Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry
This journal is © The Royal Society of Chemistry 2013

Vipin Kumar,[a] Kiran R. Gore,[b] P. I. Pradeepkumar,[b] and Venkitasamy Kesavan*[a]

[a] Department of Biotechnology, Indian Institute of Technology, Madras,

Chennai-600036, India

[b] Department of Chemistry, Indian Institute of Technology, Bombay

Mumbai-400076, India

vkesavan@iitm.ac.in*

Supporting Information

This journal is © The Royal Society of Chemistry 2013

S1: Temperature-dependent CD profile of single strands of (*S*)-BuNA and UV-melting studies

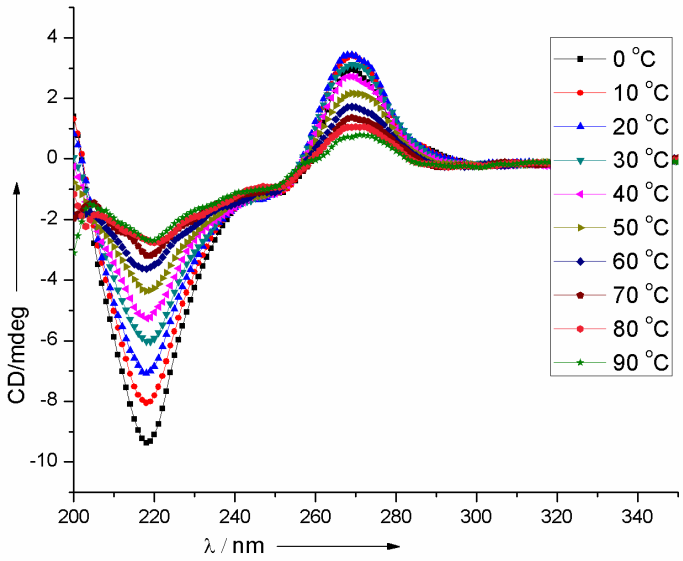


Figure S1a: Temperature-dependent CD spectrum of ON-1. Experimental condition: 20 µM oligo , 10 mM phosphate buffer, 150 mM sodium chloride, pH 7 in 1 mm quartz cuvette.

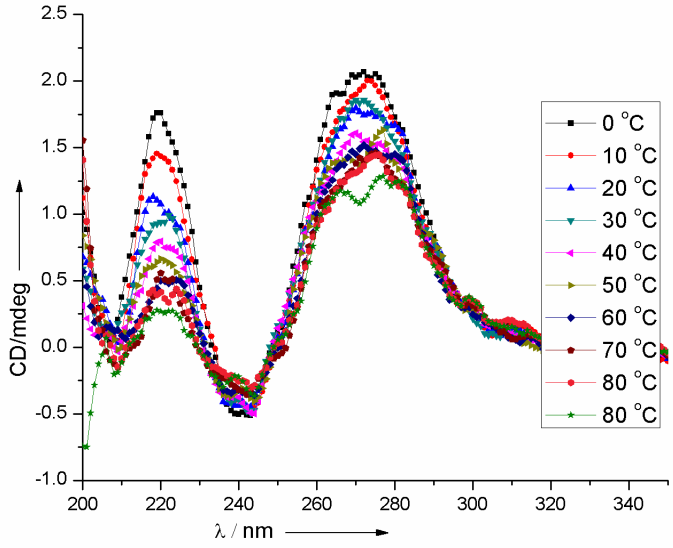


Figure S1b: Temperature-dependent CD spectrum of ON-2. Experimental condition: 20 µM, 10 mM phosphate buffer, 150 mM sodium chloride, pH 7 in 1 mm quartz cuvette.

This journal is © The Royal Society of Chemistry 2013

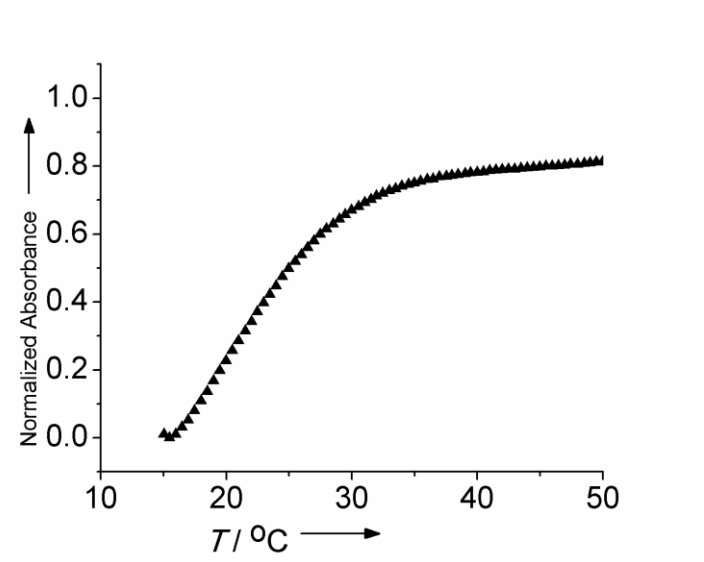


Figure S1c: UV-melting profile at 260 nm of ON-1/ON-2 duplex in 10 mM phosphate buffer containing 1 M sodium chloride at pH 7.0 with 4 µM each oligomer concentration.

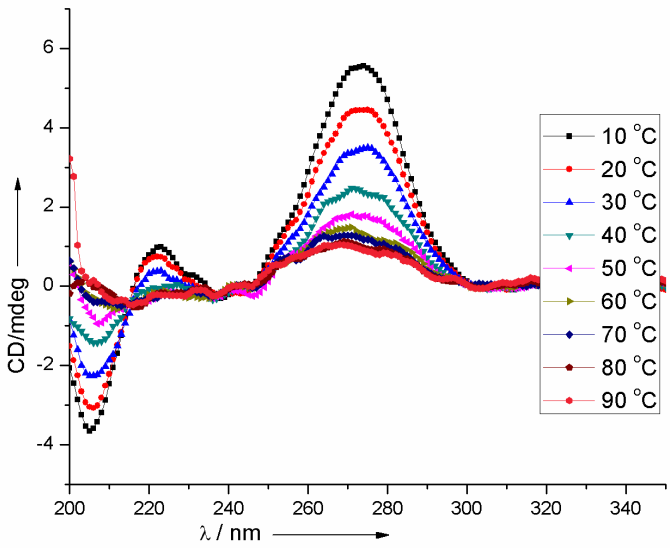


Figure S1d: Temperature-dependent CD spectrum of ON-10. Experimental condition: 20 µM, 10 mM phosphate buffer, 150 mM sodium chloride, pH 7 in 1 mm quartz cuvette.

This journal is © The Royal Society of Chemistry 2013

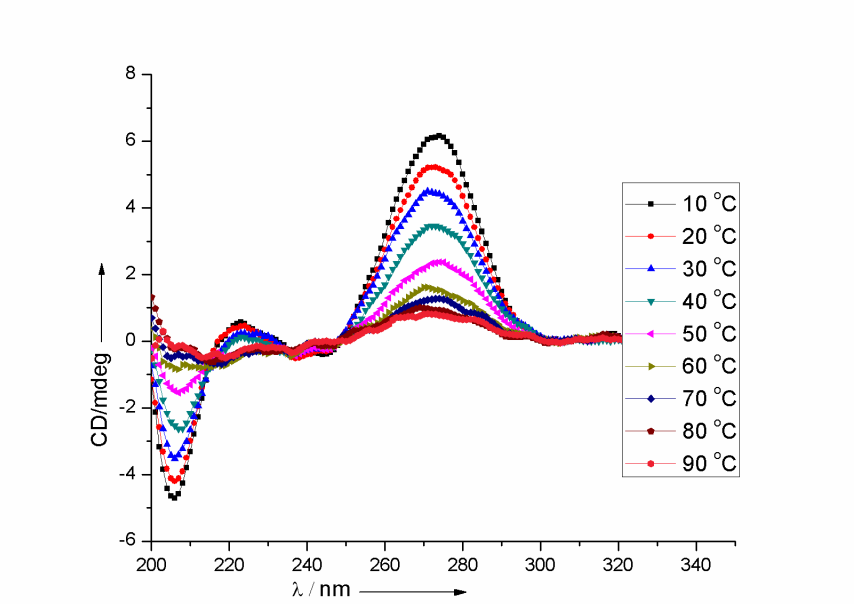


Figure S1e: Temperature-dependent CD spectrum of ON-8. Experimental condition: 20 µM, 10 mM phosphate buffer, 150 mM sodium chloride, pH 7 in 1 mm quartz cuvette.

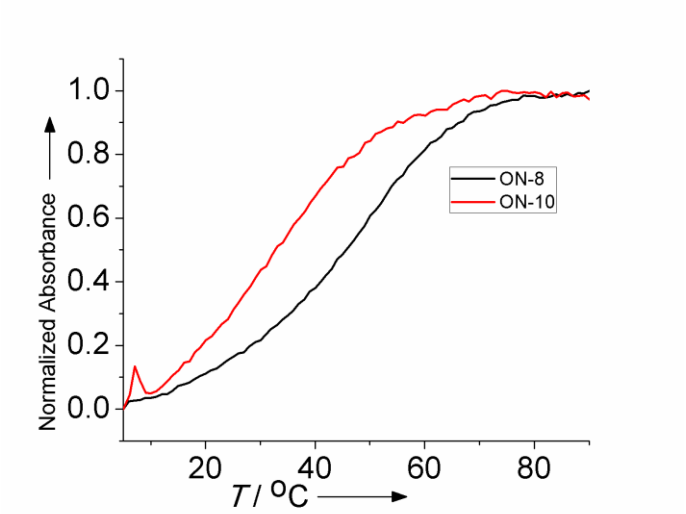


Figure S1f: UV-melting profile of single strands (ON-8 and ON-10). Experimental condition: 2 µM each oligo, 10 mM phosphate buffer at pH 7 in 150 mM sodium chloride.

This journal is © The Royal Society of Chemistry 2013

S2. Synthesis of oligonucleotides:

All the oligonucleotides were synthesized on automated DNA synthesizer using standard phosphoramidite chemistry. Adenine, thymine and cytosine attached controlled pore glass (CPG) solid supports were used to synthesize BuNA. All the synthesized oligomers were purified by preparative PAGE or preparative HPLC. Resulting oligomers characterized by MALDI-TOF-MS or LC-MS. Some of the unmodified oligonucleotides were purchased from Sigma Aldrich. Oligonucleotides containing G, g, C and c were synthesized and characterized by Alnylam Pharmaceuticals Cambridge, MA 02142.

S3. MALDI-TOF-MS

The MALDI matrix was 3-hydroxypicolinic acid (HPA), 1:1 acetonitrile/aq. ammonium citrate solution. On a spot of matrix 1 µL of oligonucleotide sample was placed on MALDI plate and dried at room temperature. MALDI-TOF-MS was analyzed on positive mode.

S3a. LC-MS (ES-API negative mode)

Column: Waters X-Bridge BEH, C8, 2.5 um particles, 2.1 * 50 mm
Flow rate 0.7 ml/min
Temperature 75 ^{o}C
Gradient: 0% B – 40% B in 9 min
Buffer A; 200 mM Hexafluoroisopropanol, 16 mM triethyl amine in water for MS
Buffer B; 100% Methanol for MS

Table S1. MALDI-TOF

Entry	Oligonucleotides	Mass calculated	Mass observed
ON-1	4’-aaaaaaaaaaaaaA-3’	4244.33	4243.692
ON-2	4’-ttttttttttttT-3’	4108.43	4112.345
ON-3	4’-atatatatatatataT-3’	4457.07	4455.859
ON-4	4’-aaaaaaaatttttT-3’	4457.07	4460.590
ON-5	4’-aaaatttatattattA-3’	4457.07	4541.629 (MW+2Na^{+})
ON-6	4’-taataatataaatttT-3’	4457.07	4459.934
ON-7	4’-gtgtaataacaacaT-3’	4191.913	4189.9
ON-8	4’-gtgttataacaacat-2’	4163.899	4162.7
ON-9	4’-atgttgttattacac-3’	4136.839	4134.9
ON-10	4’-atgttgttattacaC-2’	4164.852	4162.8
ON-11	5’-AAAAAAAAAAAAAAA-3’	4636.2	Nd
ON-12	5’-TTTTTTTTTTTTTTT-3’	4501.0	Nd

This journal is © The Royal Society of Chemistry 2013

ON-13	5’-GTGTAATAACAACAT-3	4584.037	4582.7
ON-14	5’-ATGTTGTTATTACAC-3’	4556.995	4555.7
ON-15	5’-AAAAAAAaAAAAAAA-3’	4608.20	4605.818
ON-16	5’-AAAaAAAAAAAaAAA-3’	4580.22	4578.368
ON-17	5’-TTTTTTTtTTTTTTT-3’	4472.30	4476.363
ON-18	5’-TTTtTTTTTTTtTTT-3’	4445.02	4452.519
ON-19	5’-ATGTTGTtATTACAC-3’	4528.982	4527.7
ON-20	5’-GTGTAATaACAACAT-3’	4556.03	4554.7
ON-21	5’-ATGTTGttaTTACAC-3’	4572.962	4471.7
ON-22	5’-GTGTAAtaaCAACAT-3’	4500.01	4498.01

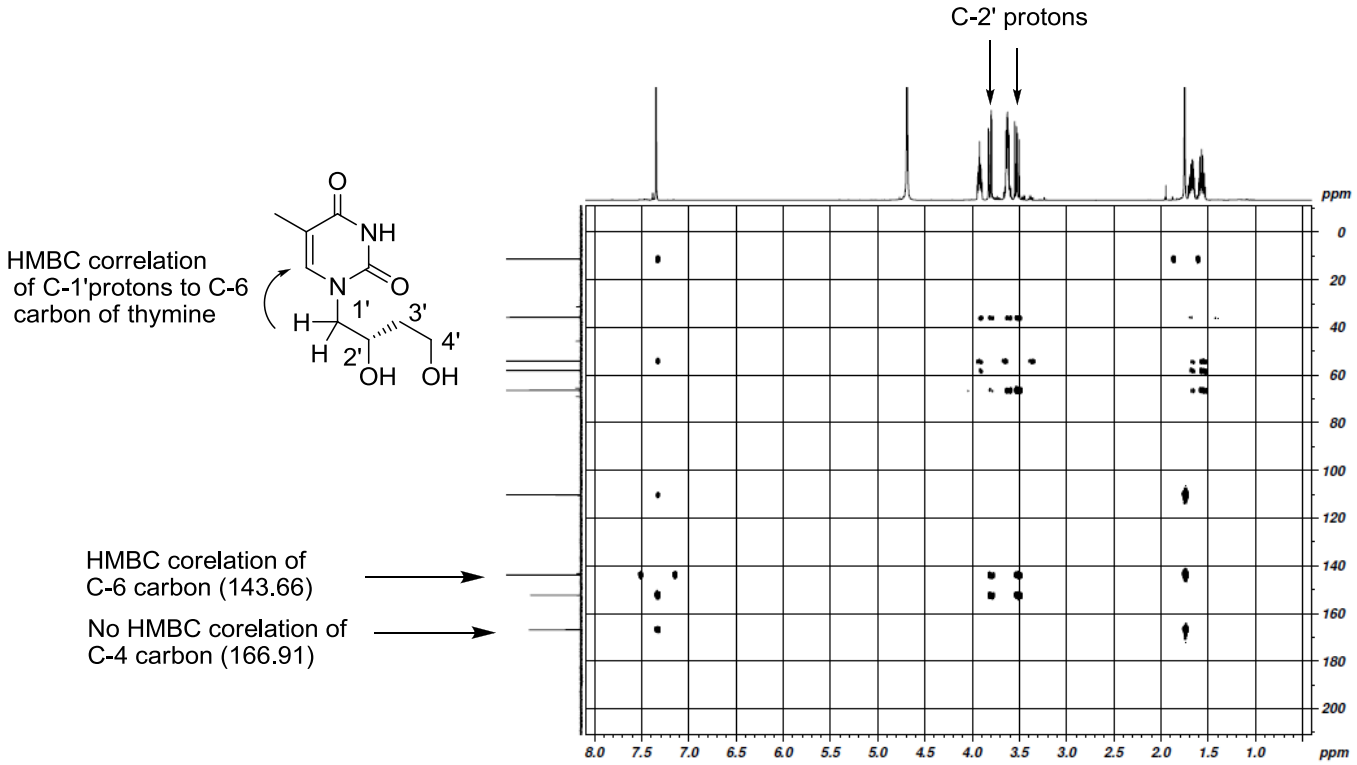
Nd = Oligonucleotides were purchased from Sigma-Aldrich

This journal is © The Royal Society of Chemistry 2013

S4. Elucidation of regioselectivity of N-alkylation of nucleobases

Thymine nucleoside

Regioselectivity of C-N bond can be elucidated by the HMBC. Three bonds coupling as shown in figure S4a clearly indicate that diol is attached at N-1 position of thymine ring. On the other hand no coupling was observed of C-1' protons with C-3 of the ring, which rule out the N-3 alkylation of thymine.

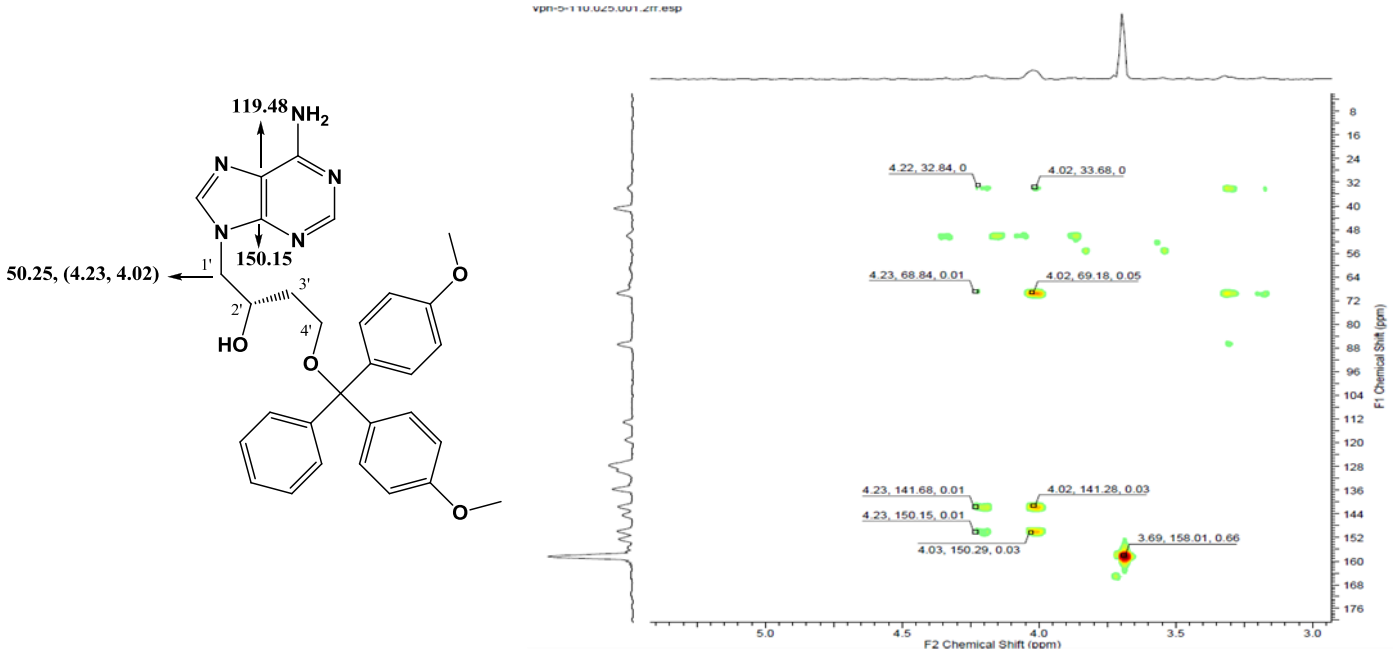


S4a. HMBC

This journal is © The Royal Society of Chemistry 2013

DMT-adenine nucleoside

C-9 alkylation of adenine was also confirmed by HMBC. Figure S4b clearly indicate that C-1' protons show coupling with C-4 carbon of the ring. No coupling was observed with C-5 of the adenine. This result clearly indicates that regioselectivity of the N-9 alkylation of adenine, which rule out the N-7 alkylation of adenine.

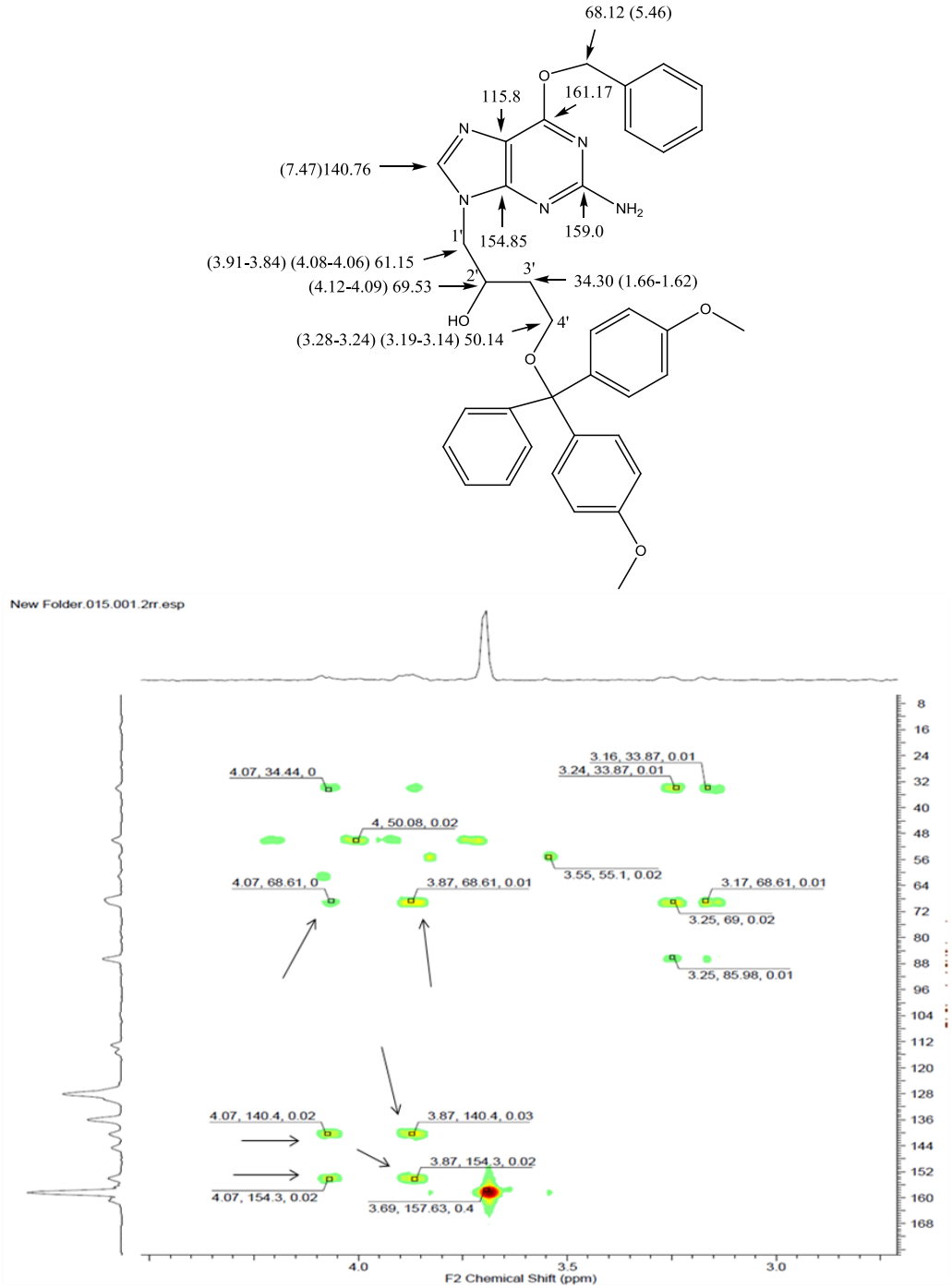


S4b. HMBC

This journal is © The Royal Society of Chemistry 2013

DMT-Guanine nucleoside

C-9 alkylation of guanine was confirmed by HMBC. Figure 4c clearly indicate that C-1' protons show coupling with C-4 (δ = 154.85) carbon of the ring. No coupling was observed with C-5 (δ = 115.8) of the heterocyclic ring. This result clearly indicates that regioselectivity of the N-9 alkylation of guanine, which rule out the N-7 alkylation.

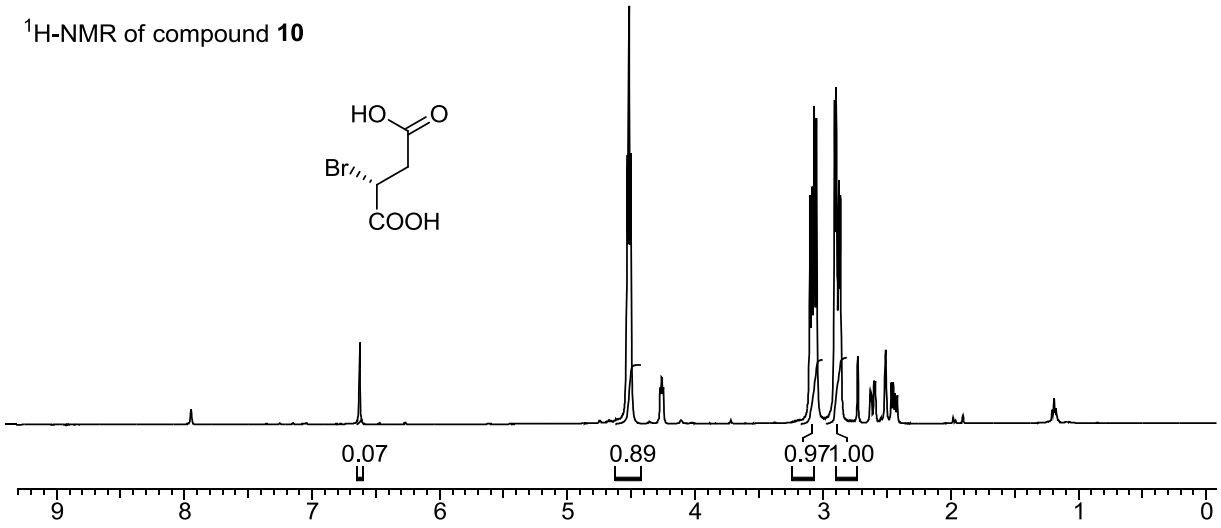


S4c. HMBC

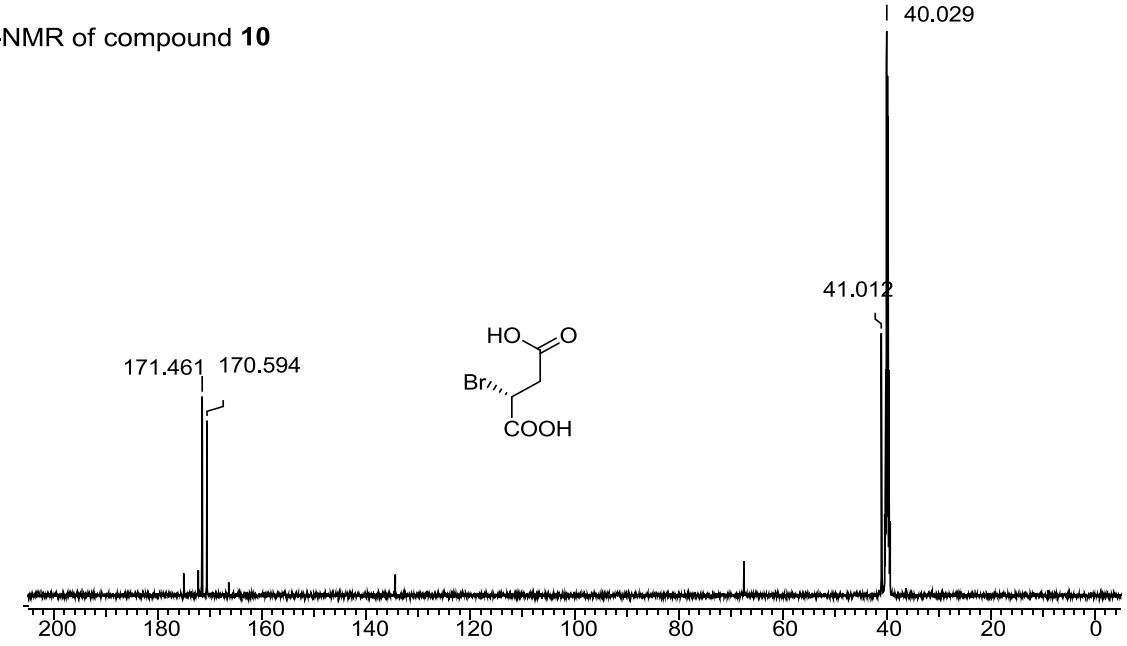
This journal is © The Royal Society of Chemistry 2013

S5. **NMR spectra**

^{1}H-NMR of compound **10**

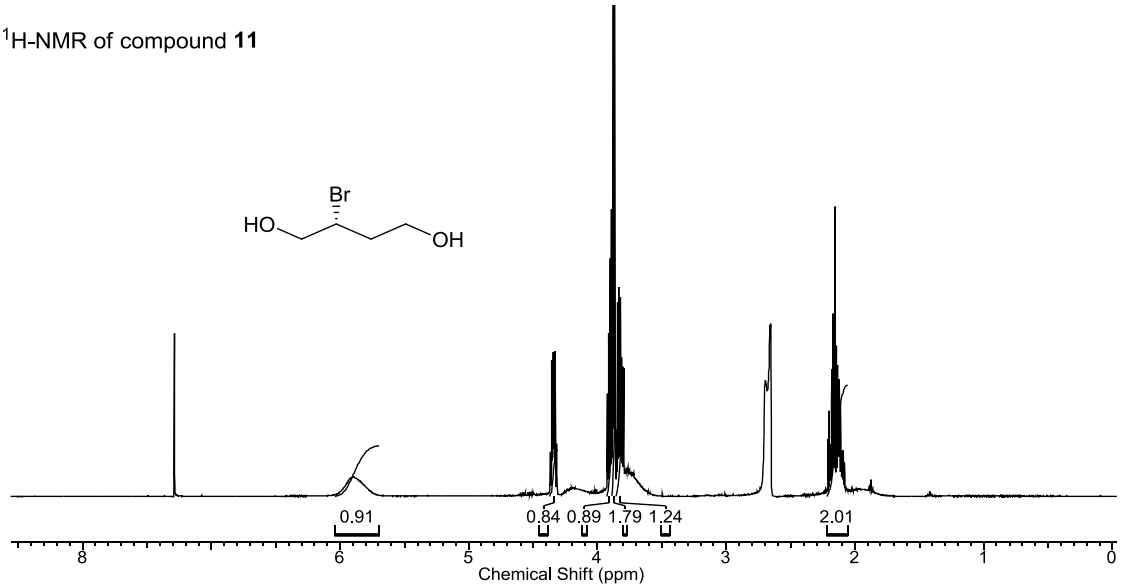


^{13}C-NMR of compound **10**

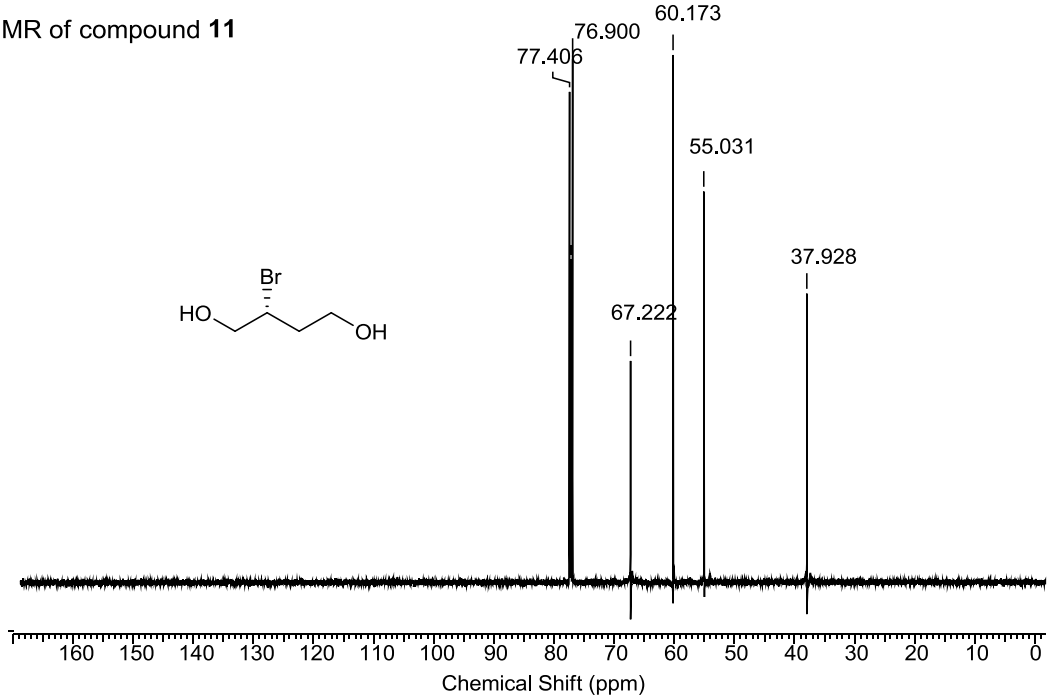


This journal is © The Royal Society of Chemistry 2013

^{1}H-NMR of compound **11**

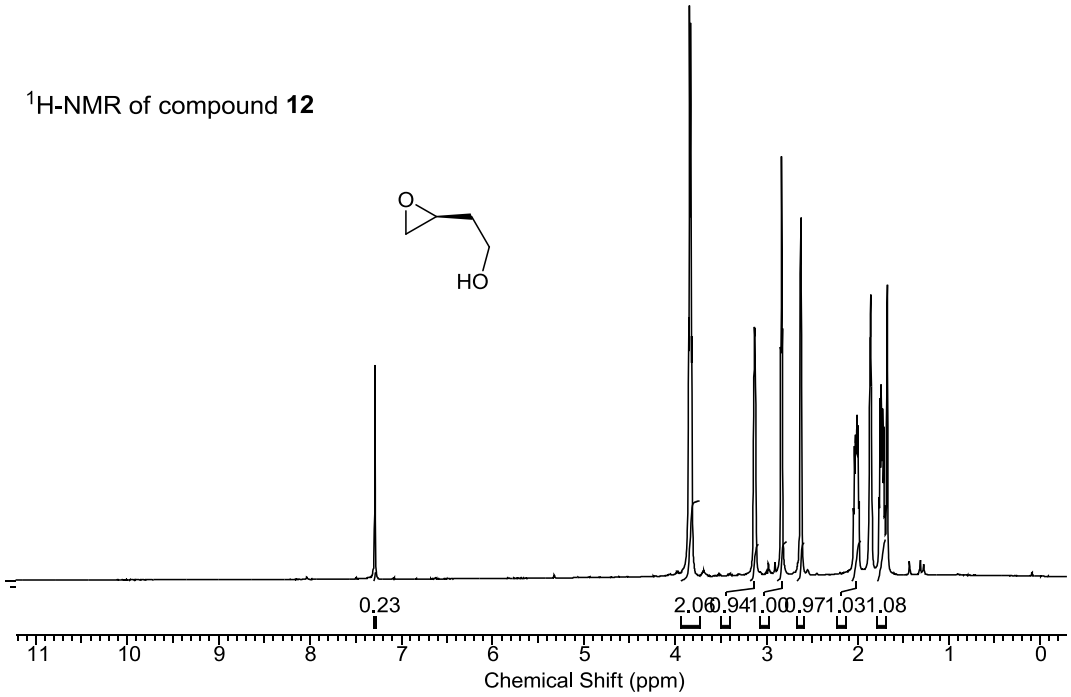


^{13}C-NMR of compound **11**

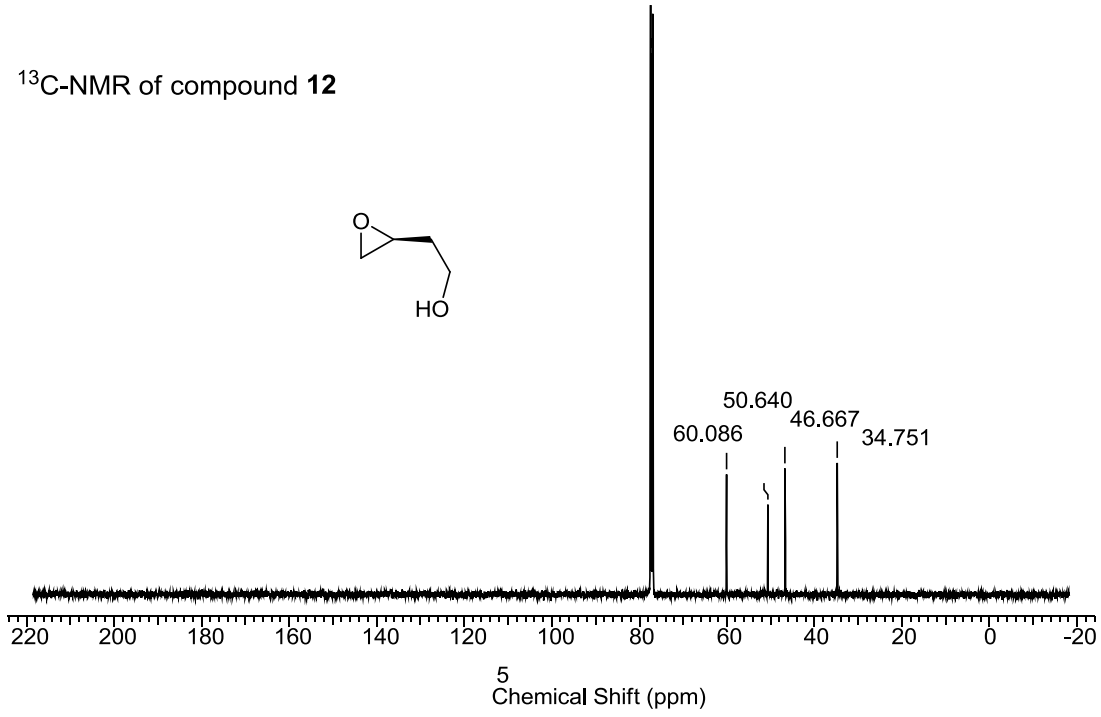


This journal is © The Royal Society of Chemistry 2013

^{1}H-NMR of compound **12**

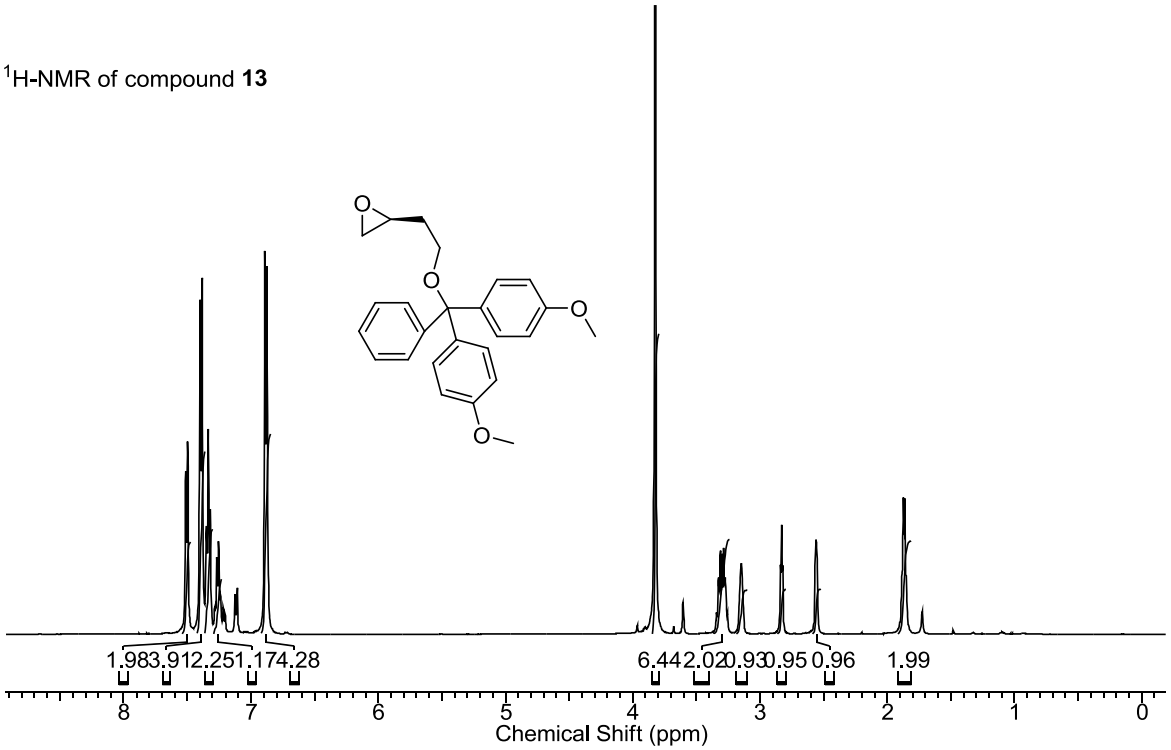


^{13}C-NMR of compound **12**

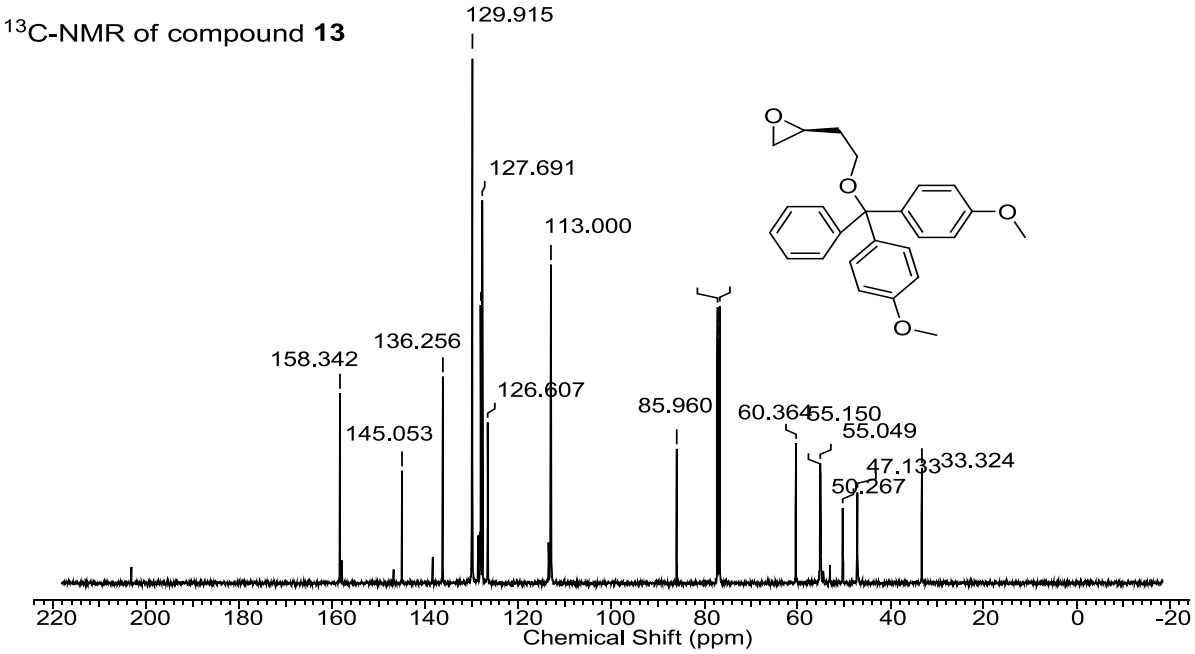


This journal is © The Royal Society of Chemistry 2013

^{1}H-NMR of compound **13**

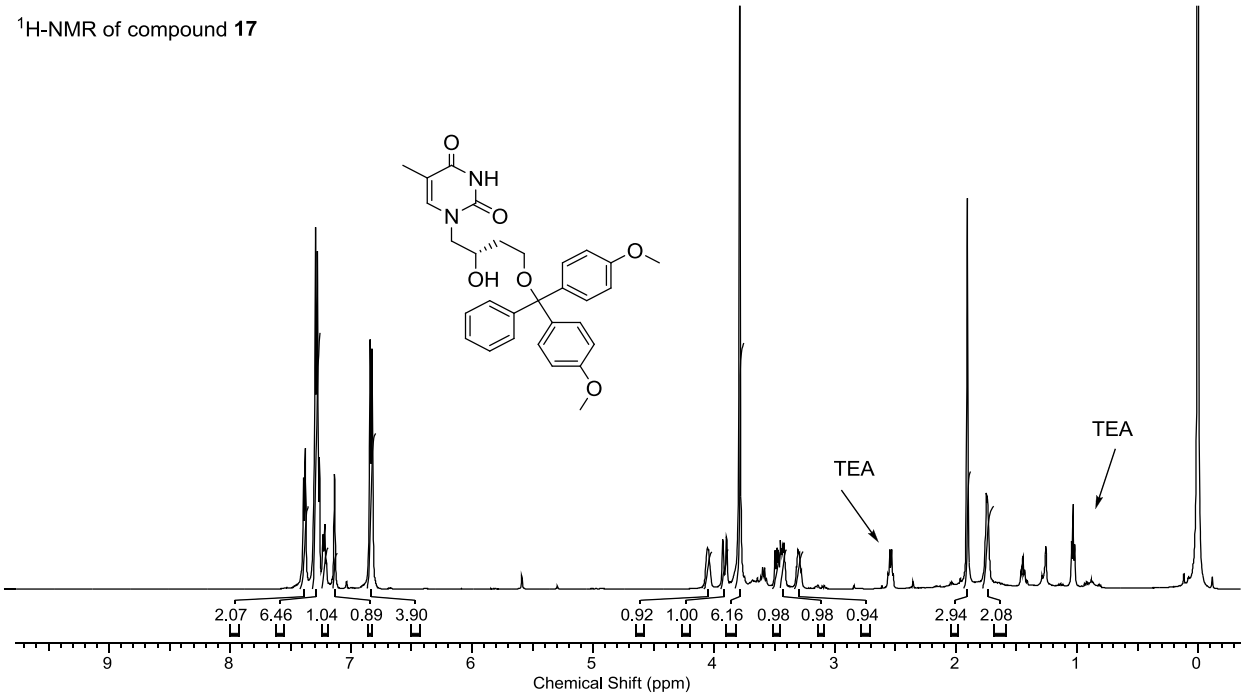


^{13}C-NMR of compound **13**

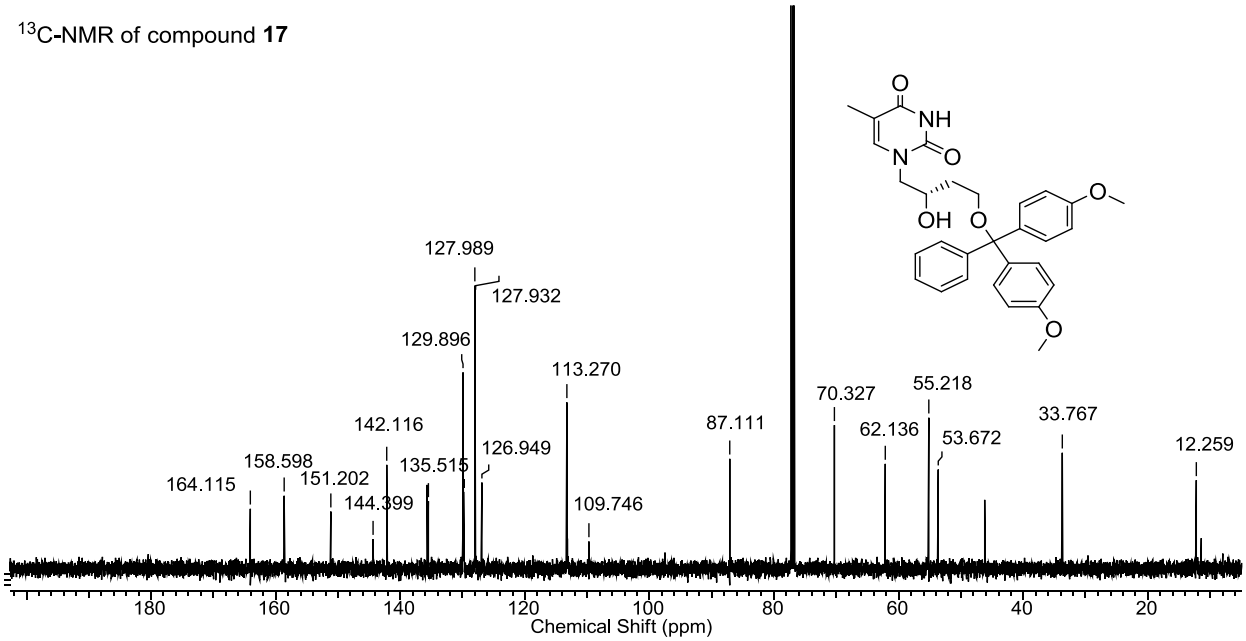


This journal is © The Royal Society of Chemistry 2013

^{1}H-NMR of compound **17**

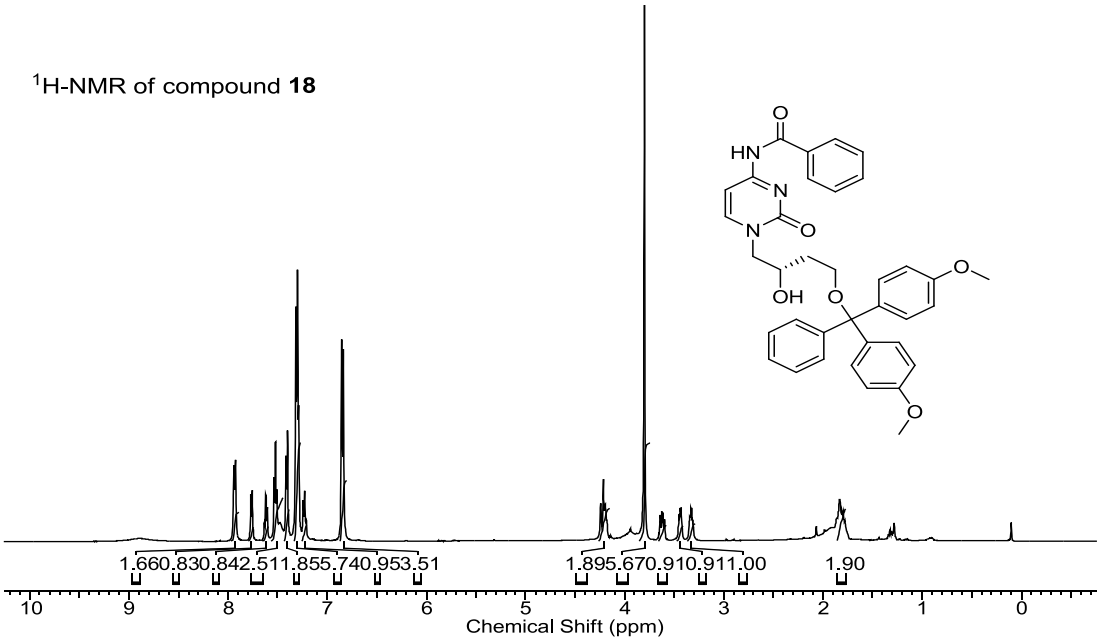


^{13}C-NMR of compound **17**

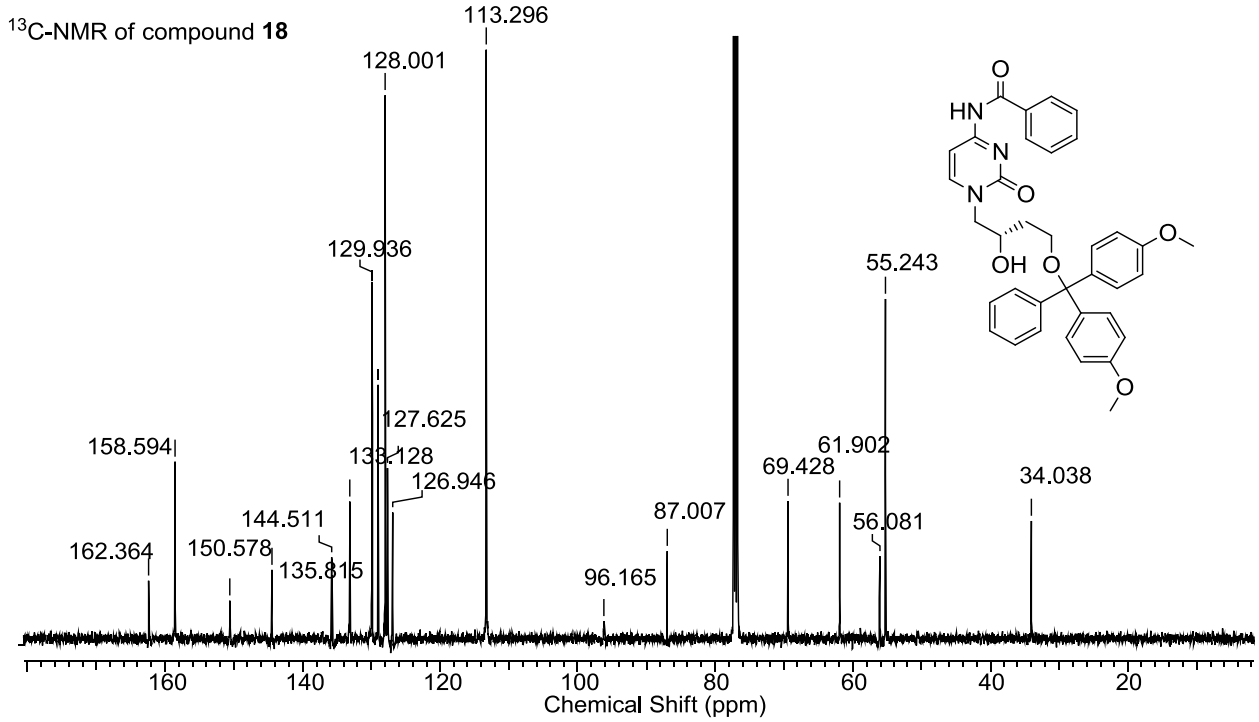


This journal is © The Royal Society of Chemistry 2013

^{1}H-NMR of compound **18**

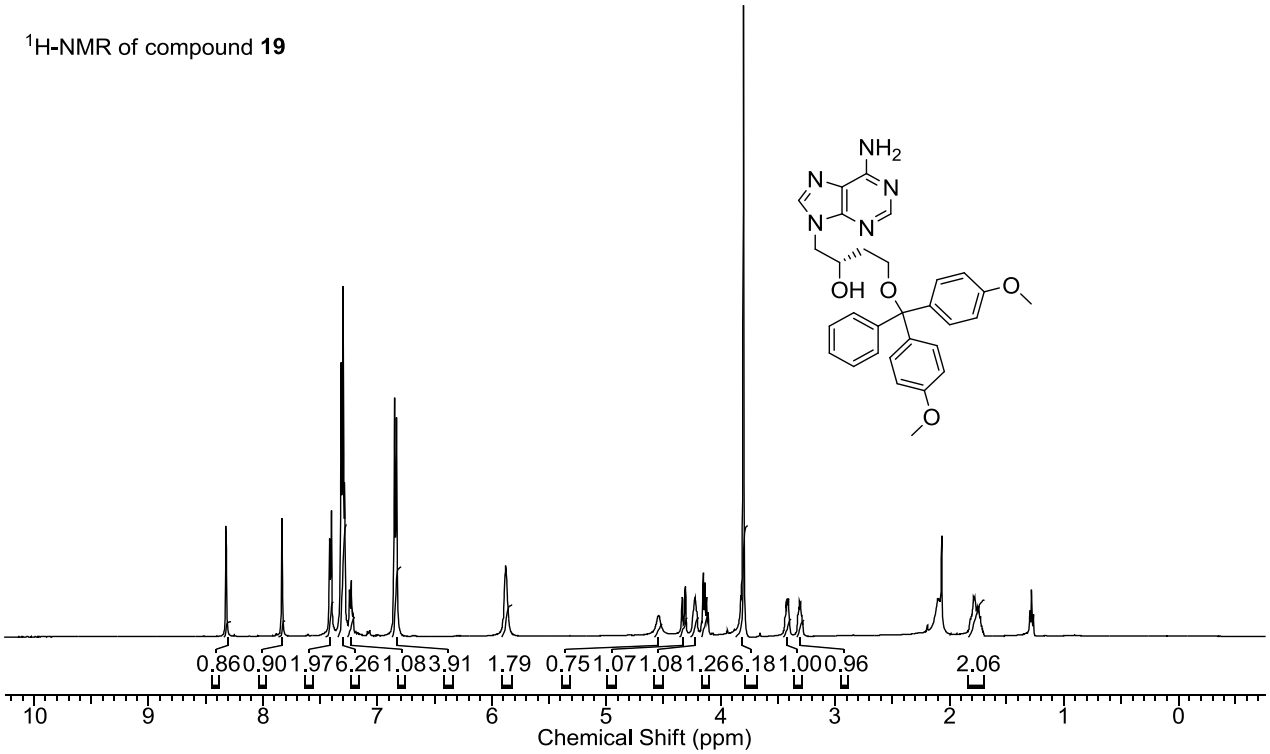


^{13}C-NMR of compound **18**

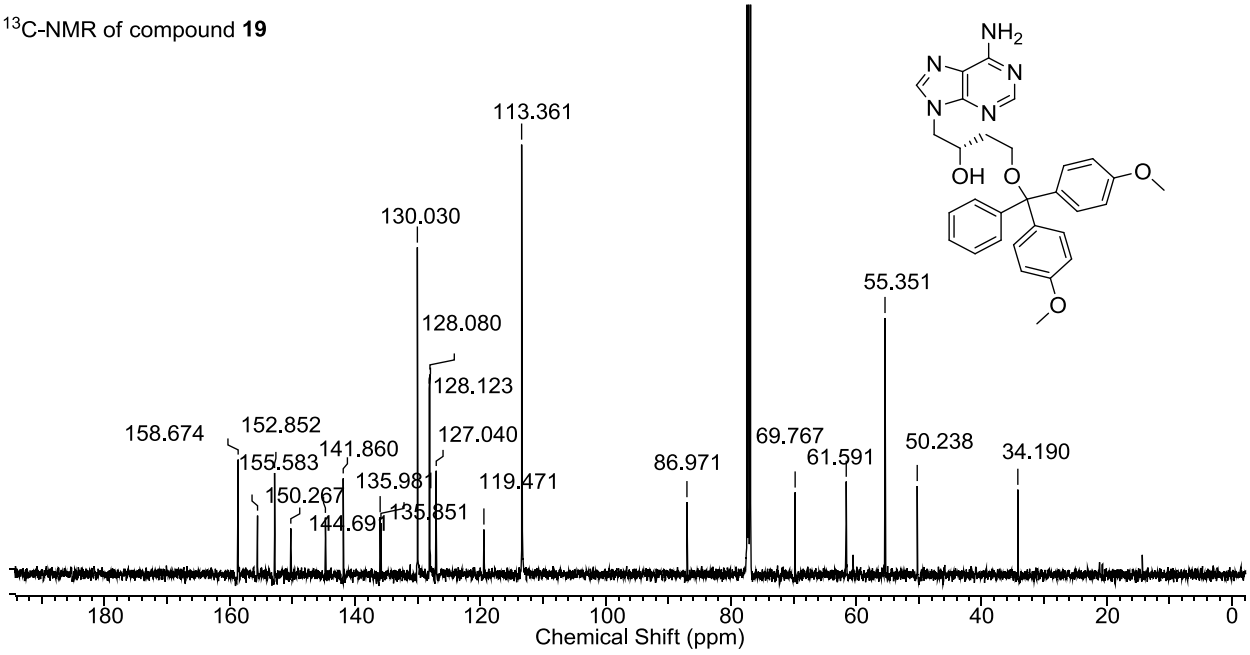


This journal is © The Royal Society of Chemistry 2013

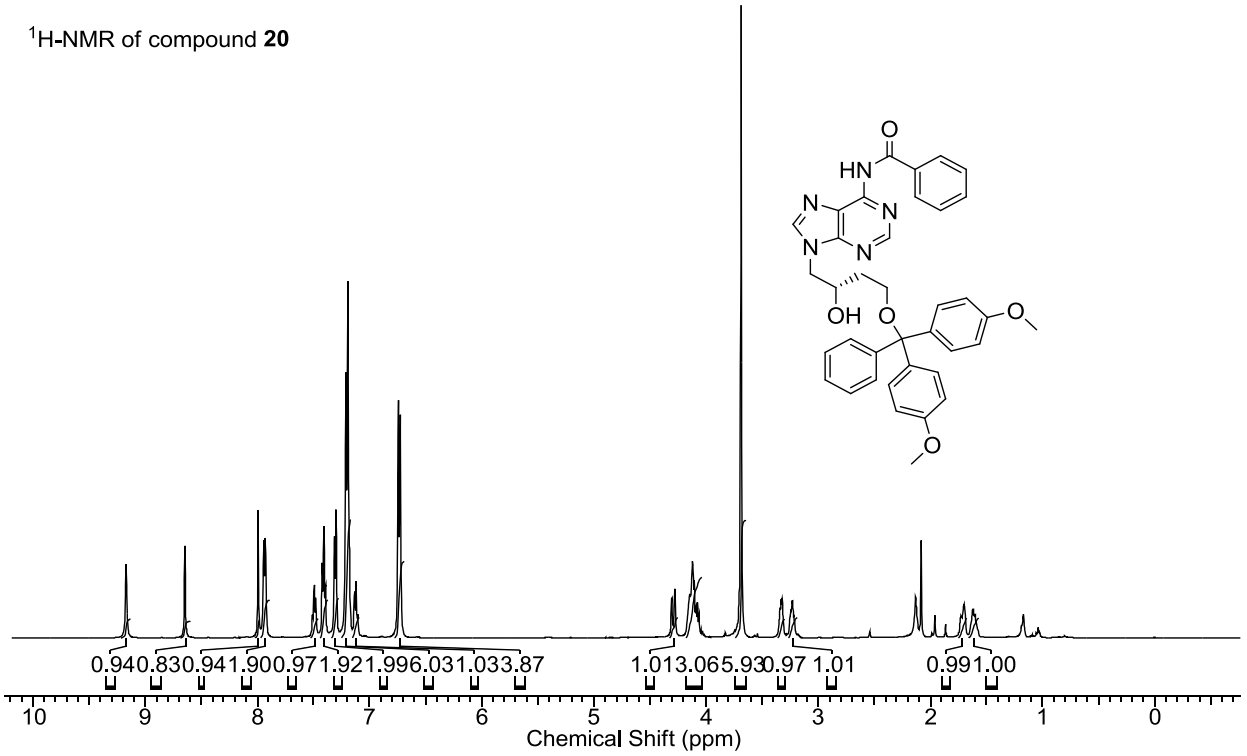
^{1}H-NMR of compound **19**



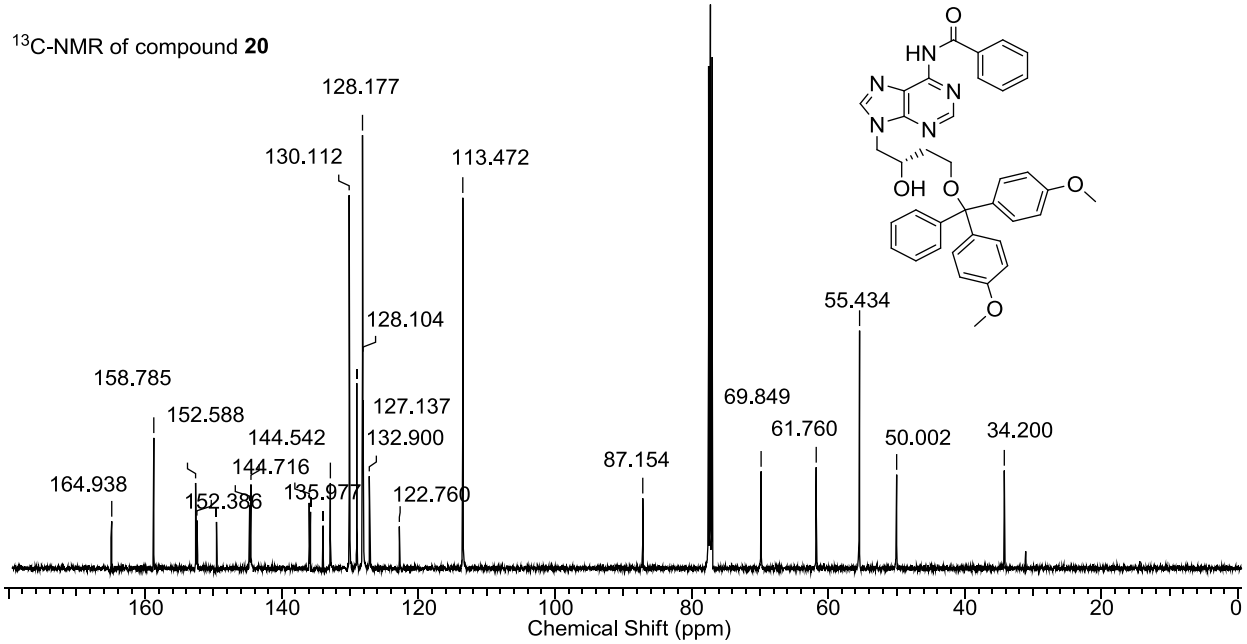
^{13}C-NMR of compound **19**



^{1}H-NMR of compound **20**

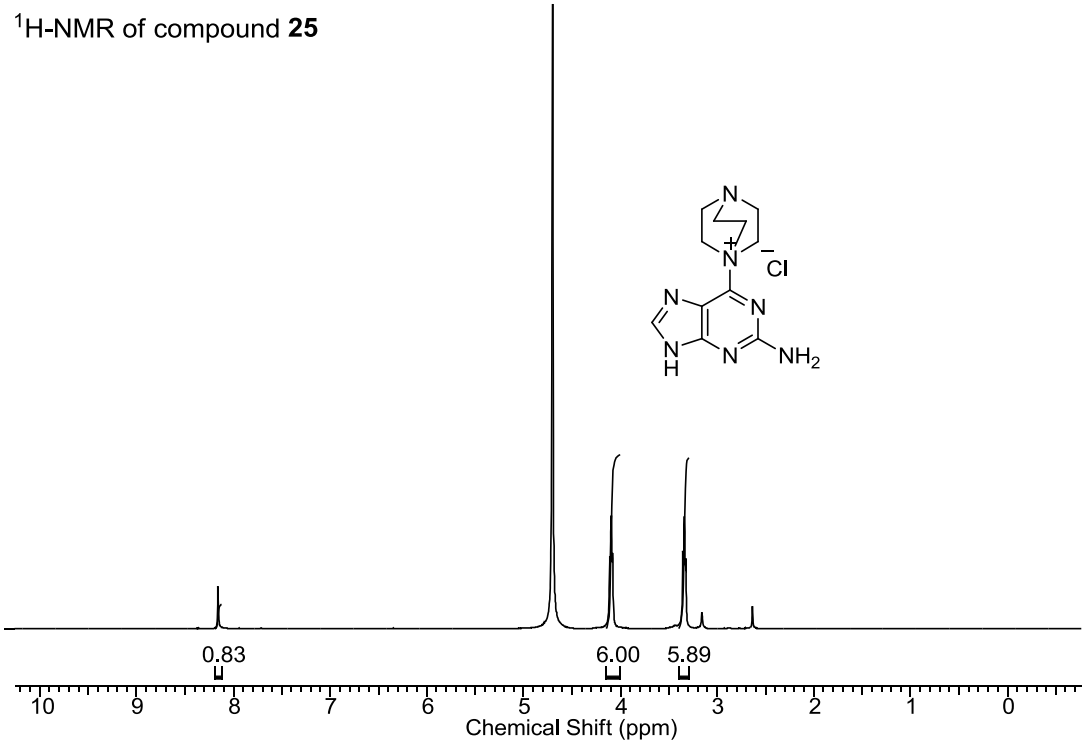


^{13}C-NMR of compound **20**

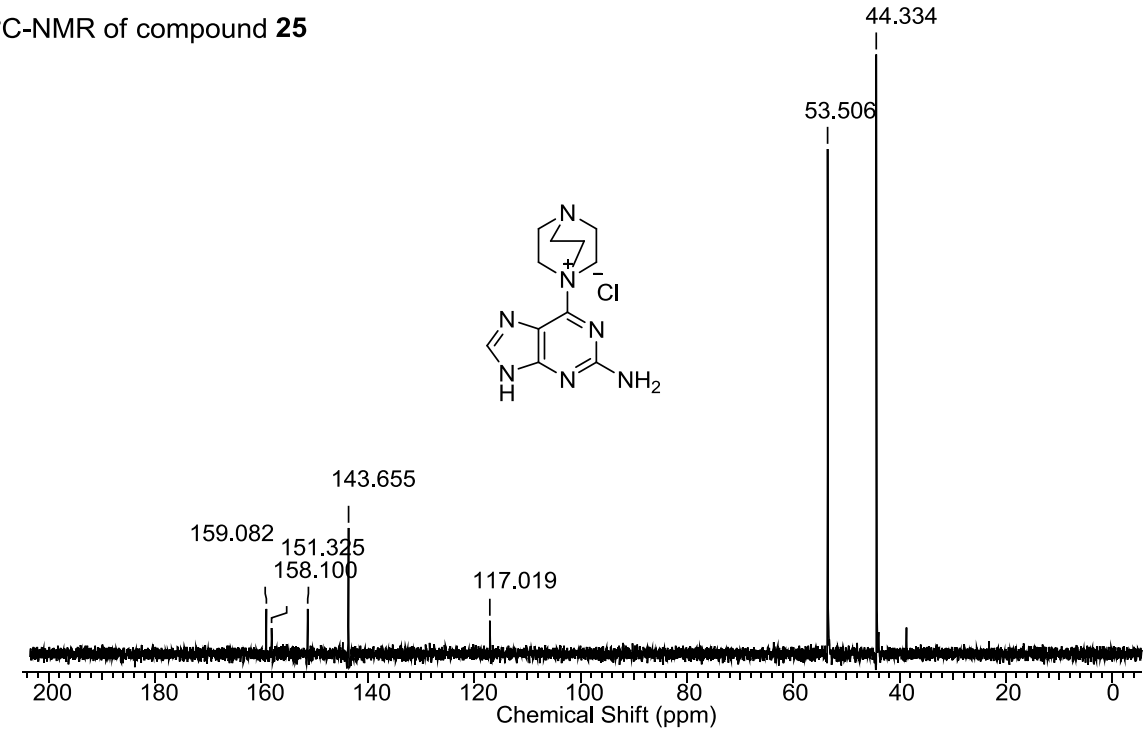


This journal is © The Royal Society of Chemistry 2013

^{1}H-NMR of compound **25**

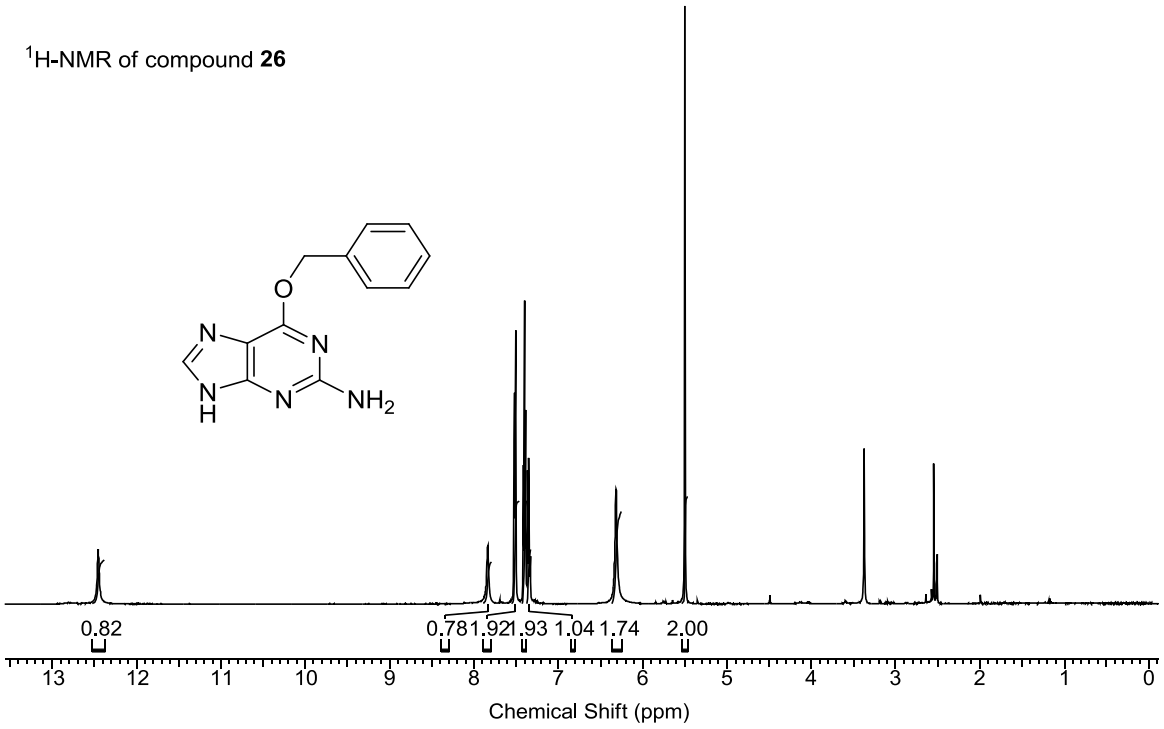


^{13}C-NMR of compound **25**

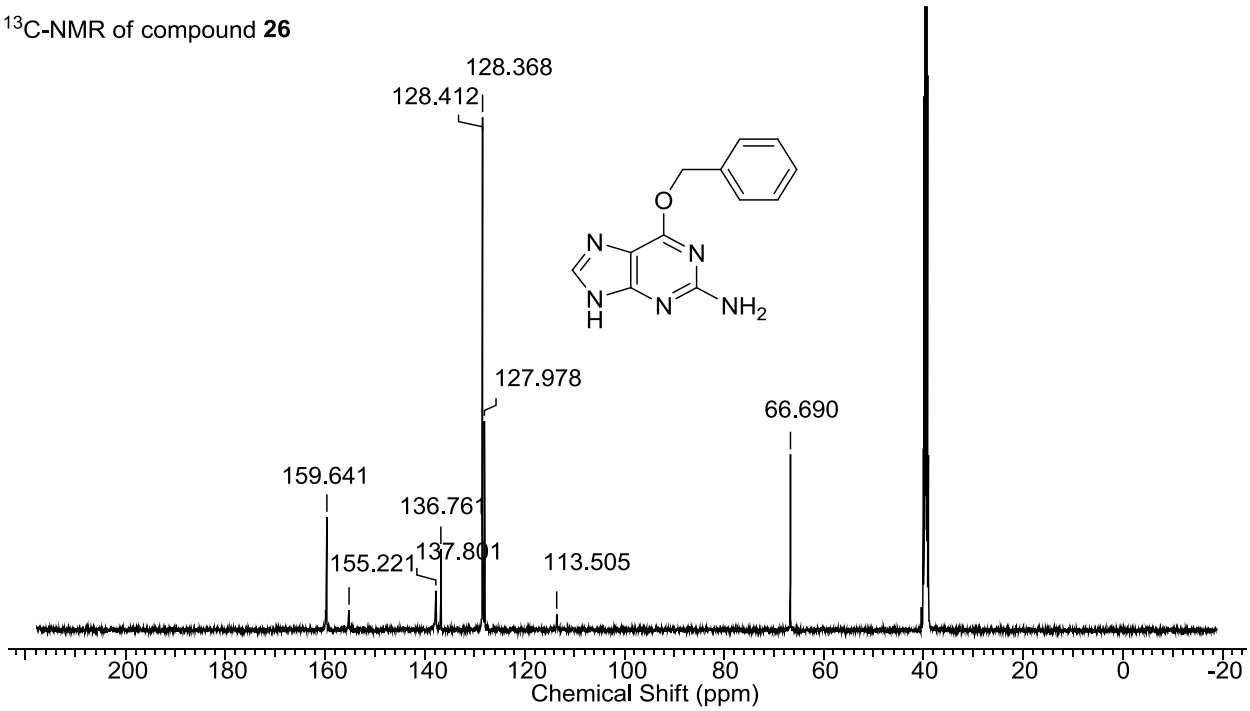


This journal is © The Royal Society of Chemistry 2013

^{1}H-NMR of compound **26**

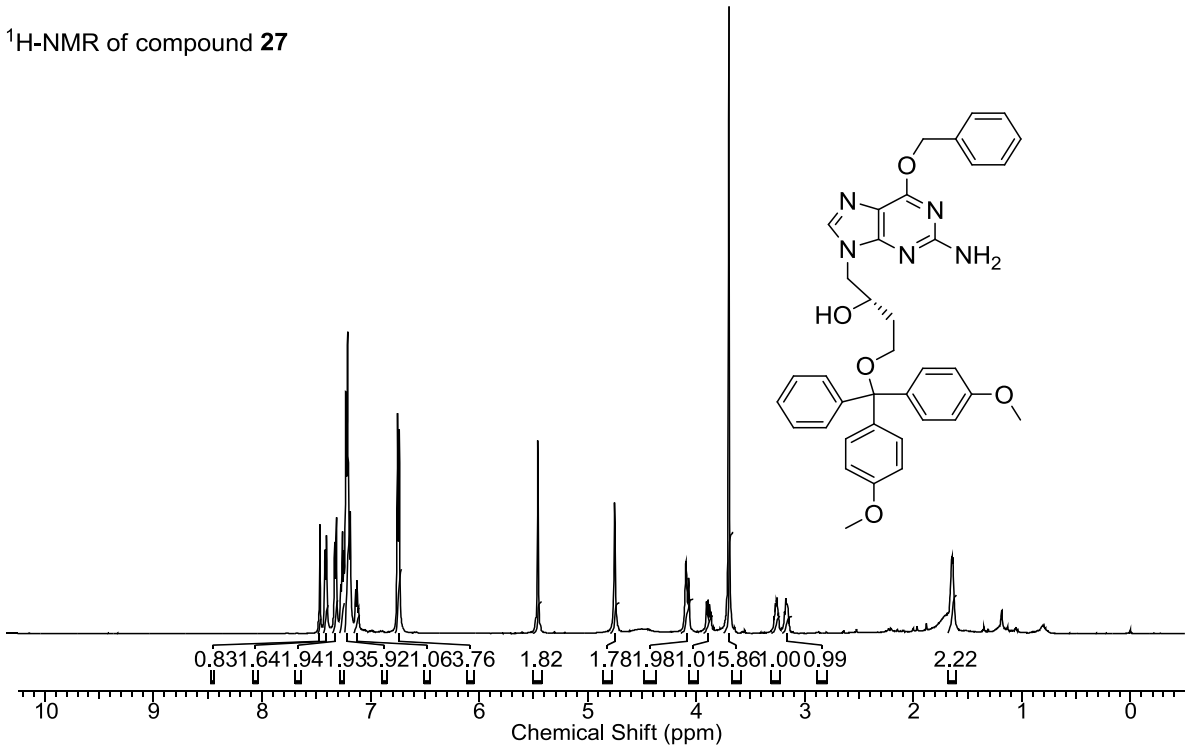


^{13}C-NMR of compound **26**

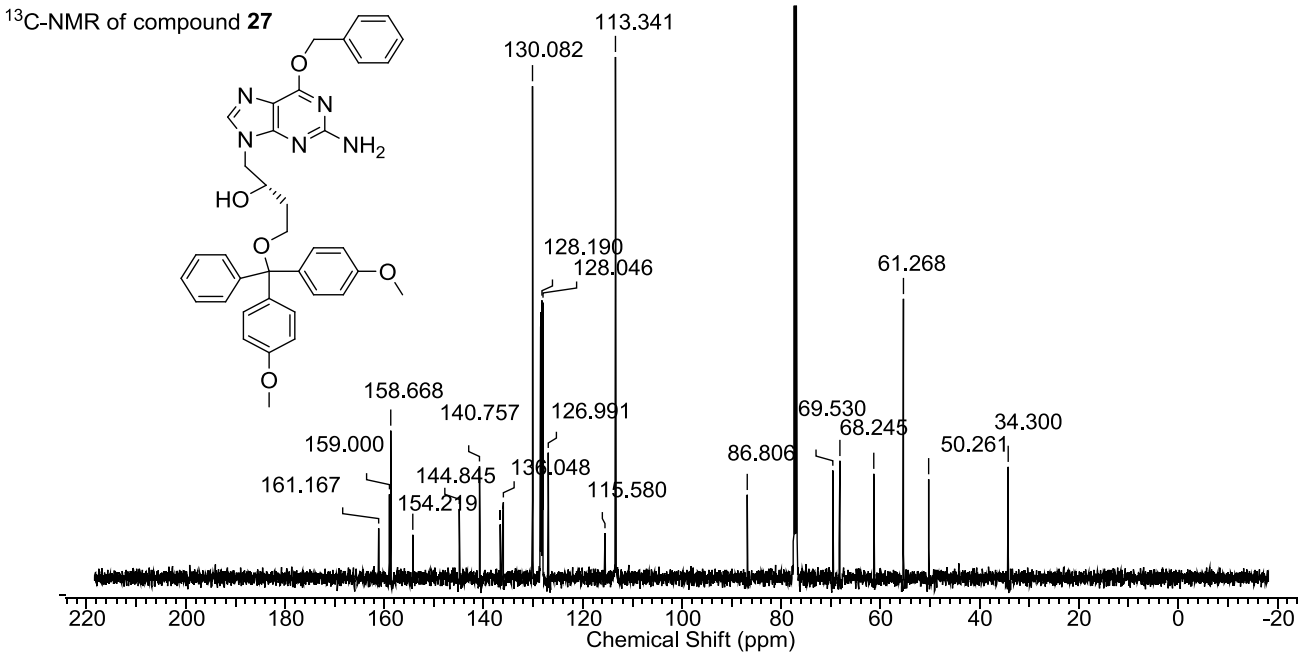


This journal is © The Royal Society of Chemistry 2013

^{1}H-NMR of compound **27**

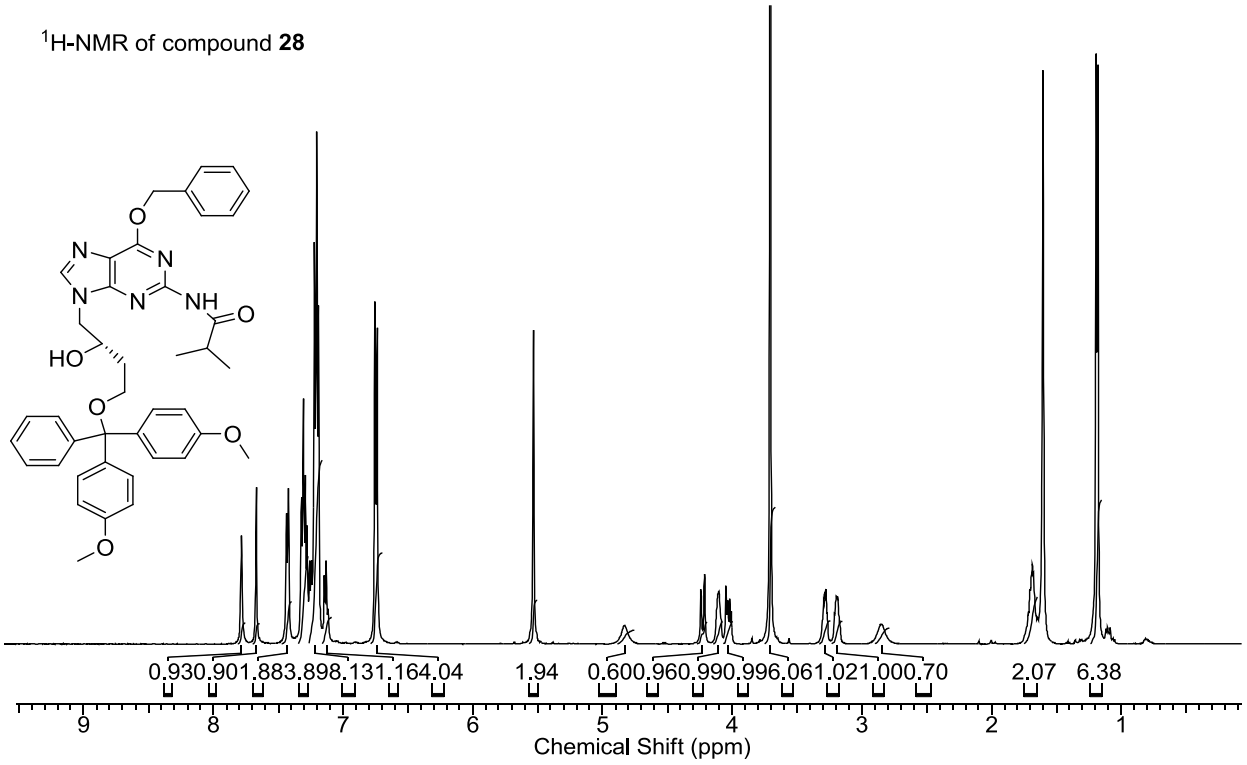


^{13}C-NMR of compound **27**

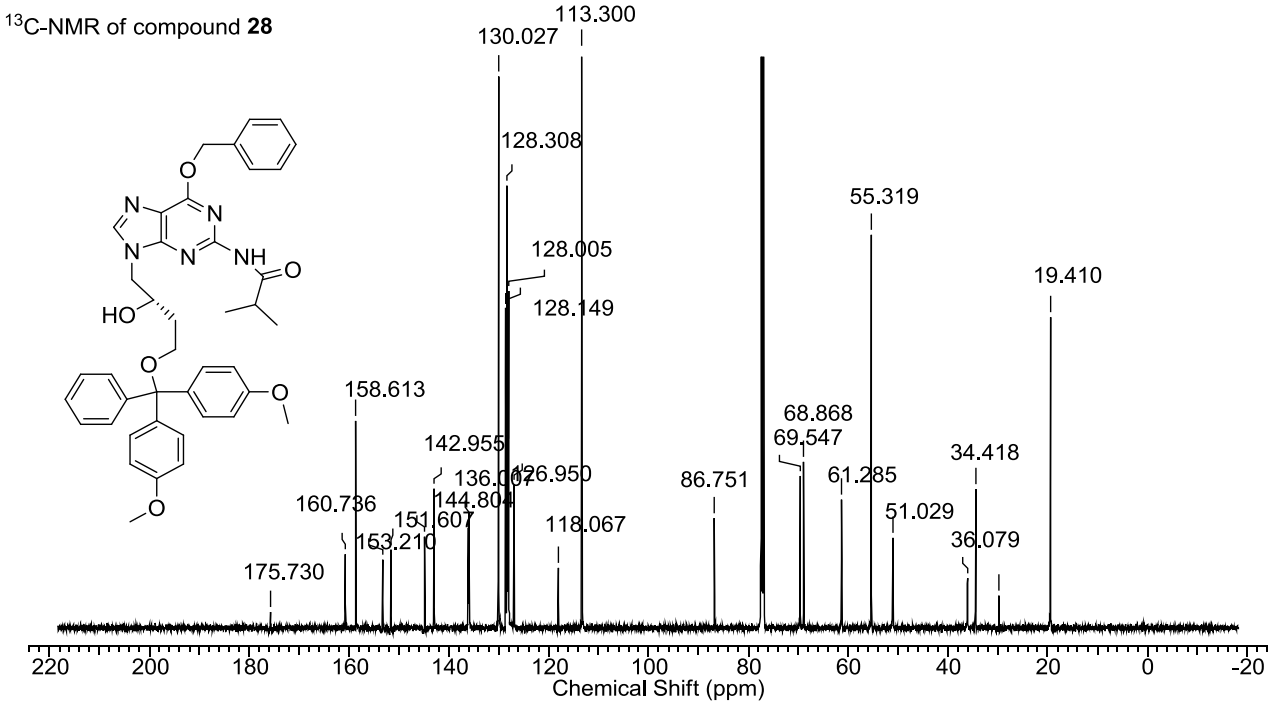


This journal is © The Royal Society of Chemistry 2013

^{1}H-NMR of compound **28**

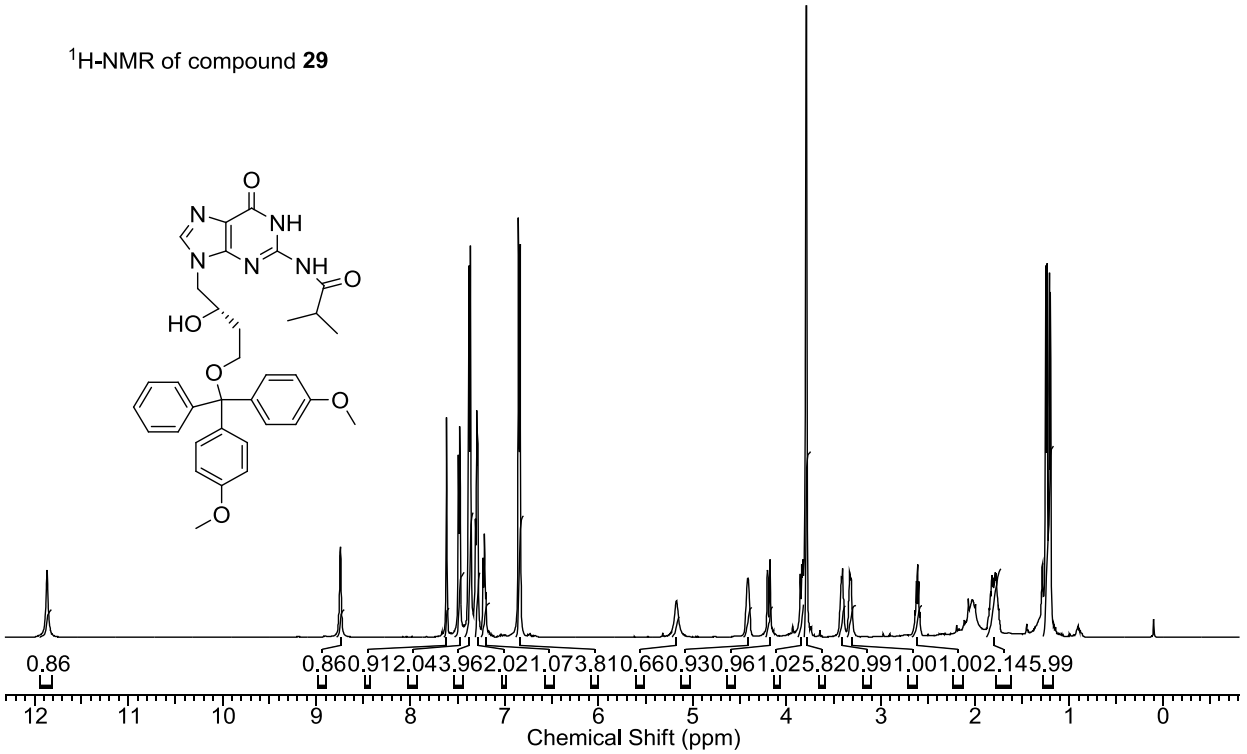


^{13}C-NMR of compound **28**

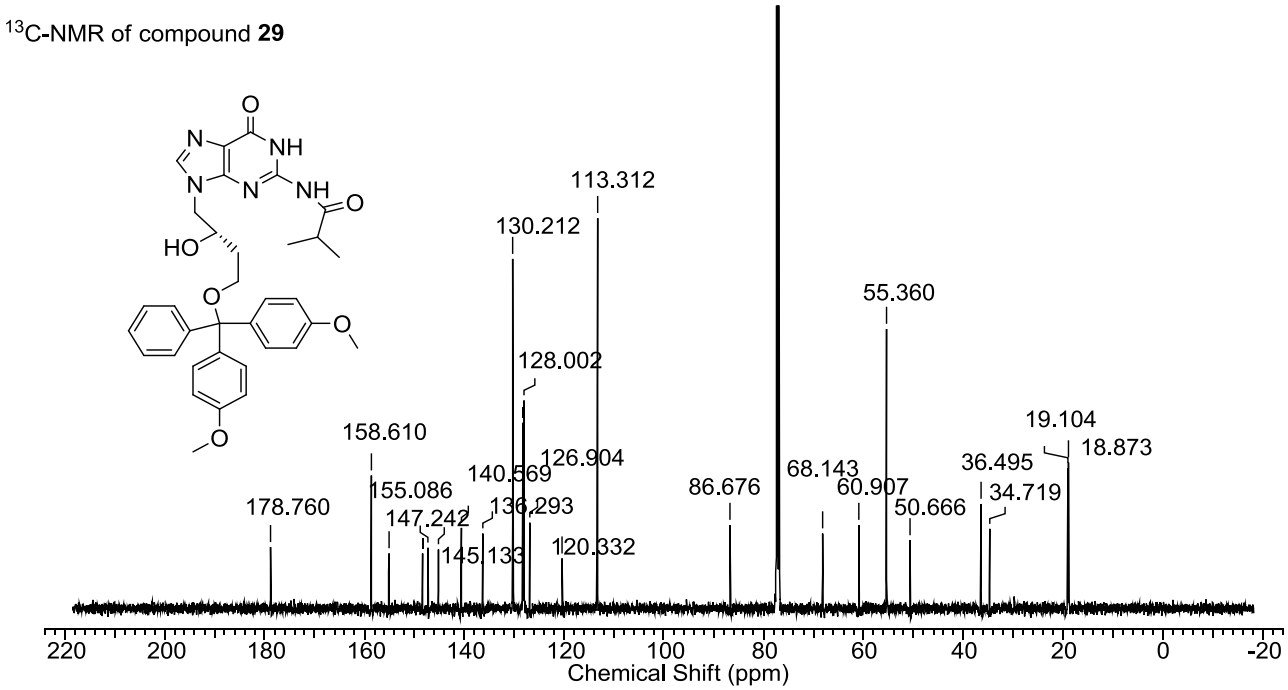


This journal is © The Royal Society of Chemistry 2013

^{1}H-NMR of compound **29**

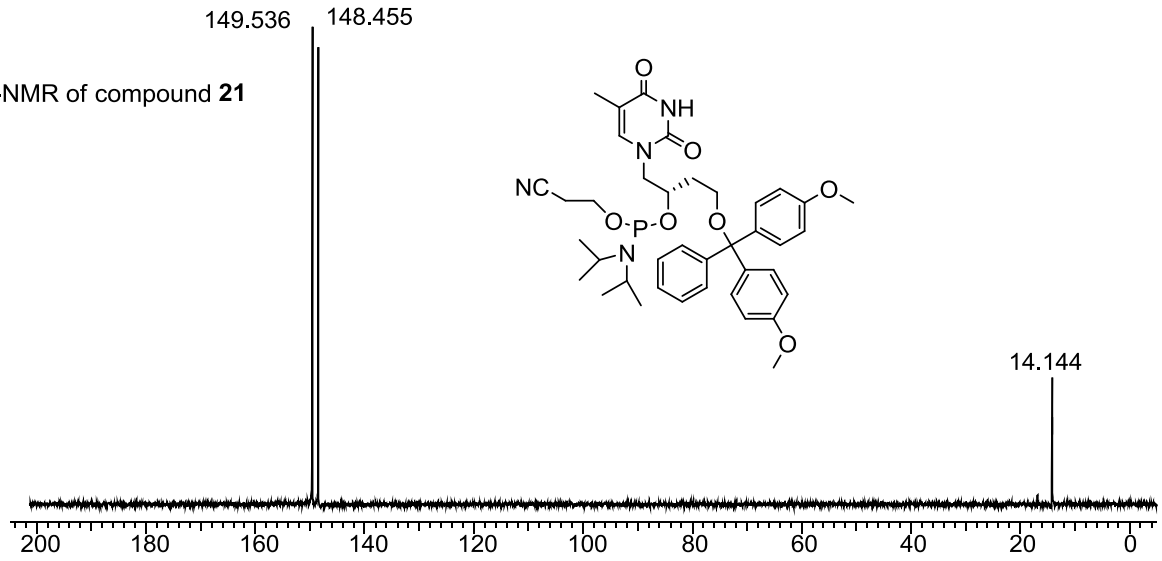


^{13}C-NMR of compound **29**

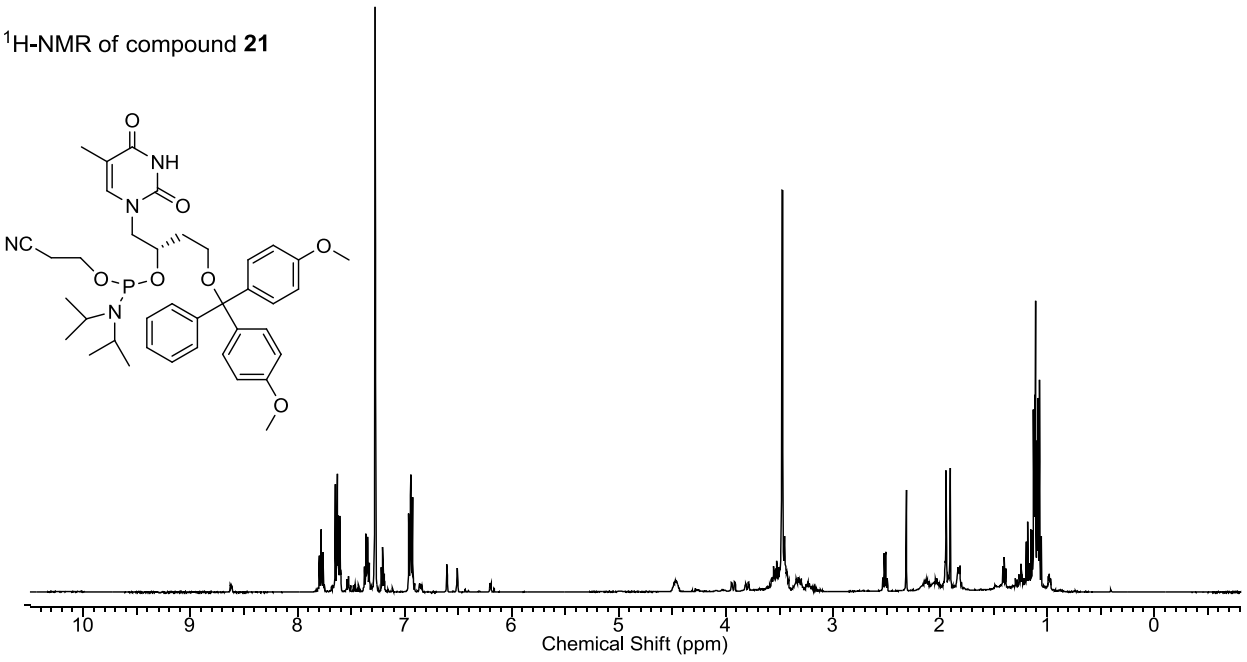


This journal is © The Royal Society of Chemistry 2013

^{31}P-NMR of compound **21**

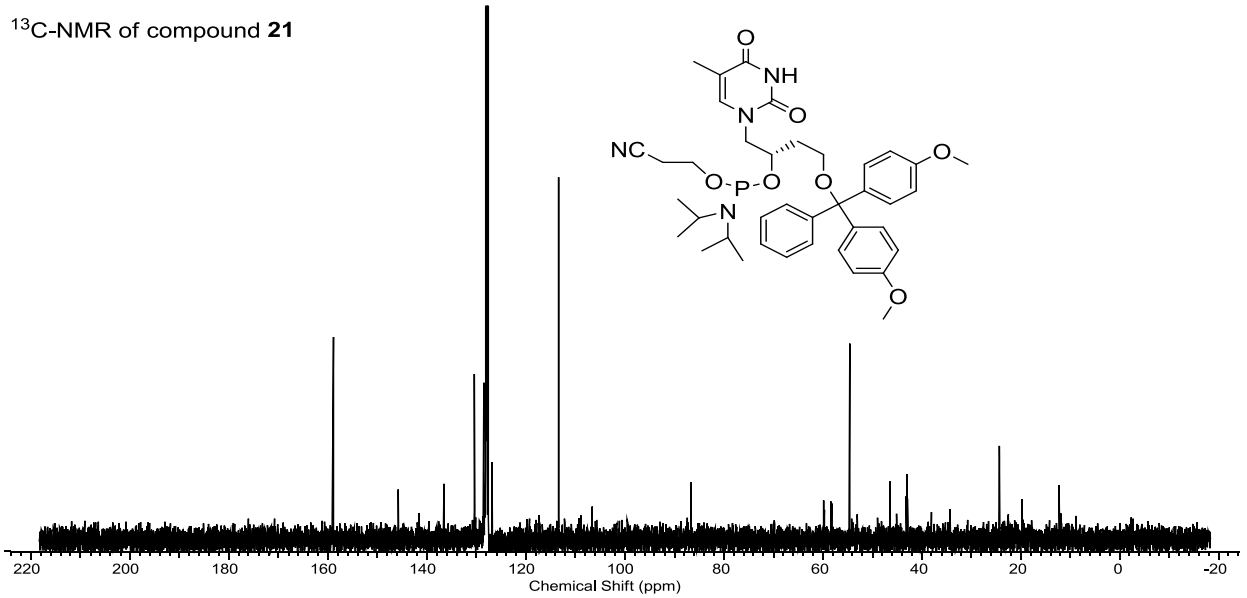


^{1}H-NMR of compound **21**

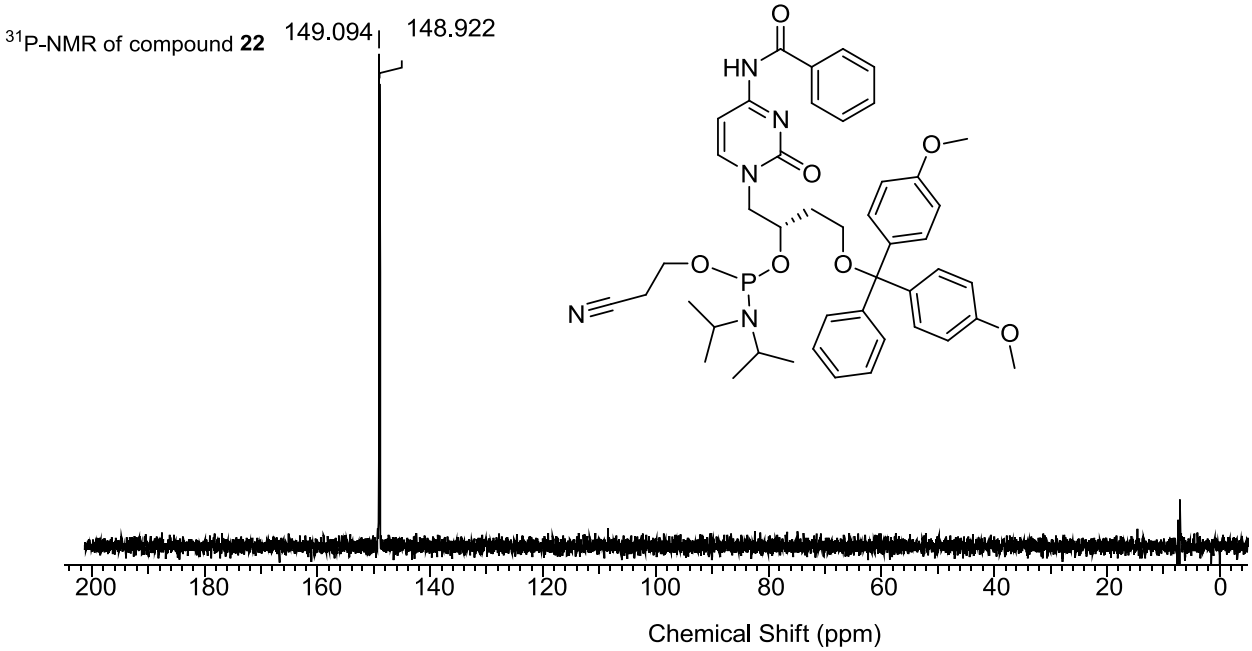


This journal is © The Royal Society of Chemistry 2013

^{13}C-NMR of compound **21**

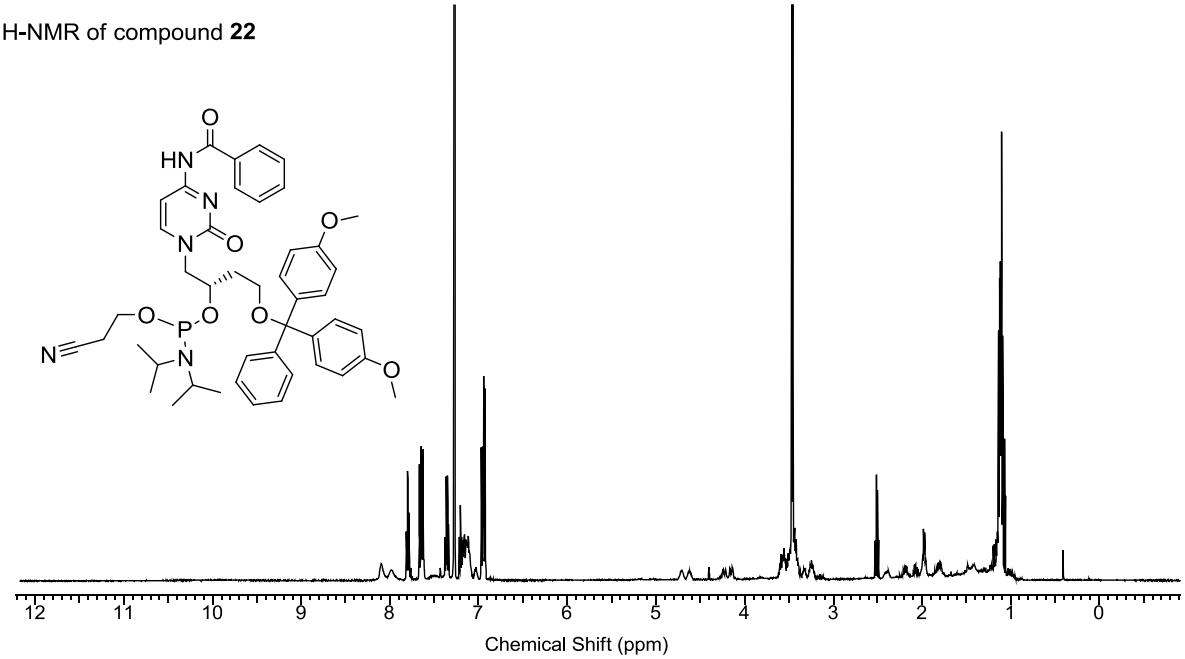


^{31}P-NMR of compound **22**

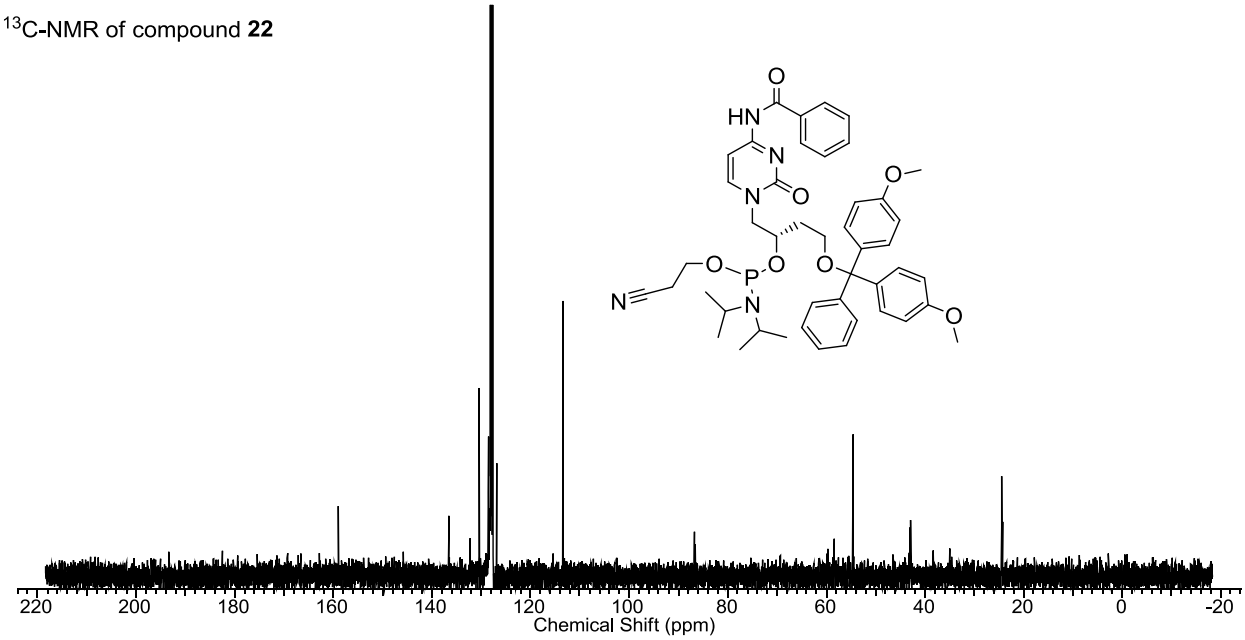


This journal is © The Royal Society of Chemistry 2013

^{1}H-NMR of compound **22**

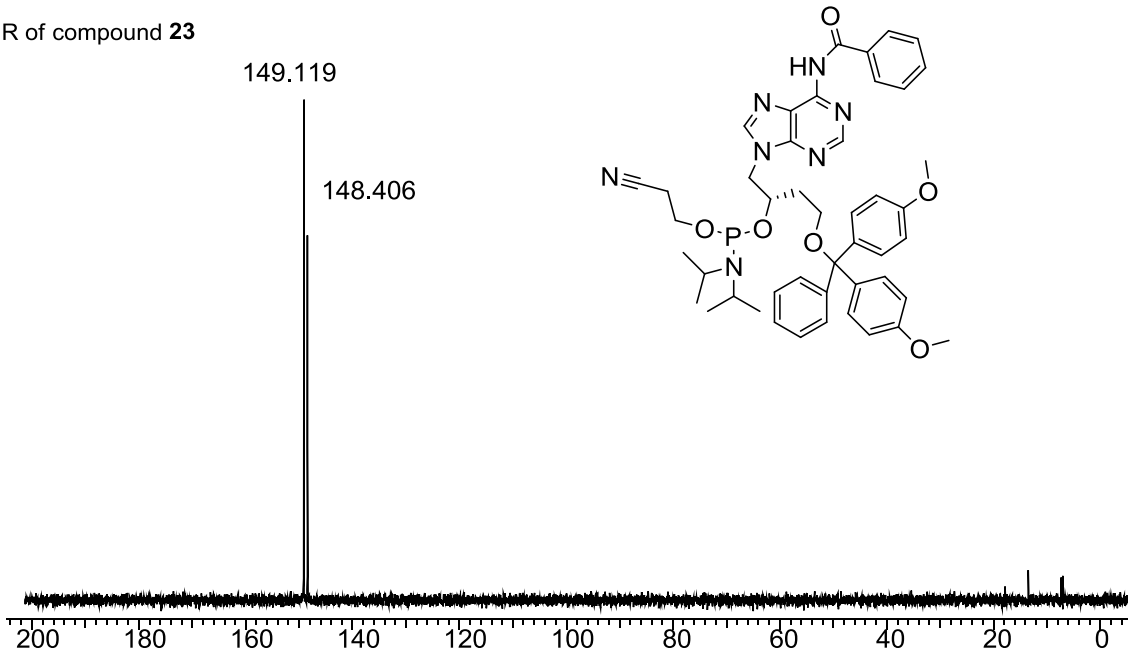


^{13}C-NMR of compound **22**

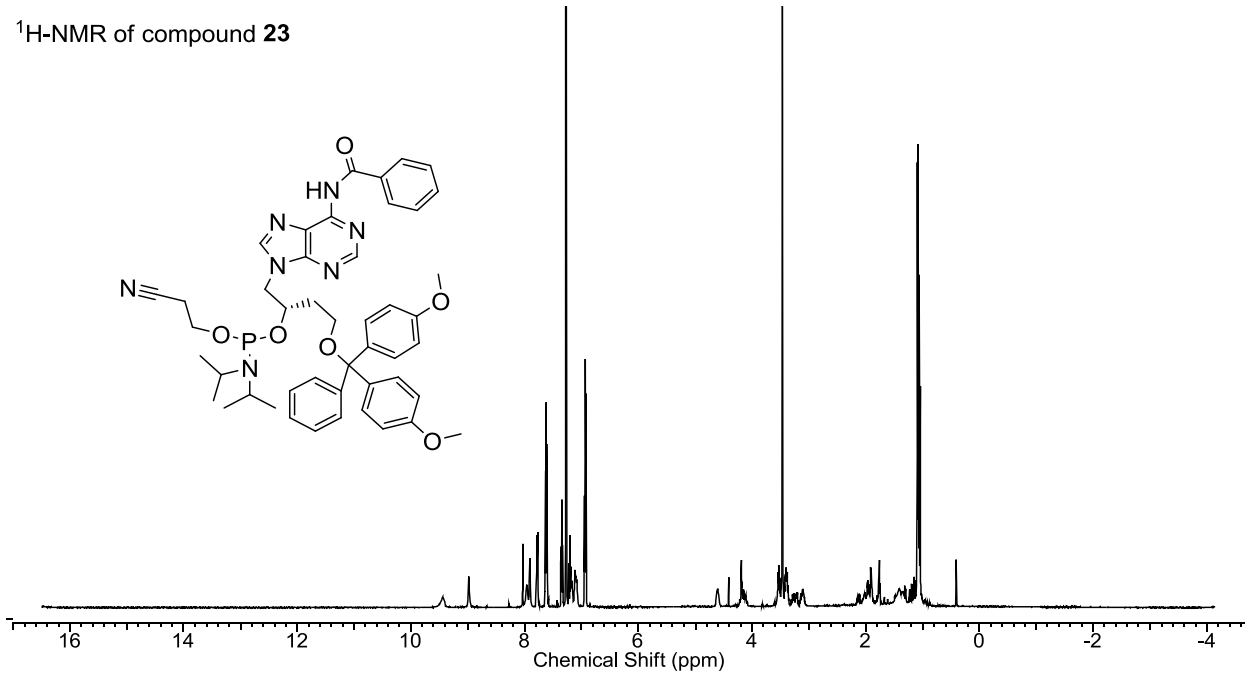


This journal is © The Royal Society of Chemistry 2013

^{31}P-NMR of compound **23**

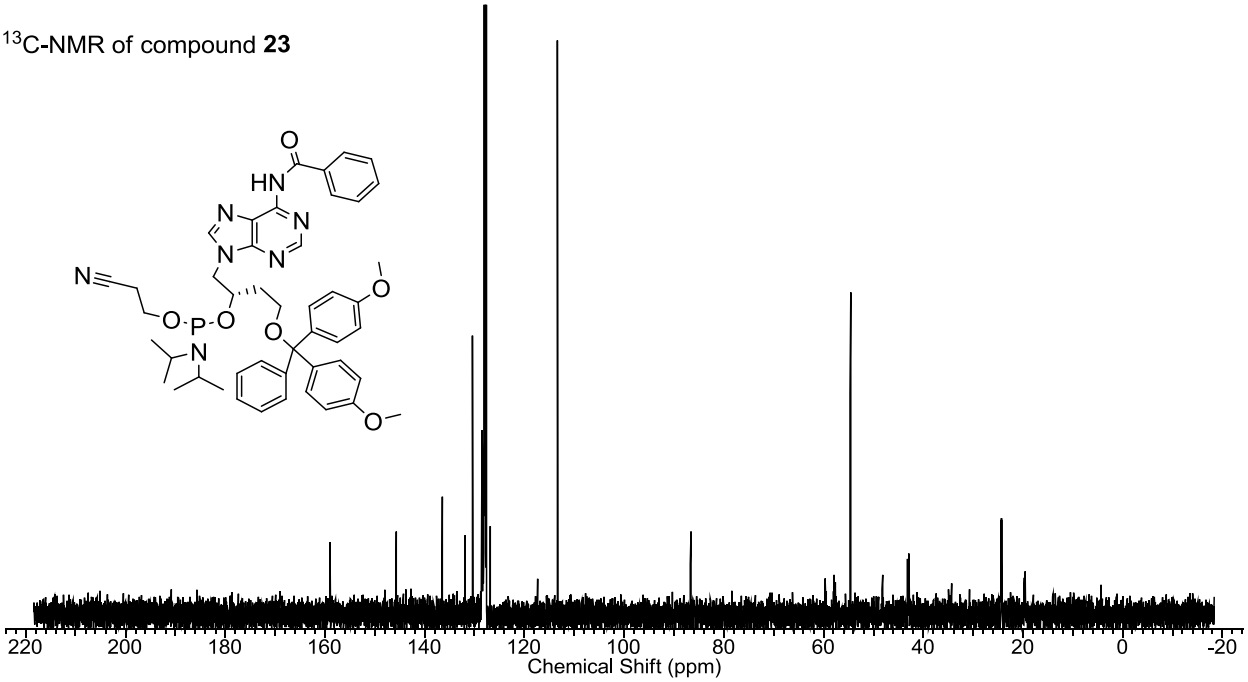


^{1}H-NMR of compound **23**

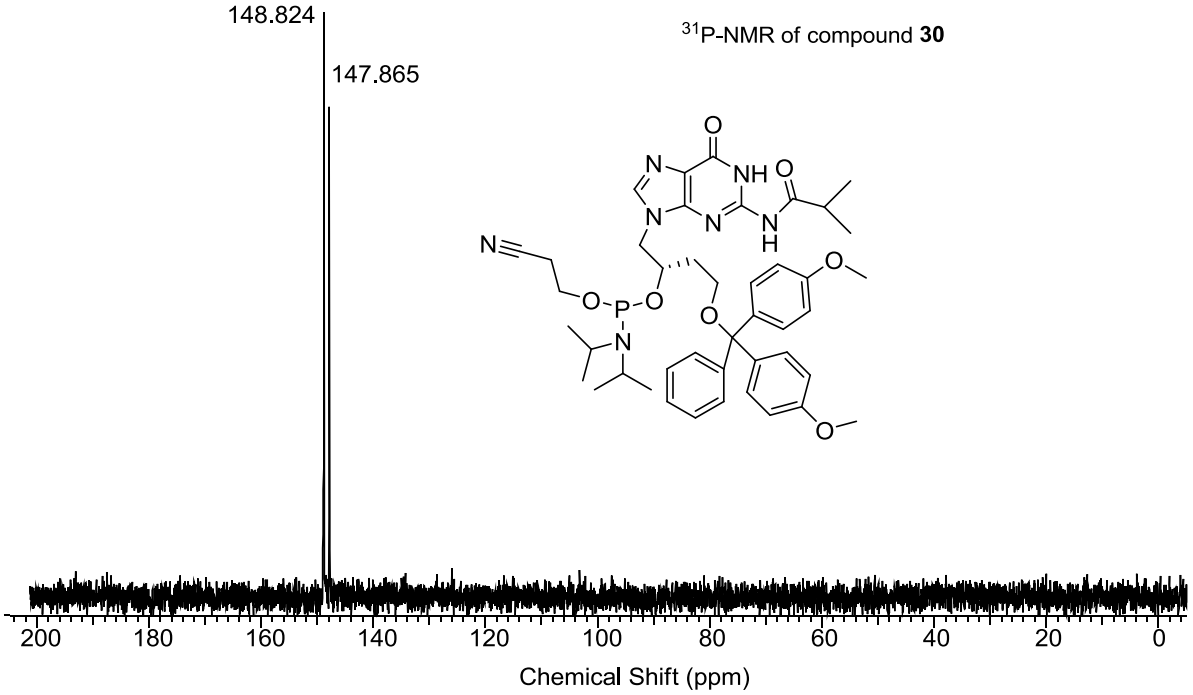


This journal is © The Royal Society of Chemistry 2013

^{13}C-NMR of compound **23**

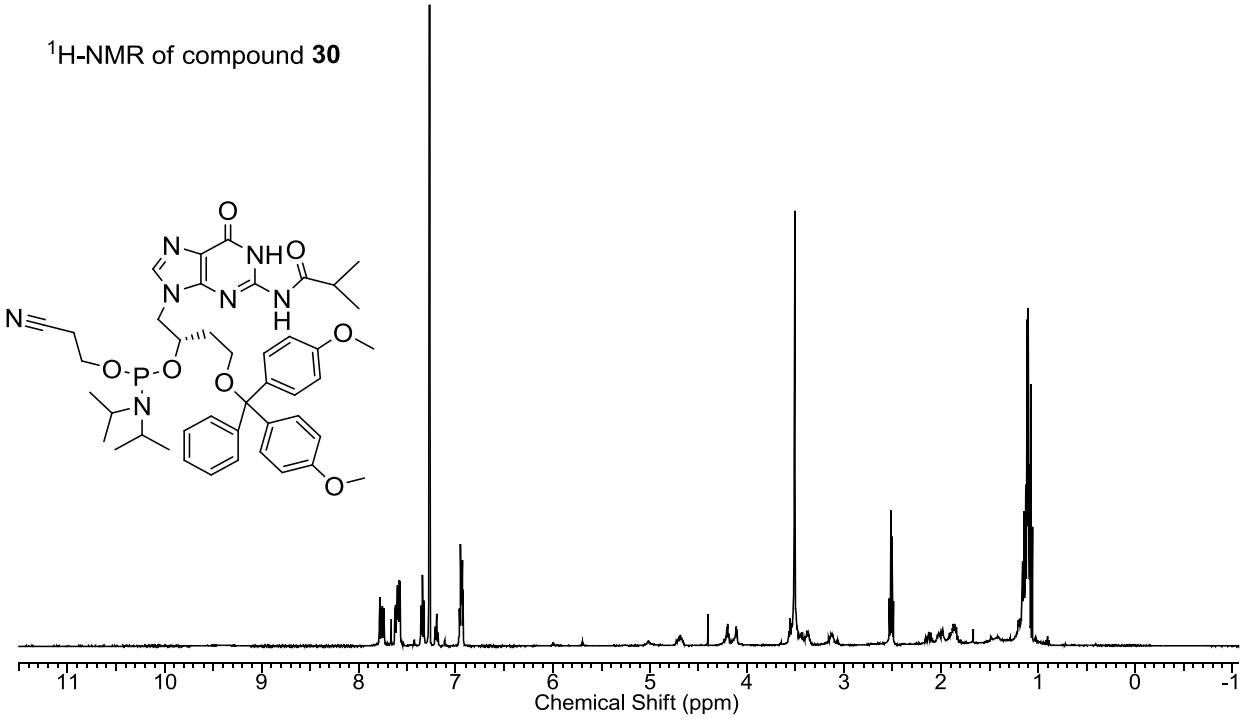


^{31}P-NMR of compound **30**



This journal is © The Royal Society of Chemistry 2013

^{1}H-NMR of compound **30**



^{13}C-NMR of compound **30**