[Electronic Supplementary Information]

Reverse-direction synthesis of oligonucleotides containing a 3′-*S*-phosphorothiolate linkage and 3′-terminal 3′-thionucleosides

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1. Experimental – ^{13}C NMR data

All ^{13}C NMR spectra were recorded on a Bruker spectrometer operating at 100 MHz . $CDCl_3$ was used for all samples and spectra were recorded relative to an internal standard of tetramethylsilane. ^{13}C spectra were ^{1}H decoupled and were singlets unless otherwise stated.

5′-*O*-(tert-Butyldiphenylsilyl)-3′-*S*-acetyl-3′-thiothymidine (6a)

^{13}C NMR δ = 12.1(Me); 19.4(CMe_3); 27.0(CMe_3); 30.6(SCOMe); 39.0(C2′); 40.1(C3′); 63.5(C5′); 84.3(C1′); 84.9(C4′); 111.4(C5); 127.91 & 127.94(*m*-Ph); 130.0 & 130.1(*p*-Ph); 132.4 & 133.0(*i*-Ph); 135.1(C6); 135.3 & 135.6(*o*-Ph); 150.5(C2); 164.0(C4); 194.4(SCOMe).

5′-*O*-(4,4′-Dimethoxytrityl)- 3′-*S*-acetyl-3′-thiothymidine (6b)

^{13}C NMR δ = 11.9(Me); 30.5(SCOMe); 39.4(C2′); 40.0(C3′); 55.2(OMe); 62.5(C5′); 84.0(C4′); 84.6(C1′); 86.95(C$PhAr_2$); 111.1(C5); 113.1(*m*-Ar_2); 127.8(*p*-Ph); 128.5 & 129.2(*o*-Ph, *m*-Ph); 130.1(*o*-Ar_2); 135.4(*i*-Ar_2); 135.8(C6); 144.2(*i*-Ph); 150.4(C2); 158.7(*p*-Ar_2); 163.9(C4); 194.4(SCOMe).

3′-*S*-(4,4′-Dimethoxytrityl)-3′-thiothymidine (7T)

^{13}C NMR δ = 12.8(Me); 41.1(C3′); 41.7 (C2′); 55.7(OMe); 61.1(C5′); 67.4(C$\underline{}$PhAr$_2$); 85.43 & 85.45(C1′, C4′); 110.8(C5); 113.7(*m*-Ar$_2$); 127.3(*p*-Ph); 128.4(*m*-Ph); 129.7(*o*-Ph); 131.0(*o*-Ar$_2$); 136.5(C6); 137.2(*i*-Ar$_2$); 145.5(*i*-Ph); 150.6(C2); 158.8(*p*-Ar$_2$); 164.2(C4).

3′-*S*-(4,4′-Dimethoxytrityl)-2′-deoxy-4-*N*-benzoyl-3′-thiocytidine (7C)

^{13}C NMR δ = 39.4(C3′); 42.6(C2′); 55.7(OMe); 60.4(C5′); 67.5(C$\underline{}$PhAr$_2$); 86.2(C1′); 87.4(C4′); 113.7(*m*-Ar$_2$); 127.4(*p*-Ph); 127.9(*o*-Bz); 128.5(*m*-Ph); 129.5(*m*-Bz, *o*-Ph); 130.8(*o*-Ar$_2$); 133.6(*p*-Bz); 137.1(*i*-Ar$_2$); 145.5(C6); 158.8(*p*-Ar$_2$); 162.5(C4). C2, C5, *i*-Ph and *i*-Bz not observed.

3′-*S*-(4,4′-Dimethoxytrityl)-3′-thiothymidine-5′-*O*-[(2-cyanoethyl)-(*N*,*N*-diisopropyl)]-phosphoramidite (2T)

^{13}C NMR δ = 12.7 & 12.8(^{a}Me, ^{b}Me); 20.7 & 20.8(^{a}C$\underline{}$H$_2$CN, ^{b}C$\underline{}$H$_2$CN); 24.9, 25.0 & 25.1(^{a}CH$\underline{}$Me$_2$, ^{b}CH$\underline{}$Me$_2$); 41.1 & 41.5(^{a}C2′, ^{b}C2′); 42.5(^{a}C3′, ^{b}C3′); 43.4, 43.6, 43.7 & 43.8(^{a}C$\underline{}$HMe$_2$, ^{b}C$\underline{}$HMe$_2$); 55.7(^{a}OMe, ^{b}OMe); 58.8 & 59.0(2xd, $^2J_{OCH2\text{-}P}$ = 81 & 87, ^{a}OCH$_2$, ^{b}OCH$_2$); 62.4 & 64.7(2xd, $^2J_{C5'\text{-}P}$ = 61 x 2, ^{a}C5′, ^{b}C5′); 67.3&67.4(^{a}C$\underline{}$PhAr$_2$, ^{b}C$\underline{}$PhAr$_2$); 84.6 & 85.0(2xd, $^3J_{C4'\text{-}P}$ = 41 & 35, ^{a}C4′, ^{b}C4′); 84.8 & 85.4(^{a}C1′, ^{b}C1′); 110.9(^{a}C5, ^{b}C5); 113.76 & 113.79(*m*-^{a}Ar$_2$, *m*-^{b}Ar$_2$); 117.67 & 117.73(^{a}CN, ^{b}CN); 127.2 & 127.3(*p*-^{a}Ph, *p*-^{b}Ph); 128.46 & 128.50(*m*-^{a}Ph, *m*-^{b}Ph); 129.68 & 129.7(*o*-^{a}Ph, *o*-^{b}Ph); 131.0(*o*-^{a}Ar$_2$, *o*-^{b}Ar$_2$); 135.8 & 136.1(^{a}C6, ^{b}C6); 137.1 & 137.2(*i*-^{a}Ar$_2$, *i*-^{b}Ar$_2$); 145.4 & 145.5(*i*-^{a}Ph, *i*-^{b}Ph); 150.47 & 150.54(^{a}C2, ^{b}C2); 158.70 & 158.72(*p*-^{a}Ar$_2$, *p*-^{b}Ar$_2$); 164.06 & 164.08(^{a}C4, ^{b}C4).

3′-*S*-(4,4′-Dimethoxytrityl)-2′-deoxy-4*N*-benzoyl-3′thiocytidine-5′-*O*-[(2-cyanoethyl)-(*N*,*N*-diisopropyl)]-phosphoramidite (2C)

^{13}C NMR (weak spectrum) δ = 20.7 & 20.8(^{a}C$\underline{}$H$_2$CN, ^{b}C$\underline{}$H$_2$CN); 25.0, 25.08, 25.13 & 25.2(^{a}CH$\underline{}$Me$_2$, ^{b}CH$\underline{}$Me$_2$); 41.1-43.7(^{a}C2′, ^{b}C2′, ^{a}C3′, ^{b}C3′, ^{a}C$\underline{}$HMe$_2$, ^{b}C$\underline{}$HMe$_2$); 55.67 & 55.67(^{a}OMe, ^{b}OMe); 59.0-67.31(^{a}OCH$_2$, ^{b}OCH$_2$, ^{a}C5′, ^{b}C5′, ^{a}C$\underline{}$PhAr$_2$, ^{b}C$\underline{}$PhAr$_2$); 85.7(^{a}C1′, ^{b}C1′, ^{a}C4′, ^{b}C4′); 100.0(^{a}C5, ^{b}C5); 113.7 & 113.8(*m*-^{a}Ar$_2$, *m*-^{b}Ar$_2$); 117.8(^{a}CN, ^{b}CN); 127.2-133.6(*o*-^{a}Ar$_2$, *o*-^{b}Ar$_2$, *p*-^{a}Ph, *p*-^{b}Ph, *m*-^{a}Ph, *m*-^{b}Ph, *o*-^{a}Ph, *o*-^{b}Ph, *m*-^{a}Bz, *m*-^{b}Bz, *o*-^{a}Bz, *o*-^{b}Bz); 133.6(*p*-^{a}Bz, *p*-^{b}Bz); 137.0 & 137.1(*i*-^{a}Ar$_2$, *i*-^{b}Ar$_2$);

145.2 & 145.4(^{a}C6, ^{b}C6); 158.7(*p*-^{a}Ar$_2$, *p*-^{b}Ar$_2$). C2, C4, *i*-Ph, *i*-Bz and COPh not observed.

5-*O*-(4,4-dimethoxytrityl)-3-*O*-(4*N*-benzoyl-4-aminopyrimidin-2-yl)-2-deoxy-alpha-D-threo-pentofuranosyl-1-*S*-thiobenzoate (11)

^{13}C NMR δ = 40.6(C2′); 55.5(OMe); 61.2(C5′); 76.0(C3′); 81.4(C4′); 81.9(C1′); 86.6(CPhAr$_2$); 104.8(C5); 113.4(*m*-Ar$_2$); 127.0-129.4(*o*-Ph, *m*-Ph, *p*-Ph, *o*-NBz, *m*-NBz, *o*-SBz, *m*-SBz); 130.4 & 130.5(*o*-Ar$_2$); 133.3(*p*-NBz); 133.6(*i*-NBz); 134.0(*p*-SBz); 136.2 & 136.4(*i*-Ar$_2$); 137.3(*i*-SBz); 145.2(*i*-Ph); 158.8(*p*-Ar$_2$); 159.7(C2); 160.8(C6); 164.2(C4); 166.3(NHCOPh); 191.0(SCOPh).

5-*O*-(4,4-dimethoxytrityl)-3-*O*-(4*N*-benzoyl-4-aminopyrimidin-2-yl)-2-deoxy-alpha-D-threo-pentofuranosyl-1-mercaptan (12)

^{13}C NMR δ = 43.4(C2′); 55.5(OMe); 61.3(C5′); 76.4(C3′); 78.4(C1′); 80.2(C4′); 86.5(CPhAr$_2$); 104.7(C5); 113.4(*m*-Ar$_2$); 127.0(*o*-Bz); 127.7-130.5(*m*-Ph, *p*-Ph, *o*-Ph, *o*-Ar$_2$, *m*-Bz); 133.4 & 133.7(*p*-Bz, *i*-Bz); 136.1 & 136.4(*i*-Ar$_2$); 145.2(*i*-Ph); 158.7(C2); 159.5(*p*-Ar$_2$); 160.8(C6); 164.0(C4); 166.3(COPh).

General procedure for 3′-*S*-trityl-3′-thionucleosides (14)

3′-Thio-3′-*S*-tritylthymidine (14T). ^{13}C NMR δ = 12.9(Me); 40.9(C2′); 41.6(C3′); 60.8(C5′); 68.3(CPh$_3$); 85.3 & 85.4(C1′, C4′); 110.9(C5); 127.5(*p*-Ph); 128.5(*o*-Ph); 129.9(*m*-Ph); 136.5(C6); 144.9(*i*-Ph); 150.5(C2); 164.2(C4).

3′-*S*-trityl-2′-deoxy-4*N*-benzoyl-3′-thiocytidine (14C). ^{13}C NMR δ = 39.3(C3′); 42.5(C2′); 60.3(C5′); 68.4(CPh$_3$); 86.2&87.3(C1′, C4′); 127.6(*p*-Ph); 127.9(*o*-Bz); 128.5(*o*-Ph); 129.4(*m*-Bz); 129.8(*m*-Ph); 133.6(*p*-Bz); 144.8(*i*-Ph/C6); C2, C4, *i*-Bz and C5 not observed.

3′-*S*-trityl-2′-deoxy-6*N*-benzoyl-3′-thioadenosine (14A). ^{13}C NMR δ = 41.0(C2′); 43.4(C3′); 63.0(C5′); 68.3(CPh$_3$); 86.7(C4′); 87.8(C1′); 124.3(C5); 127.5(*p*-Ph); 128.3(*o*-Bz); 128.6(*o*-Ph); 129.3(*m*-Bz); 129.9(*m*-Ph); 133.3(*p*-Bz); 133.9(*i*-Bz); 142.4(C2); 144.5(*i*-Ph); 150.3(C4); 151.1(C6); 152.6(C8); 165.0(COPh).

5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxy-6*N*-benzoyl-3′-thioadenosine (9A)

^{13}C NMR δ = 35.4(C3′); 42.9(C2′); 55.6(OMe); 62.1(C5′); 84.9(C1′); 87.0(CPhAr$_2$) 88.9(C4′); 113.6(*m*-Ar$_2$); 127.4(p-Ph); 128.3, 128.5 & 128.8(*o*-Ph, *m*-Ph, *o*-Bz); 129.3(*m*-Bz); 130.0 & 130.4(*o*-Ar$_2$); 133.2 & 133.3(*i*-Bz & *p*-Bz); 136.0(*i*-Ar$_2$); 142.0(C2); 144.8 (*i*-Ph); 153.0(C8); 158.9(*p*-Ar$_2$); 164.9(NHCOPh). C4, C5 & C6 not observed.

3′-*S*-Trityl-3′-thiothymidine-5′-*O*-[(2-cyanoethyl)-(*N*,*N*-diisopropyl)]-phosphoramidite (3T)

^{13}C NMR δ = 12.7 & 12.9(^{a}Me, ^{b}Me); 20.7 & 20.8(^{a}CH$_2$CN, ^{b}CH$_2$CN); 25.0 & 25.1(^{a}CHMe$_2$, ^{b}CHMe$_2$); 40.9 & 41.3(^{a}C2′, ^{b}C2′); 42.3(^{a}C3′, ^{b}C3′); 43.4, 43.5, 43.6 & 43.8(^{a}CHMe$_2$, ^{b}CHMe$_2$); 58.7 &59.0(2xd, $^2J_{OCH2\text{-}P}$ = 81, 85, ^{a}OCH$_2$, ^{b}OCH$_2$); 62.2 & 64.6(2xd, $^2J_{C5'\text{-}P}$ = 61 & 62, ^{a}C5′, ^{b}C5′); 68.1 & 68.2(^{a}CPh$_3$, ^{b}CPh$_3$); 84.5 & 84.9(2xd, $^3J_{C4'\text{-}P}$ = 40 & 35, ^{a}C4′, ^{b}C4′); 84.7 & 85.3(^{a}C1′, ^{b}C1′); 110.9 & 111.0(^{a}C5, ^{b}C5); 117.7 & 117.8(^{a}CN, ^{b}CN); 127.38 & 127.42(*p*-^{a}Ph, *p*-^{b}Ph); 128.56 & 128.61(*o*-^{a}Ph, *o*-^{b}Ph); 129.86 & 129.88(*m*-^{a}Ph, *m*-^{b}Ph); 135.9 & 136.1(^{a}C6, ^{b}C6); 144.7 & 1 44.8(*i*-^{a}Ph, *i*-^{b}Ph); 150.46 & 150.53(^{a}C2, ^{b}C2); 164.08 &164.11(^{a}C4, ^{b}C4).

3′-*S*-Trityl-2′-deoxy-4*N*-benzoyl-3’-thiocytidine-5′-*O*-[(2-cyanoethyl)-(*N*,*N*-diisopropyl)]-phosphoramidite (3C)

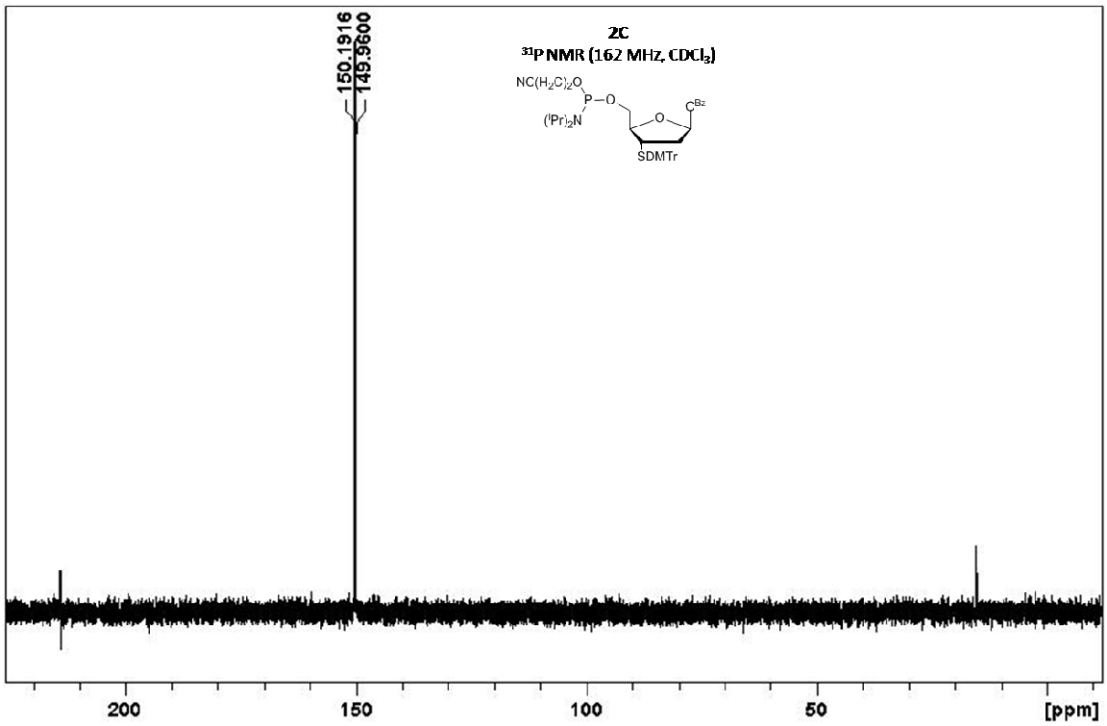
^{13}C NMR (weak spectrum) δ = 20.8(^{a}CH$_2$CN, ^{b}CH$_2$CN); 25.0, 25.08, 25.15 & 25.2(^{a}CHMe$_2$, ^{b}CHMe$_2$); 40.9(^{a}C2′, ^{b}C2′,); 42.4 & 42.7(^{a}C3′, ^{b}C3′); 43.5-43.7(^{a}CHMe$_2$, ^{b}CHMe$_2$); 58.9(^{a}OCH$_2$, ^{b}OCH$_2$); 68.1 & 68.2(^{a}CPh$_3$, ^{b}CPh$_3$); 87.6(^{a}C1′, ^{b}C1′, ^{a}C4′, ^{b}C4′); 127.4, 127.5(*p*-^{a}Ph, *p*-^{b}Ph); 127.9(*o*-^{a}Bz, *o*-^{b}Bz) 128.5 & 128.6(*o*-^{a}Ph, *o*-^{b}Ph); 129.5(*m*-^{a}Bz, *m*-^{b}Bz); 129.8 & 129.9(*m*-^{a}Ph, *m*-^{b}Ph); 133.6(*p*-^{a}Bz, *p*-^{b}Bz); 144.7(*i*-^{a}Ph, *i*-^{b}Ph, ^{a}C6 & ^{b}C6). C5′, C2, C4, C5, CN, *i*-^{b}Bz and NCOPh not observed.

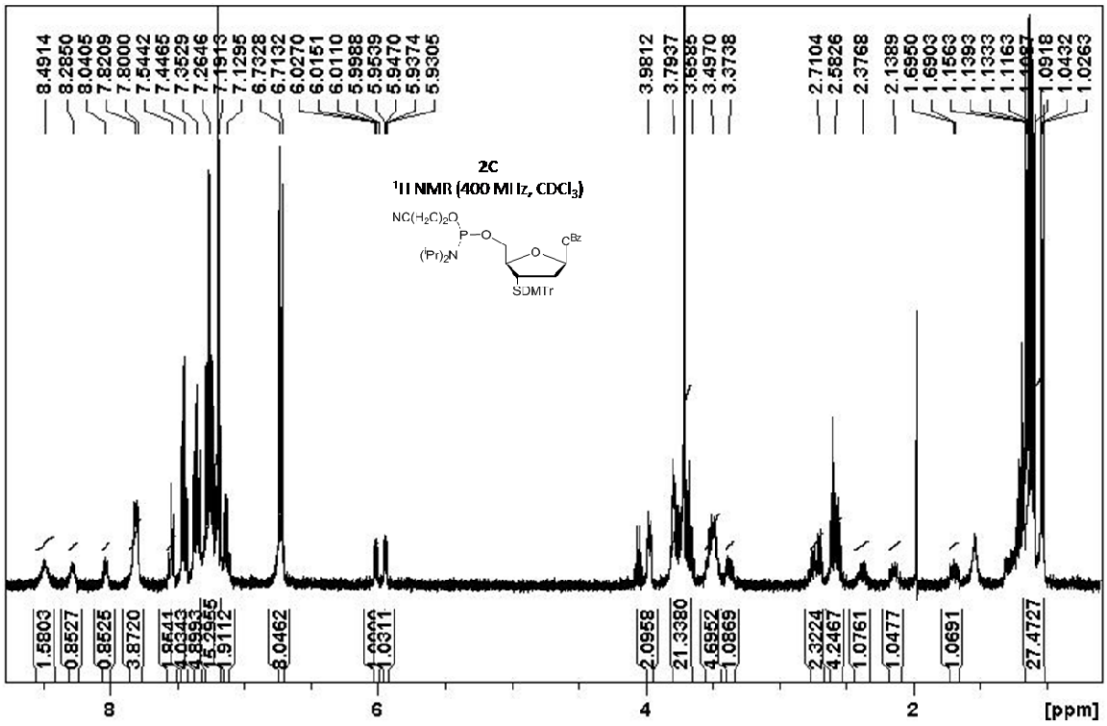
3′-*S*-Trityl-2′-deoxy-*6N*-benzoyl-3′-thioadenosine-5′-*O*-[(2-cyanoethyl)-(*N*,*N*-diisopropyl)]-phosphoramidite (3A)

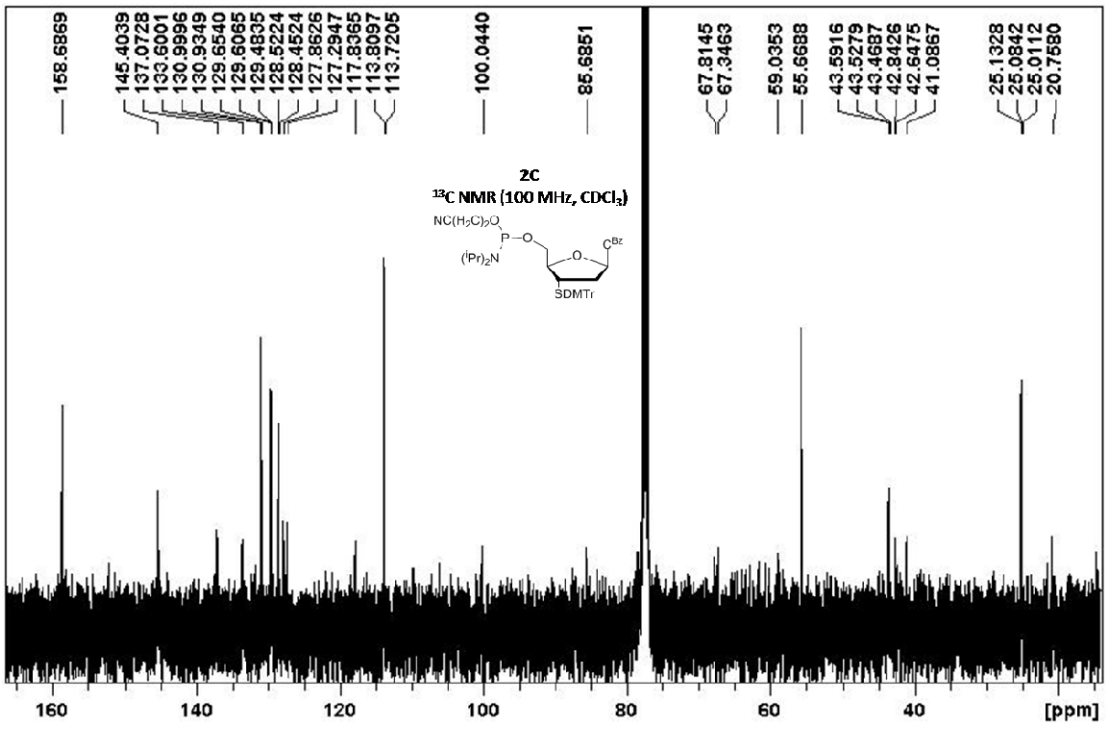
^{13}C NMR (weak spectrum) δ = 20.7(^{a}CH$_2$CN, ^{b}CH$_2$CN); 24.8 & 25.0(^{a}CHMe$_2$, ^{b}CHMe$_2$); 41.1-43.7(^{a}C2′, ^{b}C2′, ^{a}C3′, ^{b}C3′, ^{a}CHMe$_2$, ^{b}CHMe$_2$); 58.8(^{a}OCH$_2$, ^{b}OCH$_2$); 63.2(^{a}C5′, ^{b}C5′); 68.0(^{a}CPh$_3$, ^{b}CPh$_3$); 84.7(^{a}C1′, ^{b}C1′, ^{a}C4′, ^{b}C4′); 127.4 & 127.5(*p*-^{a}Ph, *p*-^{b}Ph); 128.2(*o*-^{a}Bz, *o*-^{b}Bz) 128.5(*o*-^{a}Ph, *o*-^{b}Ph); 129.3(*m*-^{a}Bz, *m*-^{b}Bz); 129.8 & 129.9(*m*-^{a}Ph, *m*-^{b}Ph); 144.8(*i*-^{a}Ph, *i*-^{b}Ph); 149.6(^{a}C4, ^{b}C4); 152.6(^{a}C8, ^{b}C8). C2, C5, C6, CN, *i*-Bz, *p*-Bz & COPh not observed.

2. Experimental – All spectra

All spectra were analysed using TopSpin v2.1



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113.7205
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80
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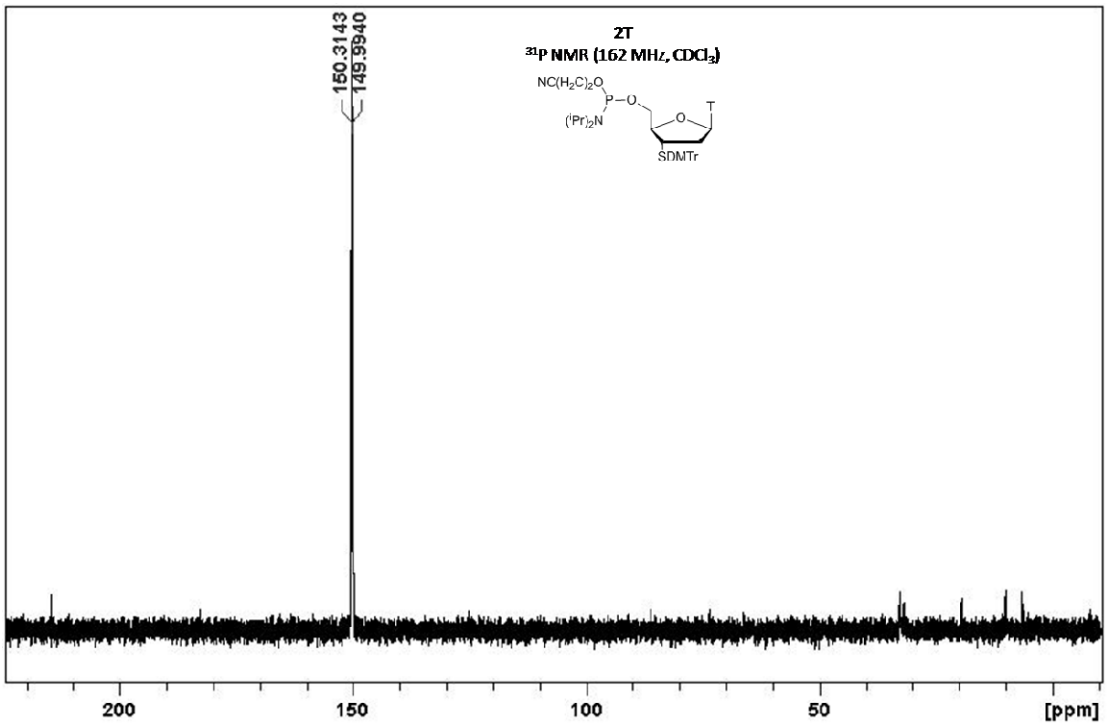
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150
100
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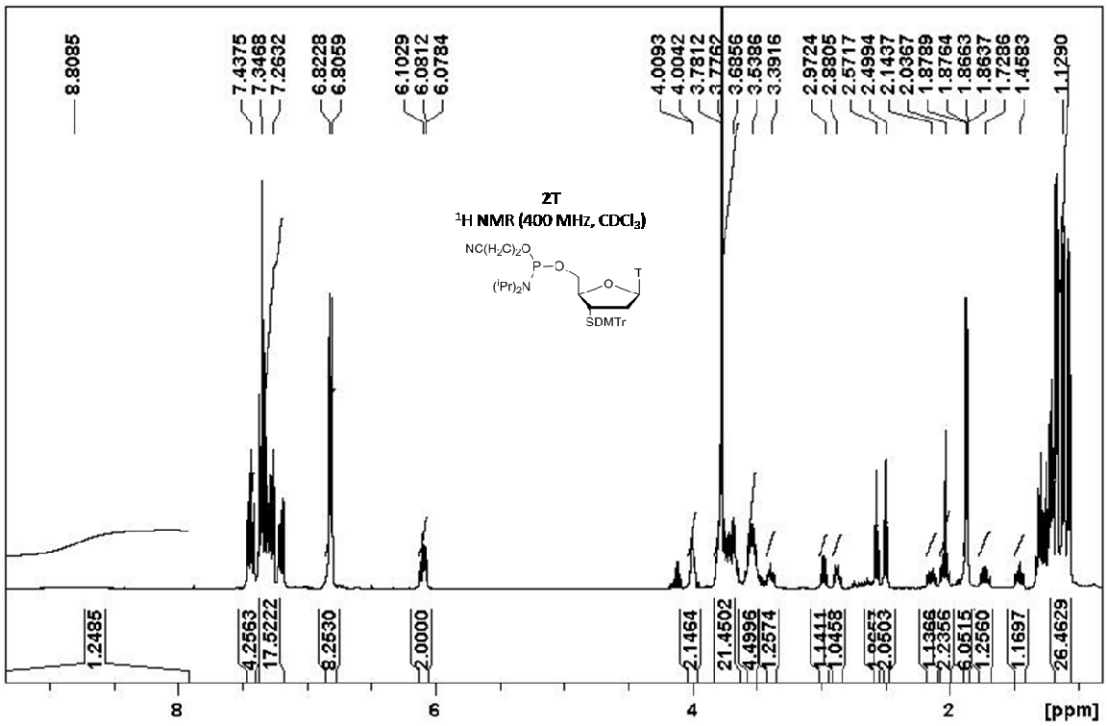
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3.5386
3.3916
2.9724
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2.5717
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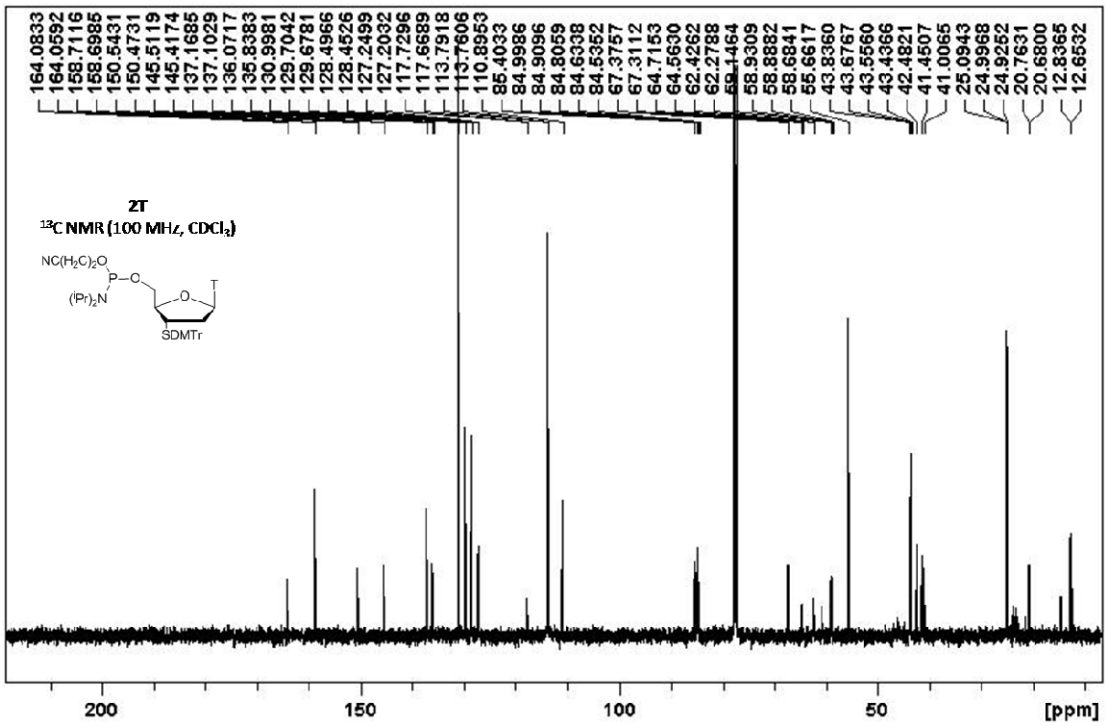
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129.7042
129.6781
128.4966
128.4526
127.2499
127.2032
117.7296
117.6689
113.7918
113.7606
110.8953
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84.9986
84.9096
84.8059
84.6038
84.5352
67.3757
67.3112
64.7153
64.5630
62.4262
62.2788
59.1464
58.9309
58.8882
58.6841
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43.8360
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43.5560
43.4366
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24.9252
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100
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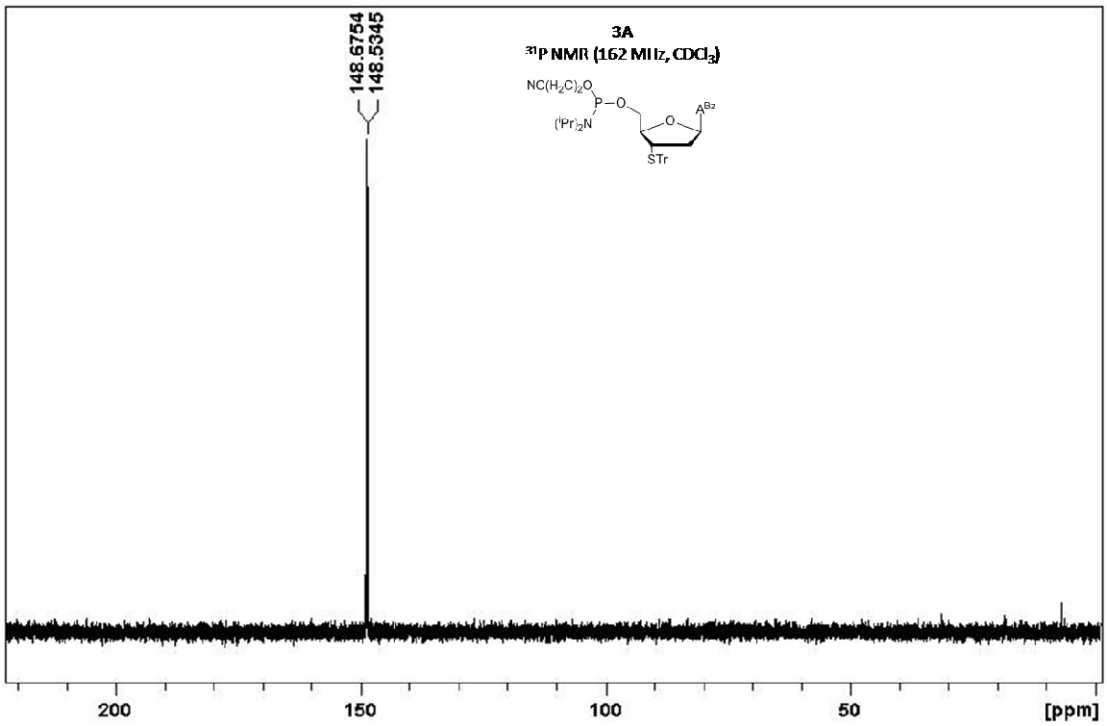
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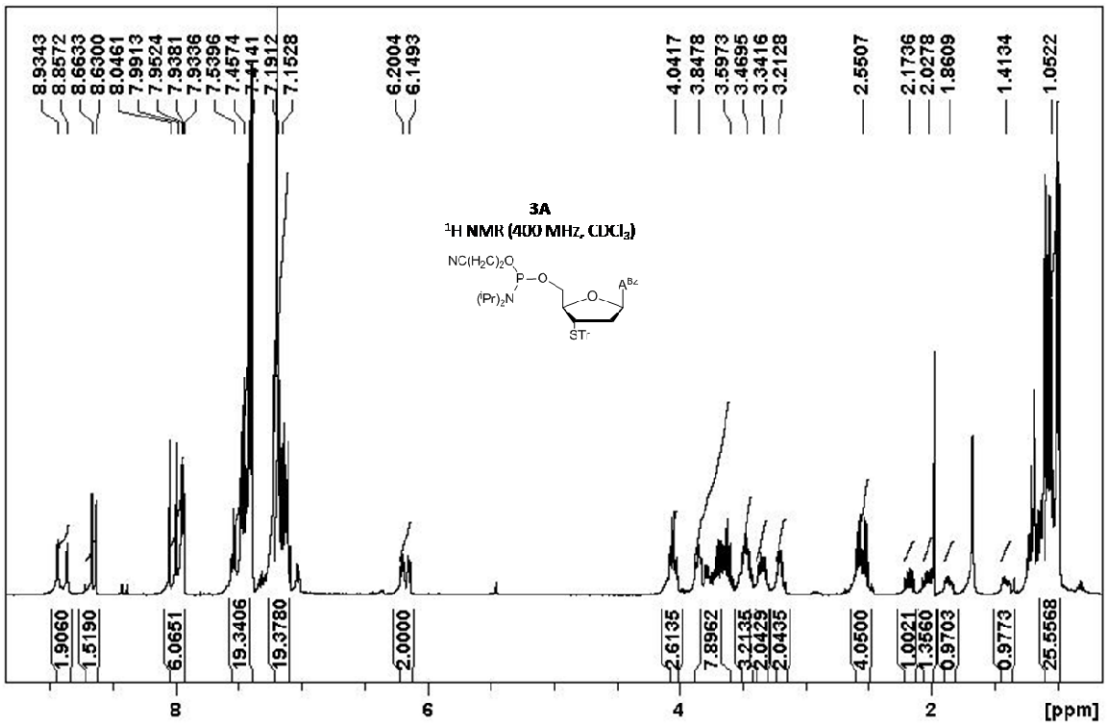
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1.0522
3A
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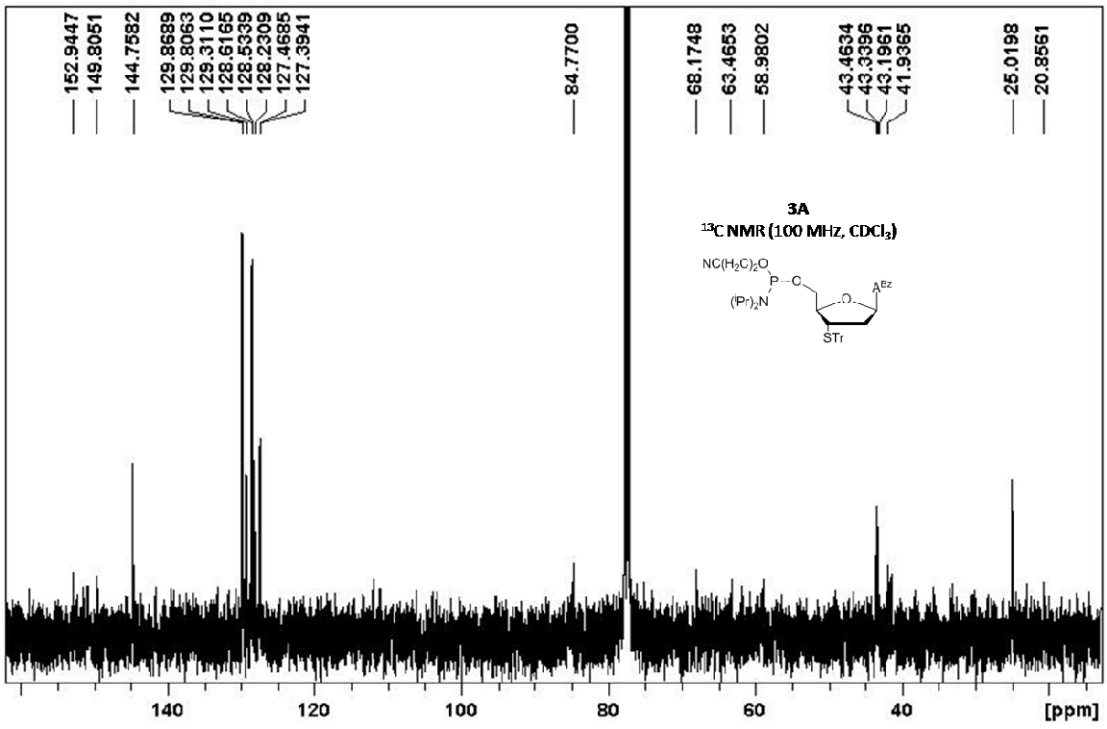
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43.3396
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80
60
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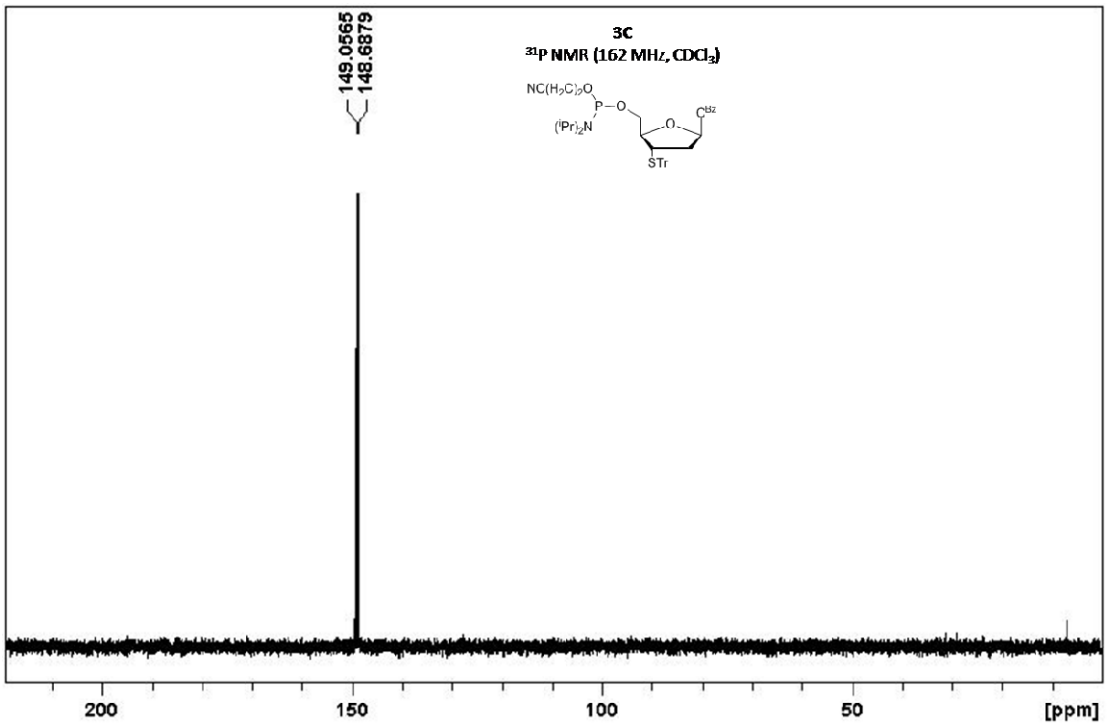
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3C
31P NMR (162 MHz, CDCl3)
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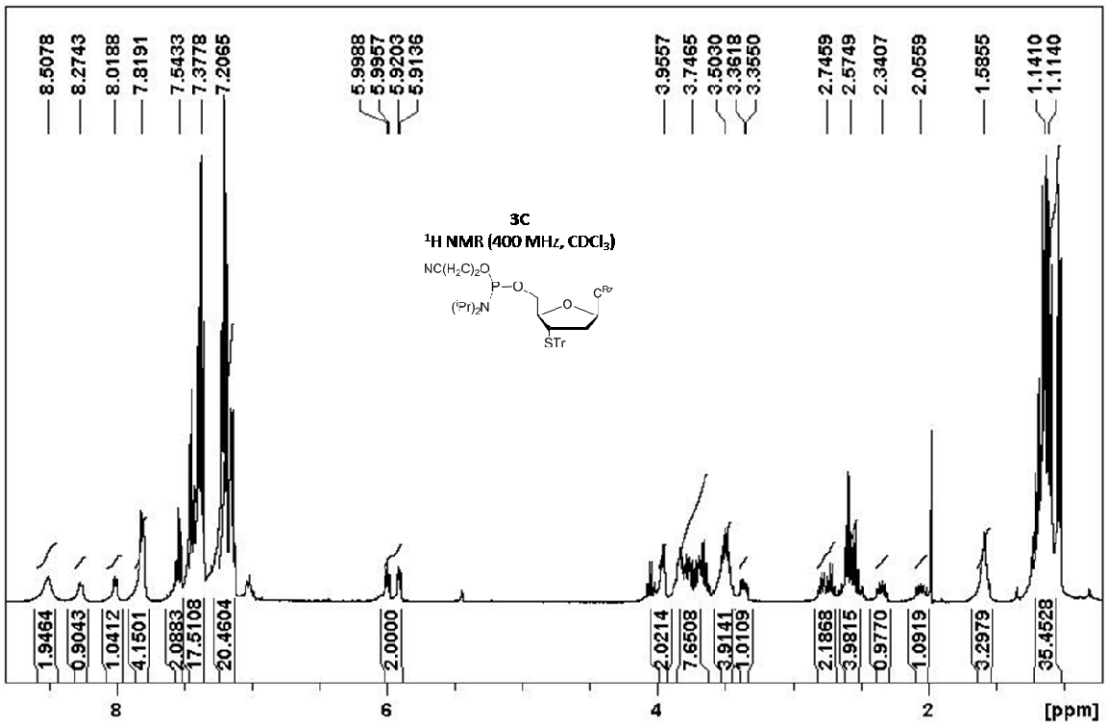
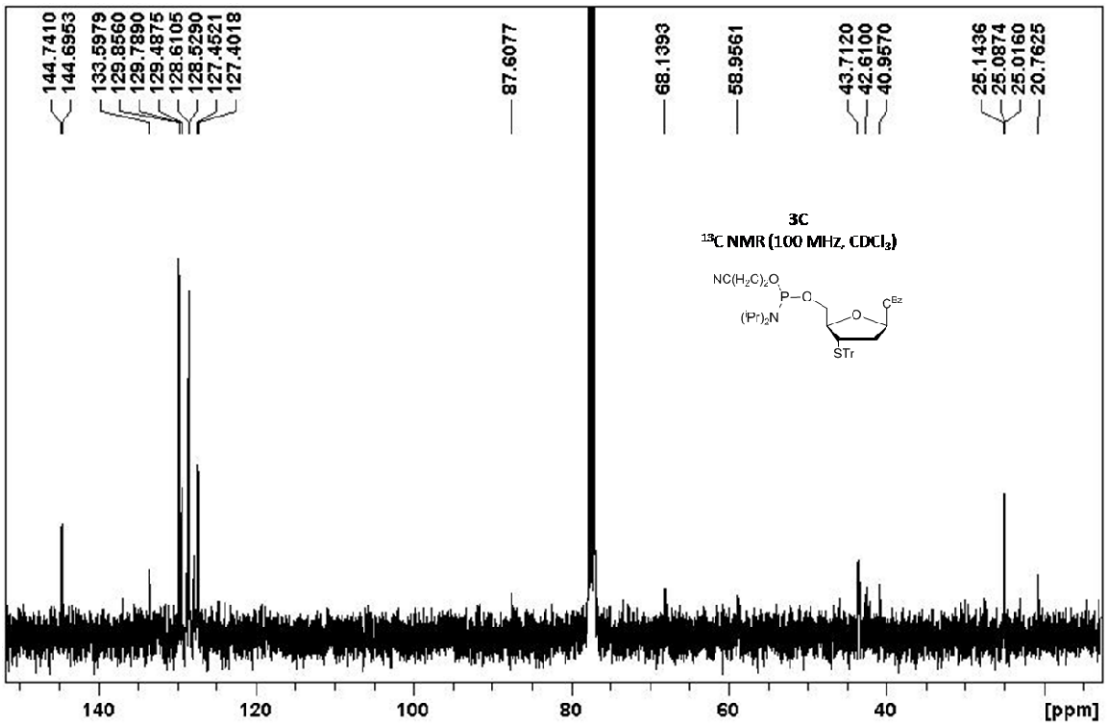
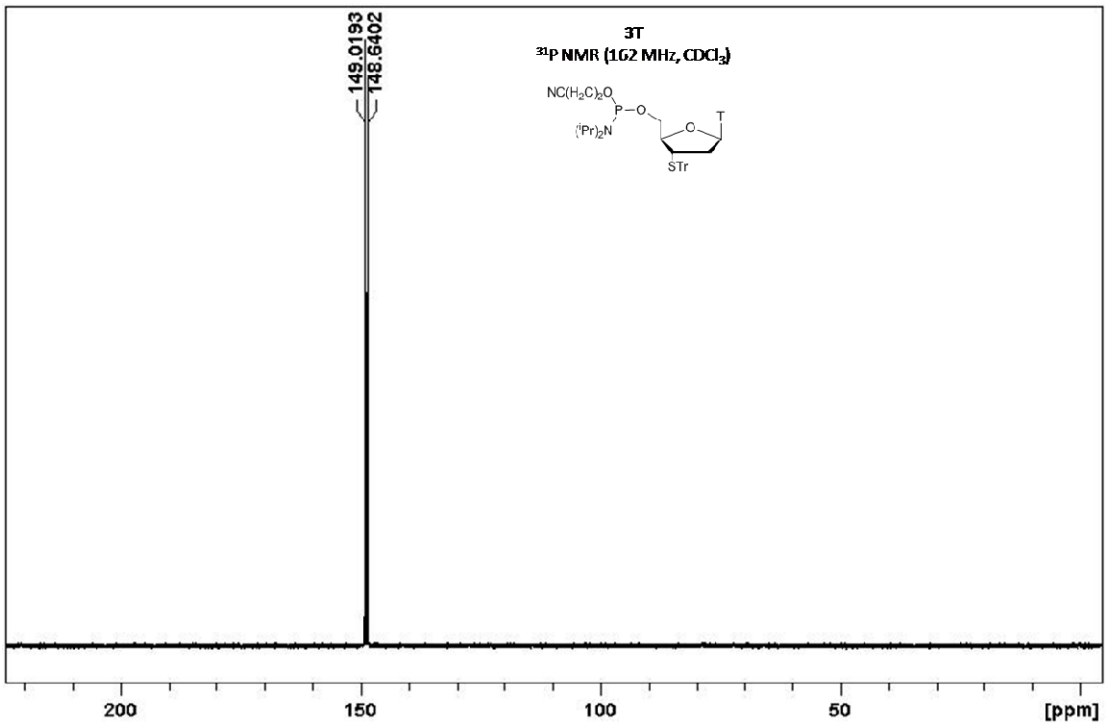
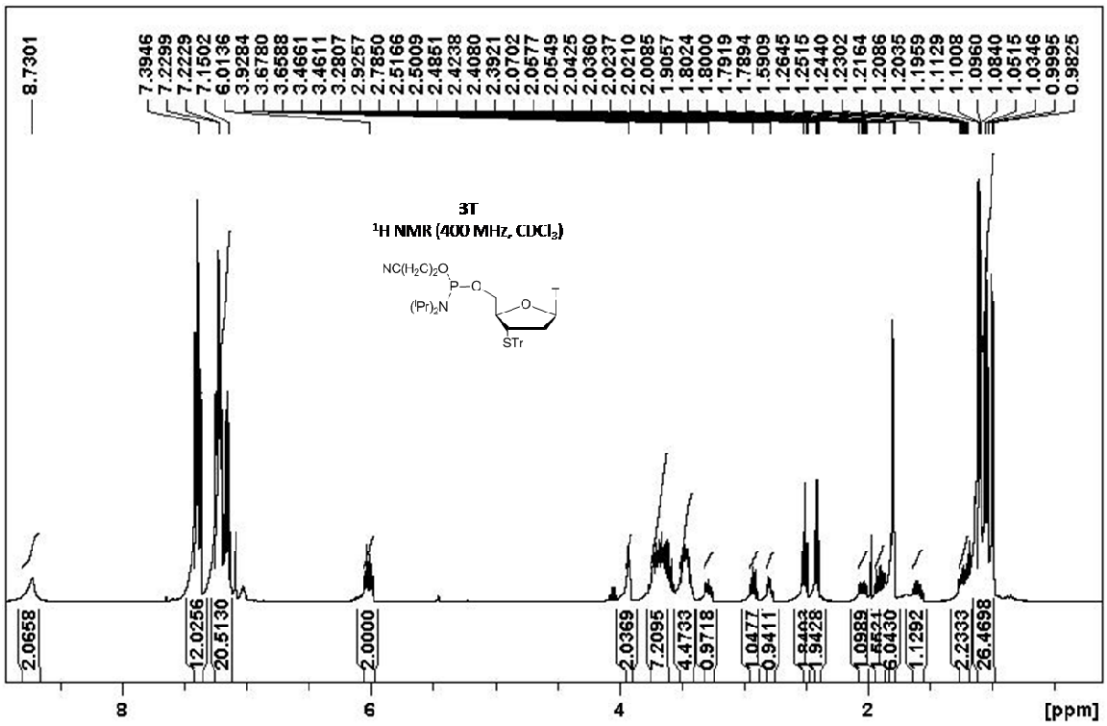
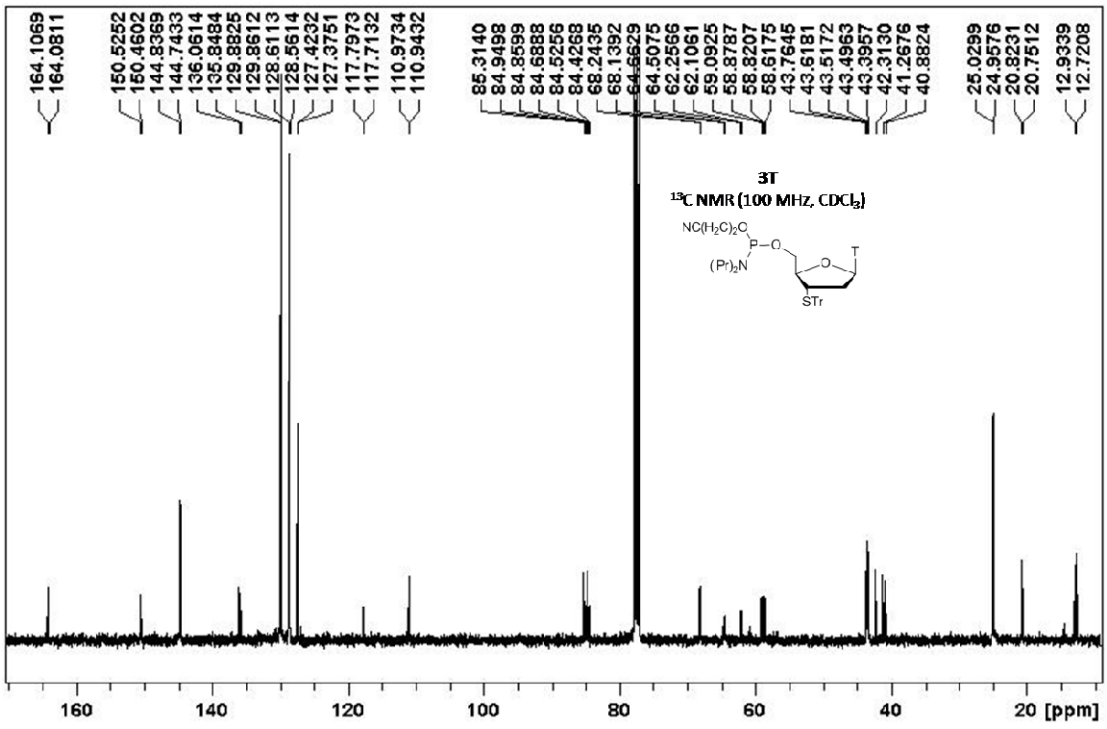
150
100
50
[ppm]

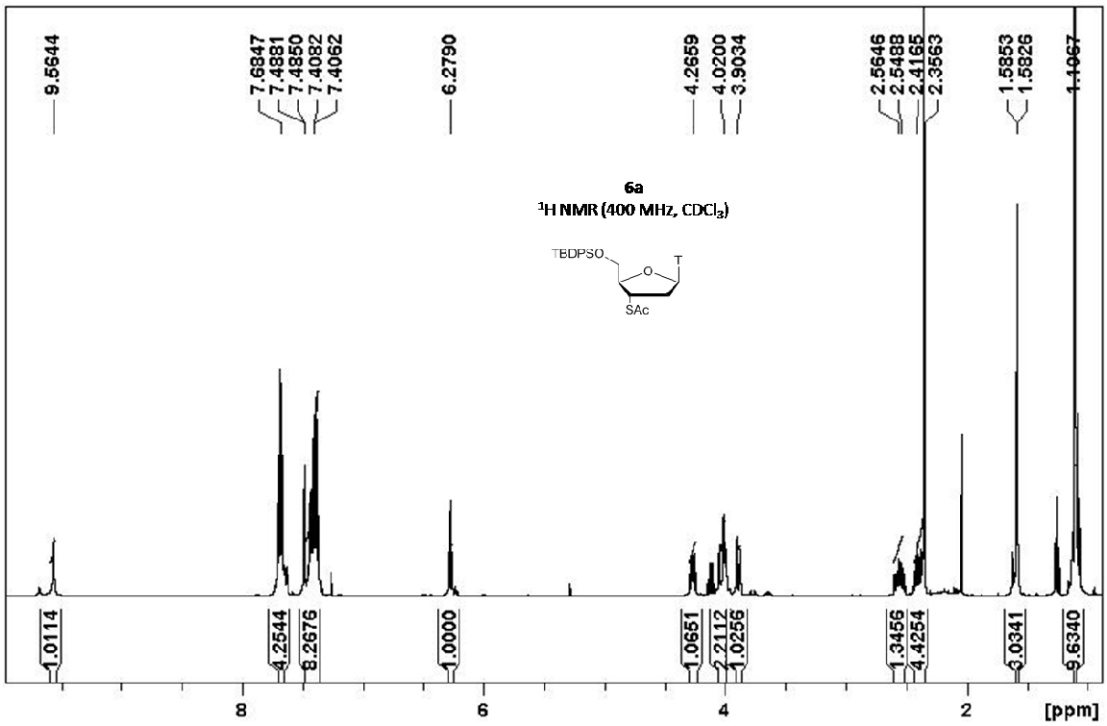

8.5078
8.2743
8.0188
7.8191
7.5433
7.3778
7.2065
5.9988
5.9957
5.9203
5.9136
3.9557
3.7465
3.5030
3.3618
3.3550
2.7459
2.5749
2.3407
2.0559
1.5855
1.1410
1.1140
3C
1H NMR (400 MHz, CDCl3)
1.9464
0.9043
1.0412
4.1501
2.0883
17.5108
20.4604
2.0000
2.0214
7.6508
3.9141
1.0109
2.1868
3.9815
0.9770
1.0919
3.2979
35.4528
8
6
4
2
[ppm]

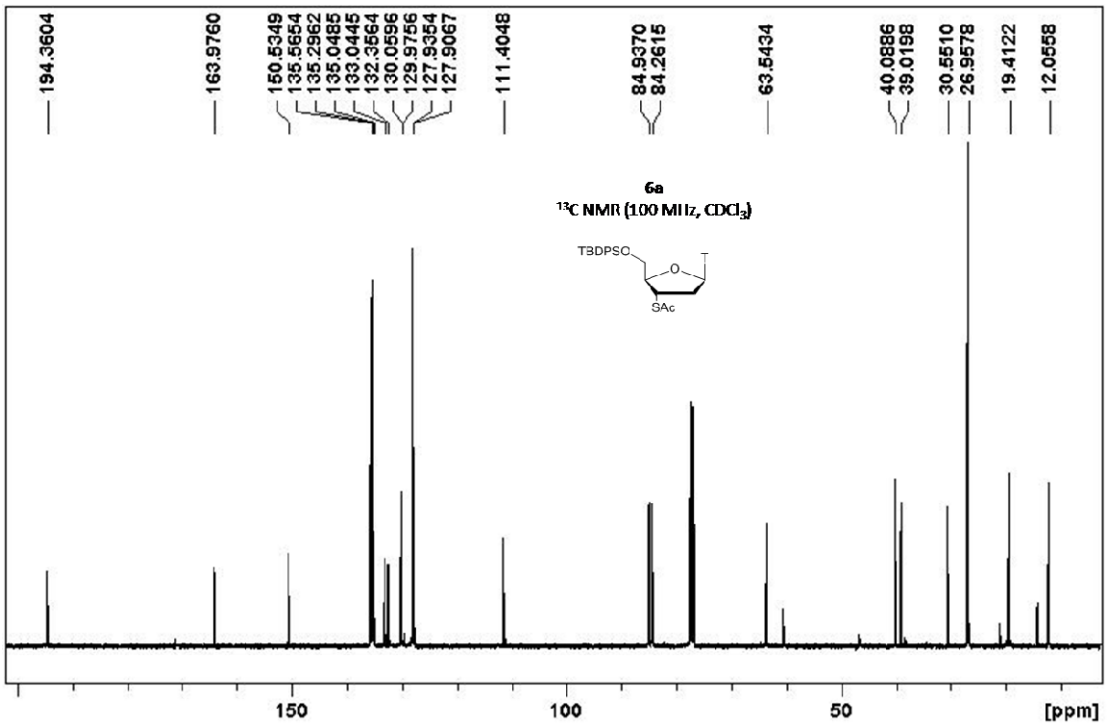

144.7410
144.6953
133.5979
129.8560
129.7890
129.4875
128.6105
128.5290
127.4521
127.4018
87.6077
68.1393
58.9561
43.7120
42.6100
40.9570
25.1436
25.0874
25.0160
20.7625
3C
13C NMR (100 MHz, CDCl3)
140
120
100
80
60
40
[ppm]

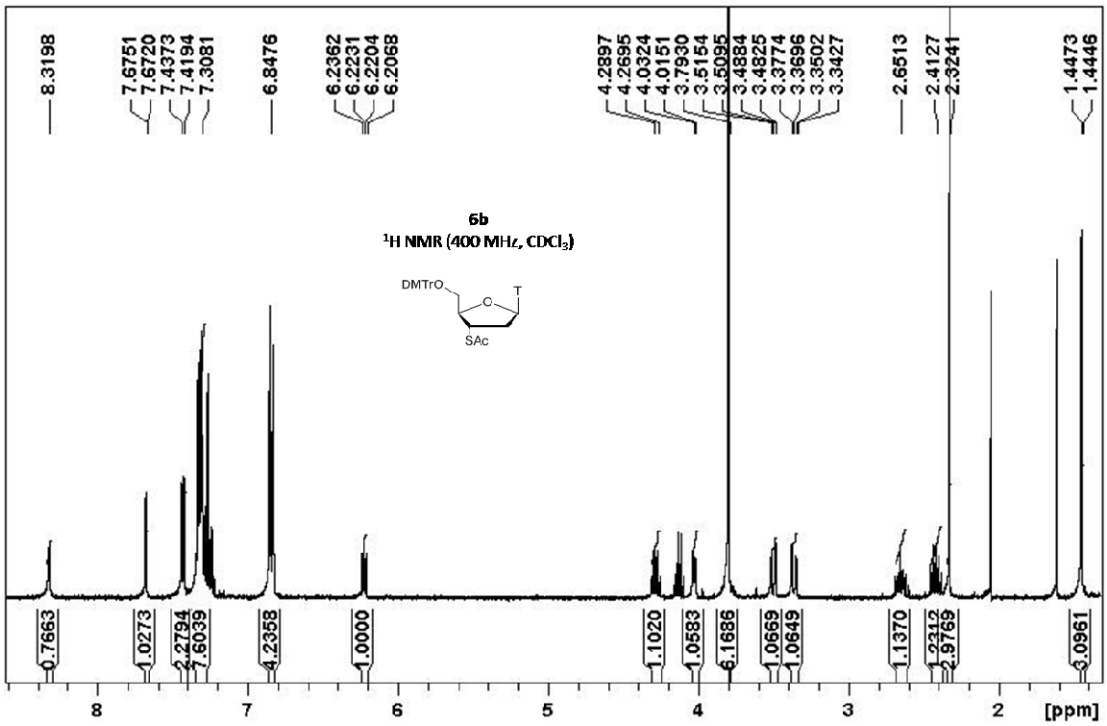

3T
31P NMR (162 MHz, CDCl3)
149.0193
148.6402
200
150
100
50
[ppm]

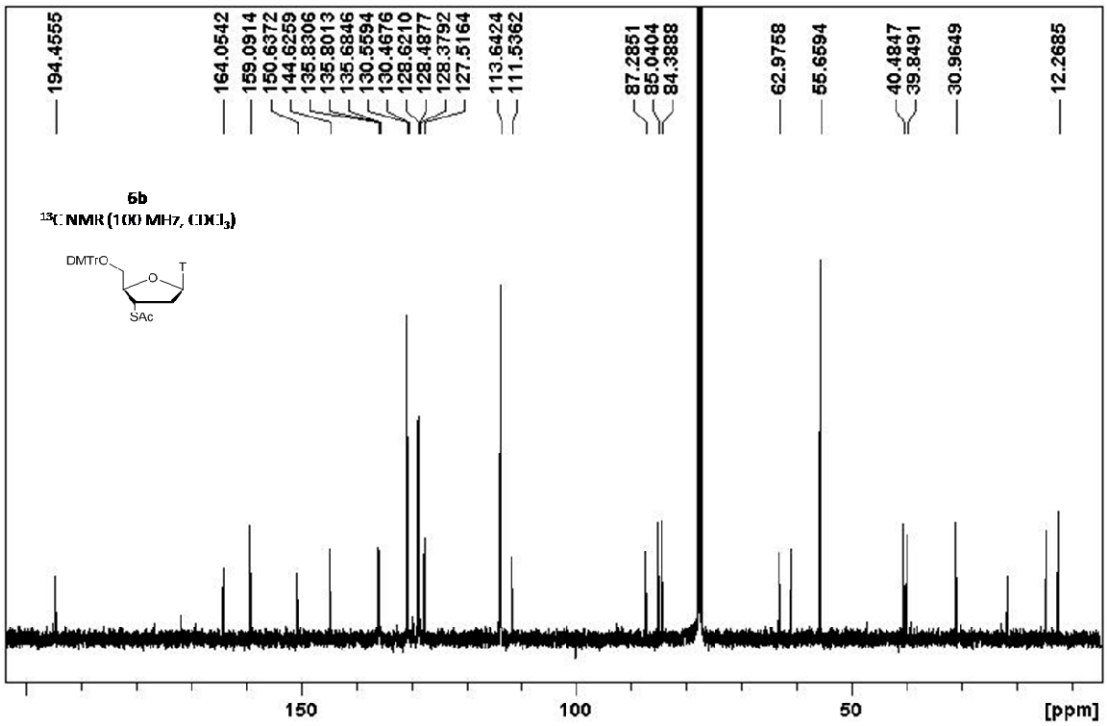

3T
1H NMR (400 MHz, CDCl3)
8.7301
7.3946
7.2299
7.2229
7.1502
6.0136
2.0658
12.0256
20.5130
2.0000
2.0369
7.2095
4.4733
0.9718
1.0477
0.9411
1.8443
1.9428
1.0989
6.0430
1.1292
2.2333
26.4698
8
6
4
2
[ppm]

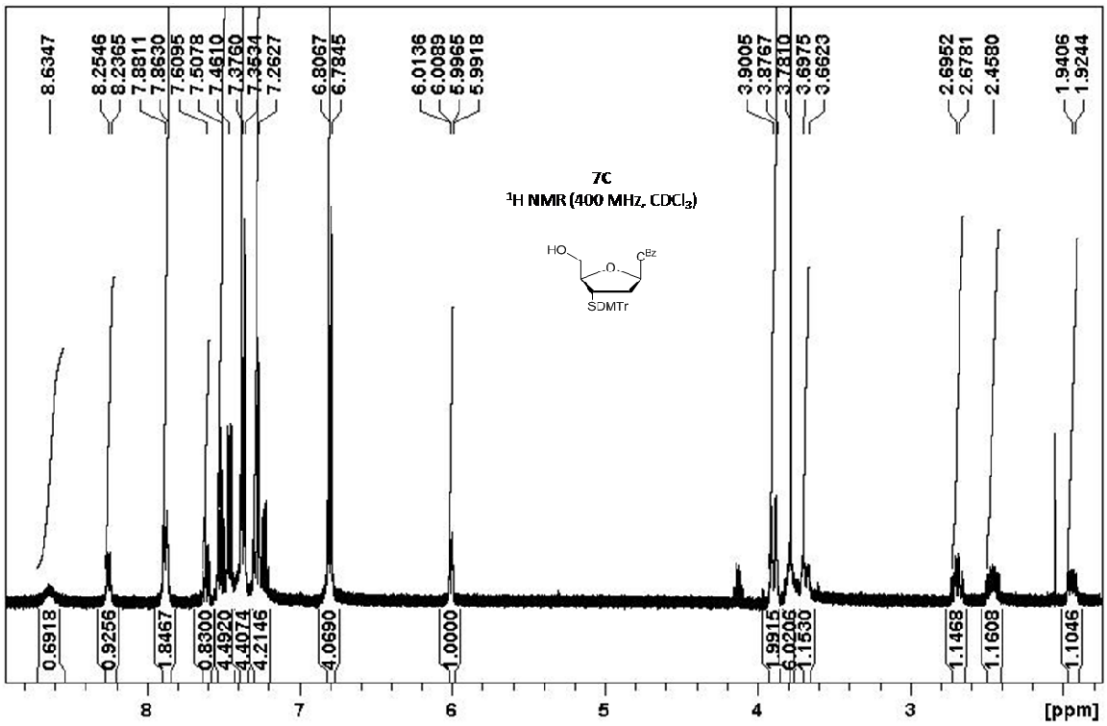

164.1069
164.0811
150.5252
150.4602
144.8369
144.7433
136.0614
135.8484
129.8825
129.8612
128.6113
128.5614
127.4232
127.3751
117.7973
117.7132
110.9734
110.9432
85.3140
84.9498
84.8599
84.6888
84.5256
84.4268
68.2435
68.1392
64.5629
64.5075
62.2566
62.1061
59.0925
58.8787
58.8207
58.6175
43.7645
43.6181
43.5172
43.4963
43.3957
42.3130
41.2676
40.8824
25.0299
24.9576
20.8231
20.7512
12.9339
12.7208
3T
13C NMR (100 MHz, CDCl3)
NC(H2C)2C
P
O
(Pr)2N
O
T
STr
160
140
120
100
80
60
40
20 [ppm]

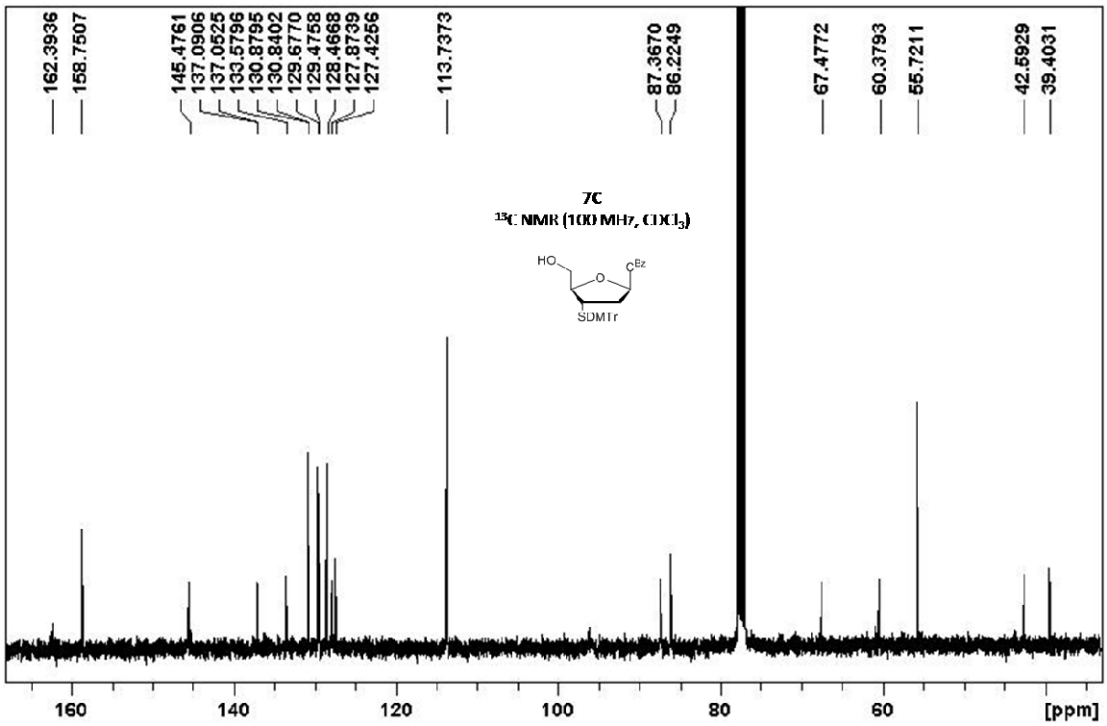

9.5644
7.6847
7.4881
7.4850
7.4082
7.4062
6.2790
4.2659
4.0200
3.9034
2.5646
2.5488
2.4165
2.3563
1.5853
1.5826
1.1067
6a
^{1}H NMR (400 MHz, CDCl$_3$)
TBDPSO
T
SAc
1.0114
4.2544
8.2676
1.0000
1.0651
2.2112
1.0256
1.3456
4.4254
3.0341
9.6340
8
6
4
2
[ppm]

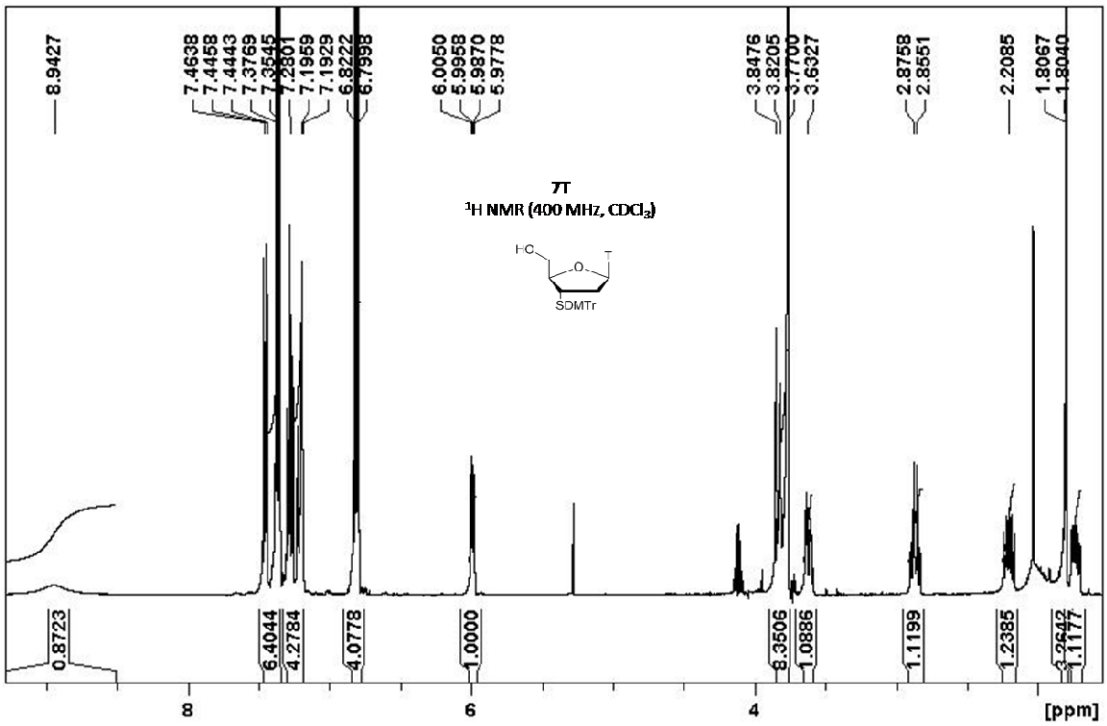

194.3604
163.9760
150.5349
135.5654
135.2962
135.0485
133.0445
132.3564
130.0596
129.9756
127.9354
127.9067
111.4048
84.9370
84.2615
63.5434
40.0886
39.0198
30.5510
26.9578
19.4122
12.0558
6a
^{13}C NMR (100 MHz, CDCl$_3$)
TBDPSO
T
SAc
150
100
50
[ppm]

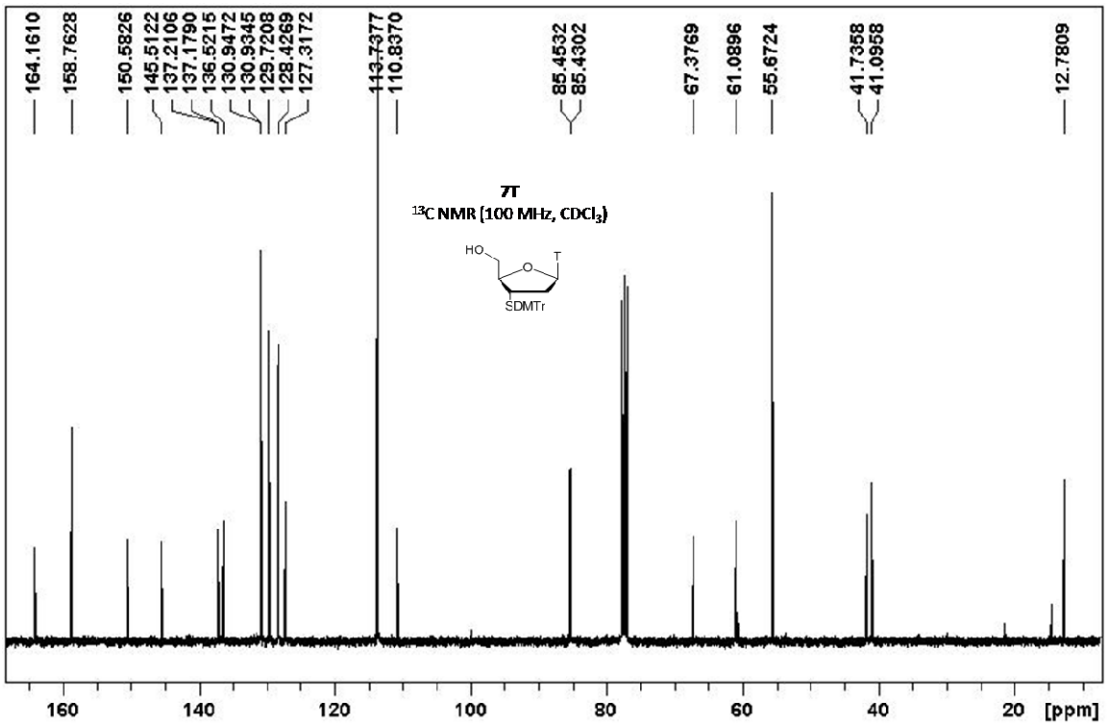

8.3198
7.6751
7.6720
7.4373
7.4194
7.3081
6.8476
6.2362
6.2231
6.2204
6.2068
4.2897
4.2695
4.0324
4.0151
3.7930
3.5154
3.5095
3.4884
3.4825
3.3774
3.3696
3.3502
3.3427
2.6513
2.4127
2.3241
1.4473
1.4446
6b
^{1}H NMR (400 MHz, CDCl$_3$)
DMTrO
T
SAc
0.7663
1.0273
2.2794
7.6039
4.2358
1.0000
1.1020
1.0583
6.1686
1.0669
1.0649
1.1370
1.2312
2.9769
3.0961
8
7
6
5
4
3
2
[ppm]

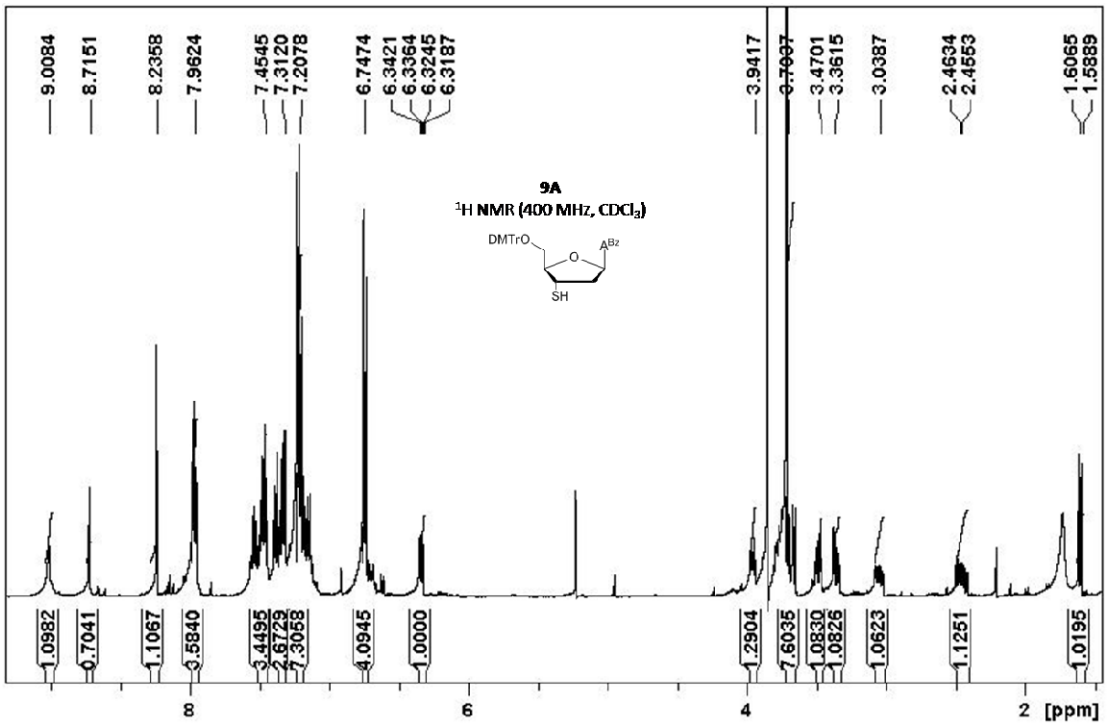

194.4555
164.0542
159.0914
150.6372
144.6259
135.8306
135.8013
135.6846
130.5594
130.4676
128.6210
128.4877
128.3792
127.5164
113.6424
111.5362
87.2851
85.0404
84.3888
62.9758
55.6594
40.4847
39.8491
30.9649
12.2685
6b
^{13}C NMR (100 MHz, CDCl$_3$)
DMTrO
T
SAc
150
100
50
[ppm]

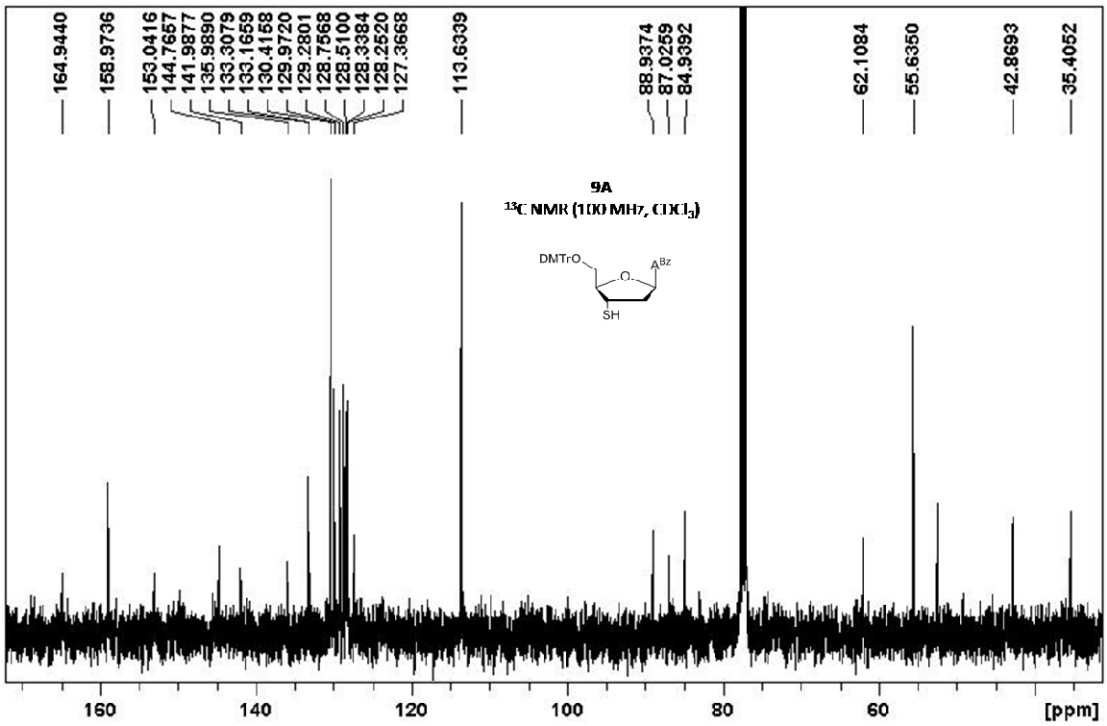

8.6347
8.2546
8.2365
7.8811
7.8630
7.6095
7.5078
7.4610
7.3760
7.3534
7.2627
6.8067
6.7845
6.0136
6.0089
5.9965
5.9918
3.9005
3.8767
3.7810
3.6975
3.6623
2.6952
2.6781
2.4580
1.9406
1.9244
0.6918
0.9256
1.8467
0.8300
4.4920
4.4074
4.2146
4.0690
1.0000
1.9915
6.0206
1.1530
1.1468
1.1608
1.1046
8
7
6
5
4
3
[ppm]
7C
^{1}H NMR (400 MHz, CDCl$_3$)
HO
OBz
O
SDMTr

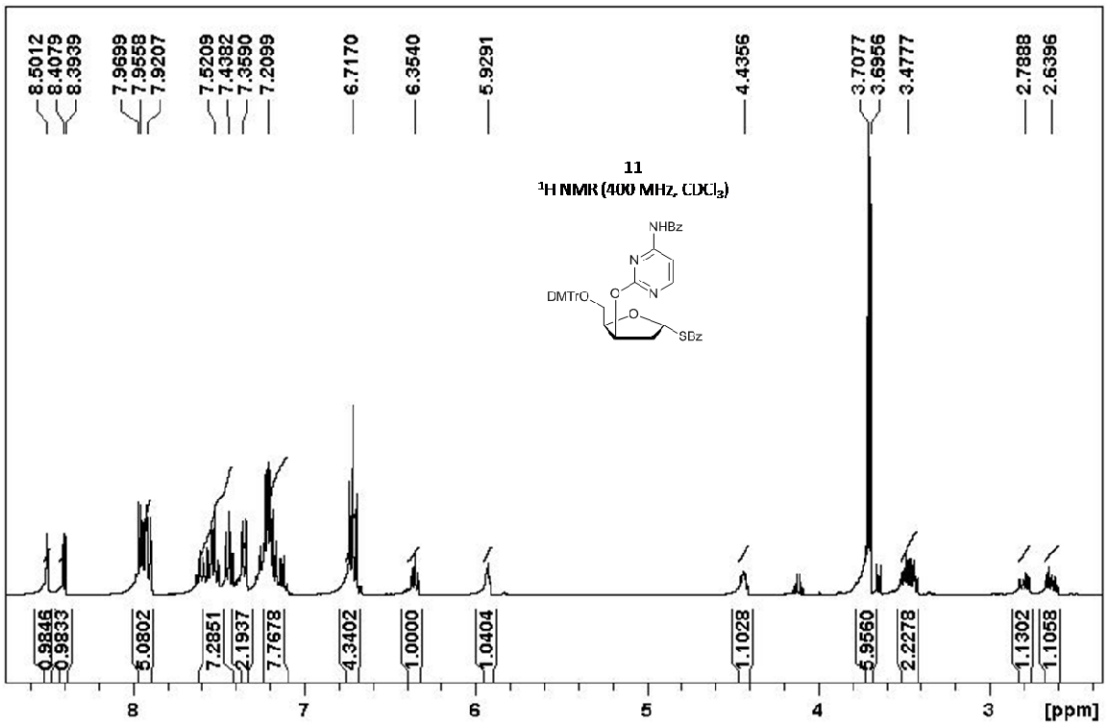

162.3936
158.7507
145.4761
137.0906
137.0525
133.5796
130.8795
130.8402
129.6770
129.4758
128.4668
127.8739
127.4256
113.7373
87.3670
86.2249
67.4772
60.3793
55.7211
42.5929
39.4031
160
140
120
100
80
60
[ppm]
7C
^{13}C NMR (100 MHz, CDCl$_3$)
HO
OBz
O
SDMTr

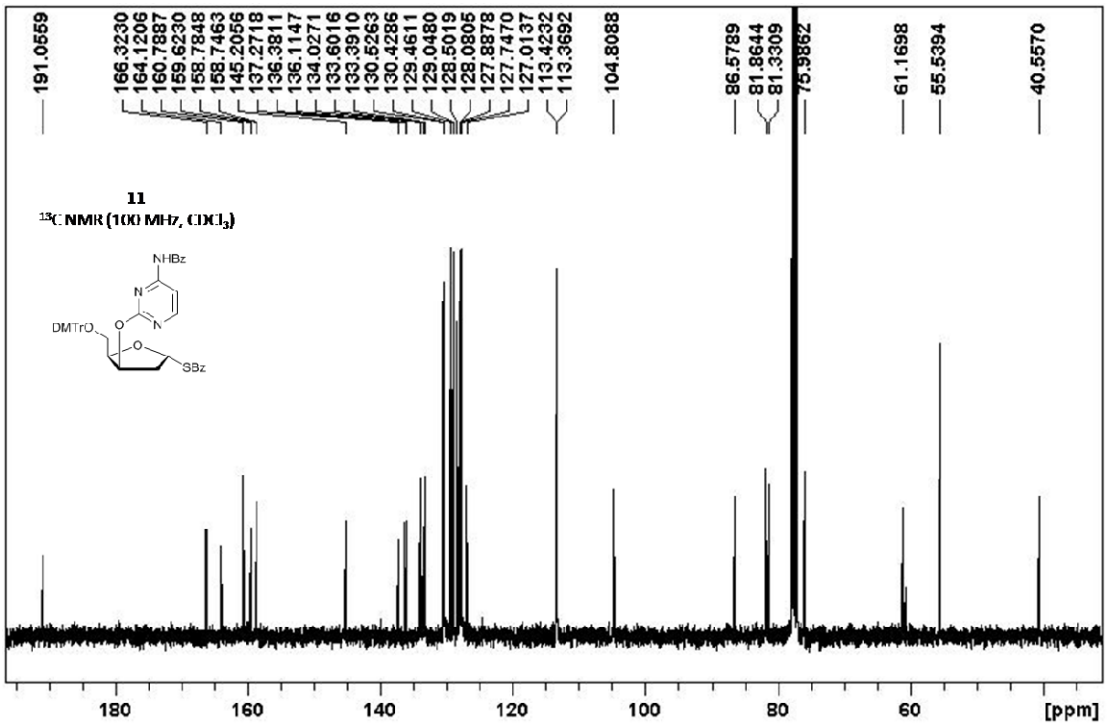

8.9427
7.4638
7.4458
7.4443
7.3769
7.3545
7.2801
7.1959
7.1929
6.8222
6.7998
6.0050
5.9958
5.9870
5.9778
3.8476
3.8205
3.7700
3.6327
2.8758
2.8551
2.2085
1.8067
1.8040
7T
1H NMR (400 MHz, CDCl3)
HO
O
T
SDMTr
0.8723
6.4044
4.2784
4.0778
1.0000
8.3506
1.0886
1.1199
1.2385
3.2642
1.1177
8
6
4
[ppm]

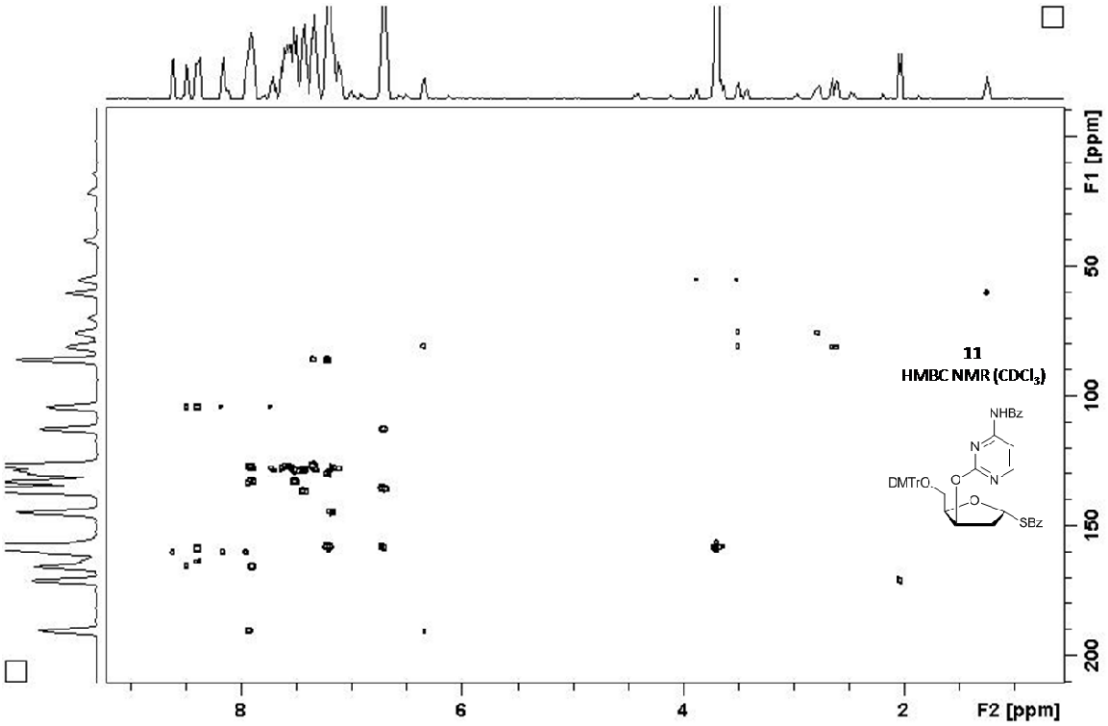

164.1610
158.7628
150.5826
145.5122
137.2106
137.1790
136.5215
130.9472
130.9345
129.7208
128.4269
127.3172
113.7377
110.8370
85.4532
85.4302
67.3769
61.0896
55.6724
41.7358
41.0958
12.7809
7T
13C NMR (100 MHz, CDCl3)
HO
O
T
SDMTr
160
140
120
100
80
60
40
20
[ppm]

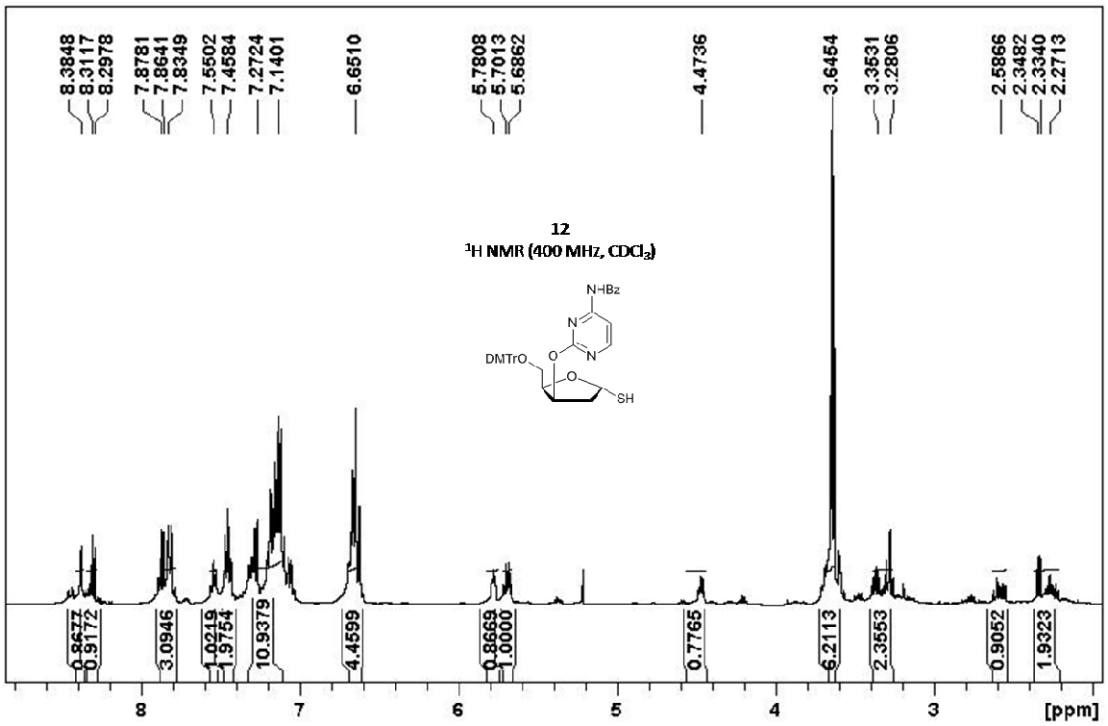

9A
^{1}H NMR (400 MHz, CDCl$_3$)
DMTrO
ABz
O
SH
9.0084
8.7151
8.2358
7.9624
7.4545
7.3120
7.2078
6.7474
6.3421
6.3364
6.3245
6.3187
3.9417
3.7037
3.4701
3.3615
3.0387
2.4634
2.4553
1.6065
1.5889
1.0982
0.7041
1.1067
0.5840
3.4495
2.8722
7.3058
4.0945
1.0000
1.2904
7.6035
1.0830
1.0826
1.0623
1.1251
1.0195
8
6
4
2
[ppm]

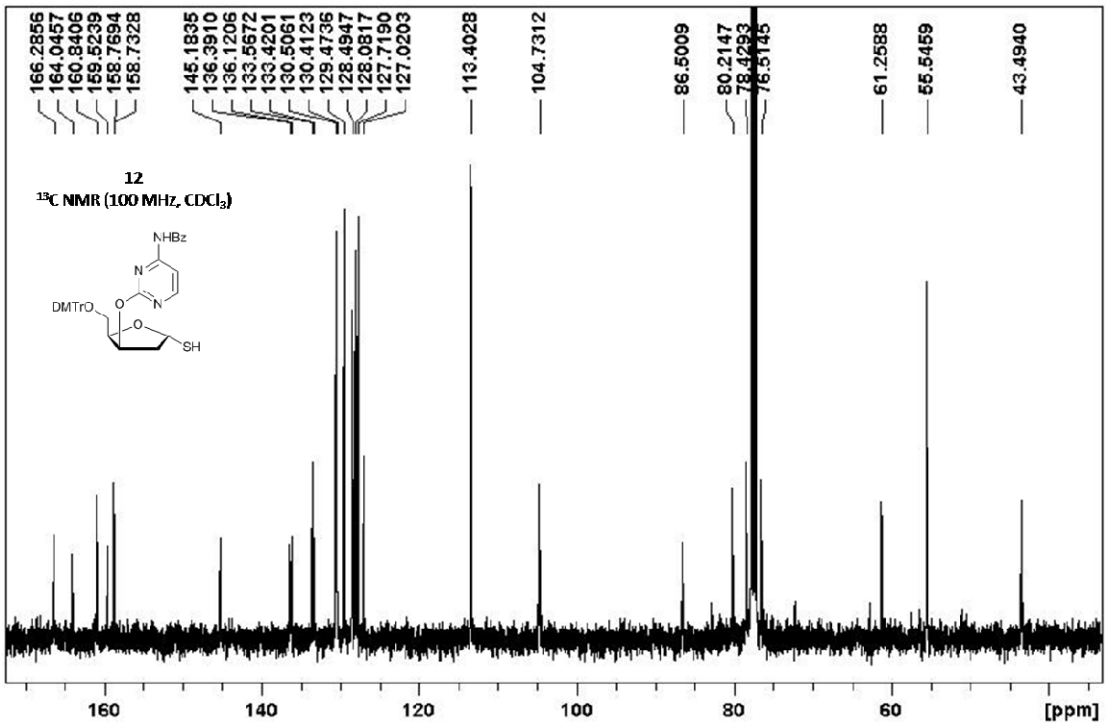

9A
^{13}C NMR (100 MHz, CDCl$_3$)
DMTrO
ABz
O
SH
164.9440
158.9736
153.0416
144.7657
141.9877
135.9890
133.3079
133.1659
130.4158
129.9720
129.2801
128.7568
128.5100
128.3384
128.2520
127.3668
113.6339
88.9374
87.0259
84.9392
62.1084
55.6350
42.8693
35.4052
160
140
120
100
80
60
[ppm]

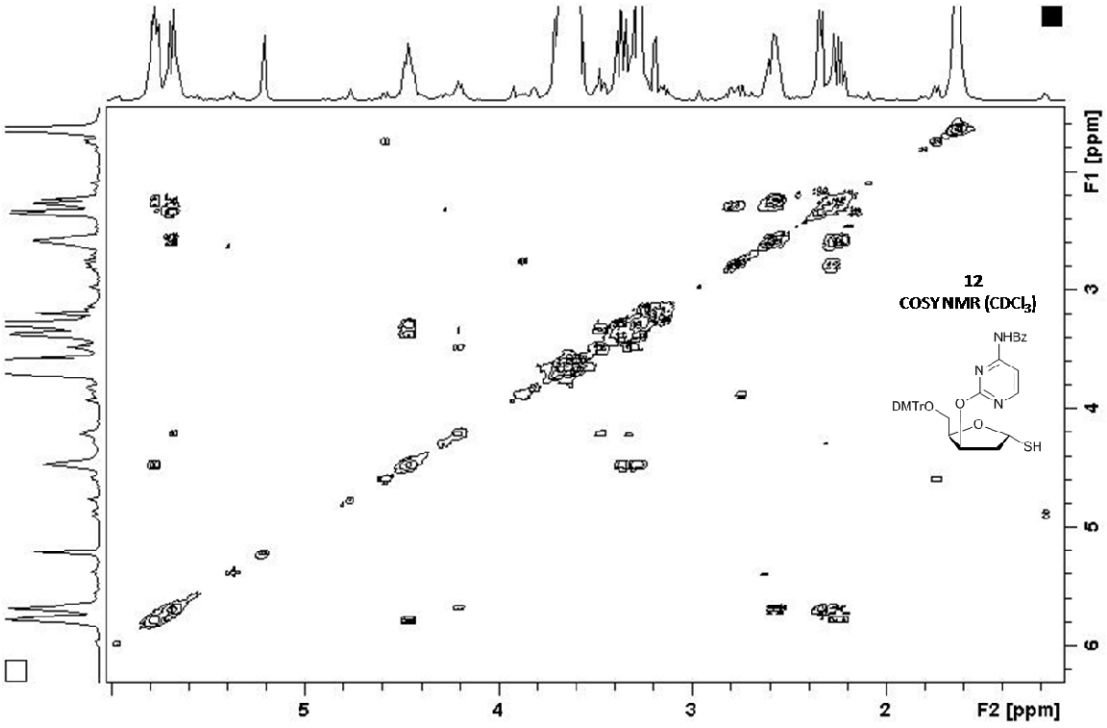

8.5012
8.4079
8.3939
7.9699
7.9558
7.9207
7.5209
7.4382
7.3590
7.2099
6.7170
6.3540
5.9291
4.4356
3.7077
3.6956
3.4777
2.7888
2.6396
11
^{1}H NMR (400 MHz, CDCl$_3$)
NHBz
DMTrO
SBz
0.9846
0.9833
5.0802
7.2851
2.1937
7.7678
4.3402
1.0000
1.0404
1.1028
5.9560
2.2278
1.1302
1.1058
8
7
6
5
4
3
[ppm]

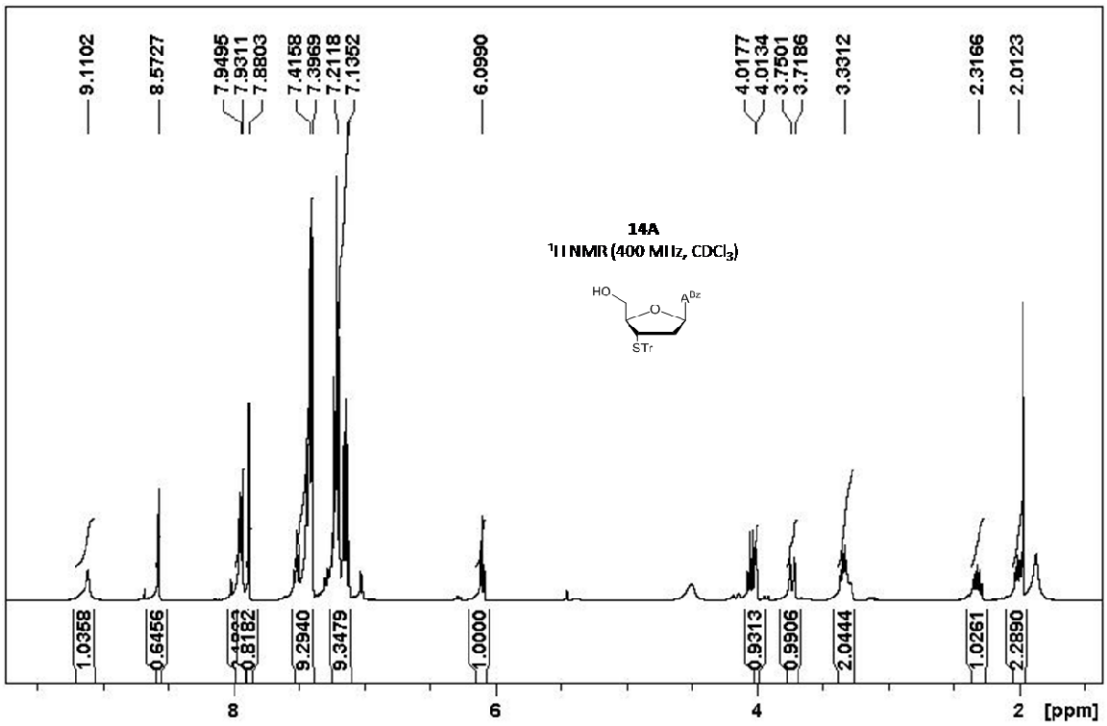

191.0559
166.3230
164.1206
160.7887
159.6230
158.7848
158.7463
145.2056
137.2718
136.3811
136.1147
134.0271
133.6016
133.3910
130.5263
130.4286
129.4611
129.0480
128.5019
128.0805
127.8878
127.7470
127.0137
113.4232
113.3692
104.8088
86.5789
81.8644
81.3309
75.9862
61.1698
55.5394
40.5570
11
^{13}C NMR (100 MHz, CDCl$_3$)
NHBz
DMTrO
SBz
180
160
140
120
100
80
60
[ppm]

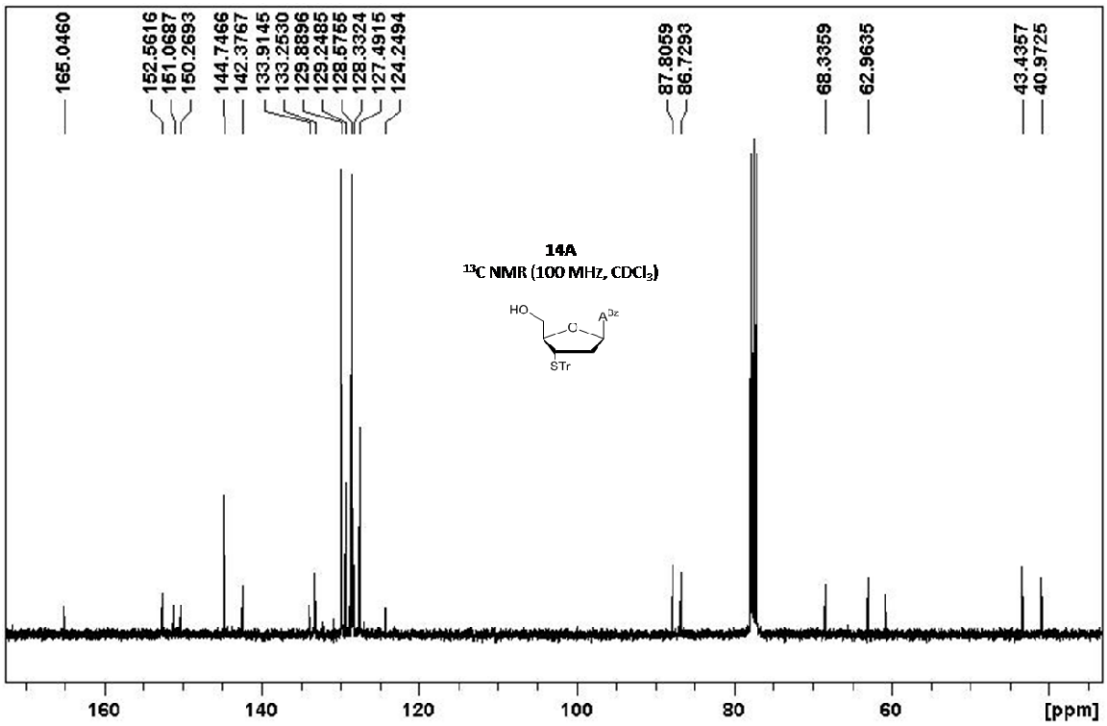

11
HMBC NMR (CDCl3)
NHBz
DMTrO
SBz
F1 [ppm]
F2 [ppm]

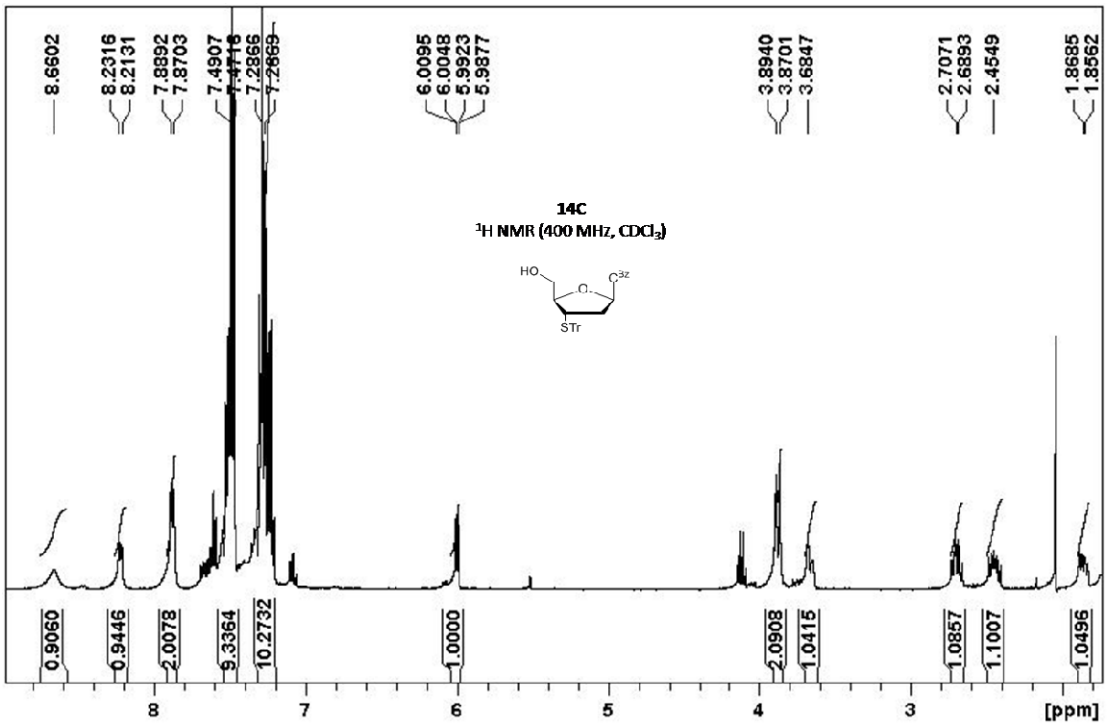

12
1H NMR (400 MHz, CDCl3)
NHBz
DMTrO
SH
8.3848
8.3117
8.2978
7.8781
7.8641
7.8349
7.5502
7.4584
7.2724
7.1401
6.6510
5.7808
5.7013
5.6862
4.4736
3.6454
3.3531
3.2806
2.5866
2.3482
2.3340
2.2713
0.8677
0.9172
3.0946
1.0219
1.9754
10.9379
4.4599
0.8669
1.0000
0.7765
6.2113
2.3553
0.9052
1.9323
[ppm]

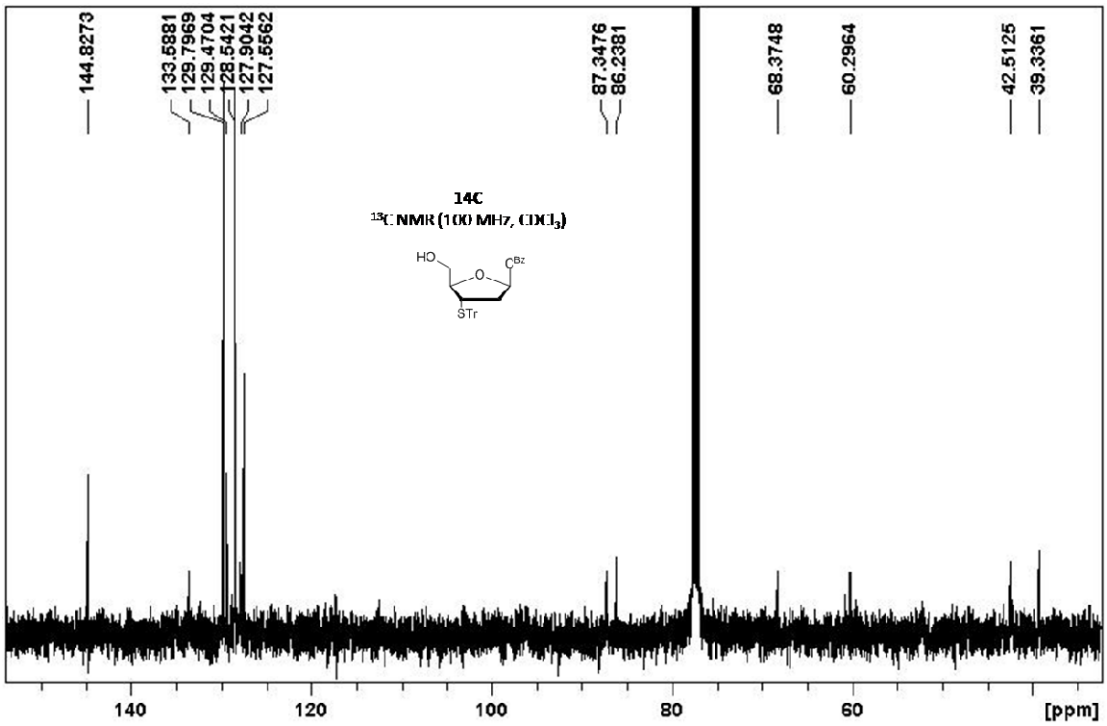

166.2856
164.0457
160.8406
159.5239
158.7694
158.7328
145.1835
136.3910
136.1206
133.5672
133.4201
130.5061
130.4123
129.4736
128.4947
128.0817
127.7190
127.0203
113.4028
104.7312
86.5009
80.2147
78.4293
76.5145
61.2588
55.5459
43.4940
12
13C NMR (100 MHz, CDCl3)
NHBz
N
O
N
DMTrO
O
SH
160
140
120
100
80
60
[ppm]


12
COSY NMR (CDCl3)
NHBz
N
O
N
DMTrO
O
SH
F1 [ppm]
F2 [ppm]
5
4
3
2
6


9.1102
8.5727
7.9495
7.9311
7.8803
7.4158
7.3969
7.2118
7.1952
6.0990
4.0177
4.0134
3.7501
3.7186
3.3312
2.3166
2.0123
14A
^{1}H NMR (400 MHz, CDCl$_3$)
1.0358
0.6456
0.8182
9.2940
9.3479
1.0000
0.9313
0.9906
2.0444
1.0261
2.2890
8
6
4
2
[ppm]


165.0460
152.5616
151.0687
150.2693
144.7466
142.3767
133.9145
133.2530
129.8896
129.2485
128.5755
128.3324
127.4915
124.2494
87.8059
86.7293
68.3359
62.9635
43.4357
40.9725
14A
^{13}C NMR (100 MHz, CDCl$_3$)
160
140
120
100
80
60
[ppm]


8.6602
8.2316
8.2131
7.8892
7.8703
7.4907
7.4716
7.2866
7.2669
6.0095
6.0048
5.9923
5.9877
3.8940
3.8701
3.6847
2.7071
2.6893
2.4549
1.8685
1.8562
14C
1H NMR (400 MHz, CDCl3)
HO
O
C3z
STr
0.9060
0.9446
2.0078
9.3364
10.2732
1.0000
2.0908
1.0415
1.0857
1.1007
1.0496
8
7
6
5
4
3
[ppm]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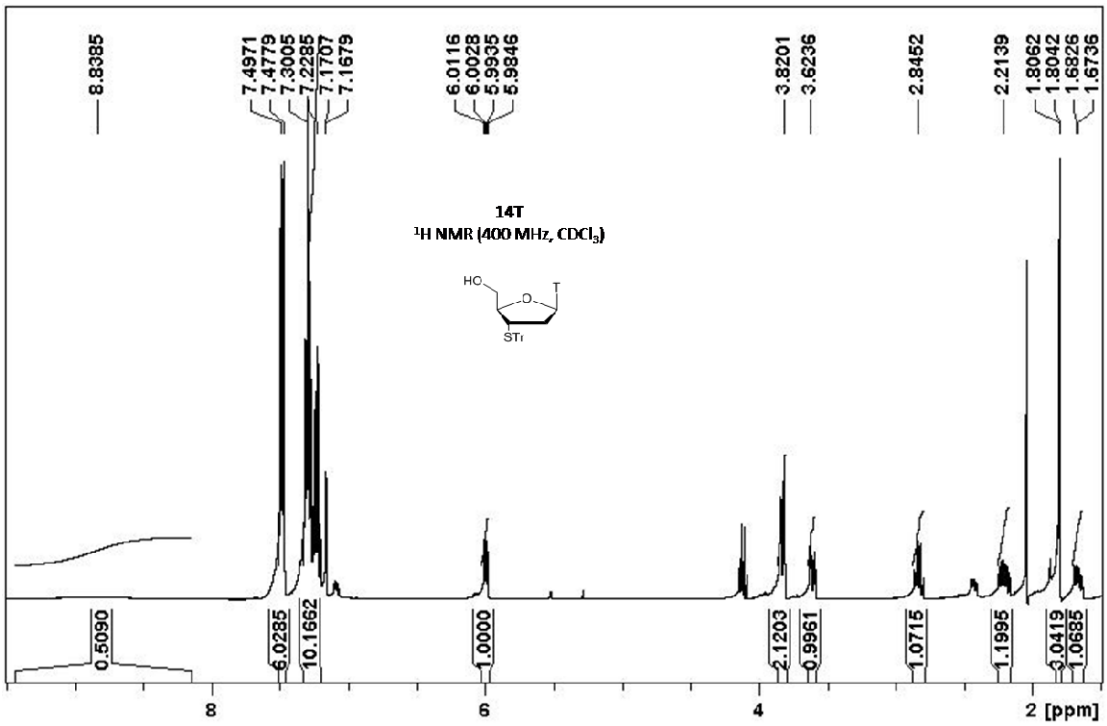
60.2964
42.5125
39.3361
14C
13C NMR (100 MHz, CDCl3)
HO
O
CBz
STr
140
120
100
80
60
[ppm]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5.9846
3.8201
3.6236
2.8452
2.2139
1.8062
1.8042
1.6826
1.6736
14T
^{1}H NMR (400 MHz, CDCl$_3$)
HO
O
T
STr
0.5090
6.0285
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1.0000
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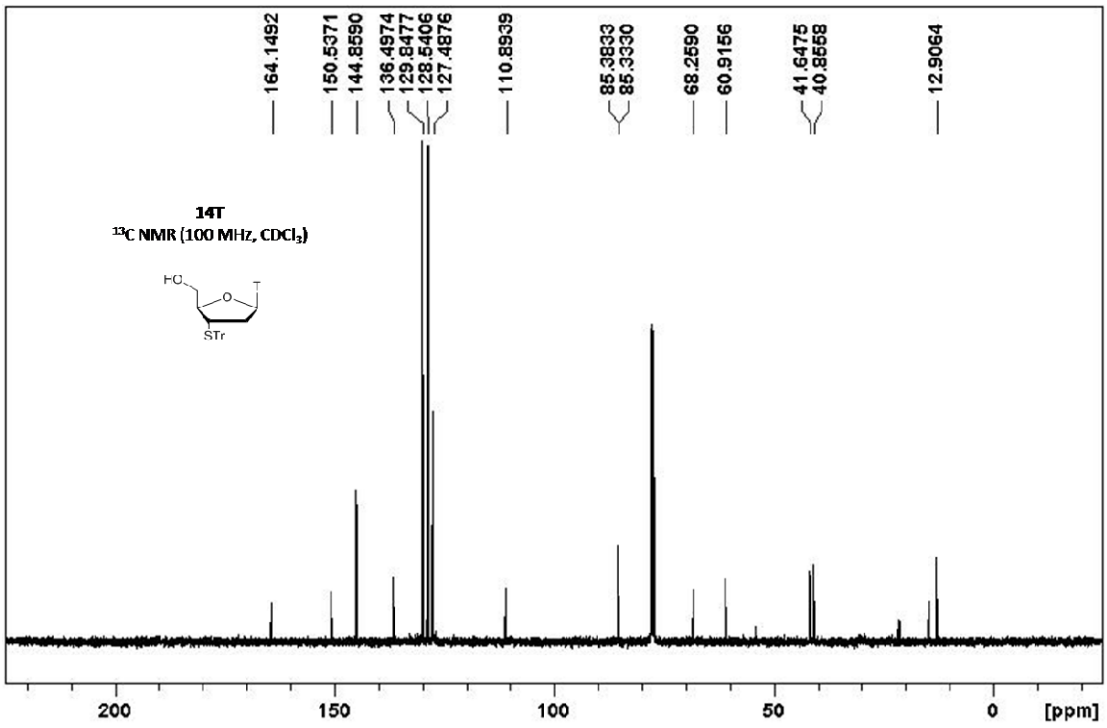
8
6
4
2 [ppm]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85.3330
68.2590
60.9156
41.6475
40.8558
12.9064
14T
^{13}C NMR (100 MHz, CDCl$_3$)
HO
O
T
STr
200
150
100
50
0
[ppm]

3. Expedite 8909 protocols for synthesis of ON1

For function definitions and general reagents, see 3.5. Modified thymidine phosphoramidite (**2T**) was placed in phosphoramidite port 5. DTT was placed in the auxillary port. Dichloromethane was placed in amidite port 6.

3.1. Cycle for the incorporation of the reverse thymidine phosphoramidite into DNA.

Function	Mode	Amount (pulse)	Time (sec)	Description
$Deblocking				
144 /*Index Fract. Coll.	*/NA	1	0	"Event out ON"
0 /*Default	*/WAIT	0	1.5	"Wait"
141 /*Trityl Mon. On/Off	*/NA	1	1	"START data collection
16 /*Dblk	*/PULSE	10	0	"Dblk to column"
16 /*Dblk	*/PULSE	50	135	"Deblock"
38 /*Diverted Wsh A	*/PULSE	40	0	"Flush system with Wsh A"
141 /*Trityl Mon. On/Off	*/NA	0	1	"STOP data collection"
38 /*Diverted Wsh A	*/PULSE	40	0	"Flush system with Wsh A"
144 /*Index Fract. Coll.	*/NA	2	0	"Event out OFF"
$Coupling				
1 /*Wsh	*/PULSE	5	0	"Flush system with Wsh"
2 /*Act	*/PULSE	5	0	"Flush system with Act"
21 /*T + Act	*/PULSE	6	0	"T + Act to column"
21 /*T + Act	*/PULSE	1	15	"Couple monomer"
2 /*Act	*/PULSE	4	60	"Couple monomer"
1 /*Wsh	*/PULSE	7	105	"Couple monomer"
1 /*Wsh	*/PULSE	8	0	"Flush system with Wsh"
$Capping				
12 /*Wsh A	*/PULSE	20	0	"Flush system with Wsh A"
13 /*Caps	*/PULSE	8	0	"Caps to column"
12 /*Wsh A	*/PULSE	6	15	"Cap"
12 /*Wsh A	*/PULSE	14	0	"Flush system with Wsh A"
$Oxidizing				
15 /*Ox	*/PULSE	15	0	"Ox to column"
12 /*Wsh A	*/PULSE	6	15	"Oxidise"
12 /*Wsh A	*/PULSE	15	0	"Flush system with Wsh A"
$Capping				
13 /*Caps	*/PULSE	7	0	"Caps to column"
12 /*Wsh A	*/PULSE	6	5	"Cap"
12 /*Wsh A	*/PULSE	30	0	"End of cycle wash"

3.2. Incorporation of modified thymidine phosphoramidite (2T) using the X-cycle

This cycle was used for the introduction of a modified thymidine phosphoramidite (**2T**) after a natural derivative, where the 3′-terminal residue bound to the solid support is a masked hydroxyl.

Function	Mode	Amount (pulse)	Time (sec)	Description
$Deblocking				
144 /*Index Fract. Coll.	*/NA	1	0	"Event out ON"
0 /*Default	*/WAIT	0	1.5	"Wait"
141 /*Trityl Mon. On/Off	*/NA	1	1	"START data collection
16 /*Dblk	*/PULSE	10	0	"Dblk to column"

16 /*Dblk	*/PULSE	50	135	"Deblock"
38 /*Diverted Wsh A	*/PULSE	40	0	"Flush system with Wsh A"
141 /*Trityl Mon. On/Off	*/NA	0	1	"STOP data collection"
38 /*Diverted Wsh A	*/PULSE	40	0	"Flush system with Wsh A"
144 /*Index Fract. Coll.	*/NA	2	0	"Event out OFF"
12 /*Wsh A	*/PULSE	5	0	"Flush system with Wsh A"
17 /*Aux	*/PULSE	6	0	"DTT to column"
17 /*Aux	*/PULSE	54	600	"DTT reduction"
$Coupling				
1 /*Wsh	*/PULSE	5	0	"Flush system with Wsh"
2 /*Act	*/PULSE	5	0	"Flush system with Act"
22 /*5 + Act	*/PULSE	6	0	"5 + Act to column"
22 /*5 + Act	*/PULSE	1	15	"Couple monomer"
2 /*Act	*/PULSE	4	60	"Couple monomer"
1 /*Wsh	*/PULSE	7	105	"Couple monomer"
1 /*Wsh	*/PULSE	8	0	"Flush system with Wsh"
$Capping				
12 /*Wsh A	*/PULSE	20	0	"Flush system with Wsh A"
13 /*Caps	*/PULSE	8	0	"Caps to column"
12 /*Wsh A	*/PULSE	6	15	"Cap"
12 /*Wsh A	*/PULSE	14	0	"Flush system with Wsh A"
$Oxidizing				
15 /*Ox	*/PULSE	15	0	"Ox to column"
12 /*Wsh A	*/PULSE	6	15	"Oxidise"
12 /*Wsh A	*/PULSE	15	0	"Flush system with Wsh A"
$Capping				
13 /*Caps	*/PULSE	7	0	"Caps to column"
12 /*Wsh A	*/PULSE	6	5	"Cap"
12 /*Wsh A	*/PULSE	30	0	"End of cycle wash"

3.3. Incorporation of modified thymidine phosphoramidite (22) using the Y-cycle

This cycle was used for the introduction of a modified thymidine phosphoramidite (22) after another modified residue, where the 3′-terminal residue bound to the solid support is a masked thiol.

Function	**Mode**	**Amount (pulse)**	**Time (sec)**	**Description**
$Deblocking				
144 /*Index Fract. Coll.	*/NA	1	0	"Event out ON"
0 /*Default	*/WAIT	0	1.5	"Wait"
141 /*Trityl Mon. On/Off	*/NA	1	1	"START data collection
16 /*Dblk	*/PULSE	10	0	"Dblk to column"
16 /*Dblk	*/PULSE	50	135	"Deblock"
38 /*Diverted Wsh A	*/PULSE	40	0	"Flush system with Wsh A"
141 /*Trityl Mon. On/Off	*/NA	0	1	"STOP data collection"
38 /*Diverted Wsh A	*/PULSE	40	0	"Flush system with Wsh A"
144 /*Index Fract. Coll.	*/NA	2	0	"Event out OFF"
12 /*Wsh A	*/PULSE	5	0	"Flush system with Wsh A"
17 /*Aux	*/PULSE	6	0	"DTT to column"
17 /*Aux	*/PULSE	54	600	"DTT reduction"
$Coupling				
1 /*Wsh	*/PULSE	60	0	"Flush system with Wsh"
4 /*6	*/PULSE	60	0	"Flush system with Dichloromethane"
1 /*Wsh	*/PULSE	10	0	"Flush system with Wsh"
2 /*Act	*/PULSE	5	0	"Flush system with Act"

22 /*5 + Act	*/PULSE	6	0	"5 + Act to column"
22 /*5 + Act	*/PULSE	1	25	"Couple monomer"
2 /*Act	*/PULSE	4	100	"Couple monomer"
1 /*Wsh	*/PULSE	7	175	"Couple monomer"
1 /*Wsh	*/PULSE	8	0	"Flush system with Wsh"
$Capping				
12 /*Wsh A	*/PULSE	20	0	"Flush system with Wsh A"
13 /*Caps	*/PULSE	8	0	"Caps to column"
12 /*Wsh A	*/PULSE	6	15	"Cap"
12 /*Wsh A	*/PULSE	14	0	"Flush system with Wsh A"
$Oxidizing				
15 /*Ox	*/PULSE	15	0	"Ox to column"
12 /*Wsh A	*/PULSE	6	15	"Oxidise"
12 /*Wsh A	*/PULSE	15	0	"Flush system with Wsh A"
$Capping				
13 /*Caps	*/PULSE	7	0	"Caps to column"
12 /*Wsh A	*/PULSE	6	5	"Cap"
12 /*Wsh A	*/PULSE	30	0	"End of cycle wash"

3.4. Incorporation of thymidine phosphoramidite using the Z-cycle

This cycle was used for the introduction of a natural thymidine phosphoramidite (**2T**) after a modified residue, where the 3′-terminal residue bound to the solid support is a masked thiol.

Function	**Mode**	**Amount (pulse)**	**Time (sec)**	**Description**
$Deblocking				
144 /*Index Fract. Coll.	*/NA	1	0	"Event out ON"
0 /*Default	*/WAIT	0	1.5	"Wait"
141 /*Trityl Mon. On/Off	*/NA	1	1	"START data collection
16 /*Dblk	*/PULSE	10	0	"Dblk to column"
16 /*Dblk	*/PULSE	50	135	"Deblock"
38 /*Diverted Wsh A	*/PULSE	40	0	"Flush system with Wsh A"
141 /*Trityl Mon. On/Off	*/NA	0	1	"STOP data collection"
38 /*Diverted Wsh A	*/PULSE	40	0	"Flush system with Wsh A"
144 /*Index Fract. Coll.	*/NA	2	0	"Event out OFF"
$Coupling				
1 /*Wsh	*/PULSE	60	0	"Flush system with Wsh"
4 /*6	*/PULSE	60	0	"Flush system with Dichloromethane"
1 /*Wsh	*/PULSE	10	0	"Flush system with Wsh"
2 /*Act	*/PULSE	5	0	"Flush system with Act"
21 /*T + Act	*/PULSE	6	0	"T + Act to column"
21 /*T + Act	*/PULSE	1	25	"Couple monomer"
2 /*Act	*/PULSE	4	100	"Couple monomer"
1 /*Wsh	*/PULSE	7	175	"Couple monomer"
1 /*Wsh	*/PULSE	8	0	"Flush system with Wsh"
$Capping				
12 /*Wsh A	*/PULSE	20	0	"Flush system with Wsh A"
13 /*Caps	*/PULSE	8	0	"Caps to column"
12 /*Wsh A	*/PULSE	6	15	"Cap"
12 /*Wsh A	*/PULSE	14	0	"Flush system with Wsh A"
$Oxidizing				
15 /*Ox	*/PULSE	15	0	"Ox to column"
12 /*Wsh A	*/PULSE	6	15	"Oxidise"
12 /*Wsh A	*/PULSE	15	0	"Flush system with Wsh A"

$Capping				
13 /*Caps	*/PULSE	7	0	"Caps to column"
12 /*Wsh A	*/PULSE	6	5	"Cap"
12 /*Wsh A	*/PULSE	30	0	"End of cycle wash"

3.5. Function definitions and general reagents used for DNA Reverse Synthesis Protocol and DNA Modified Reverse Synthesis Protocol.

Function Definitions	**Function**	**Reagent**
Dblk	Deblock	Trichloroacetic acid in dichloromethane
Wsh	Anhydrous wash	Anhydrous acetonitrile
Act	Activator	1*H*-Tetrazole
Wsh A	General wash	Acetonitrile
Caps	Capping reagents	Cap A: Acetic anhydride in pyridine/THF
		Cap B: *N*-Methylimidazole in pyridine/THF
Ox	Oxidiser	Iodine solution in THF/H_2O/pyridine.