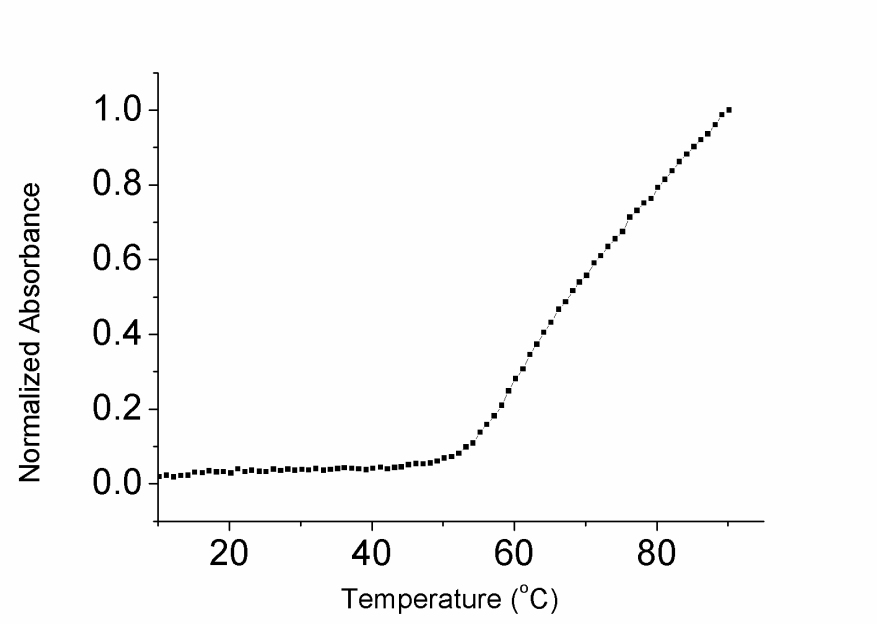


Figure S1 a) Melting-profile of ON-1 at 260 nm. Experimental conditions: Sample was incubated at 3 ºC for overnight in 10 mM phosphate buffer containing 100 mM sodium chloride at pH 7 with each oligo concentration of 2 µM.

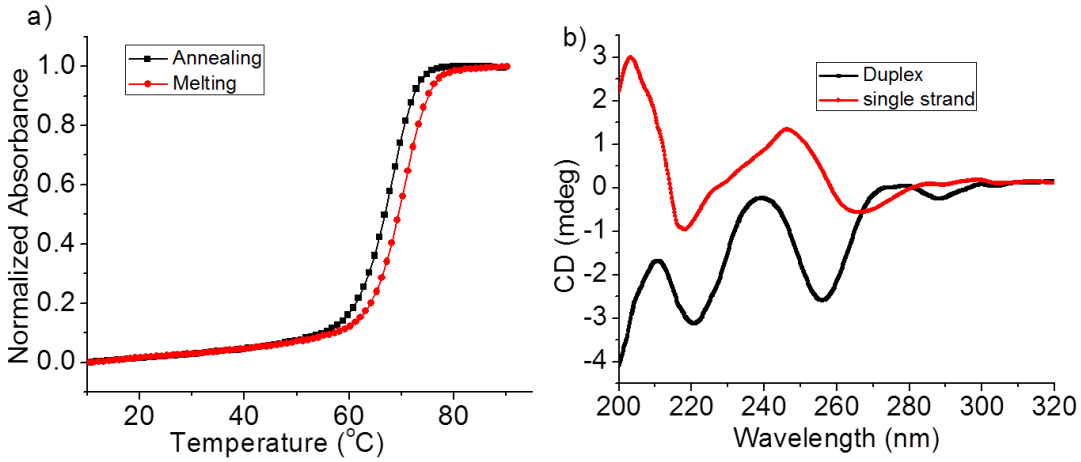


Figure S2 a) Melting-profile of (D)-aTNA duplex (ON-3/ON-4) at 260 nm; b) CD profile of (D)-aTNA duplex (ON-3/ON-4) and ON-3 at 20 ^{o}C. Experimental conditions: 10 mM phosphate buffer containing 100 mM sodium chloride at pH 7 with each oligo concentration of 0.5 µM for CD studies and 2 µM for UV-melting.

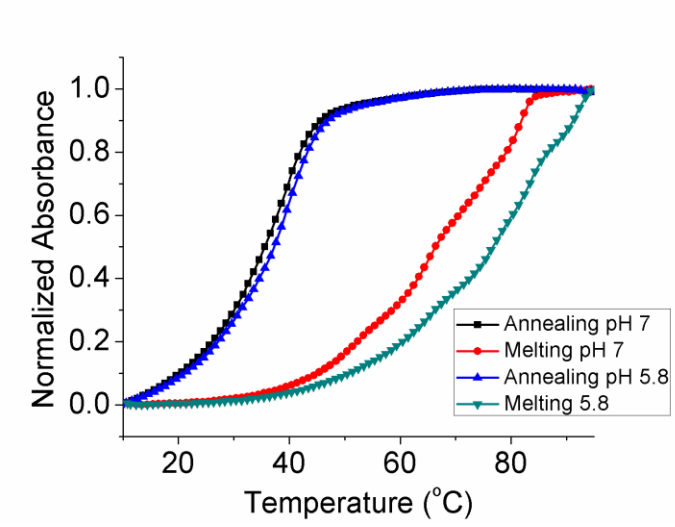


Figure S3 Melting-profile of ON-7/ON-8/ON-9 triple helix at 260 nm. (T_m = 64 ^{o}C, 81 ^{o}C, at pH 7.0) and (T_m = 76 ^{o}C, > 83 ^{o}C, at pH 5.8). Experimental conditions: 10 mM phosphate buffer containing 100 mM sodium chloride for each oligo of 1 µM.

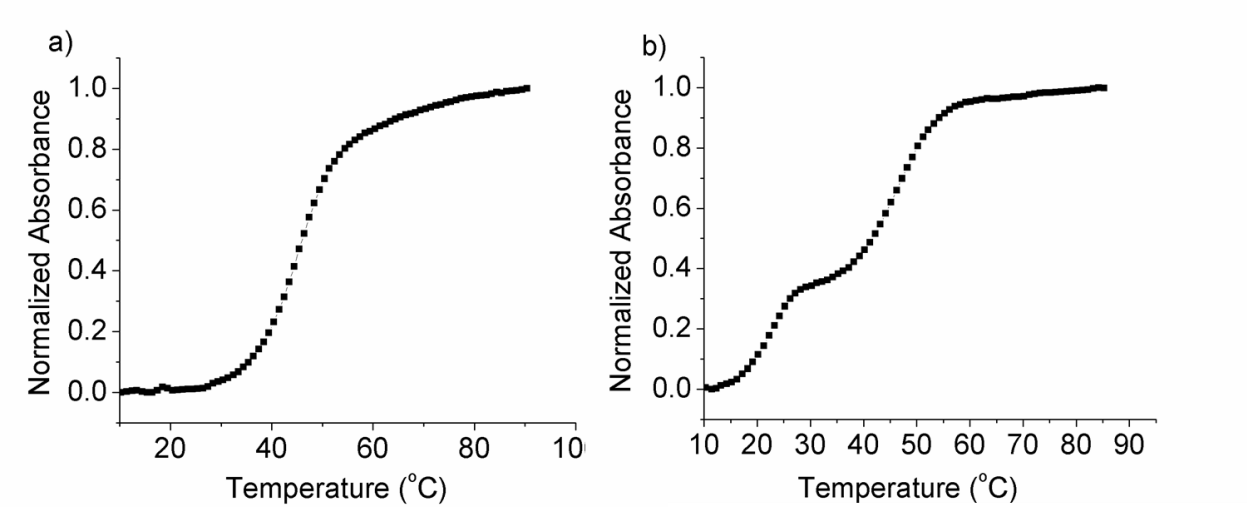


Figure S4. a) Melting-profile of (L)-aTNA $(t)_8$ (ON-10) with $d(A)_8$ in 10 mM phosphate buffer containing 100 mM sodium chloride at pH 7.0 for (L)-aTNA (2 µM) for DNA (1 µM); b) Melting-profile of (L)-aTNA $(c)_8$ (ON-11) with $d(G)_8$ in 10 mM phosphate buffer containing 100 mM sodium chloride at pH 5.8 for (L)-aTNA (2 µM) for DNA (1 µM).

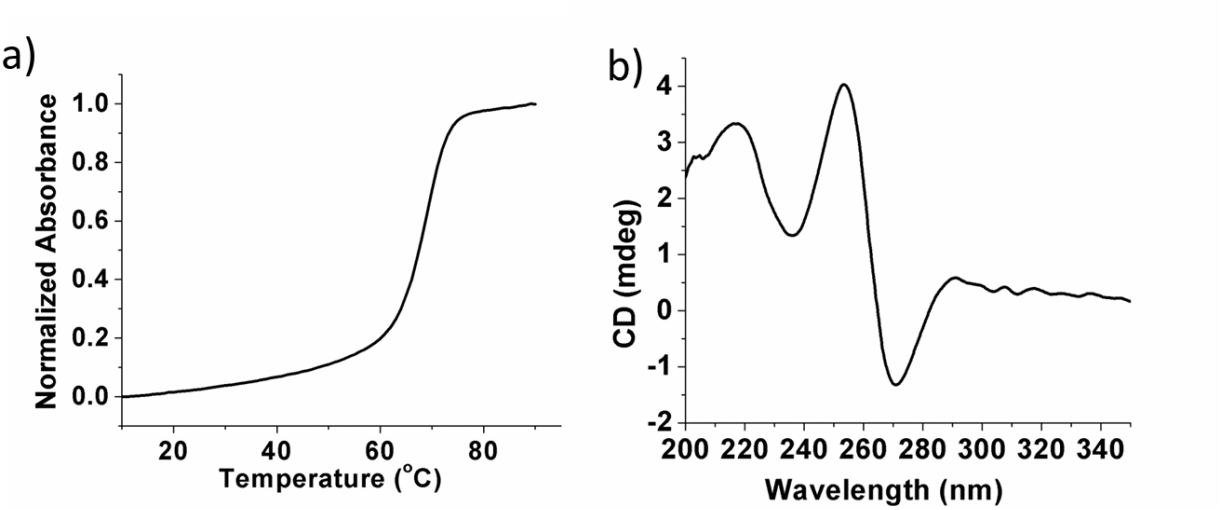


Figure S5. a) Melting profile of (L)-aTNA duplex (ON-12/ON-13) at 260 nm; b) CD profile (L)-aTNA duplex (ON-12/ON-13) at 20 ºC. Experimental conditions: 10 mM phosphate buffer containing 100 mM sodium chloride at pH 7 with each oligo concentration of 0.5 µM for CD studies and 2 µM for melting study.

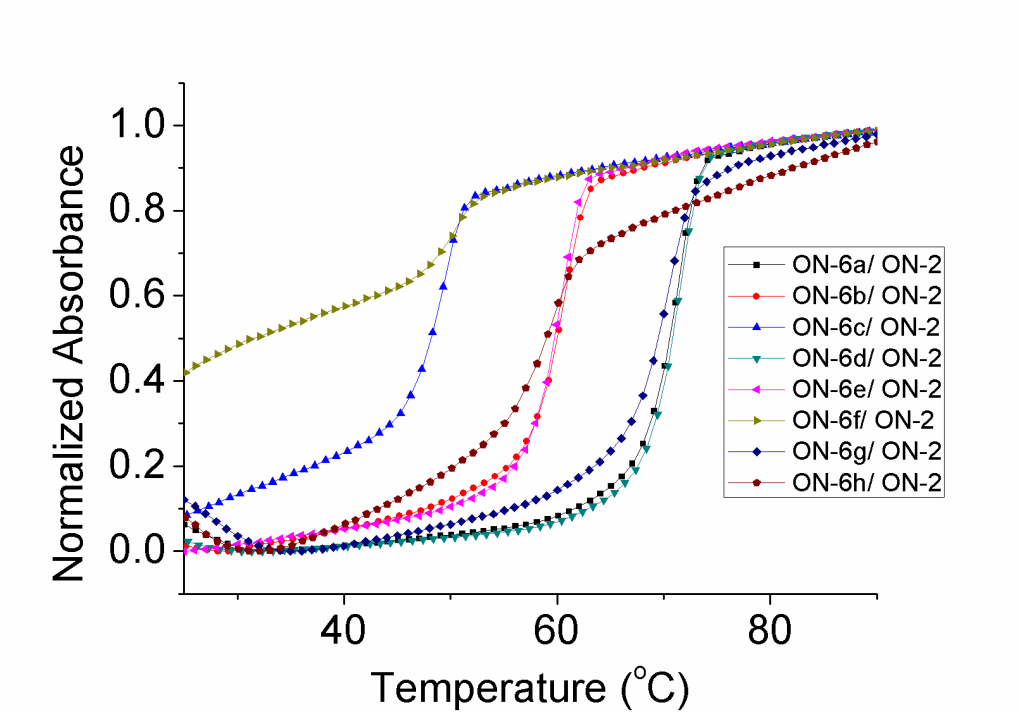


Figure S6. UV-melting profiles of triple helix at 260 nm. Experimental conditions: 10 mM phosphate buffer containing 100 mM sodium chloride at pH 7.0 with DNA strands (2 μM) and (L)-aTNA strand (4 μM) concentration.