CONFORMATIONALLY CONSTRAINED NUCLEOSIDE PHOSPHONIC ACIDS – POTENT INHIBITORS OF HUMAN MITOCHONDRIAL AND CYTOSOLIC 5'(3')-NUCLEOTIDASES

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Table 1S Calculated geometry and NMR parameters for *C3'-endo* and *C2'-endo* conformations of [6'R] and [6'S]-epimer of compound **9i**. The observed NMR parameters and and differences between calculated and observed values are presented as MAE values.

	[6'R]				[6'S]		
		C3'-endo	***C2'-endo***			***C3'-endo***	***C2'-endo***
Phase angle (P)		**20**	**174**			**36**	**172**
Max.pucker (ϕ_{max})		**34**	**37**			**33**	**37**
Torsion angle χ		**-157**	**-156**			**-162**	**-153**
Rel. energy [kcal/mol]		**0**	**8.47**			**0**	**3.08**
	***J*obs**	***J*calc**	***J*calc**		***J*obs**	***J*calc**	***J*calc**
***J*(H1',H2')**	1.8	1.3	10.4		3.0	2.2	10.5
***J*(H1',H2")**	8.3	8.4	5.6		8.4	9.2	5.7
***J*(H2',H3')**	1.0	1.1	10.7		1.0	0.9	10.6
***J*(H2",H3')**	4.9	5.7	7.2		5.4	6.7	7.3
***J*(H3',H4')**	2.5	3.3	8.4		3.2	3.4	8.5
***J*(H4',H5'a)**	1.2	1.0	7.0		2.4	0.9	7.8
***J*(H4',H5'b)**	2.1	3.0	10.7		3.9	3.3	10.2
MAE		**0.49**	**6.23**			**0.76**	**5.53**
	δH (obs)	**δH (cal.)**	**δH (cal.)**		**δH (obs)**	**δH (cal.)**	**δH (cal.)**
H1'	6.12	5.83	5.89		6.16	5.69	5.99
H2'	2.08	2.34	1.76		2.17	2.30	2.28
H2"	2.73	2.60	2.20		2.71	2.68	2.73
H3'	4.71	4.61	5.06		4.28	4.39	4.67
H4'	4.06	3.94	4.31		3.85	3.58	4.25
H5'a	4.21	4.20	3.60		4.22	4.45	4.23
H5'b	4.37	4.70	4.09		4.00	4.08	4.11
OMe	3.02	3.39	3.46		3.26	3.51	3.10
MAE		**0.20**	**0.38**			**0.20**	**0.28**
	δC (obs)	**δC (cal.)**	**δC (cal.)**		**δC (obs)**	**δC (cal.)**	**δC (cal.)**
C1'	83.97	87.00	82.10		83.64	87.04	81.88
C2'	39.36	42.05	35.84		38.47	42.93	37.07
C3'	69.48	70.27	74.51		72.89	74.28	72.06
C4'	74.08	75.23	65.84		73.81	73.89	64.76
C5'	59.74	61.00	63.62		62.79	64.39	60.42
>C<	109.58	109.97	111.59		111.31	111.38	110.98
OMe	51.01	50.28	47.80		50.20	48.85	50.58
MAE		**1.25**	**3.97**			**1.76**	**2.30**

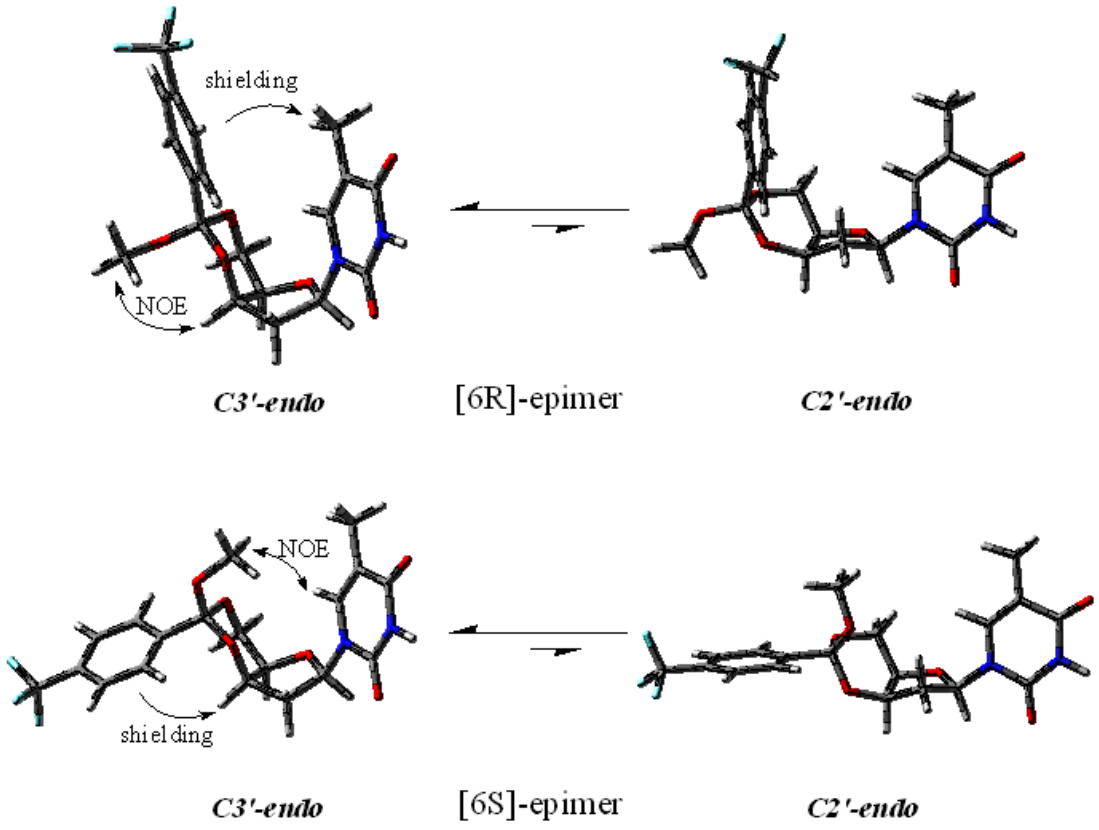

Figure 1S Geometry optimized conformers C3'-endo and C2'-endo of [6R]- and [6S]-epimer of compound **9i**. The observed characteristic NOE and shieldings with aromatic ring are indicated with arrows.

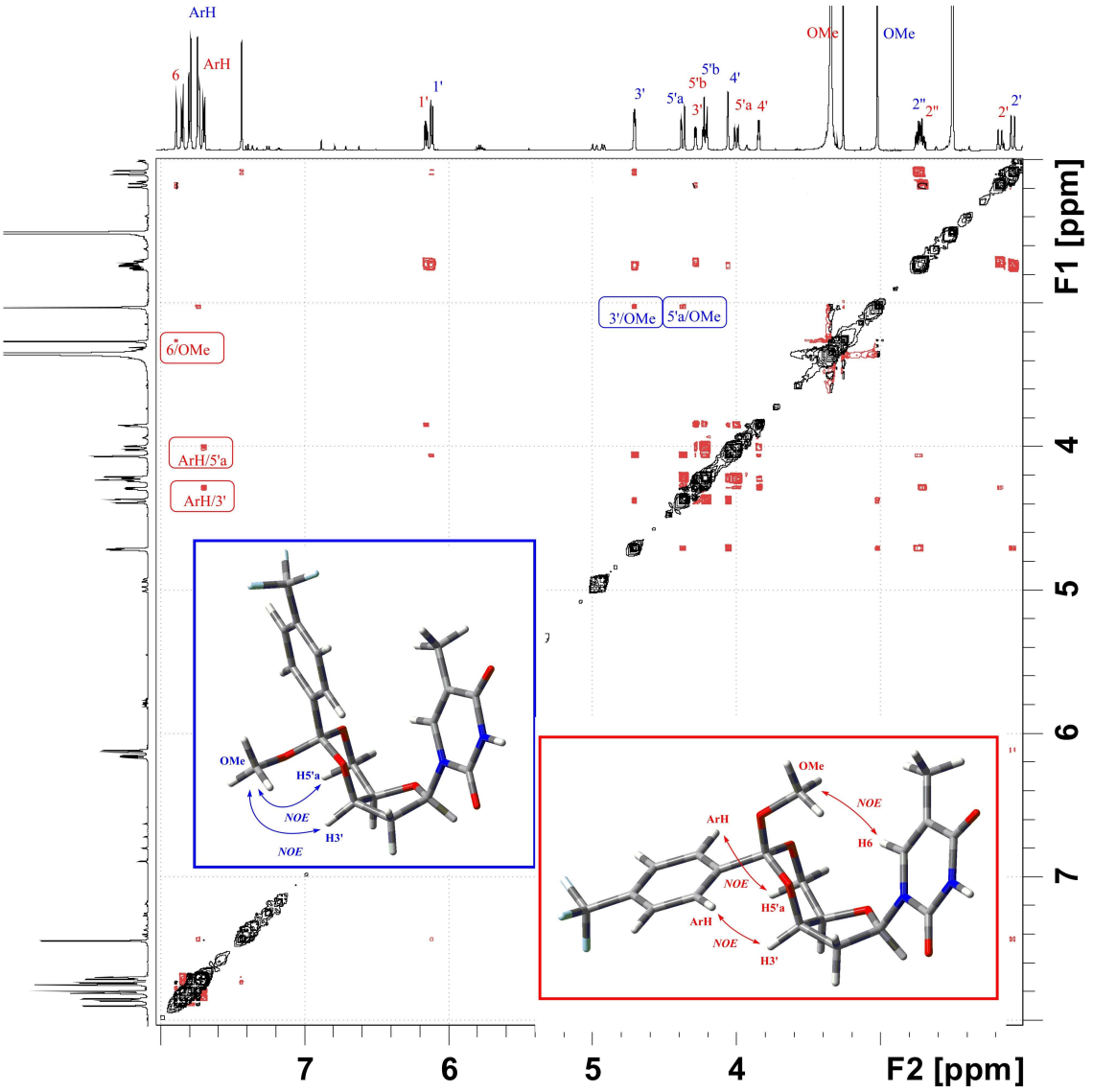

Figure 2S The 2D-H,H-ROESY spectrum of compound **9i**. The preferred conformations of 6R- and 6S-epimer are shown in blue and red frames. The NOE cross-peaks used for the structural assignment of both epimers are marked with corresponding color frames.

2. Backbone NMR chemical shift perturbations obtained for cdN complexes.

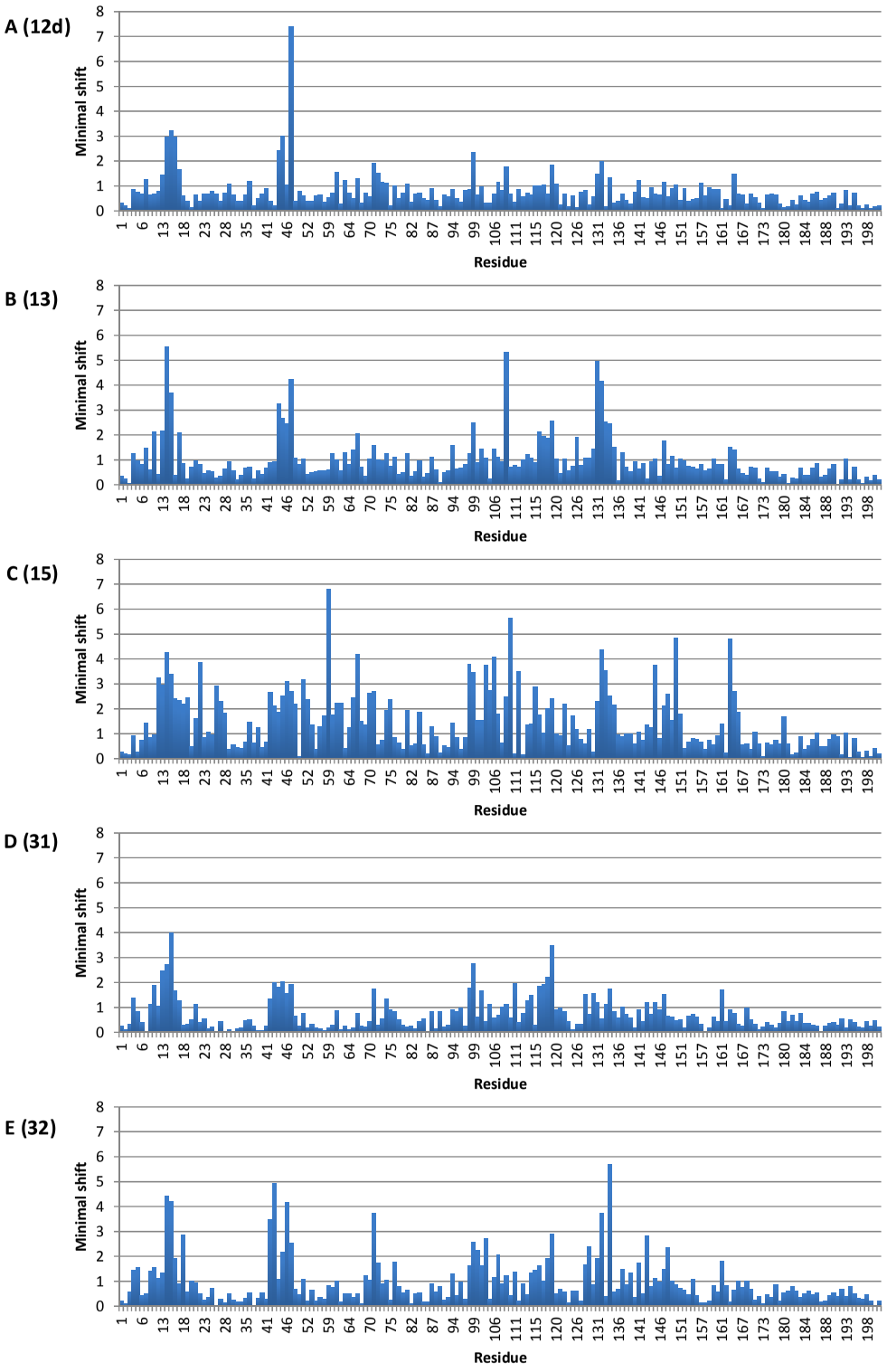

Figure 3S Graphs summarizing the cdN backbone (H^N, N) minimal shift values obtained for compounds **12d** (A), **13** (B), **15** (C), **31** (D) and **32** (E).

3. Calculated distances of metal ion and coordinating atoms in 3 different structures.

Table 2S. Calculated distances in Å of metal ion and coordinating atoms

PDB code	4L6C	4NFL	4MWO
metal atom	Mg	Mg	K
Asp41	1.99	2.10	2.49
Asp43	2.01	2.02	2.28
Asp176	2.10	2.05	2.35
water	2.11	2.00	2.44
water	1.99	2.10	2.37
phosphate ion	2.08	2.09	2.33

4. Selectivity graph of measured compounds

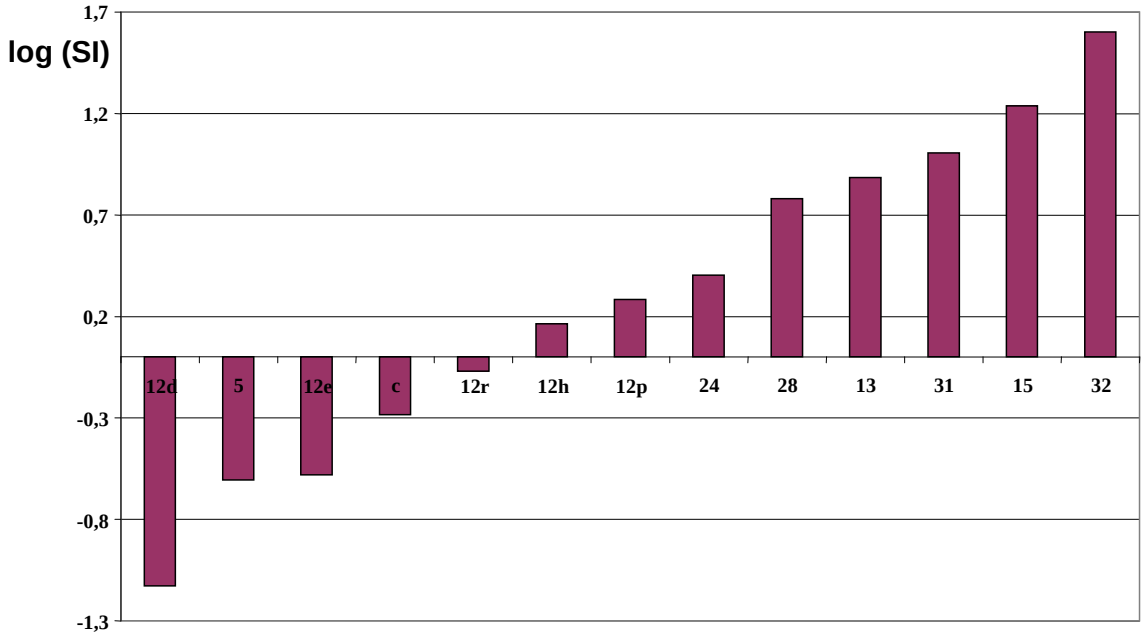

Figure 4S Graphs summarizing the range of selectivity index of measured compounds. **c** stands for mono-ethyl ester of **12d**.

5. Crystal Data and Diffraction Data Collection and Refinement Statistics

Table 3S. Crystal Data and Diffraction Data Collection and Refinement Statistics

Data collection statistics			
Compound	**12d**	**12e**	**13**
PDB code	4L6C	4NFL	4MWO
Space group	$P4_32_12$	$P4_32_12$	$P4_32_12$
Cell parameters (Å, º)	a = b = 73.82 c = 105.49 α = β = γ= 90	a = b = 73.65 c = 105.91 α = β = γ= 90	a = b = 73.97 c = 105.95 α = β = γ= 90
Number of molecules in AU	1	1	1
Wavelength (Å)	0.915	0.978	0.918
Resolution (Å)	34.84-1.78 (1.82-1.78)	46.75-1.36 (1.45-1.36)	46.90-1.67 (1.77-1.67)
Number of unique reflections	27,276 (2,751)	56,940 (6,176)	34,275 (4991)
Multiplicity	16.1 (16.0)	7.6 (4.3)	7.5 (5.1)
Completeness (%)	100.0 (100.0)	90.9 (61.9)	98.3 (90.1)
R_{meas}[a]	7.9 (63.4)	4.3 (75.8)	7.1 (56.1)
Average $I/\sigma(I)$	33.3 (4.0)	26.1 (1.91)	21.0 (3.0)
Wilson B ($Å^2$)[b]	30.8	22.5	26.2
Refinement statistics			
Resolution range (Å)	34.84-1.80	46.78-1.37	46.90-1.67
No. of reflections in working set	26,199	53,953	32,559
No. of reflections in test set	1,382	2,886	1,714
R value (%)[b]	15.1	16.1	16.3
R_{free} value (%)[c]	17.2	18.8	19.1
RMSD bond length (Å)	0.021	0.018	0.019
RMSD angle (º)	2.120	2.120	2.059
Number of atoms in AU	1,949	2142	1,933
Number of protein atoms in AU	1,688	1701	1,634
Number of water molecules in AU	176	305	234
Mean B value ($Å^2$)	26.8	22.2	20.0
Ramachandran plot statistics[d]:			
Residues in favored regions (%)	98.5	97.3	97.2
Residues in allowed regions (%)	1.5	2.7	2.8

The data in parentheses refer to the highest-resolution shell.

[a] R_{meas} defined in ref. [1]. [b] Wilson B by Sfcheck program from CCP4 suite.[2, 3] [b] R-value = $||F_o| - |F_c||/|F_o|$, where F_o and F_c are the observed and calculated structure factors, respectively. [c] R_{free} is equivalent to the R value but is calculated for 5% of the reflections chosen at random and omitted from the refinement process.[4] [d] as determined by MolProbity.[5]

6. Experimental Section

Preparation of nucleoside orthoesters - general procedures.

Unless otherwise stated, all used solvents were anhydrous. TLC was performed on TLC plates precoated with silica gel (silica gel/TLC-cards, UV 254, Merck) using the following mobile phases: C-1 (chloroform-ethanol 19:1), T-1 (toluene-acetone 1:2), IPAV (isopropanol-25% ammonia-water 7:1:2). Compounds were detected using UV light (254 nm), spraying with a 1% solution of 4-(4-nitrobenzyl) pyridine in ethanol followed by heating and treating with gaseous ammonia (for the detection of alkylating agents, such as mesyl derivatives and phosphonic acid diesters; blue color). The purity of the final compounds was greater than 95% as determined by LC-MS using a Waters AutoPurification System with 2545 Quarternary Gradient Module and 3100 Single Quadrupole Mass Detector using Luna C18 column (100 x 4.6 mm, 3 µm, Phenomenex) at a flow rate of 1 ml/min. The A, B, and C mobile phases were used representing 50 mM NH_4HCO_3, 50 mM NH_4HCO_3 in aq. 50% CH_3CN, and CH_3CN, respectively (A→B over 10 min, B→C over 10 min, and C for 5 min). Preparative RP HPLC was performed on an LC5000 Liquid Chromatograph (INGOS-PIKRON, CR) using a Luna C18 (2) column (Axia 250 x 21.2 mm, 5 µm, Phenomenex) at a flow rate of 10 ml/min. A gradient elution of methanol in 0.1 M TEAB (pH 7.5) (A, 0.1 M TEAB; B, 0.1 M TEAB in aq. 50% methanol; C, methanol) was used. All final compounds were lyophilised. The mass spectra were collected on an LTQ Orbitrap XL (Thermo Fisher Scientific) using ESI ionization. The phosphorus content in the compounds was determined using a simultaneous energy-dispersive X-ray fluorescence spectrometer SPECTRO iQ II. NMR spectra were collected in DMSO-d_6 or D_2O on a Bruker AVANCE 600 (^{1}H at 600.13 MHz, ^{13}C at 150.9 MHz). Chemical shifts (in ppm, δ scale) were referenced to the residual DMSO-d_6 signal (2.5 ppm for ^{1}H and 39.7 ppm for ^{13}C) or to the 1,4-dioxane signal (3.75 ppm for ^{1}H and 69.3 ppm for ^{13}C) as an internal standard in D_2O. Coupling constants (*J*) are given in Hz. The complete assignment of ^{1}H and ^{13}C signals was performed by an analysis of the correlated homonuclear H,H-COSY and heteronuclear H,C-HSQC and H,C-HMBC spectra. The relative

configuration of the compounds was checked using 2D-H,H-ROESY experiments. The numbering for signal assignment is shown below. UV-VIS spectra were collected on spectrophotometer CARY 100 Bio UV Spectrophotometer (Varian Inc.), samples were disolved in 50% MeOH. The purity of the final compounds was greater than 95%.

Method A

A solution of xyloT **7** (dried by co-evaporation with DMF) and trimethyl orthobenzoate (2-3 eq.) in dry DMF (0.1M solution) was carefully acidified with the 10M HCl in dry Et_2O (slightly red color on a wet pH paper), and the mixture was stirred under an argon atmosphere at r.t. for 12 h (TLC in H-3). The reaction was quenched by the addition of Et_3N and then Amberlyst A21 was added, the suspension was filtered and concentrated under reduced pressure. The resulting crude nucleoside orthoester **9** was purified on a silica gel column by elution with a linear gradient of chloroform in toluene (0-100%) followed by ethanol in chloroform (0-10%).

Method B

A solution of xyloT **7** and trimethyl orthobenzoate (2-3 eq.) dried by co-evaporation with DMF in dry DMF (0.1M solution) was treated with pyridinium tosylate (6 eq.) under an argon atmosphere and the mixture was stirred at r.t. overnight (TLC in H-3). The reaction was quenched by the addition of solid Na_2CO_3 and the suspension was concentrated under reduced pressure. Residue was extracted between $CHCl_3$ and saturated solution of Na_2CO_3. The organic layer was dried with anhydrous K_2CO_3, filtered and concentrated under reduced pressure. The resulting crude nucleoside orthoester **9** was purified on a silica gel column by elution with a linear gradient of chloroform in toluene (0-100%) followed by ethanol in chloroform (0-10%).

Preparation of nucleoside diethyl phosphonates

Method C

Orthoester **9** dried by co-evaporation with acetonitrile was treated with diethyl chlorophosphite (2 eq) in dry acetonitrile (0.05M solution) in argon atmosphere at -40 °C. The heterogenous mixture was stirred under slowly increased temperature to 0 °C (TLC in T-1).

When finish, the reaction mixture was cooled to -40 °C and 1M TEAB in 50% EtOH was added and the solution was concentrated *in vacuo*. The obtained crude phosphonate **11** was purified by silica gel chromatography (elution with a linear gradient of EtOH in $CHCl_3$).

Preparation of nucleoside phosphonic acids

Method D

Diethyl phosphonate **11** dried by co-evaporation with acetonitrile was treated under an argon atmosphere with bromotrimethylsilane (10 eq.) and 2,6-lutidine (4 eq.) in dry MeCN (0.05M solution) at r.t. overnight (TLC IPAV). When finished, the reaction was concentrated *in vacuo*, the residue was treated shortly with 2M TEAB and the obtained solution was evaporated to dryness. The residue was co-evaporated with EtOH and purified by the preparative HPLC on the C18 column using a linear gradient of methanol in 0.1M TEAB. buffer. The product was converted into the sodium salt on Dowex 50 in Na^+ form.

(*R*)-1-[2-Deoxy-3,5-*O*-(4-fluoro-methoxybenzylidene)-β-D-threo-pentofuranosyl]thymine (9a)

Compound **9a** was prepared from orthoester **6a** (248 mg, 1.24 mmol) and **7** (0.1 g, 0.4 mmol) using Method A as colorless oil in a yield of 130 mg (83%). HRMS $(M+Na)^-$ for $C_{18}H_{19}O_6N_2FNa$: calcd. *m/z* 401.11194, found 401.11176; IR ($CHCl_3$, cm^{-1}) 3397, 3087, 2931, 2855, 2839, 1702, 1684, 1612, 1605, 1512, 1471, 1449, 1435, 1407, 1384, 1360, 1320, 1306, 1297, 1272, 1239, 1194, 1157, 1133, 1111, 1094, 1078, 1056, 1040, 1016, 996, 981, 956, 945, 916, 844. ^{1}H NMR (d_6DMSO) δ 11.22 (br s, NH), 7.55 (m, 2x ArH), 7.50 (q, *J* = 1.2 Hz, H6), 7.24 (m, 2x ArH), 6.13 (dd, *J* = 8.4 and 1.8 Hz, H1´), 4.67 (br dd, *J* = 4.9, 2.6 and <1 Hz, H3´), 4.33 (dd, *J* = 13.0 and 2.2 Hz, H5´a), 4.17 (dd, *J* = 13.0 and 1.0 Hz, H5´b), 4.03 (ddd, *J* = 2.6, 2.2 and 1.0 Hz, H4´), 3.00 (s, OCH_3), 2.72 (ddd, *J* = 15.1, 8.4 and 4.9 Hz, H2´´), 2.06 (br dd, *J* = 15.1, 1.8 and <1 Hz, H2´), 1.17 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.88 (C4), 162.66 (d, *J* = 245.6 Hz, Ar-C), 150.62 (C2), 136.65 (C6), 133.83 (d, *J* = 3.0 Hz, Ar-C), 128.68 (d, *J* = 8.4 Hz, 2x Ar-CH), 115.17 (d, *J* = 21.6 Hz, 2x Ar-CH),

109.94 (>C<), 108.48 (C5), 83.86 (C1´), 74.05 (C4´), 69.38 (C3´), 59.71 (C5´), 50.92 (OCH_3), 39.70 (C2´), 11.89 (5-CH_3).

(*R*)-1-[2-Deoxy-3,5-*O*-(4-chloro-methoxybenzylidene)-β-D-threo-pentofuranosyl]thymine (9b)

Compound **9b** was prepared from orthoester **6b** (1.34 g, 6.2 mmol) and nucleoside **7** (0.5 g, 2 mmol) using Method A as colorless oil in a yield of 697 mg (86%). HRMS (M-Na)- for $C_{18}H_{19}O_6N_2ClNa$: calcd. *m/z* 417.08239, found 417.08221; IR ($CHCl_3$, cm^{-1}) 3397, 3175, 2946, 2930, 2855, 2839, 1684, 1602, 1492, 1470, 1449, 1434, 1402, 1383, 1360, 1319, 1306, 1298, 1272, 1240, 1194, 1176, 1133, 1112, 1093, 1078, 1055, 1041, 1016, 996, 981, 956, 950, 917, 840. ^{1}H NMR (d_6DMSO) δ 11.22 (br s, NH), 7.52 (m, 2x ArH), 7.48 (q, *J* = 1.2 Hz, H6), 7.48 (m, 2x ArH), 6.12 (dd, *J* = 8.3 and 1.8 Hz, H1´), 4.67 (br dd, *J* = 4.8, 2.6 and <1 Hz, H3´), 4.34 (dd, *J* = 13.0 and 2.2 Hz, H5´a), 4.17 (dd, *J* = 13.0 and 1.0 Hz, H5´b), 4.03 (ddd, *J* = 2.6, 2.2 and 1.0 Hz, H4´), 3.00 (s, OCH_3), 2.72 (ddd, *J* = 15.1, 8.3 and 4.8 Hz, H2´´), 2.07 (br dd, *J* = 15.1, 1.8 and <1 Hz, H2´), 1.16 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.86 (C4), 150.59 (C2), 136.59 (C6), 136.38 (Ar-C), 134.14 (Ar-C), 128.40 (2x Ar-CH), 128.28 (2x Ar-CH), 109.86 (>C<), 108.41 (C5), 83.93 (C1´), 74.08 (C4´), 69.40 (C3´), 59.74 (C5´), 50.96 (OCH_3), 39.70 (C2´), 11.86 (5-CH_3).

(*R*)-1-[2-Deoxy-3,5-*O*-(4-bromo-methoxybenzylidene)-β-D-threo-pentofuranosyl]thymine (9c)

Compound **9c** was prepared from orthoester **6c** (1.74 g, 6.6 mmol) and nucleoside **7** (0.5 g, 2 mmol) using Method A as colorless oil in a yield of 427 mg (47%). HRMS $(M+Na)^-$ for $C_{18}H_{19}O_6N_2BrNa$: calcd. *m/z* 461.03187, found 461.03190; IR ($CHCl_3$, cm^{-1}) 3397, 3174, 2946, 2931, 2931, 2855, 2839, 1684, 1596, 1485, 1470, 1449, 1434, 1397, 1383, 1360, 1319, 1306, 1299, 1272, 1240, 1194, 1177, 1132, 1111, 1095, 1079, 1055, 1040, 1012, 996, 981, 956, 950, 917, 838. ^{1}H NMR (d_6DMSO) δ 11.23 (br s, NH), 7.62 (m, 2x ArH), 7.48 (q, *J* = 1.2 Hz, H6), 7.45 (m, 2x ArH), 6.11 (dd, *J* = 8.3 and 1.8 Hz, H1´), 4.67 (br dd, *J* = 4.8, 2.6 and <1 Hz, H3´), 4.34 (dd, *J* = 13.0 and 2.2 Hz, H5´a), 4.17 (dd, *J* = 13.0 and 1.0 Hz, H5´b), 4.03

(ddd, *J* = 2.6, 2.2 and 1.0 Hz, H4´), 3.00 (s, OCH_3), 2.72 (ddd, *J* = 15.1, 8.3 and 4.8 Hz, H2´´), 2.06 (br dd, *J* = 15.1, 1.8 and <1 Hz, H2´), 1.16 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.83 (C4), 150.55 (C2), 136.73 (Ar-C), 136.54 (C6), 131.30 (2x Ar-CH), 128.52 (2x Ar-CH), 122.79 (Ar-C), 109.89 (>C<), 108.35 (C5), 83.92 (C1´), 74.06 (C4´), 69.36 (C3´), 59.69 (C5´), 50.92 (OCH_3), 39.70 (C2´), 11.81 (5-CH_3).

(*R*)-1-[2-Deoxy-3,5-*O*-(4-iodo-methoxybenzylidene)-β-D-threo-pentofuranosyl]thymine (9d)

Compound **9d** was prepared from orthoester **6d** (632 mg, 2.05 mmol) and nucleoside **7** (0.1 g, 0.41 mmol) using Method A as colorless oil in a yield of 175 mg (88%). HRMS $(M-H)^-$ for $C_{18}H_{20}O_6N_2I$: calcd *m/z* 487.03606, found 487.03598; IR ($CHCl_3$, cm^{-1}) 3397, 3177, 3089, 2841, 1700, 1684, 1592, 1486, 1470, 1450, 1435, 1406, 1392, 1382, 1361, 1306, 1300, 1272, 1182, 1133, 1112, 1079, 1060, 1008, 954, 686. ^{1}H NMR (d_6DMSO) δ 11.24 (br s, NH), 7.78 (m, 2x ArH), 7.45 (q, *J* = 1.2 Hz, H6), 7.29 (m, 2x ArH), 6.11 (dd, *J* = 8.3 and 1.8 Hz, H1´), 4.66 (dd, *J* = 4.8 and 2.6 Hz, H3´), 4.33 (dd, *J* = 13.1 and 2.2 Hz, H5´a), 4.17 (dd, *J* = 13.1 and 1.1 Hz, H5´b), 4.05 (ddd, *J* = 2.6, 2.2 and 1.1 Hz, H4´), 3.00 (s, OCH_3), 2.71 (ddd, *J* = 15.0, 8.3 and 4.2 Hz, H2´´), 2.06 (dd, *J* = 15.0 and 1.8 Hz, H2´), 1.15 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.84 (C4), 150.55 (C2), 137.17 (2x Ar-CH), 137.09 (Ar-C), 136.56 (C6), 128.49 (2x Ar-CH), 110.02 (>C<), 108.35 (C5), 96.16 (Ar-C), 83.94 (C1´), 74.08 (C4´), 69.33 (C3´), 59.66 (C5´), 50.93 (OCH_3), 39.38 (C2´), 11.82 (5-CH_3).

(*R*)-1-[2-Deoxy-3,5-*O*-(4-nitro-methoxybenzylidene)-β-D-threo-pentofuranosyl]thymine (9e)

Compound **9e** was prepared from orthoester **6e** (469 mg, 2.05 mmol) and nucleoside **7** (0.1 g, 0.41 mmol) using Method A as colorless oil in a yield of 155 mg (93%). HRMS $(M-H)^-$ for $C_{18}H_{20}N_3O_8$: calcd *m/z* 406.12449, found 406.12471; IR ($CHCl_3$, cm^{-1}) 3396, 3174, 3087, 2841, 1703, 1685, 1610, 1528, 1470, 1450, 1435, 1408, 1383, 1355, 1318, 1304, 1296, 1273, 1133, 1115, 1108, 1096, 1082, 1016, 964, 955, 835, 687, 414. ^{1}H NMR ($CDCl_3$) δ 8.60 (br s, NH), 7.53 (q, *J* = 1.2 Hz, H6), 8.24 (m, 2x ArH), 7.76 (m, 2x ArH), 6.24 (dd, *J* = 8.0 and 1.7

Hz, H1´), 4.79 (br dd, *J* = 4.8, 2.6 and <1 Hz, H3´), 4.47 (dd, *J* = 13.0 and 2.3 Hz, H5´a), 4.34 (dd, *J* = 13.0 and 1.0 Hz, H5´b), 4.02 (ddd, *J* = 2.6, 2.3 and 1.0 Hz, H4´), 3.08 (s, OCH_3), 2.70 (ddd, *J* = 15.4, 8.0 and 4.8 Hz, H2´´), 2.28 (br dd, *J* = 15.4, 1.7 and <1 Hz, H2´); 1.29 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR ($CDCl_3$) δ 163.58 (C4), 163.44 (Ar-C), 150.18 (C2), 143.22 (Ar-C), 136.35 (C6), 127.52 (2x Ar-CH), 123.34 (2x Ar-CH), 109.81 (>C<), 109.57 (C5), 84.79 (C1´), 74.84 (C4´), 69.35 (C3´), 59.96 (C5´), 51.30 (OCH_3), 40.20 (C2´), 12.00 (5-CH_3).

(*R*)-1-[2-Deoxy-3,5-*O*-(4-methoxycarbonyl-methoxybenzylidene)-β-D-threo-pentofuranosyl]thymine (9f)

Compound **9f** was prepared from orthoester **6f** (469 mg, 2.05 mmol) and **7** (0.1 g, 0.41 mmol) using Method A as colorless oil in 151 mg (87%). HRMS $(M-H)^-$ for $C_{20}H_{23}O_8N_2$: calcd *m/z* 419.14489, found 419.14501; IR ($CHCl_3$, cm^{-1}) 3397, 3170, 3089, 2955, 2841, 1720, 1701, 1684, 1619, 1581, 1511, 1470, 1449, 1438, 1408, 1383, 1306, 1298, 1282, 1178, 1133, 1115, 1080, 1019, 836, 712, 687. ^{1}H NMR (d_6DMSO) δ 11.24 (br s, NH), 8.00 (m, 2x ArH), 7.66 (m, 2x ArH), 7.48 (q, *J* = 1.2 Hz, H6), 6.12 (dd, *J* = 8.4 and 1.8 Hz, H1´), 4.70 (dd, *J* = 4.8 and 2.6 Hz, H3´), 4.37 (dd, *J* = 13.0 and 2.2 Hz, H5´a), 4.20 (dd, *J* = 13.0 and 1.1 Hz, H5´b), 4.05 (ddd, *J* = 2.6, 2.2 and 1.1 Hz, H4´), 3.87 (s, $COOCH_3$), 3.00 (s, OCH_3), 2.73 (ddd, *J* = 15.1, 8.4 and 4.8 Hz, H2´´), 2.09 (dd, *J* = 15.1 and 1.8 Hz, H2´), 1.10 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 166.04 (C=O), 163.83 (C4), 150.56 (C2), 142.01 (Ar-C), 136.51 (C6), 130.49 (Ar-C), 129.27 (2x Ar-CH), 126.75 (2x Ar-CH), 109.81 (>C<), 108.35 (C5), 83.94 (C1´), 74.10 (C4´), 69.45 (C3´), 59.76 (C5´), 52.54 (COO$\underline{C}H_3$), 50.98 (OCH_3), 40.00 (C2´), 11.86 (5-CH_3).

(*R*)-1-[2-Deoxy-3,5-*O*-(4-methylthio-methoxybenzylidene)-β-D-threo-pentofuranosyl]thymine (9g)

Compound **9g** was prepared from orthoester **6g** (282 mg, 1.24 mmol) and **7** (0.1 g, 0.41 mmol) using Method A as colorless oil in a yield of 90 mg (54%). HRMS $(M+Na)^-$ for $C_{19}H_{22}O_6N_2NaS$: calcd. *m/z* 429.10908, found 429.10881; IR ($CHCl_3$, cm^{-1}) 3396, 3178, 3087; 2956, 2946, 2927, 2899, 2855, 2838, 1684, 1603, 1494, 1470, 1449, 1435, 1401, 1383,

1360, 1319, 1307, 1302, 1273, 1240, 1194, 1184, 1133, 1113, 1099, 1078, 1036, 1022, 1015, 995, 981, 956, 948, 916, 837. ^{1}H NMR (d_6DMSO) δ 11.22 (br s, NH), 7.52 (q, *J* = 1.2 Hz, H6), 7.43 (m, 2x ArH), 7.28 (m, 2x ArH), 6.12 (dd, *J* = 8.3 and 1.8 Hz, H1´), 4.66 (dd, *J* = 4.9 and 2.6 Hz, H3´), 4.32 (dd, *J* = 13.0 and 2.2 Hz, H5´a), 4.16 (dd, *J* = 13.0 and 1.1 Hz, H5´b), 4.02 (ddd, *J* = 2.6, 2.2 and 1.1 Hz, H4´), 3.00 (s, OCH_3), 2.71 (ddd, *J* = 15.2, 8.3 and 4.9 Hz, H2´´), 2.47 (s, SCH_3), 2.05 (br dd, *J* = 15.2, 1.8 and <1 Hz, H2´), 1.18 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.83 (C4), 150.55 (C2), 139.70 (Ar-C), 136.64 (C6), 133.86 (Ar-C), 126.80 (2x Ar-CH), 125.43 (2x Ar-CH), 110.16 (>C<), 108.43 (C5), 83.84 (C1´), 74.08 (C4´), 69.23 (C3´), 59.59 (C5´), 50.84 (OCH_3), 39.38 (C2´), 14.70 (SCH_3), 11.87 (5-CH_3).

(*R*)-1-[2-Deoxy-3,5-*O*-(4-cyano-methoxybenzylidene)-β-D-threo-pentofuranosyl]thymine (9h)

Compound **9h** was prepared from orthoester **6h** (1 g, 4.8 mmol) and **7** (0.5 g, 2 mmol) using Method A as colorless oil in 423 mg (53%). HRMS $(M+Na)^-$ for $C_{19}H_{19}O_6N_3Na$: calcd. *m/z* 406.12449, found 406.12471; IR ($CHCl_3$, cm^{-1}) 3396, 3180, 2931, 2855, 2840; 2233, 1685 1605, 1509, 1504, 1470, 1441, 1406, 1383, 1360, 1346, 1319, 1303, 1273, 1239, 1194, 1133, 1097, 1055, 1042, 846. ^{1}H NMR (d_6DMSO) δ 11.23 (br s, NH), 7.91 (m, 2x ArH), 7.69 (m, 2x ArH), 7.43 (q, *J* = 1.2 Hz, H6), 6.11 (dd, *J* = 8.3 and 1.8 Hz, H1´), 4.70 (dd, *J* = 4.8 and 2.6 Hz, H3´), 4.36 (dd, *J* = 13.1 and 2.2 Hz, H5´a), 4.21 (dd, *J* = 13.1 and 1.0 Hz, H5´b), 4.05 (ddd, *J* = 2.6, 2.2 and 1.0 Hz, H4´), 2.73 (ddd, *J* = 15.1, 8.3 and 4.8 Hz, H2´´), 2.09 (br dd, *J* = 15.1, 1.8 and <1 Hz, H2´), 1.12 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.80 (C4), 150.53 (C2), 142.12 (Ar-C), 136.36 (C6), 132.52 (2x Ar-CH), 127.30 (2x Ar-CH), 118.62 (CN), 112.28 (Ar-C), 109.48 (>C<), 108.33 (C5), 83.94 (C1´), 74.02 (C4´), 69.54 (C3´), 59.83 (C5´), 51.02 (OCH_3), 39.70 (C2´), 11.85 (5-CH_3).

(*R,S*)-1-[2-Deoxy-3,5-*O*-(4-trifluoromethyl-methoxybenzylidene)-β-D-threo-pentofuranosyl]thymine (9i)

Compound **9i** was prepared from orthoester **6i** (0.715 g, 2 mmol) and **7** (0.25 g, 1 mmol) using Method B as colorless oil in a yield of 101 mg (23%). Ratio of *R*/*S* epimers is 58:42.

HRMS $(M+Na)^-$ for $C_{19}H_{19}O_6N_2F_3Na$: calcd. *m/z* 451,10874, found 451,10838; IR ($CHCl_3$, cm^{-1}) 3172, 3041, 2925, 2853, 1688, 1519, 1469, 1410, 1380, 1364, 1328, 1299, 1196, 1169, 1133, 1105, 1065, 846. ^{1}H NMR (d_6DMSO) δ 11.23 (br s, NH), 7.80 (m, 2x ArH), 7.74 (m, 2x ArH), 7.44 (q, *J* = 1.2 Hz, H6), 6.12 (dd, *J* = 8.3 and 1.8 Hz, H1´), 4.71 (br dd, *J* = 4.9, 2.5 and <1 Hz, H3´), 4.37 (dd, *J* = 13.1 and 2.1 Hz, H5´a), 4.21 (dd, *J* = 13.1 and 1.2 Hz, H5´b), 4.06 (ddd, *J* = 2.5, 2.1 and 1.2 Hz, H4´), 3.02 (s, OCH_3), 2.73 (ddd, *J* = 15.1, 8.3 and 4.9 Hz, H2´´), 2.08 (br dd, *J* = 15.1, 1.8 and <1 Hz, H2´), 1.05 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.79 (C4), 150.54 (C2), 141.59 (Ar-C), 136.44 (C6), 129.89 (q, *J* = 31.8 Hz, Ar-C), 127.25 (2x Ar-CH), 125.37 (q, *J* = 3.6 Hz, 2x Ar-CH), 124.24 (q, *J* = 272.2 Hz, CF_3), 109.58 (>C<), 108.26 (C5), 83.97 (C1´), 74.08 (C4´), 69.48 (C3´), 59.74 (C5´), 51.01 (OCH_3), 39.36 (C2´), 11.59 (5-CH_3).

(*R*)-1-[2-Deoxy-3,5-*O*-(4-methoxy-methoxybenzylidene)-β-D-threo-pentofuranosyl]thymine (9j)

Compound **9j** was prepared from orthoester **6j** (180 mg, 0.52 mmol) and **7** (50 mg, 0.21 mmol) using Method B as colorless oil in a yield of 58 mg (70%). HRMS $(M+Na)^-$ for $C_{19}H_{22}O_7N_2Na$: calcd. *m/z* 413.13192, found 413.13186; IR ($CHCl_3$, cm^{-1}) 3397, 3177, 2840; 1703, 1684, 1614, 1587, 1515, 1468, 1407, 1383, 1373, 1362, 1307, 1302, 1197, 1181, 1133, 1097, 1077, 1045, 841. ^{1}H NMR (d_6DMSO) δ 11.22 (br s, NH), 7.55 (q, *J* = 1.2 Hz, H6), 7.43 (m, 2x ArH), 6.94 (m, 2x ArH), 6.13 (dd, *J* = 8.4 and 1.9 Hz, H1´), 4.65 (br dd, *J* = 5.0, 2.6 and <1 Hz, H3´), 4.31 (dd, *J* = 13.0 and 2.2 Hz, H5´a), 4.14 (dd, *J* = 13.0 and 1.0 Hz, H5´b), 4.00 (ddd, *J* = 2.6, 2.2 and 1.0 Hz, H4´), 3.76 (s, OCH_3), 2.99 (s, OCH_3), 2.71 (ddd, *J* = 15.2, 8.4 and 5.0 Hz, H2´´), 2.03 (br dd, *J* = 15.2, 1.9 and <1 Hz, H2´), 1.20 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.87 (C4), 159.90 (Ar-C), 150.60 (C2), 136.77 (C6), 129.58 (Ar-C), 127.70 (2x Ar-CH), 113.50 (2x Ar-CH), 110.35 (>C<), 108.53 (C5), 83.78 (C1´), 74.08 (C4´), 69.19 (C3´), 59.55 (C5´), 55.40 (OCH_3), 50.79 (OCH_3), 39.39 (C2´), 11.93 (5-CH_3).

(*R,S*)-1-[2-Deoxy-3,5-*O*-(4-vinyl-methoxybenzylidene)-β-D-threo-pentofuranosyl]thymine (9k)

Compound **9k** was prepared from orthoester **6i** (1.95 g, 6.19 mmol) and **7** (0.5 g, 2 mmol) using Method B as colorless oil in a yield of 515 mg (65%). Ratio of *R*/*S* epimers is 77:23. HRMS $(M+Na)^-$ for $C_{20}H_{22}O_6N_2Na$: calcd. *m/z* 409.13701, found 409.13699; IR (KBr, cm^{-1}) 3436, 3089, 3045, 2837, 1684, 1571, 1511, 1472, 14021362, 1276, 1195, 1134, 912. ^{1}H NMR (d_6DMSO) δ 11.12 (br s, NH), 7.53 (q, *J* = 1.2 Hz, H6), 7.50 (m, 2x ArH), 7.49 (m, 2x ArH), 6.75 (dd, *J* = 17.7 and 10.9 Hz, -CH=), 6.12 (dd, *J* = 8.4 and 1.8 Hz, H1´), 5.87 (dd, *J* = 17.7 and 1.0 Hz, =CHaHb), 5.30 (dd, *J* = 10.9 and 1.0 Hz, =CHaHb), 4.67 (br dd, *J* = 4.9, 2.6 and <1 Hz, H3´), 4.35 (dd, *J* = 13.1 and 2.3 Hz, H5´a), 4.16 (dd, *J* = 13.1 and 1.0 Hz, H5´b), 4.03 (ddd, *J* = 2.6, 2.3 and 1.0 Hz, H4´), 3.01 (s, OCH_3), 2.72 (ddd, *J* = 15.1, 8.4 and 4.9 Hz, H2´´), 2.08 (br dd, *J* = 15.1, 1.8 and <1 Hz, H2´), 1.14 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 164.64 (C4), 151.09 (C2), 138.68 (Ar-C), 138.79 (Ar-C), 137.56 (C6), 136.72 (-CH=), 127.10 (2x Ar-CH), 126.53 (2x Ar-CH), 116.17 (=CH_2), 110.69 (>C<), 109.07 (C5), 84.65 (C1´), 74.90 (C4´), 69.76 (C3´), 60.15 (C5´), 51.42 (OCH_3), 39.91 (C2´), 12.27 (5-CH_3).

(*R,S*)-1-[2-Deoxy-3,5-*O*-(4-phenyl-methoxybenzylidene)-β-D-threo-pentofuranosyl]thymine (9l)

Compound **9l** was prepared from orthoester **6l** (756 mg, 2 mmol) and **7** (0.25 g, 1 mmol) using Method B as colorless oil in a yield of 210 mg (48%). Ratio of *R*/*S* epimers is 72:28. HRMS $(M+Na)^-$ for $C_{24}H_{24}O_6N_2Na$: calcd. *m/z* 459.15266, found 459.15244; IR ($CHCl_3$, cm-1) 3397, 3173, 3080, 3060, 2955, 2946, 2929, 2875, 2855, 2840, 1684, 1615, 1601, 1567, 1486, 1470, 1449, 1434, 1401, 1383, 1362, 1347, 1305, 1272, 1254, 1241, 1194, 1133, 1117, 1097, 1078, 1048, 1037, 1025, 1020, 1009, 995, 981, 957, 916, 846. ^{1}H NMR (d_6DMSO) δ 11.23 (br s, NH), 7.70 (m, 2x ArH), 7.66 (m, 2x ArH), 7.60 (m, 2x ArH), 7.55 (q, *J* = 1.2 Hz, H6), 7.48 (m, 2x ArH), 7.39 (m, ArH), 6.14 (dd, *J* = 8.5 and 1.8 Hz, H1´), 4.70 (br dd, *J* = 4.9, 2.6 and <1 Hz, H3´), 4.36 (dd, *J* = 13.0 and 2.3 Hz, H5´a), 4.19 (dd, *J* = 13.0 and 1.0 Hz, H5´b), 4.04 (ddd, *J* = 2.6, 2.3 and 1.0 Hz, H4´), 3.06 (s, OCH_3), 2.74 (ddd, *J* = 15.2, 8.5 and

4.9 Hz, H2´´), 2.08 (br dd, *J* = 15.2, 1.8 and <1 Hz, H2´), 1.16 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.84 (C4), 150.58 (C2), 141.15 (Ar-C), 139.78 (Ar-C), 136.67 (C6), 136.45 (Ar-C), 129.22 (2x Ar-CH), 127.00 (Ar-CH), 126.96 (2x Ar-CH), 126.89 (2x Ar-CH), 126.60 (2x Ar-CH), 110.22 (>C<), 108.49 (C5), 83.86 (C1´), 74.11 (C4´), 69.30 (C3´), 59.62 (C5´), 50.96 (OCH_3), 39.20 (C2´), 11.81 (5-CH_3).

(*R,S*)-1-[2-Deoxy-3,5-*O*-(3-phenyl-methoxybenzylidene)-β-D-threo-pentofuranosyl]thymine (9m)

Compound **9m** was prepared from orthoester **6m** (635 mg, 1.74 mmol) and **7** (235 mg, 0.97 mmol) using Method B as colorless oil in a yield of 257 mg (61%). Ratio of *R*/*S* epimers is 75:25. HRMS $(M+Na)^-$ for $C_{24}H_{24}O_6N_2Na$: calcd. *m/z* 459,15266, found 459,15256; IR ($CHCl_3$, cm-1) 3397, 3175, 3066, 2963, 2947, 2930, 2893, 2858, 2839, 1685, 1597, 1576, 1470, 1454, 1434, 1419, 1408, 1383, 1366, 1346, 1300, 1271, 1233, 1194, 1133, 1120, 1077, 1060, 1037, 995, 982, 957, 917, 841. ^{1}H NMR (d_6DMSO) δ 11.18 (br s, NH), 7.75 (m, ArH), 7.72 (m, 2x ArH), 7.68 (m, ArH), 7.61 (m, 2x ArH), 7.55 (q, *J* = 1.2 Hz, H6), 7.47 (m, 2x ArH), 7.38 (m, ArH), 6.15 (dd, *J* = 8.4 and 1.9 Hz, H1´), 4.71 (br dd, *J* = 4.9, 2.6 and <1 Hz, H3´), 4.37 (dd, *J* = 13.0 and 2.3 Hz, H5´a), 4.20 (dd, *J* = 13.0 and 1.0 Hz, H5´b), 4.04 (ddd, *J* = 2.6, 2.3 and 1.0 Hz, H4´), 3.06 (s, OCH_3), 2.73 (ddd, *J* = 15.2, 8.4 and 4.9 Hz, H2´´), 2.10 (br dd, *J* = 15.2, 1.9 and <1 Hz, H2´), 1.02 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.78 (C4), 150.59 (C2), 140.28 (Ar-C), 140.00 (Ar-C), 138.10 (Ar-C), 136.72 (C6), 129.22 (2x Ar-CH), 129.11 (Ar-CH), 127.85 (Ar-CH), 127.70 (Ar-CH), 127.08 (Ar-CH), 126.88 (2x Ar-CH), 124.38 (Ar-CH), 110.19 (>C<), 108.48 (C5), 83.77 (C1´), 74.04 (C4´), 69.37 (C3´), 59.64 (C5´), 51.00 (OCH_3), 39.00 (C2´), 11.61 (5-CH_3).

(*R,S*)-1-[2-Deoxy-3,5-*O*-(3-iodo-methoxybenzylidene)-β-D-threo pentofuranosyl]thymine (9n)

Compound **9n** was prepared from orthoester **6n** (226 mg, 0.93 mmol) and **7** (664 mg, 1.6 mmol) using Method B as colorless oil in a yield of 136 mg (30%). Ratio of *R*/*S* epimers is

57:43. HRMS $(M+Na)^-$ for $C_{18}H_{19}O_6N_2I$: calcd. *m/z* 509,01800, found 509,01780; IR (KBr, cm^{-1}) 3176, 3062, 3042, 2835, 1687, 1568, 1470, 1409, 1380, 1361, 1303, 1289, 1276, 1240, 1078, 1043, 999, 983,833. ^{1}H NMR (d_6DMSO) δ 11.10 (br s, NH), 7.82 (t, *J* = 1.2 Hz, ArH), 7.80 (ddd, *J* = 7.8, 1.2 and 1.0 Hz, ArH), 7.52 (ddd, *J* = 7.8, 1.2 and 1.0 Hz, ArH), 7.48 (q, *J* = 1.2 Hz, H6), 7.24 (t, *J* = 7.8 Hz, ArH), 6.13 (dd, *J* = 8.4 and 1.8 Hz, H1´), 4.67 (br dd, *J* = 4.8, 2.6 and <1 Hz, H3´), 4.33 (dd, *J* = 13.0 and 2.2 Hz, H5´a), 4.17 (dd, *J* = 13.0 and 1.0 Hz, H5´b), 4.02 (ddd, *J* = 2.6, 2.2 and 1.0 Hz, H4´), 3.00 (s, OCH_3), 2.72 (ddd, *J* = 15.2, 8.4 and 4.8 Hz, H2´´), 2.09 (br dd, *J* = 15.2, 1.8 and <1 Hz, H2´); 1.22 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.79 (C4), 150.58 (C2), 139.66 (Ar-C), 138.17 (Ar-CH), 136.55 (C6), 134.68 (Ar-CH), 130.69 (Ar-CH), 125.77 (Ar-CH), 109.37 (>C<), 108.49 (C5), 94.72 (Ar-C), 83.79 (C1´), 73.96 (C4´), 69.46 (C3´), 59.71 (C5´), 51.01 (OCH_3), 39.22 (C2´), 12.03 (5-CH_3).

(*R*,*S*)-1-[2-Deoxy-3,5-*O*-(3-bromo-5-iodo-methoxybenzylidene)-β-D-threo-pentofuranosyl]thymine (9o)

Compound **9o** was prepared from orthoester **6o** (390 mg, 0.8 mmol) and **7** (0.1 g, 0.41 mmol) using Method B as colorless oil in a yield of 70 mg (30%). Ratio of *R*/*S* epimers is 65:35. HRMS $(M+Na)^-$ for $C_{18}H_{18}O_6N_2BrINa$: calcd. *m/z* 586,92851, found 586,92828 IR (KBr, cm-1) 3171, 3066, 3040, 2955, 2922, 2852, 1687, 1583, 1554, 1461, 1410, 1363, 1291, 1275, 1237, 1193, 1133,1109, 1080, 1045, 861, 835. ^{1}H NMR (d_6DMSO) δ 11.27 (br s, NH), 8.06 (t, *J* = 1.7 Hz, ArH), 7.79 (t, *J* = 1.7 Hz, ArH), 7.62 (t, *J* = 1.7 Hz, ArH), 7.44 (q, *J* = 1.2 Hz, H6), 6.11 (dd, *J* = 8.4 and 1.8 Hz, H1´), 4.66 (br dd, *J* = 4.8, 2.6 and <1 Hz, H3´), 4.33 (dd, *J* = 13.1 and 2.2 Hz, H5´a), 4.18 (dd, *J* = 13.1 and 1.0 Hz, H5´b), 4.03 (ddd, *J* = 2.6, 2.2 and 1.0 Hz, H4´), 3.02 (s, OCH_3), 2.72 (ddd, *J* = 15.2, 8.4 and 4.8 Hz, H2´´), 2.14 (br dd, *J* = 15.2, 1.8 and <1 Hz, H2´); 1.27 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.77 (C4), 150.57 (C2), 141.43 (Ar-C), 139.95 (Ar-CH), 136.45 (C6), 133.96 (Ar-CH), 128.58 (Ar-CH), 122.67 (Ar-C), 108.76 (>C<), 108.39 (C5), 96.09 (Ar-C), 83.89 (C1´), 73.91 (C4´), 69.65 (C3´), 59.89 (C5´), 51.16 (OCH_3), 38.53 (C2´), 12.07 (5-CH_3).

(*R*)-1-[2-Deoxy-3,5-*O*-(4-iodo-methoxybenzylidene)-β-D-threo-pentofuranosyl]uracil (9p)

Compound **9p** was prepared from orthoester **6d** (407 mg, 1.32 mmol) and **8** (0.2 g, 0.88 mmol) using Method A as colorless oil in a yield of 346 mg (84%). HRMS $(M+Na)^-$ for $C_{17}H_{17}O_6N_2INa$: calcd. *m/z* 495.00235, found 495.00241 IR ($CHCl_3$, cm-1) 3394, 3170, 3065, 2962, 2928, 2855, 2840, 1709, 1690, 1587, 1483, 1462, 1441, 1433, 1394, 1318, 1306, 1300, 1272, 1179, 1122, 1103, 1099, 1079, 1060, 1009, 950, 809, 683, 627, 573, 551. ^{1}H NMR (d_6DMSO) δ 11.26 (br s, NH), 7.79 (m, 2x ArH), 7.70 (d, *J* = 8.2 Hz, H6), 7.26 (m, 2x ArH), 6.04 (dd, *J* = 8.0 and 1.6 Hz, H1´), 5.18 (d, *J* = 8.2 Hz, H5), 4.67 (br dd, *J* = 4.8, 2.6 and <1 Hz, H3´), 4.32 (dd, *J* = 13.2 and 2.2 Hz, H5´a), 4.18 (dd, *J* = 13.2 and 1.1 Hz, H5´b), 4.07 (ddd, *J* = 2.6, 2.2 and 1.1 Hz, H4´), 3.01 (s, OCH_3), 2.70 (ddd, *J* = 15.1, 8.0 and 4.8 Hz, H2´´), 2.06 (br dd, *J* = 15.1, 1.6 and <1 Hz, H2´); ^{13}C NMR (d_6DMSO) δ 163.26 (C4), 150.47 (C2), 140.53 (C6), 137.281 (2x Ar-CH), 137.00 (Ar-C), 128.54 (2x Ar-CH), 110.29 (>C<), 100.52 (C5), 96.25 (Ar-C), 84.55 (C1´), 74.39 (C4´), 69.35 (C3´), 59.77 (C5´), 51.05 (OCH_3), 39.70 (C2´).

(*R*)-1-[2-Deoxy-3,5-*O*-(4-methoxycarbonyl-methoxybenzylidene)-β-D-threo-pentofuranosyl]uracil (9q)

Compound **9q** was prepared from orthoester **6f** (274 mg, 1.14 mmol) and **8** (0.2 g, 0.88 mmol) using Method A as colorless oil in a yield of 155 mg (44%). HRMS $(M+Na)^-$ for $C_{19}H_{20}O_8N_2Na$: calcd. *m/z* 427.11119, found 427.11100; IR ($CHCl_3$, cm-1) 3395, 3168, 2954, 2894, 1725, 1708, 1686, 1615, 1581, 1511, 1462, 1438, 1408, 1399, 1319, 1306, 1298, 1282, 1275, 1194, 1177, 1123, 1079, 1018, 999, 836, 809, 712. ^{1}H NMR (d_6DMSO) δ 11.24 (br s, NH), 8.01 (m, 2x ArH), 7.70 (d, *J* = 8.1 Hz, H6), 7.62 (m, 2x ArH), 6.04 (dd, *J* = 8.0 and 1.6 Hz, H1´), 5.13 (d, *J* = 8.1 Hz, H5), 4.70 (br dd, *J* = 4.8, 2.6 and <1 Hz, H3´), 4.35 (dd, *J* = 13.2 and 2.2 Hz, H5´a), 4.22 (dd, *J* = 13.2 and 1.1 Hz, H5´b), 4.10 (ddd, *J* = 2.6, 2.2 and 1.1 Hz, H4´), 3.87 (s, $COOCH_3$), 3.02 (s, OCH_3), 2.71 (ddd, *J* = 15.1, 8.0 and 4.8 Hz, H2´´), 2.09 (br dd, *J* = 15.1, 1.6 and <1 Hz, H2´); ^{13}C NMR (d_6DMSO) δ 166.04 (C=O), 163.29 (C4), 150.49 (C2), 141.91 (Ar-C), 140.52 (C6), 130.54 (Ar-C), 129.34 (2x Ar-CH), 126.80 (2x Ar-

CH), 110.04 (>C<), 100.46 (C5), 84.66 (C1´), 74.49 (C4´), 69.46 (C3´), 59.84 (C5´), 52.47 (OCH_3), 51.11 (OCH_3), 39.70 (C2´).

(*R*)-1-[2-Deoxy-3,5-*O*-(ethoxybenzylidene)-β-D-threo-pentofuranosyl]uracil (9r)

Compound **9r** was prepared from orthoester **10** (462 mg, 2.04 mmol) and **8** (0.31 g, 1.36 mmol) using Method A as colorless oil in a yield of 370 mg (76%). HRMS $(M+Na)^-$ for $C_{18}H_{20}O_6N_2Na$: calcd. *m/z* 383.12136, found 383.12158; IR ($CHCl_3$, cm-1) 3396, 3174, 3063, 3027, 2981, 1709, 1686, 1630, 1605, 1580, 1495, 1463, 1452, 1433, 1395, 1360, 1355, 1299, 1274, 1124, 1105, 1080, 1062, 1031, 915, 702, 612, 527. ^{1}H NMR (d_6DMSO) δ 11.24 (br s, NH), 7.75 (d, *J* = 8.1 Hz, H6), 7.49 (m, 2x ArH), 7.41 (m, 2x ArH), 7.40 (m, ArH), 6.05 (dd, *J* = 8.1 and 1.6 Hz, H1´), 5.07 (d, *J* = 8.1 Hz, H5), 4.68 (br dd, *J* = 4.8, 2.6 and <1 Hz, H3´), 4.35 (dd, *J* = 13.1 and 2.3 Hz, H5´a), 4.18 (br dd, *J* = 13.1, 1.0 and <1 Hz, H5´b), 4.08 (ddd, *J* = 2.6, 2.3 and 1.0 Hz, H4´), 3.21 (dq, *J* = 9.6 and 7.0 Hz, O-CHaHb), 3.16 (dq, *J* = 9.6 and 7.0 Hz, O-CHaHb), 2.69 (ddd, *J* = 15.1, 8.1 and 4.8 Hz, H2´´), 2.06 (br dd, *J* = 15.1, 1.6 and <1 Hz, H2´), 1.10 (t, *J* = 7.0 Hz, CH_3); ^{13}C NMR (d_6DMSO) δ 163.28 (C4), 150.52 (C2), 140.81 (C6), 137.86 (Ar-C), 129.34 (Ar-CH), 128.28 (2x Ar-CH), 126.18 (2x Ar-CH), 110.00 (>C<), 100.40 (C5), 84.61 (C1´), 74.56 (C4´), 69.27 (C3´), 59.74 (C5´), 58.92 (O-CH_2), 39.66 (C2´), 14.79 (CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-fluoro-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (11a)

Compound **11a** was prepared from orthoester **9a** (100 mg, 0.26 mmol) using Method C as colorless oil in a yield of 118 mg (92%). HRMS $(M+Na)^+$ for $C_{21}H_{26}O_8N_2FNaP$ calcd. 507.13030 *m/z* found 507.12999; IR ($CHCl_3$, cm^{-1}) 3397, 3176, 2965, 2930, 2855, 1684, 1608, 1509, 1471, 1434, 1410, 1392, 1369, 1385, 1314, 1299, 1269, 1237, 1193, 1160, 1133, 1083, 1064, 1041, 1017, 973, 948, 917, 842, 687, 485, 413. ^{1}H NMR (d_6DMSO) δ 11.20 (br s, NH), 7.55 (m, 2x ArH), 7.29 (q, *J* = 1.2 Hz, H6), 7.21 (m, 2x ArH), 6.10 (dd, *J* = 8.3 and 1.9 Hz, H1´), 5.18 (ddd, *J* = 5.1, 2.7 and 2.1 Hz, H3´), 4.87 (ddd, *J* = 12.9, 2.7 and 2.1 Hz, H5´a), 4.26 (br dd, *J* = 12.9, 1.6 and <1 Hz, H5´b), 4.11 (td, *J* = 2.7, 2.7 and 1.6 Hz, H4´), 4.04 –

3.95 (m, 2x P-O-CH_2), 2.80 (ddd, *J* = 15.2, 8.3 and 5.1 Hz, H2´´), 2.15 (br dd, *J* = 15.2, 1.9 and <1 Hz, H2´); 1.18 (t, *J* = 7.0 Hz, CH_3), 1.15 (t, *J* = 7.0 Hz, CH_3), 0.92 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.71 (C4), 162.51 (dd, *J* = 245.4 and 2.2 Hz, Ar-C), 150.48 (C2), 136.41 (C6), 134.36 (dd, *J* = 8.5 and 2.8 Hz, Ar-C), 128.89 (dd, *J* = 8.8 and 1.3 Hz, 2x Ar-CH), 114.58 (dd, *J* = 21.5 and 0.9 Hz, 2x Ar-CH), 108.48 (C5), 96.70 (d, *J* = 190.8 Hz, >C<), 83.84 (C1´), 74.55 (C4´), 72.02 (C3´), 63.10 (d, *J* = 6.9 Hz, P-O-CH_2), 63.02 (d, *J* = 7.2 Hz, P-O-CH_2), 62.99 (C5´), 39.45 (C2´), 16.38 (d, *J* = 5.2 Hz, CH_3), 16.36 (d, *J* = 5.2 Hz, CH_3), 11.18 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-chloro-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (11b)

Compound **11b** was prepared from orthoester **9b** (100 mg, 0.25 mmol) using Method C as colorless oil in a quantitative yield of 130 mg. HRMS $(M+Na)^+$ for $C_{21}H_{26}O_8N_2ClNaP$ calcd. 523.10075 *m/z* found 523.10076; IR ($CHCl_3$, cm^{-1}) 3396, 3173, 3089, 2929, 2961, 2855, 1683, 1598, 1576, 1490, 1470, 1434, 1402, 1393, 1369, 1385, 1364, 1314, 1300, 1269, 1241, 1192, 1179, 1163, 1133, 1115, 1094, 1083, 1064, 1041, 1029, 1017, 987, 973, 949, 935, 917, 835, 829, 686, 633, 625, 486, 413. ^{1}H NMR (d_6DMSO) δ 11.20 (br s, NH), 7.52 (m, 2x ArH), 7.45 (m, 2x ArH), 7.26 (q, *J* = 1.2 Hz, H6), 6.08 (dd, *J* = 8.3 and 1.9 Hz, H1´), 5.18 (ddd, *J* = 5.0, 2.5 and 2.1 Hz, H3´), 4.88 (ddd, *J* = 12.8, 2.6 and 2.1 Hz, H5´a), 4.27 (br dd, *J* = 12.8, 1.6 and <1 Hz, H5´b), 4.12 (ddd, *J* = 2.6, 2.5 and 1.6 Hz, H4´), 4.06 – 3.95 (m, 2x P-O-CH_2), 2.80 (ddd, *J* = 15.3, 8.3 and 5.0 Hz, H2´´), 2.15 (br dd, *J* = 15.3, 1.9 and <1 Hz, H2´); 1.18 (t, *J* = 7.1 Hz, CH_3), 1.16 (t, *J* = 7.1 Hz, CH_3), 0.90 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.71 (C4), 150.46 (C2), 137.37 (d, *J* = 8.5 Hz, Ar-C), 136.32 (C6), 133.81 (d, *J* = 2.3 Hz, Ar-C), 128.53 (2x Ar-CH), 127.89 (2x Ar-CH), 108.38 (C5), 96.63 (d, *J* = 190.4 Hz, >C<), 83.98 (C1´), 74.60 (C4´), 72.06 (C3´), 63.16 (d, *J* = 6.9 Hz, P-O-CH_2), 63.07 (d, *J* = 7.1 Hz, P-O-CH_2), 63.03 (C5´), 40.00 (C2´), 16.38 (d, *J* = 5.0 Hz, CH_3), 16.36 (d, *J* = 5.1 Hz, CH_3), 11.09 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-bromo-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (11c)

Compound **11c** was prepared from orthoester **9b** (100 mg, 0.23 mmol) using Method C as colorless oil in a quantitative yield of 131 mg. HRMS $(M+Na)^+$ for $C_{21}H_{26}O_8N_2BrNaP$ calcd. 567.05024 *m/z* found 567.05024; IR (KBr, cm^{-1}) 3397, 3173, 3089, 2961, 2929, 2856, 1684, 1591, 1575, 1485, 1470, 1434, 1395, 1385, 1369, 1363, 1314, 1300, 1269, 1241, 1192, 1181, 1163, 1133, 1094, 1083, 1074, 1064, 1041, 1030, 1013, 987, 973, 949, 917, 827, 686, 630, 623, 486, 413. ^{1}H NMR (d_6DMSO) δ 11.20 (br s, NH), 7.58 (m, 2x ArH), 7.45 (m, 2x ArH), 7.25 (q, *J* = 1.2 Hz, H6), 6.07 (dd, *J* = 8.3 and 1.9 Hz, H1´), 5.18 (ddd, *J* = 5.0, 2.5 and 2.1 Hz, H3´), 4.87 (ddd, *J* = 12.8, 2.5 and 2.1 Hz, H5´a), 4.27 (br dd, *J* = 12.8 and 1.6 Hz, H5´b), 4.12 (td, *J* = 2.5, 2.5 and 1.6 Hz, H4´), 4.06 – 3.96 (m, 2x P-O-CH_2), 2.79 (ddd, *J* = 15.2, 8.3 and 5.0 Hz, H2´´), 2.14 (br dd, *J* = 15.2 and 1.9 Hz, H2´), 1.18 (t, *J* = 7.0 Hz, CH_3), 1.16 (t, *J* = 7.0 Hz, CH_3), 0.89 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.74 (C4), 150.47 (C2), 137.80 (d, *J* = 8.4 Hz, Ar-C), 136.32 (C6), 130.73 (2x Ar-CH), 128.82 (2x Ar-CH), 122.53 (d, *J* = 2.4 Hz, Ar-C),108.39 (C5), 96.67 (d, *J* = 190.4 Hz, >C<), 84.03 (C1´), 74.65 (C4´), 72.06 (C3´), 63.20 (d, *J* = 6.9 Hz, P-O-CH_2), 63.11 (d, *J* = 7.0 Hz, P-O-CH_2), 63.05 (C5´), 40.09 (C2´), 16.41 (d, *J* = 5.1 Hz, CH_3), 16.38 (d, *J* = 4.9 Hz, CH_3), 11.08 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-iodo-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (11d)

Compound **11d** was prepared from orthoester **9d** (155 mg, 0.32 mmol) using Method C as colorless oil in a yield of 118 mg (62%). HRMS $(M-H)^-$ for $C_{21}H_{27}O_8N_2IP$: calcd *m/z* 593.05442, found 593.05471; IR ($CHCl_3$, cm^{-1}) 3397, 3170, 3089, 2988, 1700, 1683, 1587, 1484, 1471, 1434, 1406, 1392, 1385, 1369, 1299, 1269, 1242, 1185, 1163, 1133, 1116, 1097, 1084, 1064, 1041, 1029, 1009, 973, 949, 685. ^{1}H NMR (d_6DMSO) δ 11.18 (br s, NH), 7.75 (m, 2x ArH), 7.29 (m, 2x ArH), 7.24 (q, *J* = 1.2 Hz, H6), 6.07 (dd, *J* = 8.3 and 1.9 Hz, H1´), 5.17 (dd, *J* = 5.0 and 2.6 Hz, H3´), 4.87 (ddd, *J* = 13.0, 2.6 and 2.0 Hz, H5´a), 4.26 (br dd, *J* = 13.0 and 1.6 Hz, H5´b), 4.12 (td, *J* = 2.6, 2.6 and 1.6 Hz, H4´), 4.05 – 3.96 (m, 2x P-O-CH_2),

2.79 (ddd, *J* = 15.2, 8.3 and 5.0 Hz, H2´´), 2.14 (br dd, *J* = 15.2 and 1.9 Hz, H2´), 1.19 (t, *J* = 7.1 Hz, CH_3), 1.16 (t, *J* = 7.1 Hz, CH_3), 0.89 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.64 (C4), 150.39 (C2), 138.16 (d, *J* = 8.6 Hz, Ar-C), 136.52 (2x Ar-CH), 136.22 (C6), 128.72 (2x Ar-CH), 108.35 (C5), 96.70 (d, *J* = 190.4 Hz, >C<), 95.66 (d, *J* = 2.0 Hz, Ar-C), 83.97 (C1´), 74.61 (C4´), 71.96 (C3´), 63.08 (d, *J* = 6.7 Hz, P-O-CH_2), 62.99 (d, *J* = 7.1 Hz, P-O-CH_2), 62.94 (C5´), 40.00 (C2´), 16.32 (d, *J* = 5.2 Hz, CH_3), 16.30 (d, *J* = 5.2 Hz, CH_3), 10.98 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-nitro-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (11e)

Compound **11e** was prepared from orthoester **9e** (120 mg, 0.3 mmol) using Method C as colorless oil in a yield of 106 mg (70%). HRMS $(M-H)^-$ for $C_{21}H_{27}O_{10}N_3P$: calcd *m/z* 512.14286, found 512.14300; IR ($CHCl_3$, cm^{-1}) 3396, 3175, 3089, 2987, 1703, 1685, 1610, 1603, 1527, 1492, 1470, 1435, 1407, 1392, 1384, 1369, 1353, 1314, 1299, 1269, 1245, 1164, 1133, 1111, 1095, 1083, 1064, 1043, 1029, 1018, 972, 946, 850, 691, 413. ^{1}H NMR (d_6DMSO) δ 11.23 (br s, NH), 8.25 (m, 2x ArH), 7.79 (m, 2x ArH), 7.21 (q, *J* = 1.2 Hz, H6), 6.07 (dd, *J* = 8.3 and 1.9 Hz, H1´), 5.23 (dd, *J* = 5.0 and 2.5 Hz, H3´), 4.92 (dd, *J* = 12.8 and 2.6 Hz, H5´a), 4.33 (dd, *J* = 12.8 and 1.6 Hz, H5´b), 4.16 (ddd, *J* = 2.6, 2.5 and 1.6 Hz, H4´), 4.10 – 3.98 (m, 2x P-O-CH_2), 2.82 (ddd, *J* = 15.2, 8.3 and 5.0 Hz, H2´´), 2.20 (dd, *J* = 15.2 and 1.9 Hz, H2´), 1.19 (t, *J* = 7.1 Hz, CH_3), 1.16 (t, *J* = 7.1 Hz, CH_3), 0.82 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.69 (C4), 150.46 (C2), 147.87 (Ar-C), 145.23 (d, *J* = 8.4 Hz, Ar-C), 136.09 (C6), 128.25 (2x Ar-CH), 122.96 (2x Ar-CH), 108.35 (C5), 96.64 (d, *J* = 189.0 Hz, >C<), 84.11 (C1´), 74.59 (C4´), 72.37 (C3´), 63.47 (d, *J* = 6.9 Hz, P-O-CH_2), 63.36 (d, *J* = 7.2 Hz, P-O-CH_2), 63.32 (C5´), 40.06 (C2´), 16.40 (d, *J* = 5.0 Hz, CH_3), 16.37 (d, *J* = 5.0 Hz, CH_3), 11.22 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-methoxycarbonyl-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (11f)

Compound **11f** was prepared from orthoester **9f** (142 mg, 0.34 mmol) using Method C as colorless oil in a yield of 96 mg (54%). HRMS $(M-H)^-$ for $C_{23}H_{30}O_{10}N_2P$: calcd *m/z* 525.16326, found 525.16334; IR ($CHCl_3$, cm^{-1}) 3397, 3091, 1722, 1703, 1683, 1655, 1613, 1578, 1509, 1470, 1438, 1392, 1385, 1369, 1312, 1283, 1243, 1197, 1164, 1133, 1114, 1097, 1083, 1065, 1020, 714, 624, 555. ^{1}H NMR (d_6DMSO) δ 11.23 (br s, 1 H), 7.96 (m, 2x ArH), 7.66 (m, 2x ArH), 7.25 (q, *J* = 1.2 Hz, H6), 6.08 (dd, *J* = 8.3 and 2.0 Hz, H1´), 5.21 (dd, *J* = 5.0 and 2.6 Hz, H3´), 4.90 (dd, *J* = 12.8 and 2.6 Hz, H5´a), 4.30 (dd, *J* = 12.8 and 1.5 Hz, H5´b), 4.14 (td, *J* = 2.6, 2.6 and 1.5 Hz, H4´), 4.03 – 3.96 (m, 2x P-O-CH_2), 3.86 (s, OCH_3), 2.80 (ddd, *J* = 15.2, 8.3 and 5.0 Hz, H2´´), 2.17 (dd, *J* = 15.2 and 2.0 Hz, H2´), 1.17 (t, *J* = 7.0 Hz, 3 H), 1.14 (t, *J* = 7.0 Hz, 3 H), 0.78 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 166.11 (C=O), 163.71 (C4), 150.46 (C2), 143.18 (d, *J* = 8.2 Hz, Ar-C), 136.27 (C6), 130.03 (Ar-C), 128.62 (2x Ar-CH), 127.06 (2x Ar-CH), 108.33 (C5), 96.86 (d, *J* = 188.8 Hz, >C<), 84.02 (C1´), 74.66 (C4´), 72.17 (C3´), 63.28 (d, *J* = 6.0 Hz, P-O-CH_2), 63.18 (d, *J* = 6.0 Hz, P-O-CH_2), 63.16 (C5´), 52.50 (OCH_3), 40.02 (C2´), 16.38 (d, *J* = 4.8 Hz, CH_3), 16.35 (d, *J* = 4.9 Hz, CH_3), 11.12 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-methylthio-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (11g)

Compound **11g** was prepared from orthoester **9g** (81 mg, 0.2 mmol) using Method C as colorless oil in a yield of 73 mg (71%). HRMS $(M+Na)^+$ for $C_{22}H_{29}O_8N_2NaPS$ calcd. 535.12744 *m/z* found 535.12766; IR (KBr, cm^{-1}) 3428, 3165, 3063, 2987, 2956, 2922, 1697, 1685, 1597, 1468, 1444, 1400, 1363, 1313, 1270, 1246, 1187, 1162, 1128, 1095, 1082, 1078, 1038, 1027, 972, 724. ^{1}H NMR (d_6DMSO) δ 11.23 (br s, NH), 7.44 (m, 2x ArH), 7.29 (q, *J* = 1.2 Hz, H6), 7.26 (m, 2x ArH), 6.10 (dd, *J* = 8.4 and 2.0 Hz, H1´), 5.17 (ddd, *J* = 5.1, 2.6 and 2.1 Hz, H3´), 4.86 (ddd, *J* = 13.0, 2.6 and 2.1 Hz, H5´a), 4.22 (br dd, *J* = 13.0, 1.8 and <1 Hz, H5´b), 4.11 (td, *J* = 2.6, 2.6 and 1.8 Hz, H4´), 4.04 – 3.95 (m, 2x P-O-CH_2), 2.79 (ddd, *J* = 15.1, 8.4 and 5.1 Hz, H2´´), 2.47 (s, SCH_3), 2.13 (bdd, *J* = 15.1, 2.0 and <1 Hz, H2´); 1.19 (t, *J* = 7.1 Hz, CH_3), 1.17 (t, *J* = 7.1 Hz, CH_3), 0.92 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO)

δ 163.63 (C4), 150.42 (C2), 139.20 (d, *J* = 2.2 Hz, Ar-C), 136.33 (C6), 134.84 (d, *J* = 8.4 Hz, Ar-C), 127.01 (d, *J* = 1.8 Hz, 2x Ar-CH), 125.08 (2x Ar-CH), 108.53 (C5), 96.83 (d, *J* = 191.2 Hz, >C<), 83.85 (C1´), 74.66 (C4´), 71.84 (C3´), 62.95 (d, *J* = 6.9 Hz, P-O-CH_2), 62.87 (d, *J* = 7.1 Hz, P-O-CH_2), 62.80 (C5´), 39.88 (C2´), 16.32 (d, *J* = 5.3 Hz, CH_3), 16.31 (d, *J* = 5.3 Hz, CH_3), 14.79 (SCH_3), 11.03 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-cyano-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (11h)

Compound **11h** was prepared from orthoester **9h** (100 mg, 0.26 mmol) using Method C as colorless oil in a quantitative yield of 131 mg. HRMS $(M+Na)^+$ for $C_{22}H_{26}O_8N_3NaP$ calcd. 514.13497 *m/z* found 514.13508; IR ($CHCl_3$, cm^{-1}) 3396, 3173, 2960, 2929, 2856, 2233, 1685, 1470, 1434, 1405, 1392, 1369, 1300, 1245, 1192, 1180, 1162, 1133, 1120, 1096, 1083, 1063, 1043, 1029, 972, 958, 947, 916, 840, 687, 646, 628, 552, 486, 413. ^{1}H NMR (d_6DMSO) δ 11.21 (br s, NH), 7.88 (m, 2x ArH), 7.69 (m, 2x ArH), 7.21 (q, *J* = 1.2 Hz, H6), 6.07 (dd, *J* = 8.2 and 1.9 Hz, H1´), 5.21 (ddd, *J* = 5.0, 2.5 and 2.1 Hz, H3´), 4.89 (ddd, *J* = 12.9, 2.5 and 2.1 Hz, H5´a), 4.30 (br dd, *J* = 12.9, 1.6 and <1 Hz, H5´b), 4.14 (td, *J* = 2.5, 2.5 and 1.6 Hz, H4´), 4.08 – 3.97 (m, 2x P-O-CH_2), 2.80 (ddd, *J* = 15.3, 8.2 and 5.0 Hz, H2´´), 2.18 (bdd, *J* = 15.3, 1.9 and <1 Hz, H2´); 1.18 (t, *J* = 7.1 Hz, CH_3), 1.15 (t, *J* = 7.1 Hz, CH_3), 0.85 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.69 (C4), 150.45 (C2), 143.42 (d, *J* = 8.4 Hz, Ar-C), 136.15 (C6), 131.84 (2x Ar-C), 127.66 (2x Ar-CH), 118.67 (CN), 111.76 (d, *J* = 1.6 Hz, Ar-C), 108.30 (C5), 96.54 (d, *J* = 189.1 Hz, >C<), 84.04 (C1´), 74.57 (C4´), 72.28 (C3´), 63.37 (d, *J* = 6.9 Hz, P-O-CH_2), 63.28 (d, *J* = 7.1 Hz, P-O-CH_2), 63.25 (d, *J* = 1.8 Hz, C5´), 40.02 (C2´), 16.38 (d, *J* = 5.2 Hz, CH_3), 16.34 (d, *J* = 5.3 Hz, CH_3), 11.16 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-trifluoromethyl-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (11i)

Compound **11i** was prepared from orthoester **9i** (91 mg, 0.21 mmol) using Method C as colorless oil in a yield of 78 mg (69%). HRMS $(M+Na)^+$ for $C_{22}H_{26}O_8N_2F_3NaP$ calcd. 557.12711 *m/z* found 557.12691; IR (KBr, cm^{-1}) 3396, 3177, 1701, 1684, 1621, 1469, 1434,

1393, 1326, 1313, 1300, 1269, 1188, 1134, 1038, 1070, 1042, 1029, 1020, 842, 605. ^{1}H NMR (d_6DMSO) δ 11.20 (br s, NH), 7.76 (m, 2x ArH), 7.73 (m, 2x ArH), 7.20 (q, *J* = 1.2 Hz, H6), 6.08 (dd, *J* = 8.3 and 1.9 Hz, H1´), 5.21 (ddd, *J* = 5.0, 2.6 and 2.1 Hz, H3´), 4.92 (ddd, *J* = 13.0, 2.5 and 2.1 Hz, H5´a), 4.32 (br dd, *J* = 13.0, 1.5 and <1 Hz, H5´b), 4.14 (ddd, *J* = 2.6, 2.5 and 1.5 Hz, H4´), 4.08 – 3.99 (m, 2x P-O-CH_2), 2.81 (ddd, *J* = 15.2, 8.3 and 5.0 Hz, H2´´), 2.17 (bdd, *J* = 15.2, 1.9 and <1 Hz, H2´); 1.19 (t, *J* = 7.1 Hz, CH_3), 1.16 (t, *J* = 7.1 Hz, CH_3), 0.77 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.69 (C4), 150.46 (C2), 142.84 (d, *J* = 8.3 Hz, Ar-C), 136.18 (C6), 129.44 (q, *J* = 30.4 Hz, Ar-C), 127.57 (2x Ar-CH), 124.76 (q, *J* = 2.8 Hz, 2x Ar-CH), 124.31 (q, *J* = 272.2 Hz, CF_3), 108.27 (C5), 96.57 (d, *J* = 189.4 Hz, >C<), 84.06 (C1´), 74.61 (C4´), 72.21 (C3´), 63.31 (d, *J* = 6.8 Hz, P-O-CH_2), 63.22 (d, *J* = 7.1 Hz, P-O-CH_2), 63.18 (C5´), 40.15 (C2´), 16.39 (d, *J* = 4.4 Hz, CH_3), 16.36 (d, *J* = 4.8 Hz, CH_3), 10.90 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-methoxy-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (11j)

Compound **11j** was prepared from orthoester **9j** (100 mg, 0.26 mmol) using Method C as colorless oil in a yield of 89 mg (69%). HRMS $(M+Na)^+$ for C_{22} H_{29} O_9 N_2 Na P calcd. 519.15029 *m/z* found 519.14998; IR ($CHCl_3$, cm^{-1}) 3397, 3177, 2855, 2840, 1699, 1684, 1612, 1585, 1512, 1468, 1406, 1392, 1304, 1269, 1240, 1192, 1176, 1096, 1065, 973, 837. ^{1}H NMR (d_6DMSO) δ 11.19 (br s, NH), 7.43 (m, 2x ArH), 7.33 (q, *J* = 1.2 Hz, H6), 6.92 (m, 2x ArH), 6.11 (dd, *J* = 8.4 and 2.0 Hz, H1´), 5.15 (ddd, *J* = 5.1, 2.7 and 2.1 Hz, H3´), 4.84 (ddd, *J* = 12.9, 2.7 and 2.1 Hz, H5´a), 4.20 (br dd, *J* = 12.9, 1.7 and <1 Hz, H5´b), 4.09 (td, *J* = 2.7, 2.7 and 1.7 Hz, H4´), 4.01 – 3.92 (m, 2x P-O-CH_2), 3.75 (s, OCH_3), 2.79 (ddd, *J* = 15.2, 8.4 and 5.1 Hz, H2´´), 2.12 (bdd, *J* = 15.2, 2.0 and <1 Hz, H2´); 1.17 (t, *J* = 7.1 Hz, CH_3), 1.15 (t, *J* = 7.1 Hz, CH_3), 0.91 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.77 (C4), 159.77 (Ar-C), 150.53 (C2), 136.60 (C6), 130.33 (d, *J* = 8.4 Hz, Ar-C), 127.96 (2x Ar-CH), 113.10 (2x Ar-CH), 108.69 (C5), 96.98 (d, *J* = 191.6 Hz, >C<), 83.78 (C1´), 74.68 (C4´), 71.84

(C3´), 62.96 (d, *J* = 6.9 Hz, P-O-CH_2), 62.88 (d, *J* = 7.0 Hz, P-O-CH_2), 62.80 (C5´), 55.41 (OCH_3), 40.00 (C2´), 16.45 (d, *J* = 5.2 Hz, CH_3), 16.43 (d, *J* = 5.2 Hz, CH_3), 11.25 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-vinyl-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (11k)

Compound **11k** was prepared from orthoester **9k** (400 mg, 1 mmol) using Method C as colorless oil in a yield of 245 mg (48%). HRMS $(M+Na)^+$ for $C_{23}H_{29}O_8N_2NaP$ calcd. 515.15537 *m/z* found 515.15535; IR (KBr, cm^{-1}) 3173, 1687, 1470, 1435, 1369, 1315, 1271, 1245, 1132, 1094, 1083, 1065, 1030, 989, 972, 764. ^{1}H NMR (d_6DMSO) δ 11.05 (br s, NH), 7.49 – 7.46 (m, 4x ArH), 7.30 (q, *J* = 1.2 Hz, H6), 6.75 (dd, *J* = 17.6 and 11.0 Hz, -CH=), 6.10 (dd, *J* = 8.4 and 2.0 Hz, H1´), 5.86 (dd, *J* = 17.6 and 1.1 Hz, =CHaHb), 5.29 (dd, *J* = 11.0 and 1.1 Hz, =CHaHb), 5.18 (ddd, *J* = 5.1, 2.7 and 1.9 Hz, H3´), 4.87 (ddd, *J* = 13.0, 2.8 and 1.9 Hz, H5´a), 4.24 (br dd, *J* = 13.0, 1.6 and <1 Hz, H5´b), 4.11 (ddd, *J* = 2.8, 2.7 and 1.6 Hz, H4´), 4.05 – 3.93 (m, 2x P-O-CH_2), 2.80 (ddd, *J* = 15.1, 8.4 and 5.1 Hz, H2´´), 2.14 (bdd, *J* = 15.1, 2.0 and <1 Hz, H2´); 1.18 (t, *J* = 7.1 Hz, CH_3), 1.15 (t, *J* = 7.1 Hz, CH_3), 0.84 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.74 (C4), 150.49 (C2), 137.76 (d, *J* = 8.2 Hz, Ar-C), 137.70 (Ar-C), 136.45 (C6), 136.30 (-CH=), 126.84 (2x Ar-CH), 125.44 (2x Ar-CH), 115.30 (=CH_2), 108.56 (C5), 96.98 (d, *J* = 190.1 Hz, >C<), 83.89 (C1´), 74.00 (C4´), 71.94 (C3´), 63.07 (d, *J* = 6.9 Hz, P-O-CH_2), 62.97 (d, *J* = 7.0 Hz, P-O-CH_2), 62.92 (C5´), 40.00 (C2´), 16.41 (d, *J* = 5.2 Hz, CH_3), 16.39 (d, *J* = 5.3 Hz, CH_3), 11.05 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-phenyl-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (11l)

Compound **11l** was prepared from orthoester **9l** (100 mg, 0.23 mmol) using Method C as colorless oil in a quantitative yield of 122 mg. HRMS $(M+Na)^+$ for C27H31N2NaO8P calcd. 565,17102 *m/z* found 565.17074; IR (KBr, cm^{-1}) 3177, 3061, 2979, 2958, 2923, 2853, 1687, 1565, 1517, 1487, 1469, 1402, 1368, 1314, 1271, 1245, 1192, 1162, 1133, 1082, 1039, 972, 766, 699, 617, 417. ^{1}H NMR (d_6DMSO) δ 11.14 (br s, NH), 7.66 (m, 2x ArH), 7.64 (m, 2x ArH), 7.60 (m, 2x ArH), 7.48 (m, 2x ArH), 7.39 (m, ArH), 7.32 (q, *J* = 1.2 Hz, H6), 6.11 (dd,

J = 8.4 and 1.9 Hz, H1´), 5.20 (ddd, *J* = 5.1, 2.6 and 2.0 Hz, H3´), 4.90 (ddd, *J* = 12.8, 2.6 and 2.0 Hz, H5´a), 4.28 (br dd, *J* = 12.8, 1.6 and <1 Hz, H5´b), 4.13 (td, *J* = 2.6, 2.6 and 1.6 Hz, H4´), 4.08 – 3.99 (m, 2x P-O-CH_2), 2.81 (ddd, *J* = 15.2, 8.4 and 5.1 Hz, H2´´), 2.17 (bdd, *J* = 15.2, 1.9 and <1 Hz, H2´); 1.20 (t, *J* = 7.1 Hz, CH_3), 1.18 (t, *J* = 7.1 Hz, CH_3), 0.84 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.74 (C4), 150.51 (C2), 140.79 (d, *J* = 1.8 Hz, Ar-C), 139.88 (Ar-C), 137.48 (d, *J* = 8.2 Hz, Ar-C), 136.46 (C6), 129.21 (2x Ar-CH), 127.90 (Ar-CH), 127.18 (2x Ar-CH), 126.93 (2x Ar-CH), 126.07 (2x Ar-CH), 108.60 (C5), 96.92 (d, *J* = 190.2 Hz, >C<), 83.92 (C1´), 74.69 (C4´), 71.96 (C3´), 63.11 (d, *J* = 6.9 Hz, P-O-CH_2), 63.02 (d, *J* = 6.9 Hz, P-O-CH_2), 62.95 (C5´), 39.10 (C2´), 16.46 (d, *J* = 5.1 Hz, CH_3), 16.43 (d, *J* = 5.1 Hz, CH_3), 11.06 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(3-phenyl-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (11m)

Compound **11m** was prepared from orthoester **9m** (119 mg, 0.27 mmol) using Method C as colorless oil in a yield of 124 mg (85%). HRMS $(M-H)^-$ for $C_{27}H_{32}O_8N_2P$ calcd. 543.18908 *m/z* found 543.18909; IR (KBr, cm^{-1}) 3171, 3061, 3035, 2981, 2928, 2854, 1687, 1600, 1575, 1477, 1454, 1393, 1368, 1314, 1298, 1273, 1245, 1195, 1163, 1133, 1082, 1038, 974, 761, 701, 616, 555, 417. ^{1}H NMR (d_6DMSO) δ 11.20 (br s, NH), 7.75 (q, *J* = 1.7 Hz, ArH), 7.66 (ddt, *J* = 7.6, 1.7, 1.1 and 1.1 Hz, ArH), 7.57 (m, 2x ArH), 7.48 (dd, *J* = 7.9 and 7.6 Hz, ArH), 7.54 (dtd, *J* = 7.9, 1.7, 1.7 and 1.1 Hz, ArH), 7.48 (m, 2x ArH), 7.38 (m, ArH), 7.34 (q, *J* = 1.2 Hz, H6), 6.13 (dd, *J* = 8.5 and 2.0 Hz, H1´), 5.22 (ddd, *J* = 5.0, 2.8 and 2.2 Hz, H3´), 4.90 (ddd, *J* = 12.8, 2.4 and 2.2 Hz, H5´a), 4.28 (br dd, *J* = 12.8, 1.6 and <1 Hz, H5´b), 4.13 (ddd, *J* = 2.8, 2.4 and 1.6 Hz, H4´), 4.07 – 3.97 (m, 2x P-O-CH_2), 2.81 (ddd, *J* = 15.3, 8.5 and 5.0 Hz, H2´´), 2.20 (bdd, *J* = 15.3, 2.0 and <1 Hz, H2´); 1.18 (t, *J* = 7.1 Hz, CH_3), 1.16 (t, *J* = 7.1 Hz, CH_3), 0.79 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.69 (C4), 150.52 (C2), 140.32 (Ar-C), 139.88 (Ar-C), 139.00 (d, *J* = 8.6 Hz, Ar-C), 136.56 (C6), 129.27 (2x Ar-CH), 128.47 (Ar-CH), 127.79 (Ar-CH), 127.37 (Ar-CH), 126.84 (2x Ar-CH), 125.74 (Ar-CH), 124.86 (Ar-CH), 108.61 (C5), 96.97 (d, *J* = 189.8 Hz, >C<), 83.77 (C1´), 74.59 (C4´), 72.08 (C3´), 63.12

(d, *J* = 6.9 Hz, P-O-CH_2), 63.04 (d, *J* = 7.0 Hz, P-O-CH_2), 63.00 (C5´), 39.70 (C2´), 16.43 (d, *J* = 5.2 Hz, 2x CH_3), 11.03 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(3-iodo-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (11n)

Compound **11n** was prepared from orthoester **9n** (116 mg, 0.24 mmol) using Method C as colorless oil in a yield of 98 mg (84%). HRMS $(M+Na)^+$ for $C_{21}H_{27}O_8N_2IP$ calcd. 593.05442 *m/z* found 593.05444; IR (KBr, cm^{-1}) 3175, 3064, 2980, 2927, 2854, 1687, 1590, 1566, 1469, 1408, 1368, 1314, 1270, 1246, 1194, 1163, 1133, 1096, 1082, 1040, 1028, 975, 892, 826, 789, 765, 697, 688, 554, 417. ^{1}H NMR (d_6DMSO) δ 11.20 (br s, NH), 7.82 (q, *J* = 1.7 Hz, ArH), 7.75 (ddt, *J* = 7.9, 1.7, 1.1 and 1.1 Hz, ArH), 7.53 (dtd, *J* = 7.9, 1.7, 1.7 and 1.1 Hz, ArH), 7.28 (q, *J* = 1.2 Hz, H6), 7.20 (t, *J* = 7.9 Hz, ArH), 6.10 (dd, *J* = 8.5 and 2.0 Hz, H1´), 5.18 (ddd, *J* = 5.0, 2.7 and 2.2 Hz, H3´), 4.85 (ddd, *J* = 13.0, 2.4 and 2.2 Hz, H5´a), 4.26 (br dd, *J* = 13.0, 1.5 and <1 Hz, H5´b), 4.10 (ddd, *J* = 2.7, 2.4 and 1.5 Hz, H4´), 4.06 – 3.96 (m, 2x P-O-CH_2), 2.79 (ddd, *J* = 15.3, 8.5 and 5.0 Hz, H2´´), 2.19 (bdd, *J* = 15.3, 2.0 and <1 Hz, H2´); 1.18 (t, *J* = 7.1 Hz, CH_3), 1.16 (t, *J* = 7.1 Hz, CH_3), 0.94 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.71 (C4), 150.52 (C2), 140.57 (d, *J* = 8.7 Hz, Ar-C), 137.57 (Ar-CH), 136.40 (C6), 135.11 (Ar-CH), 130.01 (Ar-CH), 126.03 (Ar-CH), 108.60 (C5), 96.20 (d, *J* = 190.0 Hz, >C<), 94.04 (d, *J* = 1.7 Hz, Ar-C), 83.79 (C1´), 74.47 (C4´), 72.16 (C3´), 63.25 (d, *J* = 6.9 Hz, P-O-CH_2), 63.15 (d, *J* = 7.1 Hz, P-O-CH_2), 63.15 (C5´), 39.70 (C2´), 16.35 (d, *J* = 5.4 Hz, CH_3), 16.32 (d, *J* = 5.4 Hz, CH_3), 11.30 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(3-bromo-5-iodo-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (11o)

Compound **11o** was prepared from orthoester **9o** (59 mg, 0.1 mmol) using Method C as colorless oil in a yield of 30 mg (43%). HRMS $(M+Na)^+$ for $C_{21}H_{25}O_8N_2BrINaP$ calcd. 692.94688 *m/z* found 692.94684; IR ($CHCl_3$, cm^{-1}) 3396, 3172, 2983, 2959, 2928, 2855, 1685, 1580, 1552, 1469, 1434, 1411, 1393, 1369, 1384, 1364, 1314, 1299, 1268, 1244, 1192, 1163, 1134, 1113, 1095, 1082, 1063, 1043, 1032, 978, 893, 862, 833, 554, 414. ^{1}H NMR

(d_6DMSO) δ 11.22 (br s, NH), 8.01 (q, *J* = 1.5 Hz, ArH), 7.80 (q, *J* = 1.5 Hz, ArH), 7.62 (q, *J* = 1.5 Hz, ArH), 7.26 (q, *J* = 1.2 Hz, H6), 6.09 (dd, *J* = 8.4 and 2.0 Hz, H1´), 5.16 (ddd, *J* = 5.0, 2.6 and 2.0 Hz, H3´), 4.83 (ddd, *J* = 12.8, 2.6 and 2.0 Hz, H5´a), 4.28 (br dd, *J* = 12.8, 1.5 and <1 Hz, H5´b), 4.11 (td, *J* = 2.6, 2.6 and 2.0 Hz, H4´), 4.09 – 3.99 (m, 2x P-O-CH_2), 2.77 (ddd, *J* = 15.3, 8.4 and 5.0 Hz, H2´´), 2.27 (bdd, *J* = 15.3, 2.0 and <1 Hz, H2´), 1.20 (t, *J* = 7.1 Hz, CH_3), 1.17 (t, *J* = 7.1 Hz, CH_3), 1.03 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.68 (C4), 150.50 (C2), 142.32 (d, *J* = 8.9 Hz, Ar-C), 139.24 (Ar-CH), 136.32 (C6), 134.36 (Ar-CH), 128.82 (Ar-CH), 121.96 (d, *J* = 1.5 Hz, Ar-C), 108.42 (C5), 95.63 (d, *J* = 190.5 Hz, >C<), 95.18 (Ar-C), 83.88 (C1´), 74.38 (C4´), 72.37 (C3´), 63.44 (d, *J* = 6.8 Hz, P-O-CH_2), 63.31 (d, *J* = 7.0 Hz, P-O-CH_2), 63.15 (C5´), 39.28 (C2´), 16.30 (d, *J* = 5.4 Hz, CH_3), 16.27 (d, *J* = 5.4 Hz, CH_3), 11.37 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-iodo-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]uracil (11p)

Compound **11p** was prepared from orthoester **9p** (260 mg, 0.55 mmol) using Method C as colorless oil in a yield of 200 mg (63%). HRMS $(M+Na)^-$ for $C_{20}H_{24}O_8N_2INaP$: calcd *m/z* 601.02072, obs. 601.02045; IR ($CHCl_3$, cm^{-1}) 3395, 3164, 3060, 1708, 1688, 1587, 1568, 1484, 1462, 1432, 1393, 1369, 1313, 1299, 1242, 1184, 1126, 1110, 1096, 1084, 1063, 1058, 1040, 1026, 1009, 990, 972, 951, 935, 810, 689, 631, 551, 416. ^{1}H NMR (d_6DMSO) δ 7.76 (m, 2x ArH), 7.46 (d, *J* = 8.1 Hz, H6), 7.26 (m, 2x ArH), 6.05 (dd, *J* = 8.2 and 1.9 Hz, H1´), 5.13 (ddd, *J* = 5.0, 2.8 and 2.1 Hz, H3´), 4.80 (ddd, *J* = 12.8, 3.1 and 2.1 Hz, H5´a), 4.69 (d, *J* = 8.1 Hz, H5), 4.17 (ddd, *J* = 3.1, 2.8 and 2.2 Hz, H4´), 4.12 (br dd, *J* = 12.8, 2.2 and <1 Hz, H5´b), 4.06 – 3.97 (m, 2x P-O-CH_2), 2.78 (ddd, *J* = 15.2, 8.2 and 5.0 Hz, H2´´), 2.19 (bdd, *J* = 15.2, 1.9 and <1 Hz, H2´); 1.18 (t, *J* = 7.0 Hz, CH_3), 1.16 (t, *J* = 7.0 Hz, CH_3); ^{13}C NMR (d_6DMSO) δ 163.09 (C4), 150.51 (C2), 140.64 (C6), 137.94 (d, *J* = 9.1 Hz, Ar-C), 126.52 (2x Ar-CH), 128.78 (2x Ar-CH), 100.18 (C5), 96.86 (d, *J* = 193.1 Hz, >C<), 95.77 (d, *J* = 2.1 Hz, Ar-C), 84.47 (C1´), 75.15 (C4´), 72.03 (C3´), 63.24 (d, *J* = 6.8 Hz, P-O-CH_2), 63.12 (d, *J* =

7.0 Hz, P-O-CH_2), 62.85 (C5´), 39.70 (C2´), 16.42 (d, *J* = 5.1 Hz, CH_3), 16.41 (d, *J* = 5.1 Hz, CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-methoxymethyl-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]uracil (11q)

Compound **11q** was prepared from orthoester **9q** (140 mg, 0.35 mmol) using Method C as colorless oil in a yield of 149 mg (84%). HRMS $(M+Na)^-$ for $C_{22}H_{27}O_{10}N_2NaP$: calcd *m/z* 533.12955, obs. 533.12940; IR ($CHCl_3$, cm^{-1}) 3395, 3170, 2955, 1725, 1707, 1687, 1613, 1578, 1507, 1462, 1438, 1408, 1393, 1393, 1370, 1312, 1312, 1302, 1283, 1270, 1243, 1195, 1181, 1164, 1126, 1113, 1099, 1099, 1084, 1041, 1028, 1021, 972, 833, 809, 713, 552, 415. ^{1}H NMR (d_6DMSO) δ 11.20 (d, *J* = 2.2 Hz, NH), 7.97 (m, 2x ArH), 7.65 (m, 2x ArH), 7.46 (d, *J* = 8.1 Hz, H6), 6.05 (dd, *J* = 8.1 and 1.9 Hz, H1´), 5.17 (ddd, *J* = 5.0, 2.8 and 2.0 Hz, H3´), 4.85 (ddd, *J* = 13.2, 3.3 and 2.0 Hz, H5´a), 4.59 (dd, *J* = 8.1 and 2.2 Hz, H5), 4.19 (ddd, *J* = 3.3, 2.8 and 2.0 Hz, H4´), 4.19 (br dd, *J* = 13.2, 2.0 and <1 Hz, H5´b), 4.06 – 3.96 (m, 2x P-O-CH_2), 3.87 (s, $COOCH_3$), 2.79 (ddd, *J* = 15.3, 8.1 and 5.0 Hz, H2´´), 2.23 (bdd, *J* = 15.3, 1.9 and <1 Hz, H2´); 1.17 (t, *J* = 7.1 Hz, CH_3), 1.15 (t, *J* = 7.1 Hz, CH_3); ^{13}C NMR (d_6DMSO) δ 166.12 (C=O), 163.07 (C4), 150.49 (C2), 142.99 (d, *J* = 8.7 Hz, Ar-C), 140.58 (C6), 129.99 (Ar-C), 128.53 (2x Ar-CH), 127.01 (2x Ar-CH), 100.02 (C5), 96.87 (d, *J* = 191.6 Hz, >C<), 84.54 (C1´), 75.12 (C4´), 72.11 (C3´), 63.32 (d, *J* = 6.7 Hz, P-O-CH_2), 63.21 (d, *J* = 7.0 Hz, P-O-CH_2), 62.99 (C5´), 52.44 (OCH_3), 39.70 (C2´), 16.36 (d, *J* = 5.1 Hz, 2x CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]uracil (11r)

Compound **11r** was prepared from orthoester **9r** (356 mg, 0.99 mmol) using Method C as colorless oil in a yield of 347 mg (78%). HRMS $(M+Na)^-$ for $C_{20}H_{25}O_8N_2NaP$: calcd *m/z* 475.12407, obs. 475.12468; IR ($CHCl_3$, cm^{-1}) 3396, 3171, 3062, 3029, 1705, 1686, 1631, 1605, 1580, 1495, 1462, 1451, 1432, 1393, 1370, 1313, 1299, 1270, 1241, 1164, 1127, 1109, 1095, 1084, 1060, 1060, 1036, 1025, 971, 914, 697, 613, 528. ^{1}H NMR (d_6DMSO) δ 11.20 br

s, NH), 7.50 (d, *J* = 8.1 Hz, H6), 7.49 (m, 2x ArH), 7.38 (m, 3x ArH), 6.06 (dd, *J* = 8.2 and 1.9 Hz, H1´), 5.14 (ddd, *J* = 5.0, 2.7 and 2.1 Hz, H3´), 4.82 (ddd, *J* = 12.8, 3.0 and 2.1 Hz, H5´a), 4.55 (dd, *J* = 8.1 and 1.4 Hz, H5), 4.17 (ddd, *J* = 3.0, 2.7 and 2.2 Hz, H4´), 4.13 (br dd, *J* = 12.8, 2.2 and <1 Hz, H5´b), 2.78 (ddd, *J* = 15.3, 8.2 and 5.0 Hz, H2´´), 2.20 (bdd, *J* = 15.3, 1.9 and <1 Hz, H2´); ^{13}C NMR (d_6DMSO) δ 163.08 (C4), 150.53 (C2), 140.83 (C6), 138.08 (d, *J* = 9.0 Hz, Ar-C), 128.91 (Ar-CH), 127.63 (2x Ar-CH), 126.57 (2x Ar-CH), 100.04 (C5), 96.97 (d, *J* = 192.8 Hz, >C<), 84.47 (C1´), 75.23 (C4´), 71.88 (C3´), 63.13 (d, *J* = 6.8 Hz, P-O-CH_2), 63.02 (d, *J* = 7.0 Hz, P-O-CH_2), 62.77 (C5´), 39.50 (C2´), 16.42 (d, *J* = 5.0 Hz, 2x CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-fluoro-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (12a)

Compound **12a** was prepared from diethyl phosphonate **11a** (52 mg, 0.11 mmol) using Method D as colorless oil in a yield of 26 mg (49%). HRMS $(M-H)^-$ for $C_{17}H_{17}O_8N_2FP$ calcd. 427.07120 *m/z* found 427.07132; IR (KBr, cm^{-1}) 3071, 1692, 1606, 1507, 1479, 1441, 1313, 1278, 1260, 1199, 1136, 1075, 973, 841. Elem. anal.: calcd P 6.88% found P 6.43% (MW 481.80). ε_{269} = 8442 $L.mol^{-1}.cm^{-1}$. ^{1}H NMR (D_2O) δ 7.67 (q, *J* = 1.2 Hz, H6), 7.56 (m, 2x ArH), 7.07 (m, 2x ArH), 6.13 (dd, *J* = 8.0 and 1.5 Hz, H1´), 5.35 (ddd, *J* = 4.7, 2.5 and 1.1 Hz, H3´), 5.02 (ddd, *J* = 13.2, 2.5 and 1.1 Hz, H5´a), 4.22 (br dd, *J* = 13.2 and 1.5 Hz, H5´b), 4.20 (td, *J* = 2.5, 2.5 and 1.5 Hz, H4´), 2.77 (ddd, *J* = 15.3, 8.0 and 4.7 Hz, H2´´), 2.44 (br dd, *J* = 15.3, 1.5 and <1 Hz, H2´), 1.07 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 169.29 (C4), 165.08 (d, *J* = 245.3 Hz, Ar-C),154.30 (C2), 141.58 (C6), 139.73 (dd, *J* = 7.7 and 2.8 Hz, Ar-C), 131.07 (d, *J* = 7.1 Hz, 2x Ar-CH), 116.98 (d, *J* = 21.4 Hz, 2x Ar-CH), 112.58 (C5), 101.71 (d, *J* = 176.2 Hz, >C<), 88.33 (C1´), 79.55 (C4´), 73.64 (C3´), 65.30 (C5´), 42.61 (C2´), 13.35 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-chloro-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (12b)

Compound **12b** was prepared from diethyl phosphonate **11b** (30 mg, 60 μmol) using Method D as colorless oil in a yield of 10 mg (35%). HRMS $(M-H)^-$ for $C_{17}H_{17}O_8N_2ClP$ calcd. 443.04165 *m/z* found 443.04198; IR (KBr, cm^{-1}) 1690, 1596, 1488, 1437, 1313, 1277, 1256, 1199, 1136, 1076. Elem. anal.: calcd P 6.64% found P 6.48% (MW 478.26). ε_{270} = 8274 $L.mol^{-1}.cm^{-1}$. ^1H NMR (D_2O) δ 7.63 (q, *J* = 1.2 Hz, H6), 7.52 (m, 2x ArH), 7.35 (m, 2x ArH), 6.11 (dd, *J* = 8.0 and 1.4 Hz, H1´), 5.33 (ddd, *J* = 4.7, 2.7 and 1.1 Hz, H3´), 5.01 (ddd, *J* = 13.2, 2.5 and 1.1 Hz, H5´a), 4.23 (br dd, *J* = 13.2 and 1.0 Hz, H5´b), 4.21 (ddd, *J* = 2.7, 2.5 and 1.0 Hz, H4´), 2.76 (ddd, *J* = 15.2, 8.0 and 4.7 Hz, H2´´), 2.45 (br dd, *J* = 15.2, 1.4 and <1 Hz, H2´), 1.06 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 169.24 (C4), 154.27 (C2), 143.36 (d, *J* = 7.5 Hz, Ar-C), 141.48 (C6), 136.05 (d, *J* = 1.9 Hz, Ar-C), 130.74 (2x Ar-CH), 130.33 (2x Ar-CH), 112.53 (C5), 101.53 (d, *J* = 176.2 Hz, >C<), 88.47 (C1´), 79.59 (C4´), 73.66 (C3´), 65.32 (C5´), 42.65 (C2´), 13.30 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-bromo-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (12c)

Compound **12c** was prepared from diethyl phosphonate **11c** (30 mg, 60 μmol) using Method D as colorless oil in a yield of 10 mg (29%). HRMS $(M-H)^-$ for $C_{17}H_{17}O_8N_2BrP$ calcd. 486.99114 *m/z* found 486.99096; IR (KBr, cm^{-1}) 3068, 1690, 1589, 1484, 1312, 1277, 1259, 1198, 1136, 1075, 1011, 814. Elem. anal.: calcd P 6.06% found P 5.44% (MW 569.45). ε_{270} = 8063 $L.mol^{-1}.cm^{-1}$. ^1H NMR (d_6DMSO) δ 7.64 (q, *J* = 1.2 Hz, H5), 7.50 (m, 2x ArH), 7.46 (m, 2x ArH), 6.10 (dd, *J* = 7.9 and 1.5 Hz, H1´), 5.35 (ddd, *J* = 4.7, 2.6 and 1.1 Hz, H3´), 5.03 (ddd, *J* = 13.2, 2.4 and 1.1 Hz, H5´a), 4.22 (br dd, *J* = 13.2, 1.5 and <1 Hz, H5´b), 4.20 (ddd, *J* = 2.6, 2.4 and 1.5 Hz, H4´), 2.76 (ddd, *J* = 15.3, 7.9 and 4.7 Hz, H2´´), 2.45 (bdd, *J* = 15.3, 1.5 and <1 Hz, H2´), 1.06 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 169.26 (C4), 154.28 (C2), 143.22 (d, *J* = 7.5 Hz, Ar-C), 141.53 (C6), 133.24 (2x Ar-CH), 131.10 (2x Ar-CH), 124.22 (d, *J* = 2.0 Hz, Ar-C), 112.53 (C5), 101.76 (d, *J* = 174.4 Hz, >C<), 88.49 (C1´), 79.69 (C4´), 73.57 (C3´), 65.28 (C5´), 42.68 (C2´), 13.29 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-iodo-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (12d)

Compound **12d** was prepared from diethyl phosphonate **11d** (23 mg, 40 μmol) using Method D as colorless oil in a yield of 14 mg (61%). HRMS $(M-H)^-$ for $C_{17}H_{17}O_8N_2IP$: calcd *m/z* 534.97727, found 534.97782; IR (KBr, cm^{-1}) 3065, 1695, 1658, 1586, 1482, 1437, 1386, 1312, 1278, 1219, 1136, 1117, 1080, 1051, 1007, 970, 940, 814, 767, 691, 628. Elem. anal.: calcd P 5.56%; found P 5.26% (MW 588.73). ε_{268} = 7896 $L.mol^{-1}.cm^{-1}$ ^1H NMR (D_2O) δ 7.71 (m, 2x ArH), 7.61 (q, *J* = 1.2 Hz, H6), 7.32 (m, 2x ArH), 6.10 (dd, *J* = 7.9 and 1.4 Hz, H1´), 5.32 (ddd, *J* = 4.7, 2.7 and 1.2 Hz, H3´), 4.99 (ddd, *J* = 13.2, 2.5 and 1.2 Hz, H5´a), 4.23 (br dd, *J* = 13.2 and 1.7 Hz, H5´b), 4.21 (ddd, *J* = 2.7, 2.5 and 1.7 Hz, H4´), 2.76 (ddd, *J* = 15.3, 7.9 and 4.7 Hz, H2´´), 2.45 (ddd, *J* = 15.3, 1.4 and 1.2 Hz, H2´), 1.06 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 169.24 (C4), 154.26 (C2), 143.43 (d, *J* = 7.6 Hz, Ar-C), 141.44 (C6), 139.40 (d, *J* = 1.1 Hz, 2x Ar-CH), 131.18 (d, *J* = 1.6 Hz, 2x Ar-CH), 112.55 (C5), 101.50 (d, *J* = 176.4 Hz, >C<), 96.39 (d, *J* = 2.3 Hz, Ar-C), 88.56 (C1´), 79.62 (C4´), 73.62 (C3´), 65.30 (C5´), 42.69 (C2´), 13.35 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-nitro-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (12e)

Compound **12e** was prepared from diethyl phosphonate **11e** (20 mg, 40 μmol) using Method D as colorless oil in a yield of 9 mg (48%). HRMS $(M-H)^-$ for $C_{17}H_{19}O_{10}N_3P$: calcd *m/z* 456.08026, found 456.08029; IR (KBr, cm^{-1}) 3075, 1691, 1656, 1608, 1600, 1520, 1477, 1438, 1384, 1351, 1315, 1276, 1177, 1136, 1108, 1075, 1015, 973, 954, 856, 822, 769, 753, 693, 517. Elem. anal.: calcd P 6.50%; found P 6.44% (MW 480.49). ε_{269} = 14255 $L.mol^{-1}.cm^{-1}$ ^1H NMR (D_2O) δ 8.20 (m, 2x ArH), 7.79 (m, 2x ArH), 7.60 (q, *J* = 1.2 Hz, H6), 6.10 (dd, *J* = 7.8 and 1.4 Hz, H1´), 5.36 (ddd, *J* = 4.6, 2.6 and 1.2 Hz, H3´), 5.04 (ddd, *J* = 13.3, 2.6 and 1.2 Hz, H5´a), 4.30 (br dd, *J* = 13.3 and 1.5 Hz, H5´b), 4.24 (td, *J* = 2.6, 2.6 and 1.5 Hz, H4´), 2.78 (ddd, *J* = 15.4, 7.8 and 4.6 Hz, H2´´), 2.50 (ddd, *J* = 15.4, 1.4 and 1.2 Hz, H2´), 0.98 (d, *J*

= 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 169.16 (C4), 154.22 (C2), 150.86 (d, *J* = 7.7 Hz, Ar-C), 149.98 (d, *J* = 1.9 Hz, Ar-C), 141.30 (C6), 130.37 (2x Ar-CH), 125.60 (2x Ar-CH), 112.21 (C5), 101.40 (d, *J* = 173.9 Hz, >C<), 88.61 (C1´), 79.55 (C4´), 73.92 (C3´), 65.58 (C5´), 42.64 (C2´), 13.37 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-methoxycarbonyl-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (12f)

Compound **12f** was prepared from diethyl phosphonate **11f** (20 mg, 40 μmol) using Method D as colorless oil in a yield of 12 mg (58%). HRMS $(M-H)^-$ for $C_{19}H_{20}O_{10}N_2P$: calcd *m/z* 467.08610, obs. 467.08650; IR (KBr, cm^{-1}) 1720, 1698, 1655, 1612, 1578, 1508, 1476, 1437, 1407, 1386, 1281, 1197, 1118, 1076, 1035, 1020, 972, 771, 700, 623. Elem. anal.: calcd P 6.33%; found P 5.83% (MW 525.60). ε_{243} = 16879 $L.mol^{-1}.cm^{-1}$ ^{1}H NMR (D_2O) δ 7.97 (m, 2x ArH), 7.69 (m, 2x ArH), 7.60 (q, *J* = 1.2 Hz, H6), 6.11 (dd, *J* = 7.9 and 1.4 Hz, H1´), 5.36 (ddd, *J* = 4.7, 2.8 and 1.2 Hz, H3´), 5.04 (ddd, *J* = 13.2, 2.5 and 1.2 Hz, H5´a), 4.27 (br dd, *J* = 13.2 and 1.6 Hz, H5´b), 4.22 (ddd, *J* = 2.8, 2.5 and 1.6 Hz, H4´), 3.92 (s, OCH_3), 2.78 (ddd, *J* = 15.3, 7.9 and 4.7 Hz, H2´´), 2.47 (ddd, *J* = 15.3, 1.4 and 1.2 Hz, H2´), 0.92 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 172.06 (C=O), 169.18 (C4), 154.24 (C2), 149.02 (d, *J* = 7.5 Hz, Ar-C), 141.38 (C6), 131.68 (d, *J* = 1.7 Hz, Ar-C), 131.50 (d, *J* = 1.0 Hz, 2x Ar-CH), 129.44 (d, *J* = 1.6 Hz, 2x Ar-CH), 112.42 (C5), 101.63 (d, *J* = 174.4 Hz, >C<), 88.50 (C1´), 79.58 (C4´), 73.78 (C3´), 65.45 (C5´), 55.37 (OCH_3), 42.67 (C2´), 13.24 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-methylthio-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (12g)

Compound **12g** was prepared from diethyl phosphonate **11g** (52 mg, 0.1 mmol) using Method D as colorless oil in a yield of 25 mg (49%). HRMS $(M-H)^-$ for $C_{18}H_{20}O_8N_2PS$ calcd. 455.06835 *m/z* found 455.06832; IR (KBr, cm^{-1}) 1690, 1597, 1490, 1478, 1438, 1313, 1276, 1256, 1197, 1135, 1076, 971, 707. Elem. anal.: calcd P 6.47% found P 6.07% (MW 509.91). ε_{259} = 21798 $L.mol^{-1}.cm^{-1}$. ^{1}H NMR (D_2O) δ 7.65 (q, *J* = 1.2 Hz, H6), 7.50 (m, 2x ArH), 7.26

(m, 2x ArH), 6.12 (dd, *J* = 7.9 and 1.5 Hz, H1´), 5.35 (ddd, *J* = 4.7, 2.4 and 1.1 Hz, H3´), 5.03 (ddd, *J* = 13.1, 2.7 and 1.1 Hz, H5´a), 4.21 (br dd, *J* = 13.1 and 1.6 Hz, H5´b), 4.19 (ddd, *J* = 2.7, 2.4 and 1.6 Hz, H4´), 2.76 (ddd, *J* = 15.2, 7.9 and 4.7 Hz, H2´´), 2.50 (s, S-CH_3), 2.43 (br dd, *J* = 15.2, 1.5 and <1 Hz, H2´), 1.02 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 169.24 (C4), 154.29 (C2), 141.60 (C6), 141.21 (d, *J* = 7.4 Hz, Ar-C), 139.65 (d, *J* = 1.7 Hz, Ar-C), 129.82 (d, *J* = 1.4 Hz, 2x Ar-CH), 128.27 (2x Ar-CH), 112.59 (C5), 101.88 (d, *J* = 175.2 Hz, >C<), 88.42 (C1´), 79.71 (C4´), 73.51 (C3´), 65.21 (C5´), 42.70 (C2´), 17.43 (S-CH_3), 13.27 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-cyano-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (12h)

Compound **12h** was prepared from diethyl phosphonate **11h** (150 mg, 0.31 mmol) using Method D as colorless oil in a yield of 65 mg (45%). HRMS $(M-H)^-$ for $C_{18}H_{17}O_8N_3P$ calcd. 434.07587 *m/z* found 434.07576; IR (KBr, cm^{-1}) 3077, 2955, 2229, 1691, 1477, 1437, 1405, 1383, 1313, 1275, 1199, 1180, 1137, 1075, 1020, 972, 830, 769, 432. Elem. anal.: calcd P 6.77% found P 6.52% (MW 474.84). ε_{260} = 9975 $L.mol^{-1}.cm^{-1}$. ^{1}H NMR (D_2O) δ 7.75 (m, 2x ArH), 7.72 (m, 2x ArH), 7.57 (q, *J* = 1.2 Hz, H6), 6.09 (dd, *J* = 7.8 and 1.9 Hz, H1´), 5.32 (ddd, *J* = 4.8, 2.8 and 1.3 Hz, H3´), 4.99 (ddd, *J* = 13.4, 2.6 and 1.3 Hz, H5´a), 4.30 (br dd, *J* = 13.2 and 1.6 Hz, H5´b), 4.24 (ddd, *J* = 2.8, 2.6 and 1.6 Hz, H4´), 2.78 (ddd, *J* = 15.5, 7.8 and 4.8 Hz, H2´´), 2.49 (br dd, *J* = 15.5, 1.9 and <1 Hz, H2´), 1.00 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 169.15 (C4), 154.20 (C2), 148.48 (d, *J* = 7.9 Hz, Ar-C), 141.22 (C6), 134.63 (d, *J* = 1.3 Hz, 2x Ar-CH), 129.94 (d, *J* = 1.7 Hz, 2x Ar-CH), 122.26 (-CN), 113.52 (d, *J* = 1.9 Hz, Ar-C), 112.31 (C5), 101.00 (d, *J* = 176.8 Hz, >C<), 88.59 (C1´), 79.45 (C4´), 73.96 (C3´), 65.57 (d, *J* = 1.0 Hz, C5´), 42.63 (C2´), 13.32 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-trifluoromethyl-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (12i)

Compound **12i** was prepared from diethyl phosphonate **11i** (60 mg, 1 □mol) using Method D as colorless oil in a yield of 24 mg (43%). HRMS $(M-H)^-$ for $C_{18}H_{17}O_8N_2F_3P$ calcd. 439.09119 *m/z* found 439.09126; IR (KBr, cm^{-1}) 3070, 2965, 2884, 1691, 1620, 1477, 1409, 1327, 1276, 1198, 1165, 1128, 1019, 973, 842, 769, 682, 606. Elem. anal.: calcd P 6.19% found P 5.52% (MW 561.02). ε_{270} = 8768 $L.mol^{-1}.cm^{-1}$. ^1H NMR (D_2O) δ 7.73 (m, 2x ArH), 7.66 (m, 2x ArH), 7.60 (q, *J* = 1.2 Hz, H6), 6.10 (dd, *J* = 7.9 and 1.4 Hz, H1´), 5.37 (ddd, *J* = 4.7, 2.7 and 1.2 Hz, H3´), 5.04 (ddd, *J* = 13.2, 2.5 and 1.2 Hz, H5´a), 4.26 (br dd, *J* = 13.2 and 1.5 Hz, H5´b), 4.22 (ddd, *J* = 2.7, 2.5 and 1.5 Hz, H4´), 2.77 (ddd, *J* = 15.3, 7.9 and 4.7 Hz, H2´´), 2.47 (br dd, *J* = 15.3, 1.4 and <1 Hz, H2´), 0.95 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 169.16 (C4), 154.25 (C2), 147.86 (d, *J* = 7.7 Hz, Ar-C), 141.40 (C6), 131.96 (q, *J* = 32.0 Hz, Ar-C), 129.66 (2x Ar-CH), 127.24 (q, *J* = 3.2 Hz, 2x Ar-CH), 127.04 (q, *J* = 271.5 Hz, -CF_3), 112.40 (C5), 101.60 (d, *J* = 173.7 Hz, >C<), 88.58 (C1´), 79.67 (C4´), 73.69 (C3´), 65.40 (C5´), 42.74 (C2´), 13.16 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-methoxy-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (12j)

Compound **12j** was prepared from diethyl phosphonate **11j** (50 mg, 1 mmol) using Method D as colorless oil in a yield of 17 mg (33%). HRMS $(M-H)^-$ for $C_{18}H_{20}O_9N_2P$ calcd. 439.09119 *m/z* found 439.09126; IR (KBr, cm^{-1}) 1691, 1610, 1584, 1476, 1301, 1277, 1249, 1135, 1076, 1037, 827. Elem. anal.: calcd P 6.70% found P 5.97% (MW 518.85). ε_{271} = 8136 $L.mol^{-1}.cm^{-1}$. ^1H NMR (D_2O) δ 7.68 (q, *J* = 1.2 Hz, H6), 7.50 (m, 2x ArH), 6.93 (m, 2x ArH), 6.13 (dd, *J* = 8.0 and 1.5 Hz, H1´), 5.35 (ddd, *J* = 4.7, 2.5 and 1.0 Hz, H3´), 5.03 (ddd, *J* = 13.2, 2.5 and 1.0 Hz, H5´a), 4.20 (br dd, *J* = 13.2 and 1.6 Hz, H5´b), 4.18 (td, *J* = 2.5, 2.5 and 1.6 Hz, H4´), 3.84 (s, OCH_3), 2.77 (ddd, *J* = 15.3, 8.0 and 4.7 Hz, H2´´), 2.43 (br dd, *J* = 15.3, 1.5 and <1 Hz, H2´), 1.04 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 169.29 (C4), 161.12 (d, *J* = 1.5 Hz, Ar-C), 154.31 (C2), 141.70 (C6), 136.85 (d, *J* = 7.3 Hz, Ar-C), 130.57 (d, *J* = 1.4 Hz, 2x Ar-CH), 115.76 (2x Ar-CH), 112.63 (C5), 101.95 (d, *J* = 176.3 Hz, >C<), 88.32 (C1´), 79.65 (C4´), 73.49 (C3´), 65.17 (C5´), 58.14 (OCH_3), 42.68 (C2´), 13.31 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-vinyl-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (12k)

Compound **12k** was prepared from diethyl phosphonate **11k** (100 mg, 210 μmol) using Method D as colorless oil in a yield of 52 mg (60%). HRMS $(M-H)^-$ for $C_{19}H_{20}O_8N_2P$: calcd *m/z* 435.09628, obs. 435.09644; IR (KBr, cm^{-1}) 3427, 3159, 3064, 3040, 1691, 1633, 1509, 1477, 1407, 1384, 1349, 1312, 1278, 1198, 1182, 1135, 1076, 1076, 990, 974, 909, 827, 768, 569. ^{1}H NMR (D_2O) δ 7.67 (q, *J* = 1.2 Hz, H6), 7.53 (m, 2x ArH), 7.43 (m, 2x ArH), 6.80 (dd, *J* = 17.8 and 10.9 Hz, -CH=), 6.13 (dd, *J* = 8.0 and 1.5 Hz, H1´), 5.84 (dd, *J* = 17.8 and 1.0 Hz, =CHaHb), 5.37 (ddd, *J* = 4.8, 2.7 and 1.0 Hz, H3´), 5.29 (dd, *J* = 10.9 and 1.0 Hz, =CHaHb), 5.03 (ddd, *J* = 13.2, 2.9 and 1.0 Hz, H5´a), 4.20 (br dd, *J* = 13.5 and 1.8 Hz, H5´b), 4.20 (ddd, *J* = 2.9, 2.7 and 1.8 Hz, H4´), 2.77 (ddd, *J* = 15.3, 8.0 and 4.8 Hz, H2´´), 2.44 (br dd, *J* = 15.3, 1.5 and <1 Hz, H2´), 1.00 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 169.25 (C4), 154.32 (C2), 143.62 (d, *J* = 7.2 Hz, Ar-C), 141.58 (C6), 139.63 (d, *J* = 1.8 Hz, Ar-C), 139.32 (-CH=), 129.45 (d, *J* = 1.5 Hz, 2x Ar-CH), 128.05 (2x Ar-CH), 116.96 (=CH_2), 112.72 (C5), 102.13 (d, *J* = 174.4 Hz, >C<), 88.36 (C1´), 79.73 (C4´), 73.54 (C3´), 65.22 (C5´), 42.62 (C2´), 13.21 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-phenyl-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (12l)

Compound **12l** was prepared from diethyl phosphonate **11l** (172 mg, 310 μmol) using Method D as colorless oil in a yield of 59 mg (31%). HRMS $(M-H)^-$ for $C_{23}H_{22}O_8N_2P$ calcd. 485.11193 *m/z* found 485.11188; IR (KBr, cm^{-1}) 3059, 3030, 2954, 1689, 1563, 1516, 1486, 1446, 1438, 1403, 1384, 1312, 1275, 1197, 1136, 1075, 1008, 841, 766, 731, 698, 618, 553, 416. Elem. anal.: calcd P 6.09% found P 5.11% (MW 605.89). ε_{256} = 30672 $L.mol^{-1}.cm^{-1}$. ^{1}H NMR (D_2O) δ 7.66 (m, 4x ArH), 7.59 (m, 2x ArH), 7.59 (q, *J* = 1.2 Hz, H6), 7.48 (m, 2x ArH), 7.40 (m, ArH), 6.15 (dd, *J* = 8.1 and 1.6 Hz, H1´), 5.35 (ddd, *J* = 4.8, 2.7 and 1.1 Hz, H3´), 5.03 (ddd, *J* = 13.2, 2.7 and 1.1 Hz, H5´a), 4.26 (br dd, *J* = 13.2 and 1.7 Hz, H5´b), 4.23

(td, *J* = 2.7, 2.7 and 1.7 Hz, H4´), 2.80 (ddd, *J* = 15.4, 8.1 and 4.8 Hz, H2´´), 2.40 (br dd, *J* = 15.4, 1.6 and <1 Hz, H2´), 0.89 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 168.99 (C4), 154.27 (C2), 143.01 (Ar-C), 142.88 (d, *J* = 1.6 Hz, Ar-C), 142.65 (d, *J* = 7.7 Hz, Ar-C), 141.40 (C6), 131.84 (2x Ar-CH), 130.43 (Ar-CH), 129.81 (2x Ar-CH), 129.62 (2x Ar-CH), 128.83 (2x Ar-CH), 112.74 (C5), 101.72 (d, *J* = 177.8 Hz, >C<), 88.20 (C1´), 79.52 (C4´), 73.74 (C3´), 65.30 (C5´), 42.61 (C2´), 13.26 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(3-phenyl-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (12m)

Compound **12m**was prepared from diethyl phosphonate **11m** (114 mg, 210 μmol) using Method D as colorless oil in a yield of 73 mg (60%). HRMS $(M-H)^-$ for $C_{23}H_{22}O_8N_2P$ calcd. 485.11193 *m/z* found 485.11198; IR (KBr, cm^{-1}) 3060, 3029, 2955, 1689, 1572, 1478, 1451, 1413, 1313, 1277, 1201, 1136, 1075, 1002, 860, 801, 758, 702, 554. Elem. anal.: calcd P 6.09% found P 5.39% (MW 574.41). ε_{253} = 21289 $L.mol^{-1}.cm^{-1}$. ^{1}H NMR (D_2O) δ 7.82 (m, ArH), 7.63 (m, 2x ArH), 7.62 (m, ArH), 7.61 (q, *J* = 1.2 Hz, H6), 7.58 (m, ArH), 7.49 (m, 2x ArH), 7.45 (m, ArH), 7.39 (m, ArH), 6.09 (dd, *J* = 7.9 and 1.4 Hz, H1´), 5.36 (ddd, *J* = 4.7, 2.6 and 1.2 Hz, H3´), 5.03 (ddd, *J* = 13.2, 2.5 and 1.2 Hz, H5´a), 4.30 (br dd, *J* = 13.2 and 1.6 Hz, H5´b), 4.24 (ddd, *J* = 2.6, 2.5 and 1.2 Hz, H4´), 2.79 (ddd, *J* = 15.3, 7.9 and 4.7 Hz, H2´´), 2.45 (br dd, *J* = 15.3, 1.4 and <1 Hz, H2´), 0.87 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 168.95 (C4), 154.17 (C2), 143.80 (d, *J* = 7.7 Hz, Ar-C), 143.31 (Ar-C), 142.44 (Ar-C), 141.42 (C6), 131.81 (2x Ar-CH), 131.13 (Ar-CH), 130.36 (Ar-CH), 129.58 (2x Ar-CH), 129.38 (Ar-CH), 128.39 (Ar-CH), 127.46 (Ar-CH), 112.44 (C5), 101.44 (d, *J* = 178.3 Hz, >C<), 88.57 (C1´), 79.58 (C4´), 73.71 (C3´), 65.40 (C5´), 42.93 (C2´), 13.19 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(3-iodo-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (12n)

Compound **12n** was prepared from diethyl phosphonate **11n** (88 mg, 150 μmol) using Method D as colorless oil in a yield of 48 mg (54%). HRMS $(M-H)^-$ for $C_{17}H_{17}O_8N_2IP$ calcd. 534.97727 *m/z* found 534.97752; IR (KBr, cm^{-1}) 3064, 2953, 1689, 1590, 1564, 1470, 1436,

1409, 1383, 1312, 1276, 1255, 1200, 1137, 1075, 997, 976, 894, 878, 781, 689, 648, 557, 522, 427, 421. Elem. anal.: calcd P 5.55% found P 5.27% (MW 587.84). ε_{269} = 7200 L.mol^{-1}.cm^{-1}. ^{1}H NMR (D_2O) δ 7.87 (q, *J* = 1.9, 1.6 and 1.6 Hz, ArH), 7.71 (ddt, *J* = 7.9, 1.9, 1.0 and 1.0 Hz, ArH), 7.59 (q, *J* = 1.2 Hz, H6), 7.57 (dtd, *J* = 7.9, 1.6, 1.6 and 1.0 Hz, ArH), 7.13 (t, *J* = 7.9 Hz, ArH), 6.07 (dd, *J* = 7.7 and 1.3 Hz, H1´), 5.33 (ddd, *J* = 4.6, 2.7 and 1.2 Hz, H3´), 4.99 (ddd, *J* = 13.3, 2.4 and 1.2 Hz, H5´a), 4.27 (ddd, *J* = 13.3, 1.5 and 0.5 Hz, H5´b), 4.22 (ddd, *J* = 2.7, 2.4 and 1.5 Hz, H4´), 2.76 (ddd, *J* = 15.2, 7.7 and 4.6 Hz, H2´´), 2.49 (br dd, *J* = 15.2, 1.3 and <1 Hz, H2´), 1.05 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 169.25 (C4), 154.19 (C2), 145.78 (d, *J* = 7.9 Hz, Ar-C), 141.52 (C6), 139.72 (Ar-CH), 137.87 (Ar-CH), 132.33 (Ar-CH), 128.60 (Ar-CH), 112.33 (C5), 100.78 (d, *J* = 149.6 Hz, >C<), 95.95 (d, *J* = 1.3 Hz, Ar-C), 88.83 (C1´), 79.69 (C4´), 73.62 (C3´), 65.39 (C5´), 42.93 (C2´), 13.54 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(3-bromo-5-iodo-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (12o)

Compound **12o** was prepared from diethyl phosphonate **11o** (84 mg, 125 μmol) using Method D as colorless oil in a yield of 45 mg (50%). HRMS $(M-H)^-$ for C_{17} H_{16} O_8 N_2 I Br P calcd. 612.88778 *m/z* found 612.88750; IR (KBr, cm^{-1}) 3077, 2975, 2954, 1689, 1577, 1549, 1477, 1407, 1382, 1312, 1276, 1199, 1137, 1110, 1077, 1001, 980, 857, 832, 768, 725, 678, 556, 524. Elem. anal.: calcd P 4.86% found P 4.32% (MW 716.73). ε_{270} = 8734 L.mol^{-1}.cm^{-1}. ^{1}H NMR (D_2O) δ 7.92 (td, *J* = 1.6, 1.6 and 0.9 Hz, ArH), 7.85 (q, *J* = 1.6 Hz, ArH), 7.69 (q, *J* = 1.6 Hz, ArH), 7.59 (q, *J* = 1.2 Hz, H6), 6.03 (dd, *J* = 7.6 and 1.2 Hz, H1´), 5.32 (ddd, *J* = 4.4, 2.7 and 1.1 Hz, H3´), 4.98 (ddd, *J* = 13.3, 2.4 and 1.1 Hz, H5´a), 4.27 (br dd, *J* = 13.3 and 1.5 Hz, H5´b), 4.23 (ddd, *J* = 2.7, 2.4 and 1.5 Hz, H4´), 2.73 (ddd, *J* = 15.3, 7.6 and 4.4 Hz, H2´´), 2.51 (br dd, *J* = 15.3, 1.2 and <1 Hz, H2´), 1.14 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 169.34 (C4), 154.15 (C2), 147.80 (d, *J* = 8.0 Hz, Ar-C), 141.55 (C6), 141.45 (Ar-CH), 137.04 (Ar-CH), 131.75 (Ar-CH), 124.42 (Ar-C), 111.97 (C5), 100.39 (d, *J* = 174.5 Hz,

>C<), 95.99 (Ar-C), 89.13 (C1´), 79.86 (C4´), 73.59 (C3´), 65.42 (C5´), 43.02 (C2´), 13.64 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-iodo-phosphonobenzylidene)-β-D-threo-pentofuranosyl]uracil (12p)

Compound **12p** was prepared from diethyl phosphonate **11p** (40 mg, 69 μmol) using Method D as colorless oil in a yield of 11 mg (31%). HRMS $(M-H)^-$ for $C_{16}H_{15}O_8N_2IP$: calcd *m/z* 520.96162, obs. 520.96167; IR (KBr, cm^{-1}) 3065, 1690, 1583, 1522, 1481, 1467, 1389, 1389, 1310, 1257, 1177, 1131, 1078, 1006, 971, 932, 769, 687, 628. Elem. anal.: calcd P 5.69%; found P 4.96% (MW 624.01). ε_{262} = 9103 $L.mol^{-1}.cm^{-1}$ ^{1}H NMR (D_2O) δ 7.89 (d, *J* = 8.2 Hz, H6), 7.70 (m, 2x ArH), 7.32 (m, 2x ArH), 6.10 (dd, *J* = 7.8 and 1.3 Hz, H1´), 5.33 (ddd, *J* = 4.6, 2.6 and 0.9 Hz, H3´), 5.01 (ddd, *J* = 13.1, 2.7 and 0.9 Hz, H5´a), 4.96 (d, *J* = 8.2 Hz, H5), 4.21 (ddd, *J* = 2.7, 2.6 and 2.0 Hz, H4´), 4.13 (br dd, *J* = 13.1 and 2.0 Hz, H5´b), 2.72 (ddd, *J* = 15.3, 7.8 and 4.6 Hz, H2´´), 2.48 (br dd, *J* = 15.3, 1.3 and <1 Hz, H2´); ^{13}C NMR (D_2O) δ 169.11 (C4), 154.34 (C2), 146.01 (C6), 144.66 (d, *J* = 7.5 Hz, Ar-C), 138.98 (2x Ar-CH), 131.31 (2x Ar-CH), 102.68 (C5), 102.09 (d, *J* = 171.9 Hz, >C<), 95.63 (d, *J* = 2.0 Hz, Ar-C), 88.90 (C1´), 80.18 (C4´), 73.36 (C3´), 65.03 (C5´), 42.43 (C2´).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-methoxycarbonyl-phosphonobenzylidene)-β-D-threo-pentofuranosyl]uracil (12q)

Compound **12q** was prepared from diethyl phosphonate **11q** (40 mg, 78 μmol) using Method D as colorless oil in a yield of 17 mg (47%). HRMS $(M-H)^-$ for $C_{18}H_{18}O_{10}N_2P$: calcd *m/z* 453.07045, obs. 453.07020; IR (KBr, cm^{-1}) 2955, 1725, 1698, 1609, 1580, 1523, 1475, 1436, 1412, 1311, 1283, 1260, 1179, 1123, 1080, 1019, 977, 938, 769, 704. ^{1}H NMR (D_2O) δ 7.94 (m, 2x ArH), 7.90 (d, *J* = 8.1 Hz, H6), 7.68 (m, 2x ArH), 6.10 (dd, *J* = 7.8 and 1.3 Hz, H1´), 5.39 (ddd, *J* = 4.6, 2.8 and 0.9 Hz, H3´), 5.08 (ddd, *J* = 13.1, 2.6 and 0.9 Hz, H5´a), 4.82 (d, *J* = 8.1 Hz, H5), 4.20 (ddd, *J* = 2.8, 2.6 and 1.8 Hz, H4´), 4.14 (br dd, *J* = 13.1 and 1.8 Hz, H5´b), 3.93 (s, $COOCH_3$), 2.73 (ddd, *J* = 15.3, 7.8 and 4.6 Hz, H2´´), 2.48 (br dd, *J* = 15.3,

1.3 and <1 Hz, H2´); ^{13}C NMR (D_2O) δ 172.35 (C=O), 169.31 (C4), 154.49 (C2), 151.00 (d, *J* = 7.2 Hz, Ar-C), 146.10 (C6), 131.01 (2x Ar-CH), 130.94 (Ar-C), 129.55 (2x Ar-CH), 102.61 (d, *J* = 166.9 Hz, >C<), 102.54 (C5), 88.89 (C1´), 80.31 (C4´), 73.35 (C3´), 65.06 (C5´), 55.28 (OCH_3), 42.52 (C2´).

(*S*)-1-[2-Deoxy-3,5-*O*-(phosphonobenzylidene)-β-D-threo-pentofuranosyl]uracil (12r)

Compound **12r** was prepared from diethyl phosphonate **11r** (200 mg, 440 μmol) using Method D as colorless oil in a yield of 87 mg (50%). HRMS $(M-H)^-$ for $C_{16}H_{16}O_8N_2P$: calcd *m/z* 395.06498, obs. 395.06480; IR (KBr, cm^{-1}) 3099, 3060, 3026, 1688, 1628, 1492, 1472, 1448, 1397, 1311, 1276, 1132, 1071, 1033, 968, 920, 768, 759, 701, 535. Elem. anal.: calcd P 7.41%; found P 6.91% (MW 447.23). ε_{264} = 8641 $L.mol^{-1}.cm^{-1}$ ^{1}H NMR (D_2O) δ 7.85 (d, *J* = 8.2 Hz, H6), 7.55 (m, 2x ArH), 7.37 (m, 3x ArH), 6.12 (dd, *J* = 7.9 and 1.4 Hz, H1´), 5.28 (ddd, *J* = 4.7, 2.8 and 1.4 Hz, H3´), 4.93 (ddd, *J* = 13.2, 3.0 and 1.4 Hz, H5´a), 4.92 (d, *J* = 8.2 Hz, H5), 4.26 (ddd, *J* = 3.0, 2.8 and 2.2 Hz, H4´), 4.18 (ddd, *J* = 13.2, 2.2 and 1.4 Hz, H5´b), 2.77 (ddd, *J* = 15.4, 7.9 and 4.7 Hz, H2´´), 2.51 (br dd, *J* = 15.4, 1.4 and <1 Hz, H2´); ^{13}C NMR (D_2O) δ 169.12 (C4), 154.31 (C2), 145.84 (C6), 142.91 (d, *J* = 8.2 Hz, Ar-C), 130.99 (Ar-CH), 130.31 (d, *J* = 1.2 Hz, 2x Ar-CH), 129.12 (d, *J* = 1.8 Hz, 2x Ar-CH), 102.85 (C5), 101.53 (d, *J* = 166.2 Hz, >C<), 88.84 (C1´), 79.85 (C4´), 73.68 (C3´), 65.15 (d, *J* = 1.8 Hz, C5´), 42.29 (C2´).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-carboxy-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (13)

A solution of methyl ester **12f** (13 mg, 30 μmol) in 0.05 M NaOH (5 mL) was set aside at rt overnight (TLC IPAV). The pH value of the reaction mixture was adjusted to 7.2 by a careful addition of Dowex in H^+ form. Filtration and evaporation *in vacuo* yielded **13** 12 mg (65%). HRMS $(M-H)^-$ for $C_{18}H_{18}O_{10}N_2P$: calcd *m/z* 453.07045, found 453.06996; IR (KBr, cm^{-1}) 1698, 1683, 1556, 1508, 1473, 1437, 1396, 1313, 1197, 1136, 1081, 976, 939, 770, 698. Elem. anal.: calcd P 6.23%; found P 4.63% (MW 669.13). ε_{238} = 14720 $L.mol^{-1}.cm^{-1}$ ^{1}H NMR

(D_2O) δ 7.77 (m, 2x ArH), 7.67 (q, *J* = 1.2 Hz, H6), 7.63 (m, 2x ArH), 6.16 (dd, *J* = 8.2 and 1.6 Hz, H1´), 5.44 (ddd, *J* = 4.8, 2.7 and 0.9 Hz, H3´), 5.15 (ddd, *J* = 13.0, 2.4 and 0.9 Hz, H5´a), 4.16 (ddd, *J* = 13.0, 1.5 and 0.6 Hz, H5´b), 4.14 (ddd, *J* = 2.7, 2.4 and 1.5 Hz, H4´), 2.75 (ddd, *J* = 15.2, 8.2 and 4.8 Hz, H2´´), 2.38 (ddd, *J* = 15.2, 1.6 and 0.9 Hz, H2´), 0.91 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 178.56 (C=O), 172.24 (C4), 156.55 (C2), 148.41 (d, *J* = 6.8 Hz, Ar-C), 141.50 (C6), 137.63 (d, *J* = 1.4 Hz, Ar-C), 130.64 (2x Ar-CH), 129.10 (d, *J* = 1.3 Hz, 2x Ar-CH), 112.84 (C5), 103.02 (d, *J* = 166.2 Hz, >C<), 88.17 (C1´), 79.74 (C4´), 73.44 (C3´), 65.16 (C5´), 42.86 (C2´), 13.49 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-carbamoyl-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (14)

Compound **12f** (13 mg, 30 μmol) was heated with 33% aqueous ammonia (5 mL) in a thick wall tube to 100 °C for 24 h (TLC IPAV) yielded a 4:1 mixture of amide **14** and carboxylic acid **13**. The separation of both compounds was accomplished by the preparative HPLC on the C18 column using a linear gradient of methanol in 0.1M TEAB. The product was converted into the sodium salt on Dowex 50 in Na^+ form. Yield of **14** 11 mg (67%). HRMS (M-H)- for $C_{18}H_{21}O_9N_3P$: calcd m/z 454.10099, found 454.10102; IR (KBr, cm^{-1}) 3418, 3193, 2818, 1676, 1616, 1567, 1477, 1435, 1411, 1386, 1313, 1299, 1275, 1224, 1198, 1137, 1116, 1087, 1075, 1018, 973, 827, 770, 686, 570. Elem. anal.: calcd P 6.53%; found P 5.21% (MW 594.18). ε_{243} = 14829 $L.mol^{-1}.cm^{-1}$ ^{1}H NMR (D_2O) δ 7.76 (m, 2x ArH), 7.68 (m, 2x ArH), 7.61 (q, *J* = 1.2 Hz, 5-CH_3), 6.11 (dd, *J* = 7.9 and 1.4 Hz, H1´), 5.35 (ddd, *J* = 4.6, 2.8 and 1.2 Hz, H3´), 5.04 (ddd, *J* = 13.3, 2.5 and 1.2 Hz, H5´a), 4.28 (br dd, *J* = 13.3 and 1.6 Hz, H5´b), 4.23 (ddd, *J* = 2.8, 2.5 and 1.6 Hz, H4´), 2.77 (ddd, *J* = 15.4, 7.9 and 4.6 Hz, H2´´), 2.48 (ddd, *J* = 15.4, 1.4 and 1.2 Hz, H2´), 0.92 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 175.67 ($CONH_2$), 169.25 (C4), 154.24 (C2), 147.89 (d, *J* = 7.3 Hz, Ar-C), 141.45 (C6), 134.92 (Ar-C), 129.55 (2x Ar-CH), 129.49 (2x Ar-CH), 112.39 (C5), 101.55 (d, *J* = 174.7 Hz, >C<), 88.56 (C1´), 79.60 (C4´), 73.74 (C3´), 65.43 (C5´), 42.77 (C2´), 13.14 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-carboxy-phosphonobenzylidene)-β-D-threo-pentofuranosyl]uracil (15)

A solution of methyl ester **12q** (17 mg, 37 μmol) in 0.05 M NaOH (5 mL) was set aside at rt overnight (TLC IPAV). The pH value of the reaction mixture was adjusted to 7.2 by a careful addition of Dowex in H^+ form. Filtration and evaporation *in vacuo* yielded **15** 9 mg (56%). HRMS $(M-H)^-$ for $C_{17}H_{16}O_{10}N_2P$: calcd *m/z* 439.05480, found 439.05501; IR (KBr, cm^{-1}) 3428, 1698, 1552, 1471, 1394, 1312, 1278, 1260, 1130, 1077, 970. Elem. anal.: calcd P 6.4%; found P 5.1% (MW 607.41). ε_{240} = 15740 $L.mol^{-1}.cm^{-1}$. NMR (D_2O) δ 7.92 (d, *J* = 8.1 Hz, H6), 7.78 (m, 2x ArH), 7.61 (m, 2x ArH), 6.14 (dd, *J* = 7.9 and 1.4 Hz, H1´), 5.38 (ddd, *J* = 4.7, 2.8 and 1.0 Hz, H3´), 5.04 (ddd, *J* = 13.1, 2.8 and 1.0 Hz, H5´a), 4.93 (d, *J* = 8.1 Hz, H5), 4.21 (td, *J* = 2.8, 2.8 and 1.9 Hz, H4´), 4.13 (br dd, *J* = 13.1 and 1.9 Hz, H5´b), 2.75 (ddd, *J* = 15.4, 7.9 and 4.7 Hz, H2´´), 2.47 (bdd, *J* = 15.4, 1.4 and <1 Hz, H2´); ^{13}C NMR (D_2O) δ 178.59 (COOH), 169.11 (C4), 154.42 (C2), 147.63 (d, *J* = 7.7 Hz, Ar-C), 146.11 (C6), 137.97 (Ar-C), 130.45 (2x Ar-CH), 129.10 (2x Ar-CH), 102.92 (C5), 102.50 (d, *J* = 170.9 Hz, >C<), 88.65 (C1´), 80.12 (C4´), 73.47 (C3´), 65.06 (C5´), 42.36 (C2´).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-amino-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (16)

Phosphonic acid **12e** (37 mg, 81 μmol) was dissolved in H_2O (10 mL) and 10% Palladium on activated Charcoal (15 mg) was added. Reaction was stirred in H_2 atmosphere overnight (TLC IPAV). Filtration through Celite and evaporation *in vacuo* yielded **16** 17 mg (50%). HRMS $(M-H)^-$ for $C_{17}H_{19}O_8N_3P$: calcd *m/z* 424.09152, found 424.09124; IR (KBr, cm^{-1}) 3430, 3367, 3229, 3066, 1690, 1585, 1515, 1475, 1410, 1383, 1350, 1310, 1276, 1178, 1135, 1076, 975. Elem. anal.: calcd P 6.92%; found P 6.04% (MW 512.22). ε_{267} = 9029 $L.mol^{-1}.cm^{-1}$. ^1H NMR (D_2O) δ 7.77 (q, *J* = 1.2 Hz, H5), 7.37 (m, 2x ArH), 6.75 (m, 2x ArH), 6.14 (dd, *J* = 8.1 and 1.5 Hz, H1´), 5.40 (ddd, *J* = 4.8, 2.6 and 0.9 Hz, H3´), 5.08 (ddd, *J* = 13.4, 2.8 and 0.9 Hz, H5´a), 4.14 (ddd, *J* = 2.8, 2.6 and 1.5 Hz, H4´), 4.13 (br dd, *J* = 13.4 and 1.5 Hz, H5´b), 2.74 (ddd, *J* = 15.3, 8.1 and 4.8 Hz, H2´´), 2.38 (bdd, *J* = 15.3, 1.5 and <1 Hz, H2´), 1.10 (d, *J* =

1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 169.46 (C4), 154.41 (C2), 147.84 (Ar-C), 142.00 (C6), 136.40 (d, *J* = 7.0 Hz, Ar-C), 130.22 (d, *J* = 1.3 Hz, 2x Ar-CH), 118.11 (2x Ar-CH), 112.77 (C5), 102.86 (d, *J* = 171.7 Hz, >C<), 88.19 (C1´), 79.94 (C4´), 73.21 (C3´), 64.98 (C5´), 42.72 (C2´), 13.27 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-amino-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (17)

The diethyl aminobenzylidenephosphonate derivative **17** was prepared by catalytic hydrogenation of nitro derivative **11e** (2.57 g, 5 mmol) with palladium on activated charcoal (310 mg) in EtOH (60 mL). Reaction was stirred for 3 hours at rt. The purification of crude product by silica gel chromatography (elution with a linear gradient of EtOH in $CHCl_3$) afforded a quantitative yield of **17** (2.4 g). HRMS $(M+Na)^+$ for $C_{21}H_{28}O_8N_3NaP$ calcd. 504.15062 *m/z* found 504.15048; IR (KBr, cm^{-1}) 3446, 3364, 3230, 3180, 3066, 3042, 2982, 2930, 1688, 1658, 1630, 1613, 1580, 1517, 1470, 1435, 1407, 1391, 1369, 1314, 1298, 1271, 1234, 1194, 1179, 1163, 1133, 1117, 1097, 1082, 1065, 1052, 1035, 971, 946, 840, 811, 746. ^{1}H NMR (d_6DMSO) δ 7.42 (q, *J* = 1.2 Hz, H5), 7.15 (m, 2x ArH), 6.50 (m, 2x ArH), 6.12 (dd, *J* = 8.5 and 2.1 Hz, H1´), 5.20 (br s, NH_2), 5.11 (ddd, *J* = 5.2, 2.8 and 2.0 Hz, H3´), 4.78 (ddd, *J* = 12.9, 2.8 and 2.0 Hz, H5´a), 4.13 (dd, *J* = 12.9 and 1.8 Hz, H5´b), 4.07 (td, *J* = 2.8, 2.8 and 1.8 Hz, H4´), 3.98 – 3.86 (m, 2x P-O-CH_2), 2.77 (ddd, *J* = 15.3, 8.5 and 5.2 Hz, H2´´), 2.08 (bdd, *J* = 15.3, 2.1 and <1 Hz, H2´), 1.16 (t, *J* = 7.0 Hz, CH_3), 1.15 (t, *J* = 7.0 Hz, CH_3), 1.04 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.80 (C4), 150.55 (C2), 149.27 (d, *J* = 1.3 Hz, Ar-C), 136.83 (C6), 127.33 (2x Ar-CH), 124.74 (d, *J* = 12.3 Hz, Ar-C), 112.67 (2x Ar-CH), 108.84 (C5), 97.38 (d, *J* = 192.7 Hz, >C<), 83.68 (C1´), 74.83 (C4´), 71.56 (C3´), 63.76 (d, *J* = 7.0 Hz, P-O-CH_2), 62.70 (d, *J* = 7.0 Hz, P-O-CH_2), 62.51 (C5´), 40.00 (C2´), 16.44 (d, *J* = 5.3 Hz, CH_3), 16.43 (d, *J* = 5.3 Hz, CH_3), 11.31 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-formamido-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (18)

The compound **18** was prepared from **17** (135 mg, 0.28 mmol) treating with ammonium formate (35 mg ,0.56 mmol) in CH_3CN (1 mL) overnight heated to 90°C in pressure glass tube under anhydrous conditions in a quantitative yield of 144 mg. HRMS $(M-H)^-$ for $C_{22}H_{29}O_9N_3P$ calcd. 510.16359 *m/z* found 510.16371; IR (KBr, cm^{-1}) 3263, 3193, 3063, 2982, 2929, 1690, 1611, 1523, 1471, 1408, 1369, 1313, 1300, 1271, 1243, 1183, 1163, 1133, 1083, 1040, 1031, 972, 949, 826, 766, 628, 557, 418. ^{1}H NMR (d_6DMSO), mixture of two isomers around –NH-CH=O, ratio 76 : 24, some signals are doubled δ 10.26 and 10.22 (2x bd, *J* = 1.9 and 11.0 Hz, NH), 8.27 and 8.80 (2x d, *J* = 1.9 and 11.0 Hz, -CH=O), 7.57 (m, 2x ArH), 7.45 (m, 2x ArH), 7.32 (q, *J* = 1.2 Hz, H5), 6.10 (dd, *J* = 8.4 and 2.0 Hz, H1´), 5.17 (ddd, *J* = 5.1, 3.0 and 2.1 Hz, H3´), 4.85 (ddd, *J* = 13.0, 2.6 and 2.1 Hz, H5´a), 4.22 (br dd, *J* = 13.0, 1.6 and <1 Hz, H5´b), 4.10 (ddd, *J* = 3.0, 2.6 and 1.6 Hz, H4´), 4.03 - 3.93 (m, 2x P-O-CH_2), 2.79 (ddd, *J* = 15.3, 8.4 and 5.1 Hz, H2´´), 2.13 (bdd, *J* = 15.3, 2.0 and <1 Hz, H2´), 1.18 (t, *J* = 7.0, CH_3), 1.15 (t, *J* = 7.0, CH_3), 0.90 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.74 (C4), 159.90 and 162.65 (-CH=O), 150.50 (C2), 138.73 and 138.95 (2x d, *J* = 1.8 and 1.6 Hz, Ar-C), 136.51 (C6), 133.27 and 133.45 (2x d, *J* = 8.3 Hz, Ar-C), 127.22 and 127.75 (2x Ar-CH), 118.23 and 116.46 (2x Ar-CH), 108.57 (C5), 96.92 (d, *J* = 191.0 Hz, >C<), 83.86 (C1´), 74.71 and 74.68 (C4´), 71.88 (C3´), 63.02 (d, *J* = 6.9 Hz, P-O-CH_2), 62.92 (d, *J* = 7.0 Hz, P-O-CH_2), 62.84 (C5´), 39.92 (C2´), 16.42 (d, *J* = 5.2 Hz, CH_3), 16.40 (d, *J* = 5.1 Hz, CH_3), 11.20 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-isocyano-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (19)

The isocyano derivative **19** was prepared by dehydration of **18** (140 mg, 0.275 mmol). The reaction was performed in CH_3CN/Et_3N (6 mL, 1:1) by $POCl_3$ (63 mg, 0.41 mmol). The reaction mixture was extracted in $CHCl_3/H_2O$ and the crude product was purified by silica gel chromatography (elution with a linear gradient of EtOH in $CHCl_3$) yielding 96 mg (71%) of **19**. HRMS $(M+Na)^+$ for $C_{22}H_{26}O_8N_3NaP$ calcd. 514.13497 *m/z* found 514.13514; IR (KBr, cm^{-1}) 3175, 3056, 2981, 2928, 2856, 2124, 1686, 1582, 1502, 1471, 1399, 1369, 1315, 1272,

1249, 1194, 1168, 1133, 1083, 1043, 973, 792, 775, 685, 641, 628, 417. ^{1}H NMR (d_6DMSO) δ 7.62 (m, 2x ArH), 7.59 (m, 2x ArH), 7.22 (q, *J* = 1.2 Hz, H6), 6.07 (dd, *J* = 8.3 and 1.9 Hz, H1´), 5.19 (ddd, *J* = 5.0, 2.5 and 2.0 Hz, H3´), 4.88 (ddd, *J* = 12.8, 2.6 and 2.0 Hz, H5´a), 4.29 (br dd, *J* = 12.8, 1.5 and <1 Hz, H5´b), 4.13 (ddd, *J* = 2.6, 2.5 and 1.5 Hz, H4´), 4.07 - 3.96 (m, 2x P-O-CH_2), 2.80 (ddd, *J* = 15.2, 8.3 and 5.0 Hz, H2´´), 2.17 (bdd, *J* = 15.2, 1.9 and <1 Hz, H2´), 1.18 (t, *J* = 7.1, CH_3), 1.15 (t, *J* = 7.1, CH_3), 0.88 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 164.92 (-NC), 163.69 (C4), 150.44 (C2), 140.02 (d, *J* = 8.4 Hz, Ar-C), 136.20 (C6), 128.12 (d, *J* = 1.3 Hz, 2x Ar-CH), 126.06 (Ar-C), 125.85 (2x Ar-CH), 108.29 (C5), 96.52 (d, *J* = 189.8 Hz, >C<), 84.02 (C1´), 74.58 (C4´), 72.18 (C3´), 63.27 (d, *J* = 6.9 Hz, P-O-CH_2), 63.18 (d, *J* = 6.9 Hz, P-O-CH_2), 63.16 (C5´), 39.70 (C2´), 16.37 (d, *J* = 5.0 Hz, CH_3), 16.34 (d, *J* = 5.1 Hz, CH_3), 11.14 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-formamido-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (20)

Compound **20** was prepared from diethyl phosphonate **18** (100 mg, 0.2 mmol) using Method D as colorless oil in a yield of 65 mg (65%). HRMS $(M-H)^-$ for $C_{18}H_{20}O_9N_3NaP$ calcd. 476.08294 *m/z* found 476.08302; IR (KBr, cm^{-1}) 3077, 2955, 2229, 1691, 1477, 1437, 1405, 1383, 1313, 1275, 1199, 1180, 1137, 1075, 1020, 972, 830, 769, 432. Elem. anal.: calcd P 6.52% found P 6.20% (MW 499.85). ε_{251} = 23022 $L.mol^{-1}.cm^{-1}$. ^{1}H NMR (D_2O) mixture of isomers around –NH-CH=O, ratio 64 : 36, many signals are doubled δ 8.66 and 8.25 (2x s, N-CH=O), 7.64 and 7.63 (2x q, *J* = 1.2 Hz, H6), 7.56 (m, 2x ArH), 7.44 and 7.16 (2x m, 2x ArH), 6.12 and 6.11 (2x dd, *J* = 8.0 and 1.4 Hz, H1´), 5.32 (m, H3´), 5.01 and 5.00 (2x ddd, *J* = 13.2, 2.6 and 1.1 Hz, H5´a), 4.25 and 4.24 (2x br dd, *J* = 13.2 and 1.5 Hz, H5´b), 4.21 (m, H4´), 2.78 and 2.77 (2x ddd, *J* = 15.4, 8.0 and 4.8 Hz, H2´´), 2.46 and 2.45 (2x bdd, *J* = 15.4, 1.4 and <1 Hz, H2´), 1.02 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 169.24 (C4), 165.14 and 168.10 (-N-CH=O), 154.27 and 154.25 (C2), 141.48 and 141.53 (C6), 140.54 and 140.71 (2x d, *J* = 7.6 Hz, Ar-C), 138.55 and 139.00 (2x d, *J* = 1.9 and 1.6 Hz, Ar-C), 129.94 and 130.46 (2x d, *J* = 1.7 Hz, 2x Ar-CH), 123.06 and 120.82 (2x d, *J* = 1.1 Hz, 2x Ar-CH), 112.58 and

112.46 (C5), 101.49 and 101.44 (2x d, *J* = 178.0 Hz, >C<), 88.43 and 88.52 (C1´), 79.54 and 79.59 (C4´), 73.67 and 73.63 (C3´), 65.31 (C5´), 42.67 and 42.72 (C2´), 13.29 and 13.24 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-isocyano-phosphonobenzylandidene)-β-D-threo-pentofuranosyl]thymine (21)

Compound **21** was prepared from diethyl phosphonate **19** (84 mg, 0.17 mmol) using Method D as colorless oil in a yield of 8 mg (9%). HRMS $(M-H)^-$ for $C_{18}H_{17}O_8N_3P$ calcd. 434.07587 *m/z* found 434.07593; IR (KBr, cm^{-1}) 3071, 2123, 1691, 1612, 1501, 1478, 1438, 1313, 1276, 1259, 1198, 1136, 1076, 1019, 973, 949, 771. Elem. anal.: calcd P 6.77% found P 5.97% (MW 518.59). ε_{266} = 8941 $L.mol^{-1}.cm^{-1}$. ^1H NMR (D_2O) δ 7.64 (m, 2x ArH), 7.61 (q, *J* = 1.2 Hz, H6), 7.44 (m, 2x ArH), 6.10 (dd, *J* = 7.9 and 1.4 Hz, H1´), 5.35 (ddd, *J* = 4.7, 2.7 and 1.1 Hz, H3´), 5.02 (ddd, *J* = 13.3, 2.6 and 1.1 Hz, H5´a), 4.25 (br dd, *J* = 13.3 and 1.6 Hz, H5´b), 4.23 (ddd, *J* = 2.7, 2.6 and 1.6 Hz, H4´), 2.77 (ddd, *J* = 15.2, 7.9 and 4.7 Hz, H2´´), 2.46 (bdd, *J* = 15.3, 1.4 and <1 Hz, H2´), 1.04 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 169.20 (C4), 162.49 (-NC), 154.24 (C2), 145.52 (d, *J* = 7.5 Hz, Ar-C), 141.41 (C6), 130.32 (2x Ar-CH), 128.51 (2x Ar-CH), 128.10 (Ar-C), 112.36 (C5), 101.51 (d, *J* = 174.4 Hz, >C<), 88.50 (C1´), 79.59 (C4´), 73.76 (C3´), 65.43 (C5´), 42.65 (C2´), 13.30 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-formyl-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (22)

Phosphonic acid **12k** (10 mg, 23 μmol) was dissolved in H_2O (0.5 mL) and $NaIO_4$ (10 mg, 46 μmol) and OsO_4 (31 μL of 2.5% wt solution in *t*-BuOH, 2.3 μmol) was added and the reaction mixture was stirred for 1 h. Progress of reaction was monitored by LCMS. When finished, the reaction was diluted by 0.1M TEAB and purified by the preparative HPLC on the C18 column using a linear gradient of methanol in 0.1M TEAB. The product **22** (7 mg, 67%) was converted into the sodium salt on Dowex 50 in Na^+ form. HRMS $(M-H)^-$ for $C_{18}H_{18}O_9N_2P$: calcd *m/z* 437.07554, found 437.07544; IR (KBr, cm^{-1}) 3432, 3165, 2817, 1701, 1695, 1605,

1576, 1476, 1402, 1303, 1277, 1200, 1134, 1079, 975, 823. Elem. anal.: calcd P 6.73%; found P 5.71% (MW 516.34). ε_{261} = 19504 L.mol^{-1}.cm^{-1}. ^{1}H NMR (D_2O) δ 9.92 (s, CH=O), 7.87 (m, 2x ArH), 7.79 (m, 2x ArH), 7.67 (q, *J* = 1.2 Hz, H6), 6.12 (dd, *J* = 8.0 and 1.5 Hz, H1´), 5.45 (ddd, *J* = 4.8, 2.2 and 0.8 Hz, H3´), 5.14 (ddd, *J* = 13.1, 2.4 and 0.8 Hz, H5´a), 4.22 (br dd, *J* = 13.1 and 1.5 Hz, H5´b), 4.18 (ddd, *J* = 2.4, 2.2 and 1.5 Hz, H4´), 2.76 (ddd, *J* = 15.2, 8.0 and 4.8 Hz, H2´´), 2.45 (bdd, *J* = 15.2, 1.5 and <1 Hz, H2´), 0.91 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 199.26 (CH=O), 169.25 (C4), 154.30 (C2), 152.47 (d, *J* = 6.7 Hz, Ar-C), 141.64 (C6), 137.48 (Ar-C), 132.04 (2x Ar-CH), 130.01 (2x Ar-CH), 112.38 (C5), 102.76 (d, *J* = 168.2 Hz, >C<), 88.42 (C1´), 79.93 (C4´), 73.52 (C3´), 65.34 (C5´), 42.73 (C2´), 13.19 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-methylsulfinyl-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (23)

Phosphonic acid **12g** (5 mg, 11 μmol) was dissolved in H_2O (1 mL), $NaIO_4$ (7 mg, 33 μmol) was added, and the reaction mixture was stirred for 2 h. Progress of the reaction was monitored by LCMS. When finished, the reaction was diluted by 0.1M TEAB and purified by the preparative HPLC on the C18 column using a linear gradient of methanol in 0.1M TEAB. The product **23**, 5 mg (96%), was converted into the sodium salt on Dowex 50 in Na^+ form. HRMS (M-H)$^-$ for $C_{18}H_{20}O_9N_2PS$: calcd *m/z* 471.06326, found 471.06298; IR (KBr, cm^{-1}) 3430, 1691, 1659 ,1474, 1438, 1354, 1313, 1300, 1276, 1134, 1085, 1047, 989, 975, 542. Elem. anal.: calcd P 6.27%; found P 4.92% (MW 629.61). ε_{266} = 8913 L.mol^{-1}.cm^{-1}. ^{1}H NMR (D_2O) δ 7.80 (m, 2x ArH), 7.70 (q, *J* = 1.2 Hz, H6), 7.66 (m, 2x ArH), 6.13 (dd, *J* = 8.0 and 1.4 Hz, H1´), 5.45 (ddd, *J* = 4.6, 2.6 and 0.8 Hz, H3´), 5.15 (ddd, *J* = 13.3, 2.6 and 0.8 Hz, H5´a), 4.22 (br dd, *J* = 13.3 and 1.5 Hz, H5´b), 4.18 (td, *J* = 2.6, 2.6 and 1.5 Hz, H4´), 2.89 (s, CH_3-SO-), 2.76 (ddd, *J* = 15.3, 8.0 and 4.6 Hz, H2´´), 2.44 (bdd, *J* = 15.3, 1.4 and <1 Hz, H2´); ^{13}C NMR (D_2O) some signals are doubled due to a chiral CH_3-S(=O)- group, δ 169.24 (C4), 154.32 (C2), 149.53 and 149.46 (2x d, *J* = 7.0 Hz, Ar-C), 143.71 and 143.63 (2x d, *J* = 1.7 Hz, Ar-C), 141.75 (C6), 130.60 (2x Ar-CH), 129.16 (d, *J* = 9.2 Hz, 2x Ar-CH), 112.30

and 112.29 (C5), 102.54 (d, *J* = 166.2 Hz, >C<), 88.36 and 88.35 (C1´), 79.54 (C4´), 73.51 (C3´), 65.33 (C5´), 44.02 (CH_3-SO-), 42.77 (C2´), 17.31 and 17.29 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(phosphonobenzylidene)-β-D-threo-pentofuranosyl]-5-chloro-uracil (24)

A solution of phosphonic acid **12r** (20 mg; 50 μmol) dried by co-evaporation with pyridine, in dry pyridine (1.5 mL) was treated with *N*-chlorosuccinimide (9 mg, 65 μmol) at 90 °C. After 30 min there was still starting material (TLC IPAV). Another portion of *N*-chlorosuccinimide (9 mg, 65 μmol) was added. After next 30 min the reaction was diluted by 0.1M TEAB and purified by the preparative HPLC on the C18 column using a linear gradient of methanol in 0.1M TEAB. The product **24** (14 mg; 64%) was converted into the sodium salt on Dowex 50 in Na^+ form. HRMS $(M-H)^-$ for $C_{16}H_{15}O_8N_2ClP$: calcd *m/z* 429.02600, found 429.02591; IR (KBr, cm^{-1}) 3064, 1695, 1644, 1622, 1562, 1537, 1491, 1449, 1449, 1309, 1296, 1272, 1194, 1143, 1080, 1060, 1035, 977, 945, 781, 758, 702. Elem. anal.: calcd P 6.84%; found P 5.63% (MW 549.17). ε_{279} = 7684 $L.mol^{-1}.cm^{-1}$. ^{1}H NMR (D_2O) δ 8.09 (s, H6), 7.56 (m, 2x ArH), 7.30 (m, 3x ArH), 6.10 (dd, *J* = 7.8 and 1.4 Hz, H1´), 5.44 (ddd, *J* = 4.7, 2.7 and 0.9 Hz, H3´), 5.07 (ddd, *J* = 13.1, 2.7 and 0.9 Hz, H5´a), 4.14 (br dd, *J* = 13.1 and 1.8 Hz, H5´b), 4.20 (td, *J* = 2.7, 2.7 and 1.8 Hz, H4´), 2.75 (ddd, *J* = 15.3, 7.8 and 4.7 Hz, H2´´), 2.49 (bdd, *J* = 15.3, 1.4 and <1 Hz, H2´); ^{13}C NMR (D_2O) δ 167.00 (C4), 155.09 (C2), 144.58 (d, *J* = 7.1 Hz, Ar-C), 142.41 (C6), 130.23 (2x Ar-CH), 130.19 (Ar-CH), 129.06 (d, *J* = 1.3 Hz, 2x Ar-CH), 110.86 (C5), 102.90 (d, *J* = 169.6 Hz, >C<), 89.14 (C1´), 80.40 (C4´), 73.32 (C3´), 65.21 (d, *J* = 11.5 Hz, C5´), 42.55 (C2´).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-azido-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (26)

A mixture of diethyl iodobenzylidenephosphonate **11d** (50 mg, 85 μmol), $CuSO_4 \cdot 5H_2O$ (2.2 mg, 8.5 μmol), NaN_3 (11 mg, 170 μmol), Na_2CO_3 (2 mg, 17 μmol), ascorbic acid (3 mg, 17 μmol), and L-proline (2 mg, 17 μmol) in aq. 90% DMSO (0.5 mL) was heated to 60 °C for 24

h. Since, according to the LCMS still some starting material was presented, another portion of NaN_3 (11 mg, 170 μmol), Na_2CO_3 (2 mg, 17 μmol), and ascorbic acid (3 mg, 17 μmol) was added and the heating was continued for further 24 h. When finish, reaction mixture was evaporated *in vacuo*, the residue dissolved in 0.1M TEAB and purified by the preparative HPLC on the C18 column using a linear gradient of methanol in 0.1M TEAB. The monoethyl azido phosphonate **25** (29 mg; 64%) was converted directly, without additional characterization, by Method D to the corresponding phosphonic acid **26** (11 mg; 41%). HRMS $(M-H)^-$ for $C_{17}H_{17}O_8N_5P$: calcd *m/z* 450.08202, found 450.08192; IR (KBr, cm^{-1}) 3434, 2125, 2089, 1690, 1606, 1503, 1473, 1411, 1382, 1357, 1310, 1282, 1133, 1080, 977. Elem. anal.: calcd P 5.39% found P 5.17% (MW 493.2). ε_{256} = 18347 $L.mol^{-1}.cm^{-1}$. ^1H NMR (D_2O) δ 7.73 (q, *J* = 1.2 Hz, H5), 7.57 (m, 2x ArH), 7.02 (m, 2x ArH), 6.13 (dd, *J* = 8.1 and 1.5 Hz, H1´), 5.43 (ddd, *J* = 4.8, 2.7 and 0.9 Hz, H3´), 5.12 (ddd, *J* = 13.3, 2.8 and 0.9 Hz, H5´a), 4.16 (br dd, *J* = 13.3 and 1.5 Hz, H5´b), 4.16 (ddd, *J* = 2.8, 2.7 and 1.5 Hz, H4´), 2.74 (ddd, *J* = 15.3, 8.1 and 4.8 Hz, H2´´), 2.41 (bdd, *J* = 15.3, 1.5 and <1 Hz, H2´), 1.05 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 169.44 (C4), 154.42 (C2), 142.16 (d, *J* = 6.8 Hz, Ar-C), 141.87 (C6), 141.40 (Ar-C), 130.75 (2x Ar-CH), 120.49 (2x Ar-CH), 112.55 (C5), 102.76 (d, *J* = 168.2 Hz, >C<), 88.30 (C1´), 79.96 (C4´), 73.81 (C3´), 65.13 (C5´), 42.74 (C2´), 13.32 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-diethylphosphono-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (27)

Diethyl iodobenzylidenephosphonate **11d** (50 mg, 85 μmol) was dissolved in THF (0.4 mL) and $Pd(dppf)Cl_2$ (2 mg, 2.1 μmol), $OP(Et)_2$ (17 μL, 128 μmol), KOAc (1 mg, 8.5 μmol), Et_3N (18 μL, 128 μmol) were added. The mixture was heated to 70 °C for 24 h and progress of reaction was monitored by LCMS. After this, there was still presented unreacted starting material. Another portion of $Pd(dppf)Cl_2$ (2 mg, 2.1 μmol), $OP(Et)_2$ (17 μL, 128 μmol), Et_3N (18 μL, 128 μmol) was added and the heating was continued for further 24 h. When finish,

reaction was evaporated *in vacuo* and dissolved in 0.1M TEAB and purified by the preparative HPLC on the C18 column using a linear gradient of methanol in 0.1M TEAB. The tetraethyl diphosphonate **27** was obtained as colourless oil in a yield of 26 mg (51%). HRMS $(M-H)^+$ for $C_{25}H_{37}O_{11}N_2P_2$: calcd *m/z* 603.18671, found 603.18681; IR ($CHCl_3$, cm^{-1}) 2931, 2870, 2856, 1685, 1471, 1434, 1369, 1349, 1314, 1269, 1243, 1164, 1131, 1097, 1042, 1029, 972. ^{1}H NMR (d_6DMSO) δ 11.20 (br s, NH), 7.73 (m, 2x ArH), 7.68 (m, 2x ArH), 7.25 (q, *J* = 1.2 Hz, H6), 6.10 (dd, *J* = 8.4 and 2.0 Hz, H1´), 5.19 (ddd, *J* = 5.1, 2.5 and 2.1 Hz, H3´), 4.90 (ddd, *J* = 13.0, 2.6 and 2.1 Hz, H5´a), 4.29 (br dd, *J* = 13.0, 1.5 and <1 Hz, H5´b), 4.13 (ddd, *J* = 2.6, 2.5 and 2.1 Hz, H4´), 4.07 - 3.97 (m, 4x P-O-CH_2), 2.81 (ddd, *J* = 15.3, 8.4 and 5.1 Hz, H2´´), 2.16 (bdd, *J* = 15.3, 2.0 and <1 Hz, H2´), 1.24 (t, *J* = 7.0, CH_3), 1.23 (t, *J* = 7.0, CH_3), 1.17 (t, *J* = 7.0, CH_3), 1.15 (t, *J* = 7.0, CH_3), 0.82 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.67 (C4), 150.47 (C2), 142.48 (dd, *J* = 8.4 and 3.1 Hz, Ar-C), 136.46 (C6), 130.80 (d, *J* = 10.1 Hz, 2x Ar-CH), 129.12 (d, *J* = 186.4 Hz, Ar-C), 126.88 (d, *J* = 14.9 Hz, 2x Ar-CH), 108.44 (C5), 96.75 (d, *J* = 189.0 Hz, >C<), 83.84 (C1´), 74.51 (C4´), 72.16 (C3´), 63.23 (d, *J* = 6.9 Hz, P-O-CH_2), 63.15 (d, *J* = 7.1 Hz, P-O-CH_2), 63.11 (C5´), 61.90 (d, *J* = 5.5 Hz, P-O-CH_2), 61.89 (d, *J* = 5.5 Hz, P-O-CH_2), 39.93 (C2´), 16.35 (d, *J* = 5.6 Hz, 4x CH_3), 11.11 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-phosphono-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (28)

Compound **28** was prepared from tetraethyl diphosphonate **27** (16 mg, 28 μmol) using Method D as colorless oil in a yield of 6 mg (49%). HRMS $(M-H)^-$ for $C_{17}H_{19}O_{11}N_2P_2$: calcd *m/z* 489.04696, obs. 489.04721; IR (KBr, cm^{-1}) 3433, 3246, 1692, 1481, 1384, 1357, 1313, 1278, 1129, 1078, 973. Elem. anal.: calcd P 11.6% found P 8.95% (MW 692.14). ε_{270} = 10314 $L.mol^{-1}.cm^{-1}$. ^{1}H NMR (D_2O) δ 7.72 (q, *J* = 1.2 Hz, H5), 7.63 (m, 2x ArH), 7.59 (m, 2x ArH), 6.16 (dd, *J* = 8.1 and 1.6 Hz, H1´), 5.43 (ddd, *J* = 4.8, 2.6 and 0.9 Hz, H3´), 5.12 (ddd, *J* = 13.1, 2.5 and 0.9 Hz, H5´a), 4.19 (br dd, *J* = 13.1 and 1.6, H5´b), 4.16 (ddd, *J* = 2.6, 2.5 and 1.6 Hz, H4´), 2.76 (ddd, *J* = 15.3, 8.1 and 4.8 Hz, H2´´), 2.42 (bdd, *J* = 15.3, 1.6 and <1 Hz,

H2´), 0.99 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 169.39 (C4), 154.38 (C2), 145.80 (dd, *J* = 6.9 and 2.8 Hz, Ar-C), 141.76 (C6), 139.82 (d, *J* = 173.2 Hz, Ar-C), 132.05 (d, *J* = 9.6 Hz, 2x Ar-CH), 128.66 (d, *J* = 13.0 Hz, 2x Ar-CH), 112.92 (C5), 102.68 (d, *J* = 169.2 Hz, >C<), 88.13 (C1´), 79.78 (C4´), 73.50 (C3´), 65.24 (C5´), 42.64 (C2´), 13.41 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-borono-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (29)

Diethyl iodobenzylidenephosphonate **11d** (100 mg, 170 μmol) was dissolved in dioxane (1 mL) and $Pd_2(dba)_3$ (1.2 mg, 1.7 μmol), XPhos (3.2 mg, 6.8 μmol), bis(pinacolato)diboron (128 mg, 510 μmol), and KOAc (50 mg, 510 μmol) were added. The reaction mixture was heated to 110 °C for 8 h. Progress of reaction was monitored by LCMS. When finish, the reaction mixture was concentrated *in vacuo* and the crude compound was purified by the preparative HPLC on the C18 column using a linear gradient of methanol in 0.1M TEAB. The borono diphosphonate **29** was obtained as colourless oil in a yield of 42 mg (49%). HRMS $(M-H)^+$ for $C_{21}H_{27}O_{10}N_2BP$: calcd *m/z* 509.15019, found 509.15027; IR ($CHCl_3$, cm^{-1}) 3397, 2932, 2826, 1684, 1473, 1473, 1385, 1372, 1369, 1271, 1239, 1192, 1163, 1083, 1000, 652, 572. ^{1}H NMR (d_6DMSO) δ 11.20 (br s, NH), 7.77 (m, 2x ArH), 7.48 (m, 2x ArH), 7.30 (q, *J* = 1.2 Hz, H6), 6.11 (dd, *J* = 8.5 and 2.0 Hz, H1´), 5.19 (ddd, *J* = 5.1, 2.7 and 2.1 Hz, H3´), 4.87 (ddd, *J* = 13.0, 2.6 and 2.1 Hz, H5´a), 4.22 (br dd, *J* = 13.0, 1.7 and <1 Hz, H5´b), 4.11 (ddd, *J* = 2.7, 2.6 and 1.7 Hz, H4´), 4.02 - 3.90 (m, 2x P-O-CH_2), 2.80 (ddd, *J* = 15.2, 8.5 and 5.1 Hz, H2´´), 2.15 (bdd, *J* = 15.2, 2.0 and <1 Hz, H2´), 1.17 (t, *J* = 7.0 Hz, CH_3), 1.14 (t, *J* = 7.0 Hz, CH_3), 0.81 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (d_6DMSO) δ 163.74 (C4), 150.50 (C2), 139.76 (d, *J* = 8.4 Hz, Ar-C), 136.46 (C6), 135.00 (Ar-C), 133.46 (2x Ar-CH), 125.48 (2x Ar-CH), 108.68 (C5), 97.18 (d, *J* = 167.3 Hz, >C<), 83.81 (C1´), 74.72 (C4´), 71.95 (C3´), 63.04 (d, *J* = 6.8 Hz, P-O-CH_2), 62.92 (d, *J* = 7.1 Hz, P-O-CH_2), 62.92 (C5´), 39.70 (C2´), 16.40 (d, *J* = 4.8 Hz, CH_3), 16.38 (d, *J* = 5.1 Hz, CH_3), 11.11 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-borono-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (30)

Compound **30** was prepared from tetraethyl diphosphonate **29** (22 mg, 43 μmol) using Method D as colorless oil in a yield of 9 mg (49%). HRMS $(M-H)^-$ for $C_{17}H_{19}O_{10}N_2BP$: calcd *m/z* 453.08758, obs. 453.08776; IR (KBr, cm^{-1}) 3434, 3183, 3070, 1691, 1610, 1516, 1475, 1437, 1402, 1314, 1277, 1196, 1134, 1080, 977, 937, 825, 428. Elem. anal.: calcd P 6.22% found P 5.67% (MW 546.37). ε_{270} = 8122 $L.mol^{-1}.cm^{-1}$. ^1H NMR (D_2O) δ 7.72 (q, *J* = 1.2 Hz, H6), 7.64 (m, 2x ArH), 7.60 (m, 2x ArH), 6.14 (dd, *J* = 8.1 and 1.5 Hz, H1´), 5.44 (ddd, *J* = 4.8, 2.6 and 0.7 Hz, H3´), 5.13 (ddd, *J* = 13.4, 2.7 and 0.7 Hz, H5´a), 4.17 (br dd, *J* = 13.4, 1.6 and <1 Hz, H5´b), 4.16 (ddd, *J* = 2.7, 2.6 and 1.6 Hz, H4´), 2.76 (ddd, *J* = 15.2, 8.1 and 4.8 Hz, H2´´), 2.42 (bdd, *J* = 15.2, 1.5 and <1 Hz, H2´), 0.92 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 169.40 (C4), 154.41 (C2), 147.46 (Ar-C), 141.86 (C6), 135.29 (2x Ar-CH), 134.37 (Ar-C), 128.73 (2x Ar-CH), 112.72 (C5), 103.00 (d, *J* = 167.3 Hz, >C<), 88.25 (C1´), 79.98 (C4´), 73.35 (C3´), 65.15 (C5´), 42.72 (C2´), 13.10 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-iodoxy-phosphonobenzylidene)-β-D-threo-pentofuranosyl]thymine (31)

Iodobenzylidenephosphonic acid **12d** (10 mg, 18 μmol) was dissolved in H_2O (0.5 mL) and MCPBA (18 mg, 72 μmol) was added. Then CH_3CN was added dropwise under stirring until clear solution was obtained. Reaction mixture was stirred at rt overnight. When finish (TLC IPAV), reaction mixture was evaporated *in vacuo* and crude compound was purified by the preparative HPLC on the C18 column using a linear gradient of methanol in 0.1M TEAB. The iodoxy diphosphonate **31** was obtained as colourless oil in a yield of 6 mg (57%). HRMS $(M-H)^+$ for $C_{17}H_{17}O_{10}N_2IP$: calcd *m/z* 566.96710, found 566.96687; IR (KBr, cm^{-1}) 3420, 3238, 1690, 1578, 1479, 1410, 1389, 1313, 1276, 1265, 1135, 1077, 1005, 973, 813. Elem. anal.: calcd P 5.39% found P 4.32% (MW 546,37). ε_{260} = 12722 $L.mol^{-1}.cm^{-1}$. ^1H NMR (D_2O) δ 7.91 (m, 2x ArH), 7.87 (m, 2x ArH), 7.69 (q, *J* = 1.2 Hz, H6), 6.13 (dd, *J* = 8.0 and 1.4 Hz,

H1´), 5.47 (ddd, *J* = 4.8, 2.6 and 0.8 Hz, H3´), 5.17 (ddd, *J* = 13.1, 2.4 and 0.8 Hz, H5´a), 4.22 (br dd, *J* = 13.1, 1.4 and <1 Hz, H5´b), 4.17 (ddd, *J* = 2.6, 2.4 and 1.4 Hz, H4´), 2.75 (ddd, *J* = 15.3, 8.0 and 4.8 Hz, H2´´), 2.43 (bdd, *J* = 15.3, 1.4 and <1 Hz, H2´), 0.97 (d, *J* = 1.2 Hz, 5-CH_3); ^{13}C NMR (D_2O) δ 170.00 (C4), 154.81 (C2), 151.14 (d, *J* = 6.8 Hz, Ar-C), 150.36 (Ar-C), 141.67 (C6), 131.26 (2x Ar-CH), 128.19 (2x Ar-CH), 112.41 (C5), 102.69 (d, *J* = 163.6 Hz, >C<), 88.38 (C1´), 79.92 (C4´), 73.56 (C3´), 65.38 (C5´), 42.79 (C2´), 13.55 (5-CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-iodoxy-phosphonobenzylidene)-β-D-threo-pentofuranosyl]uracil (32)

Iodobenzylidenephosphonic acid **12p** (7 mg, 13 μmol) was converted to iodoxy derivative **32** by same procedure as compound **31** in a yield of 3 mg (41%). HRMS $(M-H)^-$ for $C_{16}H_{15}O_{10}N_2IP$: calcd *m/z* 552.95145, found 552.95129; IR (KBr, cm^{-1}) 3435, 3198, 1683, 1632, 1620, 1464, 1443, 1385, 1314, 1277, 1256, 1129, 1081, 1005, 985, 970, 932, 817, 582. ^{1}H NMR (D_2O) δ 7.89 (m, 4x ArH), 7.89 (d, *J* = 8.0 Hz, H6), 6.11 (dd, *J* = 7.8 and 1.3 Hz, H1´), 5.41 (ddd, *J* = 4.6, 2.6 and 0.8 Hz, H3´), 5.12 (ddd, *J* = 13.0, 2.5 and 0.8 Hz, H5´a), 4.89 (d, *J* = 8.0 Hz, H5), 4.18 (br dd, *J* = 13.0 and 1.7 Hz, H5´b), 4.21 (ddd, *J* = 2.6, 2.5 and 1.7 Hz, H4´), 2.74 (ddd, *J* = 15.2, 7.8 and 4.6 Hz, H2´´), 2.48 (bdd, *J* = 15.2, 1.3 and <1 Hz, H2´); ^{13}C NMR (D_2O) δ 170.16 (C4), 154.99 (C2), 151.22 (d, *J* = 7.2 Hz, Ar-C), 150.19 (Ar-C), 145.99 (C6), 131.37 (2x Ar-CH), 129.85 (2x Ar-CH), 102.58 (C5), 102.45 (d, *J* = 165.3 Hz, >C<), 88.90 (C1´), 80.24 (C4´), 73.48 (C3´), 65.18 (C5´), 42.59 (C2´).

Biochemical equipment. Cultivated cells were disrupted with an EmulsifFlex C3 (Avestin). For protein purification ÄKTA FPLC (GE Healthcare) was used together with HisTrap HP column (GE Healthcare). Samples from kinetic measurements were separated and measured using Agilent 1100 HPLC system.

Protein preparation. Both enzymes were prepared according to published protocols[6], already modified in[7] for the production of cdN. Unlabelled proteins were prepared using LB broth growth media (Sigma) supplemented with 0.8% (v/v) glycerol and 20 μg/mL ampicillin

and in a final buffer containing 20 mM Tris.HCl, 20 mM $MgCl_2$, 2 mM EDTA and 2 mM DTT, pH 7.5, and 10% (v/v) glycerol in case of cdN.

Both proteins contained N-terminal hexahistidine (His_6) tag followed by the tobacco etch virus (TEV) protease recognition sequence. The cleavage with TEV protease yields products where mature proteins contain a cloning artifact sequence Ser Asn Ala Ala Ser at the N-terminus.

The yield of protein from 1L of bacterial culture was 4.16 mg and 4.29 mg for mdN and cdN, respectively. Purity was > 98%, as judged by SDS-PAGE. The protein solutions at concentration around 1 mg/mL were stored at -20 ºC. For crystallization, the proteins were further concentrated to a final concentration using ultrafiltration in Centricon YM-10 (Millipore).

Activity assay. Enzyme activity was determined by high-performance liquid chromatography with spectrophotometric detection of dUMP substrate and dU product at 262 nm. Typically, 2-4 nM enzyme in 20 mM Tris.HCl, 20 mM $MgCl_2$, 2 mM EDTA and 2 mM DTT, pH 7.5, was reacted with dUMP at a concentration comparable to enzyme K_M value (1 mM and 100 μM for cdN and mdN, respectively) for 6 min at 37 ºC in a final volume of 100 μL. The reaction was stopped by the addition of 3 μL of 30% (v/v) TCA and 5 μL of a reaction mixture was applied to a Zorbax C18 column (150 mm×3 mm, 2.5 μm particle size, Agilent) mounted to an Agilent 1100 system. The separation was done using isocratic elution in 75 mM KH_2PO_4 buffer. After each run, the column was washed with 100% methanol supplemented with 0.05% (v/v) TFA. None of the reactions reached more than 10% of the substrate conversion.

Enzymatic constants determination. To determine the K_M value of mdN and cdN, a series of activity assays were performed at different substrate concentrations. For mdN, eleven measurements were performed in triplicates with substrate concentration varying from 5 μM

to 10 mM. For cdN, nine measurements were performed in duplicates with substrate concentration varying from 100 μM to 10 mM. All data were fitted using non-linear regression with classical Michaelis-Menten equation.[8]

Inhibition assay. The inhibition effect on mdN and cdN activity was screened for all synthesized compounds by the determination of the relative activity of the enzyme in the absence and in the presence of the inhibitor (v_I/v_0). The substrate concentration in the activity reaction was 1 mM for cdN and 100 μM for mdN, with the values corresponding to enzymes K_M. The compound concentration in the reaction was 10 times lower than the substrate concentration. For the compounds with v_I/v_0 value for either enzyme below 0.6, the inhibition constant (K_i) was determined.

To determine the K_i values, a series of activity assays were performed in the presence of the selected compounds. The concentration of inhibitors in the reaction mixture was adjusted to fit a range of relative enzyme activity between 0.03 and 1. For each compound, the reaction mixtures with 11 different inhibitor concentrations were analyzed in triplicates. To estimate the apparent K_i value, the initial velocities of the inhibited reactions at varying inhibitor concentrations were fitted using the Williams-Morrison equation.[89] The true K_i value was then calculated presuming the competitive type of inhibition. This presumption was based on the high structural similarity of the tested compounds with the lead structure **5**.

To explore the mode of inhibition for compound **5**, the inhibition measurements were performed at four different substrate concentrations (corresponding to $0.1 - 2 \times K_M$ value). The data were fitted simultaneously by the Williams-Morrison equation for the specific 3 different inhibition types (competitive, noncompetitive, and uncompetitive) using a non-linear regression. The best model was selected using the Akaike information criterion. [10, 11] This analysis proved that the competitive model can satisfactorily be used to describe the inhibition mode of mdN and cdN by this type of compound.

Crystallization. For crystallization of mdN, the conditions successfully used by others were utilized.[12] The crystals of mdN were obtained using the hanging drop vapour diffusion technique with a precipitating solution containing 24 mM KH_2PO_4, PEG 8000 9.6% (w/v), and glycerol 12% (v/v). Small protein crystals grown in the drop made by mixing 1.33 μL of the protein solution (8.4 mg/mL at 20 mM Tris.HCl pH 7.5, 20 mM $MgCl_2$, 2 mM DTT, 2 mM EDTA) and 0.66 μL of the precipitating solution at 18 ºC. The crystal nuclei from this drop were seeded into a new drop containing 20 mM KH_2PO_4, PEG 8000 8% (w/v), glycerol 10% (v/v) using a treak seeding technique with the cat whisker.[13] The crystals grew to a final size of 250 × 160 × 160 μm within three days. The crystals were soaked for 12h with with 5 mM compounds **12d**, **12e**, and **13, respectively,** in 1 μL crystallization drop supplemented with 45 mM $MgCl_2$ solution. For cryoprotection, the crystals were soaked for 10 s in a reservoir solution supplemented with 30% (v/v) glycerol. Crystals were flash-cooled by plunging into liquid nitrogen and were stored in liquid nitrogen until used for X-ray diffraction experiments.

Data collection and structure determination. The diffraction data for crystal soaked with compound **12d** were collected at 100 K at the MX14.1 beamline of the BESSY, Berlin, Germany[14] at a wavelength of 0.915 Å. The diffraction data to 1.78 Å resolution were integrated and reduced using MOSFLM[15, 16]and scaled using SCALA[17] from the CCP4 suite.[2]

The diffraction data for crystal soaked with compounds **12e** and **13** were collected at 100 K at the MX14.2 beamline of the BESSY, Berlin, Germany at a wavelength of 0.919 Å and 0.978 Å, **,** respectively . The diffraction data to resolution were integrated and reduced using XDS[18] and its graphical interface XDSAPP[19].

All crystals exhibited the symmetry of space group $P4_32_12$ and contained one molecule in the asymmetric unit with a solvent content of about 60%. Crystal parameters and data collection statistics are summarized in Table 2.

The structure of mdN was determined by the difference-Fourier method using coordinates from the isomorphous structure of the identical protein (PDB code 4L6A). Refinement was carried out using the program REFMAC 5.5[20] Program Coot[21] was used for model building. The final refinement steps included translation-libration-screw (TLS) refinement[22, 23] using 3 (**12d**) or 4 (**12e** and **13**) TLS groups. The refinement statistics are given in Table 2.

The quality of the final model was validated with Molprobity[5]. All figures showing structural representations were prepared with the program PyMOL[24]. The atomic coordinates and experimental structure factors have been deposited with the Protein Data Bank under the accession codes 4L6C, 4NFL, and 4MWO.

NMR spectroscopy. Uniformly ^{15}N labelled cdN protein was prepared on minimal medium containing ^{15}N-ammonium sulfate as the sole nitrogen source as described recently.[7] All NMR data were acquired at 35^{o}C on a 600 MHz Bruker Avance spectrometer equipped with a triple-resonance (^{15}N/^{13}C/^{1}H) cryoprobe. The binding of selected inhibitors to cdN was monitored in 2D ^{15}N/^{1}H TROSY spectra of 100 μM ^{15}N labelled cdN using comprehensive backbone resonance assignments (BioMagResBank database accession code 19490).[7] In particular, minimal shift values were calculated[25, 26] to assess the changes in the positions of cdN backbone signals (H^N, N) induced by inhibitor binding. Resulting graphs of minimal shifts were used to identify the regions within cdN implicated in small molecule binding. All ^{15}N/^{1}H TROSY experiments were collected with 128 transients and acquisition times of 80 ms in F_2 (^{1}H) and 50ms in F_1 (^{15}N). The 3D NMR data were processed using Topspin 2.1 (Bruker) and analyzed using the Sparky (UCSF).

7. ^{1}H and ^{13}C-NMR spectra of final compounds tested for their inhibition activities

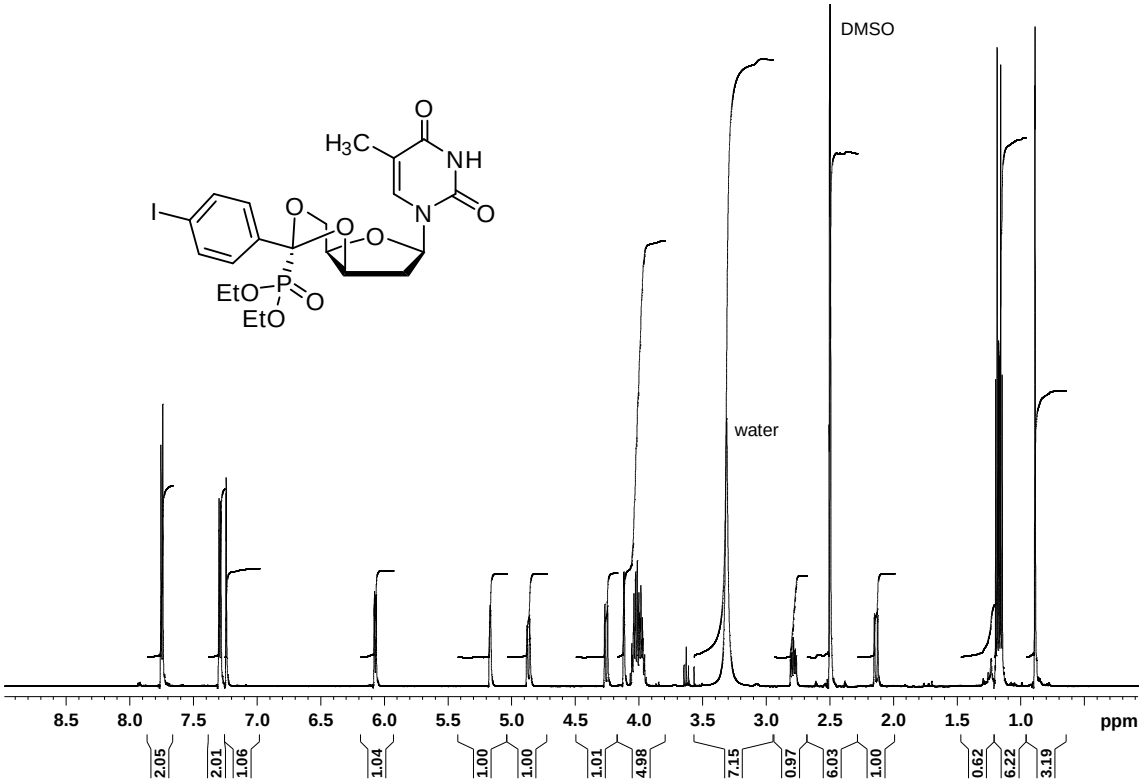

Figure 5Sa ^{1}H NMR spectrum (600.13 MHz) of compound **11d** in DMSO.

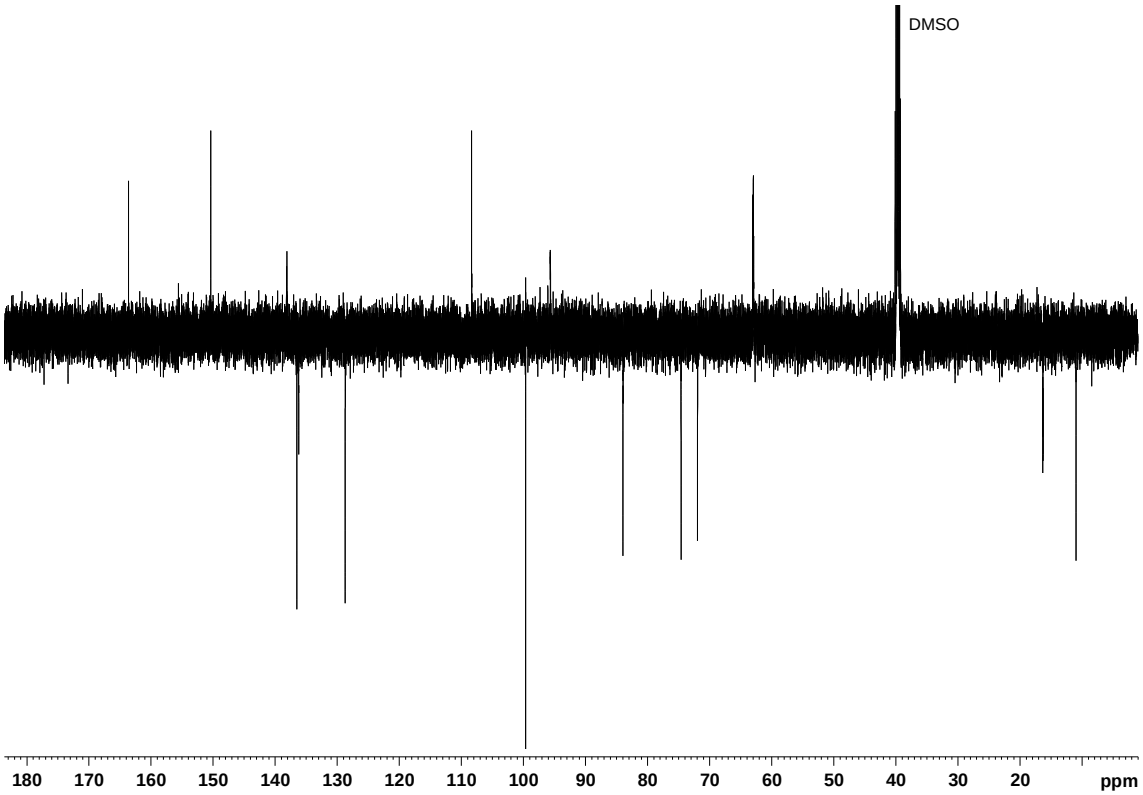

Figure 5Sb ^{13}C NMR spectrum (150.9 MHz) of compound **11d** in DMSO.

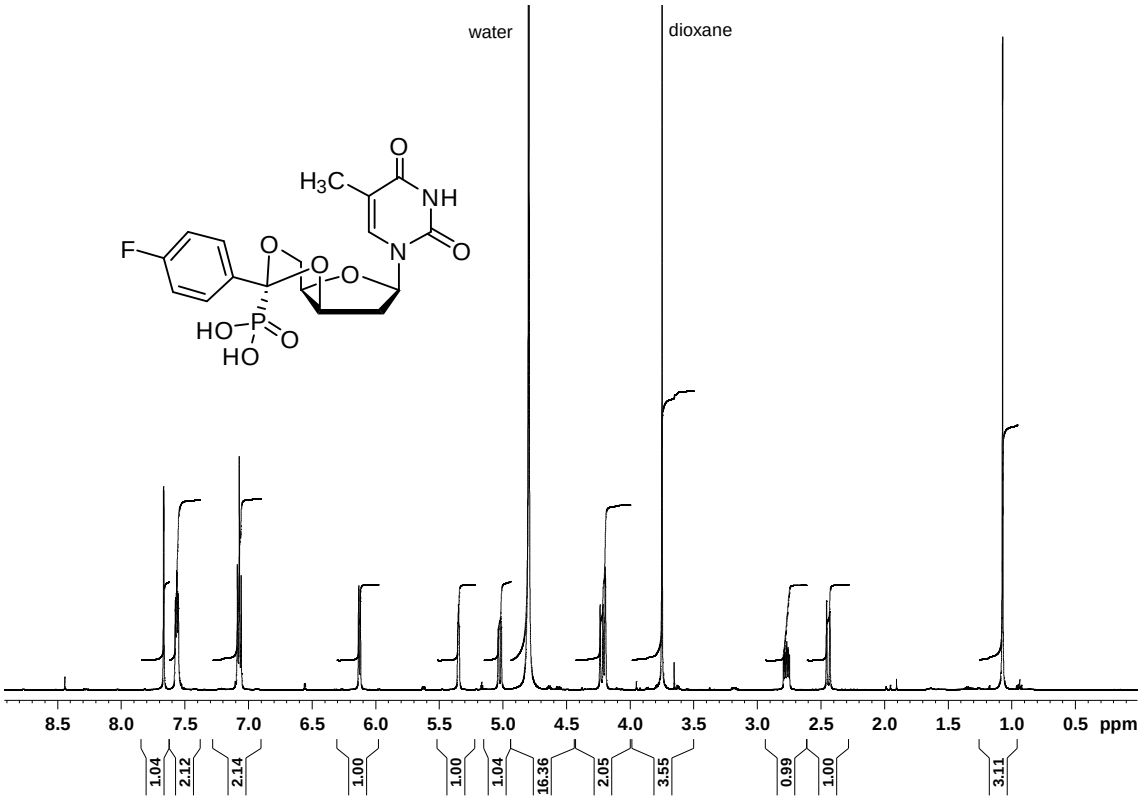

Figure 6Sa ^{1}H NMR spectrum (600.13 MHz) of compound **12a** in D_2O.

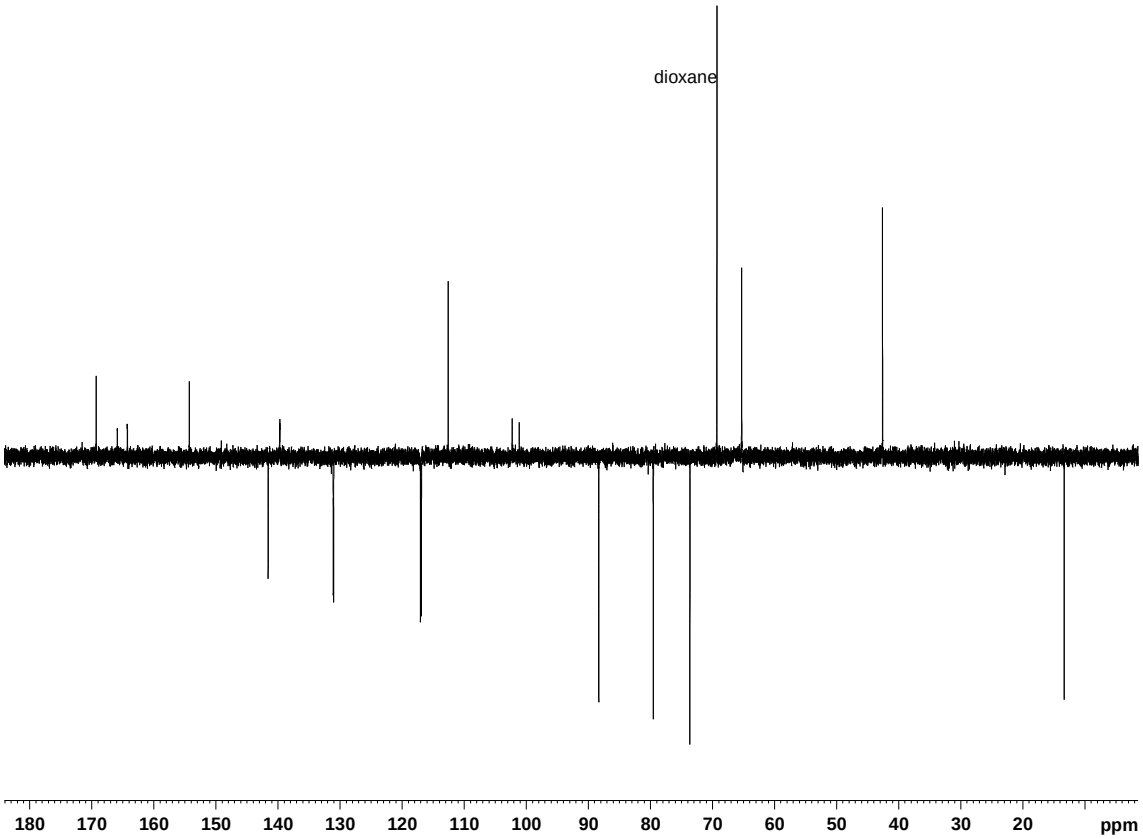

Figure 6Sb ^{13}C NMR spectrum (150.9 MHz) of compound **12a** in D_2O.

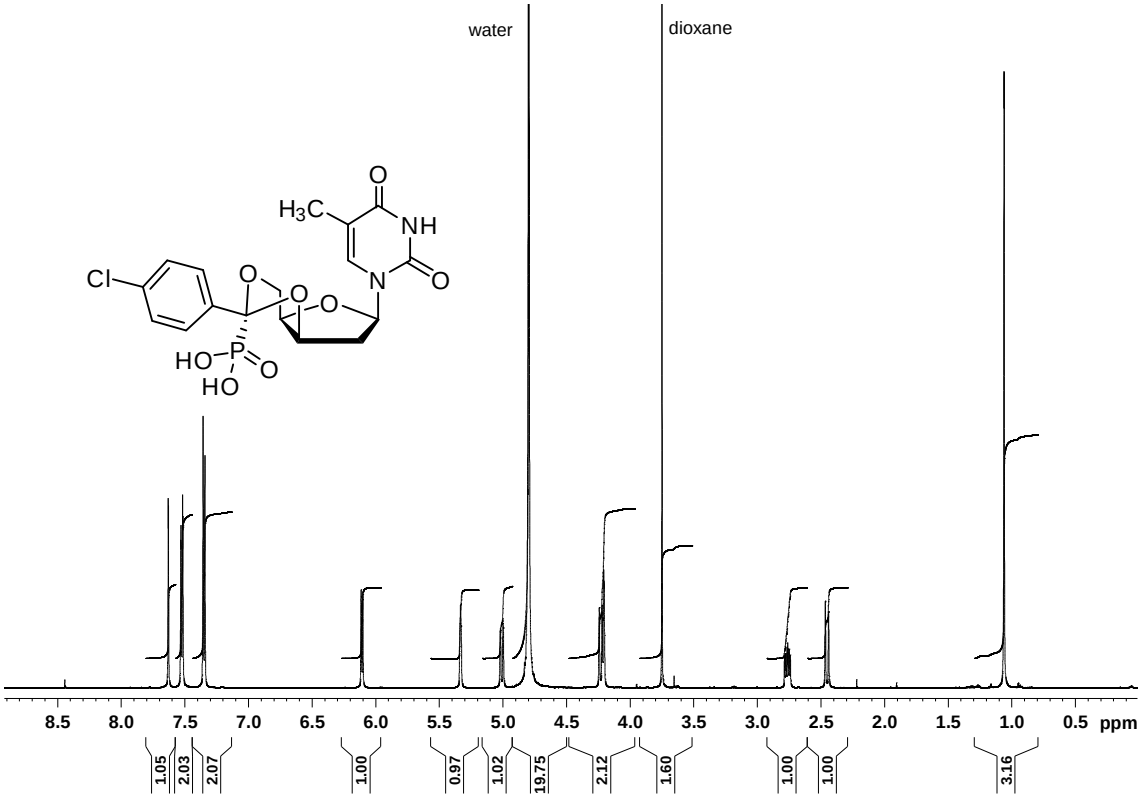

Figure 7Sa ^{1}H NMR spectrum (600.13 MHz) of compound **12b** in D_2O.

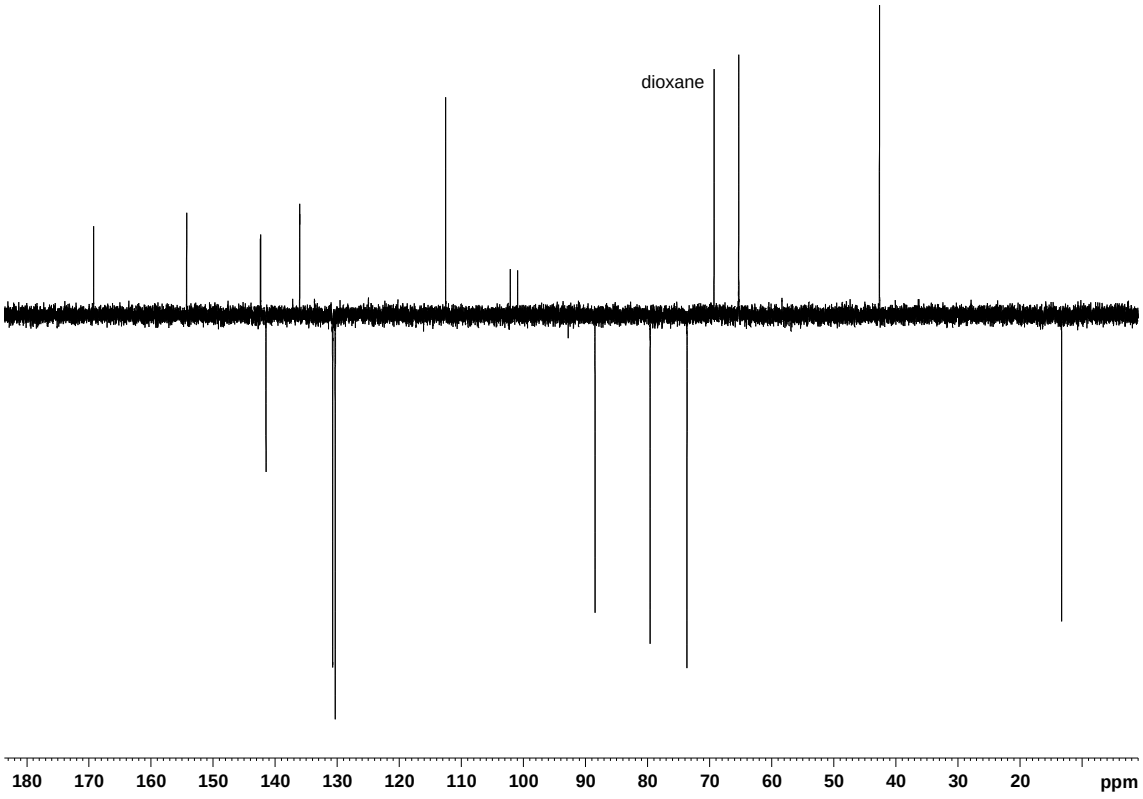

Figure 7Sb ^{13}C NMR spectrum (150.9 MHz) of compound **12b** in D_2O.

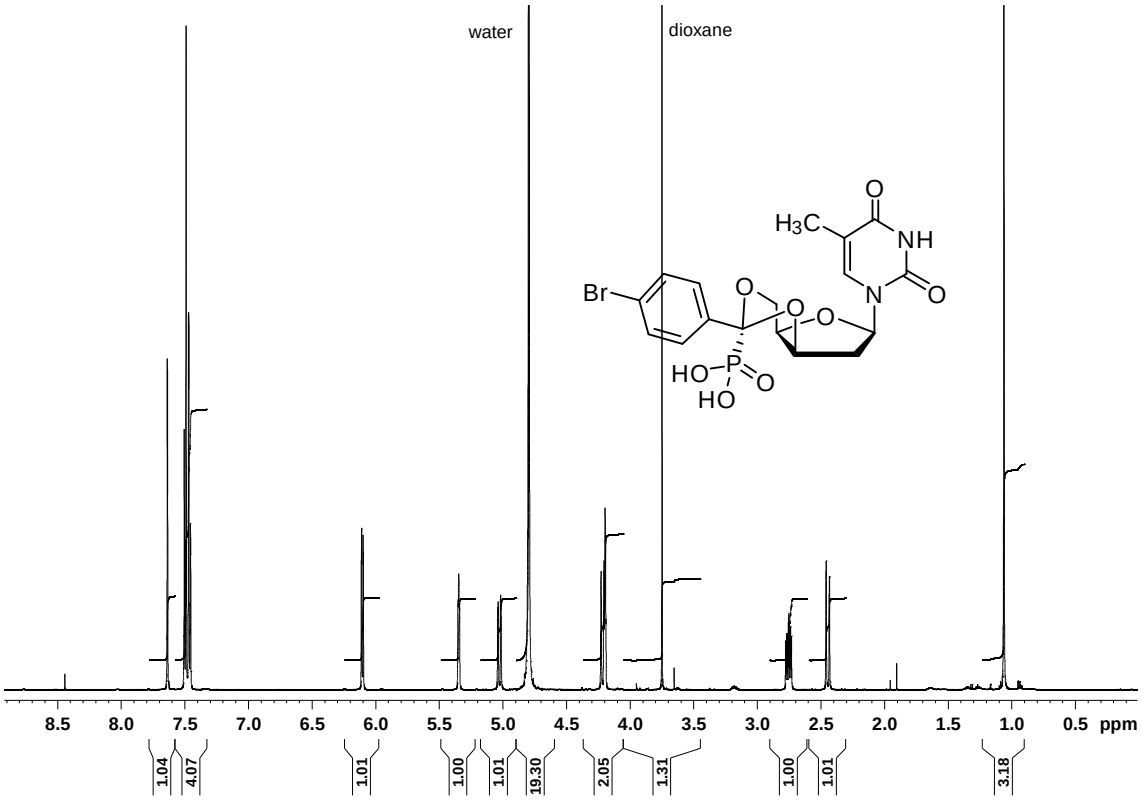

Figure 8Sa ^{1}H NMR spectrum (600.13 MHz) of compound **12c** in D_2O.

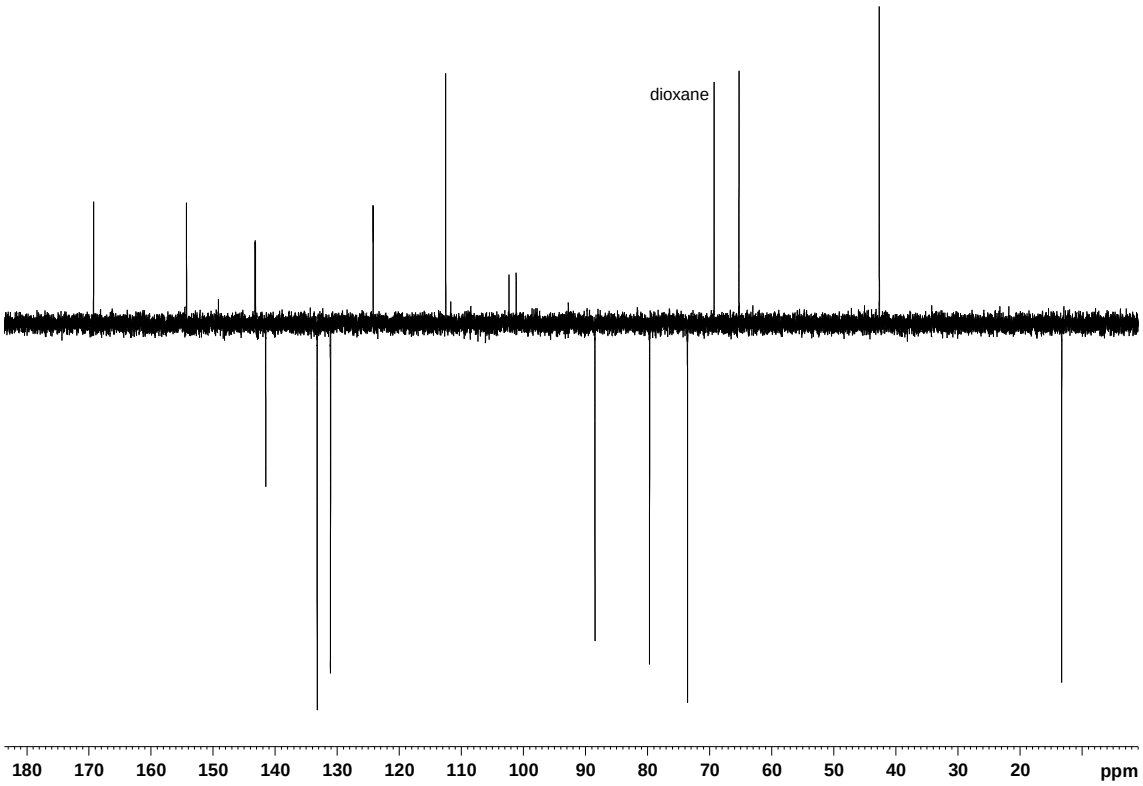

Figure 8Sb ^{13}C NMR spectrum (150.9 MHz) of compound **12c** in D_2O.

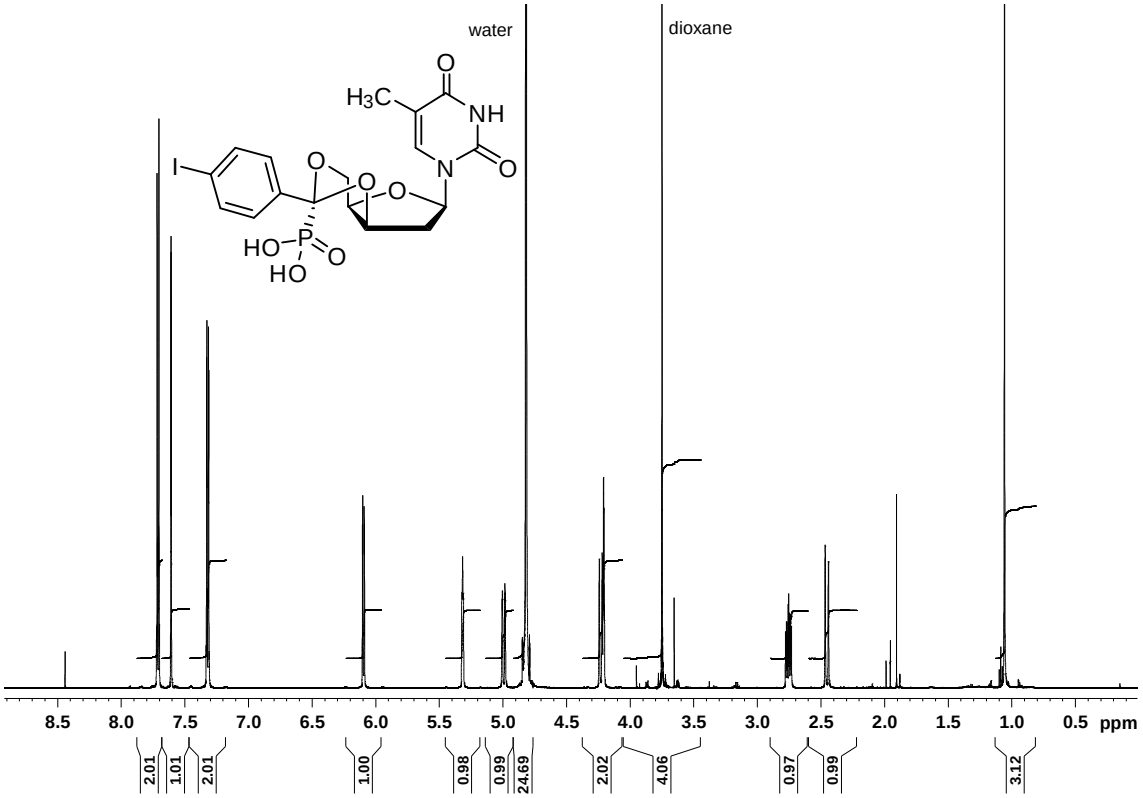

Figure 9Sa ^{1}H NMR spectrum (600.13 MHz) of compound **12d** in D_2O.

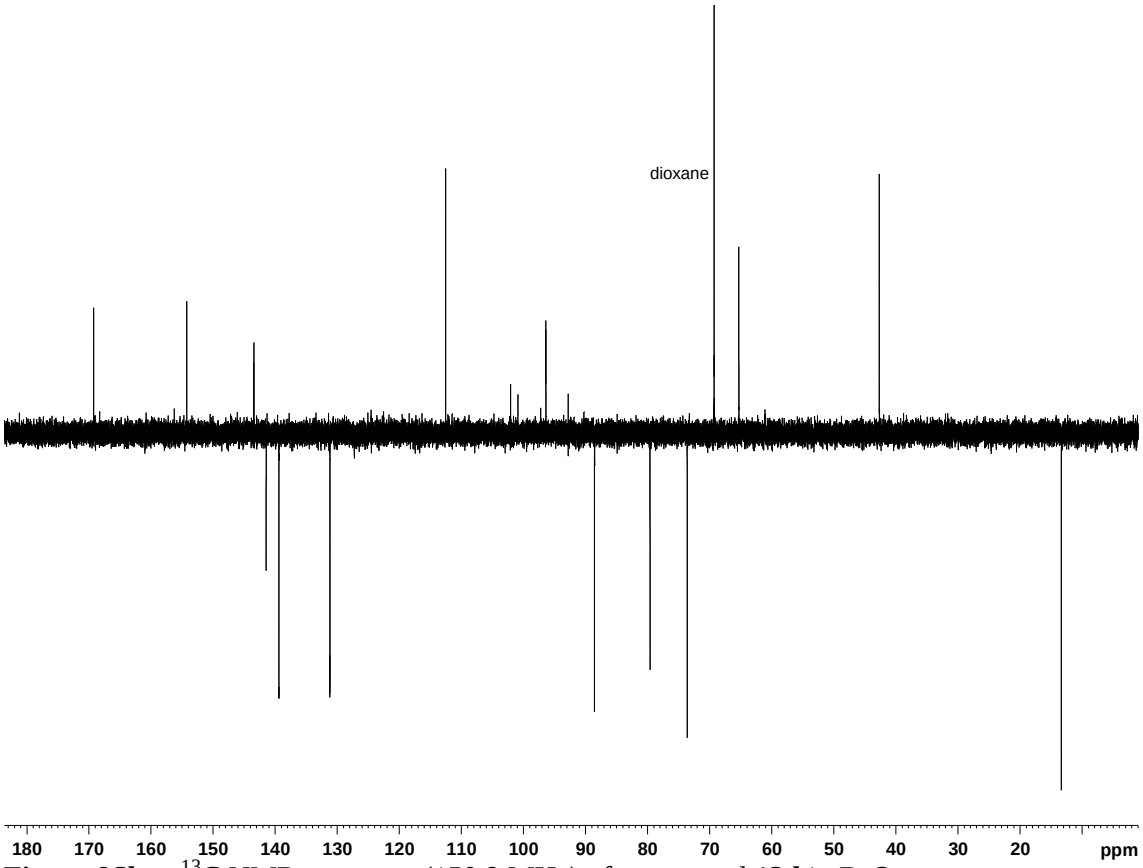

Figure 9Sb ^{13}C NMR spectrum (150.9 MHz) of compound **12d** in D_2O.

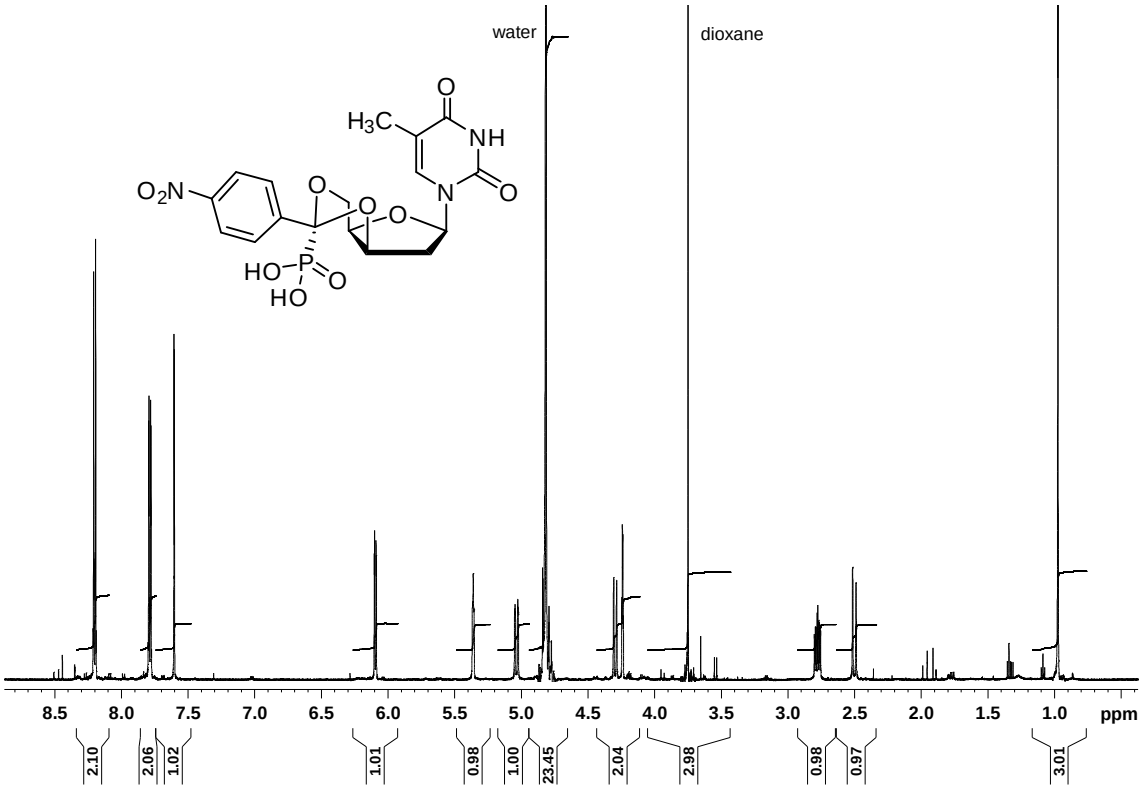

Figure 10Sa ^{1}H NMR spectrum (600.13 MHz) of compound **12e** in D_2O.

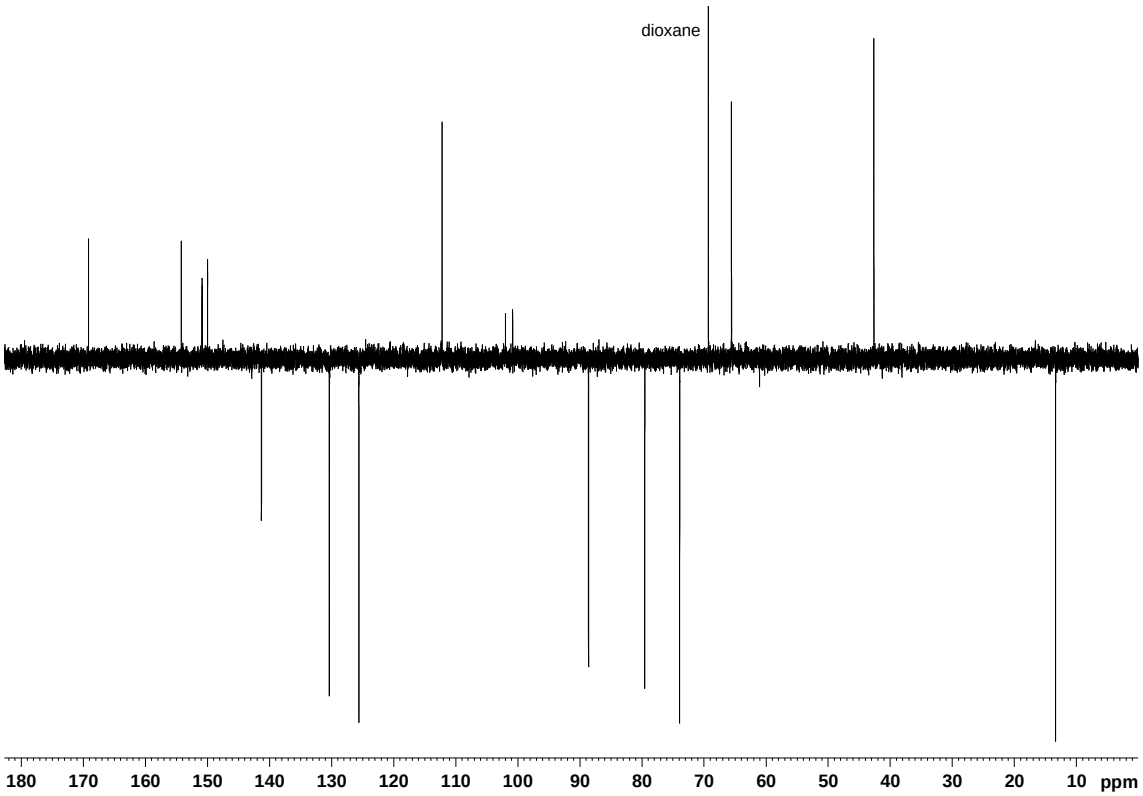

Figure 10Sb ^{13}C NMR spectrum (150.9 MHz) of compound **12e** in D_2O.

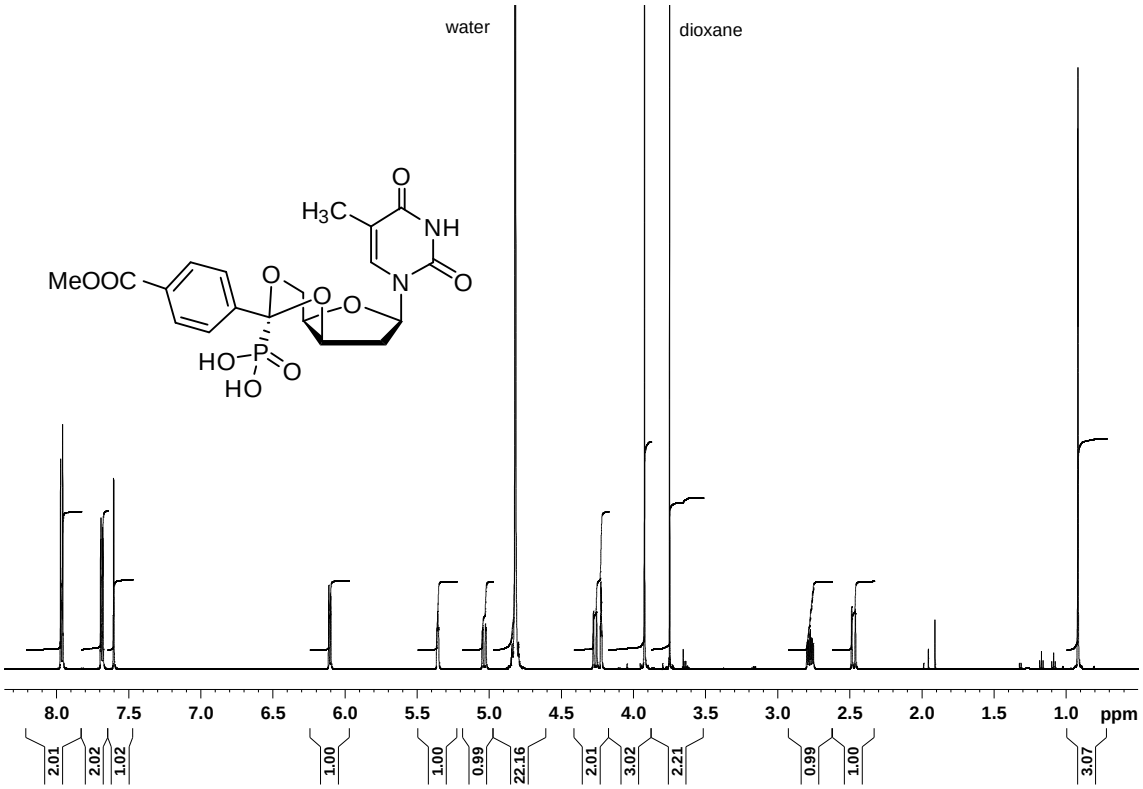

Figure 11Sa ^{1}H NMR spectrum (600.13 MHz) of compound **12f** in D_2O.

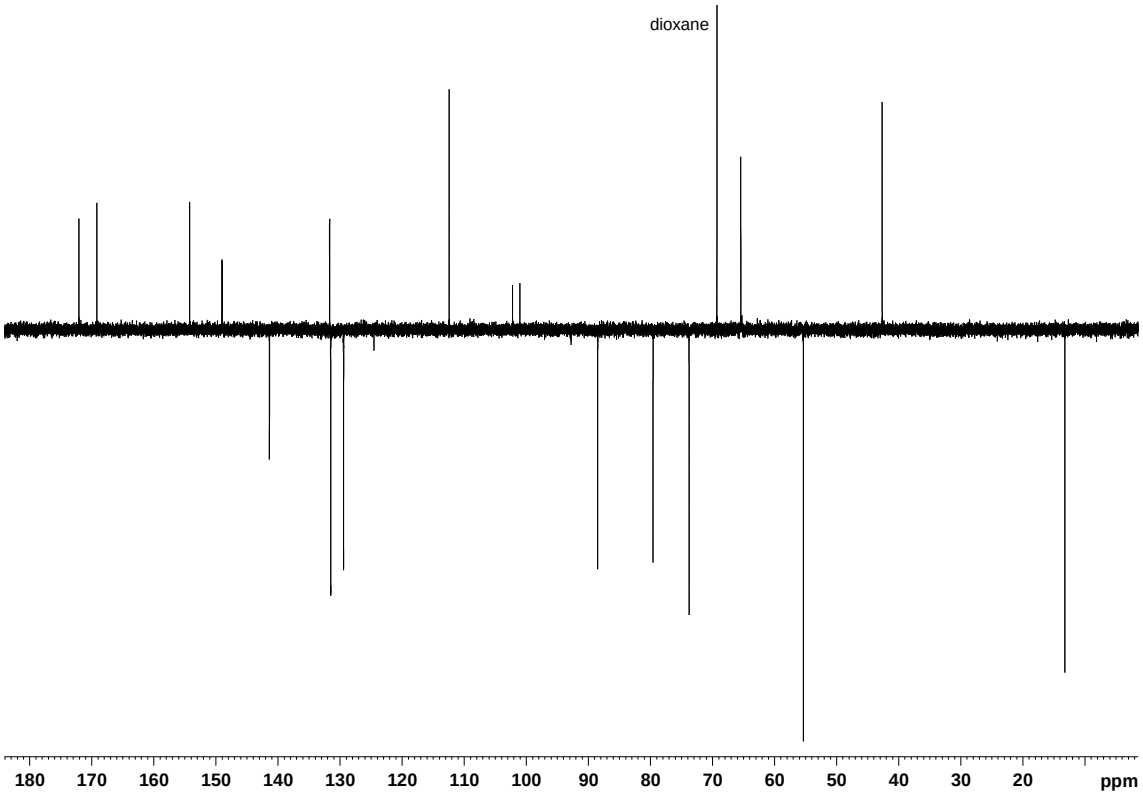

Figure 11Sb ^{13}C NMR spectrum (150.9 MHz) of compound **12f** in D_2O.

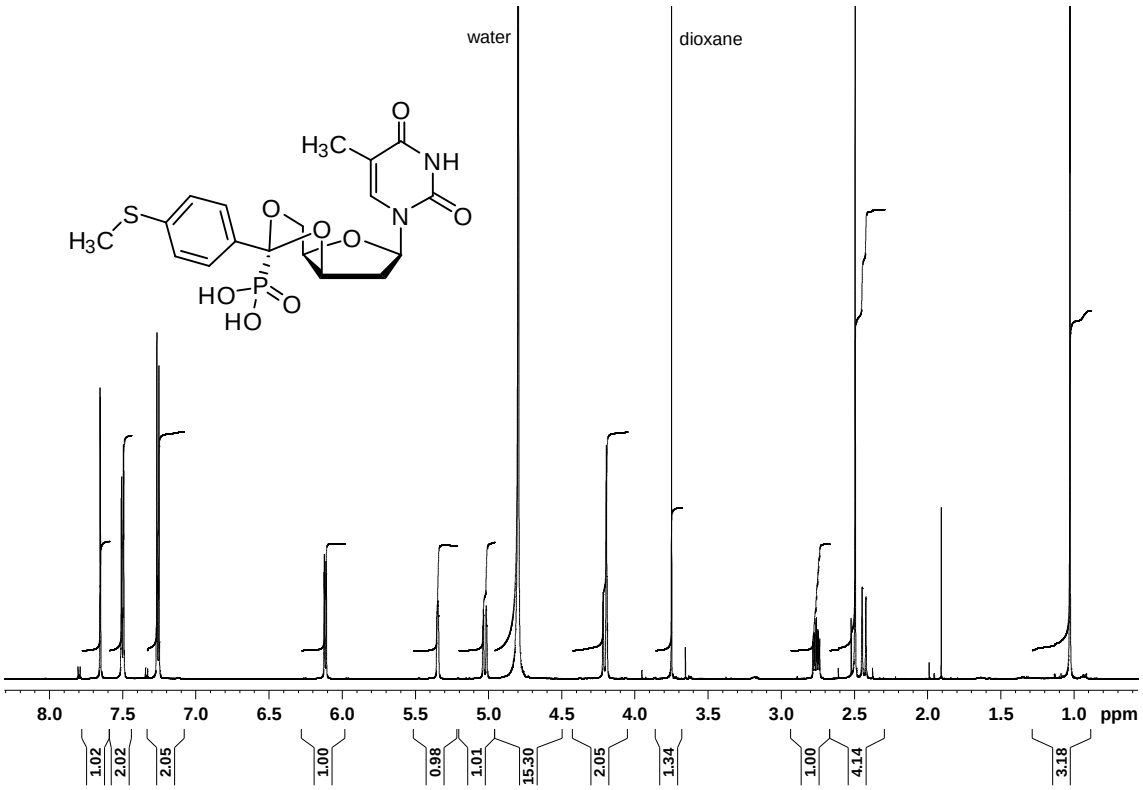

Figure 12Sa ^{1}H NMR spectrum (600.13 MHz) of compound **12g** in D_2O.

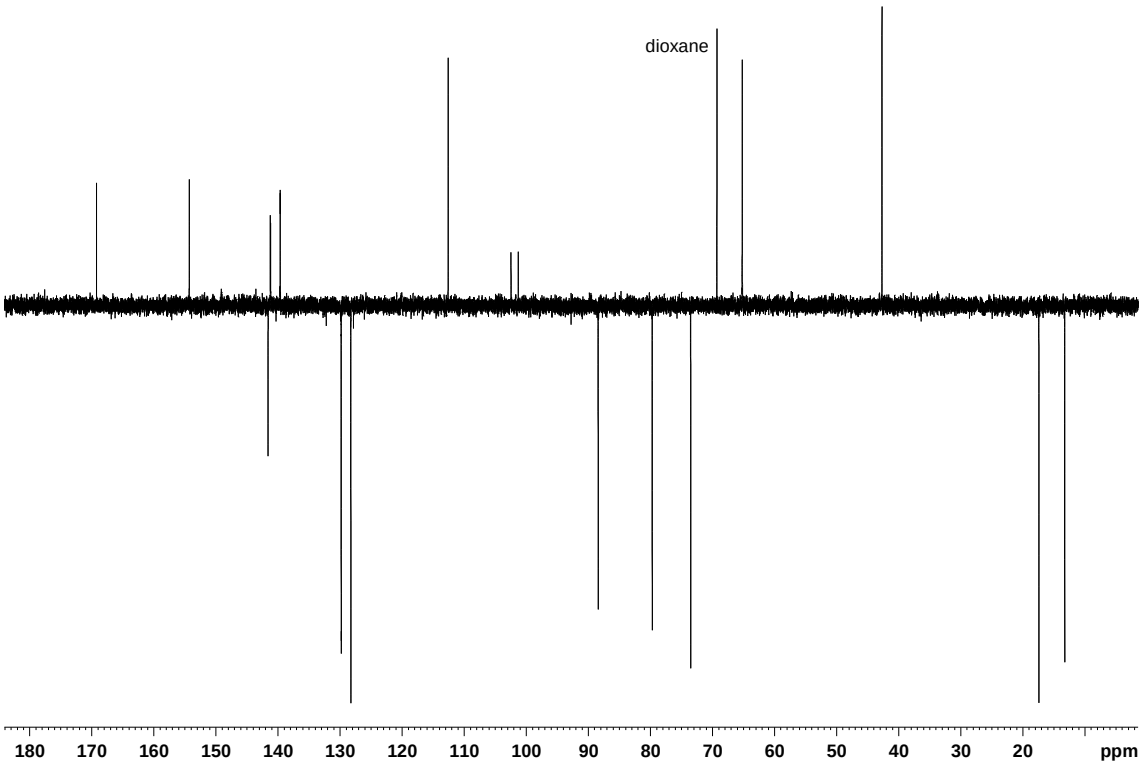

Figure 12Sb ^{13}C NMR spectrum (150.9 MHz) of compound **12g** in D_2O.

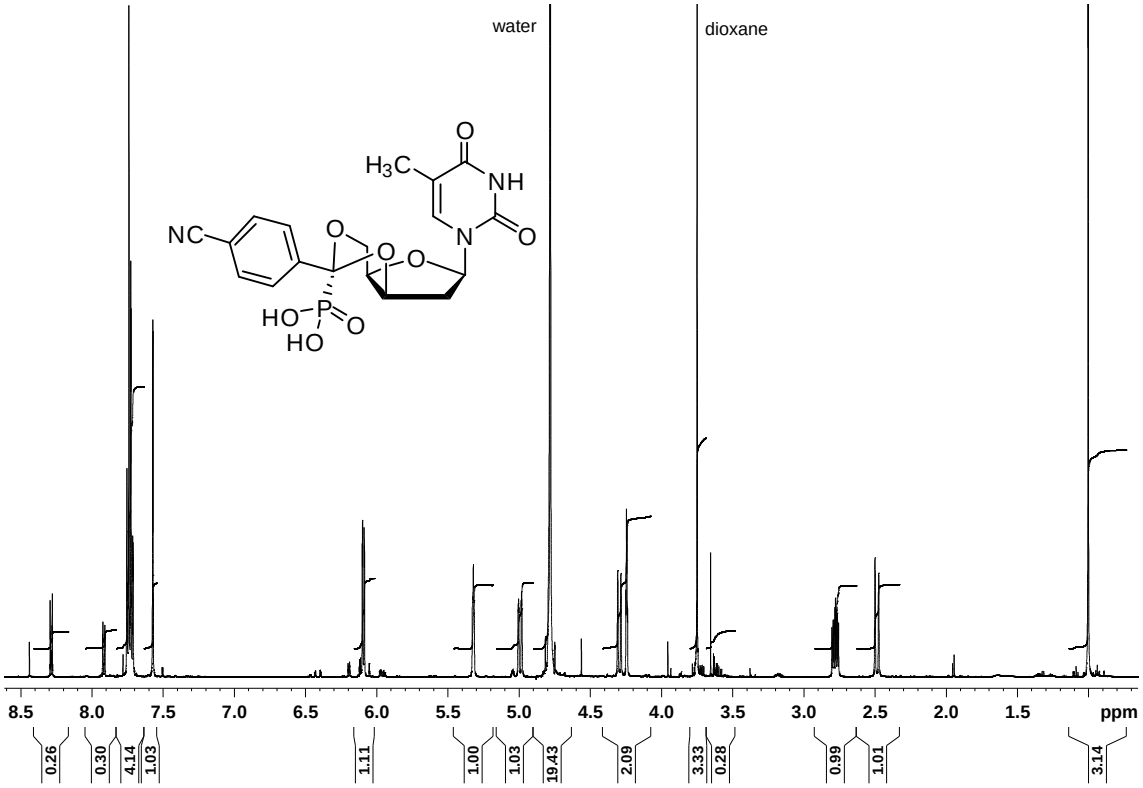

Figure 13Sa ^{1}H NMR spectrum (600.13 MHz) of compound **12h** in D_2O.

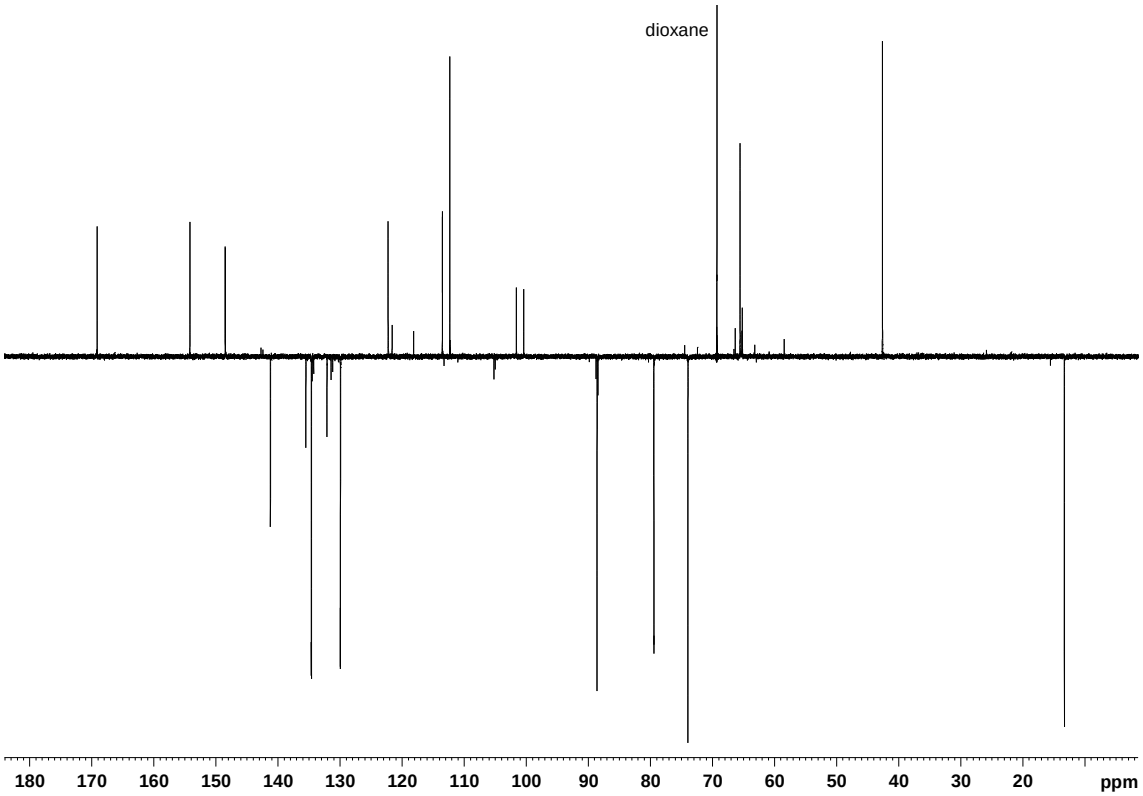

Figure 13Sb ^{13}C NMR spectrum (150.9 MHz) of compound **12h** in D_2O.

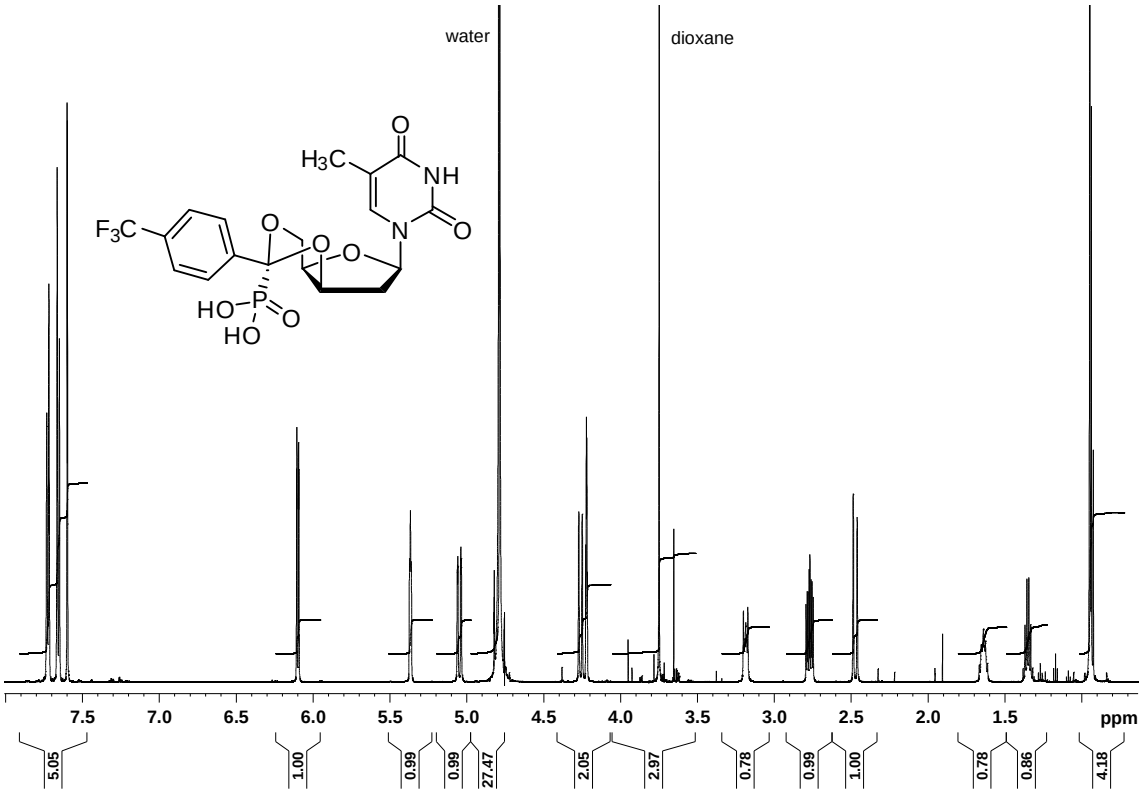

Figure 14Sa ^{1}H NMR spectrum (600.13 MHz) of compound **12i** in D_2O.

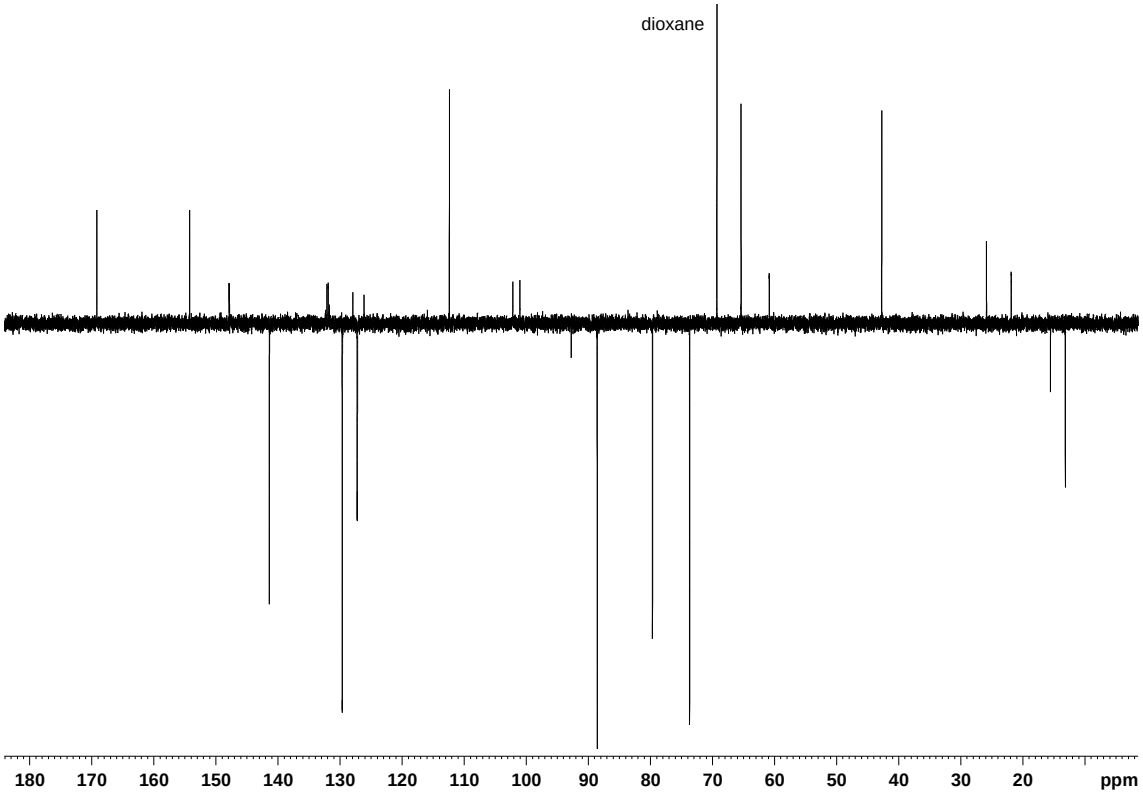

Figure 14Sb ^{13}C NMR spectrum (150.9 MHz) of compound **12i** in D_2O.

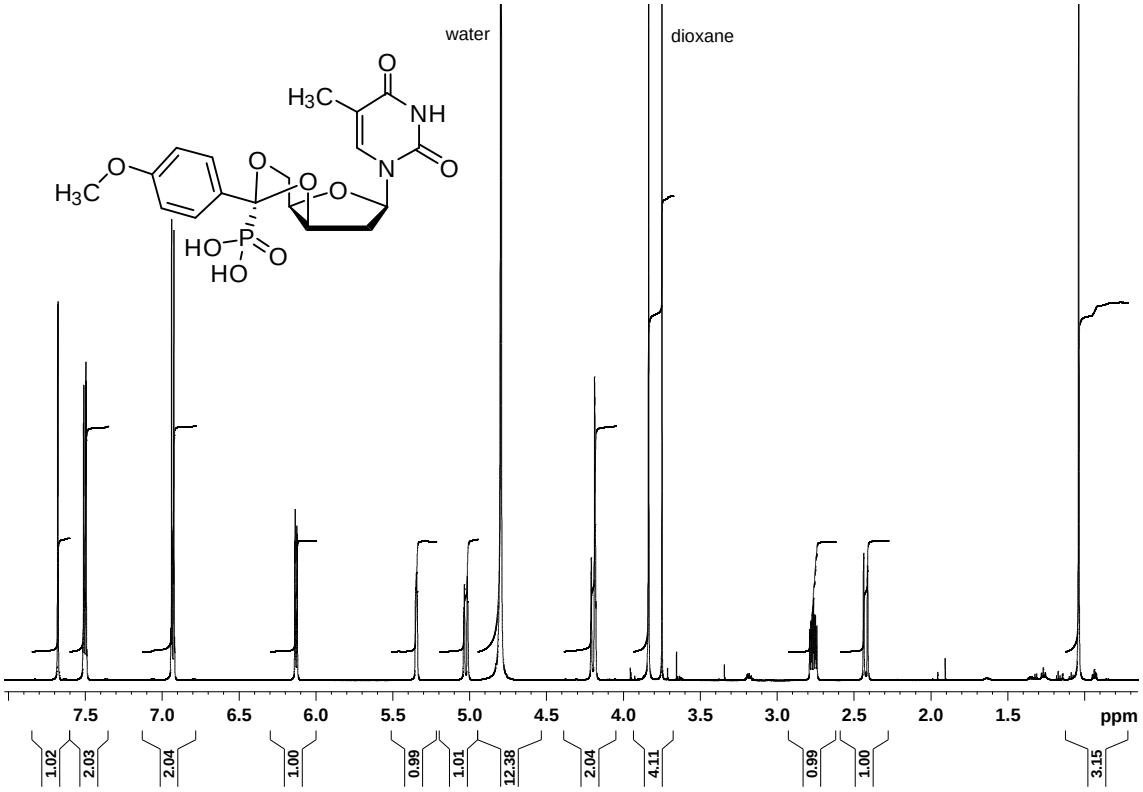

Figure 15Sa ^{1}H NMR spectrum (600.13 MHz) of compound **12j** in D_2O.

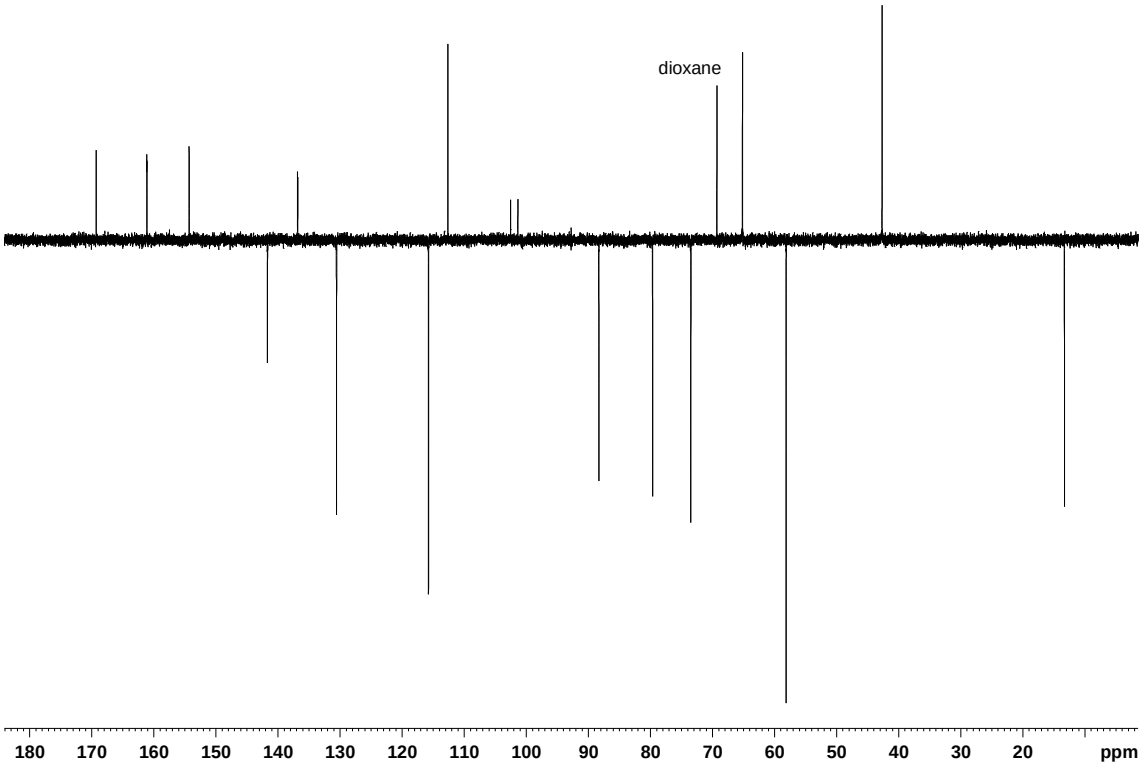

Figure 15Sb ^{13}C NMR spectrum (150.9 MHz) of compound **12j** in D_2O.

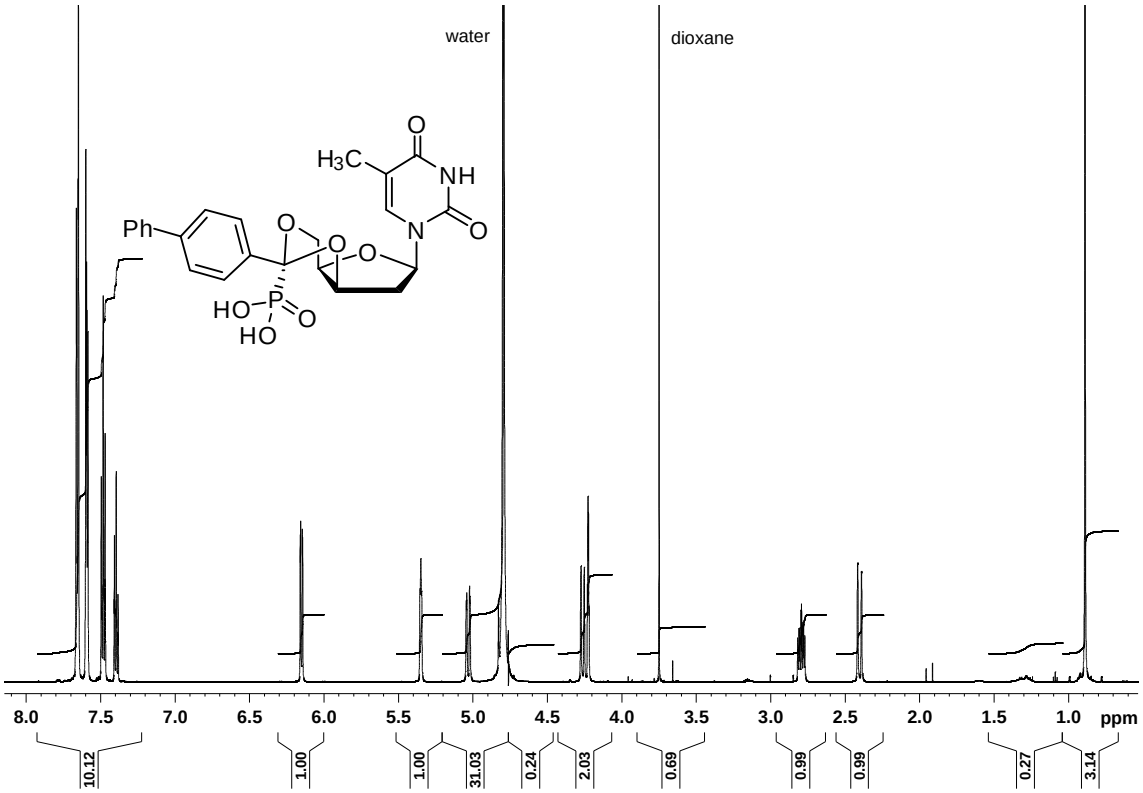

Figure 16Sa ^{1}H NMR spectrum (600.13 MHz) of compound **12l** in D_2O.

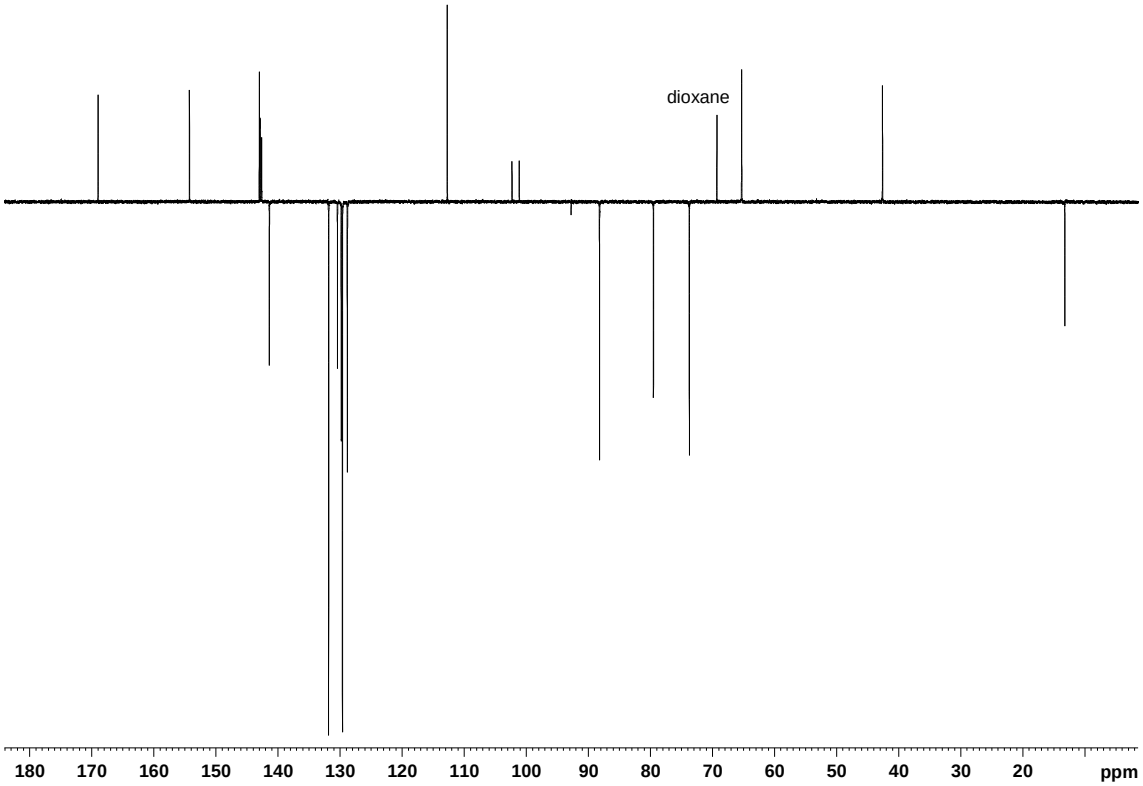

Figure 16Sb ^{13}C NMR spectrum (150.9 MHz) of compound **12l** in D_2O.

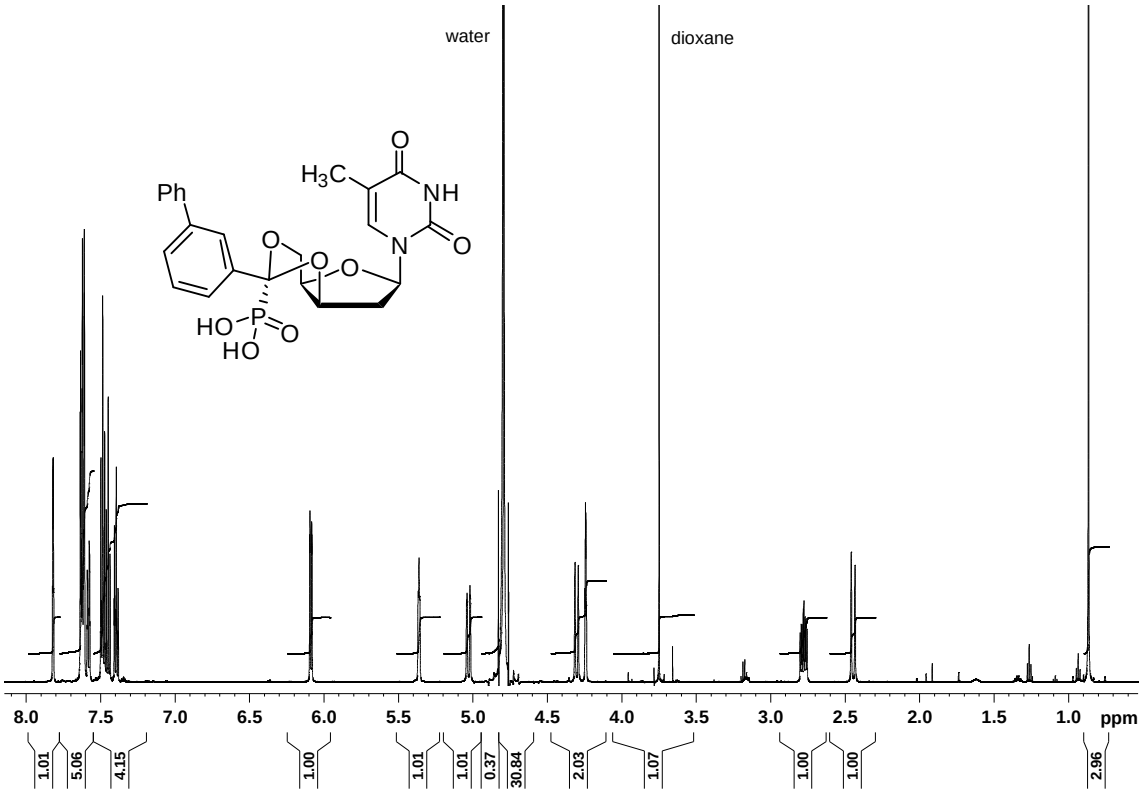

Figure 17Sa ^{1}H NMR spectrum (600.13 MHz) of compound **12m** in D_2O.

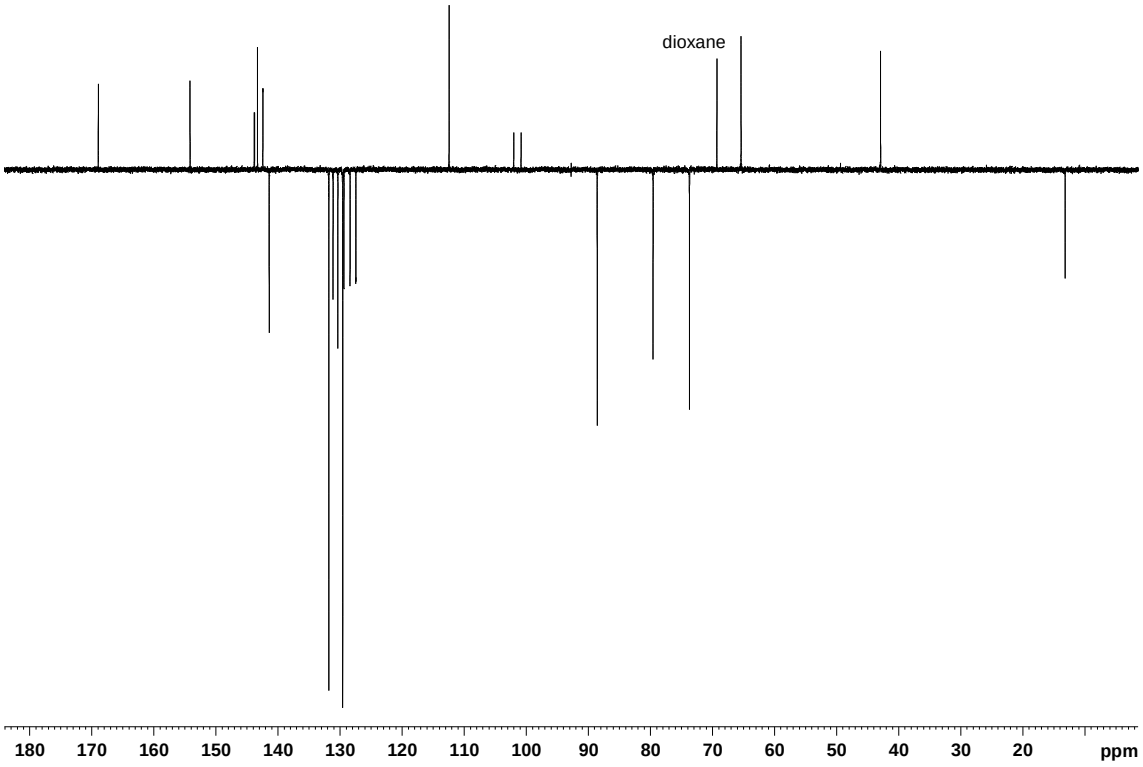

Figure 17Sb ^{13}C NMR spectrum (150.9 MHz) of compound **12m** in D_2O.

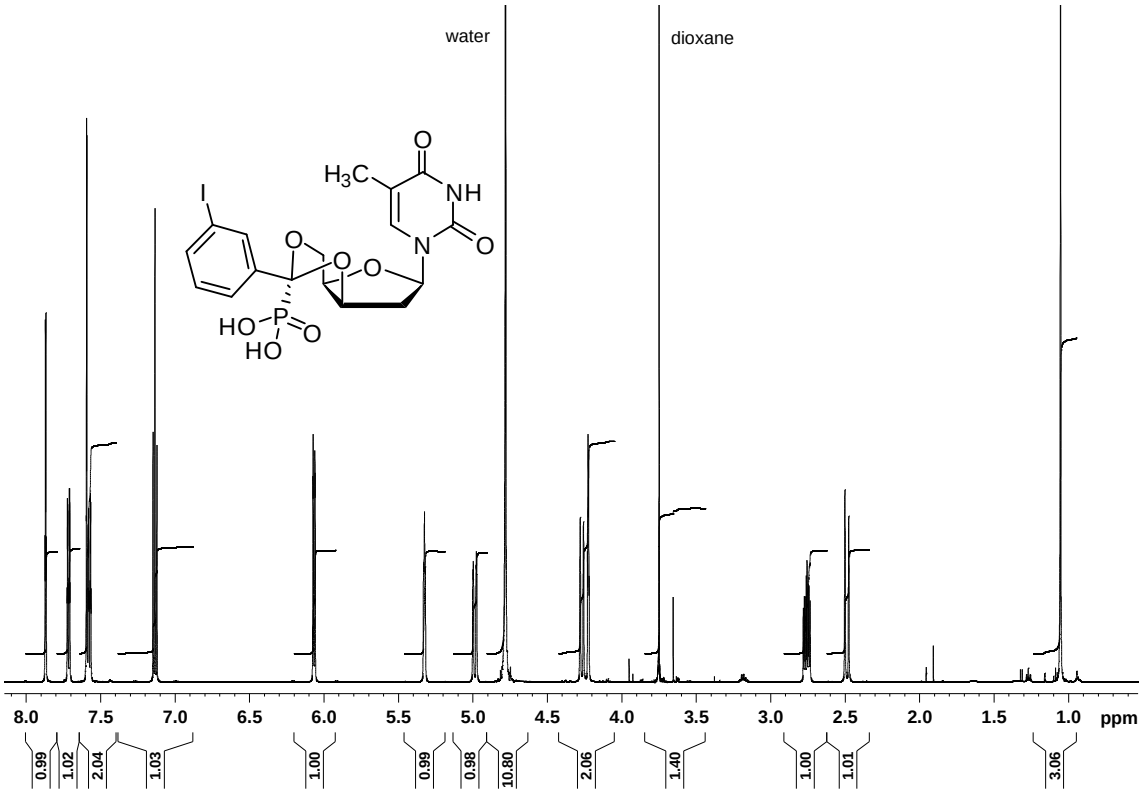

Figure 18Sa ^{1}H NMR spectrum (600.13 MHz) of compound **12n** in D_2O.

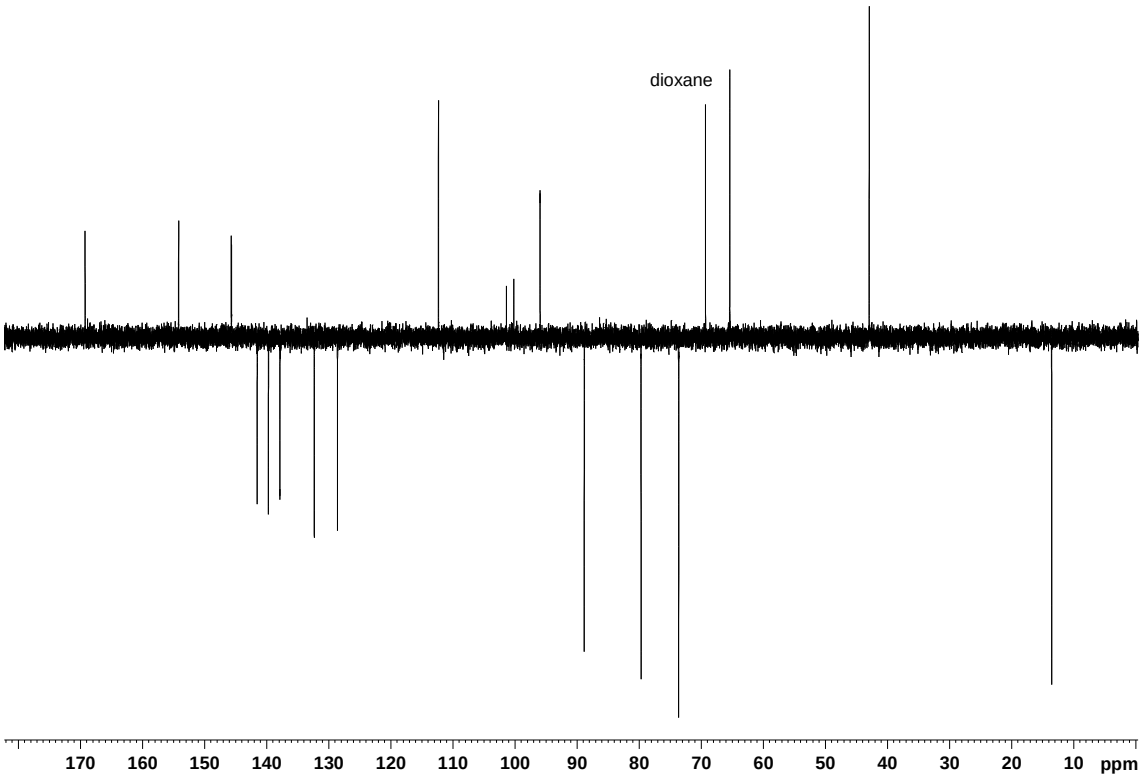

Figure 18Sb ^{13}C NMR spectrum (150.9 MHz) of compound **12n** in D_2O.

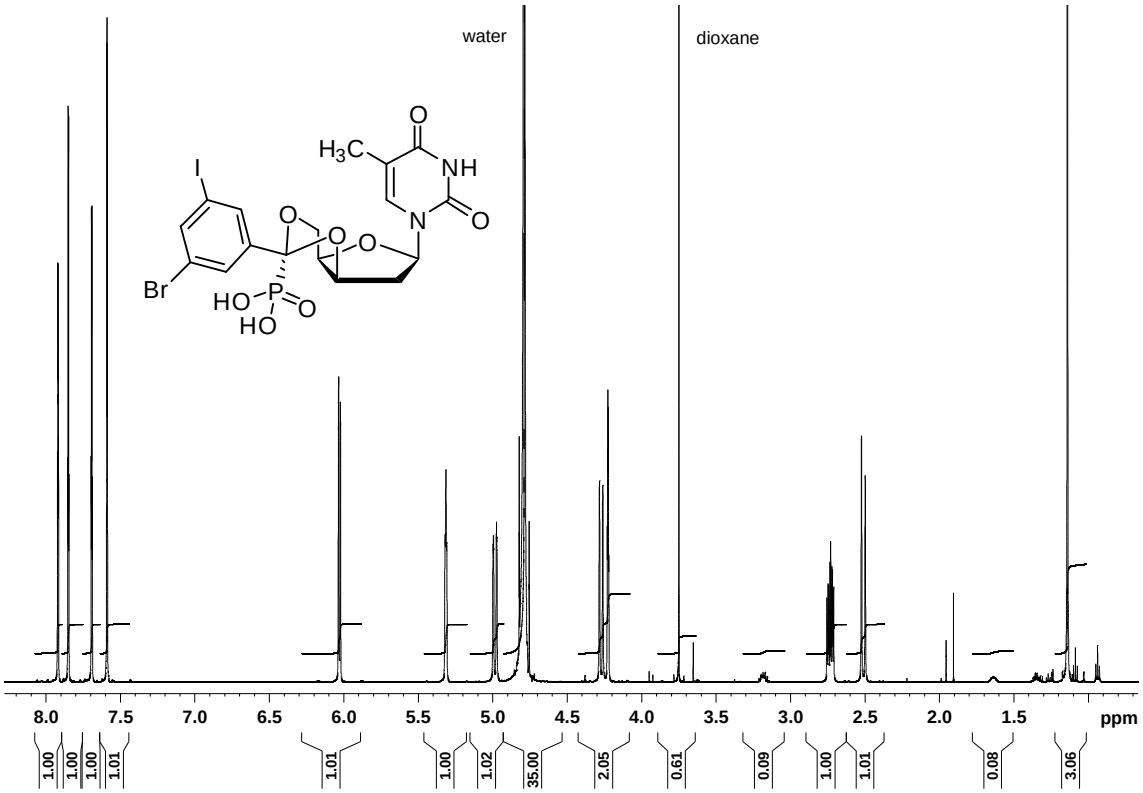

Figure 19Sa ^{1}H NMR spectrum (600.13 MHz) of compound **12o** in D_2O.

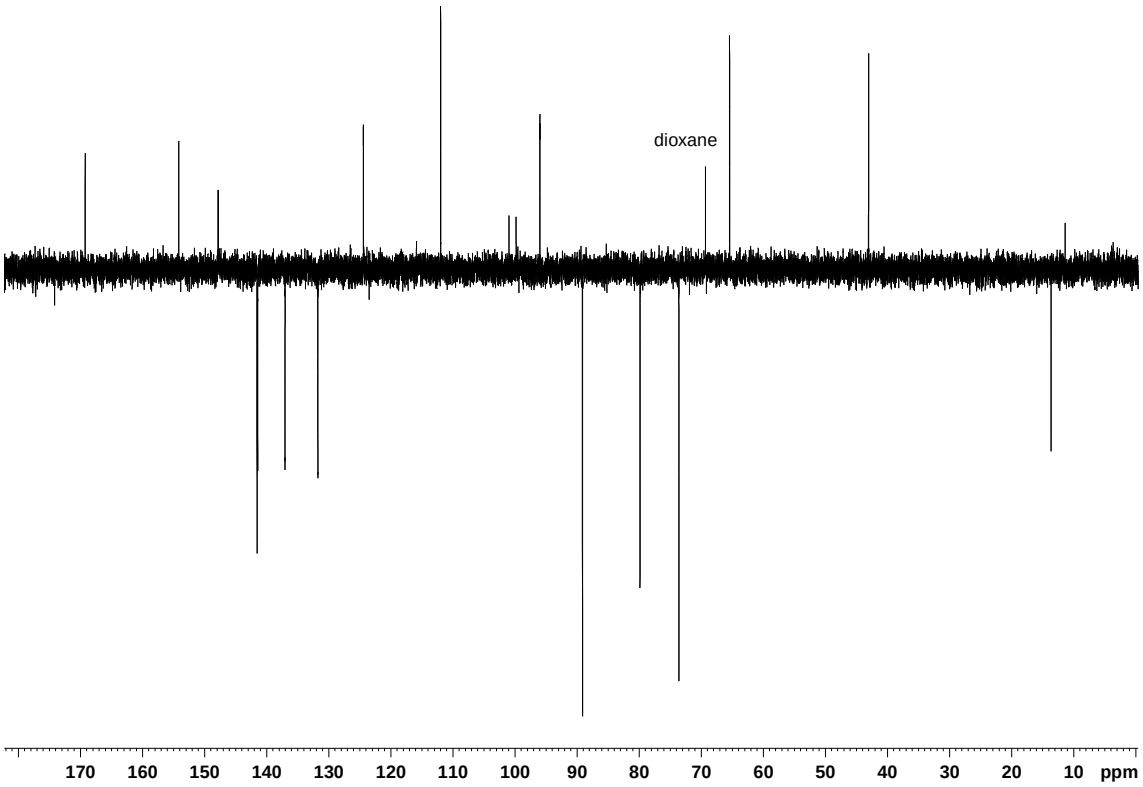

Figure 19Sb ^{13}C NMR spectrum (150.9 MHz) of compound **12o** in D_2O.

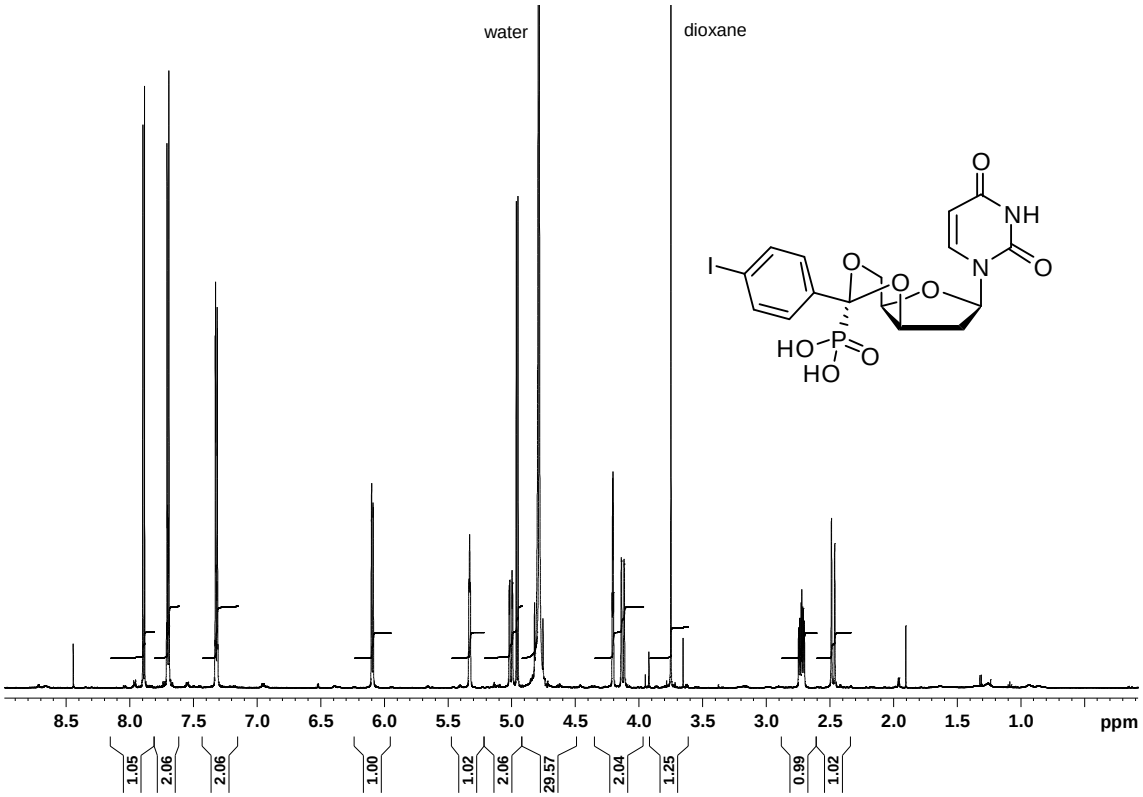

Figure 20Sa ^{1}H NMR spectrum (600.13 MHz) of compound **12p** in D_2O.

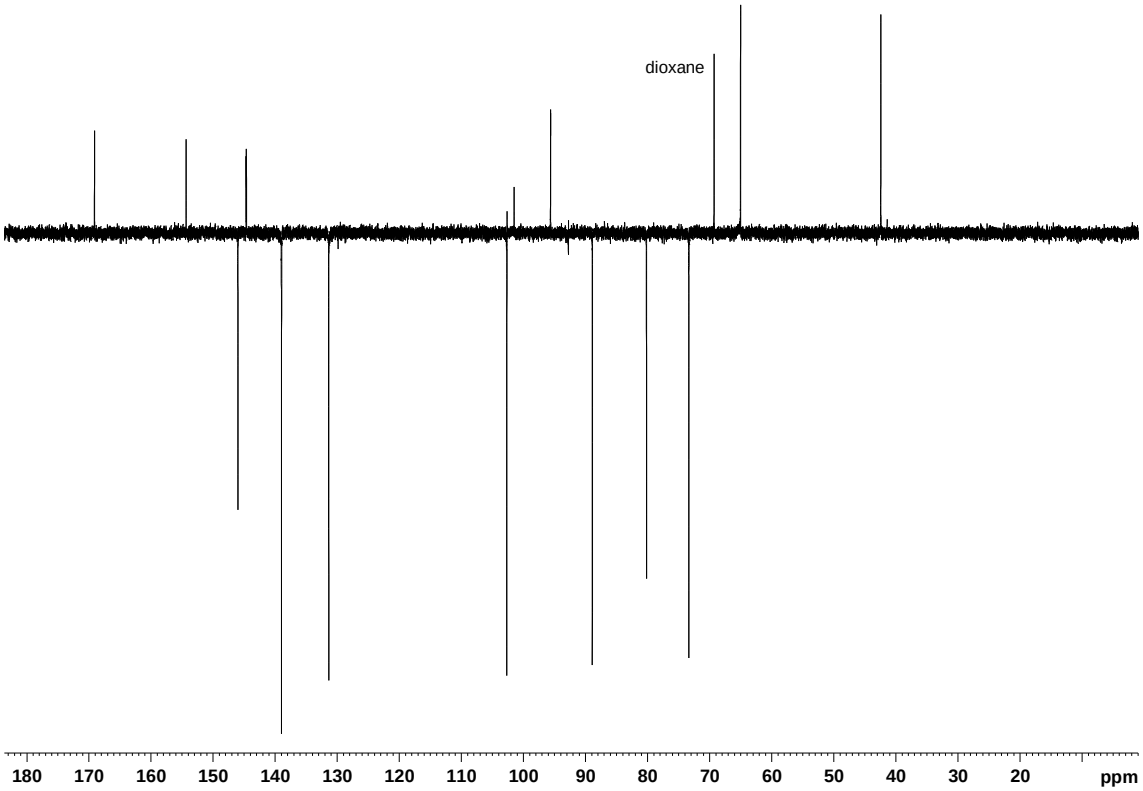

Figure 20Sb ^{13}C NMR spectrum (150.9 MHz) of compound **12p** in D_2O.

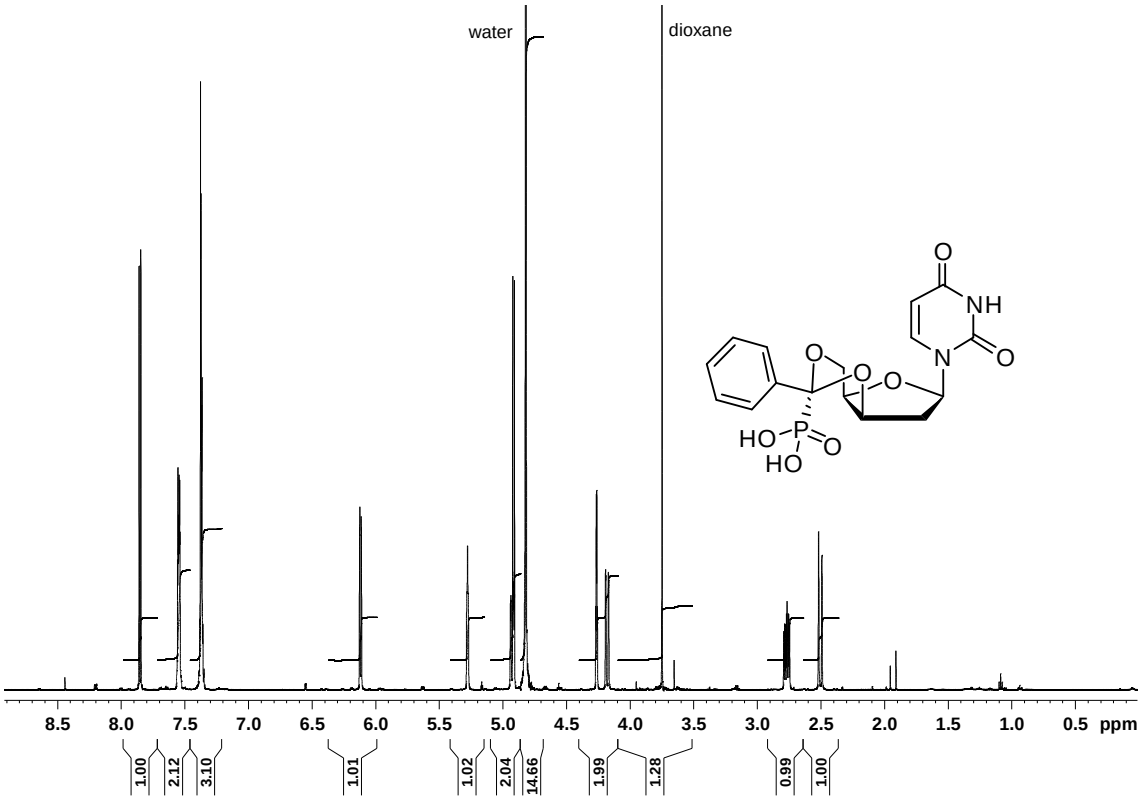

Figure 21Sa ^{1}H NMR spectrum (600.13 MHz) of compound **12r** in D_2O.

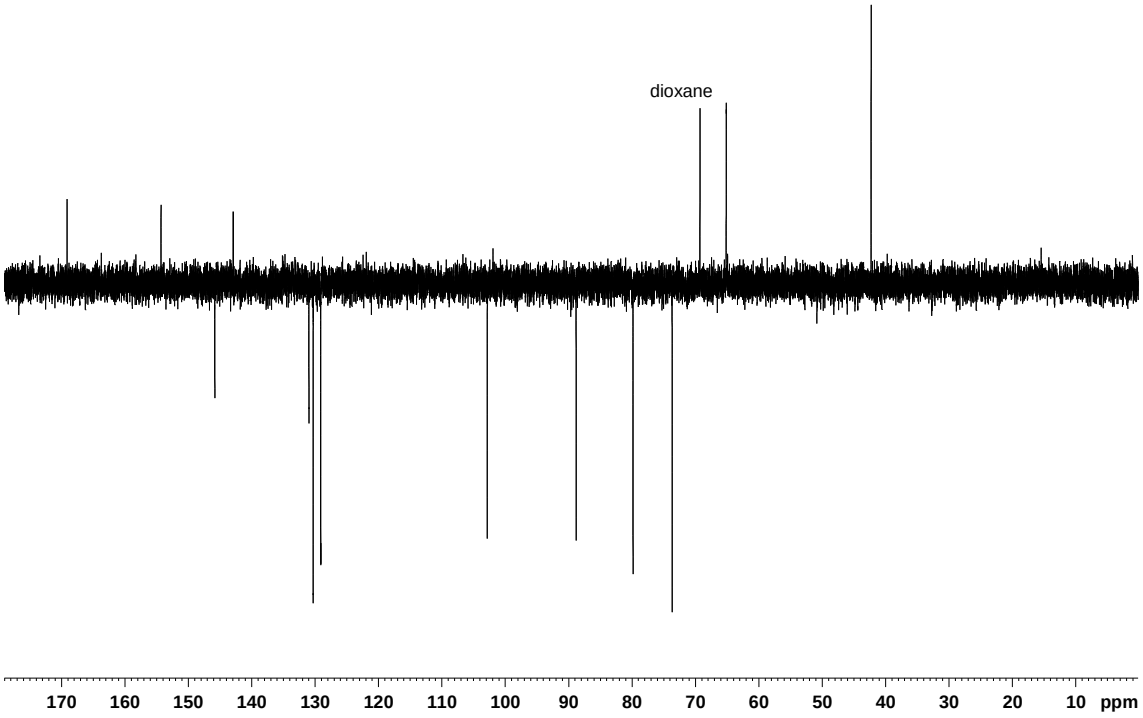

Figure 21Sb ^{13}C NMR spectrum (150.9 MHz) of compound **12r** in D_2O.

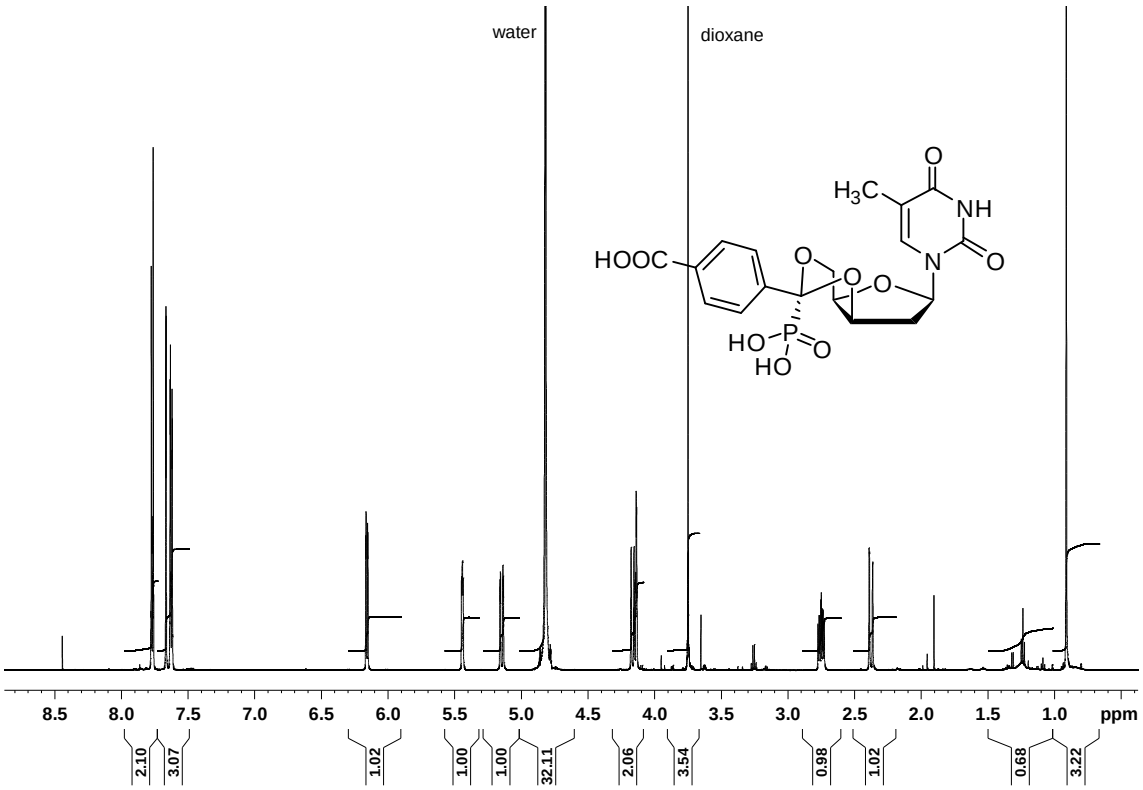

Figure 22Sa ^{1}H NMR spectrum (600.13 MHz) of compound **13** in D_2O.

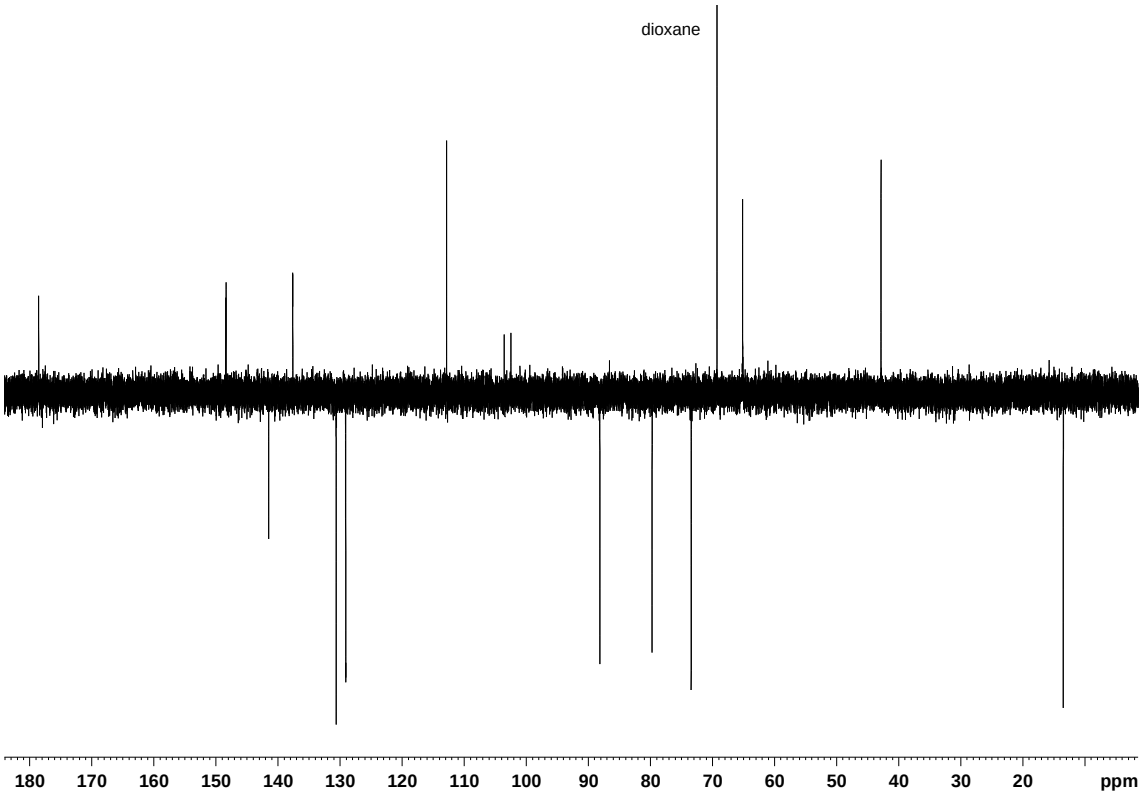

Figure 22Sb ^{13}C NMR spectrum (150.9 MHz) of compound **13** in D_2O.

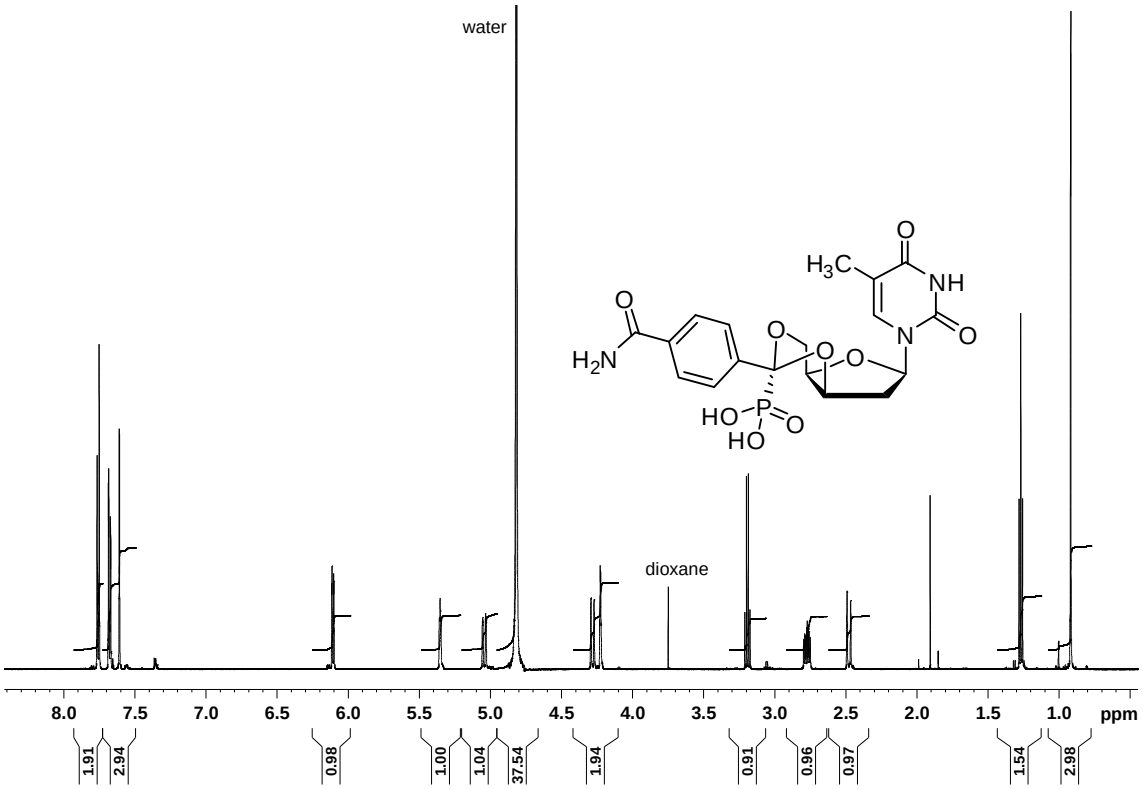

Figure 23Sa ^{1}H NMR spectrum (600.13 MHz) of compound **14** in D_2O.

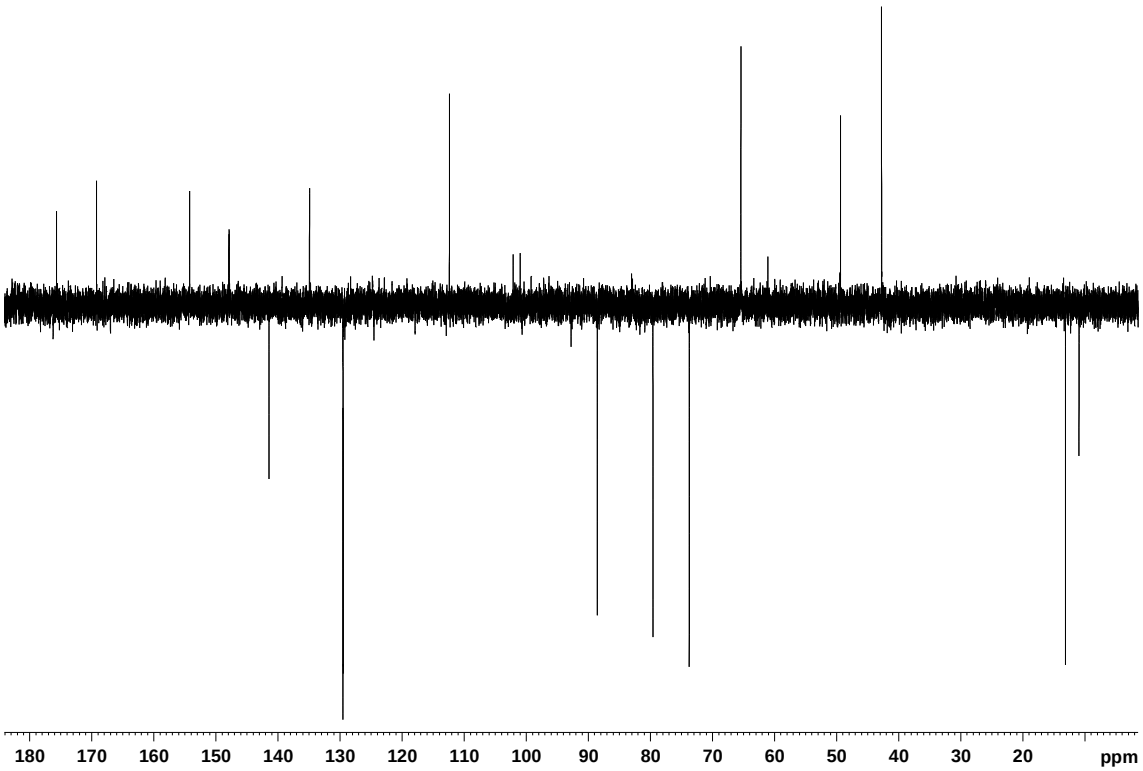

Figure 23Sb ^{13}C NMR spectrum (150.9 MHz) of compound **14** in D_2O.

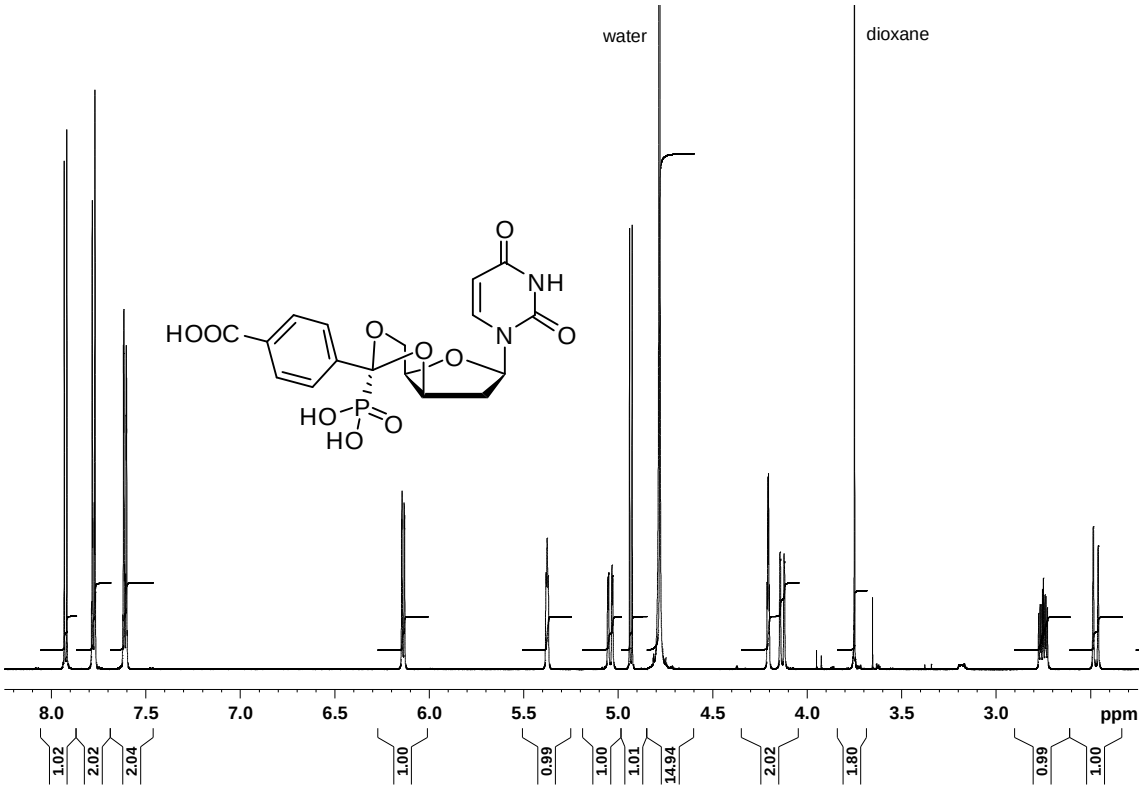

Figure 24Sa ^{1}H NMR spectrum (600.13 MHz) of compound **15** in D_2O.

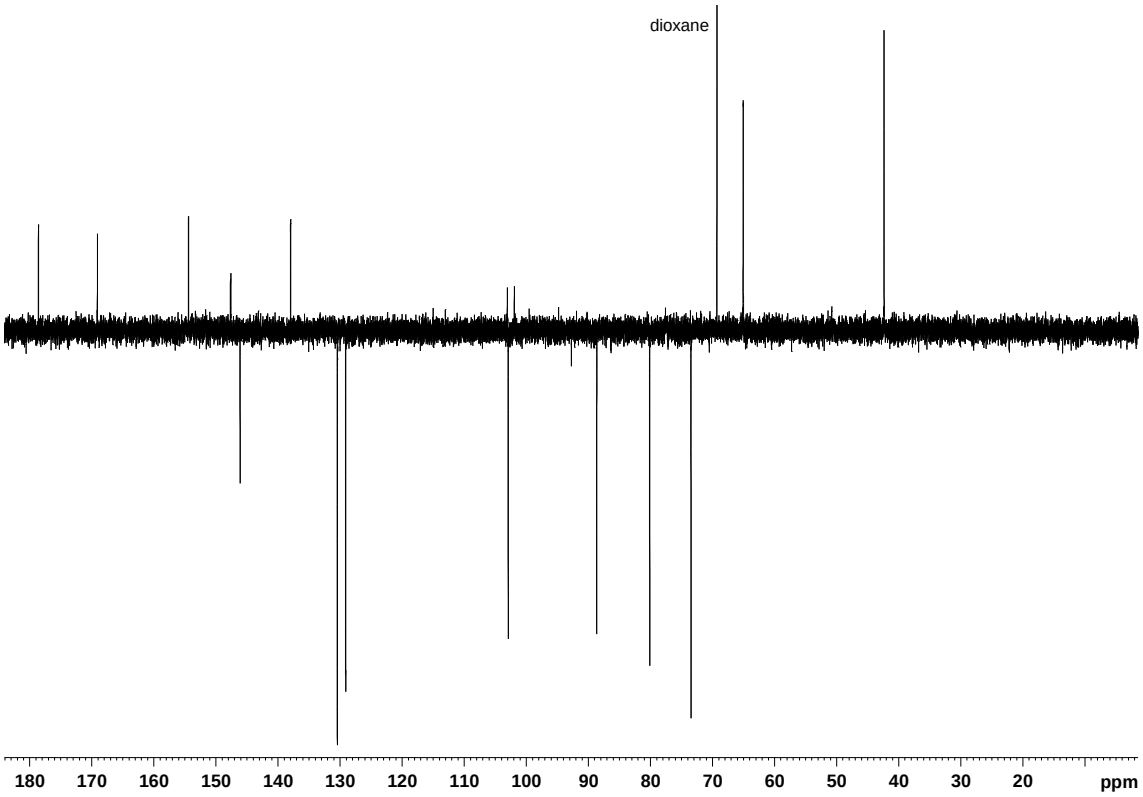

Figure 24Sb ^{13}C NMR spectrum (150.9 MHz) of compound **15** in D_2O.

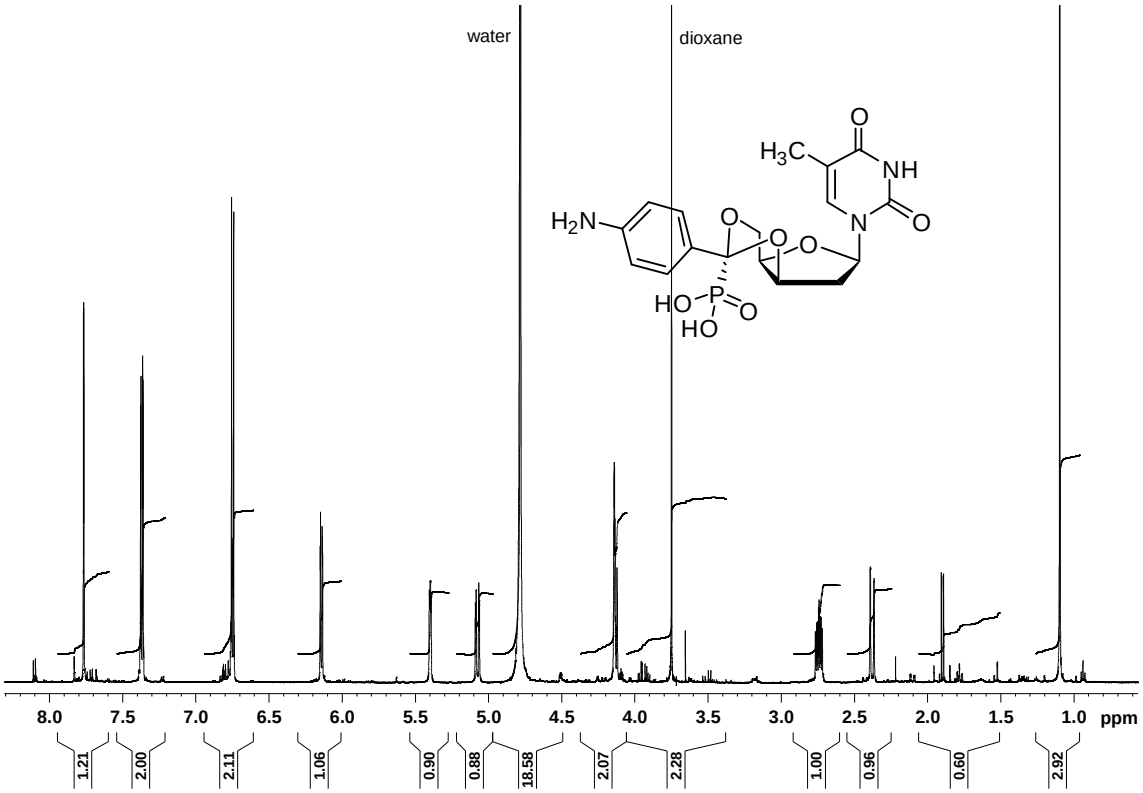

Figure 25Sa ^{1}H NMR spectrum (600.13 MHz) of compound **16** in D_2O.

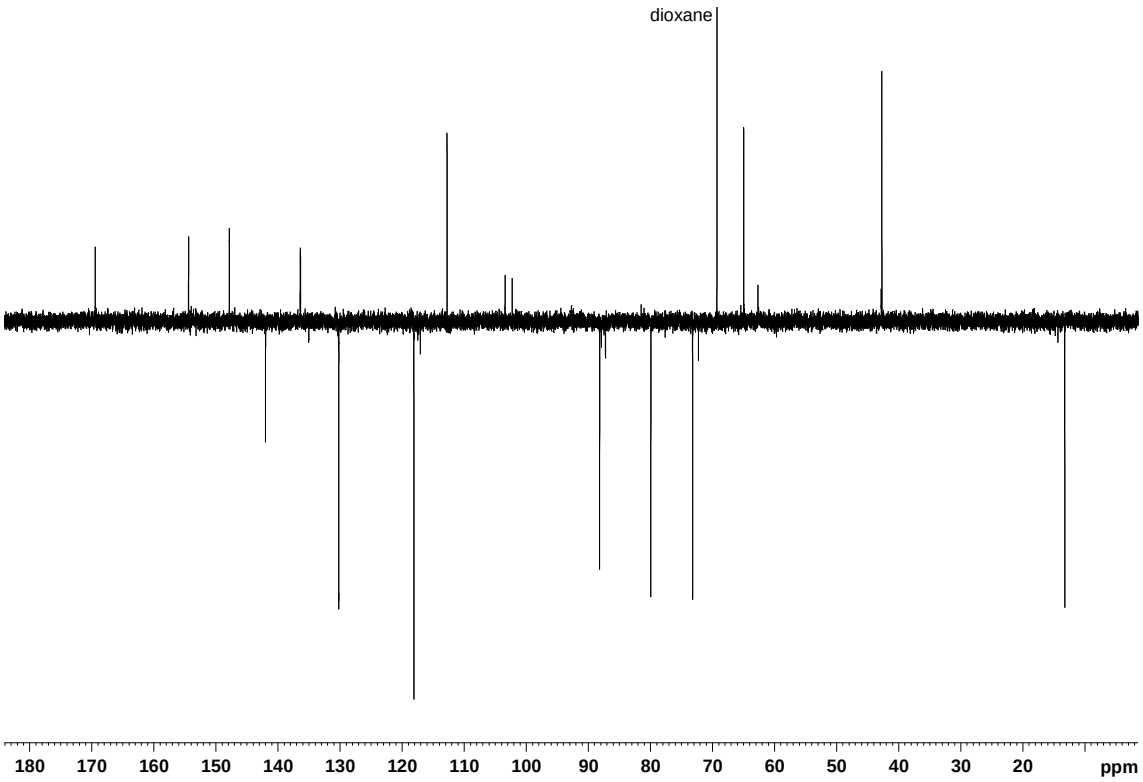

Figure 25Sb ^{13}C NMR spectrum (150.9 MHz) of compound **16** in D_2O.

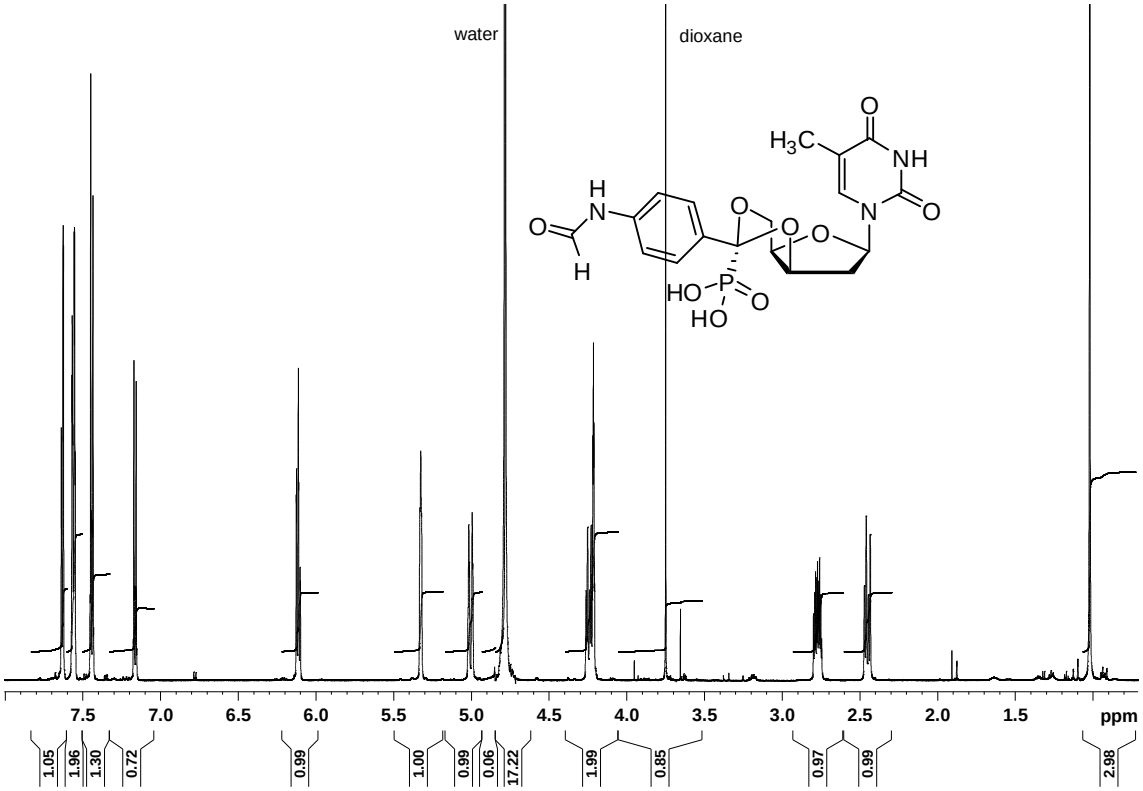

Figure 26Sa ^{1}H NMR spectrum (600.13 MHz) of compound **20** in D_2O.

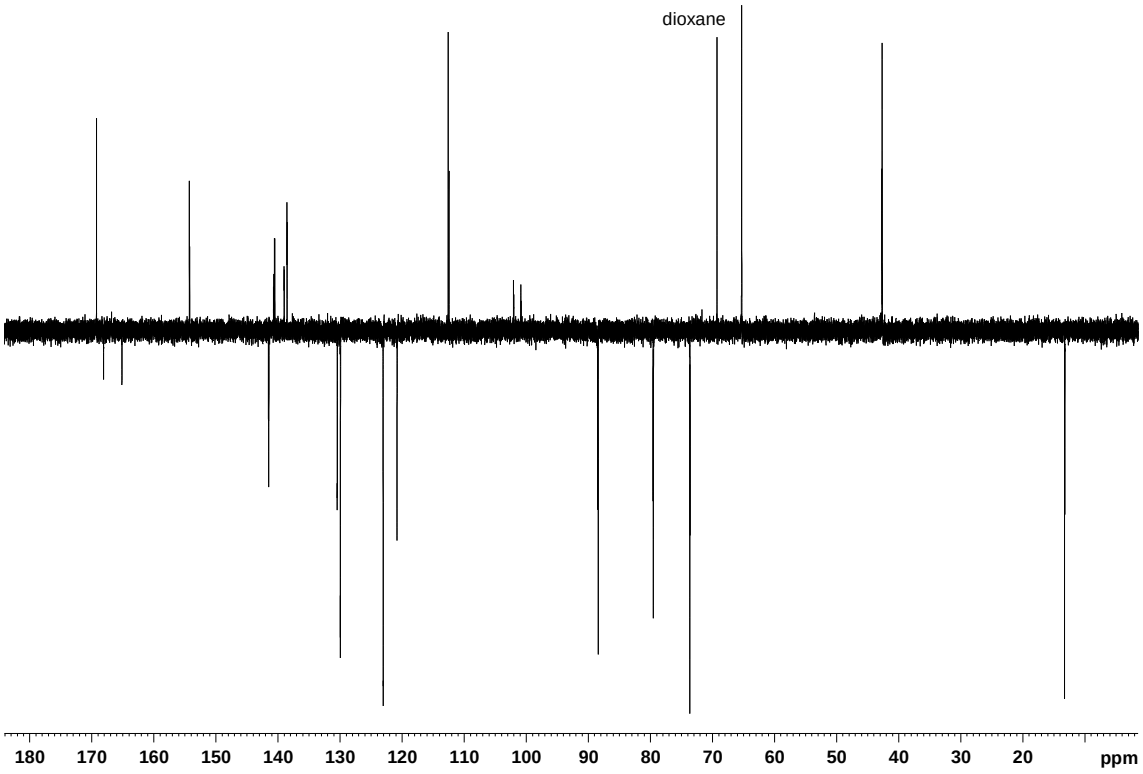

Figure 26Sb ^{13}C NMR spectrum (150.9 MHz) of compound **20** in D_2O.

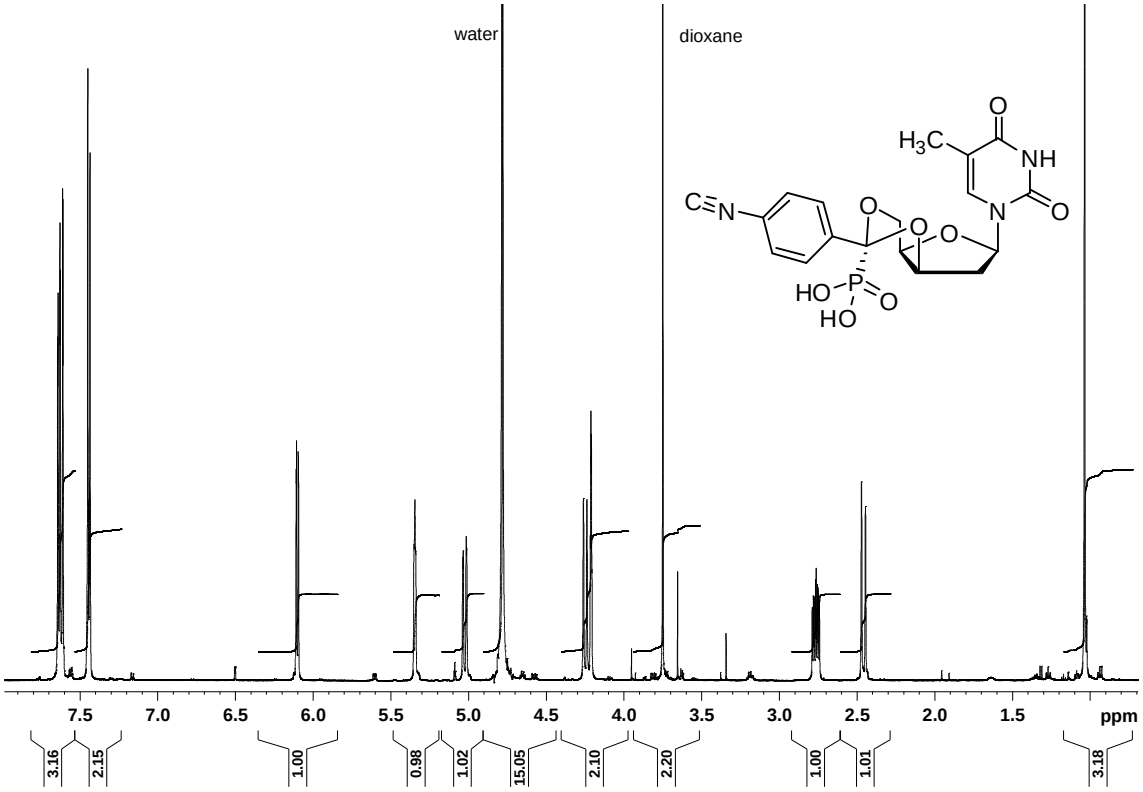

Figure 27Sa ^{1}H NMR spectrum (600.13 MHz) of compound **21** in D_2O.

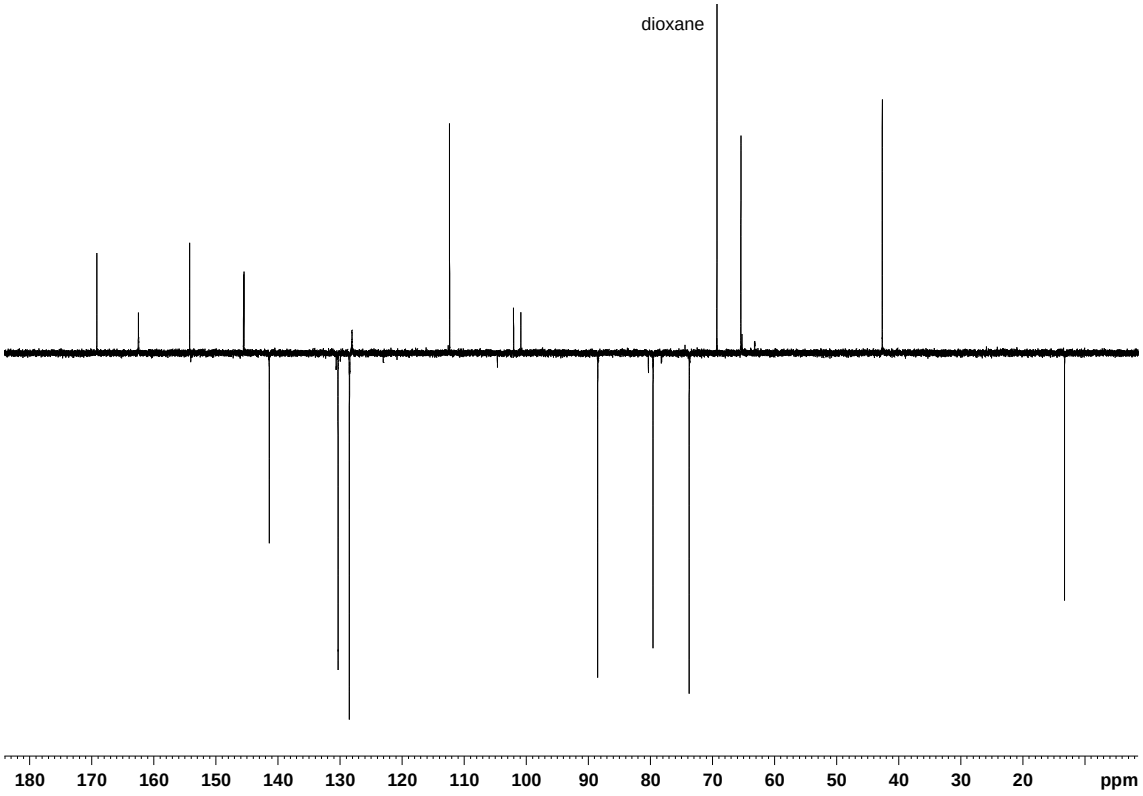

Figure 27Sb ^{13}C NMR spectrum (150.9 MHz) of compound **21** in D_2O.

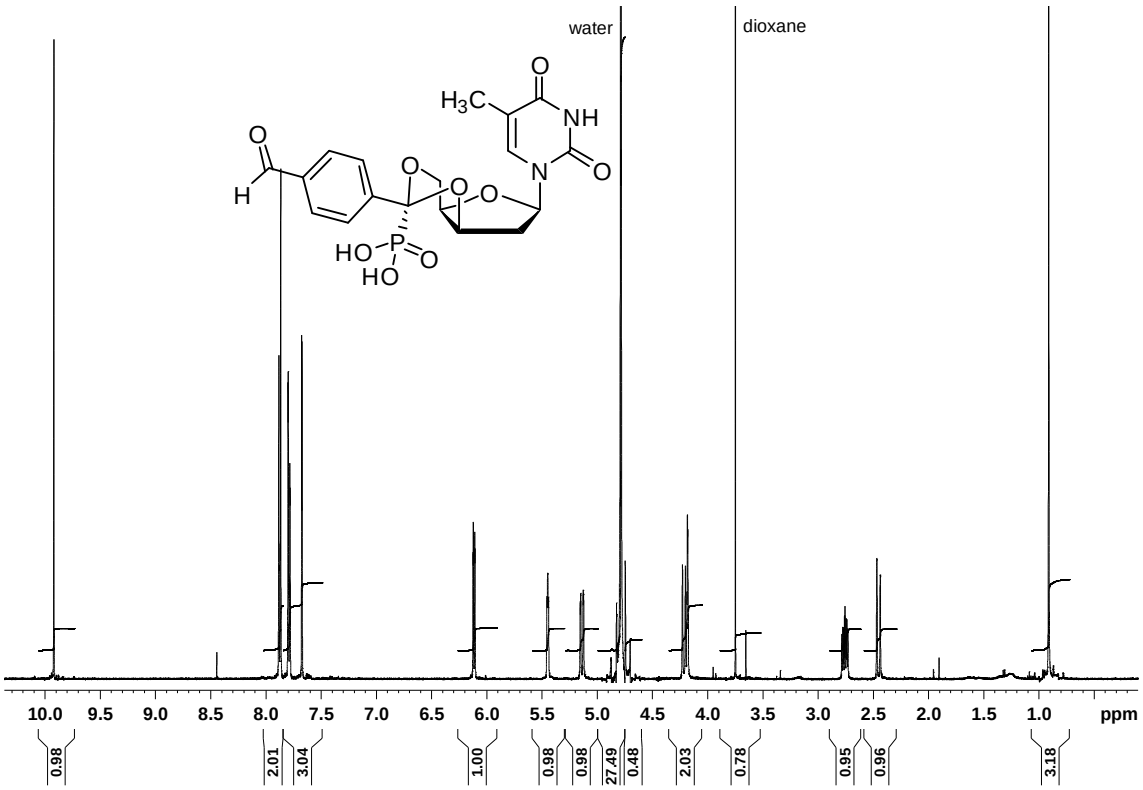

Figure 28Sa ^{1}H NMR spectrum (600.13 MHz) of compound **22** in D_2O.

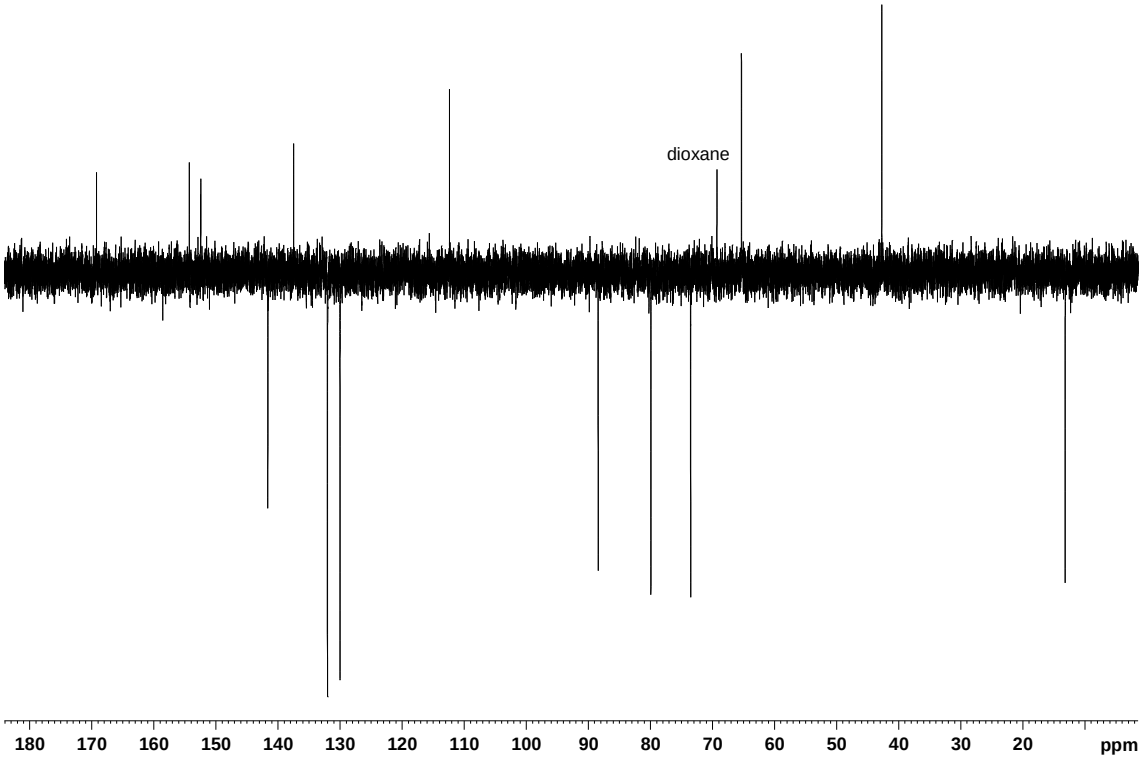

Figure 28Sb ^{13}C NMR spectrum (150.9 MHz) of compound **22** in D_2O.

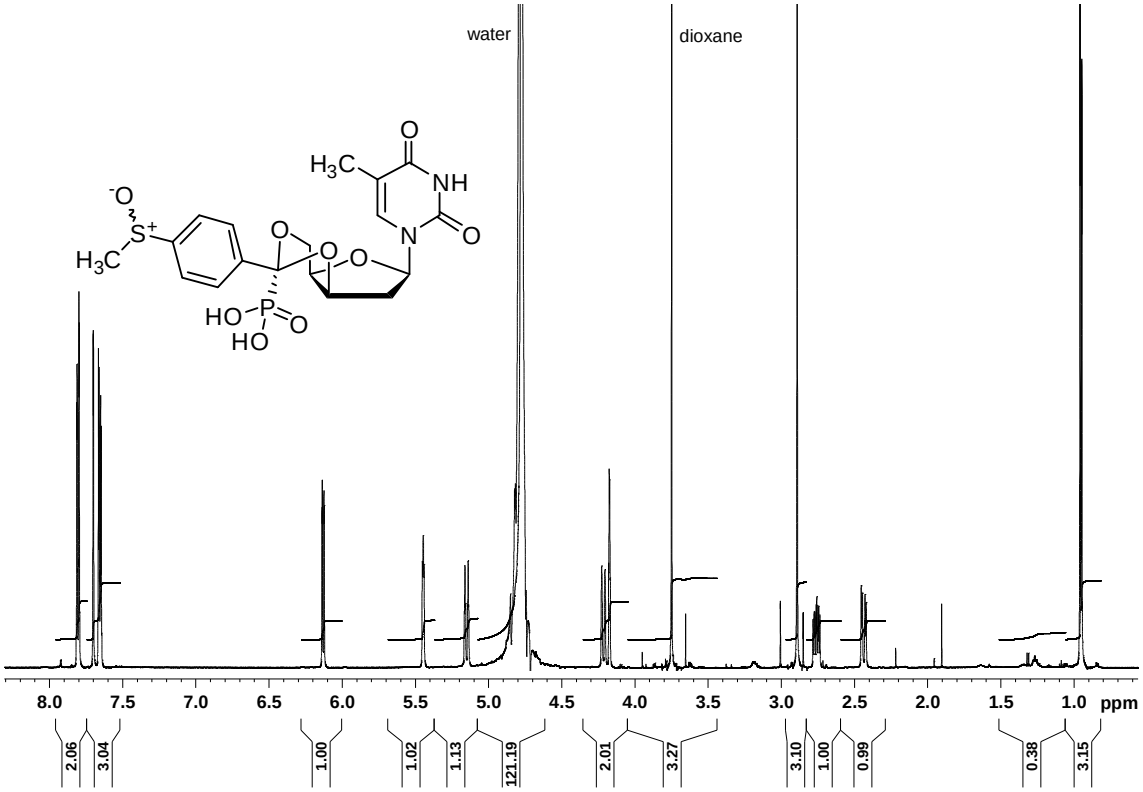

Figure 29Sa ^{1}H NMR spectrum (600.13 MHz) of compound **23** in D_2O.

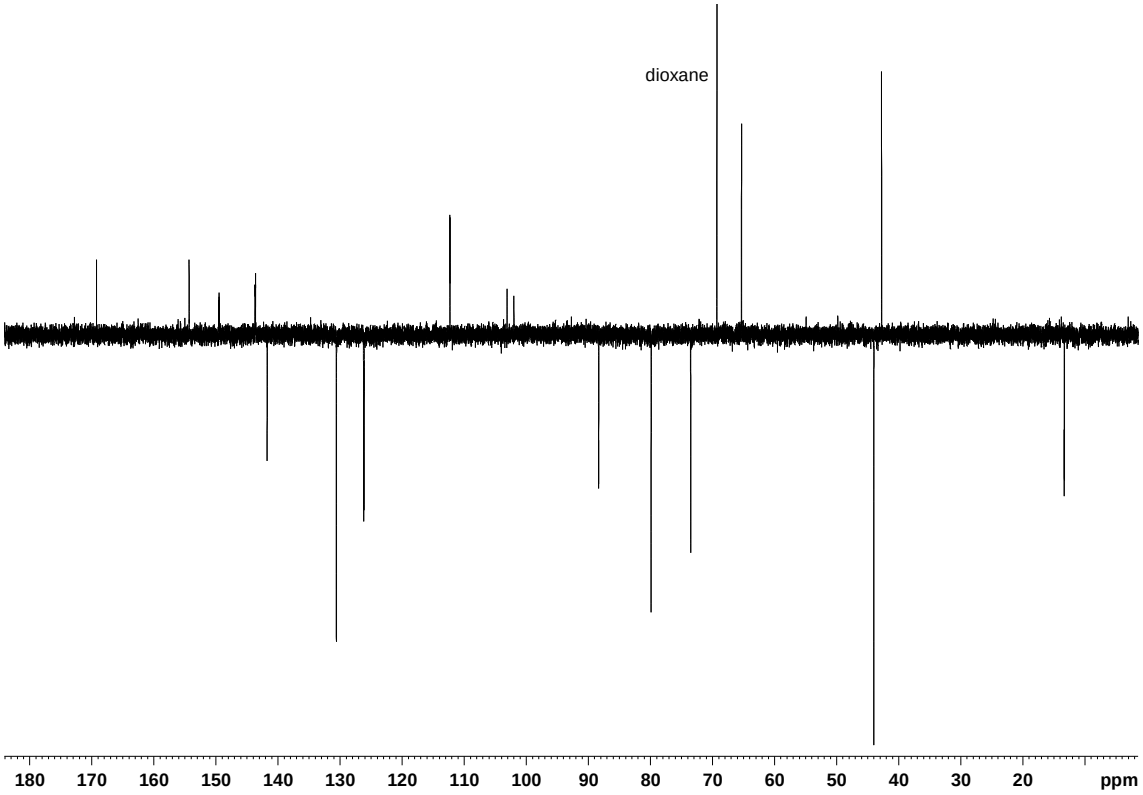

Figure 29Sb ^{13}C NMR spectrum (150.9 MHz) of compound **23** in D_2O.

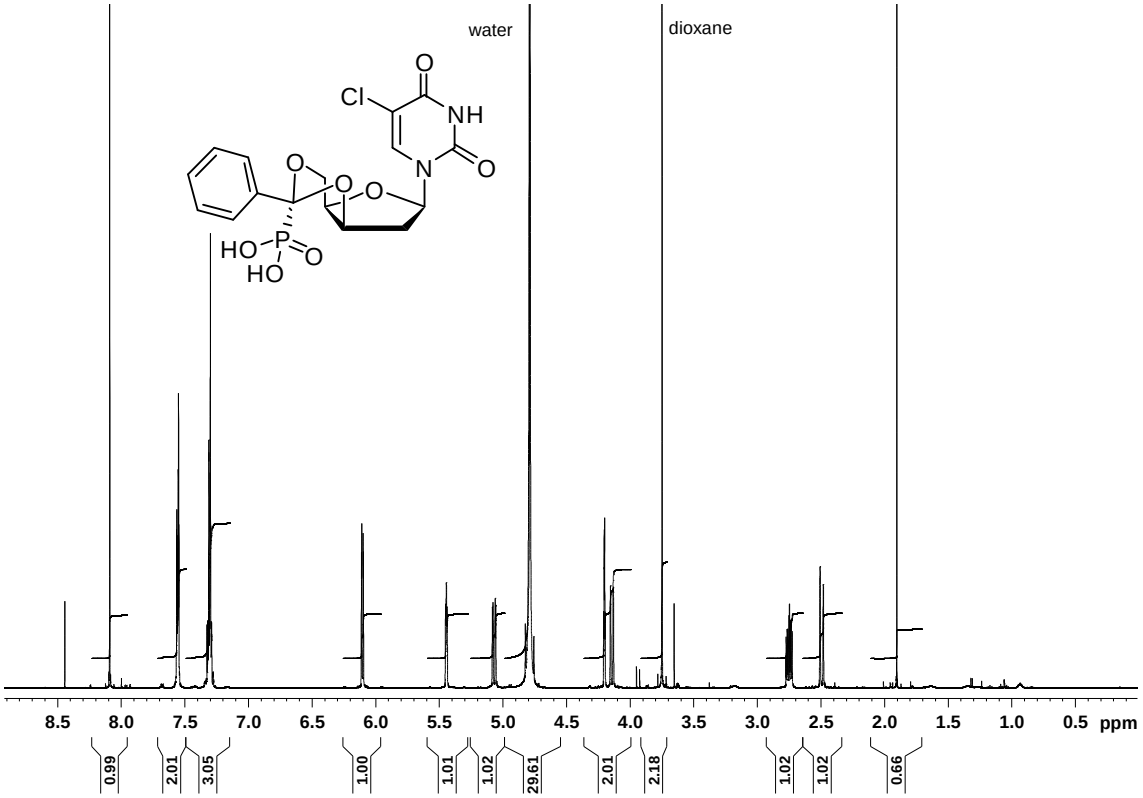

Figure 30Sa ^{1}H NMR spectrum (600.13 MHz) of compound **24** in D_2O.

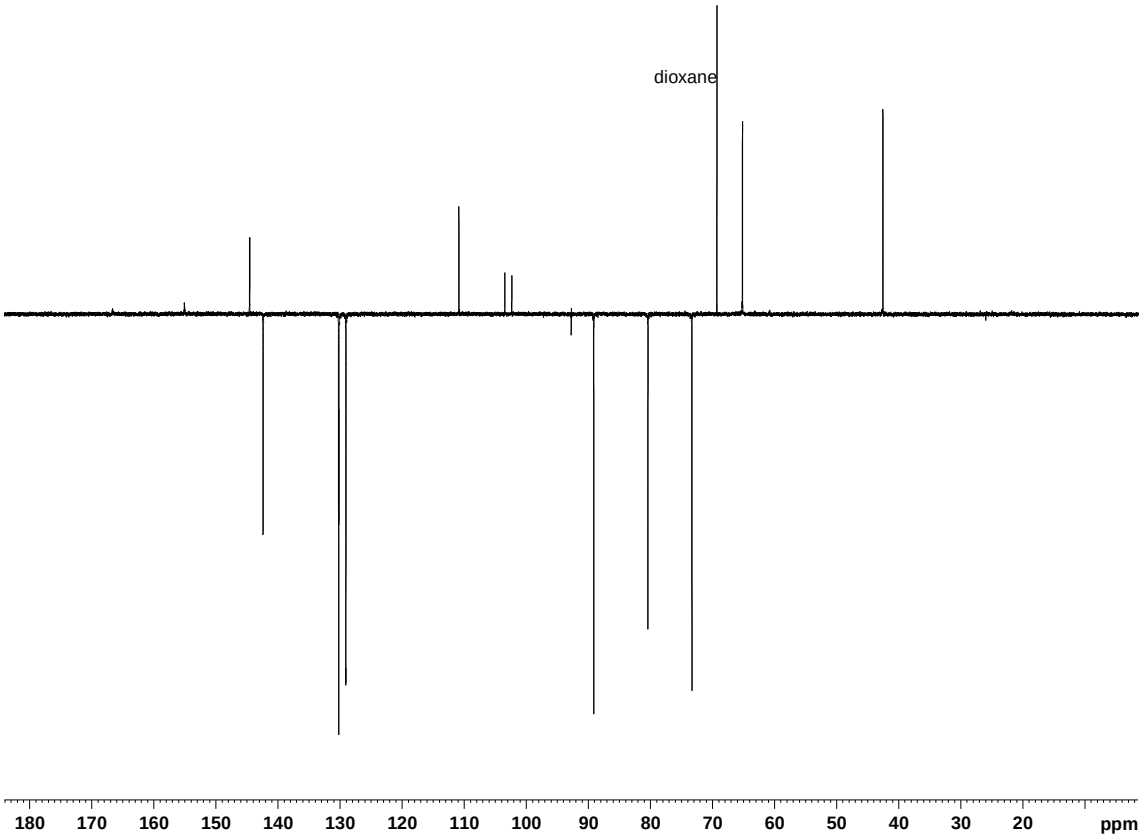

Figure 30Sb ^{13}C NMR spectrum (150.9 MHz) of compound **24** in D_2O.

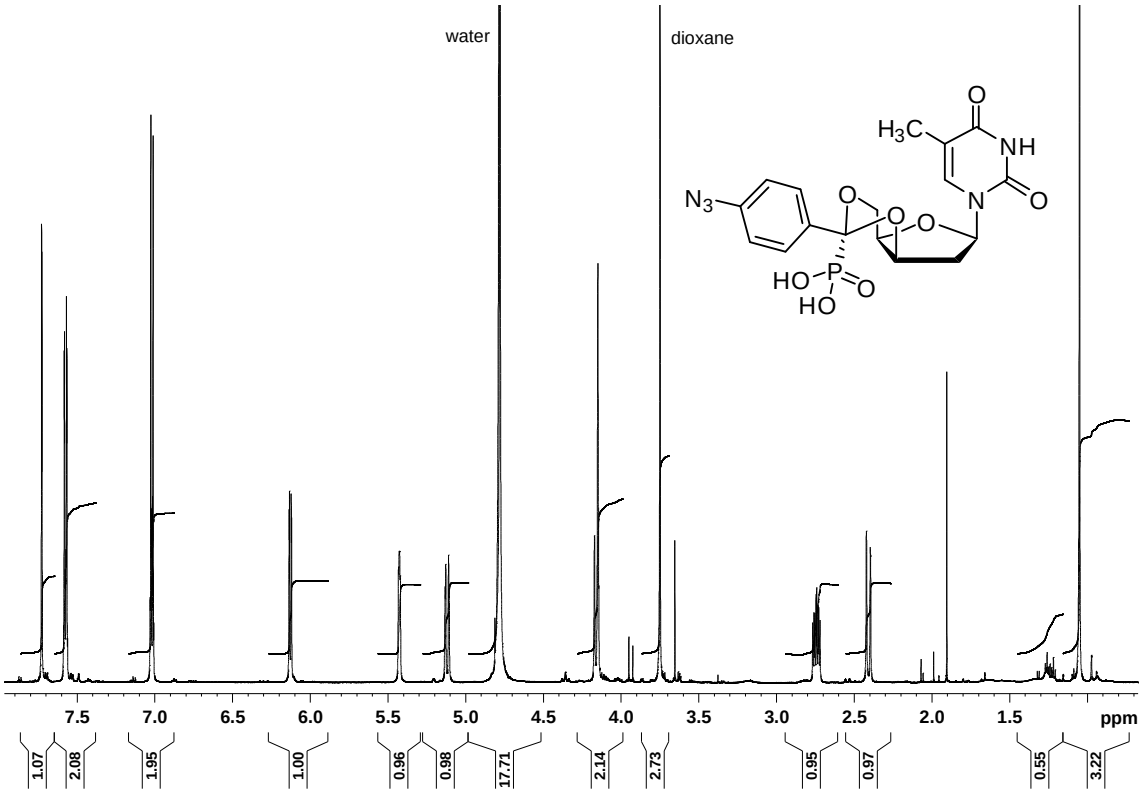

Figure 31Sa ^{1}H NMR spectrum (600.13 MHz) of compound **26** in D_2O.

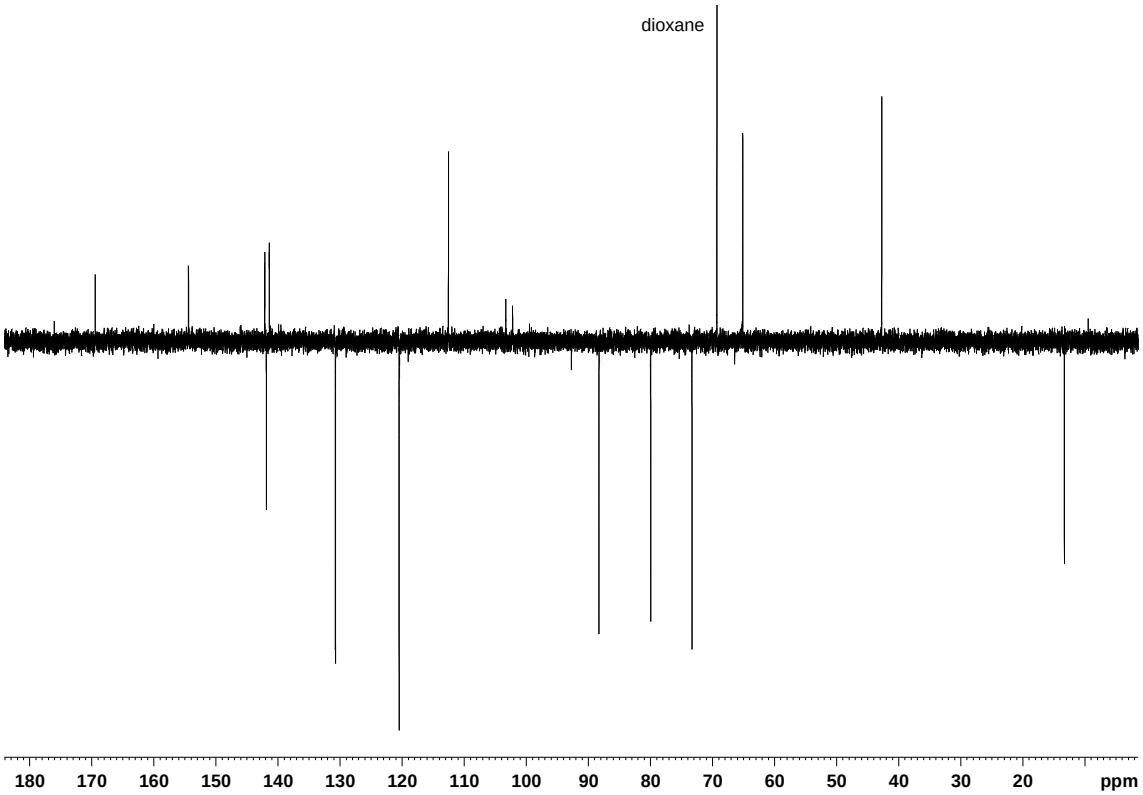

Figure 31Sb ^{13}C NMR spectrum (150.9 MHz) of compound **26** in D_2O.

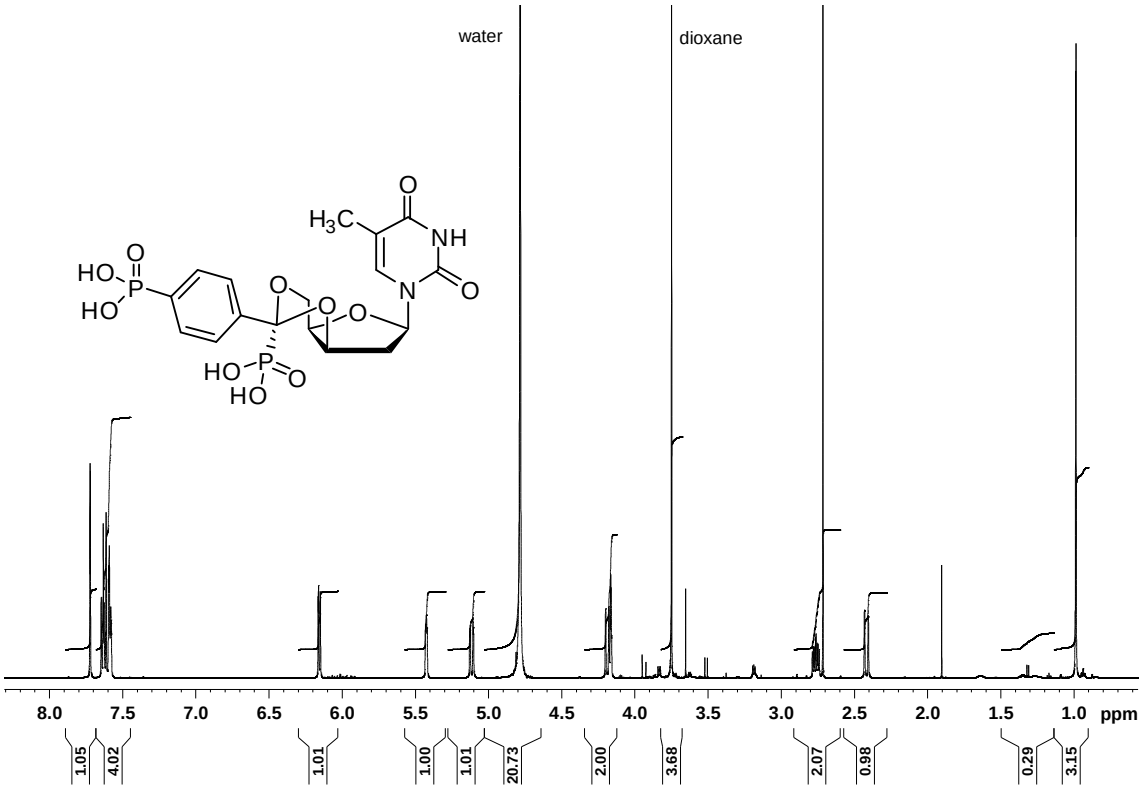

Figure 32Sa ^{1}H NMR spectrum (600.13 MHz) of compound **28** in D_2O.

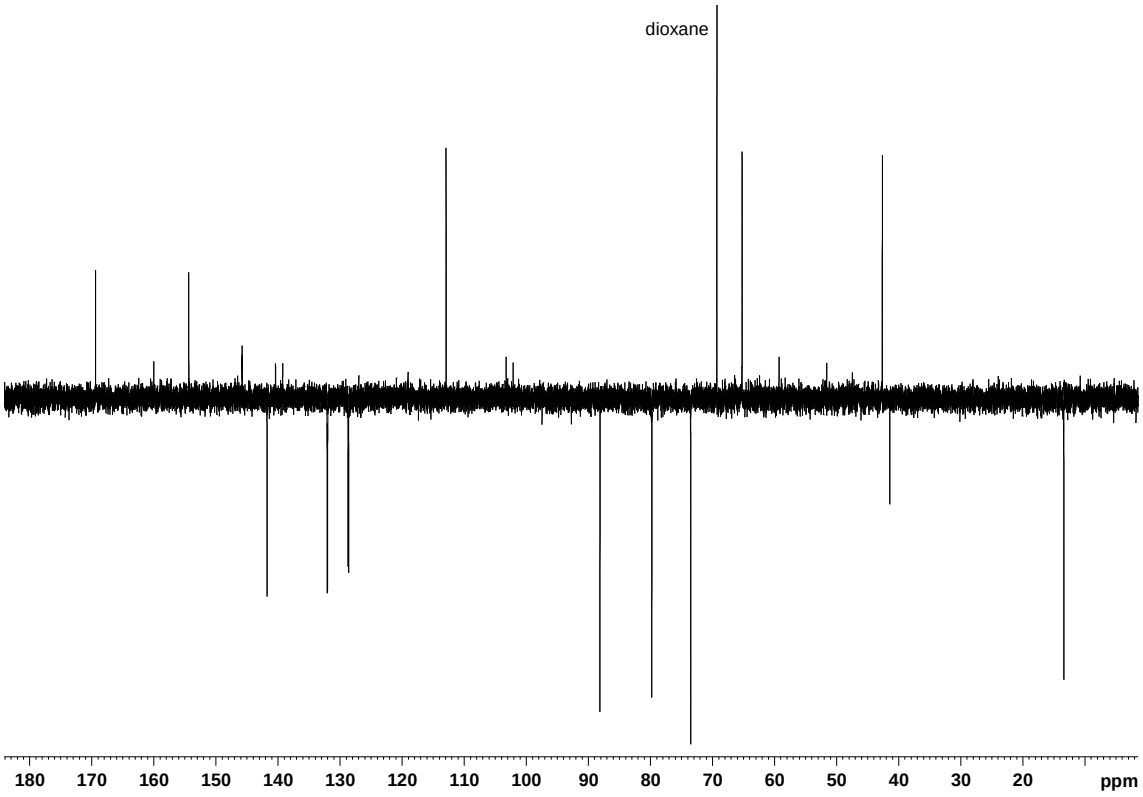

Figure 32Sb ^{13}C NMR spectrum (150.9 MHz) of compound **28** in D_2O.

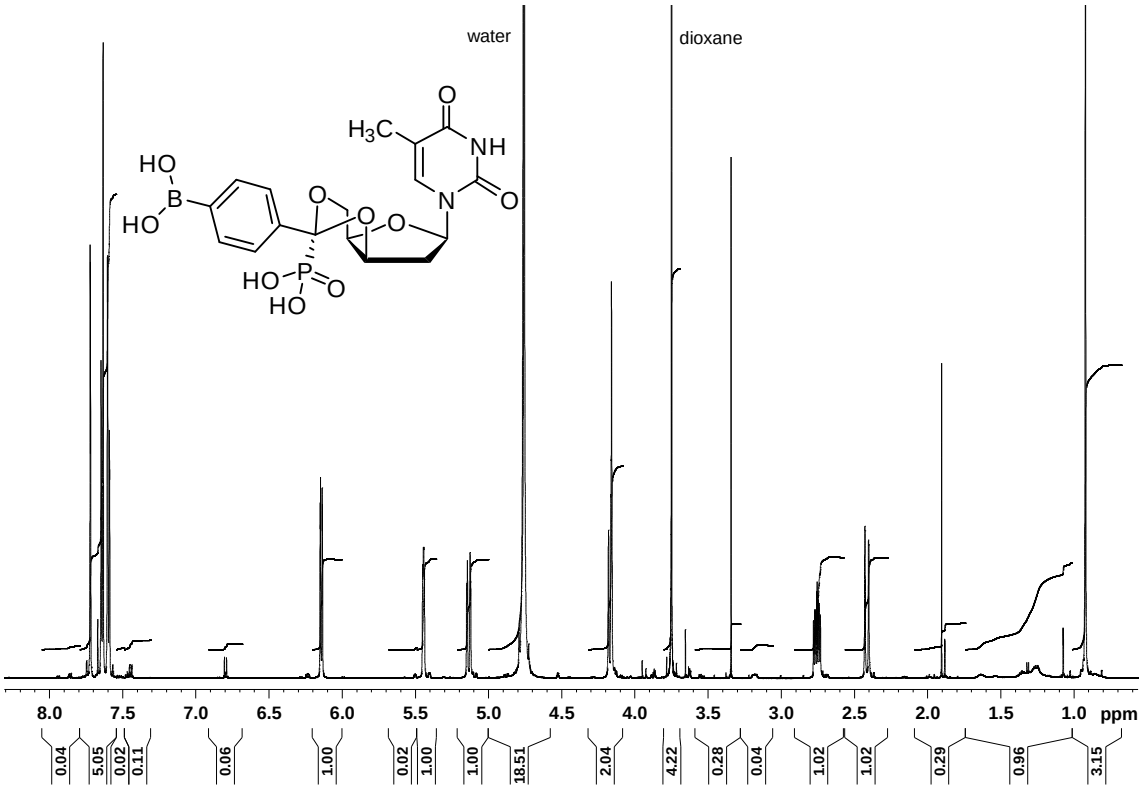

Figure 33Sa ^{1}H NMR spectrum (600.13 MHz) of compound **30** in D_2O.

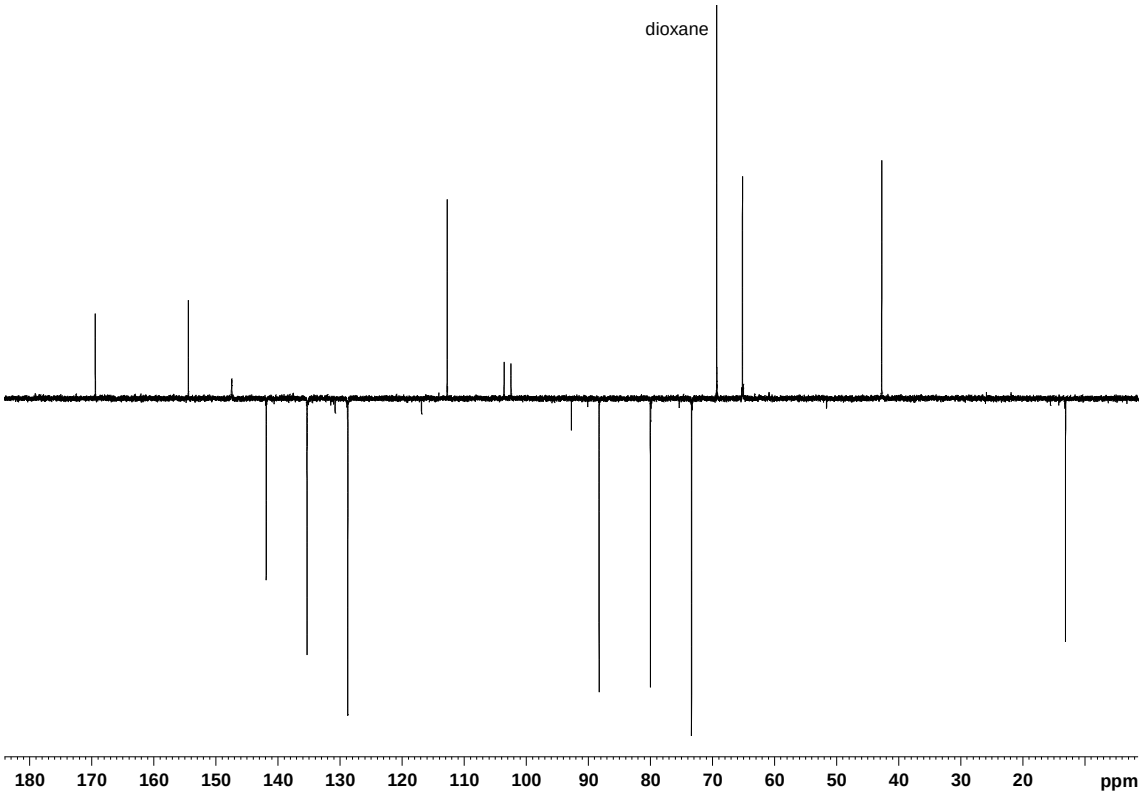

Figure 33Sb ^{13}C NMR spectrum (150.9 MHz) of compound **30** in D_2O.

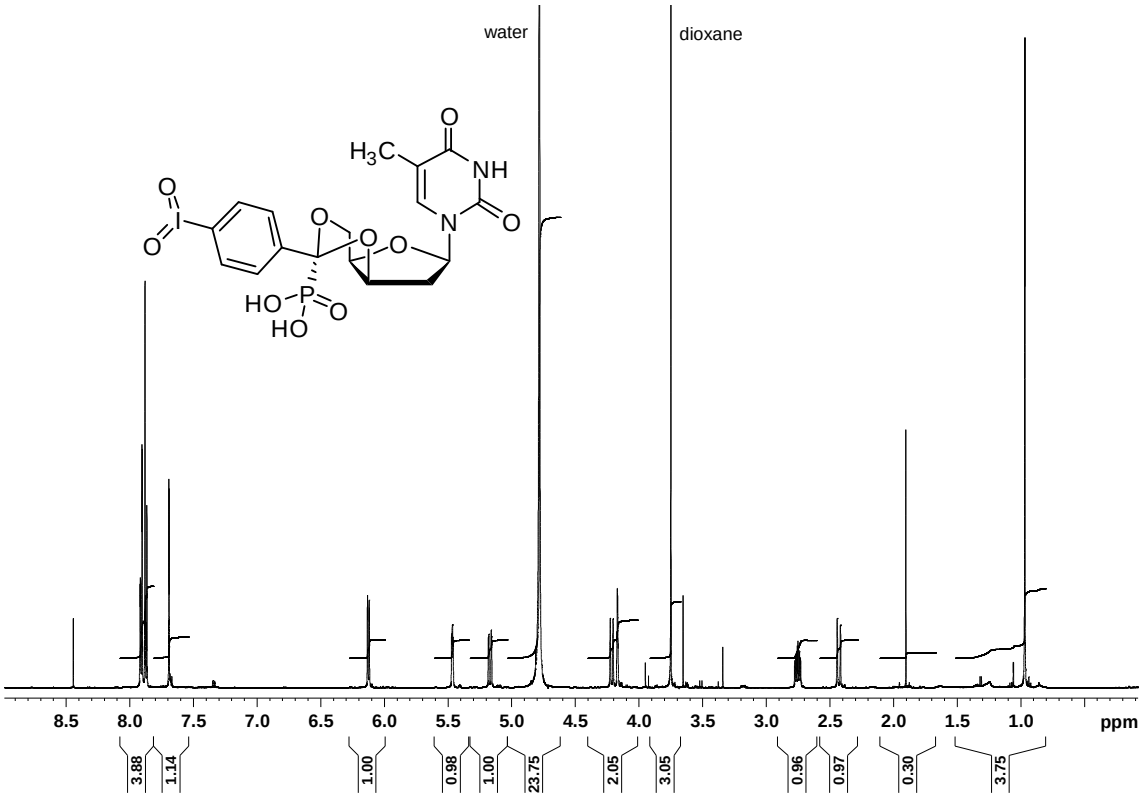

Figure 34Sa ^{1}H NMR spectrum (600.13 MHz) of compound **31** in D_2O.

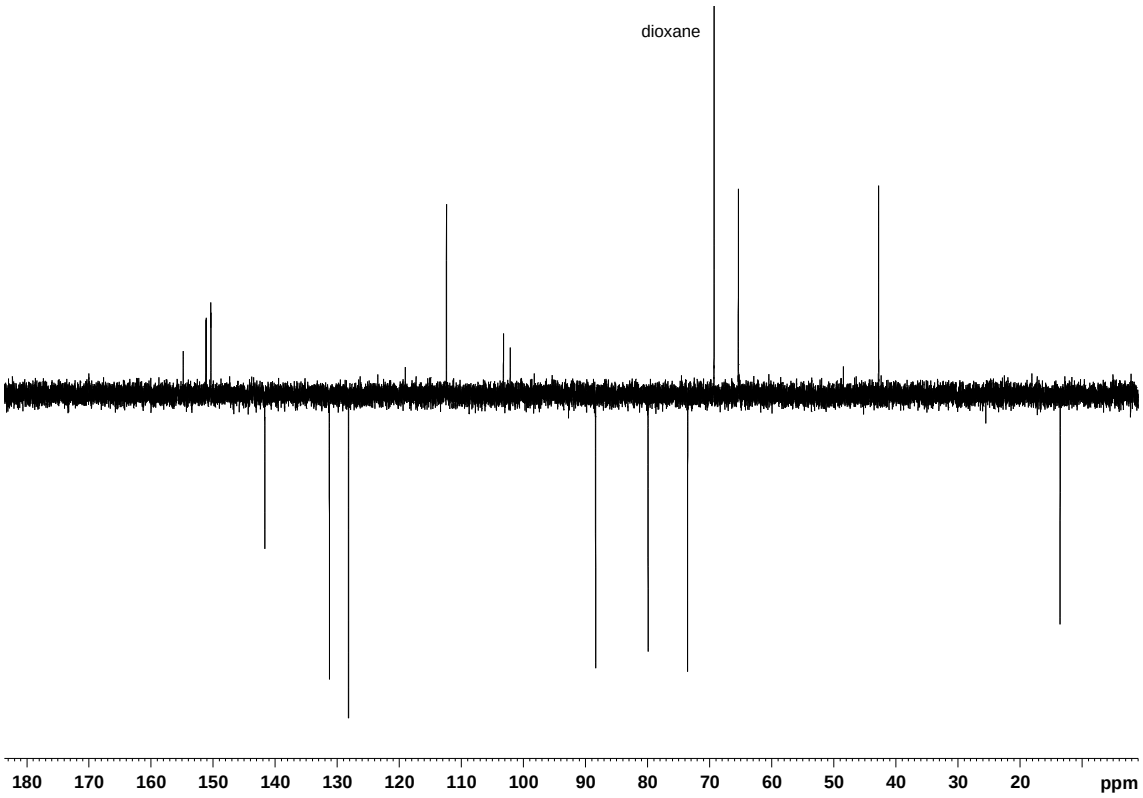

Figure 34Sb ^{13}C NMR spectrum (150.9 MHz) of compound **31** in D_2O.

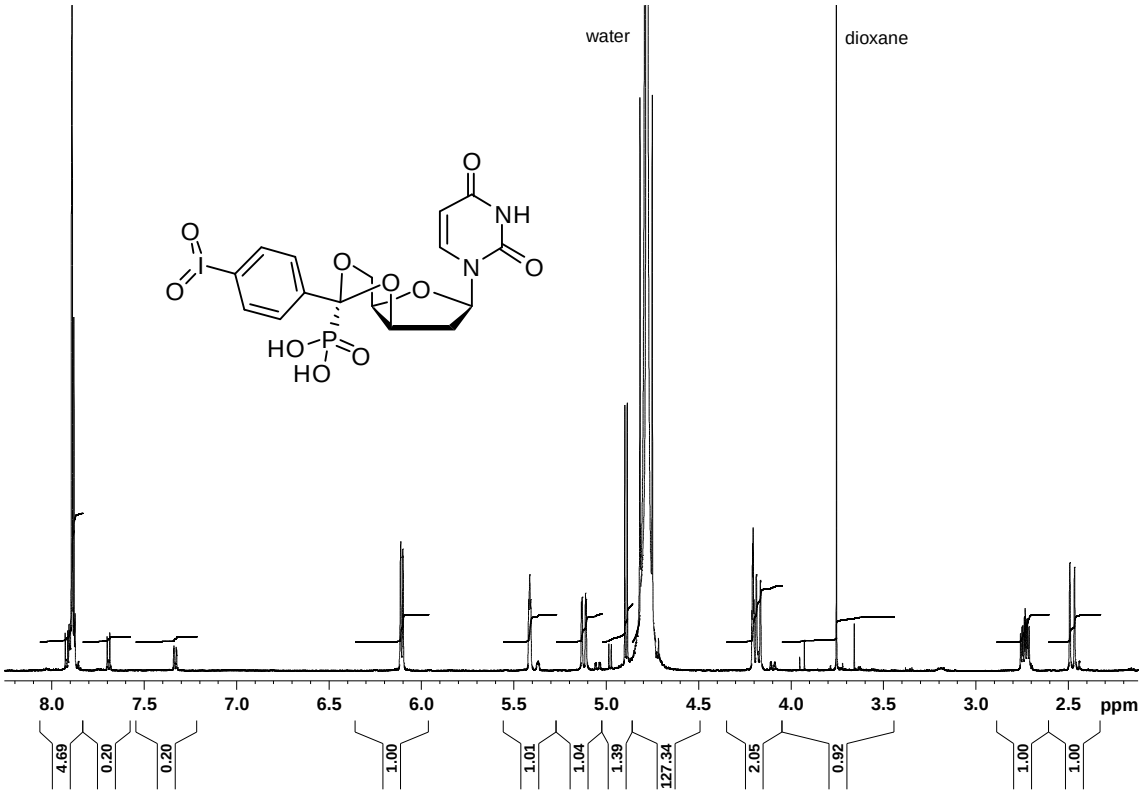

Figure 35Sa ^{1}H NMR spectrum (600.13 MHz) of compound **32** in D_2O.

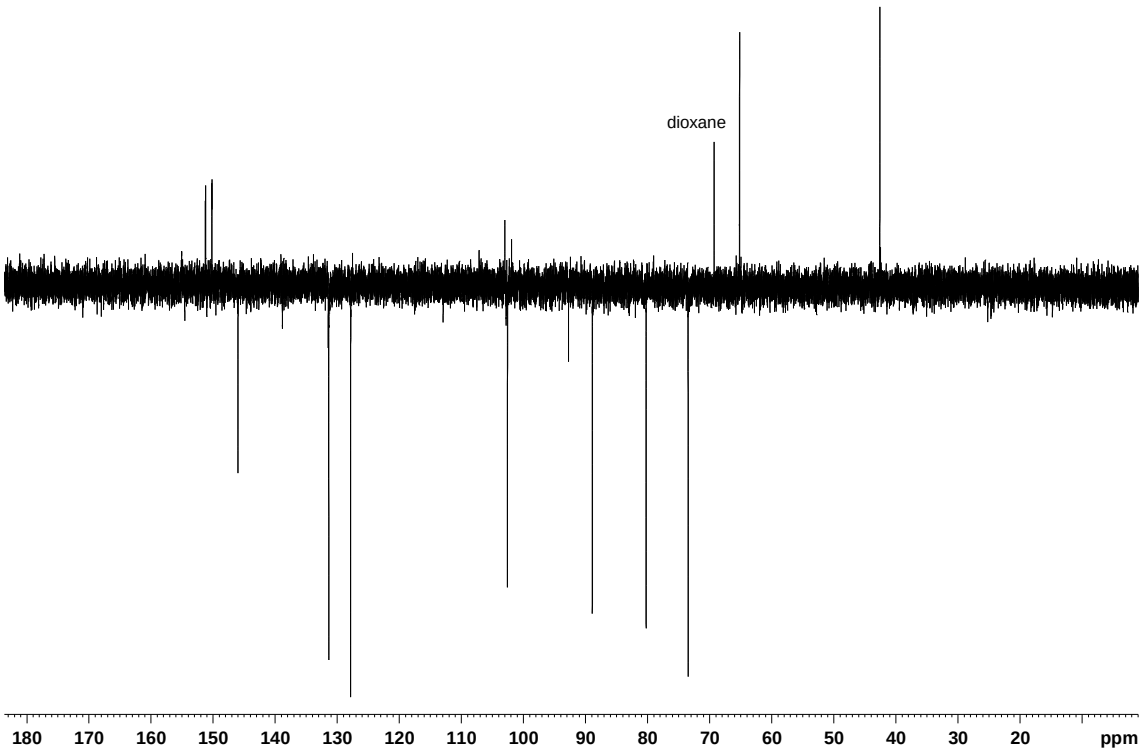

Figure 35Sb ^{13}C NMR spectrum (150.9 MHz) of compound **32** in D_2O.

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