Amido-bridged nucleic acids with small hydrophobic residues enhance hepatic tropism of antisense oligonucleotides *in vivo.*

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Figure S9-S33. ^{1}H, ^{13}C, and ^{31}P-NMR spectra of synthesized compounds

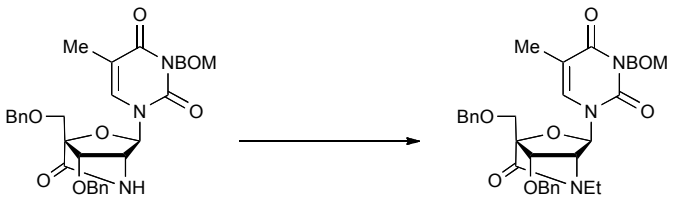
Figure S34-S50. MALDI-TOF mass data

1. General

All moisture-sensitive reactions were carried out in well-dried glassware under a N_2 atmosphere. Anhydrous dichloromethane, DMF, MeCN, and pyridine were used as purchased. ^{1}H NMR spectra were recorded at 300 and 400 MHz and 500 MHz, ^{13}C NMR were recorded at 75 and 100 MHz, and the ^{31}P spectrum was recorded at 161 MHz. Chemical shift values are expressed in δ values (ppm) relative to tetramethylsilane (TMS) as internal standard and residual solvents for ^{1}H NMR, and $CHCl_3$ (δ = 77.00 ppm), methanol (δ = 49.00 ppm), and DMSO (39.50 ppm) for ^{13}C NMR, and 85% H_3PO_4 (δ = 0 ppm) for ^{31}P NMR. Fast atom bombardment mass spectra (FAB-MS) were recorded in positive ion mode. For column chromatography, silica gel PSQ 100B was used. The progress of reaction was monitored by analytical thin layer chromatography (TLC) on pre-coated aluminium sheets (Silica gel 60 F_{254}- sheet-Merck), and the products were visualized by UV light.

2. Synthesis of AmNA monomers and phosphoramidites.

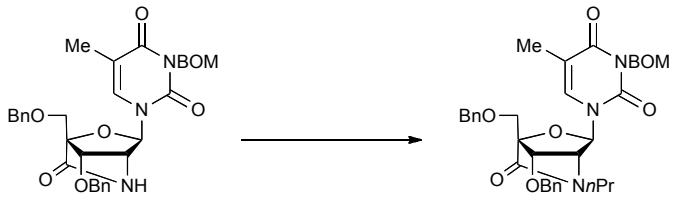
(2'R)-3',5'-Di-*O*-benzyl-N^3-benzyloxymethyl-2'-ethylamino-2'-*N*,4'-*C*-oxomethylenethymidine (2c: R = Et)



1 **2c**

To the stirring solution of **1** (100 mg, 0.171 mmol) in DMF (1.5 mL) was added NaH (8.2 mg, 0.205 mmol) at 0 °C. After stirring for 30 min, bromoethane (58.3 μL, 0.513 mmol) was added. The reaction temperature was gradually raised from 0 °C to room temperature and after completion of the reaction (approx. 30 min), ice-cold water was added. The solution was stirred for 15 min and the product was extracted with ethyl acetate (15 mL × 3). The organic phase was washed with brine (30 mL), dried (Na_2SO_4), and concentrated. The product was purified by flash column chromatography (*n*-hexane : ethyl acetate = 3 : 1) to afford **2c** (**R = Et** : 105 mg, quant) as a white amorphous solid.

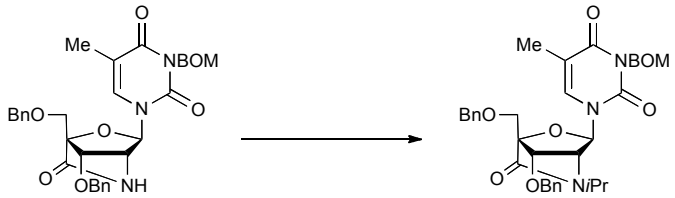
(2'R)-3',5'-Di-*O*-benzyl-N^3-benzyloxymethyl-2'-propylamino-2'-*N*,4'-*C*-oxomethylenethymidine (2d: R = *n*Pr)



1 **2d**

To the stirring solution of **1** (750 mg, 1.29 mmol) in DMF (13 mL) was added NaH (61.9 mg, 1.55 mmol) at 0 ºC. After stirring for 30 min, 1-bromopropane (141 μL, 1.55 mmol) was added. After 30 min, the reaction temperature was gradually raised from 0 ºC to room temperature and after completion of the reaction (approx. 30 min), ice-cold water was added. The solution was stirred for 10 min and the product was extracted with ethyl acetate (70 mL×3). The organic phase was washed with brine (100 mL), dried (Na_2SO_4), and concentrated. The product was purified by flash column chromatography (*n*-hexane : ethyl acetate = 2 : 1) to afford **2d** (**R = *n*Pr** : 610 mg, 76%) as a white amorphous solid.

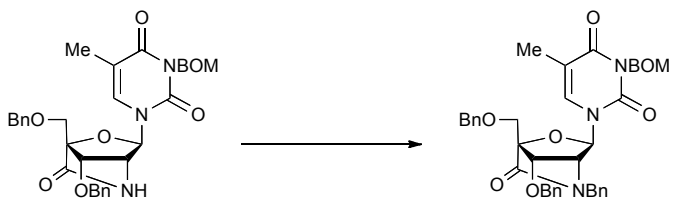
(2'R)-3',5'-Di-*O*-benzyl-N^3-benzyloxymethyl-2'-isopropylamino-2'-*N*,4'-*C*-oxomethylenethymidine (2e: R = *i*Pr)



1 **2e**

To the stirring solution of **1** (100 mg, 0.171 mmol) in DMF (1.5 mL) was added NaH (8.2 mg, 0.205 mmol) at 0 ºC. After stirring for 30 min, 2-iodopropane (51.2 μL, 0.513 mmol) was added. Afeter 30 min, the reaction temperature was gradually raised from 0 ºC to room temperature and after completion of the reaction (approx. 4 h), ice-cold water was added. The solution was stirred for 10 min and the product was extracted with ethyl acetate (15 mL×3). The organic phase was washed with brine (30 mL), dried (Na_2SO_4), and concentrated. The product was purified by flash column chromatography (*n*-hexane : ethyl acetate = 3 : 1) to afford **2e** (**R = *i*Pr** : 107 mg, quant) as a white amorphous solid.

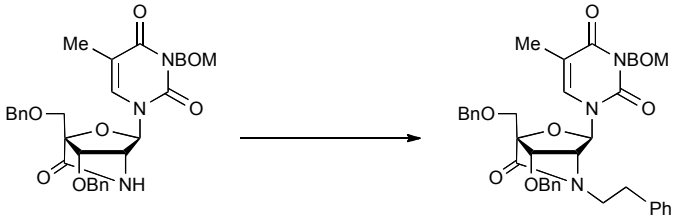
(2'R)- 3',5'-Di-*O*-benzyl- N^3-benzyloxymethyl-2'-benzylamino-2'-*N*,4'-*C*-oxomethylenethymidine (2f: R = Bn)



1 **2f**

To the stirring solution of **1** (2.00 g, 3.4 mmol) in DMF (35 mL) was added NaH (164 mg, 4.1 mmol) at 0 ºC. After stirring for 30 min, bromomethylbenzene (487 μL, 4.1 mmol) was added. After 1 hr, the reaction temperature was gradually raised from 0 ºC to room temperature and after completion of the reaction (approx. 30 min), ice-cold water was added. The solution was stirred for 10 min and the product was extracted with ethyl acetate (150 mL×3). The organic phase was washed with brine (200 mL), dried (Na_2SO_4), and concentrated. The product was purified by flash column chromatography (*n*-hexane : ethyl acetate = 2 : 1) to afford **2f (R = Bn** : 2.4 g, quant) as a white amorphous solid.

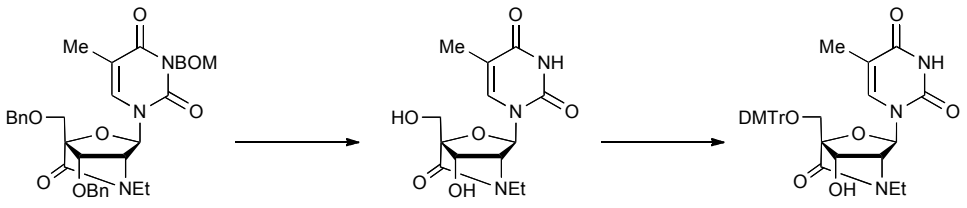
(2'R)-3',5'-Di-*O*-benzyl-N^3-benzyloxymethyl-2'-phenethylamino-2'-*N*,4'-*C*-oxomethylenethymidine (2g: R = CH_2CH_2Ph)



1 **2g**

To the stirring solution of **1** (2.00 g, 3.4 mmol) in DMF (35 mL) was added NaH (164 mg, 4.1 mmol) at 0 ºC. After stirring for 30 min, 2-bromoethylbenzene (554 μL, 4.1 mmol) was added. The reaction temperature was gradually raised from 0 ºC to room temperature and after completion of the reaction (approx. 30 min), ice-cold water was added. The solution was stirred for 10 min and the product was extracted with ethyl acetate (150 mL×3). The organic phase was washed with brine (200 mL), dried (Na_2SO_4), and concentrated. The product was purified by flash column chromatography (*n*-hexane : ethyl acetate = 2 : 1) to afford **2g** (**R = CH_2CH_2Ph** : 997 mg, 43%) as a white amorphous solid.

(2'R)-5'-*O*-(4,4'-Dimethoxytrityl)-2'-ethylamino-2'-*N*,4'-*C*-oxomethylenethymidine (3c : R = Et)



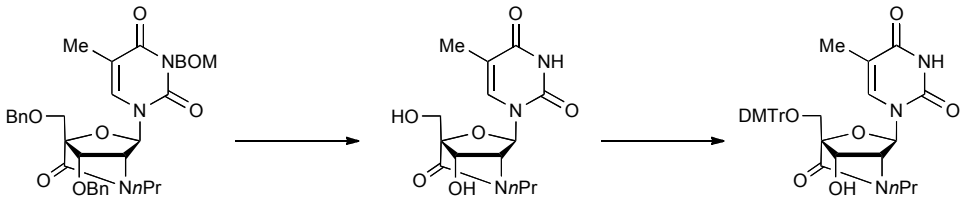
2c **S1c** **3c**

To the solution of **2c** (**R = Et**: 83.8 mg, 0.137 mmol) in THF (1.5 mL) was added 20 % palladium on carbon (83 mg) and the reaction vessel was degassed several times with hydrogen. The reaction mixture was stirred under a hydrogen atmosphere for 4.5 h at room temperature. After completion of the reaction, the reaction solution was filtered by filter paper

and washed thoroughly by methanol (30 mL). After evaporation of solvents, the product was dissolved with methanol (1.5 mL) and added 28 % ammonia solution (1.5 mL) was added and the solution was stirred at room temperature. After 5 min, the product was concentrated to afford **S1c** (**R = Et**: 42.8 mg) as a white solid.

To the solution of **S1c** (**R = Et**: 42.8 mg) in anhydrous pyridine (1.5 mL) was added DMTrCl (55.7 mg, 0.164 mmol) and the solution was stirred at room temperature. After stirring for 2.5 h, ice-cold water was added and the product was extracted with ethyl acetate (15 mL×3). The organic phase was washed with brine (30 mL), dried (Na_2SO_4), and concentrated. The product was purified by flash column chromatography (*n*-hexane : ethyl acetate = 1 : 1) to afford **3c** (**R = Et**: 38.9 mg, 45%, 2 steps) as a white amorphous solid.

(2'R)-5'-*O*-(4,4'-Dimethoxytrityl)-2'-propylamino-2'-*N*,4'-*C*-oxomethylenethymidine (3d: R = *n*Pr)

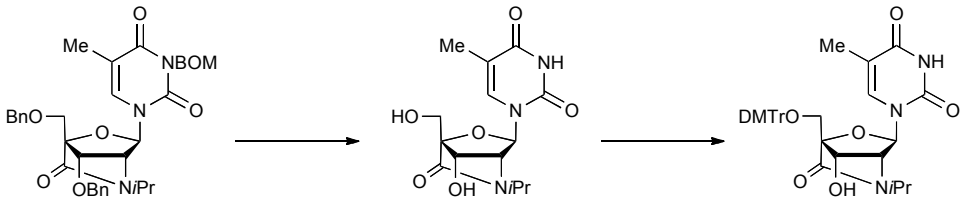


2d **S1d** **3d**

To the solution of **2d** (**R = *n*Pr**: 590 g, 0.943 mmol) in methanol (9.5 mL) was added 20 % palladium on carbon (590 mg) and the reaction vessel was degassed several times with hydrogen. The reaction mixture was stirred under a hydrogen atmosphere for 1 h at rt. After completion of the reaction, the reaction solution was filtered by filter paper and washed thoroughly by methanol. After evaporation of solvents, the product was dissolved with methanol (5 mL) and added 28 % ammonia solution (5 mL) was added and the solution was stirred at room temperature. After 5 min, the product was concentrated to afford **S1d** (**R = *n*Pr**: 311 mg) as a white solid.

To the solution of **S1d** (**R = *n*Pr**: 311 mg) in anhydrous pyridine (9.5 mL) was added DMTrCl (383 mg, 1.13 mmol) and the solution was stirred at room temperature. After stirring for 19 h, ice-cold water was added and the product was extracted with ethyl acetate (50 mL×3). The organic phase was washed with brine (70 mL), dried (Na_2SO_4), and concentrated. The product was purified by flash column chromatography (*n*-hexane : ethyl acetate = 1 : 3) to afford **3d** (**R = *n*Pr**: 573 mg, 96%, 2 steps) as a white amorphous solid.

(2'R)-5'-*O*-(4,4'-Dimethoxytrityl)-2'-isopropylamino-2'-*N*,4'-*C*-oxomethylenethymidine (3e: R = *i*Pr)

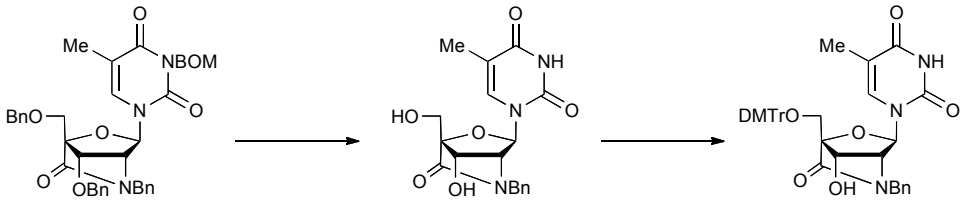


2e **S1e** **3e**

To the solution of **2e** (**R = *i*Pr**: 81.3 mg, 0.130 mmol) in THF (1.5 mL) was added 20 % palladium on carbon (81 mg) and the reaction vessel was degassed several times with hydrogen. The reaction mixture was stirred under a hydrogen atmosphere for 5 h at rt. After completion of the reaction, the reaction solution was filtered by filter paper and washed thoroughly by methanol. After evaporation of solvents, the product was dissolved with methanol (1.5 mL) and added 28 % ammonia solution (1.5 mL) was added and the solution was stirred at room temperature. After 5 min, the product was concentrated to afford **S1e** (**R = *i*Pr**: 42.2 mg) as white solid.

To the solution of **S1e** (**R = *i*Pr**: 42.2 mg) in anhydrous pyridine (1.5 mL) was added DMTrCl (52.9 mg, 0.156 mmol) and the solution was stirred at room temperature. After stirring for 2.5 h, ice-cold water was added and the product was extracted with ethyl acetate (10 mL×3). The organic phase was washed with brine (20 mL), dried (Na_2SO_4), and concentrated. The product was purified by flash column chromatography (*n*-hexane : ethyl acetate = 1 : 1) to afford **3e** (**R = *i*Pr**: 65.7 mg, 78%, 2 steps) as a white amorphous solid.

(2'R)-5'-*O*-(4,4'-Dimethoxytrityl)-2'-benzylamino-2'-*N*,4'-*C*-oxomethylenethymidine (3f: R = Bn)

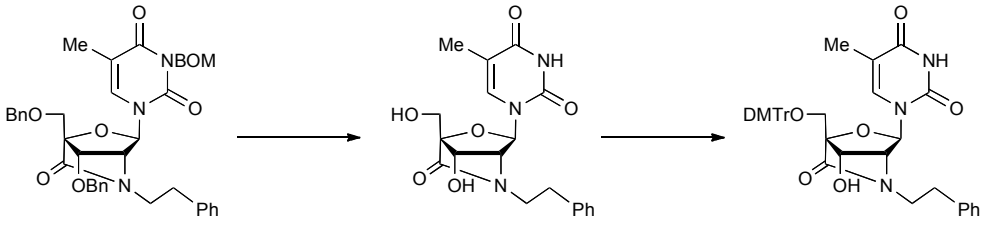


2f **S1f** **3f**

To the solution of **2f** (**R = Bn**: 2.3 g, 3.4 mmol) in methanol (30 mL) was added 20 % palladium on carbon (2.3 g) and the reaction vessel was degassed several times with hydrogen. The reaction mixture was stirred under a hydrogen atmosphere for 1 h at rt. After completion of the reaction, the reaction solution was filtered by filter paper and washed thoroughly by methanol. After evaporation of solvents, the product was dissolved with methanol (10 mL) and added 28 % ammonia solution (0.22 mL) was added and the solution was stirred at room temperature. After 5 min, the product was concentrated to afford **S1f** (**R = Bn** : 1.3 g) as a white solid.

To the solution of **S1f** (**R = Bn** : 1.3 g) in anhydrous pyridine (35 mL) was added DMTrCl (1.5 g, 4.4 mmol) and the solution was stirred at room temperature. After stirring for 1 h, ice-cold water was added and the product was extracted with ethyl acetate (100 mL×3). The organic phase was washed with brine (150 mL), dried (Na_2SO_4), and concentrated. The product was purified by flash column chromatography (*n*-hexane : ethyl acetate = 1 : 3) to afford **3f** (**R = Bn**: 2.06 g, 90%, 2 steps) as a white amorphous solid.

(2'R)-5'-*O*-(4,4'-Dimethoxytrityl)-2'-phenethyl amino-2'-*N*,4'-*C*-oxomethylenethymidine (3g: R = CH_2CH_2Ph)

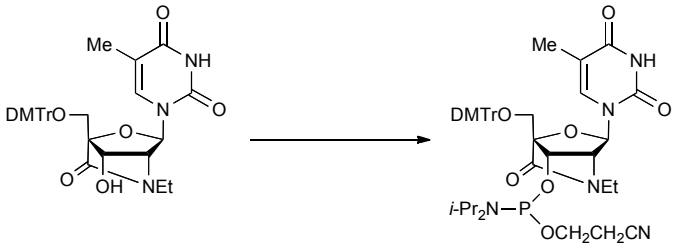


2g **S1g** **3g**

To the solution of **2g** (**R = CH_2CH_2Ph**: 997 mg, 1.45 mmol) in methanol (15 mL) was added 20 % palladium on carbon (990 mg) and the reaction vessel was degassed several times with hydrogen. The reaction mixture was stirred under a hydrogen atmosphere for 1 h at rt. After completion of the reaction, the reaction solution was filtered by filter paper and washed thoroughly by methanol. After evaporation of solvents, the product was dissolved with methanol (5 mL) and added 28 % ammonia solution (0.09 mL) was added and the solution was stirred at room temperature. After 5 min, the product was concentrated to afford **S1g** (**R = CH_2CH_2Ph**: 569 mg) as a white solid.

To the solution of **S1g** (**R = CH_2CH_2Ph**: 569 mg) in anhydrous pyridine (15 mL) was added DMTrCl (630 mg, 1.86 mmol) and the solution was stirred at room temperature. After stirring for 1 h, ice-cold water was added and the product was extracted with ethyl acetate (70 mL×3). The organic phase was washed with brine (100 mL), dried (Na_2SO_4), and concentrated. The product was purified by flash column chromatography (*n*-hexane : ethyl acetate = 1 : 3) to afford **3g** (**R = CH_2CH_2Ph**: 826 mg, 84%, 2 steps) as a white amorphous solid.

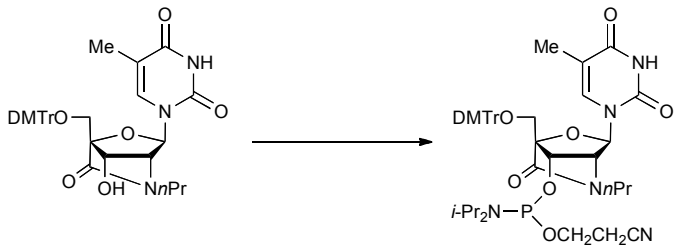
(2'R)-3'-*O*-[2-Cyanoethoxy(disopropylamino)phosphino]-5'-*O*-(4,4'-dimethoxytrityl)-2'-ethylamino-2'-*N*,4'-*C*-oxomethylenethymidine (4c: R = Et)



3c **4c**

To the solution of **3c** (**R = Et**: 38.3 mg, 0.062 mmol) in anhydrous MeCN-THF (3:1, 0.6 mL) was added *N,N*-diisopropylammoniumtetrazolide (8.04 mg, 0.047mmol) and 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphorodiamidite (23.5 μL, 0.074 mmol). After stirring at room temperature for 9 h, ice-cold water was added and the product was extracted with ethyl acetate (10 mL×3). The organic phase was washed with brine (20 mL), dried (Na_2SO_4), and concentrated. The product was purified by flash column chromatography (0.05 eq. of triethylamine in *n*-hexane : ethyl acetate = 1 : 1) to afford **4c** (**R = Et**: 39 mg, 77%) as a white amorphous solid.

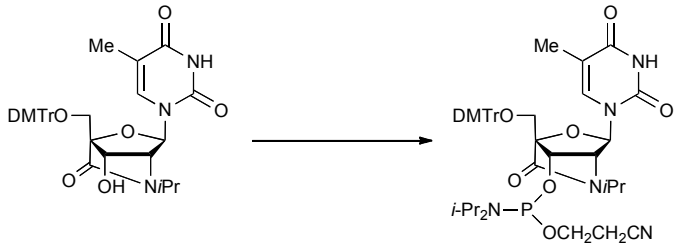
(2'R)-3'-*O*-[2-Cyanoethoxy(disopropylamino)phosphino]-5'-*O*-(4,4'-dimethoxytrityl)-2'-propylamino-2'-*N*,4'-*C*-oxo methylenethymidine (4d: R = *n*Pr)



3d **4d**

To the solution of **3d** (**R** = ***n*Pr**: 448 mg, 0.71 mmol) in anhydrous MeCN-THF (3:1, 7 mL) was added *N,N*-diisopropylammoniumtetrazolide (91.6 mg, 0.535 mmol) and 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphorodiamidite (272 μL, 0.857 mmol). After stirring at room temperature for 19 h, ice-cold water was added and the product was extracted with ethyl acetate (35 mL×3). The organic phase was washed with brine (50 mL), dried (Na_2SO_4), and concentrated. The product was purified by flash column chromatography (0.05 eq. of triethylamine in *n*-hexane : ethyl acetate = 1 : 1) to afford **4d** (**R = *n*Pr**: 522 mg, 88%) as a white amorphous solid.

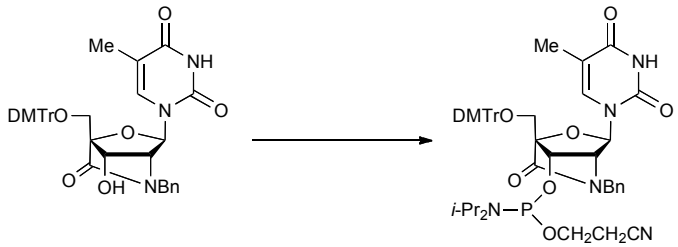
(2'R)-3'-*O*-[2-Cyanoethoxy(disopropylamino)phosphino]-5'-*O*-(4,4'-dimethoxytrityl)-2'-isopropylamino-2'-*N*,4'-*C*-o xomethylenethymidine (4e: R = *i*Pr)



3e **4e**

To the solution of **3e** (**R** = ***i*Pr**: 40.3 mg, 0.064 mmol) in anhydrous MeCN-THF (3:1, 0.6 mL) was added *N,N*-diisopropylammoniumtetrazolide (8.22 mg, 0.048 mmol) and 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphorodiamidite (24.5 μL, 0.077 mmol). After stirring at room temperature for 9 h, ice-cold water was added and the product was extracted with ethyl acetate (10 mL×3). The organic phase was washed with brine (20 mL), dried (Na_2SO_4), and concentrated. The product was purified by flash column chromatography (0.05 eq. of triethylamine in *n*-hexane : ethyl acetate = 1 : 1) to afford **4e** (**R = *i*Pr**: 17 mg, 32%) as a white amorphous solid.

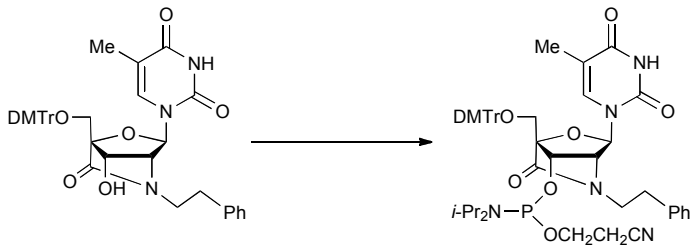
(2'R)-3'-*O*-[2-Cyanoethoxy(disopropylamino)phosphino]-5'-*O*-(4,4'-dimethoxytrityl)-2'-benzylamino-2'-*N*,4'-*C*-oxo methylenethymidine (4f: R = Bn)



3f **4f**

To the solution of **3f** (**R = Bn**: 2.05 g, 3.03 mmol) in anhydrous MeCN-THF (3:1, 30 mL) was added *N,N*-diisopropylammoniumtetrazolide (390 mg, 2.28 mmol) and 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphorodiamidite (1.15 mL, 3.64 mmol). After stirring at room temperature for 12 h, ice-cold water was added and the product was extracted with ethyl acetate (100 mL×3). The organic phase was washed with brine (150 mL), dried (Na_2SO_4), and concentrated. The product was purified by flash column chromatography (0.05 eq. of triethylamine in *n*-hexane : ethyl acetate = 1 : 1) to afford **4f** (**R = Bn**: 1.61 g, 61%) as a white amorphous solid.

(2'R)-3'-*O*-[2-Cyanoethoxy(disopropylamino)phosphino]-5'-*O*-(4,4'-dimethoxytrityl)-2'-phenethylamino-2'-*N*,4'-*C*-oxomethylenethymidine (4g: R = CH_2CH_2Ph)



3g **4g**

To the solution of **3g** (**R = CH_2CH_2Ph**: 825 mg, 1.20 mmol) in anhydrous MeCN-THF (3:1, 12 mL) was added *N,N*-diisopropylammoniumtetrazolide (154 mg, 0.90 mmol) and 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphorodiamidite (457 μL, 1.44 mmol). After stirring at room temperature for 12 h, ice-cold water was added and the product was extracted with ethyl acetate (50 mL×3). The organic phase was washed with brine (70 mL), dried (Na_2SO_4), and concentrated. The product was purified by flash column chromatography (0.05 eq. of triethylamine in *n*-hexane : ethyl acetate = 1 : 1) to afford **4g** (**R = CH_2CH_2Ph**: 509 mg, 48%) as a white amorphous solid.

Table S1. HPLC data (Retention Time) and MALDI-TOF-MASS data of AmNA[NR]-T modified oligonucleotides.

Oligonucleotide No.	R	Retention time (min)	Yield (%)	MALDI-TOF-MASS	
				Calcd. $(M-H)^-$	Found $(M-H)^-$
ON-3	Me	**10.7**[a]	38	3034.0	3033.4
ON-4	Et	**13.5**[a]	8	3048.0	3047.6
ON-5	*n*Pr	**15.5**[a]	15	3062.1	3062.8
ON-6	*i*-Pr	**17.0**[a]	5	3062.0	3062.4
ON-7	Bn	**22.4**[a]	19	3110.1	3109.9
ON-8	CH_2CH_2Ph	**25.8**[a]	16	3124.1	3124.4
ON-11	Me	**10.9**[b]	10	5150.36	5149.41
ON-16	Et	**12.9**[b]	10	5234.52	5234.89
ON-12	*i*-Pr	**15.2**[b]	11	5318.68	5319.20
ON-13	Bn	**24.2**[b]	9	5606.94	5606.81
ON-17	CH_2CH_2Ph	**25.5**[b]	10	5691.10	5693.95

Strand: **ON-3 – 8**: 5'-d(TTTTTtTTTT)-3', **ON-11 – 13, 16, 17**: 5'-d(ttAtCCAGCTTtAttA)-3', t=AmNA[NR].

[a]Conditions : eluent A : 0.1M TEAA buffer, eluent B : A/MeCN (1/1, v/v),
gradient : MeCN conc. = 8-13% (30min), 260 nm.

[b]Conditions : eluent A : 0.1M TEAA buffer, eluent B : A/MeCN (1/1, v/v),
gradient : MeCN conc. = 5-33% (30min), 260 nm.

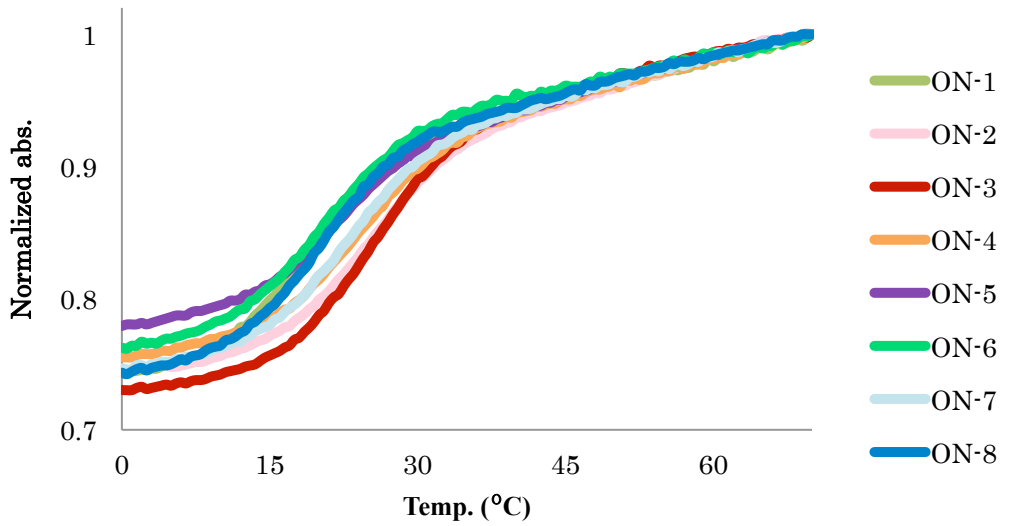


Figure S1. UV melting curves for the duplexes formed by 5'-d(TTTTTtTTTT)-3' oligonucleotides containing LNA, AmNA[*N*-Me], AmNA[*N*-Et], AmNA[*N*-*n*Pr], AmNA[*N*-*i*Pr], AmNA[*N*-Bn], AmNA[*N*-CH_2CH_2Ph] at t position, and the target strand, 3'-r(AAAAAAAAAA)-5'.

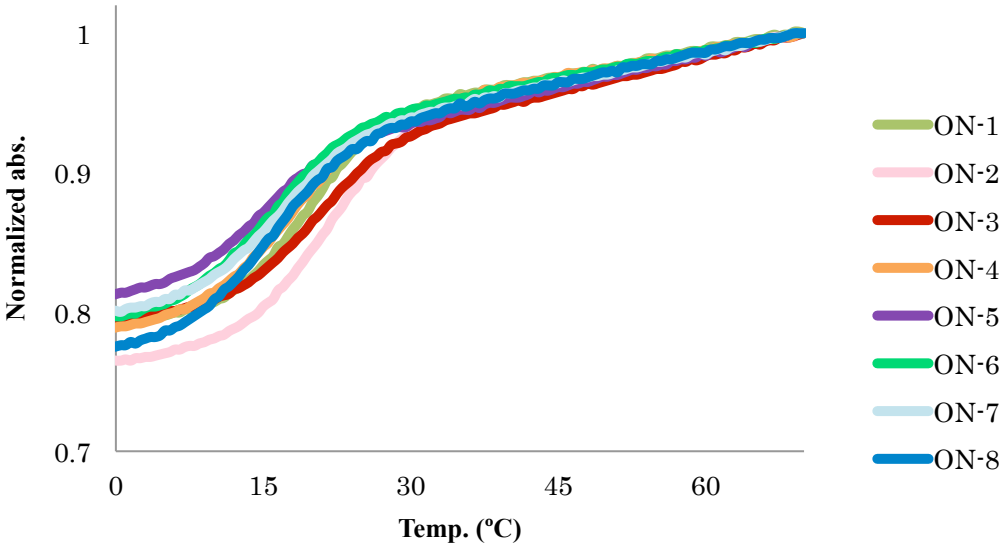


Figure S2. UV melting curves for the duplexes formed by 5'-d(TTTTTtTTTT)-3' oligonucleotides containing LNA, AmNA[*N*-Me], AmNA[*N*-Et], AmNA[*N*-*n*Pr], AmNA[*N*-*i*Pr], AmNA[*N*-Bn], AmNA[*N*-CH_2CH_2Ph] at t position, and the target strand, 3'-d(AAAAAAAAAA)-5'.

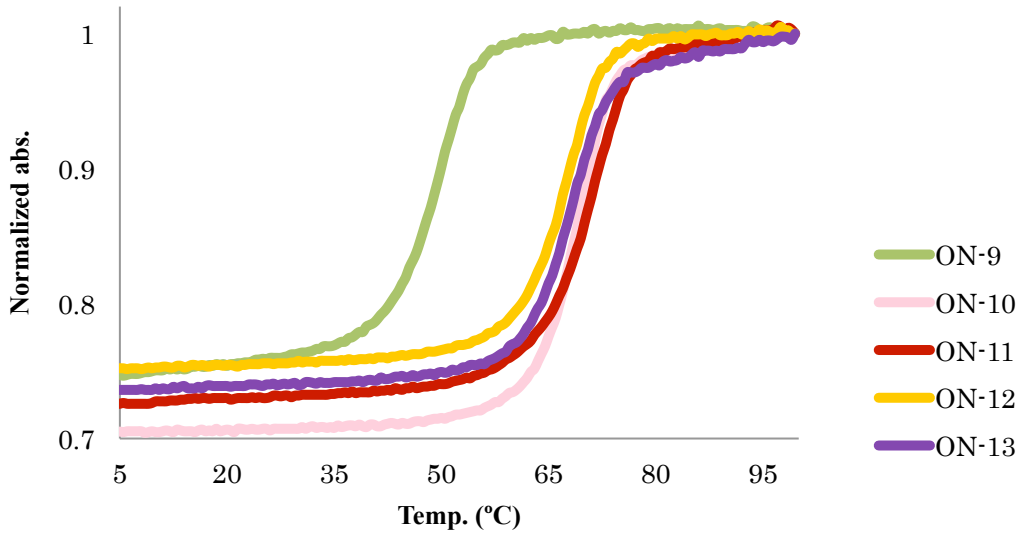


Figure S3. UV melting curves for the duplexes formed by 5'-d(ttAtCCAGCTTtAttA)-3' oligonucleotides containing LNA, AmNA[*N*-Me], AmNA[*N*-*i*Pr], AmNA[*N*-Bn] at t position, and the target strand, 3'-r(AAUAGGUCGAAAUAAU)-5'.

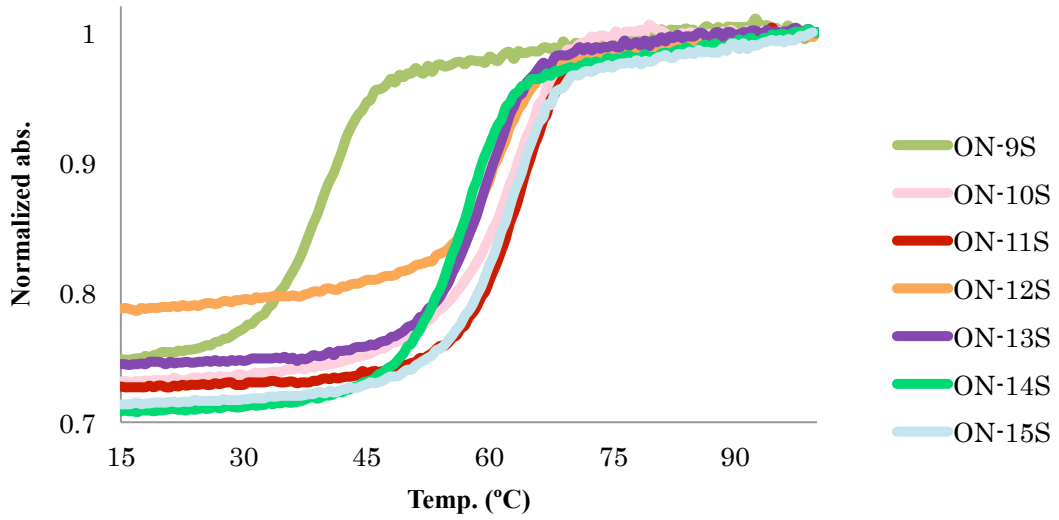


Figure S4. UV melting curves for the duplexes formed by phosphorthioate oligonucleotides 5'-d(ttAtCCAGCTTtAttA)-3' containing LNA, AmNA[*N*-Me], AmNA[*N*-*i*Pr], AmNA[*N*-Bn] at t position, and the target strand, 3'-r(AAUAGGUCGAAAUAAU)-5'.

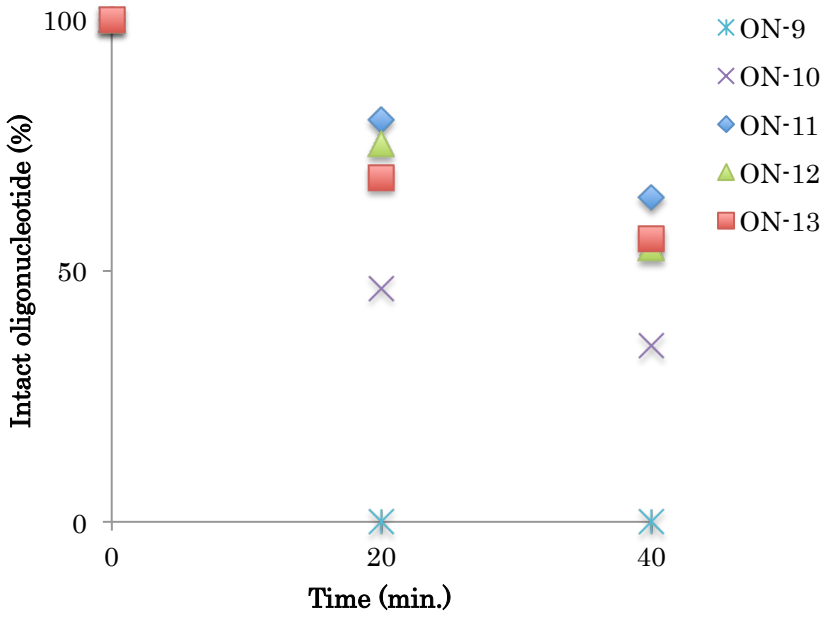


Figure S5. Nuclease resistance of 5'-d(ttAtCCAGCTTtAttA)-3' against *Crotalus admanteus* venom phosphodiesterase (CAVP). T = natural (blue asterisk) (**ON-9**), LNA (purple chros) (**ON-10**), AmNA[*N*-Me] (blue closed diamond) (**ON-11**), AmNA[*N*-*i*Pr] (green closed triangle) (**ON-12**), AmNA[*N*-Bn] (red closed square) (**ON-13**). Experiments were performed at 37°C in 100 µL of buffer containing 50 mM Tris-HCl (pH 8.0), 10 mM MgCl2, 0.175 mg/mL CAVP, and 7.5 µM of oligonucleotide.

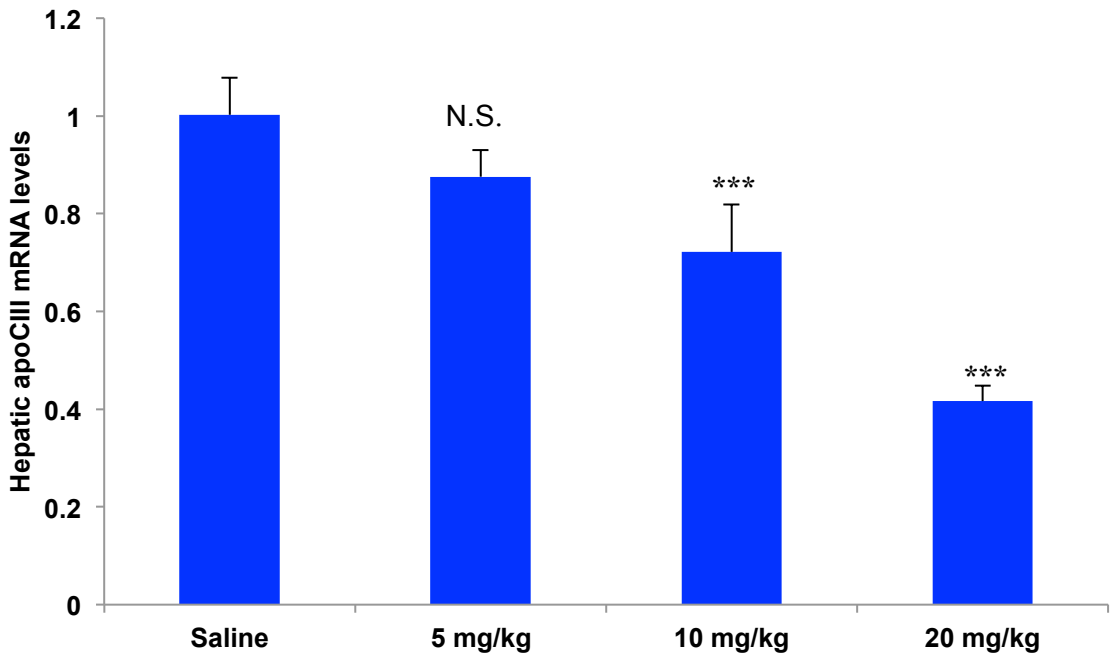


Figure S6. Dose-dependent reduction of apoC-III mRNA in the livers of mice receiving a single intravenous dose of 5 to 20 mg kg^{-1} of **ON-10S** after 72 hours post-injection. Dunnett's multiple comparison test, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, N.S.; not significant. Error bars represent group means + S.D. $n = 3$.

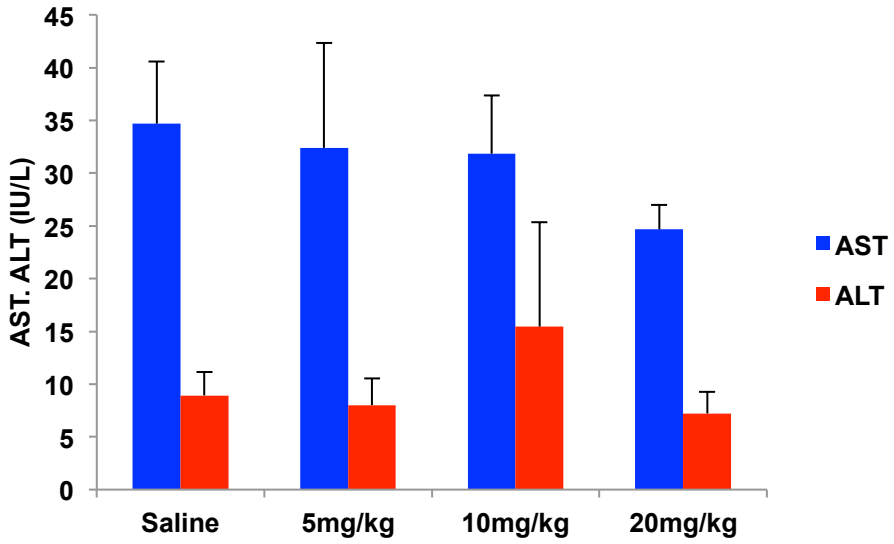


Figure S7. Dose-dependent changes of serum transaminases of mice receiving a single intravenous dose of 5 to 20 mg kg^{-1} of **ON-10S** after 72 hours post-injection. Dunnett's multiple comparison tests revealed no statistical significant changes of aspartate transaminases (AST) and alanine transaminase (ALT) from saline-treated arms. Error bars represent group means + S.D. $n = 3$.

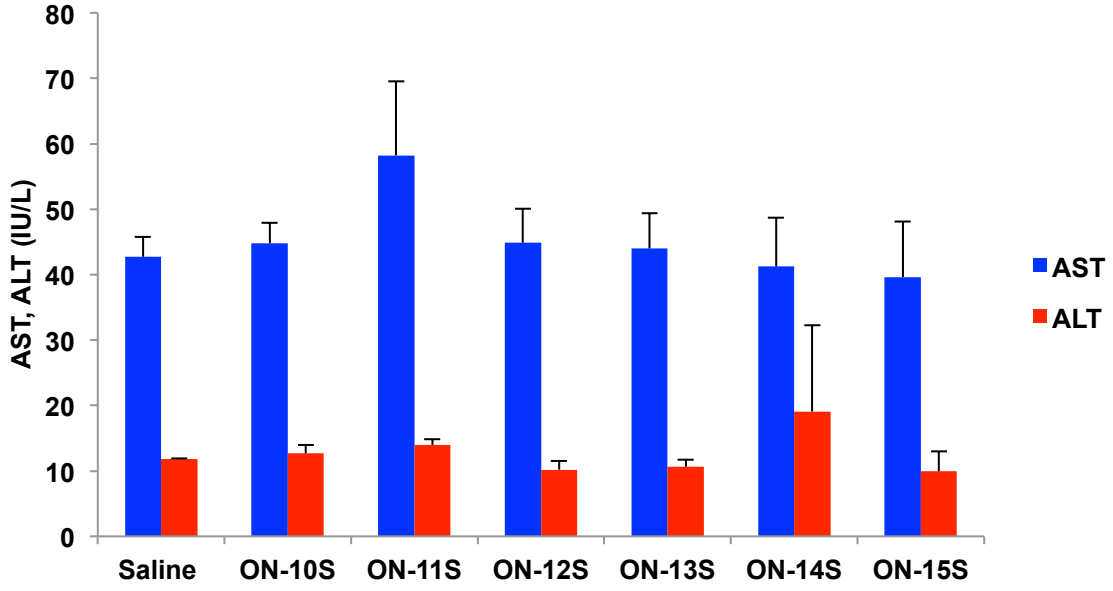
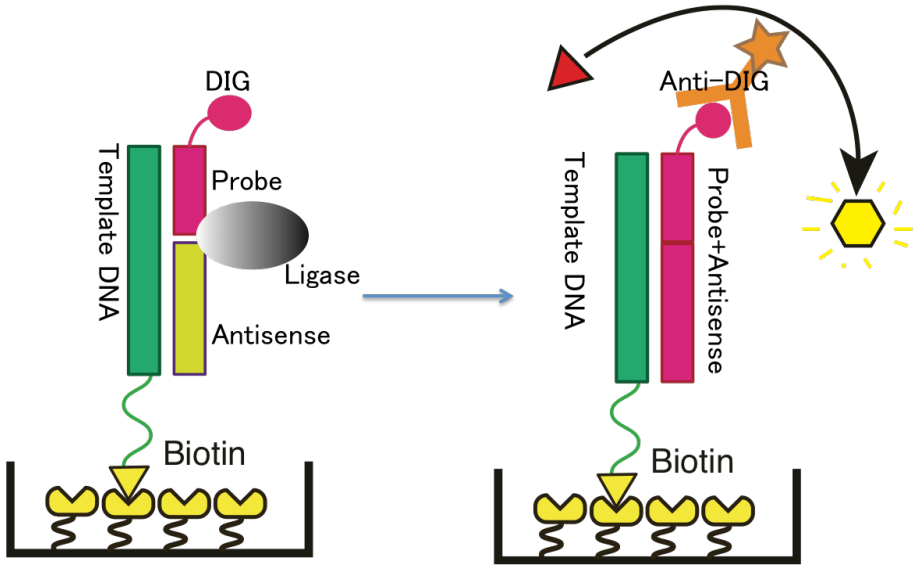


Figure S8 Changes of serum transaminases of mice receiving a single intravenous dose of 2.868 μmole kg^{-1} of a series of AONs developed here after 72 hours post-injection. Dunnett's multiple comparison tests revealed no statistical significant changes of aspartate transaminases (AST) and alanine transaminase (ALT) from saline-treated arms. Error bars represent group means + S.D. $n = 3$.

3. Illustration of ELISA method for AONs quantification in liver



Materials and reagents

The template DNA was a 25-mer DNA (5'-gaa tag cga taa taa agc tgg ata a-3'), which is complementary to ON-9S to ON-15S, with biotin at the 3'-end. The ligation probe DNA was a 9-mer DNA (5'-tcgctattc-3') with phosphate at the 5'-end and digoxigenin at the 3'-end. The template DNA and the ligation probe DNA were purchased from Japan Bio

Service. Reacti-Bind NeutrAvidin-coated polystyrene strip plates were purchased from Thermo Fisher Scientific (nunc immobilizer streptavdin F96 white, 436015). The template DNA solution (100 nM) was prepared in hybridization buffer containing 60 mM Na_2HPO_4 (pH 7.4), 0.9 M NaCl, and 0.24% Tween 20. The ligation probe DNA solution (200 nM) was prepared in 1.5 units/well of T4 DNA ligase (TaKaRa) with 66 mM Tris-HCl (pH7.6), 6.6 mM $MgCl_2$, 10 mM DTT and 0.1 mM ATP.

The washing buffer used throughout the assay contained 25 mM Tris-HCl (pH 7.2), 0.15 M NaCl and 0.1% Tween 20. Anti-digoxigenin-AP antibody (Fab fragments conjugated with alkaline phosphatase) was obtained from Roche Diagnostics. A 1:2000 dilution of the antibody with 1:10 super block buffer in TBS (Pierce) was used in the assay. The alkaline phosphatase luminous substrate was prepared in 250 μM CDP-Star (Roche) with 100 mM Tris-HCl (*p*H 7.6) and 100 mM NaCl.

Assay procedures

Frozen liver tissue was collected in a 2-mL tube with 1 mL of PBS and a zirconia ball (ø 5 mm, Irie) and mechanically homogenized for 2 min at 30 oscillations per second by a TissueLyser II apparatus (Qiagen). Total protein concentrations were measured with a detergent compatible assay kit (Bio-Rad) and adjusted to 8 mg/L with PBS. The assay was performed at the concentration range of 128 pM to 1000 nM in duplicate. For the standard curve, 7 standard solutions were prepared. To AON-untreated mice liver homogenates were added ON-10S, ON-11S, ON-12S, ON-13S, ON-14S, and ON-15S solutions to prepare 7 standard samples at a range of 128 pM to 1000 nM. Next, the template DNA solution (100 μL) and standard solution (10 μL) or liver homogenates (10 μL) containing ON-10S, ON-11S, ON-12S, ON-13S, ON-14S, and ON-15S were added to Reacti-Bind Neutr Avidin-coated polystyrene strip 96-well plates and incubated at 37°C for 1 h to allow the binding of biotin to streptavidin-coated wells and hybridization. After hybridization, the plate was washed three times with 200 μL of washing buffer. Then, ligation probe DNA solution (100 μL) was added, and the plate was incubated at room temperature (15°C) for 3 h. The plate was then washed three times with the washing buffer. Subsequently, 200 μL of a 1:2000 dilution of anti-digoxigenin-AP was added, and the plate was incubated at 37°C for 1h. After washing three times with the washing buffer, CDP-Star solution was added to the plate, and finally the luminescence intensity was determined by using a Centro XS^3 luminometer (Berthold) one second after the addition of CDP-Star. The linear range of 128 pM to 1000 nM in this ELISA system was determined as $r > 0.97$.

4. Spectral data

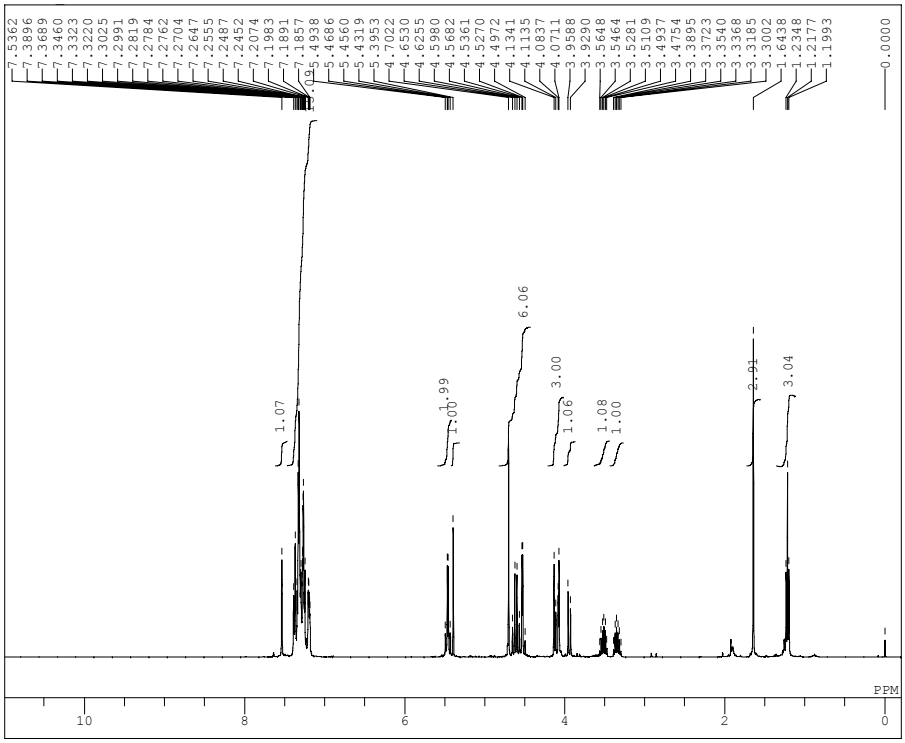


Figure S9. [1]H-NMR Spectrum of Compound **2c (R = Et)**

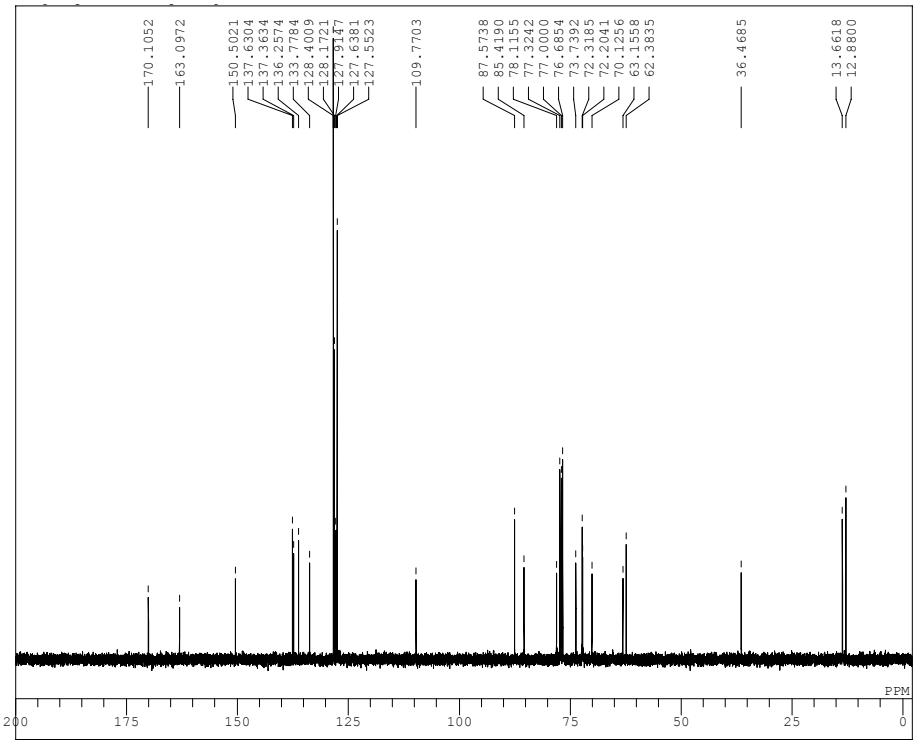


Figure S10. [13]C-NMR Spectrum of Compound **2c (R = Et)**

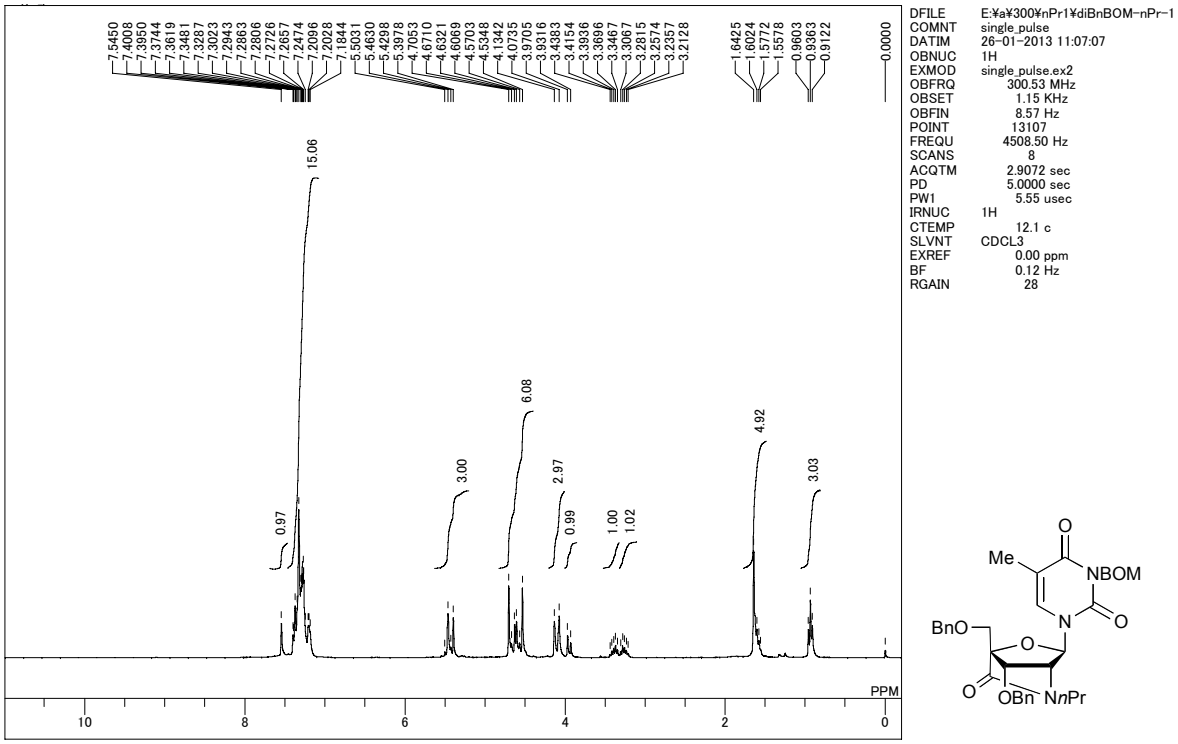


Figure S11. [1]H-NMR Spectrum of Compound **2d (R = *n*Pr)**

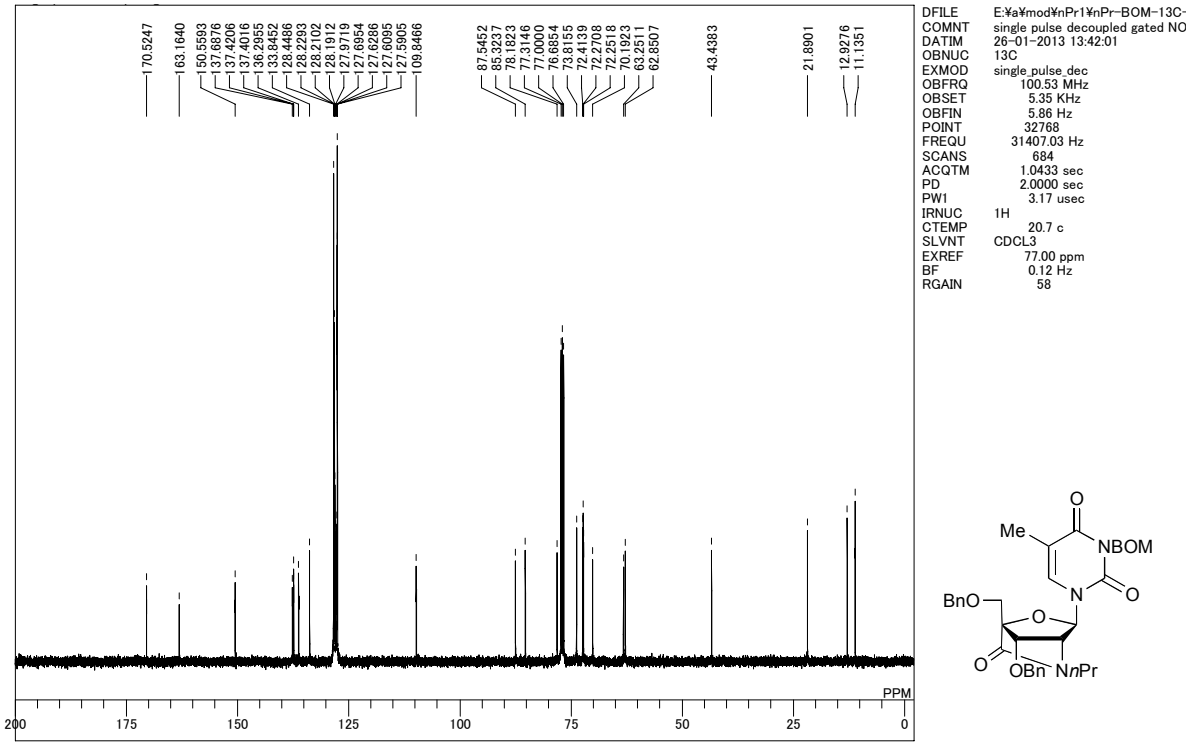


Figure S12. [13]C-NMR Spectrum of Compound **2d (R = *n*Pr)**

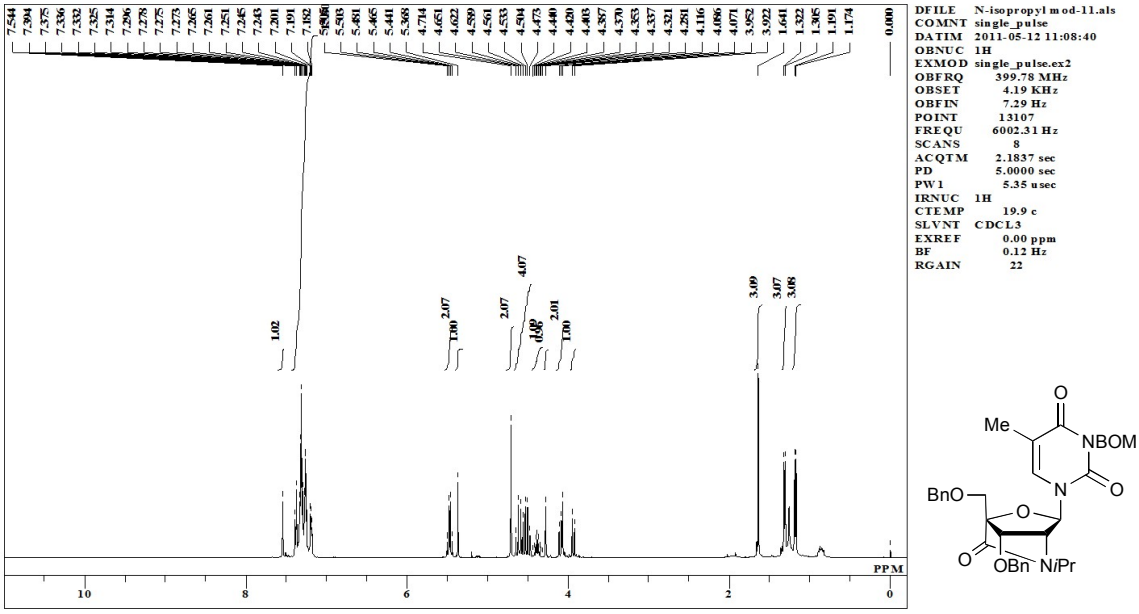


Figure S13. ^{1}H-NMR Spectrum of Compound **2e (R = *i*Pr)**

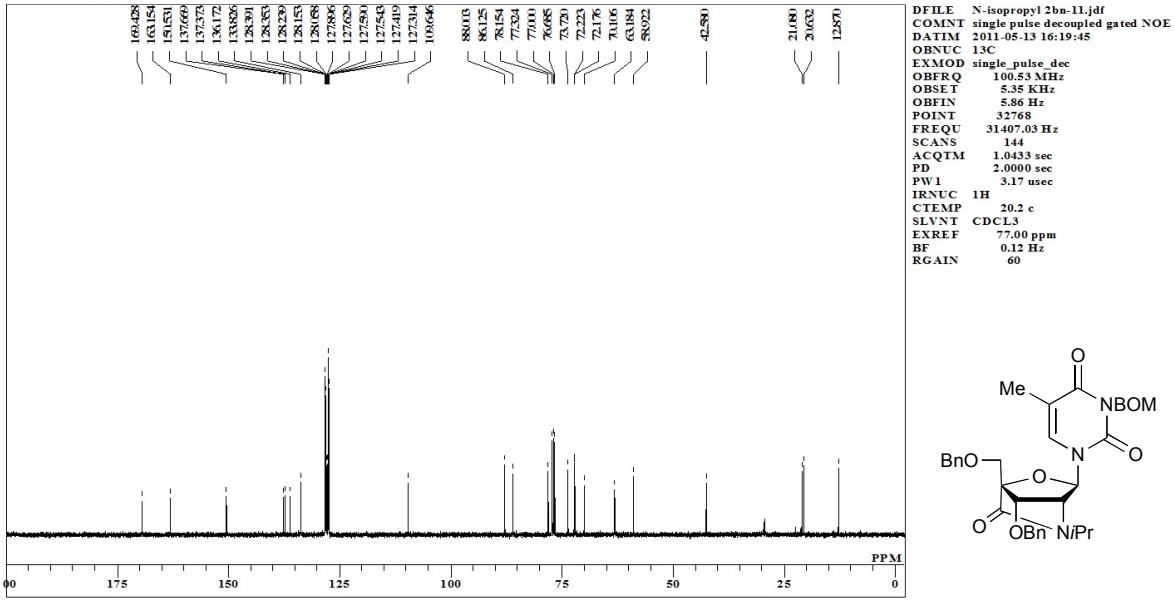


Figure S14. ^{13}C-NMR Spectrum of Compound **2e (R = *i*Pr)**

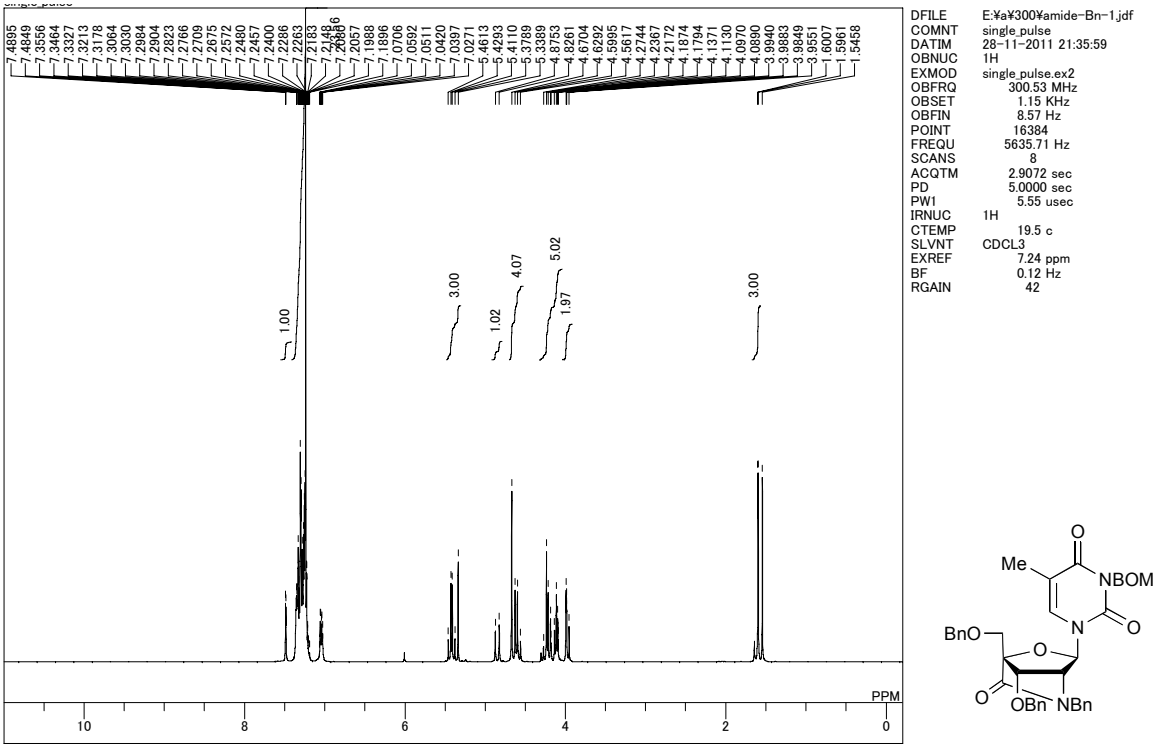


Figure S15. ^{1}H-NMR Spectrum of Compound **2f (R = Bn)**

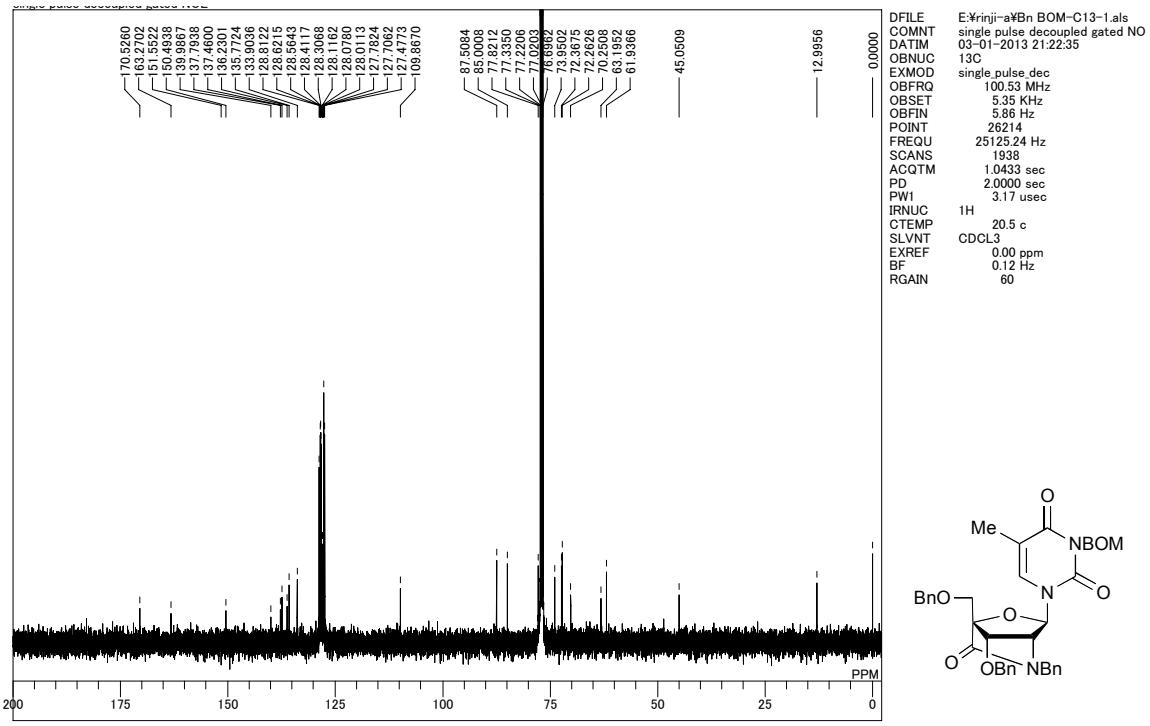


Figure S16. ^{13}C-NMR Spectrum of Compound **2f (R = Bn)**

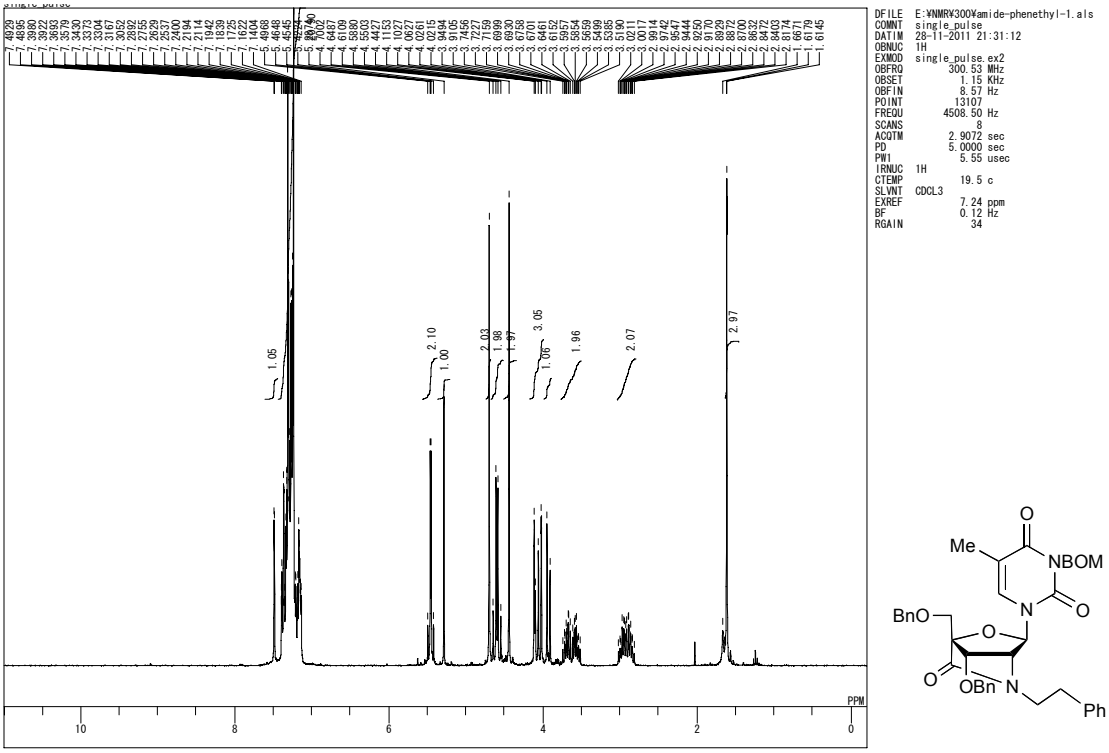


Figure S17. ^{1}H-NMR Spectrum of Compound **2g (R = CH_2CH_2Ph)**

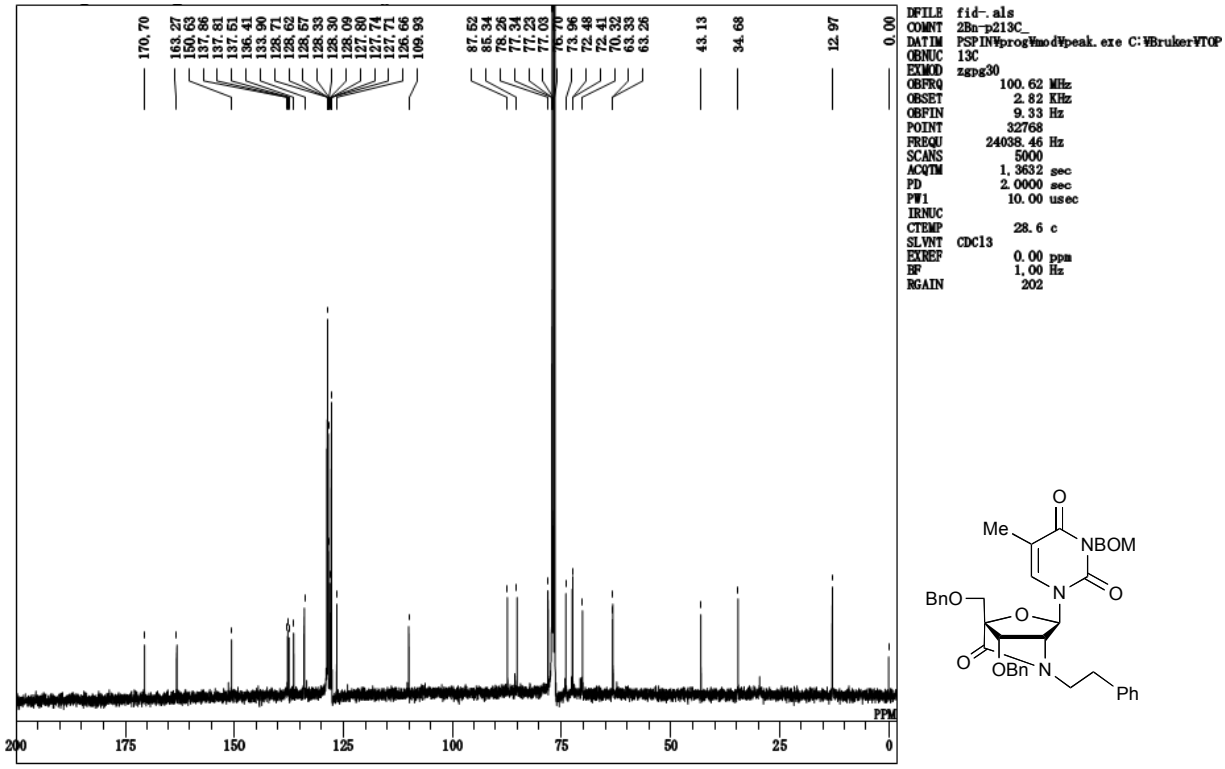


Figure S18. ^{13}C-NMR Spectrum of Compound **2g (R = CH_2CH_2Ph)**

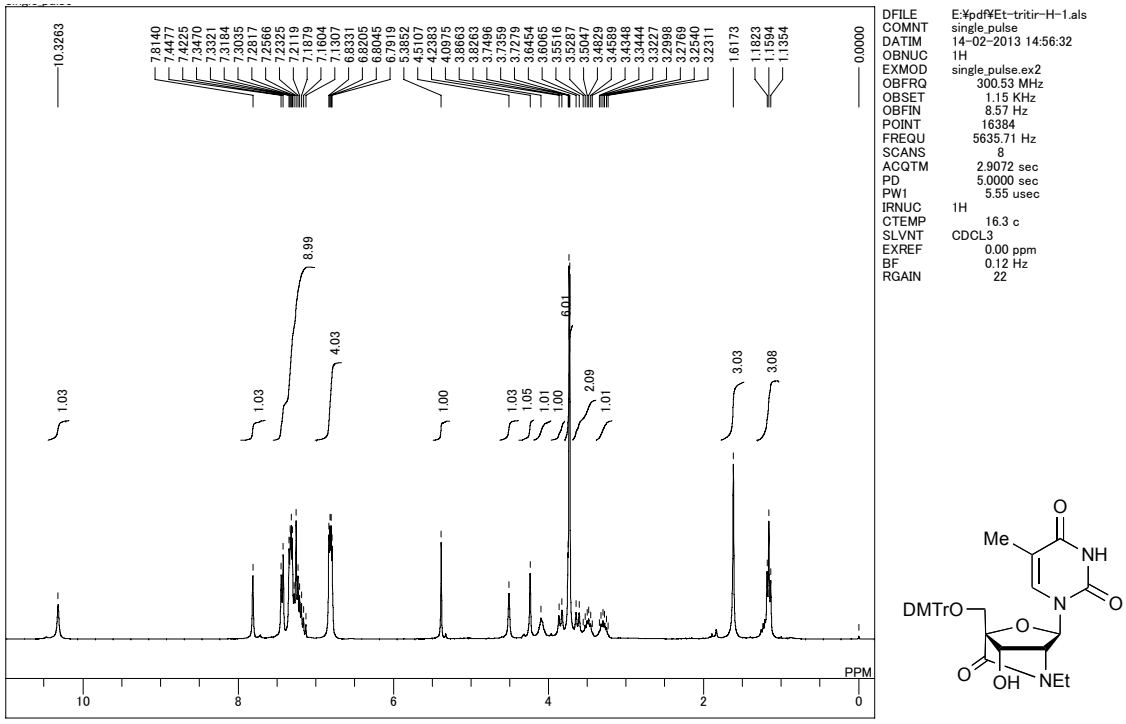


Figure S19. [1]H-NMR Spectrum of Compound **3c (R = Et)**

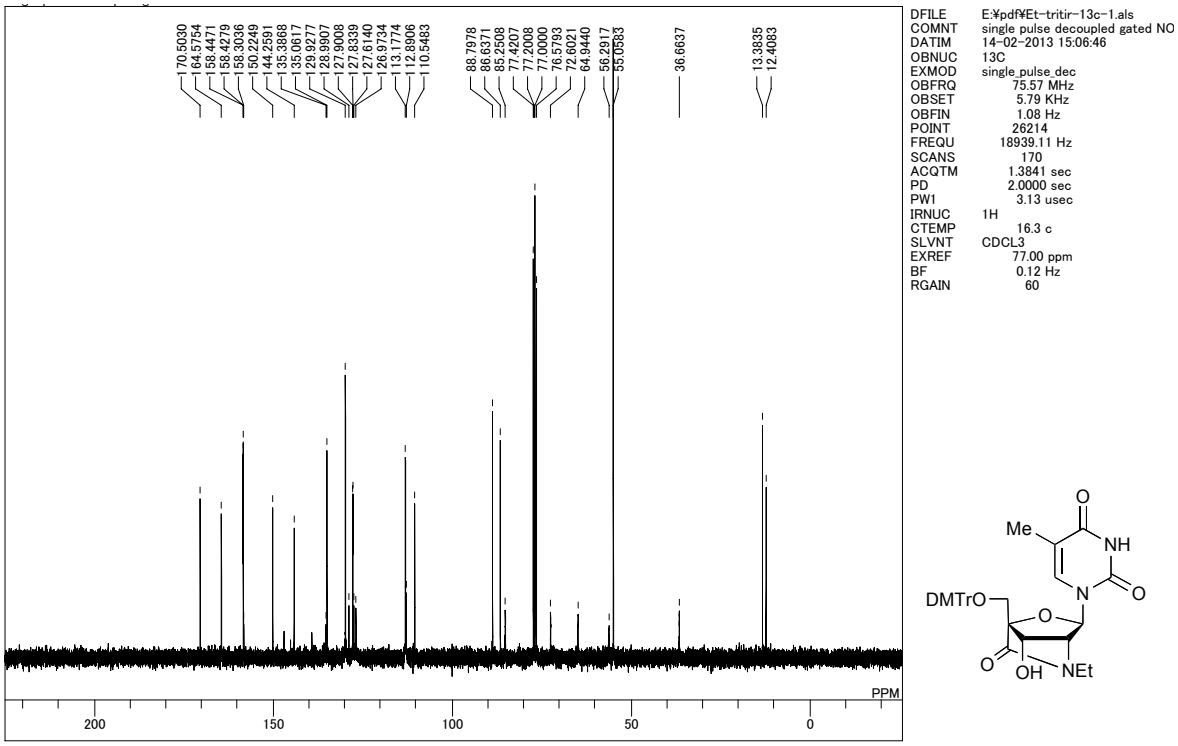


Figure S20. [13]C-NMR Spectrum of Compound **3c (R = Et)**

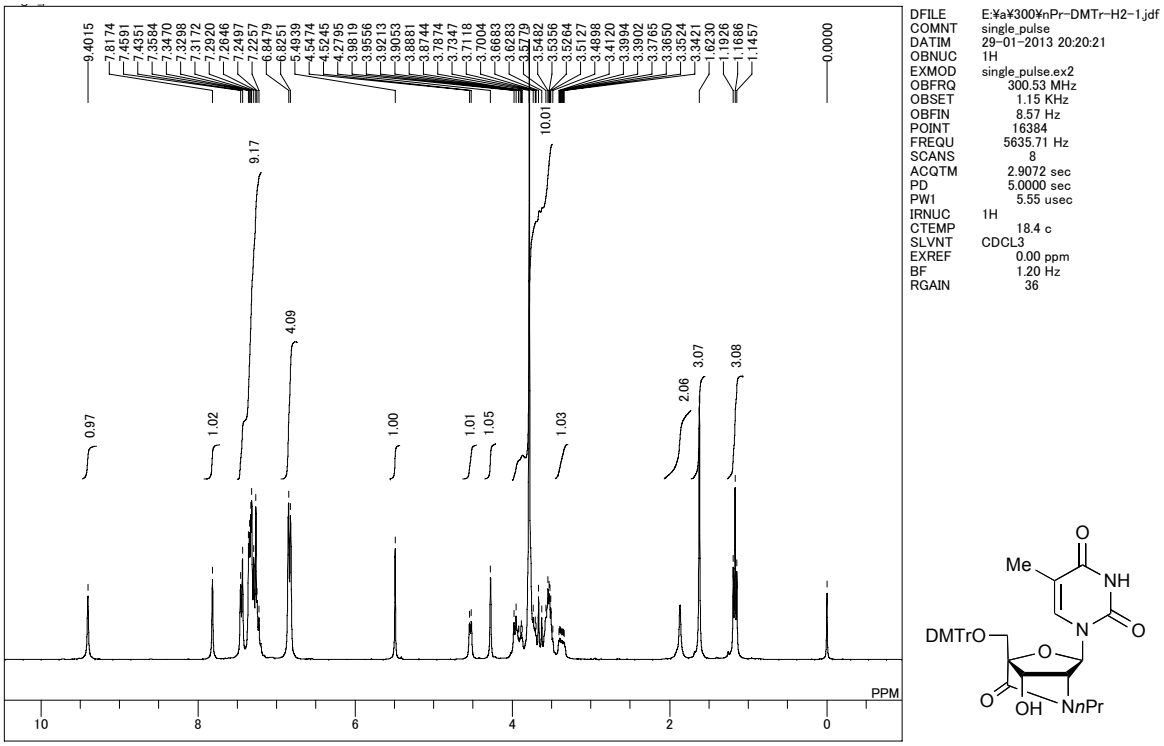


Figure S21. [1]H-NMR Spectrum of Compound **3d (R = *n*Pr)**

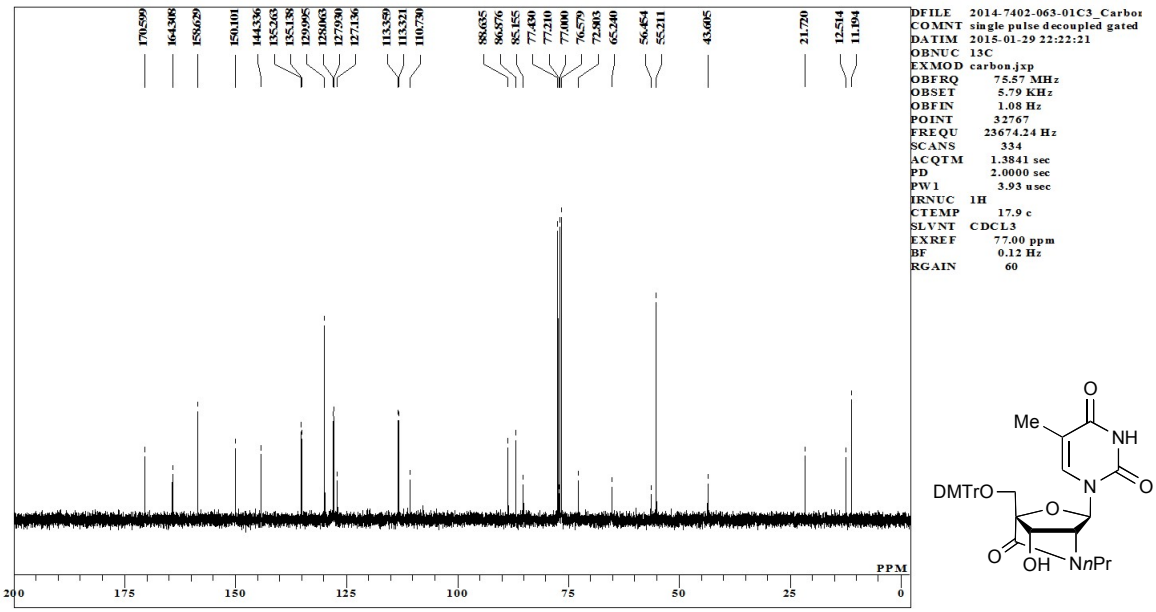


Figure S22. [13]C-NMR Spectrum of Compound **3d (R = *n*Pr)**

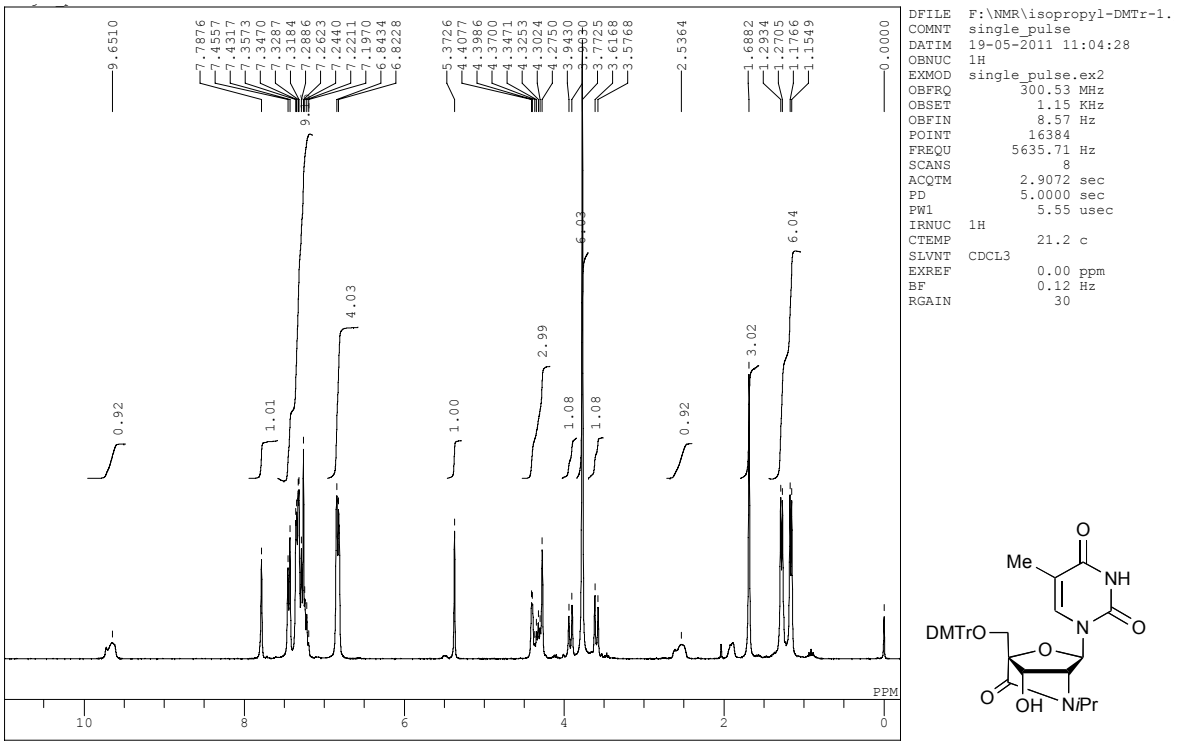


Figure S23. ^{1}H-NMR Spectrum of Compound **3e (R = *i*Pr)**

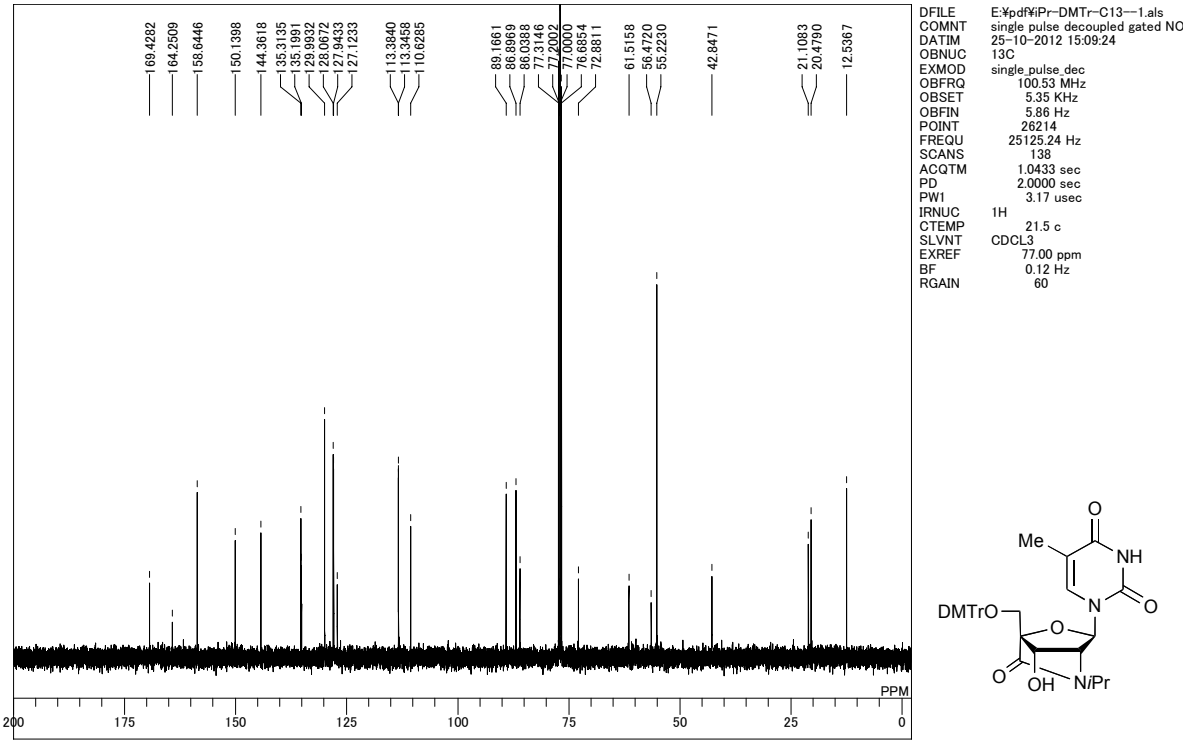


Figure S24. ^{13}C-NMR Spectrum of Compound **3e (R = *i*Pr)**

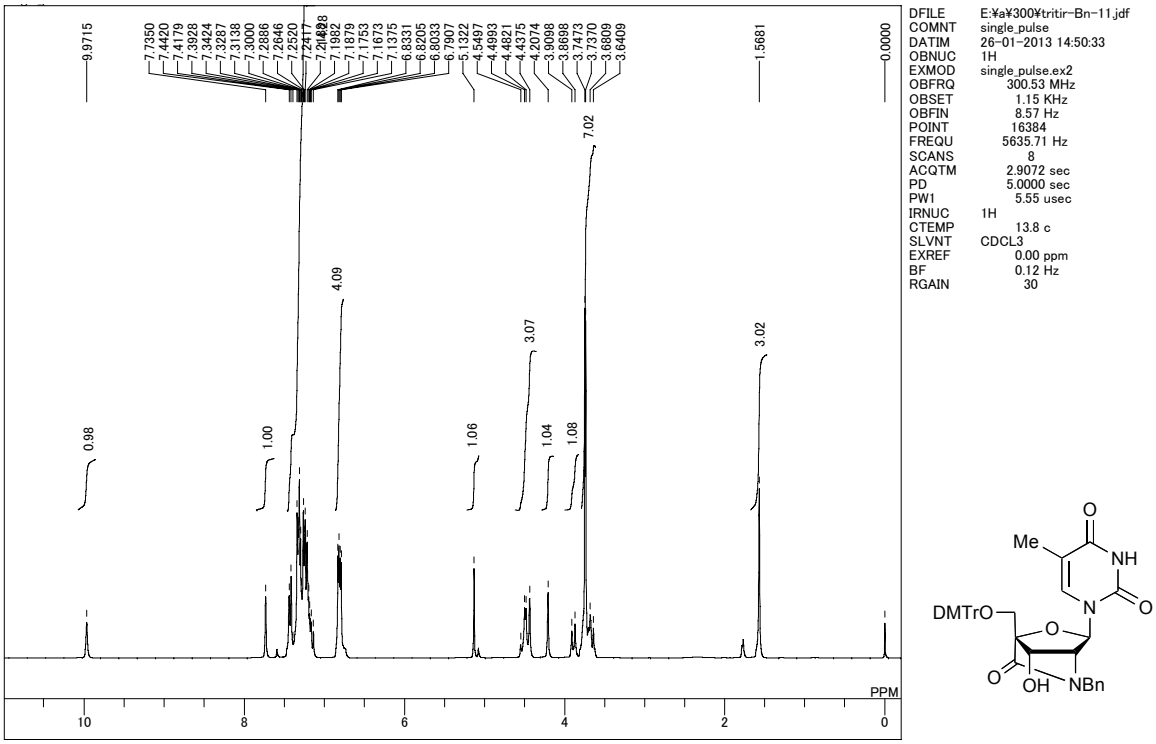


Figure S25. ^{1}H-NMR Spectrum of Compound **3f (R = Bn)**

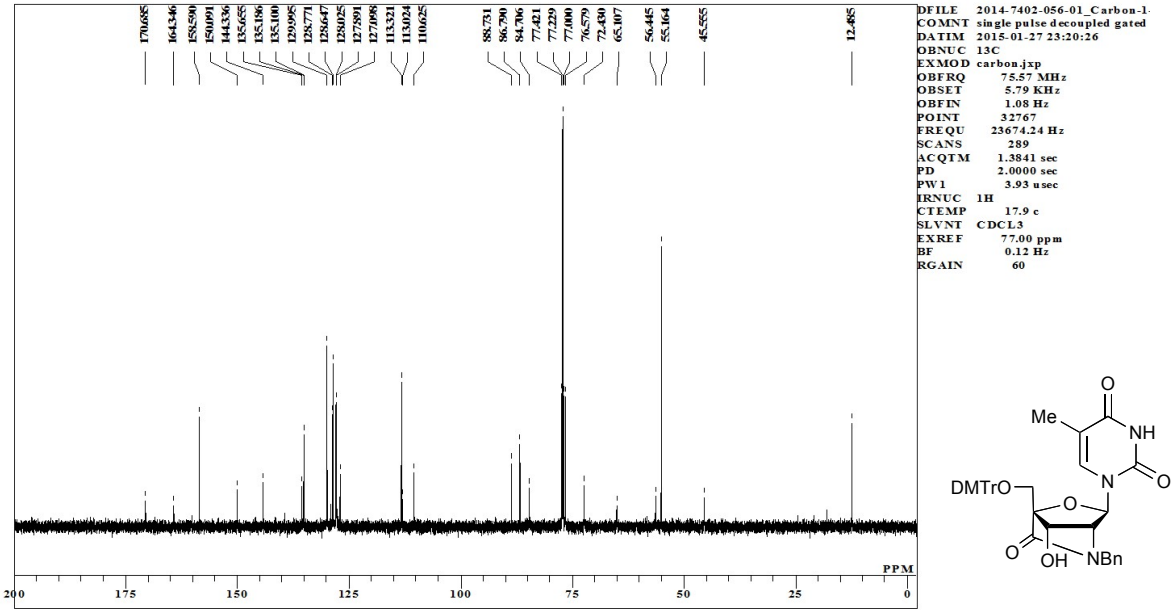


Figure S26. ^{13}C-NMR Spectrum of Compound **3f (R = Bn)**

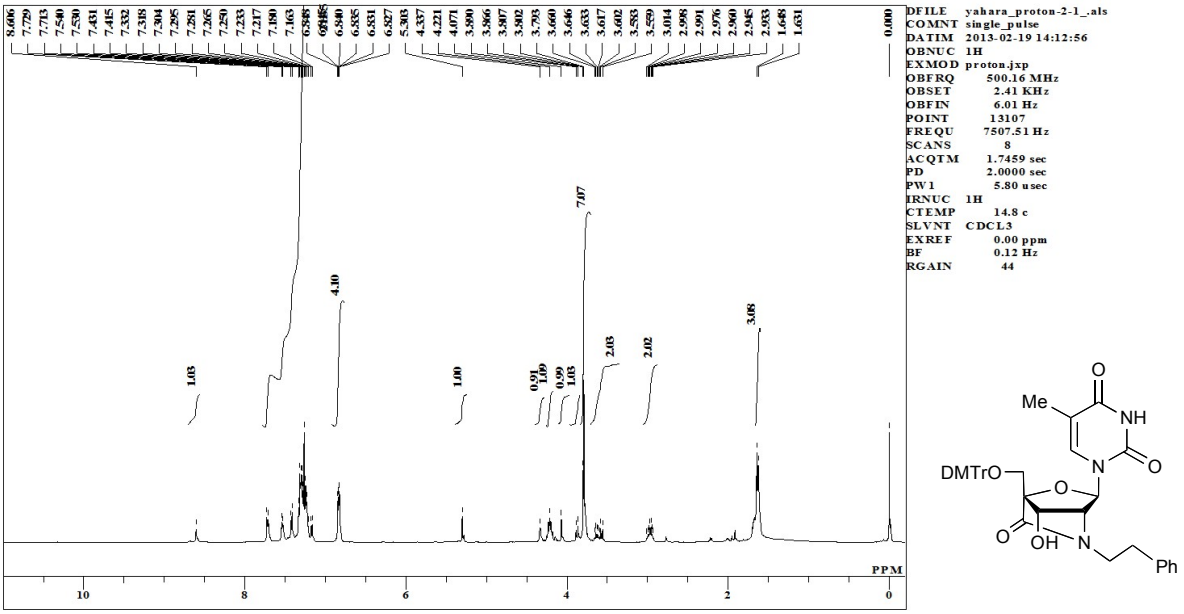


Figure S27. ^{1}H-NMR Spectrum of Compound **3g (R = CH_2CH_2Ph)**

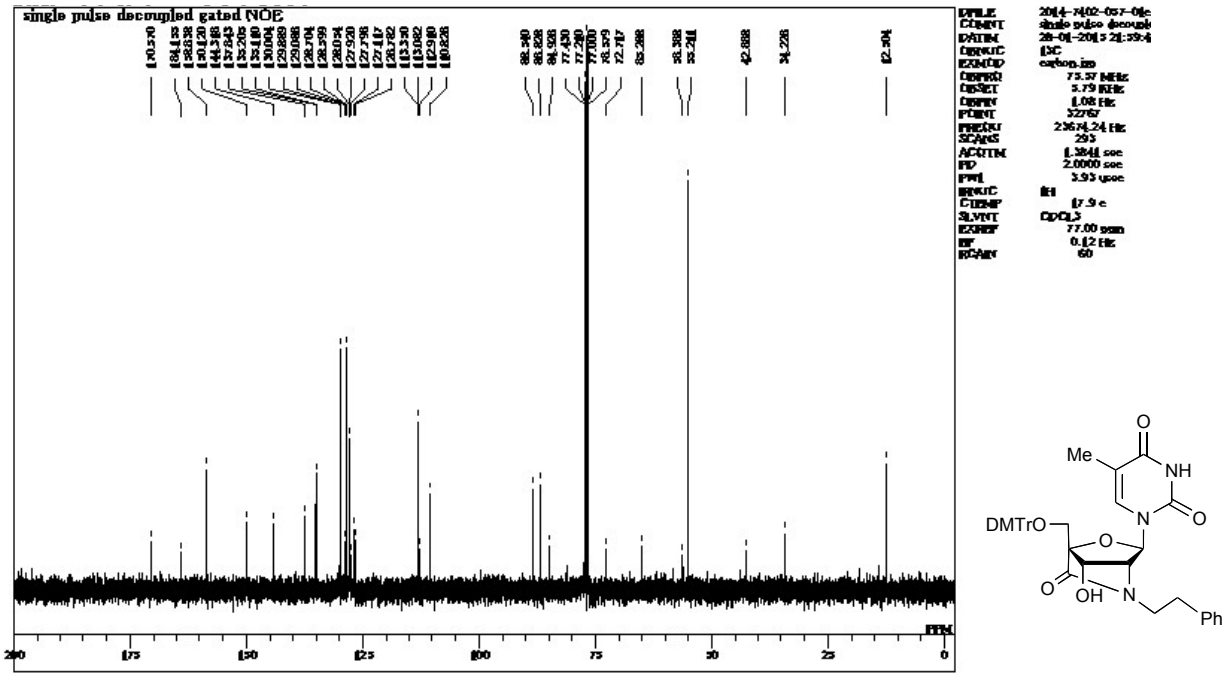


Figure S28. ^{13}C-NMR Spectrum of Compound **3g (R = CH_2CH_2Ph)**

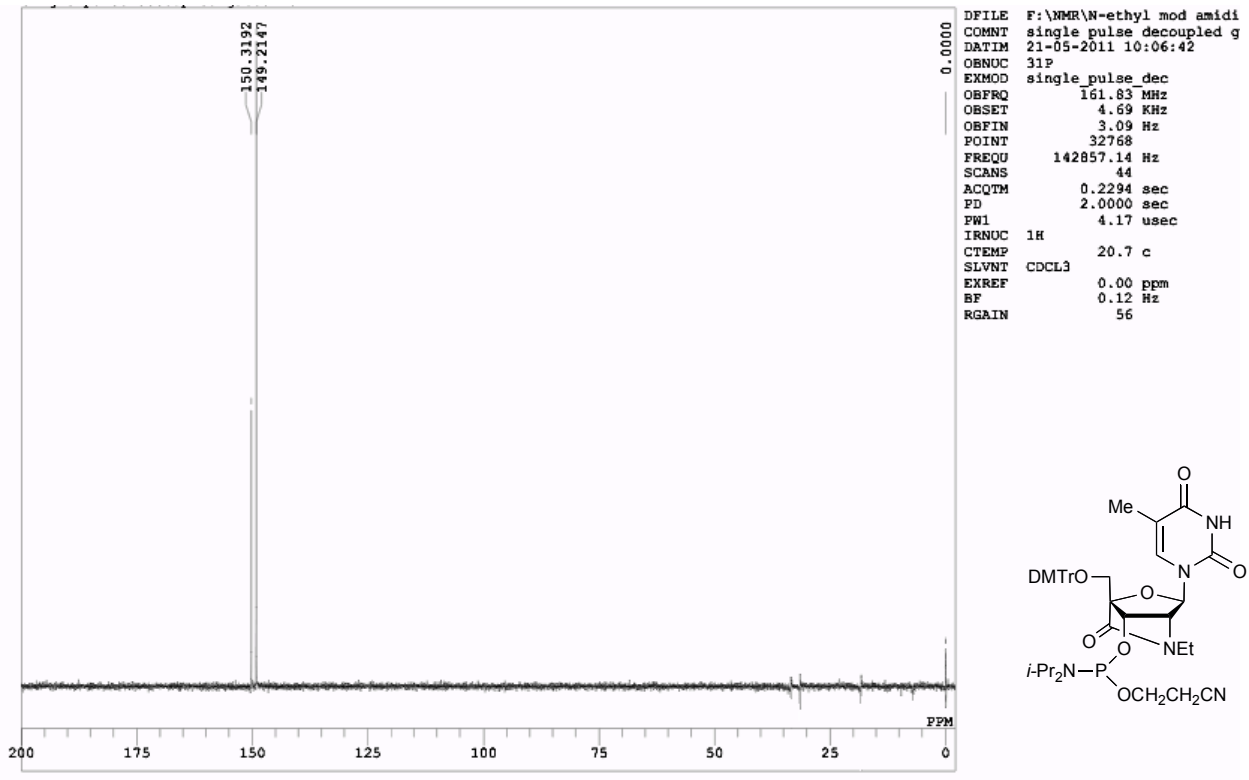


Figure S29. ^{31}P-NMR Spectrum of Compound **4c (R = Et)**

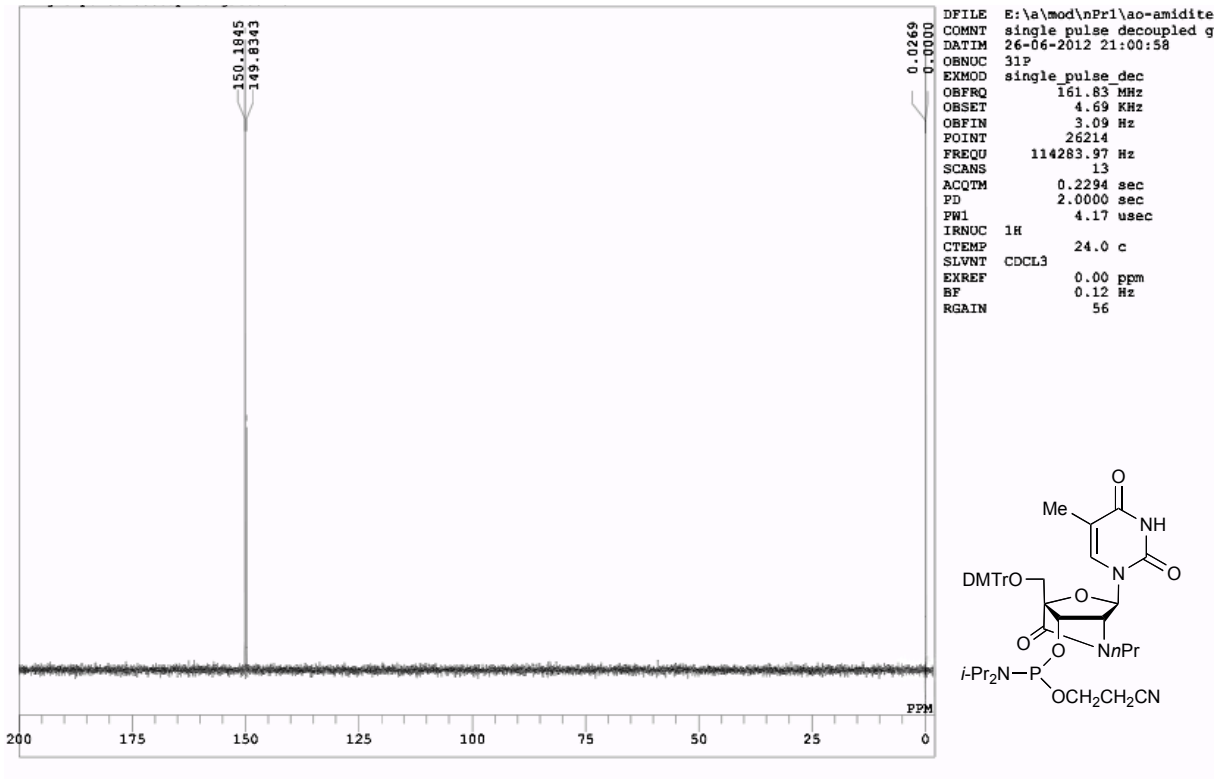


Figure S30. ^{31}P-NMR Spectrum of Compound **4d (R = *n*Pr)**

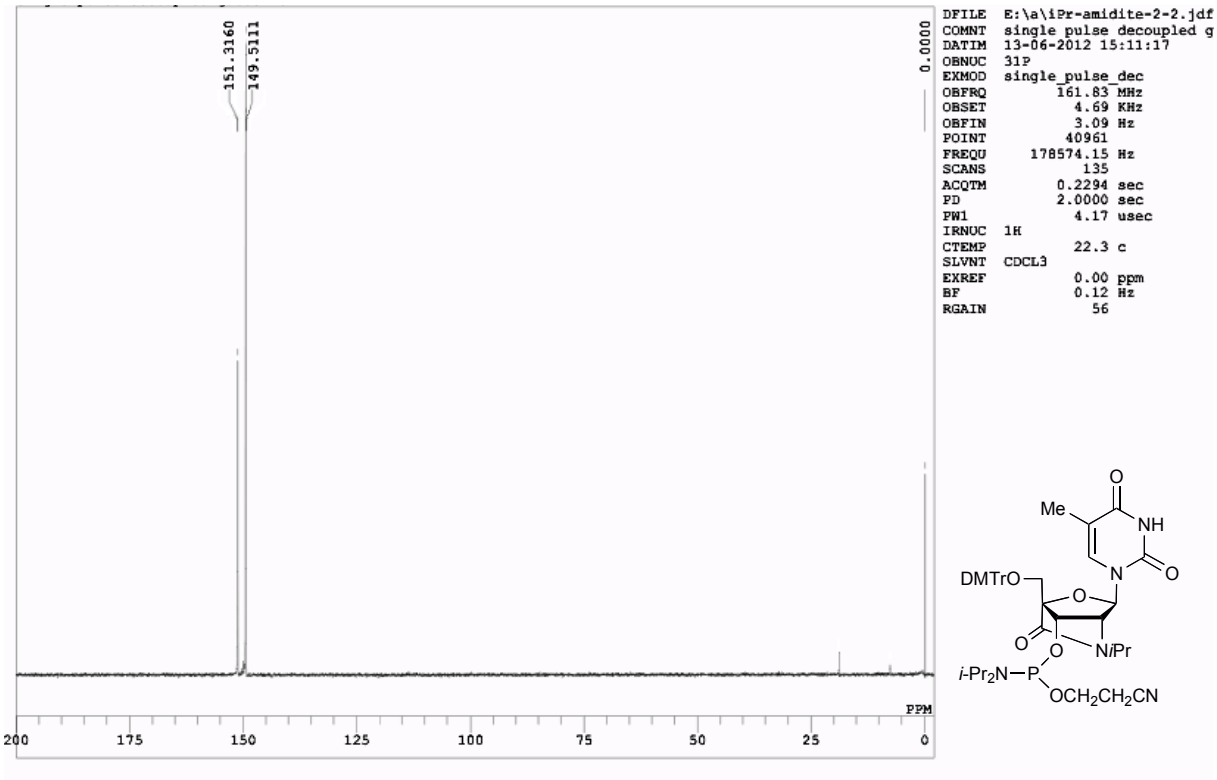


Figure S31. ^{31}P-NMR Spectrum of Compound **4e (R = *i*Pr)**

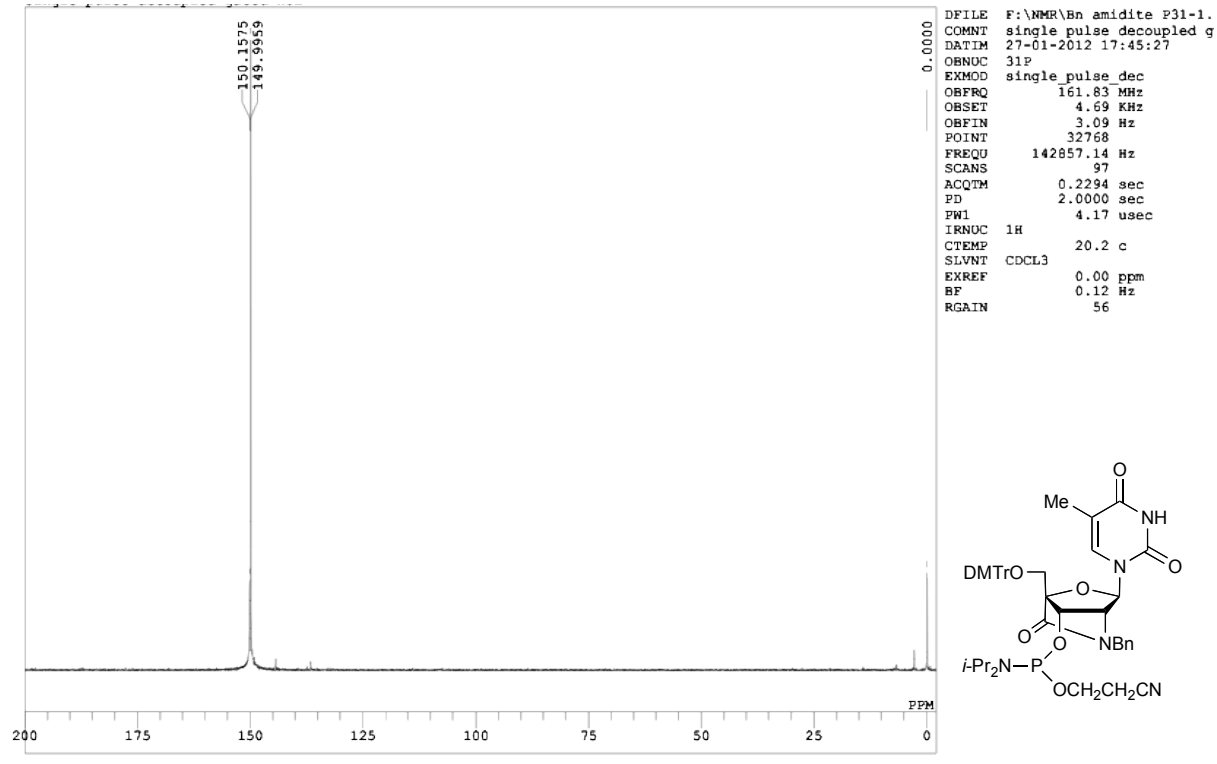


Figure S32. ^{31}P-NMR Spectrum of Compound **4f (R = Bn)**

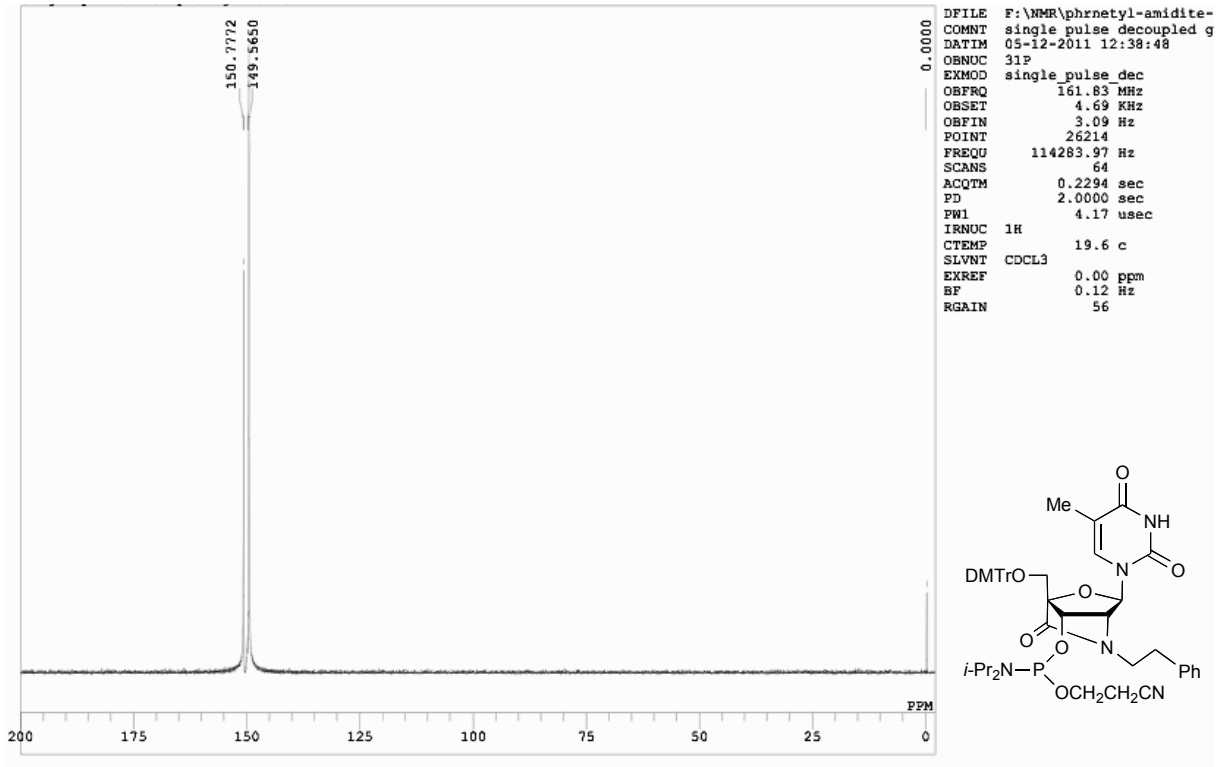


Figure S33. ^{31}P-NMR Spectrum of Compound 4**g (R = CH_2CH_2Ph)**

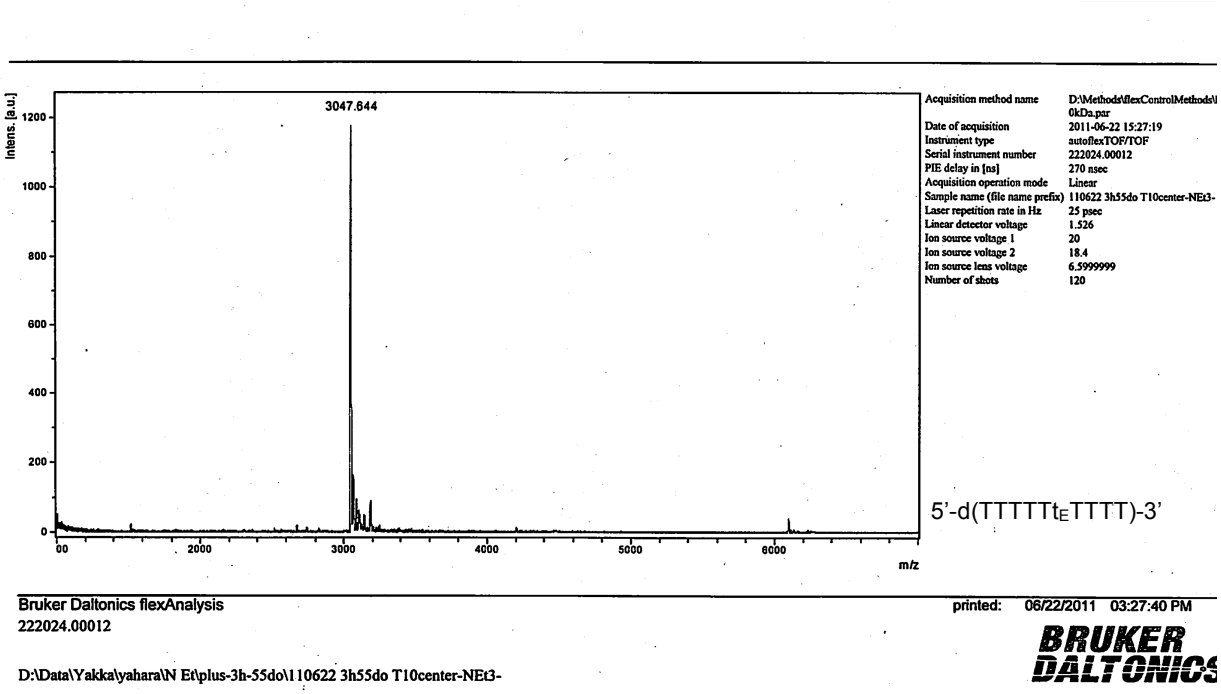


Figure S34. MALDI-TOF mass of **ON-4**.

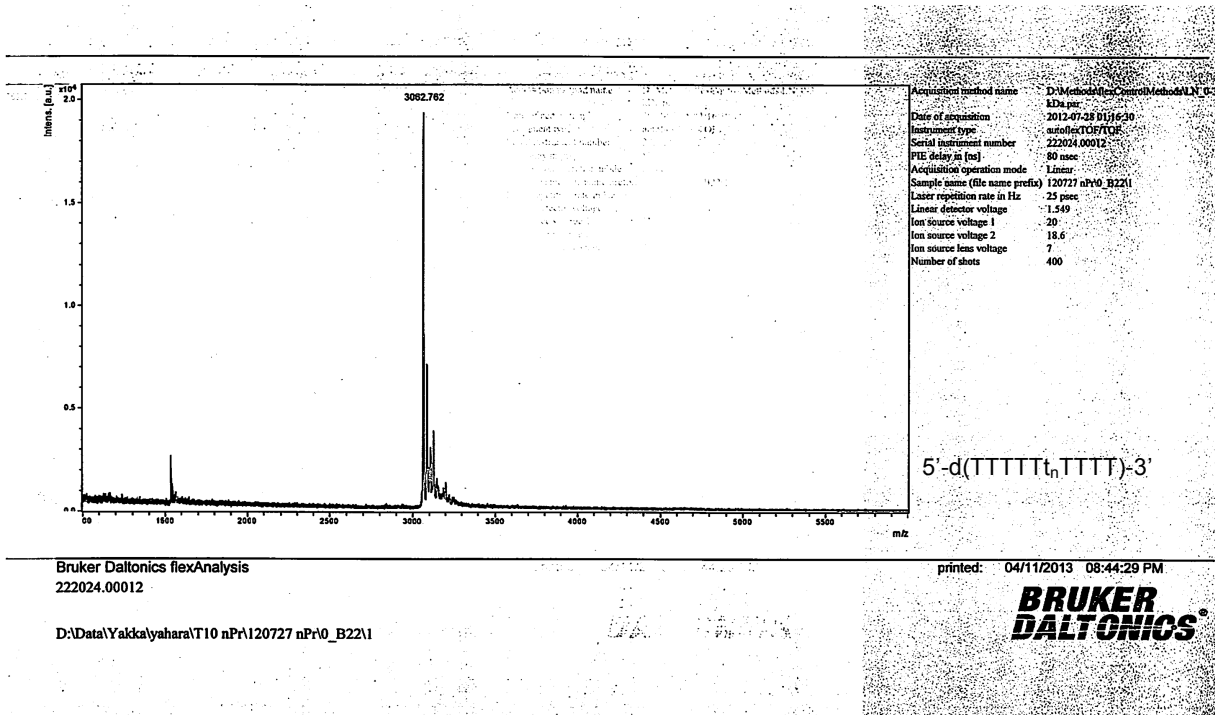


Figure S35. MALDI-TOF mass of **ON-5**.

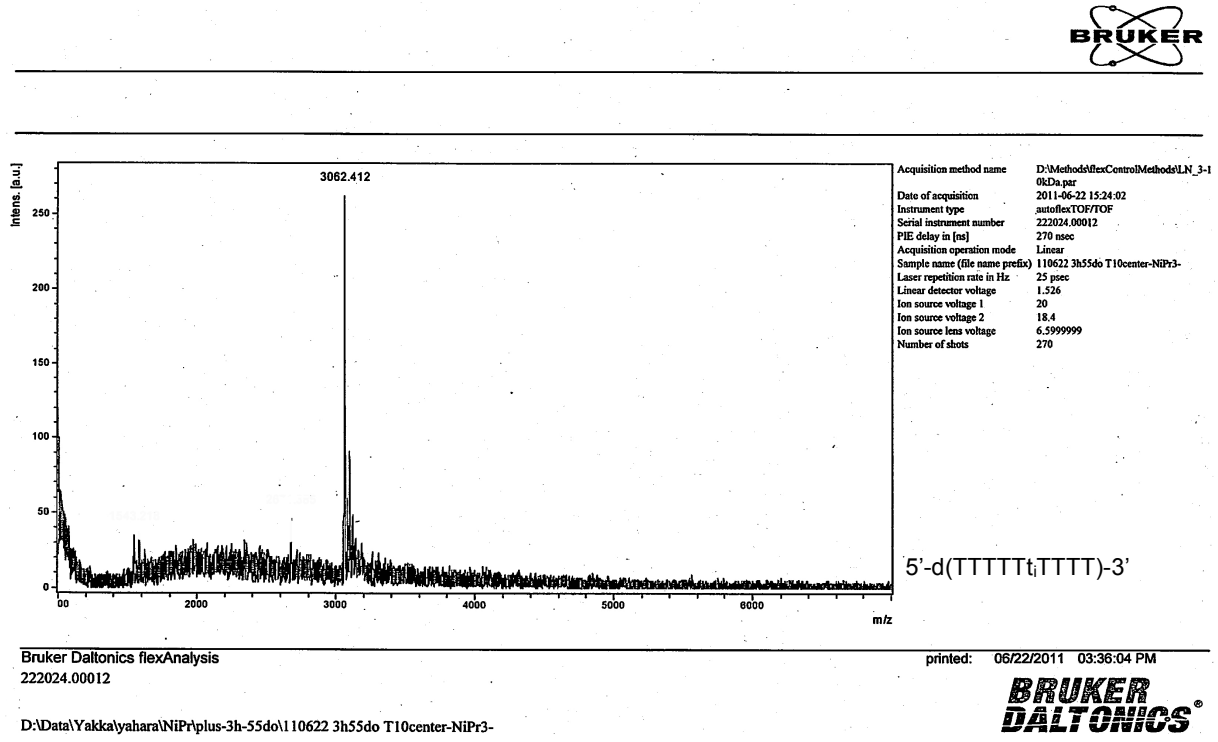


Figure S36. MALDI-TOF mass of **ON-6**.

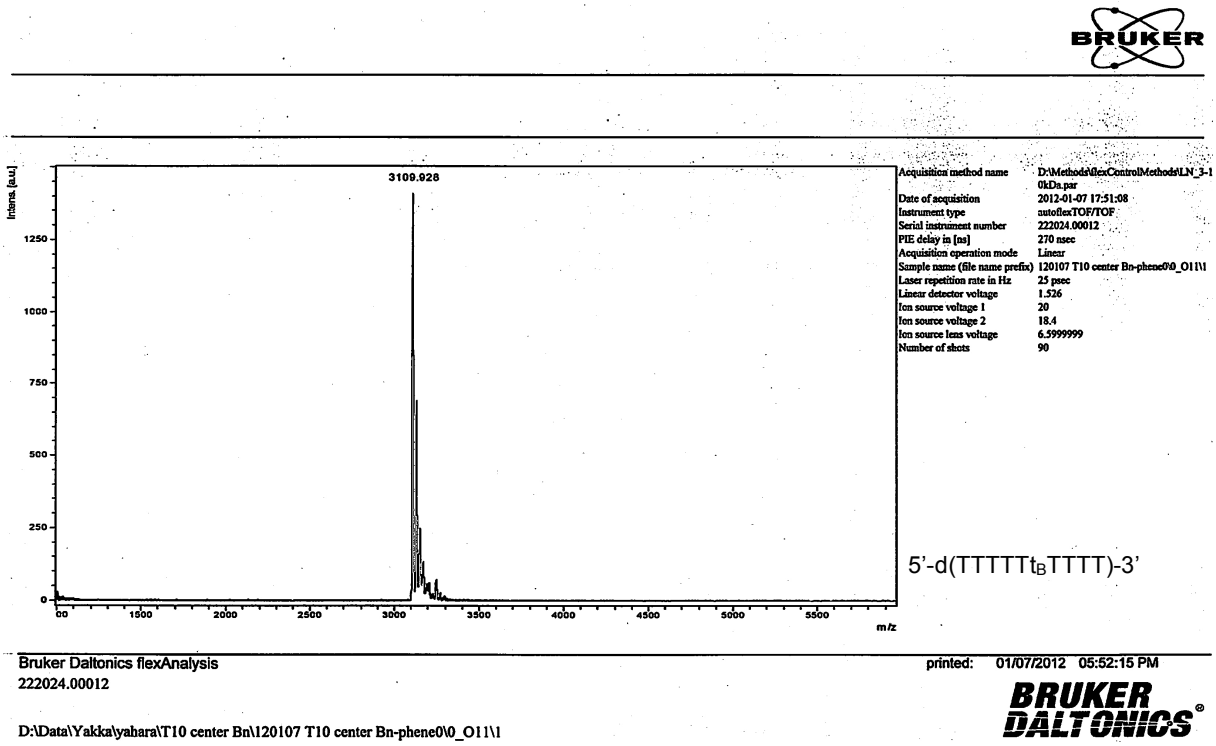


Figure S37. MALDI-TOF mass of **ON-7**.

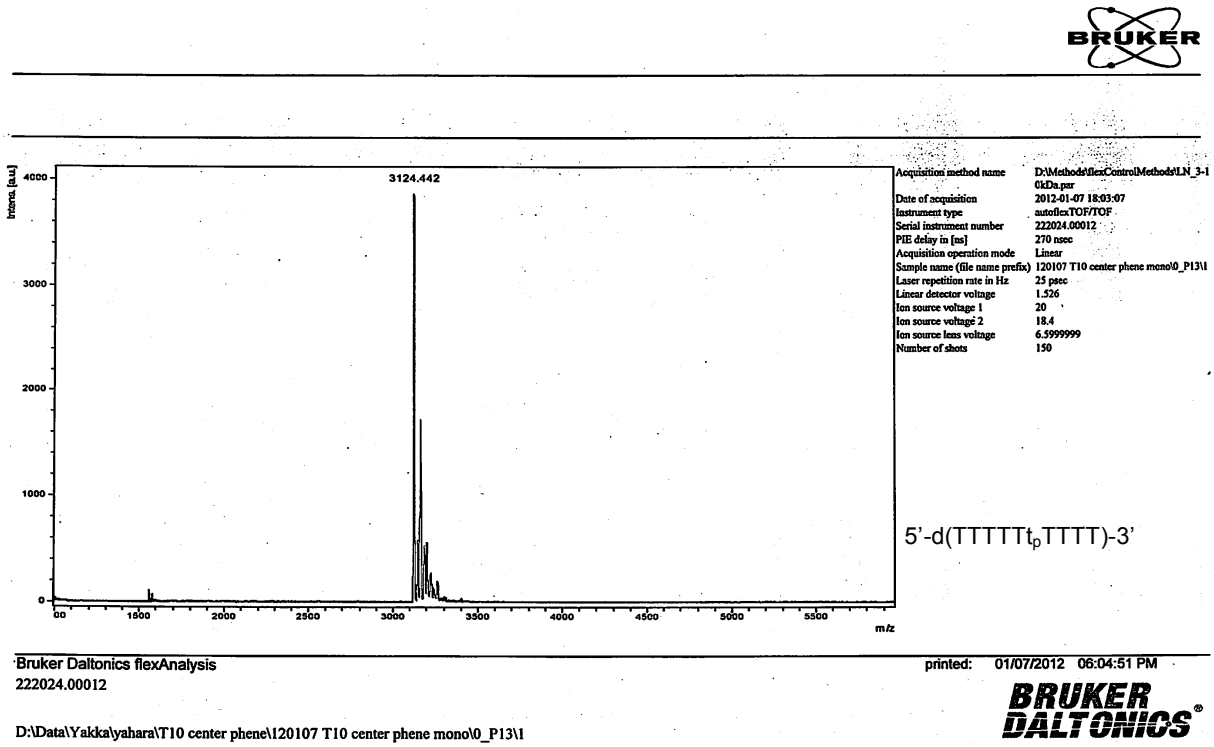


Figure S38. MALDI-TOF mass of **ON-8**.

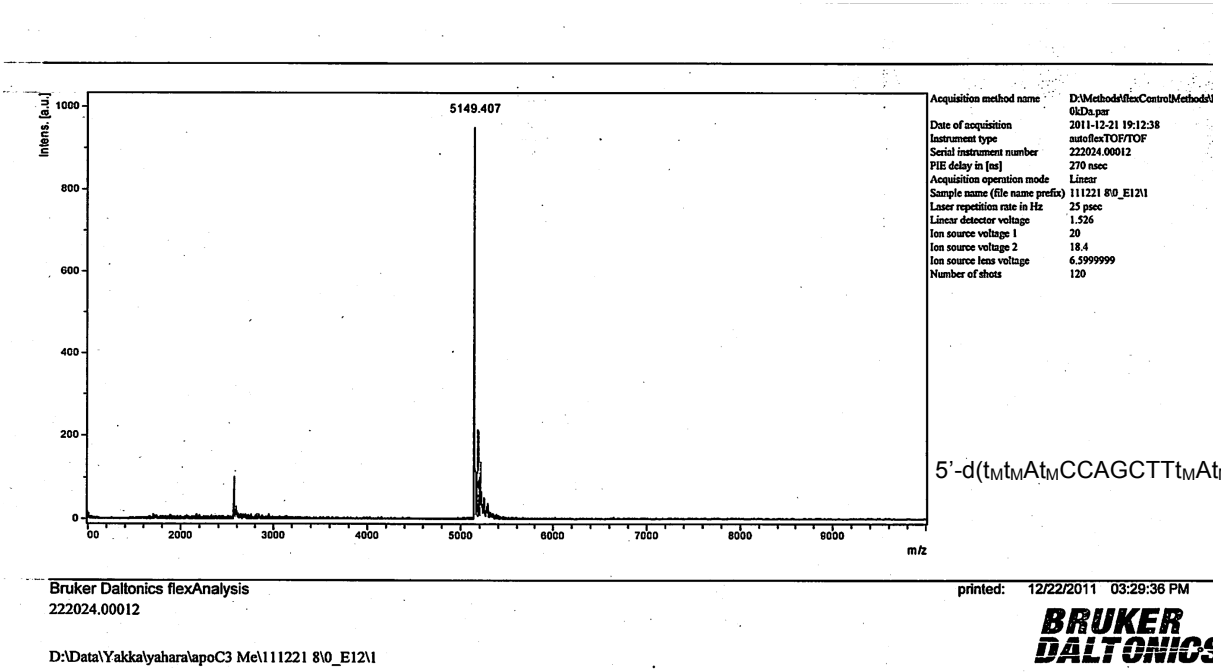


Figure S39. MALDI-TOF mass of **ON-11**.

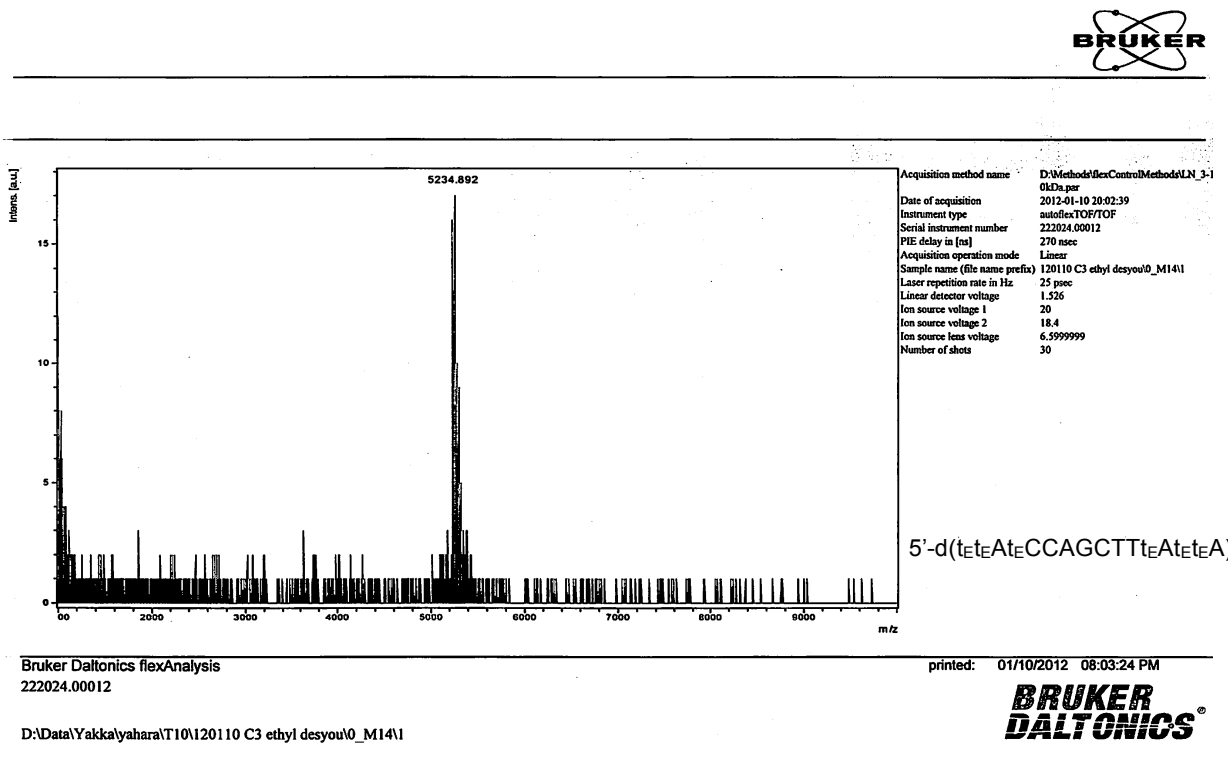


Figure S40. MALDI-TOF mass of **ON-16**.

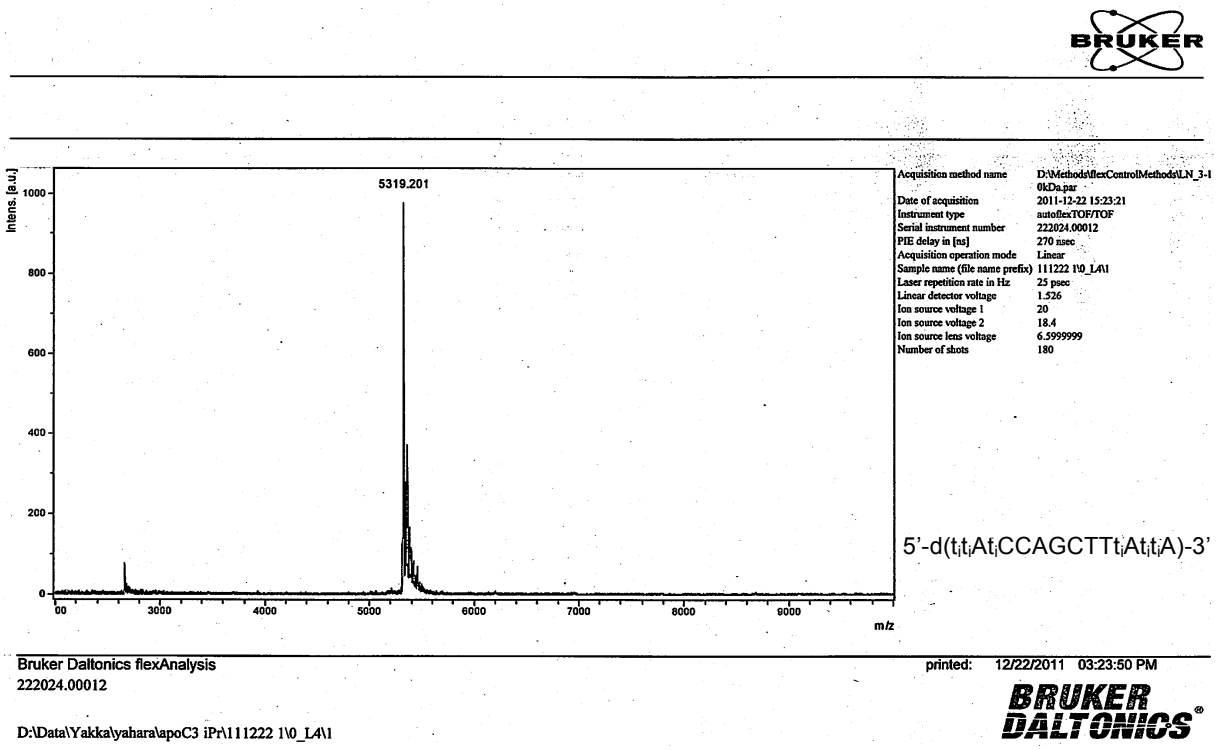


Figure S41. MALDI-TOF mass of **ON-12**.

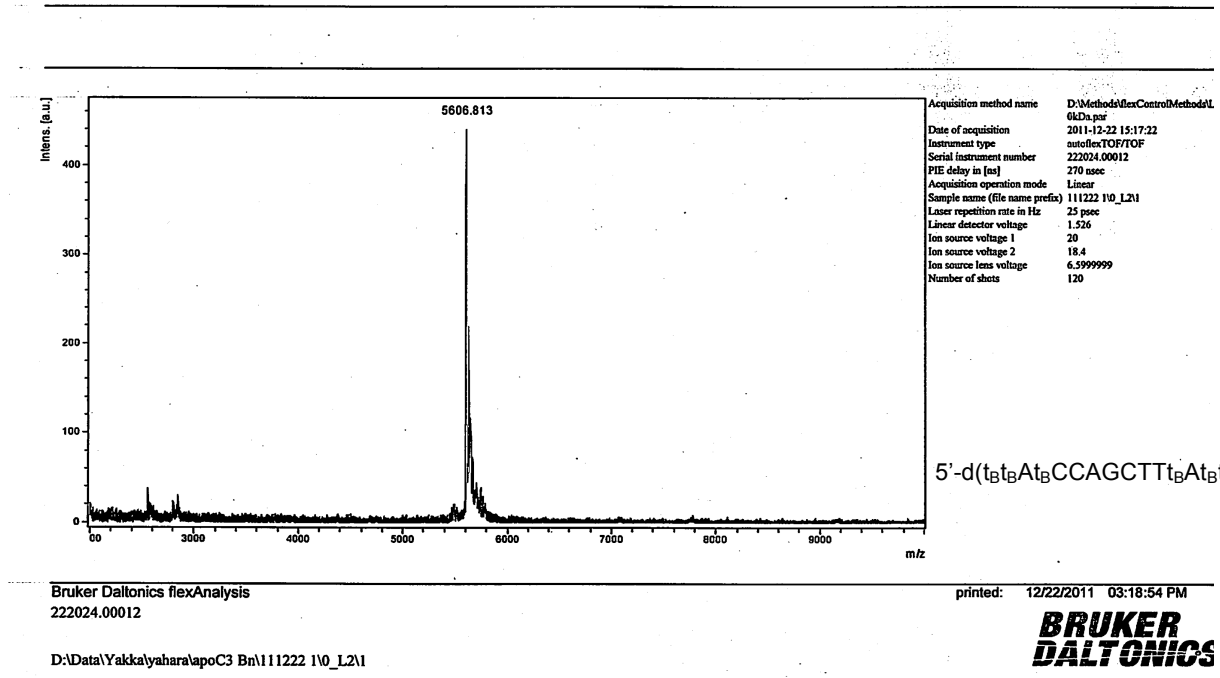


Figure S42. MALDI-TOF mass of **ON-13**.

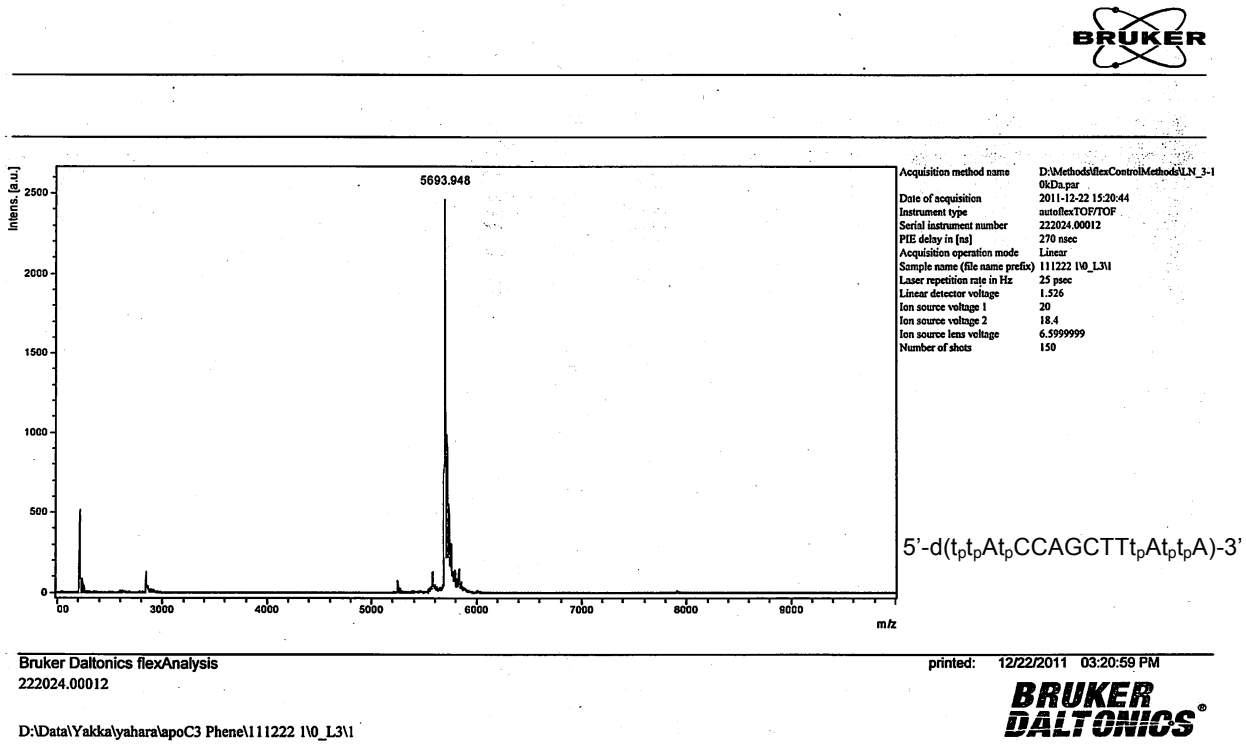


Figure S43. MALDI-TOF mass of **ON-17**.

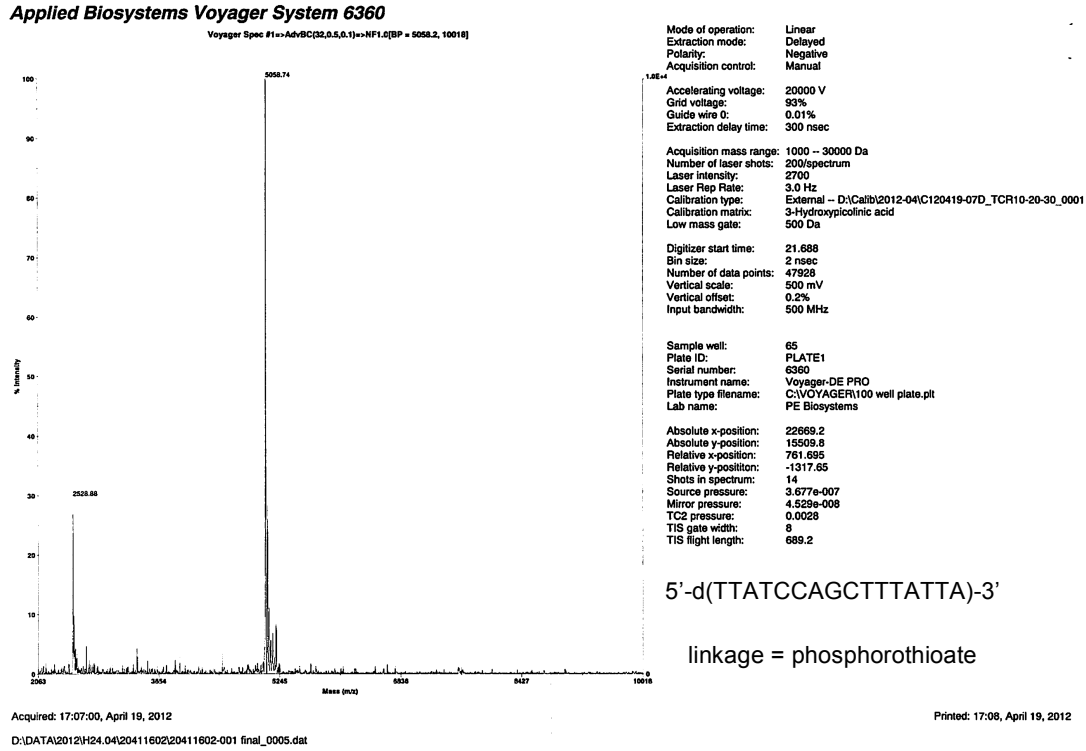
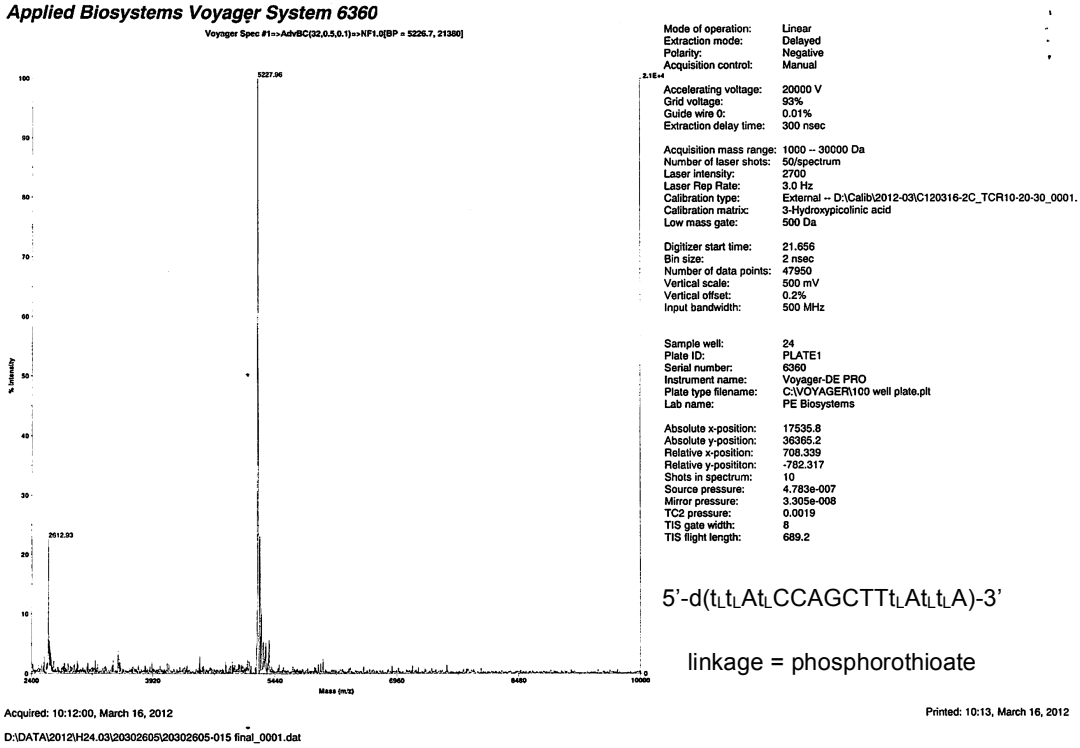


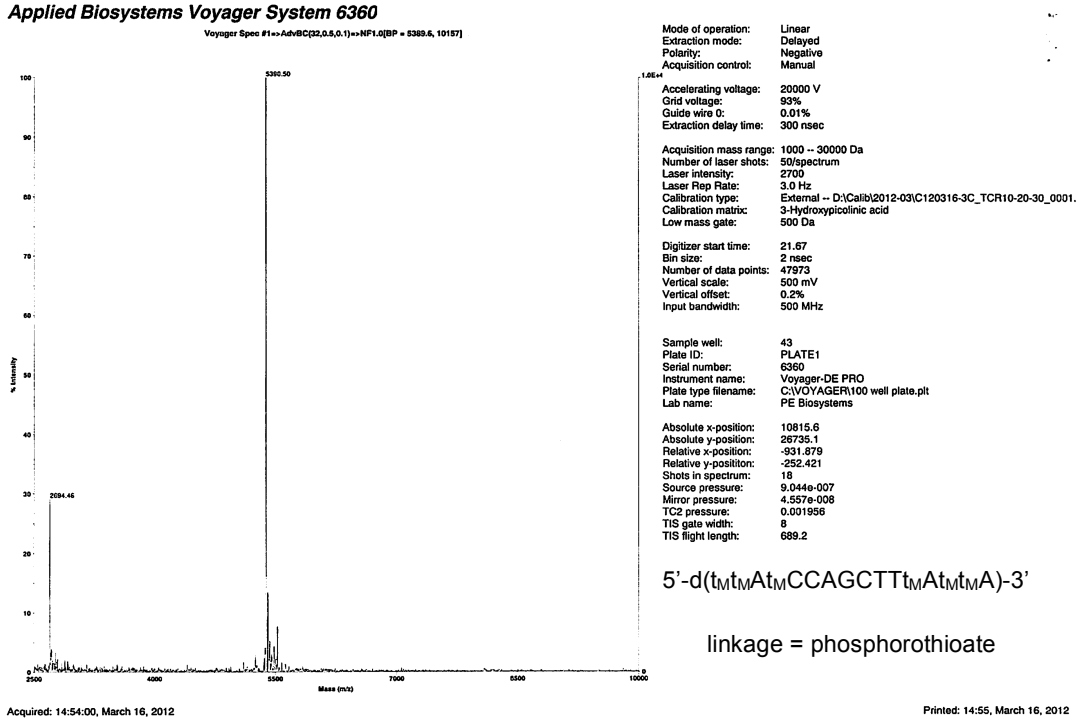
Figure S44. MALDI-TOF mass of **ON-9S**.



5'-d($t_Lt_LAt_LCCAGCTTt_LAt_Lt_LA$)-3'

linkage = phosphorothioate

Figure S45. MALDI-TOF mass of **ON-10S**.



5'-d($t_Mt_MAt_MCCAGCTTt_MAt_Mt_MA$)-3'

linkage = phosphorothioate

Figure S46. MALDI-TOF mass of **ON-11S**.

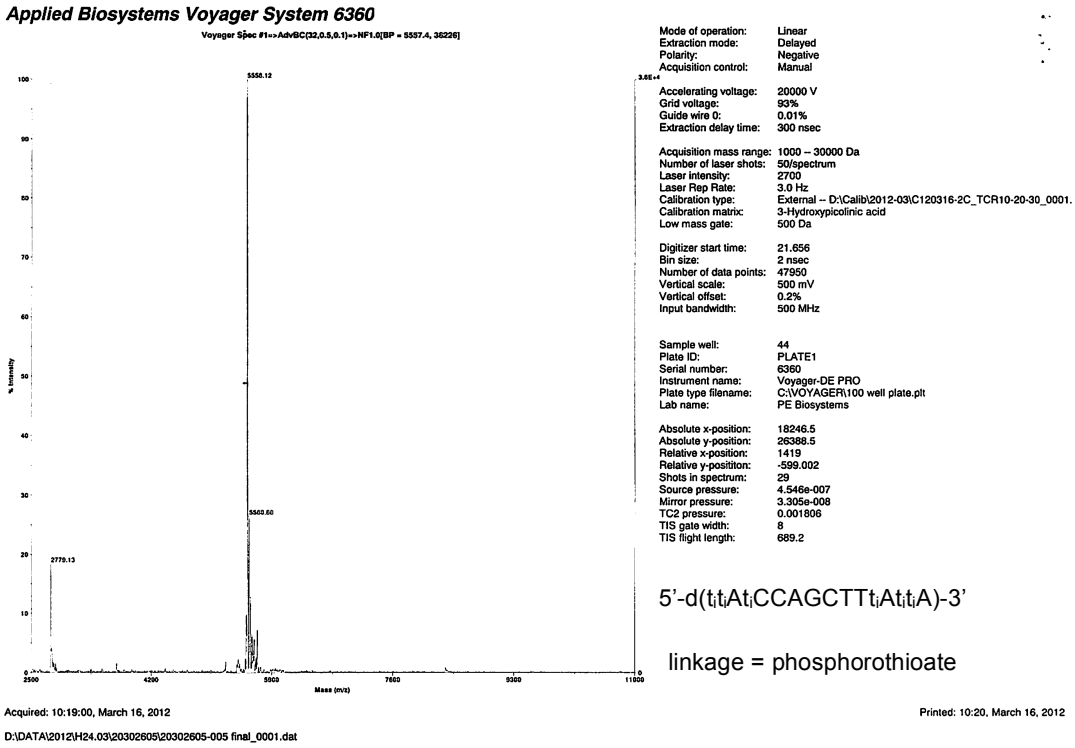


Figure S47. MALDI-TOF mass of **ON-12S**.

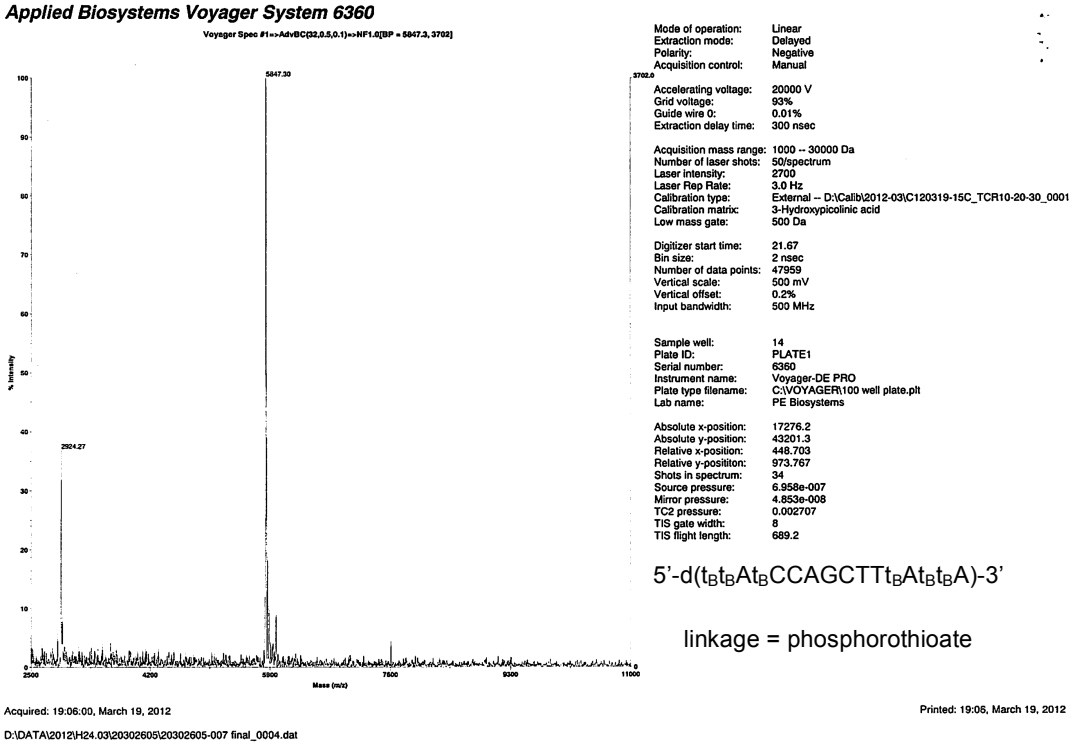


Figure S48. MALDI-TOF mass of **ON-13S**.

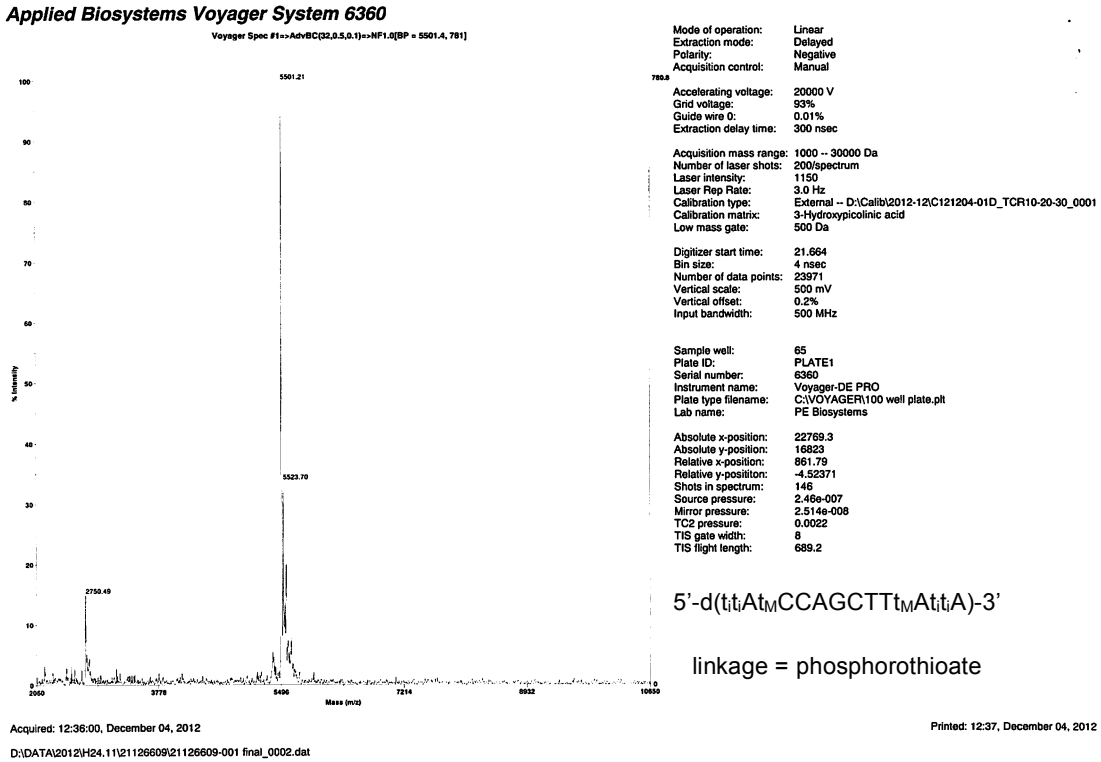


Figure S49. MALDI-TOF mass of **ON-14S**.

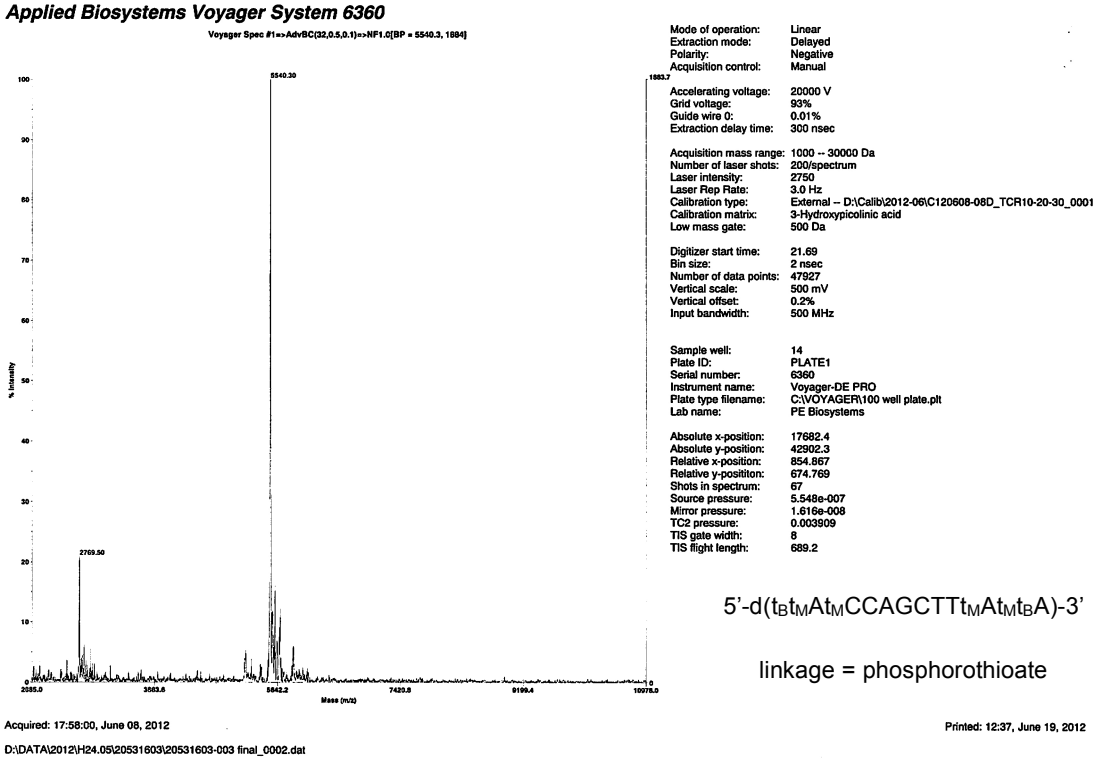


Figure S50. MALDI-TOF mass of **ON-15S**.