SUPPLEMENTARY INFORMATION

P-stereodefined morpholino dinucleoside 3',5'-phosphorothioates

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Experimental information

Supporting data

1. Materials

Silica gel chromatography media were supplied by MERCK. TLC silica gel 60 plates were used for routine analyses, while HPTLC silica gel 60 plates were used for the assessment of the chromatographic separability of P-diastereomers; all plates were with a UV F254 indicator. Silica gel 60, 230–400 mesh, was used for routine open column chromatographic purification. Unless otherwise stated, solvents of analytical grade were used. The cartridges packed with silica gel (FP ECOFLEX Si 25g) was used for flash chromatographic purification. Anhydrous acetonitrile, DBU (1,4-diazabicyclo[5.4.0]undec-7-ene), TBD (1,5,7-Triazabicyclo[4.4.0]dec-5-ene) and Verkade base (Vb, 2,8,9-Trimethyl-2,5,8,9-tetraaza-1-phosphabicyclo[3.3.3]undecane) and Bovine Pancreatic RNase A (type XII-A) were supplied by Merck. For chromatographic separation of OTP monomers, HPLC grade ethyl acetate, hexane were supplied by Witko (Lodz, Poland; solvents of a ChemSolve class). 5′-DMT-protected ribonucleosides of uridine were purchased from ChemGenes Corporation (Wilmington, MA). The 2-Chloro-“spiro”-4,4-pentamethylene-1,3,2-oxathiaphospholane (the phosphitylating reagent) was synthesized according to the published protocols[1].

2. Methods

Synthesis of *m*U-OTP (2)
The 6′-*O*-DMT-morpholino nucleoside (mU, **1**, 1.5g, 2.8mmol) in 15 mL round-bottom flask and elemental sulfur were dried overnight at a high vacuum (0.01 mmHg). To the flask anhydrous pyridine (water content < 20ppm) was added, followed by 500µl (3.4 mmol) of 2-chloro-“spiro”-4,4-pentamethylene-1,3,2-oxathiaphospholane. After ca. 5 minutes, dry elemental sulfur (180 mg, 5.6 mmol) was added and the stirring was continued for ca. 20 minutes at room temperature. TLC analysis showed no substrate remaining. Excess sulfur was filtered off and the solvent was evaporated. The crude mixture was isolated by means of silica gel chromatography using a flash chromatography system (Reveleris) eluted with chloroform containing 0.1% TEA. The appropriate fractions were combined and concentrated under reduced pressure to give **2** (as a mixture of P-diastereomers) in 75% yield (1.5g). HR MS: m/z 734, calcd MW 735.

Separation of morpholino oxathiaphospholane monomer *m*U-OTP into P-diastereomers
Approximately 1.5 mL of an ethyl acetate solution containing 100 mg of *m*U-OTP monomer was filtered through an organic solvent resistant membrane filter (0.2 µm) and the filtrate was applied onto an HPLC system. The column (Pursuit XRs 10 µm, 250×21,2 mm) was eluted at a flow rate of 10 mL min^{-1} using the eluents specified in Table 1. Under these conditions, baseline separation was achieved and after evaporation of the solvents pure P-diastereomers were obtained at ca. 90% recovery. HR MS, ^{31}P NMR, ^{1}H NMR, and ^{13}C NMR spectra for the *fast*- (**2a**) and *slow*-eluting (**2b**) P-diastereomers are given in Figures S2-12 (ESI†).

Detritylation of P-diastereomers 2a and 2b
To a solution of 100 mg (0.14 mmol) of **2a** (and **2b**) in 2 mL of anhydrous acetonitrile a sodium hydrogen sulfate/silica gel reagent (50 mg) was added and the suspension was stirred at room temperature. The reaction progress was monitored by TLC using a 9:1 (v/v) $CHCl_3$:MeOH mixture as an eluent (R_f of the substrate 0.76; R_f of the product **3a** and **3b** 0.43). After 15 minutes the reaction went to completion. The silica gel was filtered off and the solvent evaporated under vacuum. The product was isolated by semi-preparative HPLC (a silica gel column eluted with ethyl acetate) and evaporation at reduced pressure furnished white solid material in 68% yield (40 mg) for **3a** and 61% yield (36mg) for **3b**.

The synthesis of P-stereodefined dinucleotides 4a and 4b (semi-preparative scale)

To a sample of **2a** (or **2b)** (100 mg, 0.14 mmol) and 3′-O-Ac-thymidine (68 mg, 0.28 mmol), 2 mL of dry acetonitrile with activator Vb (45 mg, 0.21 mmol) was added. The reaction progress was monitored by TLC using a 9:1 (v/v) $CHCl_3$:MeOH mixture as an eluent (Rf of the substrate 0.76; Rf of the product **4a** and **4b** 0.31). The reaction was completed within 15 minutes and the product was confirmed by ^{31}P NMR. Then the reaction mixture was concentrated under reduced pressure and the crude product was purified using cartridges packed with silica gel of Reveleris Flash Chromatography and was eluted with a linear 0→50% gradient of MeOH in CH_2Cl_2 (see Figure S21, ESI†). The product was isolated in 70% yield (88 mg) for **4a** and 72% yield (90 mg) for **4b**. HR MS: m/z 890.2571 for **4a** and 890.2571 for **4b**, calcd MW 891. ^{31}P NMR, HR MS are given in Figures S22, S25, S27.

Detritylation of 4a and 4b

The dinucleotides **4a** and **4b** (0.1 mmol) were dissolved in 1.5% DCA in CH_2Cl_2 (10 mL), with stirring at room temperature. The reaction progress was monitored by TLC using a 8:2 (v/v) $CHCl_3$:MeOH mixture as an eluent (Rf of the substrate 0.58; Rf of the product **5a** and **5b** 0.34). After 10 min, the reaction mixture was concentrated under vacuum. The crude product was purified with silica gel of Reveleris Flash Chromatography and was eluted with a linear 0→80% gradient of MeOH in CH_2Cl_2 (Figure S23, ESI†). The product was isolated in 66% yield (38 mg) for **5a** and 61% yield (36 mg) for **5b**. HR MS: m/z 588.1170 for **5a** and 588.1161 for **5b**, calcd. MW 589. ^{31}P NMR, HR MS are given in Figures S24, S26, S28.

Digestion of 5 with svPDE

Dinucleotide (from mixture of P-diastereomers) 0.5 OD) was dissolved in a 25 mM Tris-Cl (pH 8.5), 5 mM $MgCl_2$ buffer (20 µL) containing 1 µL of the svPDE suspension. The sample was incubated for 24 h at 37 °C, the protein was denatured (for 1 minute at 95 °C). The solution was then filtered and analyzed by RP-HPLC. Conditions: a C18 column, 250 ×4.6 mm, 5 µm; elution at 0.5 mL min^{-1} with a gradient of 0.1 M TEAB, pH 7.3 to 40% CH_3CN in 0.1 M TEAB over 32 minutes.

Digestion of 5 with nP1

Dinucleotide (from mixture of P-diastereomers) 0.5 OD) was dissolved in a 100 mM Tris-Cl (pH 7.2), 1 mM $ZnCl_2$ buffer (20 µL) containing 1 µL of the svPDE suspension. The sample was incubated for 24 h at 37 °C, the protein was denatured (for 1 minute at 95 °C). The solution was then filtered and analyzed by RP-HPLC under the same conditions as above.

3. Equipment

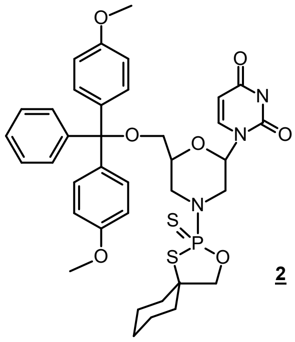
^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra were recorded using Bruker instruments (AV-200, DRX-500, or Avance Neo 400; operating for ^{1}H at 200, 500, and 400 MHz, respectively). Chemical shift(σ) values are in ppm, referenced to internal tetramethylsilane (TMS) or residual solvent protons for ^{1}H NMR and external 85% H_3PO_4 for ^{31}P NMR.

High-resolution mass spectra (HR MS) were acquired using a Synapt G2 Si mass spectrometer (Waters Corporation, Milford, MA) consisting of ESI source and a quadrupole time-of-flight mass analyzer. Measurements were performed in negative ion mode with the capillary and sampling cone voltages set at 2.7 kV and 20 V, respectively. The source temperature was 110°C. To ensure satisfactory accuracy, data were obtained in centroid mode and readings were corrected during acquisition with using leucine-enkephalin as an external reference (Lock-SprayTM), which generates reference ions at m/z 554.2615 Da ([M–H]$^{-}$) in negative ESI mode. Data sets were processed with the use of MassLynx 4.1 software (Waters).

Flash chromatography system with UV and ELSD detectors (Reveleris, Fluka).

Preparative HPLC separation of **2** was performed using a binary Varian HPLC system, consisting of two PrepStar 210 pumps (equipped with 25 mL pump heads) and a ProStar 320 UV/VIS detector set at 275 nm. A silica gel column Pursuit XRs (10 µm, 250 × 21.2 mm) was eluted at a flow rate of 10 mL min^{-1}. Gradient-grade HPLC solvents from Baker, or ChemPur were used.
UV absorption measurements were performed in a 1 cm path-length cell, using a double beam spectrophotometer (CINTRA 10e, GBC, Dandenong, Australia), equipped with a silicon photo-diode detector.
The crystallographic data were acquired using Rigaku XtaLAB Synergy-S diffractometer equipped with a HyPix-6000HE Hybrid Photon Counting (HPC) detector and Cu microfocus sealed X-ray source as well as a low-temperature Oxford Cryostream 800 liquid nitrogen cooling system at 100 K.

Figure S1. Structures of the studied compounds:



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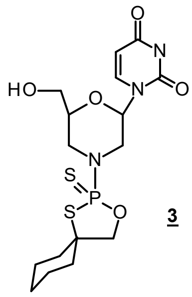

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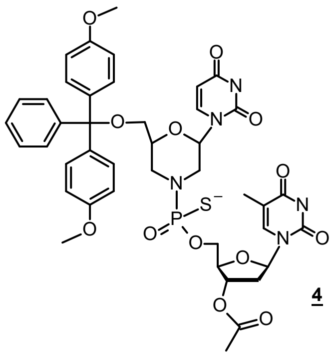

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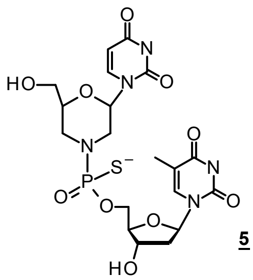

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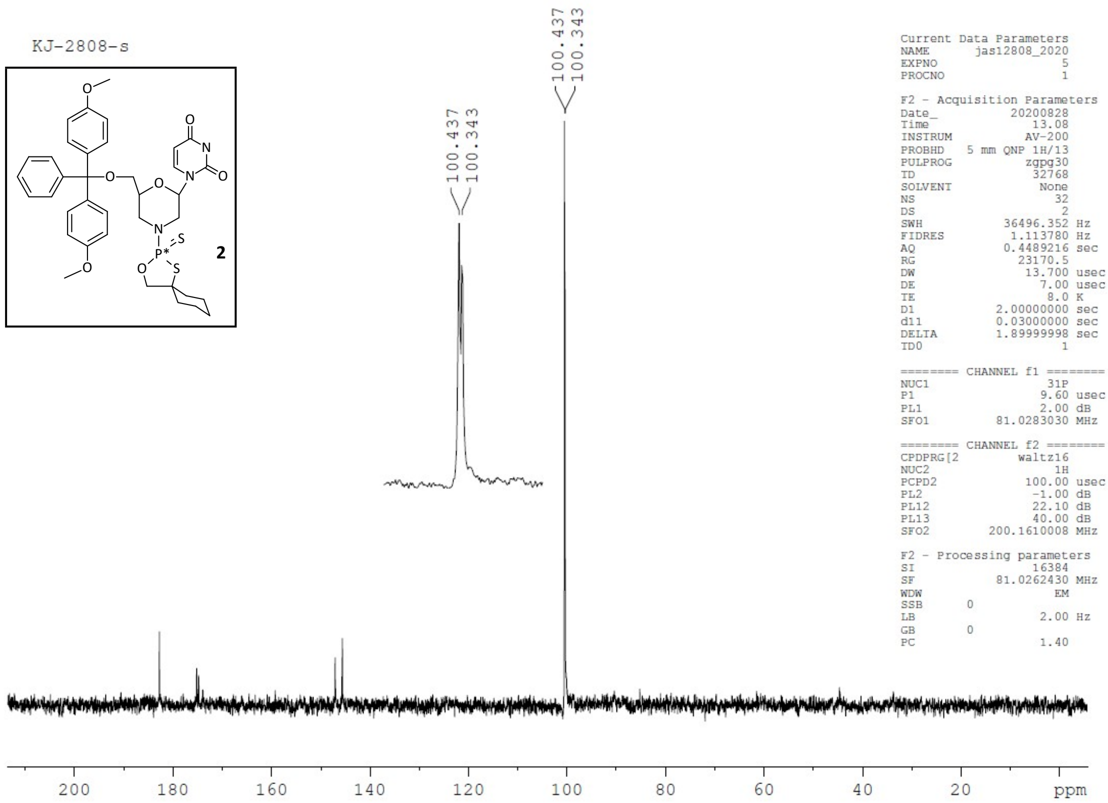


Figure S2. ^{31}P NMR spectrum of crude reaction mixture of *m*U-OTP formation (**2**) (analysis performed in non-deuterium solvent).

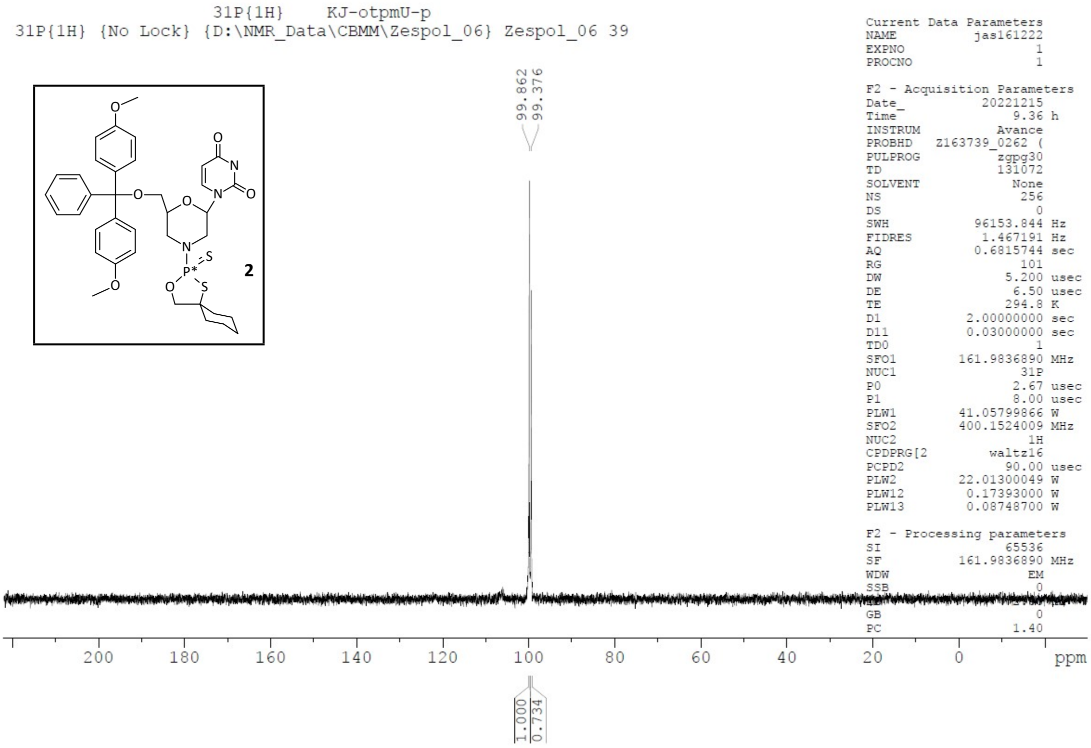


Figure S3. ^{31}P NMR spectrum of purified *m*U-OTP (**2**) (analysis performed in non-deuterium solvent).

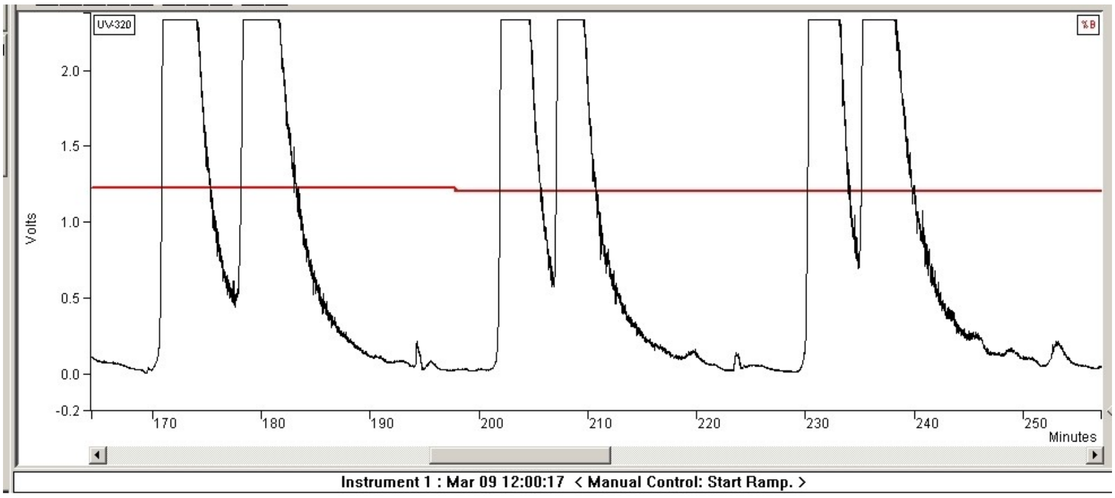


Figure S4. An HPLC sample profile for the semi-preparative separation of P-diastereomers of *m*U-OTP (**2**) using a Pursuit XRs 10 µm, 250×21,2 mm column; flow rate 10 mL min^{-1}), detector set at 275 nm, the column was loaded with ca. 100 mg of **2** (3 injections).

Figures S5-10. NMR spectra for separated P-diastereomers of *m*U-OTP

^{1}H NMR and ^{13}C NMR spectra recorded with a Bruker AV-500 spectrometer (500 MHz). ^{31}P NMR spectra recorded with a Bruker AV-200 spectrometer (200 MHz)

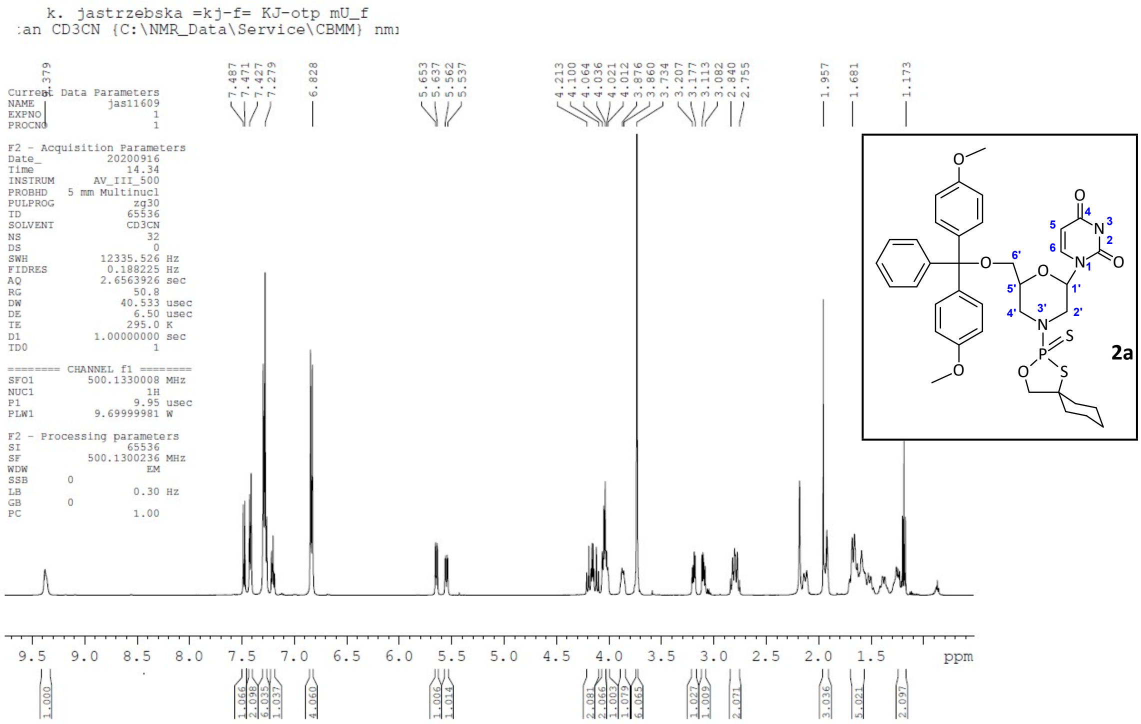


Figure S5. ^{1}H NMR *for fast*-eluting ***mU*-OTP** (**2a**); δ (ppm, CD_3CN)
9.38 (1H, N3-H), 7,49-7.47 (1H, C6-H), 7,43-6,83 (13H, DMT), 5,65-5.64 (1H, C1'-H), 5,56-5.54 (1H, C5'-H), 4,21-4.10 (2H, C2'-H), 4.06-4.03 (2H, C4'-H), 3.73 (6H, 2xOCH_3), 3.21-3.18 (2H, P-O-CH_2C-S), 2.84-2.75 (2H, 6'CH_2), 1.96-1.17 (10H, -$(CH_2)_5$-„*spiro*")

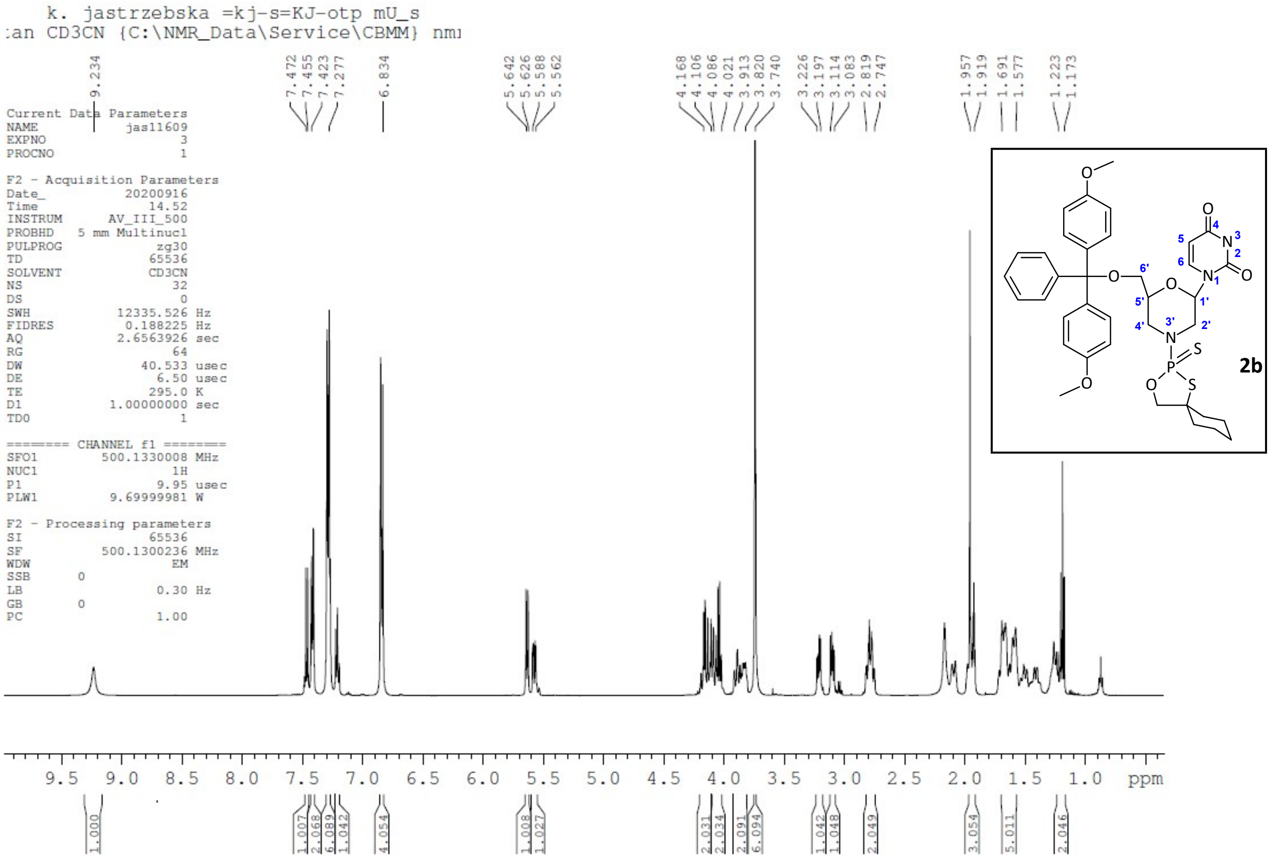


Figure S6. [1]H NMR *for slow*-eluting ***mU*-OTP** (**2b**); δ (ppm, CD_3CN)

9.23 (1H, N3-H), 7,47-7.46 (1H, C6-H), 7,42-6,83 (13H, DMT), 5,64-5.62 (1H, C1'-H), 5,59-5.56 (1H, C5'-H), 4,17-4.11 (2H, C2'-H), 4.09-4.02 (2H, C4'-H), 3.74 (6H, 2xOCH_3), 3.23-3.20 (2H, P-O-CH_2C-S), 2.82-2.75 (2H, 6'CH_2), 1.96-1.17 (10H, -$(CH_2)_5$-„*spiro*")

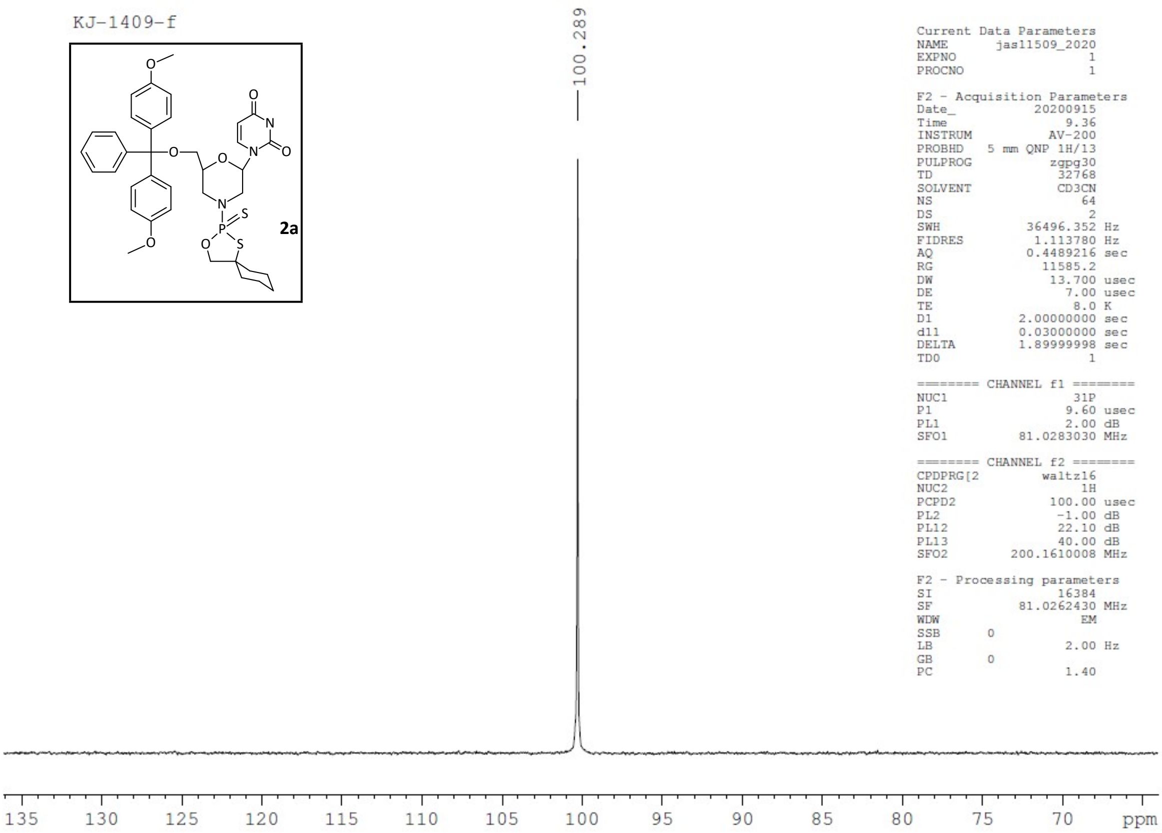


Figure S7. ^{31}P NMR *for fast*-eluting ***mU*-OTP** (**2a**); δ (ppm, CD_3CN)

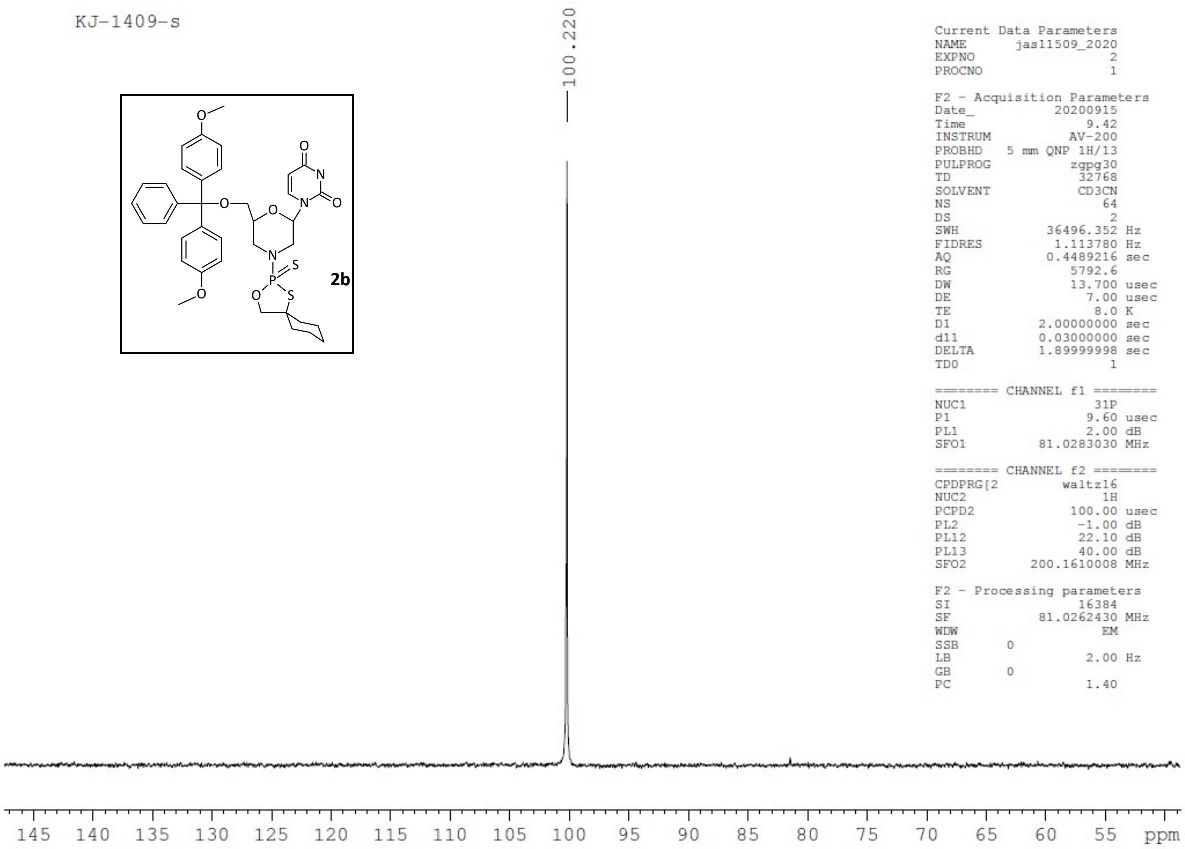


Figure S8. [31]P NMR *for slow*-eluting ***mU*-OTP** (**2b**); δ (ppm, CD_3CN)

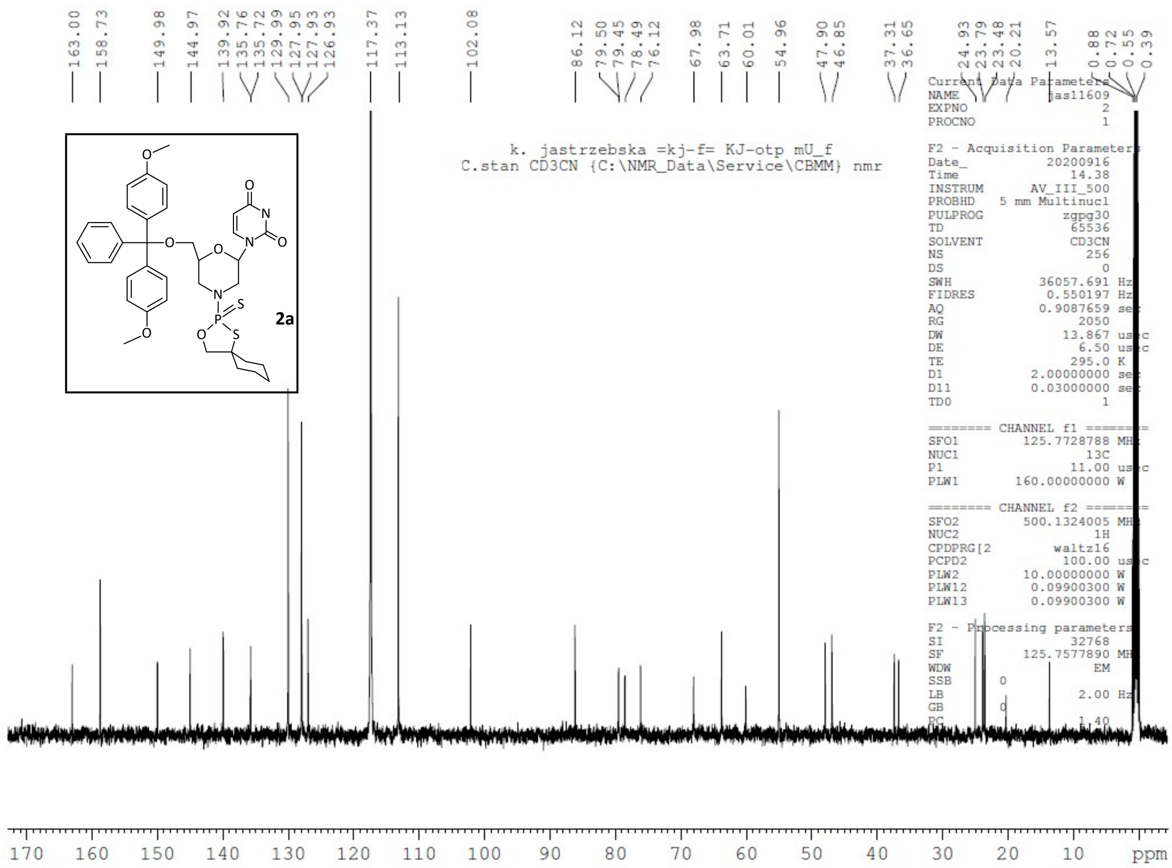


Figure S9. ^{13}C NMR *for fast*-eluting ***mU*-OTP** (**2a**); δ (ppm, CD_3CN)

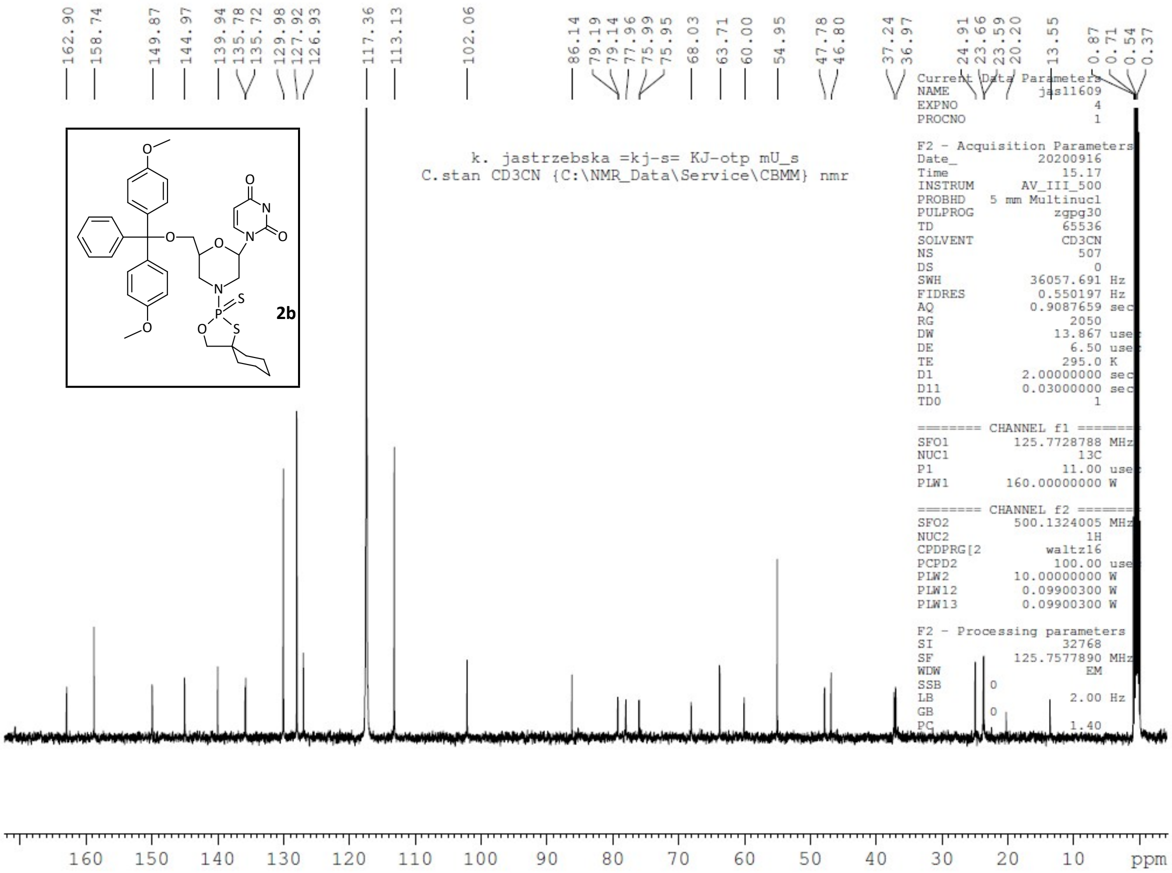


Figure S10. ^{13}C NMR *for slow*-eluting ***mU*-OTP** (**2b**); δ (ppm, CD_3CN)

Figures S11-12. HR MS spectra for separated P-diastereomers of *m*U-OTP.
HR MS spectra recorded with a SYNAPT G2-Si High Definition Mass Spectrometer (qTOF, Electro Spray Ionization; Waters)

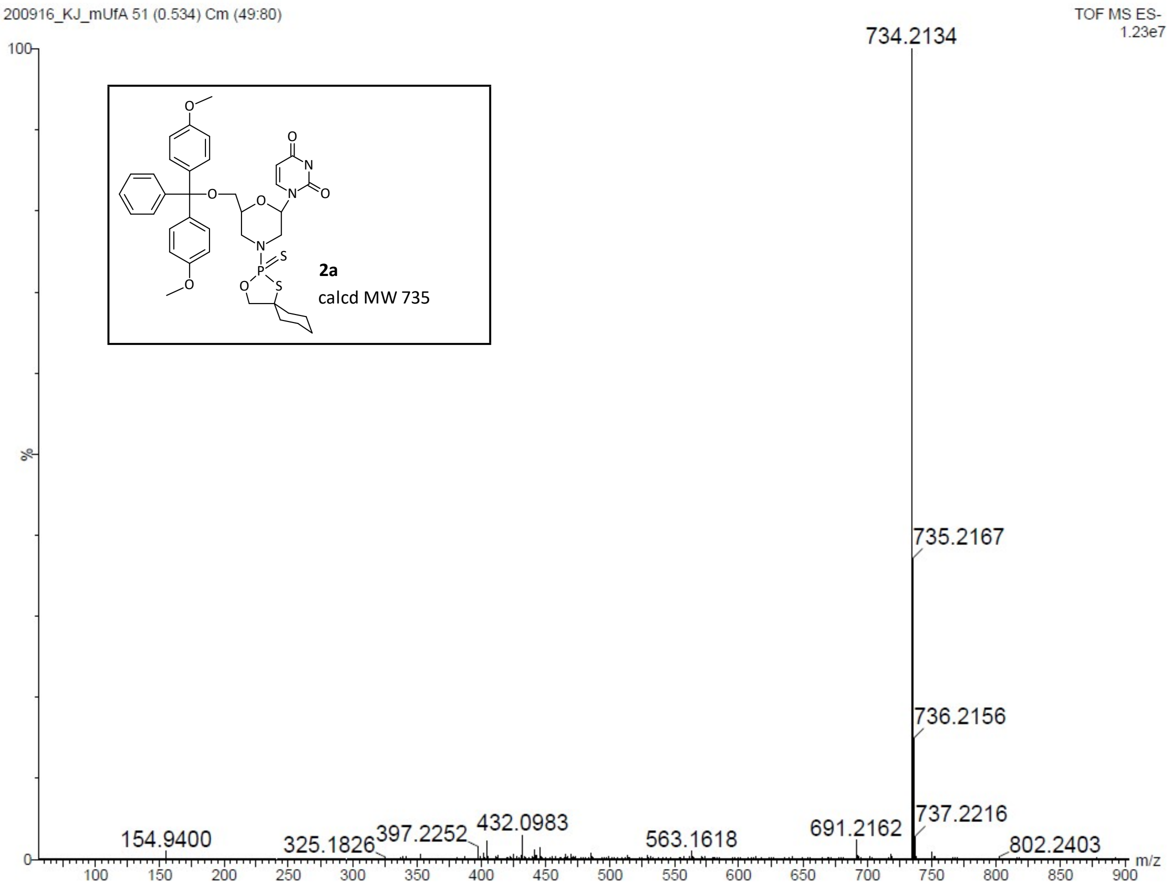


Figure S11. HR MS spectra for *fast*-eluting *m*U-OTP **2a**

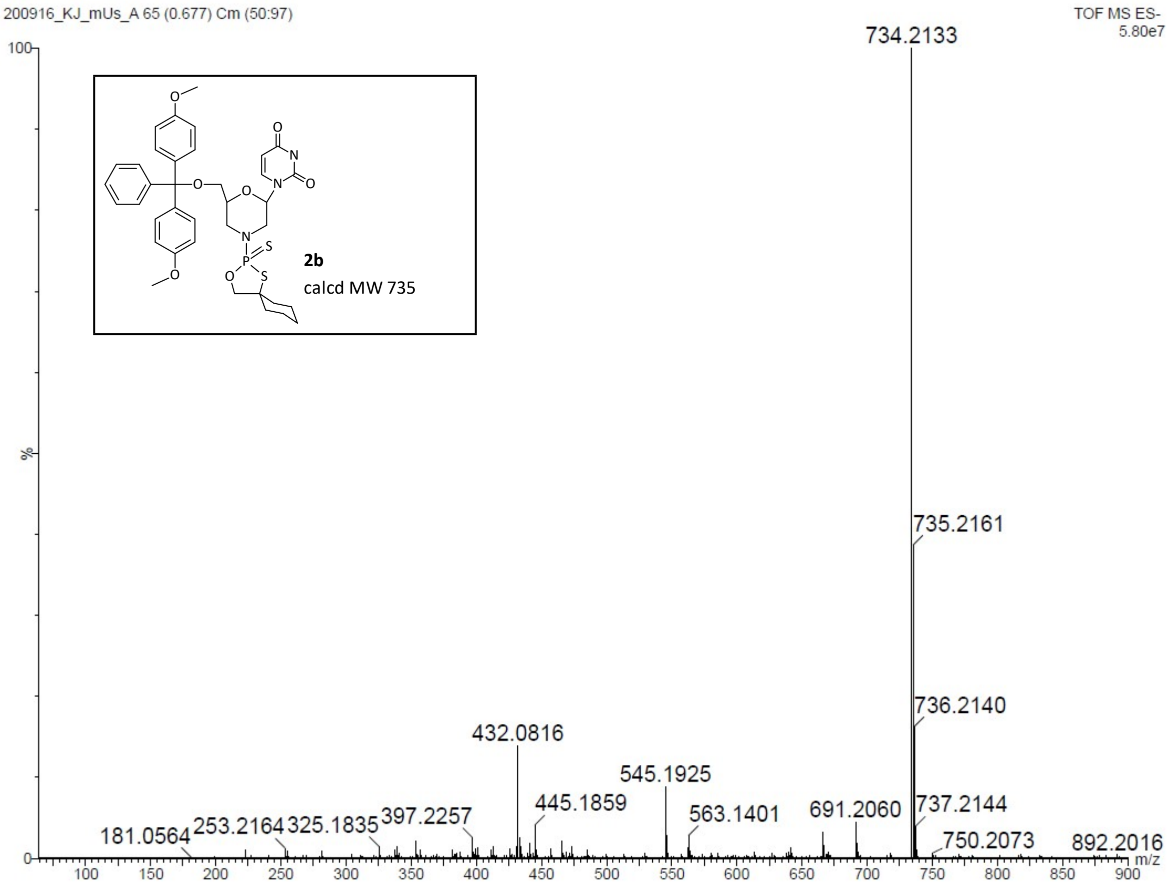
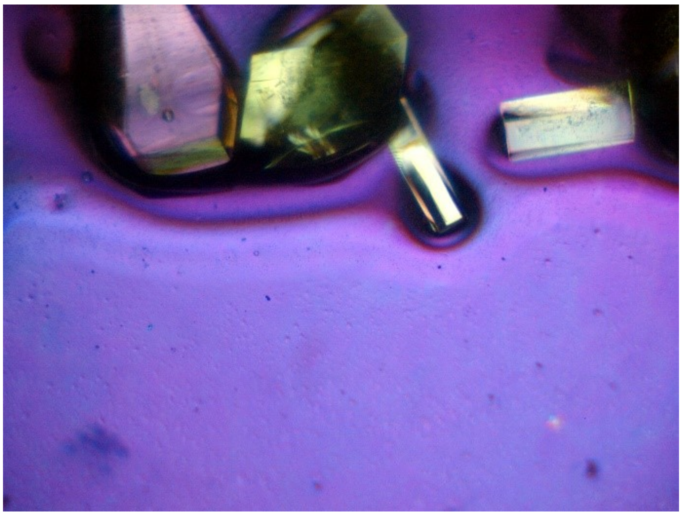


Figure S12. HR MS spectra for *slow*-eluting *m*U-OTP **2b**



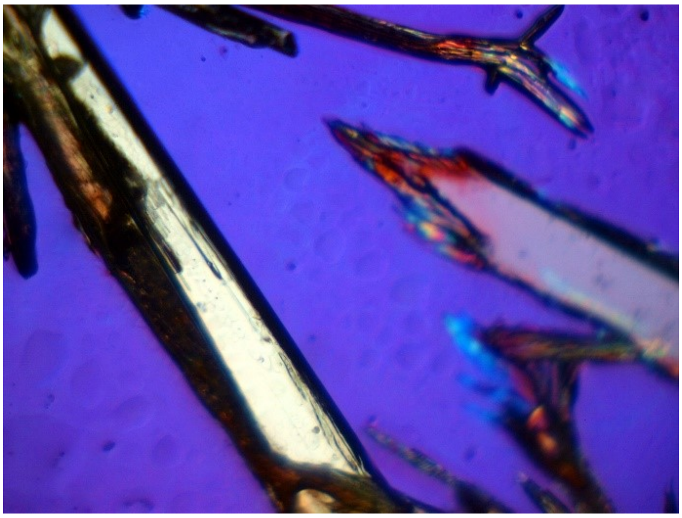


Figure S13. Crystals of **3a** (above) and **3b** (below)

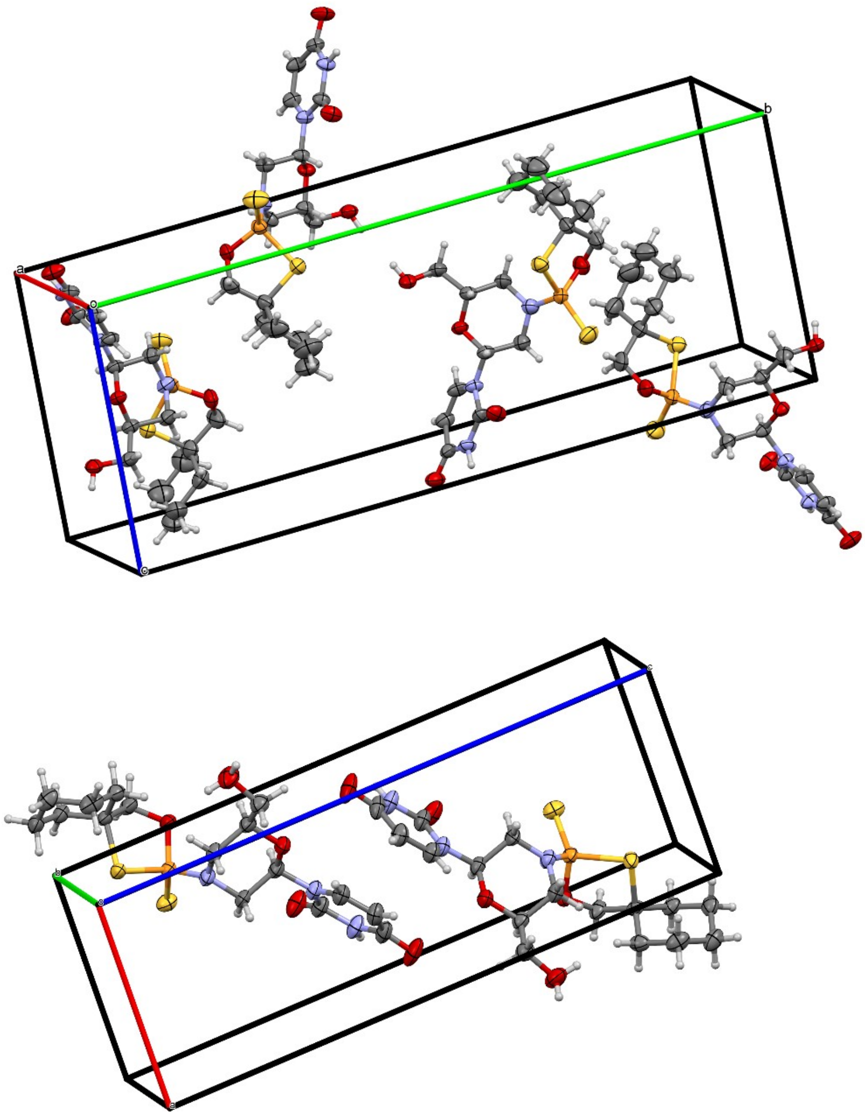


Figure S14. The crystal packing for **3a** (above) and **3b** (below)

Table S1. The data-collection, processing and refinement statistics for **3a** and **3b**

Compound	**3a**	**3b**
Crystal data		
CCDC	2079449	2079450
Chemical formula	$C_{16}H_{24}N_3O_5PS_2$	$C_{16}H_{24}N_3O_5PS_2$
Formula weight	433.47	433.47
Crystal system	monoclinic	monoclinic
Space group	$P2_1$	$P2_1$
Temperature (K)	293.88(10)	293.88(10)
a [Å]	6.80030(10)	7.4472(2)
b [Å]	28.6550(3)	6.4902(2)
c [Å]	10.60610(10)	20.4323(5)
β (°)	93.4520(10)	91.566(2)
V [Å^3]	2062.98(4)	987.20(5)
Z	4	2
d_{calc} [g/cm^3]	1.396	1.458
Crystal dimensions [mm]	0.57 × 0.57 × 0.28	0.67 × 0.15 × 0.10
Radiation type	CuK_α	CuK_α
Wavelength (Å)	1.54184	1.54184
μ [mm^{-1}]	3.357	3.508
Data collection		
Reflections measured	12241	10844
Range/indices (h, k, l)	-6, 8; -34, 35; -13, 13	-9, 9; -7, 7; -25, 24
θ (max, min) [°]	76.114, 3.084	76.662, 4.329
Total no. of unique data	6583	3894
No. of observed data, I > 2σ(I)	6454	3729
R_{int}	0.037	0.039
Refinement		
R [$F^2 > 2\sigma(F^2)$]	0.049	0.030
$wR(F^2)$	0.132	0.077
S	1.027	1.048
No. of reflections	6583	3894
No. of parameters	498	249
No. of restraints	1	1
H-atom treatment	H-atoms treated by a mixture of independent and constrained refinement	H-atoms treated by a mixture of independent and constrained refinement
$\Delta\rho$ (min, max), e/Å^3	-0.40, 0.50	-0.25, 0.19
Absolute structure parameter	-0.001(19)	-0.022(10)

Table S2.1. Fractional atomic coordinates (×10[4]) and isotropic or equivalent isotropic displacement parameters (Å^2×10^3) for **3a**. U_{eq} is defined as 1/3 of the trace of the orthogonalised U_{ij}.

Atom	*x*	*y*	*z*	U(eq)
P1A	6794.7(16)	3237.2(4)	10213.2(10)	41.0(3)
S1A	5612.8(18)	3557.7(4)	11771.0(10)	49.2(3)
S2A	4927(2)	3090.7(6)	8837.8(13)	68.8(4)
O1A	10437(4)	4396.3(11)	9392(3)	39.1(6)
O2A	6072(4)	4488.2(14)	6794(3)	49.5(8)
O3A	10256(6)	5032.2(17)	3981(3)	63.8(10)
O4A	7755(6)	2800.5(11)	10983(3)	56.8(9)
O5A	11399(5)	4965.6(12)	11500(3)	50.1(8)
N4A	8735(6)	3519.3(15)	9782(3)	45.6(8)
N7A	9363(5)	4415.2(13)	7292(3)	37.3(7)
N9A	8218(5)	4748.3(14)	5389(3)	40.3(7)
C2A	8969(5)	4219.3(15)	8526(3)	36.3(8)
C3A	8974(7)	3693.9(17)	8506(4)	44.2(9)
C5A	10199(7)	3715.8(17)	10710(4)	46.1(10)
C6A	10106(6)	4239.1(16)	10662(4)	40.0(8)
C8A	7744(6)	4545.4(15)	6511(4)	36.8(8)
C10A	10092(7)	4825.7(17)	4977(4)	42.1(9)
C11A	11657(6)	4651.2(19)	5802(4)	45.9(10)
C12A	11264(6)	4461.5(18)	6912(4)	42.2(9)
C13A	11663(7)	4474.9(18)	11516(4)	47.1(10)
C14A	6504(12)	2640.7(19)	11942(6)	70.0(16)
C15A	6018(8)	3038.1(18)	12811(5)	51.7(11)
C16A	7659(9)	3139(2)	13796(5)	60.8(13)
C17A	7095(12)	3515(3)	14725(6)	77.8(18)
C18A	5258(14)	3386(4)	15356(7)	96(2)
C19A	3566(13)	3294(4)	14402(7)	101(3)
C20A	4098(12)	2924(3)	13453(7)	84(2)
P1B	1200.2(17)	6759.2(4)	4655.3(10)	41.6(3)
S1B	-129.9(17)	6430.6(4)	3059.2(10)	48.1(3)
S2B	-524(2)	6881.6(6)	5991.2(13)	64.9(4)
O1B	5032(4)	5616.1(11)	5514(3)	40.3(6)
O2B	1247(4)	5535.8(14)	8173(3)	53.3(8)
O3B	6050(5)	4952.2(16)	10861(3)	59.2(9)
O4B	1972(6)	7203.5(12)	3929(4)	59.1(9)
O5B	5504(5)	5051.3(12)	3421(3)	47.4(7)
N4B	3237(6)	6494.3(15)	5130(4)	48.0(9)
N7B	4413(5)	5600.4(14)	7615(3)	38.7(7)
N9B	3697(5)	5270.9(15)	9535(3)	43.4(8)
C2B	3724(6)	5788.9(16)	6389(4)	38.0(8)
C3B	3708(7)	6318.6(18)	6408(4)	46.8(10)
C5B	4579(7)	6303.9(17)	4216(4)	47.1(10)
C6B	4461(6)	5772.0(17)	4257(4)	40.6(9)
C8B	2961(6)	5471.0(16)	8424(4)	39.2(8)
C10B	5637(6)	5173.3(17)	9887(4)	41.5(9)

Atom	*x*	*y*	*z*	U(eq)
C11B	7026(6)	5345.4(19)	9040(4)	45.3(10)
C12B	6374(6)	5542.5(18)	7951(4)	44.6(10)
C13B	5818(8)	5537.4(19)	3376(4)	50.0(11)
C14B	638(12)	7350.5(19)	2910(6)	71.2(17)
C15B	146(9)	6940.7(18)	2005(5)	55.5(12)
C16B	1788(10)	6861(2)	1132(5)	64.3(14)
C17B	1313(12)	6482(3)	169(6)	81.6(19)
C18B	-598(16)	6584(3)	-572(7)	99(3)
C19B	-2275(14)	6647(4)	284(8)	99(3)
C20B	-1795(13)	7033(3)	1261(8)	93(2)
H2A	7678.1	4328.4	8766.87	44
H3AA	7906.33	3581.2	7938.48	53
H3AB	10206.25	3581.6	8202.69	53
H5A	10988.39	5050.53	12173.54	75
H5AA	11507.36	3611.5	10522.9	55
H5AB	9934.91	3608.28	11549.91	55
H6A	8800.62	4342.19	10890.79	48
H9A	7245.65	4836.75	4889.88	48
H11A	12950.62	4669.34	5568.17	55
H12A	12305.47	4357.01	7447.07	51
H13A	11586.56	4360.08	12371.88	56
H16A	8817.16	3239.83	13379.61	73
H14A	7164.17	2393.97	12429.46	84
H17A	8165.08	3557.15	15361.3	93
H18A	4906.54	3638.26	15910.39	115
H19A	3217.06	3581.38	13958.15	122
H20A	3028.39	2895.01	12809.85	101
H2B	2391.89	5673.61	6165.52	46
H3BA	2733.6	6428.34	6970.22	56
H3BB	4987.57	6434.09	6717.87	56
H5B	5864.21	4930.94	2772.77	71
H5BA	5917.65	6405.37	4433.21	57
H5BB	4197.3	6415.11	3373.09	57
H6B	3102.57	5674.21	4038.44	49
H9B	2848.04	5198.37	10070.79	52
H11B	8370.76	5320.57	9245.01	54
H12B	7292.51	5644.79	7397.15	54
H13B	12958.16	4399.43	11237.34	56
H14B	5296.49	2514.07	11545.96	84
H16B	7993.03	2854.87	14256.83	73
H17B	6890.91	3807.86	14279.89	93
H18B	5502.7	3109.7	15868.24	115
H19B	2428.73	3191.75	14837.04	122
H20B	4245.81	2625.78	13880.91	101
H13C	5540(80)	5660(20)	2500(50)	47(14)

Atom	*x*	*y*	*z*	U(eq)
H14C	1228.18	7602.02	2451.63	85
H16C	2976.85	6776.3	1630.11	77
H18C	-904.39	6328.3	-1151.47	119
H20C	-1724.69	7330.43	831.5	111
H13D	7200(100)	5550(20)	3710(60)	62(17)
H14D	-563.57	7467.1	3245.46	85
H16D	2044.79	7149.63	693.52	77
H18D	-453.23	6864.77	-1067.54	119
H20D	-2845.03	7050.88	1838.97	111

Table S2.2. Fractional atomic coordinates (×10^4) and isotropic or equivalent isotropic displacement parameters (Å^2×10^3) for **3b**. U_{eq} is defined as 1/3 of the trace of the orthogonalised U_{ij}.

Atom	*x*	*y*	*z*	U(eq)
P1	7863.7(9)	6621.3(11)	8087.3(3)	34.05(18)
S1	8952.6(9)	5417.0(11)	8960.0(3)	39.01(19)
S2	6049.3(10)	8703.0(13)	8190.3(4)	47.4(2)
O1	7487(3)	3247(4)	6325.3(10)	44.2(5)
O2	3805(4)	7840(4)	6192.7(14)	62.5(7)
O3	395(4)	3916(5)	4814.4(13)	69.2(8)
O4	9728(3)	7376(4)	7794.5(10)	41.5(5)
O5	11763(3)	1313(5)	6904.6(14)	63.1(7)
N4	7232(3)	4708(4)	7612.6(12)	41.2(6)
N7	4737(3)	4551(4)	6005.5(12)	39.9(6)
N9	2154(4)	5826(4)	5489.1(14)	50.6(7)
C2	6261(4)	4825(5)	6472.9(14)	38.2(6)
C3	5644(4)	4697(5)	7174.0(14)	39.4(7)
C5	8458(4)	3020(5)	7460.4(15)	41.7(7)
C6	9048(4)	3171(5)	6759.9(15)	39.8(7)
C8	3578(5)	6195(5)	5918.2(15)	44.9(7)
C10	1764(5)	4011(6)	5164.2(16)	49.6(8)
C11	3023(5)	2387(5)	5284.9(17)	50.4(8)
C12	4412(5)	2685(5)	5702.6(16)	45.2(7)
C13	10080(4)	1302(6)	6556.5(16)	48.3(8)
C14	11003(4)	8060(5)	8292.9(15)	42.8(7)
C15	11268(4)	6390(5)	8813.0(13)	34.8(6)
C16	12493(4)	4632(5)	8592.7(16)	43.7(7)
C17	12807(5)	3033(5)	9123.4(19)	53.5(8)
C18	13584(5)	4009(5)	9747.3(19)	52.3(8)
C19	12415(4)	5730(5)	9976.0(15)	45.5(7)
C20	12056(4)	7330(5)	9443.9(15)	39.5(7)
H2	6821.68	6169.26	6400.23	46
H3A	4878.51	5861.62	7269.73	47
H3B	4961.11	3442.08	7235.67	47
H5	12310(70)	240(90)	6770(30)	87(16)
H5A	7860.32	1710.87	7526.08	50
H5B	9502.61	3075.59	7754.06	50

Atom	*x*	*y*	*z*	U(eq)
H6	9779.4	4410.67	6703.94	48
H9	1429.22	6837.73	5416.16	61
H11	2877.68	1126.05	5073.75	60
H12	5187.88	1592.27	5792.21	54
H13A	9416.47	61.86	6657.87	58
H13B	10267.24	1334.81	6088.65	58
H14A	10566.68	9310.02	8494.78	51
H14B	12142.28	8366.88	8095.82	51
H16A	11951.87	3971.94	8209.75	52
H16B	13639.19	5200.62	8468.88	52
H17A	13629.32	1992.24	8969.71	64
H17B	11679.1	2362.15	9217.49	64
H18A	13691.24	2970.31	10087.36	63
H18B	14775.85	4536.61	9666.33	63
H19A	12993.47	6392.77	10351.55	55
H19B	11280.86	5167.4	10114.77	55
H20A	13172.17	8024.39	9348.02	47
H20B	11228.56	8354.22	9604.72	47

Table S3.1. Anisotropic displacement parameters ($\times10^4$) for **3a**. The anisotropic displacement factor exponent takes the form: $-2p^2[h^2a^{*2} \times U_{11}+ ... +2hka^* \times b^* \times U_{12}]$

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
P1A	51.2(6)	34.7(5)	37.0(5)	1.9(4)	1.4(4)	-5.5(4)
P1B	51.3(6)	34.3(5)	39.8(5)	-0.6(4)	7.6(4)	2.4(4)
S1B	57.6(6)	43.1(5)	43.9(5)	1.3(4)	6.0(4)	-8.4(5)
S1A	57.5(6)	47.3(6)	42.8(5)	3.8(4)	4.2(4)	11.1(5)
S2B	63.5(7)	81.4(10)	51.1(7)	-10.9(6)	13.9(5)	16.8(7)
S2A	71.1(8)	85.5(10)	48.7(6)	-3.9(6)	-5.5(5)	-33.1(7)
O1A	39.5(14)	48.3(16)	28.9(12)	3.6(12)	-2.9(10)	-3.6(12)
O2A	28.5(13)	72(2)	47.8(16)	17.5(15)	-0.2(11)	-4.4(14)
O1B	43.0(14)	48.4(16)	30.3(13)	2.7(12)	9.4(11)	2.3(12)
O5A	71(2)	48.4(17)	32.0(14)	0.2(13)	12.2(14)	-8.7(15)
O5B	63.3(19)	49.1(17)	29.5(13)	0.3(12)	0.8(13)	2.0(14)
O2B	33.4(14)	73(2)	54.7(18)	19.1(16)	13.3(12)	8.3(14)
O4A	91(3)	30.2(15)	50.4(19)	4.6(13)	12.4(17)	10.3(16)
O4B	89(3)	30.6(15)	57(2)	0.4(14)	2.6(18)	-7.5(16)
N7A	32.2(16)	51(2)	29.1(15)	4.4(14)	1.3(12)	-0.5(14)
O3A	60(2)	93(3)	40.3(17)	20.4(18)	14.3(15)	-3(2)
N9B	39.5(17)	56(2)	36.6(17)	11.5(16)	16.1(13)	4.5(16)
O3B	60(2)	81(3)	37.8(16)	11.6(17)	9.3(14)	20.0(18)
N4A	49.2(19)	50(2)	37.3(17)	9.0(16)	-4.0(14)	-8.2(16)
N7B	36.1(16)	49.7(19)	31.3(16)	2.1(14)	9.6(13)	-3.2(14)
C8A	32.2(18)	45(2)	32.6(18)	5.5(16)	-2.0(14)	-3.4(15)
N4B	51(2)	52(2)	42.3(19)	8.2(17)	10.0(15)	5.6(17)
N9A	33.8(16)	54(2)	33.0(16)	8.5(15)	-2.3(12)	-1.9(14)
C6A	41(2)	48(2)	31.5(18)	5.5(17)	0.1(14)	0.3(17)

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C8B	34.8(19)	46(2)	38.7(19)	4.9(17)	13.3(15)	2.0(16)
C12A	32.6(18)	59(3)	34.9(19)	-1.6(18)	-0.7(15)	1.5(18)
C10B	44(2)	50(2)	31.4(18)	-4.1(17)	6.5(15)	6.9(18)
C10A	43(2)	52(2)	31.1(18)	0.1(17)	7.3(15)	-2.3(18)
C11A	31.0(18)	67(3)	40(2)	-2(2)	7.9(15)	-3.1(19)
C3B	51(2)	51(3)	39(2)	0.3(18)	4.5(17)	3.0(19)
C3A	47(2)	50(2)	35(2)	2.3(18)	3.4(17)	-6.0(18)
C6B	41(2)	50(2)	31.6(18)	4.5(17)	6.6(15)	-0.4(17)
C13A	53(2)	51(2)	36(2)	4.9(18)	-7.3(18)	-6(2)
C11B	35.6(19)	64(3)	36(2)	-4.0(19)	1.2(15)	2.6(18)
C2A	30.1(17)	50(2)	28.7(17)	5.7(16)	-1.3(13)	-1.6(16)
C5A	42(2)	50(2)	45(2)	10.5(19)	-6.1(17)	-3.7(18)
C12B	35.5(19)	66(3)	32.8(18)	-4.3(19)	7.8(15)	-10.6(19)
C2B	34.8(18)	49(2)	31.2(18)	0.6(16)	6.7(14)	-1.8(16)
C15A	70(3)	43(2)	43(2)	7.8(19)	4(2)	-5(2)
C16A	80(3)	55(3)	47(2)	10(2)	-9(2)	0(3)
C5B	53(2)	47(2)	42(2)	9.8(18)	14.3(18)	5.3(19)
C13B	60(3)	56(3)	36(2)	8(2)	14.1(19)	7(2)
C15B	84(3)	38(2)	45(2)	4.1(19)	1(2)	8(2)
C14A	120(5)	36(2)	55(3)	9(2)	9(3)	-14(3)
C16B	87(4)	54(3)	53(3)	12(2)	14(3)	-9(3)
C14B	120(5)	34(2)	58(3)	4(2)	-4(3)	10(3)
C17A	104(5)	74(4)	53(3)	-6(3)	-12(3)	0(4)
C17B	111(5)	77(4)	59(3)	-7(3)	27(3)	-10(4)
C19B	105(6)	109(7)	79(5)	7(4)	-34(4)	-2(5)
C20A	91(5)	98(5)	64(4)	9(4)	14(3)	-40(4)
C18B	150(8)	91(6)	55(4)	0(3)	-7(4)	-27(5)
C20B	107(6)	89(5)	80(5)	14(4)	-14(4)	35(4)
C18A	132(7)	104(6)	52(3)	-7(3)	10(4)	14(5)
C19A	99(5)	135(8)	74(4)	-4(5)	34(4)	-8(5)

Table S3.2.. Anisotropic displacement parameters (×10^4) for **3b**. The anisotropic displacement factor exponent takes the form: *-2p^2[h^{2}a*^{2} × U$_{11}$+ ... +2hka* × b* × U$_{12}$]*

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
P1	27.7(3)	42.0(4)	32.3(3)	2.1(3)	-3.6(2)	-4.0(3)
S1	31.0(3)	54.0(4)	31.8(3)	6.3(3)	-3.5(2)	-8.6(3)
S2	35.2(4)	47.3(4)	59.5(5)	-1.2(4)	-1.1(3)	1.2(3)
O4	31.3(10)	57.4(13)	35.6(10)	8.7(9)	-3.1(8)	-9.1(9)
O1	37.4(11)	59.9(15)	34.9(11)	-2.4(10)	-6.2(8)	4.4(10)
O2	60.9(16)	48.7(14)	75.9(17)	-9.1(13)	-31.0(13)	7.6(12)
O5	44.3(14)	73.7(18)	70.5(16)	-10.7(15)	-11.6(12)	16.8(14)
N7	38.7(13)	44.2(13)	36.3(13)	2.1(10)	-11.4(10)	-2.4(11)
O3	70.8(17)	64.7(16)	69.7(18)	15.1(14)	-44.1(14)	-15.1(14)

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
N4	35.5(13)	50.4(14)	37.0(13)	-3.0(11)	-10.2(10)	0.8(11)
N9	50.1(16)	48.4(16)	52.0(16)	6.3(12)	-24.7(12)	0.6(12)
C20	32.9(15)	40.2(16)	44.8(16)	-6.7(12)	-8.6(12)	-0.3(12)
C15	26.8(13)	39.1(15)	38.1(14)	0.0(12)	-3.3(10)	-4.4(12)
C8	43.6(17)	45.9(18)	44.3(16)	5.0(14)	-14.8(13)	-2.4(14)
C6	31.6(14)	45.8(17)	41.7(16)	1.9(12)	-6.2(12)	-2.4(12)
C14	32.4(15)	47.9(17)	47.7(17)	10.4(14)	-8.4(12)	-8.9(13)
C16	34.4(15)	48.7(17)	48.0(18)	-9.5(14)	2.5(12)	-2.3(14)
C11	56(2)	49.6(18)	44.5(17)	-4.4(14)	-11.7(15)	-4.2(16)
C12	45.8(17)	46.2(17)	43.1(17)	-1.3(14)	-8.5(13)	0.0(15)
C2	33.0(14)	43.5(16)	37.5(15)	2.5(12)	-9.6(11)	-1.1(12)
C19	41.0(16)	54.8(19)	40.1(15)	0.5(14)	-9.1(12)	-6.4(14)
C13	41.2(17)	54.9(19)	48.6(17)	-5.2(15)	-3.5(13)	3.8(15)
C3	28.3(13)	53.8(17)	35.6(14)	-2.8(13)	-8.3(11)	-4.8(13)
C10	52.7(18)	54.2(19)	40.8(17)	10.9(14)	-19.1(14)	-13.2(16)
C5	41.8(16)	42.6(16)	40.1(16)	0.4(13)	-11.3(12)	1.0(13)
C17	45.6(18)	37.3(17)	77(2)	-1.1(16)	-3.5(16)	2.4(15)
C18	48.1(19)	43.6(18)	64(2)	11.9(16)	-14.8(16)	0.8(15)

Table S4.1. Bond lengths (Å) for **3a**.

Atom — Atom	Length	Atom — Atom	Length
P1A — S1A	2.0927(16)	N7B — C12B	1.370(6)
P1A — S2A	1.9219(16)	N7B — C2B	1.460(5)
P1A — O4A	1.611(3)	C8A — N9A	1.379(5)
P1A — N4A	1.636(4)	N4B — C3B	1.463(6)
P1B — S1B	2.0943(16)	N4B — C5B	1.475(6)
P1B — S2B	1.9247(16)	N9A — C10A	1.390(6)
P1B — O4B	1.593(4)	C6A — C13A	1.511(6)
P1B — N4B	1.632(4)	C6A — C 5A	1.502(6)
S1B — C15B	1.856(5)	C12A — C11A	1.338(6)
S1A — C15A	1.864(5)	C10B — C11B	1.429(6)
O1A — C6A	1.450(5)	C10A — C11A	1.427(7)
O1A — C2A	1.410(5)	C3B — C2B	1.518(7)
O2A — C8A	1.205(5)	C3A — C2A	1.506(6)
O1B — C6B	1.437(5)	C6B — C5B	1.527(7)
O1B — C2B	1.413(5)	C6B — C13B	1.510(6)
O5A — C13A	1.417(6)	C11B — C12B	1.337(7)
O5B — C13B	1.411(6)	C15A — C16A	1.510(8)
O2B — C8B	1.194(5)	C15A — C14A	1.514(8)
O4A — C14A	1.439(7)	C15A — C20A	1.543(9)
O4B — C14B	1.431(7)	C16A — C17A	1.523(9)
N7A — C8A	1.389(5)	C15B — C16B	1.510(8)
N7A — C12A	1.383(5)	C15B — C14B	1.540(8)
N7A — C2A	1.463(5)	C15B — C20B	1.520(9)
O3A — C10A	1.222(6)	C16B — C17B	1.512(9)

Atom — Atom	Length	Atom — Atom	Length
N9B — C8B	1.377(6)	C17A — C18A	1.498(12)
N9B — C10B	1.378(6)	C17B — C18B	1.506(13)
O3B — C10B	1.230(6)	C19B — C18B	1.511(14)
N4A — C3A	1.462(5)	C19B — C20B	1.536(13)
N4A — C5A	1.469(6)	C20A — C19A	1.521(13)
N7B — C8B	1.397(5)	C18A — C19A	1.509(13)

Table S5.2. Bond lengths (Å) for **3b**.

Atom — Atom	Length	Atom — Atom	Length
P1 — S1	2.0901(9)	N4 — C5	1.465(4)
P1 — S2	1.9264(11)	N9 — C8	1.379(4)
P1 — O4	1.604(2)	N9 — C10	1.379(5)
P1 — N4	1.637(3)	C20 — C15	1.529(4)
S1 — C15	1.869(3)	C20 — C19	1.522(4)
O4 — C14	1.443(4)	C15 — C14	1.527(4)
O1 — C6	1.444(3)	C15 — C16	1.536(4)
O1 — C2	1.410(4)	C6 — C13	1.501(5)
O2 — C8	1.216(4)	C6 — C5	1.512(4)
O5 — C13	1.424(4)	C16 — C17	1.514(5)
N7 — C8	1.381(4)	C11 — C12	1.337(4)
N7 — C12	1.379(4)	C11 — C10	1.428(5)
N7 — C2	1.474(3)	C2 — C3	1.519(4)
O3 — C10	1.231(4)	C19 — C18	1.498(5)
N4 — C3	1.465(3)	C17 — C18	1.523(5)

Table S5.1. Bond angles (°) for **3a**.

Atom — Atom — Atom	Angle	Atom — Atom — Atom	Angle
S2A — P1A — S1A	115.31(8)	O3A — C10A — N9A	118.8(4)
O4A — P1A — S1A	95.96(14)	O3A — C10A — C11A	126.6(4)
O4A — P1A — S2A	116.38(16)	N9A — C10A — C11A	114.6(4)
O4A — P1A — N4A	102.5(2)	C12A — C11A — C10A	120.0(4)
N4A — P1A — S1A	111.01(16)	N4B — C3B — C2B	109.5(4)
N4A — P1A — S2A	113.76(14)	N4A — C3A — C2A	109.2(4)
S2B — P1B — S1B	115.11(8)	O1B — C6B — C5B	109.0(4)
O4B — P1B — S1B	96.16(15)	O1B — C6B — C13B	107.1(4)
O4B — P1B — S2B	116.32(17)	C13B — C6B — C5B	113.1(4)
O4B — P1B — N4B	102.9(2)	O5A — C13A — C6A	110.7(4)
N4B — P1B — S1B	110.95(16)	C12B — C11B — C10B	119.4(4)
N4B — P1B — S2B	113.62(15)	O1A — C2A — N7A	106.5(3)
C15B — S1B — P1B	94.48(17)	O1A — C2A — C3A	111.5(3)
C15A — S1A — P1A	93.81(17)	N7A — C2A — C3A	111.7(3)
C2A — O1A — C6A	110.5(3)	N4A — C5A — C6A	109.6(4)
C2B — O1B — C6B	110.8(3)	C11B — C12B — N7B	122.8(4)
C14A — O4A — P1A	111.5(4)	O1B — C2B — N7B	105.9(3)
C14B — O4B — P1B	112.8(4)	O1B — C2B — C3B	111.4(3)

Atom — Atom — Atom	Angle	Atom — Atom — Atom	Angle
C8A — N7A — C2A	117.1(3)	N7B — C2B — C3B	111.1(4)
C12A — N7A — C8A	121.4(3)	C16A — C15A — S1A	109.4(4)
C12A — N7A — C2A	121.5(3)	C16A — C15A — C14A	112.7(5)
C8B — N9B — C10B	127.6(3)	C16A — C15A — C20A	110.2(5)
C3A — N4A — P1A	124.4(3)	C14A — C15A — S1A	105.7(3)
C3A — N4A — C5A	112.4(4)	C14A — C15A — C20A	109.3(5)
C5A — N4A — P1A	121.8(3)	C20A — C15A — S1A	109.4(5)
C8B — N7B — C2B	116.4(3)	C15A — C16A — C17A	112.3(5)
C12B — N7B — C8B	121.4(4)	N4B — C5B — C6B	108.4(4)
C12B — N7B — C2B	122.1(3)	O5B — C13B — C6B	108.6(4)
O2A — C8A — N7A	122.7(4)	C16B — C15B — S1B	110.8(4)
O2A — C8A — N9A	123.1(4)	C16B — C15B — C14B	110.9(5)
N9A — C8A — N7A	114.2(3)	C16B — C15B — C20B	111.0(5)
C3B — N4B — P1B	125.5(3)	C14B — C15B — S1B	104.5(4)
C3B — N4B — C5B	111.8(4)	C20B — C15B — S1B	109.4(5)
C5B — N4B — P1B	121.1(3)	C20B — C15B — C14B	109.9(5)
C8A — N9A — C10A	127.2(3)	O4A — C14A — C15A	110.5(4)
O1A — C6A — C13A	106.0(3)	C15B — C16B — C17B	112.8(5)
O1A — C6A — C5A	109.4(4)	O4B — C14B — C15B	110.5(4)
C5A — C6A — C13A	113.6(4)	C18A — C17A — C16A	111.4(7)
O2B — C8B — N9B	123.8(4)	C18B — C17B — C16B	110.9(7)
O2B — C8B — N7B	122.5(4)	C18B — C19B — C20B	110.8(8)
N9B — C8B — N7B	113.7(3)	C19A — C20A — C15A	112.6(6)
C11A — C12A — N7A	122.3(4)	C17B — C18B — C19B	111.6(6)
N9B — C10B — C11B	114.7(4)	C15B — C20B — C19B	111.6(6)
O3B — C10B — N9B	119.9(4)	C17A — C18A — C19A	111.5(6)
O3B — C10B — C11B	125.4(4)	C18A — C19A — C20A	111.3(8)

Table S5.2. Bond angles (°) for **3b**.

Atom — Atom — Atom	Angle	Atom — Atom — Atom	Angle
S2 — P1 — S1	115.22(5)	O2 — C8 — N7	122.7(3)
O4 — P1 — S1	96.47(8)	O2 — C8 — N9	122.7(3)
O4 — P1 — S2	116.34(10)	N9 — C8 — N7	114.5(3)
O4 — P1 — N4	104.38(13)	O1 — C6 — C13	105.5(2)
N4 — P1 — S1	108.68(10)	O1 — C6 — C5	109.5(2)
N4 — P1 — S2	113.95(10)	C13 — C6 — C5	112.0(3)
C15 — S1 — P1	94.29(9)	O4 — C14 — C15	110.1(2)
C14 — O4 — P1	112.97(18)	C17 — C16 — C15	112.4(3)
C2 — O1 — C6	114.1(2)	C12 — C11 — C10	119.7(3)
C8 — N7 — C2	117.2(3)	C11 — C12 — N7	122.5(3)
C12 — N7 — C8	121.3(2)	O1 — C2 — N7	105.4(2)
C12 — N7 — C2	121.3(3)	O1 — C2 — C3	112.1(2)
C3 — N4 — P1	125.5(2)	N7 — C2 — C3	110.9(2)
C3 — N4 — C5	111.4(2)	C18 — C19 — C20	112.2(3)
C5 — N4 — P1	121.5(2)	O5 — C13 — C6	107.9(3)

Atom — Atom — Atom	Angle	Atom — Atom — Atom	Angle
C8 — N9 — C10	127.0(3)	N4 — C3 — C2	108.5(2)
C19 — C20 — C15	112.7(3)	O3 — C10 — N9	118.8(3)
C20 — C15 — S1	109.5(2)	O3 — C10 — C11	126.4(4)
C20 — C15 — C16	109.0(2)	N9 — C10 — C11	114.8(3)
C14 — C15 — S1	104.42(18)	N4 — C5 — C6	110.6(2)
C14 — C15 — C20	110.1(2)	C16 — C17 — C18	111.2(3)
C14 — C15 — C16	113.0(3)	C19 — C18 — C17	111.1(3)
C16 — C15 — S1	110.7(2)		

Table S6.1. Torsion angles (°) for **3a**.

Atom — Atom — Atom — Atom	Angle	Atom — Atom — Atom — Atom	Angle
P1A — S1A — C15A — C16A	109.0(4)	C6A — O1A — C2A — C3A	-60.8(4)
P1A — S1A — C15A — C14A	-12.7(4)	C8B — N9B — C10B — O3B	-173.0(5)
P1A — S1A — C15A — C20A	-130.3(4)	C8B — N9B — C10B — C11B	6.7(7)
P1A — O4A — C14A — C15A	-54.9(6)	C8B — N7B — C12B — C11B	2.5(7)
P1A — N4A — C3A — C2A	112.8(4)	C8B — N7B — C2B — O1B	-142.7(4)
P1A — N4A — C5A — C6A	-111.5(4)	C8B — N7B — C2B — C3B	96.2(4)
P1B — S1B — C15B — C16B	104.7(4)	C12A — N7A — C8A — O2A	176.4(5)
P1B — S1B — C15B — C14B	-14.8(4)	C12A — N7A — C8A — N9A	-4.1(6)
P1B — S1B — C15B — C20B	-132.5(4)	C12A — N7A — C2A — O1A	39.6(5)
P1B — O4B — C14B — C15B	-53.9(7)	C12A — N7A — C2A — C3A	-82.4(5)
P1B — N4B — C3B — C2B	110.9(4)	C10B — N9B — C8B — O2B	178.3(5)
P1B — N4B — C5B — C6B	-109.1(4)	C10B — N9B — C8B — N7B	-2.7(7)
S1B — P1B — O4B — C14B	35.9(4)	C10B — C11B — C12B — N7B	1.9(8)
S1B — P1B — N4B — C3B	-125.9(4)	C3B — N4B — C5B — C6B	57.1(5)
S1B — P1B — N4B — C5B	38.3(4)	C3A — N4A — C5A — C6A	55.5(5)
S1B — C15B — C16B — C17B	68.1(6)	C6B — O1B — C2B — N7B	178.6(3)
S1B — C15B — C14B — O4B	40.3(7)	C6B — O1B — C2B — C3B	-60.4(5)
S1B — C15B — C20B — C19B	-69.4(8)	C13A — C6A — C5A — N4A	-175.7(4)
S1A — P1A — O4A — C14A	38.2(4)	C2A — O1A — C6A — C13A	-176.1(4)
S1A — P1A — N4A — C3A	-125.6(4)	C2A — O1A — C6A — C5A	61.1(4)
S1A — P1A — N4A — C5A	39.8(4)	C2A — N7A — C8A — O2A	-2.6(7)
S1A — C15A — C16A — C17A	67.0(6)	C2A — N7A — C8A — N9A	177.0(4)
S1A — C15A — C14A — O4A	39.6(6)	C2A — N7A — C12A — C11A	-178.2(4)
S1A — C15A — C20A — C19A	-68.0(7)	C5A — N4A — C3A — C2A	-53.8(5)
S2B — P1B — O4B — C14B	-86.1(4)	C5A — C6A — C13A — O5A	-175.5(4)
S2B — P1B — N4B — C3B	5.6(5)	C12B — N7B — C8B — O2B	176.9(5)
S2B — P1B — N4B — C5B	169.9(3)	C12B — N7B — C8B — N9B	-2.2(6)
S2A — P1A — O4A — C14A	-83.9(4)	C12B — N7B — C2B — O1B	35.9(5)
S2A — P1A — N4A — C3A	6.5(5)	C12B — N7B — C2B — C3B	-85.2(5)
S2A — P1A — N4A — C5A	171.8(3)	C2B — O1B — C6B — C5B	62.4(4)
O1A — C6A — C13A — O5A	64.3(5)	C2B — O1B — C6B — C13B	-175.0(4)
O1A — C6A — C5A — N4A	-57.5(5)	C2B — N7B — C8B — O2B	-4.5(7)
O2A — C8A — N9A — C10A	-179.5(5)	C2B — N7B — C8B — N9B	176.4(4)
O1B — C6B — C5B — N4B	-59.6(5)	C2B — N7B — C12B — C11B	-176.0(4)

Atom — Atom — Atom — Atom	Angle	Atom — Atom — Atom — Atom	Angle
O1B — C6B — C13B — O5B	63.8(5)	C15A — C16A — C17A — C18A	56.3(7)
O4A — P1A — N4A — C3A	133.0(4)	C15A — C20A — C19A — C18A	-53.4(10)
O4A — P1A — N4A — C5A	-61.7(4)	C16A — C15A — C14A — O4A	-79.9(6)
O4B — P1B — N4B — C3B	132.2(4)	C16A — C15A — C20A — C19A	52.3(8)
O4B — P1B — N4B — C5B	-63.5(4)	C16A — C17A — C18A — C19A	-56.4(10)
N7A — C8A — N9A — C10A	0.9(7)	C5B — N4B — C3B — C2B	-54.5(5)
N7A — C12A — C11A — C10A	1.7(8)	C5B — C6B — C13B — O5B	-176.1(4)
O3A — C10A — C11A — C12A	175.1(5)	C13B — C6B — C5B — N4B	-178.7(4)
N9B — C10B — C11B — C12B	-6.0(7)	C15B — C16B — C17B — C18B	55.0(7)
O3B — C10B — C11B — C12B	173.7(5)	C14A — C15A — C16A — C17A	-175.7(5)
N4A — P1A — O4A — C14A	151.3(4)	C14A — C15A — C20A — C19A	176.7(7)
N4A — C3A — C2A — O1A	56.3(4)	C16B — C15B — C14B — O4B	-79.2(6)
N4A — C3A — C2A — N7A	175.4(3)	C16B — C15B — C20B — C19B	53.2(9)
C8A — N7A — C12A — C11A	2.9(7)	C16B — C17B — C18B — C19B	-56.0(9)
C8A — N7A — C2A — O1A	-141.5(4)	C14B — C15B — C16B — C17B	-176.3(5)
C8A — N7A — C2A — C3A	96.5(4)	C14B — C15B — C20B — C19B	176.3(7)
C8A — N9A — C10A — O3A	-176.3(5)	C17A — C18A — C19A — C20A	55.1(11)
C8A — N9A — C10A — C11A	3.2(7)	C20A — C15A — C16A — C17A	-53.3(7)
N4B — P1B — O4B — C14B	149.1(4)	C20A — C15A — C14A — O4A	157.2(6)
N4B — C3B — C2B — O1B	55.5(5)	C18B — C19B — C20B — C15B	-54.7(10)
N4B — C3B — C2B — N7B	173.3(3)	C20B — C15B — C16B — C17B	-53.7(7)
N9A — C10A — C11A — C12A	-4.5(7)	C20B — C15B — C14B — O4B	157.7(6)
C6A — O1A — C2A — N7A	177.1(3)	C20B — C19B — C18B — C17B	56.1(10)

Table S6.2. Torsion angles (°) for **3b**.

Atom — Atom — Atom — Atom	Angle	Atom — Atom — Atom — Atom	Angle
P1 — S1 — C15 — C20	136.00(19)	C8 — N9 — C10 — O3	177.4(4)
P1 — S1 — C15 — C14	18.2(2)	C8 — N9 — C10 — C11	-1.6(5)
P1 — S1 — C15 — C16	-103.71(19)	C6 — O1 — C2 — N7	-176.5(2)
P1 — O4 — C14 — C15	53.6(3)	C6 — O1 — C2 — C3	-55.7(3)
P1 — N4 — C3 — C2	108.4(3)	C14 — C15 — C16 — C17	177.6(2)
P1 — N4 — C5 — C6	-107.6(3)	C16 — C15 — C14 — O4	77.7(3)
S1 — P1 — O4 — C14	-33.5(2)	C16 — C17 — C18 — C19	55.1(4)
S1 — P1 — N4 — C3	143.8(2)	C12 — N7 — C8 — O2	178.0(3)
S1 — P1 — N4 — C5	-52.2(3)	C12 — N7 — C8 — N9	-2.7(5)
S1 — C15 — C14 — O4	-42.8(3)	C12 — N7 — C2 — O1	21.7(4)
S1 — C15 — C16 — C17	-65.7(3)	C12 — N7 — C2 — C3	-99.8(3)
S2 — P1 — O4 — C14	88.9(2)	C12 — C11 — C10 — O3	-176.8(4)
S2 — P1 — N4 — C3	13.8(3)	C12 — C11 — C10 — N9	2.0(5)
S2 — P1 — N4 — C5	177.9(2)	C2 — O1 — C6 — C13	175.3(3)
O4 — P1 — N4 — C3	-114.1(3)	C2 — O1 — C6 — C5	54.6(3)
O4 — P1 — N4 — C5	49.9(3)	C2 — N7 — C8 — O2	2.3(5)
O1 — C6 — C13 — O5	171.7(3)	C2 — N7 — C8 — N9	-178.3(3)
O1 — C6 — C5 — N4	-54.8(3)	C2 — N7 — C12 — C11	178.9(3)
O1 — C2 — C3 — N4	55.2(3)	C19 — C20 — C15 — S1	67.7(3)

Atom — Atom — Atom — Atom	Angle	Atom — Atom — Atom — Atom	Angle
N7 — C2 — C3 — N4	172.7(3)	C19 — C20 — C15 — C14	-178.0(3)
N4 — P1 — O4 — C14	-144.7(2)	C19 — C20 — C15 — C16	-53.6(3)
C20 — C15 — C14 — O4	-160.2(2)	C13 — C6 — C5 — N4	-171.5(2)
C20 — C15 — C16 — C17	54.9(3)	C3 — N4 — C5 — C6	58.5(3)
C20 — C19 — C18 — C17	-54.1(4)	C10 — N9 — C8 — O2	-178.8(4)
C15 — C20 — C19 — C18	54.7(4)	C10 — N9 — C8 — N7	1.9(5)
C15 — C16 — C17 — C18	-56.4(4)	C10 — C11 — C12 — N7	-3.0(5)
C8 — N7 — C12 — C11	3.5(5)	C5 — N4 — C3 — C2	-57.0(3)
C8 — N7 — C2 — O1	-162.6(3)	C5 — C6 — C13 — O5	-69.2(4)
C8 — N7 — C2 — C3	75.8(3)		

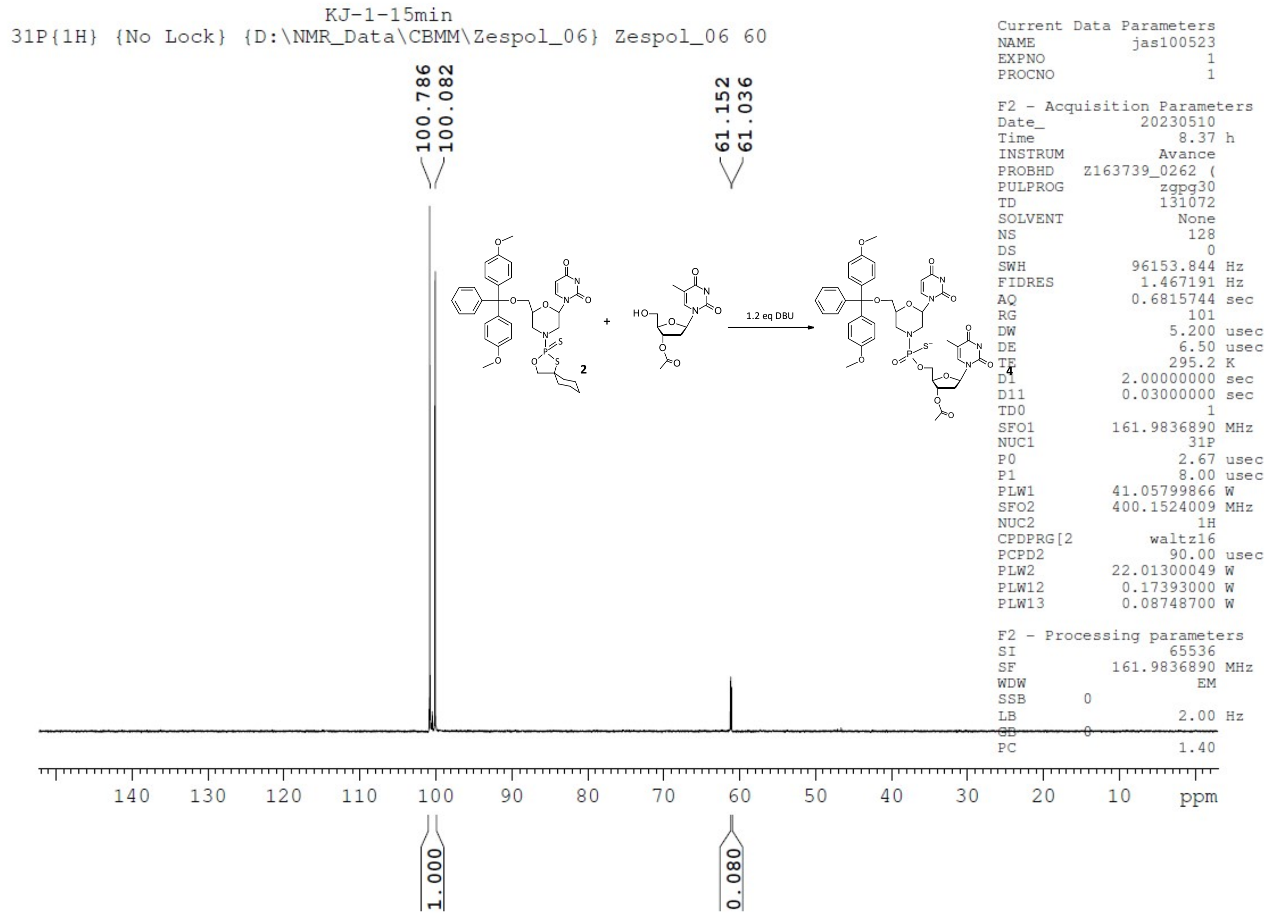


Figure S15. ^{31}P NMR spectrum of crude reaction mixture of dinucleotide formation *m*U$_{PS}$T **4** (analysis performed after 15 minutes in non-deuterium acetonitrile) with using 1.2 eq DBU

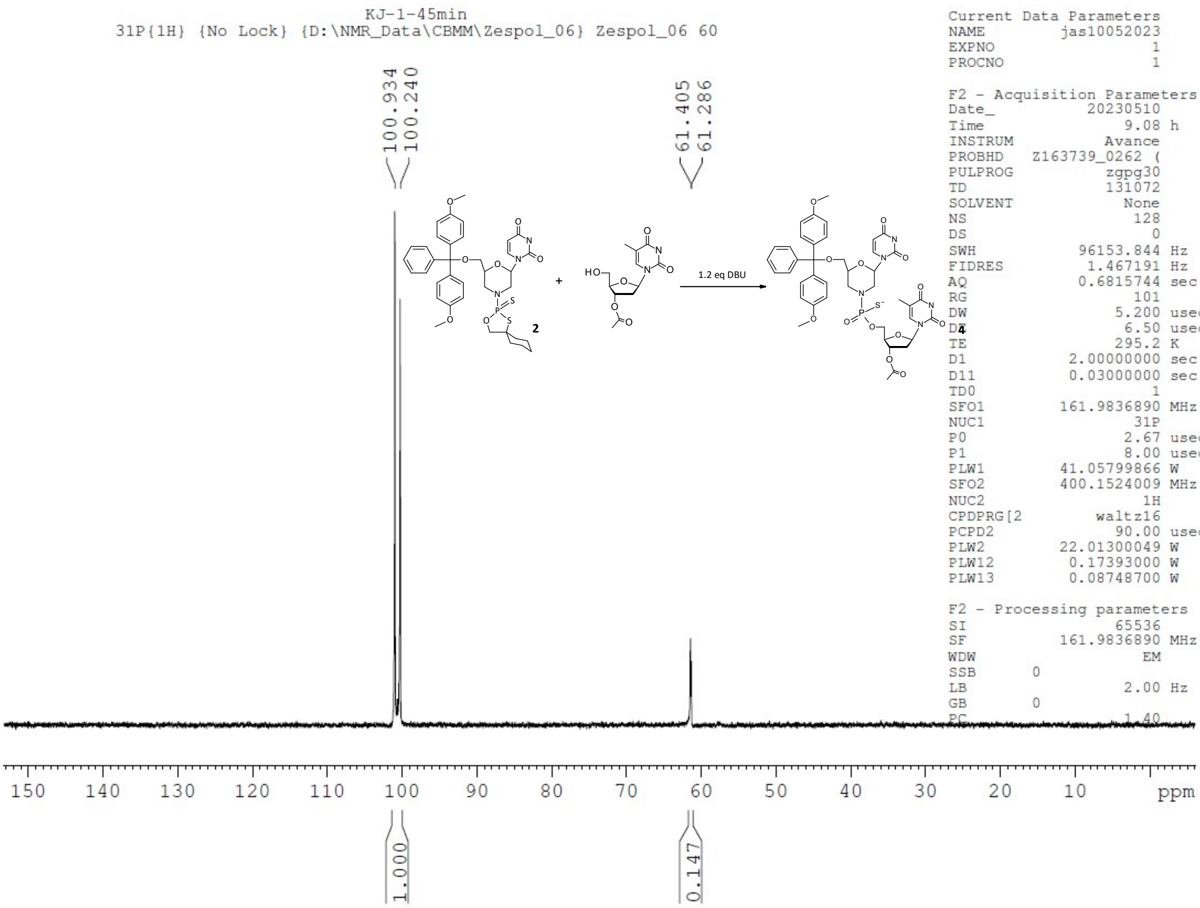


Figure S16. ^{31}P NMR spectrum of crude reaction mixture of dinucleotide formation mU$_{PS}$T **4** (analysis performed after 45 minutes in non-deuterium acetonitrile) with using 1.2 eq DBU

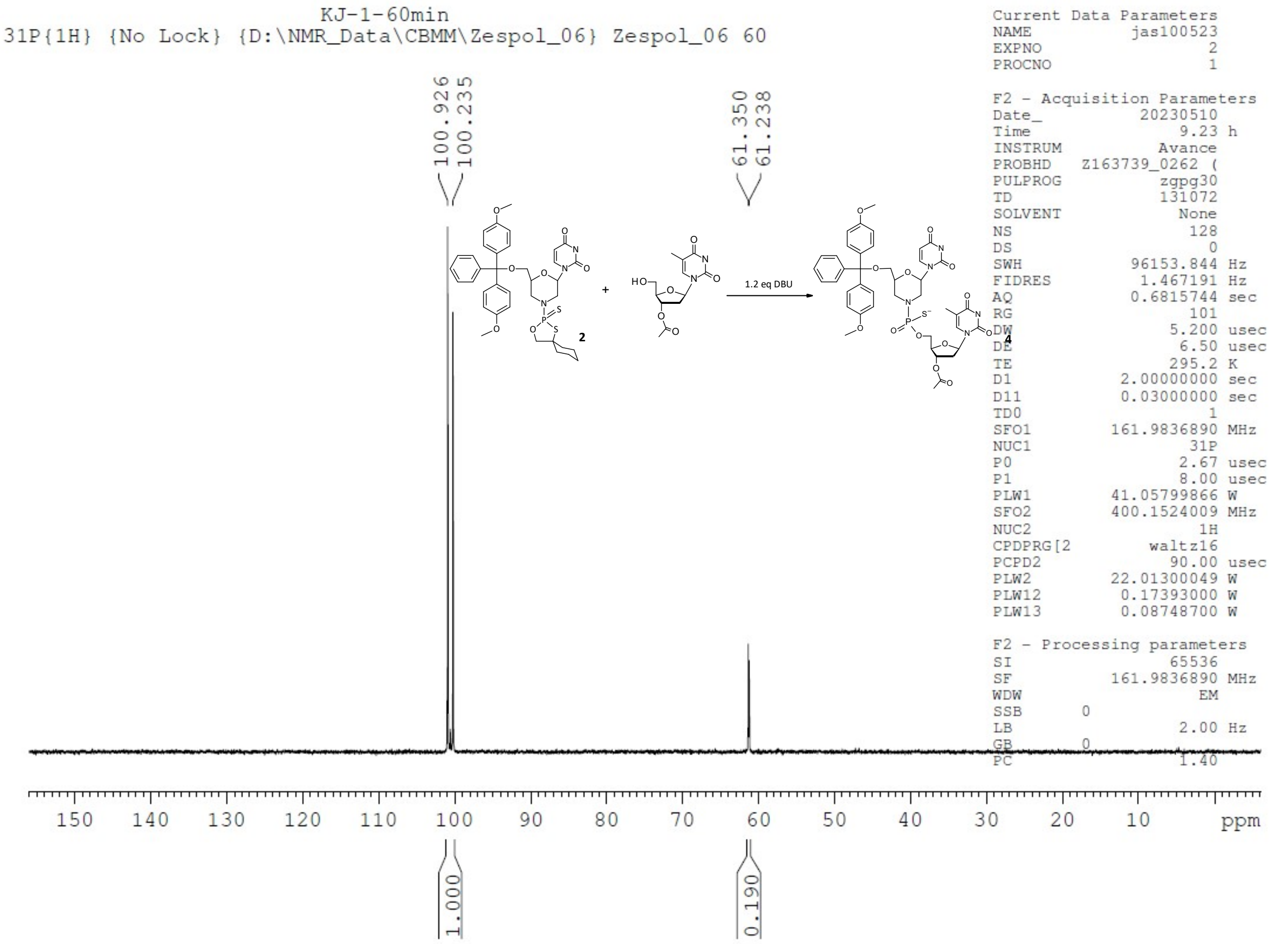


Figure S17. ^{31}P NMR spectrum of crude reaction mixture of dinucleotide formation *m*U$_{PS}$T **4** (analysis performed after 60 minutes in non-deuterium acetonitrile) with using 1.2 eq DBU

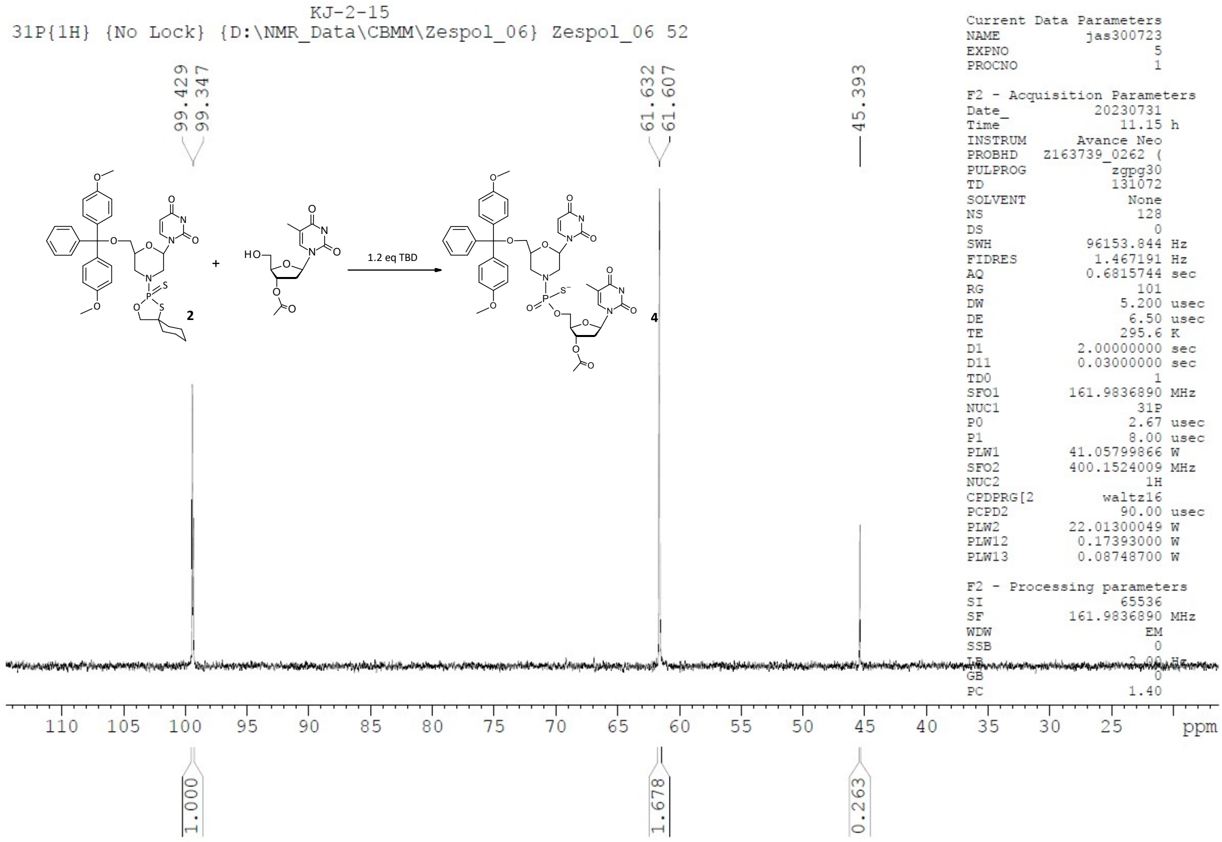


Figure S18. ^{31}P NMR spectrum of crude reaction mixture of dinucleotide formation *m*U$_{PS}$T **4** (analysis performed after 15 minutes in non-deuterium acetonitrile) with using 1.2 eq TBD

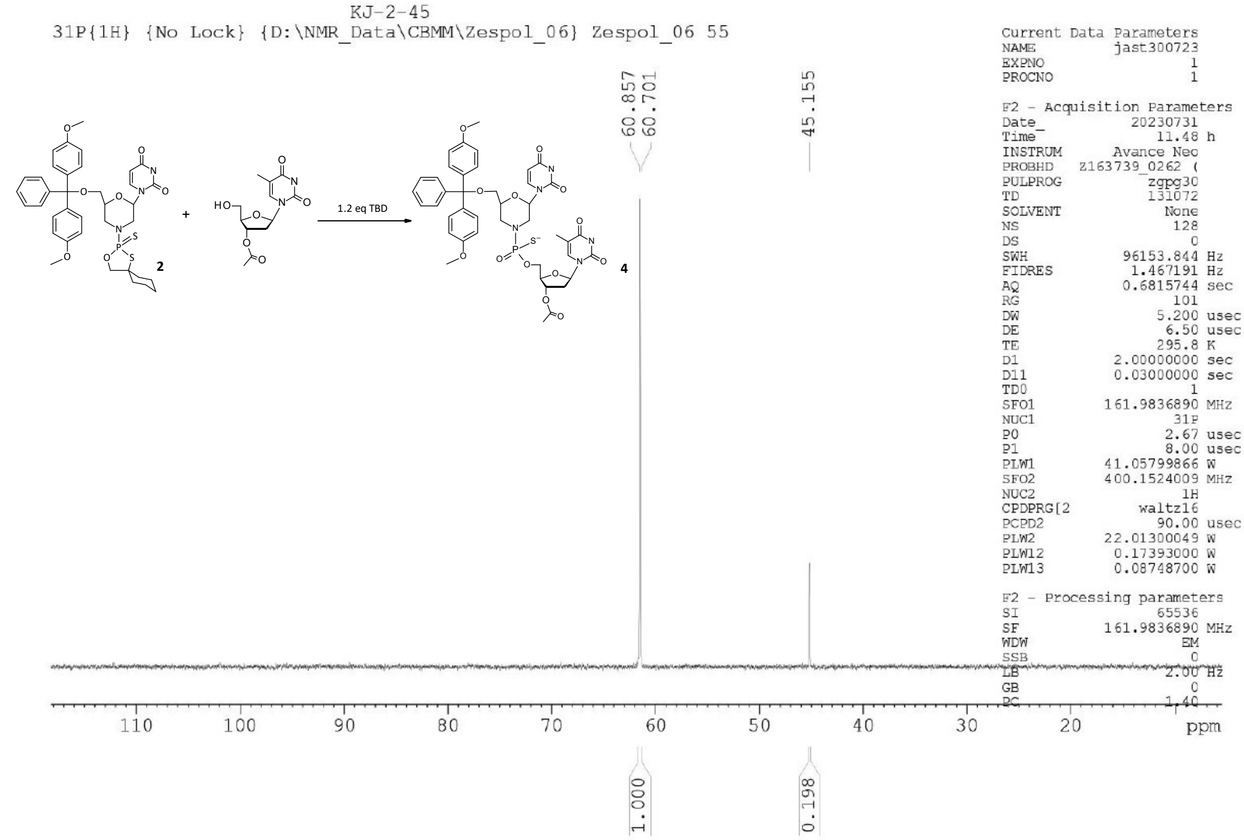


Figure S19. ^{31}P NMR spectrum of crude reaction mixture of dinucleotide formation mU$_{PS}$T **4** (analysis performed after 45 minutes in non-deuterium acetonitrile) with using 1.2 eq TBD

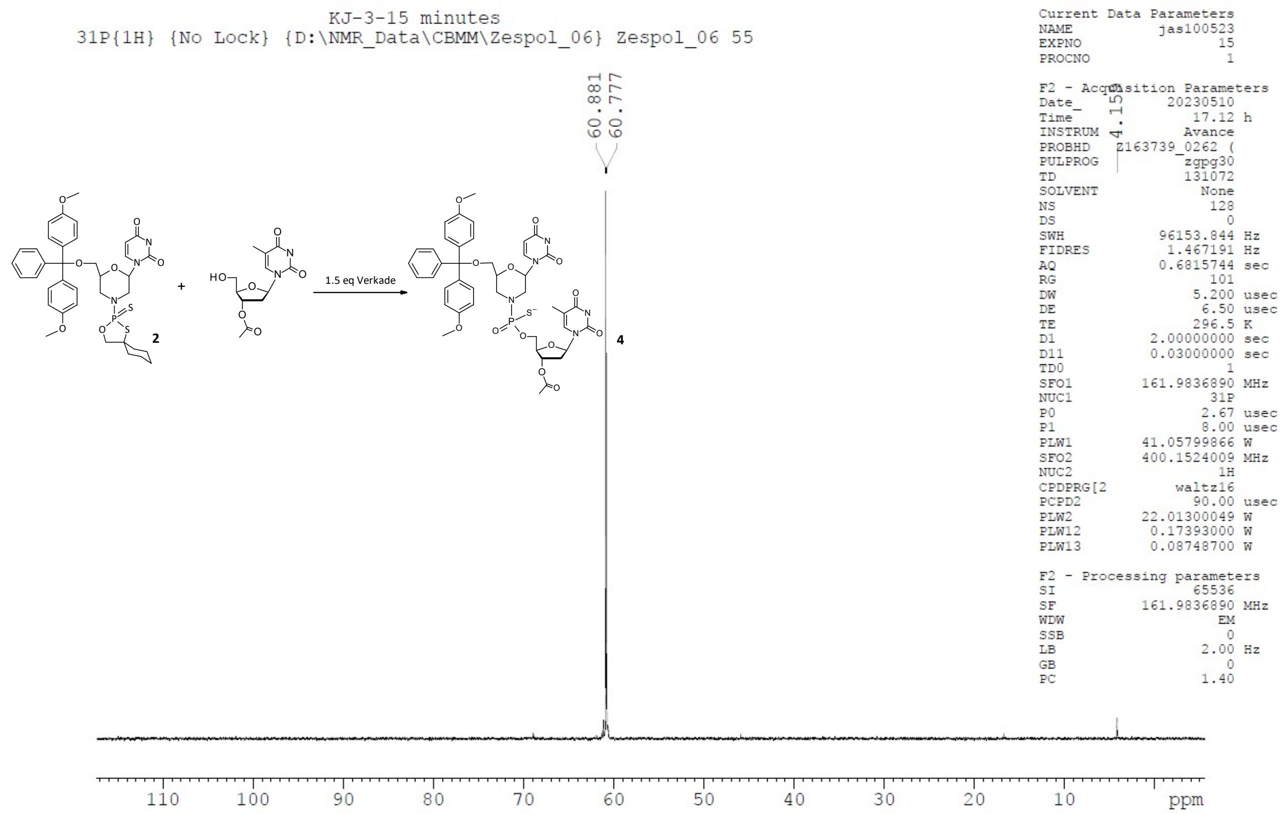


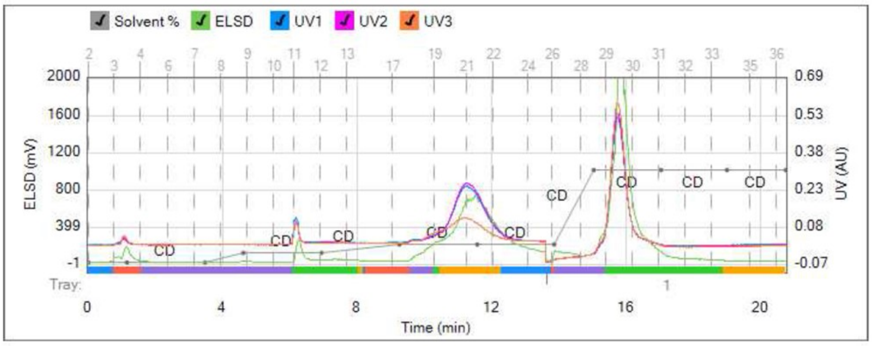
Figure S20. ^{31}P NMR spectrum of crude reaction mixture of dinucleotide formation *m*U$_{PS}$T **4** (analysis performed after 15 minutes in non-deuterium acetonitrile) with using 1.5 eq Verkade base (2,8,9-Trimethyl-2,5,8,9-tetraaza-1-phosphabicyclo[3.3.3]undecane)

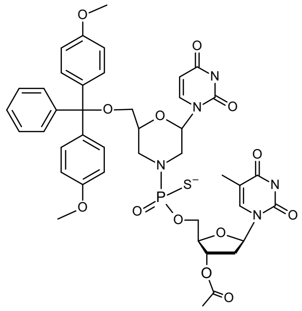
Reveleris® X2

Method Name: KJ_morfoliny
Run Name: 2023-04-14_dm crude
Run Date: 2023-04-14 01:04

BÜCHI

Column: FP ECOFLEX Si 25g	Solvent A: Empty	UV Threshold: 0.05 AU	ELSD Threshold: 20 mV
Flow Rate: 32 mL/min	Solvent B: Empty	UV Sensitivity: High	ELSD Sensitivity: High
Equilibration: 2.0 min	Solvent C: Dichloromethane	UV1 Wavelength: 254 nm	Collection: Collect All
Run Length: 20.8 min	Solvent D: Methanol	UV2 Wavelength: 265 nm	Per-Vial Volume: 25 mL
Mode: Flash Liquid	Slope Detection: Off	UV3 Wavelength: 280 nm	Non-Peak Volume: 25 mL




4b

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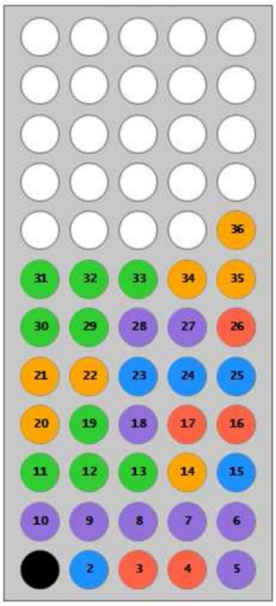


Gradient Table

	Min	Solvents	% 2nd
1	0.0	CD	0
2	1.2	CD	0
3	2.3	CD	0
4	1.2	CD	5
5	2.3	CD	5
6	2.3	CD	10
7	2.3	CD	10
8	2.3	CD	10
9	1.2	CD	50
10	2.0	CD	50
11	2.0	CD	50
12	2.0	CD	50

Vial Mapping Table

Peak #	Start Tray:Vial	End Tray:Vial
1	1:3	1:4
2	1:11	1:13
3	1:15	1:15
4	1:18	1:18
5	1:19	1:19
6	1:20	1:22
7	1:23	1:25
8	1:27	1:28
9	1:29	1:33

Page 1

Figure S21. Purification of crude product *m*U_{PS}T **4b** obtained from *slow*-eluting P-diastereomer using cartridges packed with silica gel (FP ECOFLEX Si 25g) of Reveleris Flash Chromatography system with UV and ELSD detectors (Reveleris) and was eluted with a linear 0→50% gradient of MeOH in CH_2Cl_2.

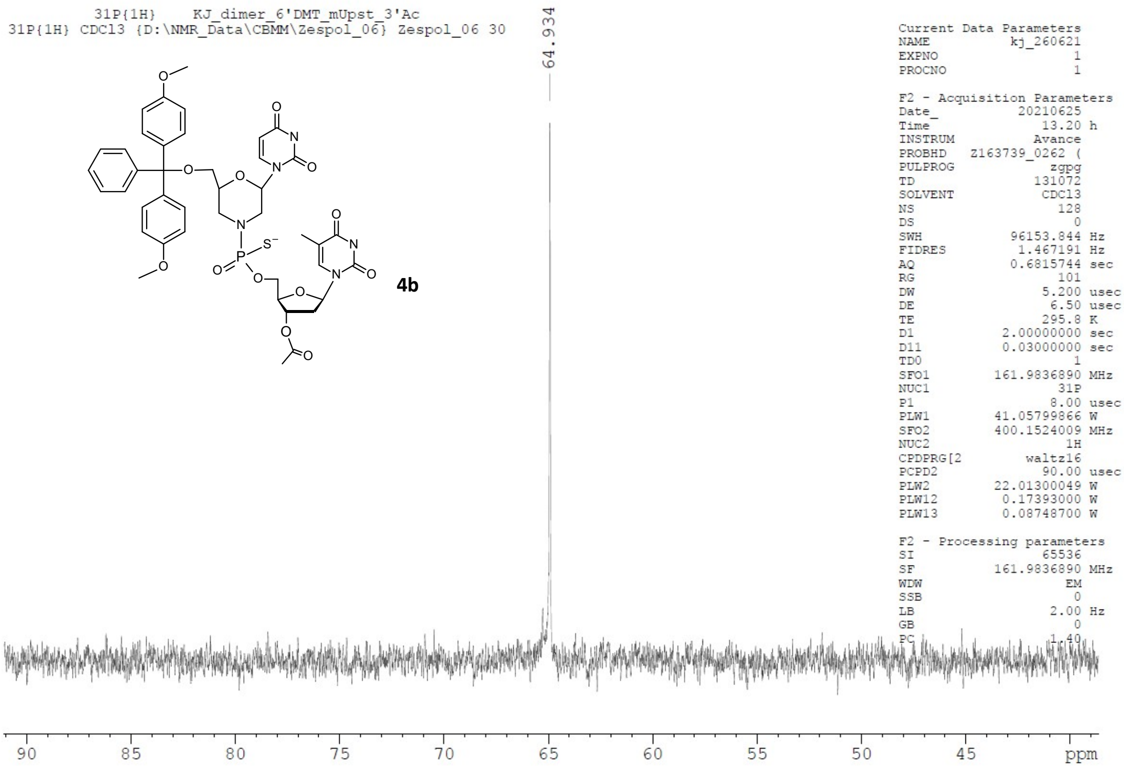


Figure S22. ^{31}P NMR spectrum of purified P-stereodefined dinucleotide *m*U$_{PS}$T **4b** obtained from *slow*-eluting P-diastereomer; δ (ppm, CDCl$_3$)

Reveleris®
X2

Method Name: KJ_dm_ON
Run Name: 2023-04-19-KJ_dm off
Run Date: 2023-04-19 23:56

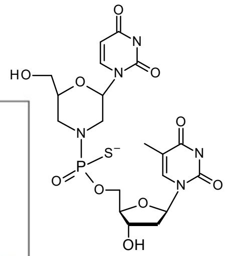
BÜCHI

Column: FP ECOFLEX Si 12g
Flow Rate: 20 mL/min
Equilibration: 2.0 min
Run Length: 13.9 min
Mode: Flash Liquid

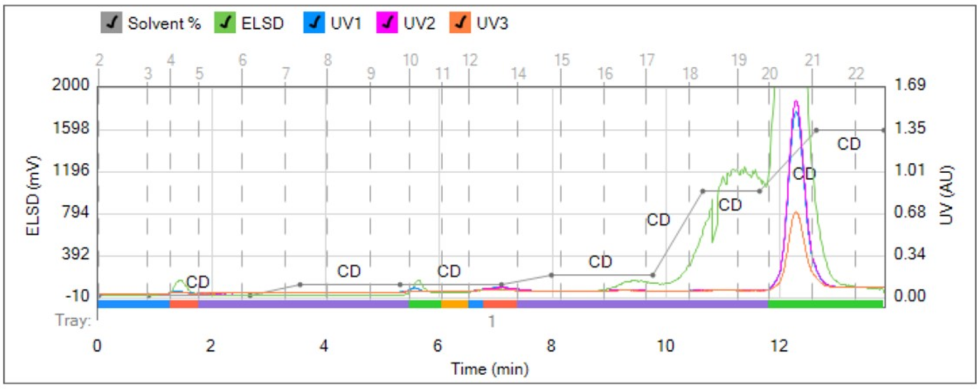
Solvent A: Empty
Solvent B: Empty
Solvent C: Dichloromethane
Solvent D: Methanol
Slope Detection: Off

UV Threshold: 0.05 AU
UV Sensitivity: High
UV1 Wavelength: 254 nm
UV2 Wavelength: 265 nm
UV3 Wavelength: 280 nm

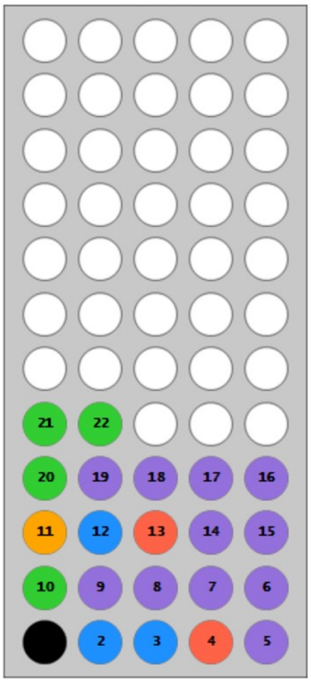
ELSD Threshold: 20 mV
ELSD Sensitivity: High
Collection: Collect All
Per-Vial Volume: 15 mL
Non-Peak Volume: 15 mL



5b



1 - 540D



Gradient Table

	Min	Solvents	% 2nd
1	0.0	CD	0
2	0.9	CD	0
3	1.8	CD	0
4	0.9	CD	5
5	1.8	CD	5
6	1.8	CD	5
7	0.9	CD	10
8	1.8	CD	10
9	0.9	CD	50
10	1.0	CD	50
11	1.0	CD	80
12	2.0	CD	80
13	2.0	CD	80

Vial Mapping Table

Peak #	Start Tray:Vial	End Tray:Vial
1	1:4	1:4
2	1:10	1:10
3	1:12	1:12
4	1:13	1:13
5	1:14	1:19
6	1:20	1:22

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Figure S23. Purification of crude product *m*U$_{PS}$T **5b** after detritylation by treatment with 1.5% DCA using cartridges packed with silica gel (FP ECOFLEX Si 25g) of Reveleris Flash Chromatography system with UV and ELSD detectors (Reveleris); elution with a linear 0→80% gradient of MeOH in CH_2Cl_2.

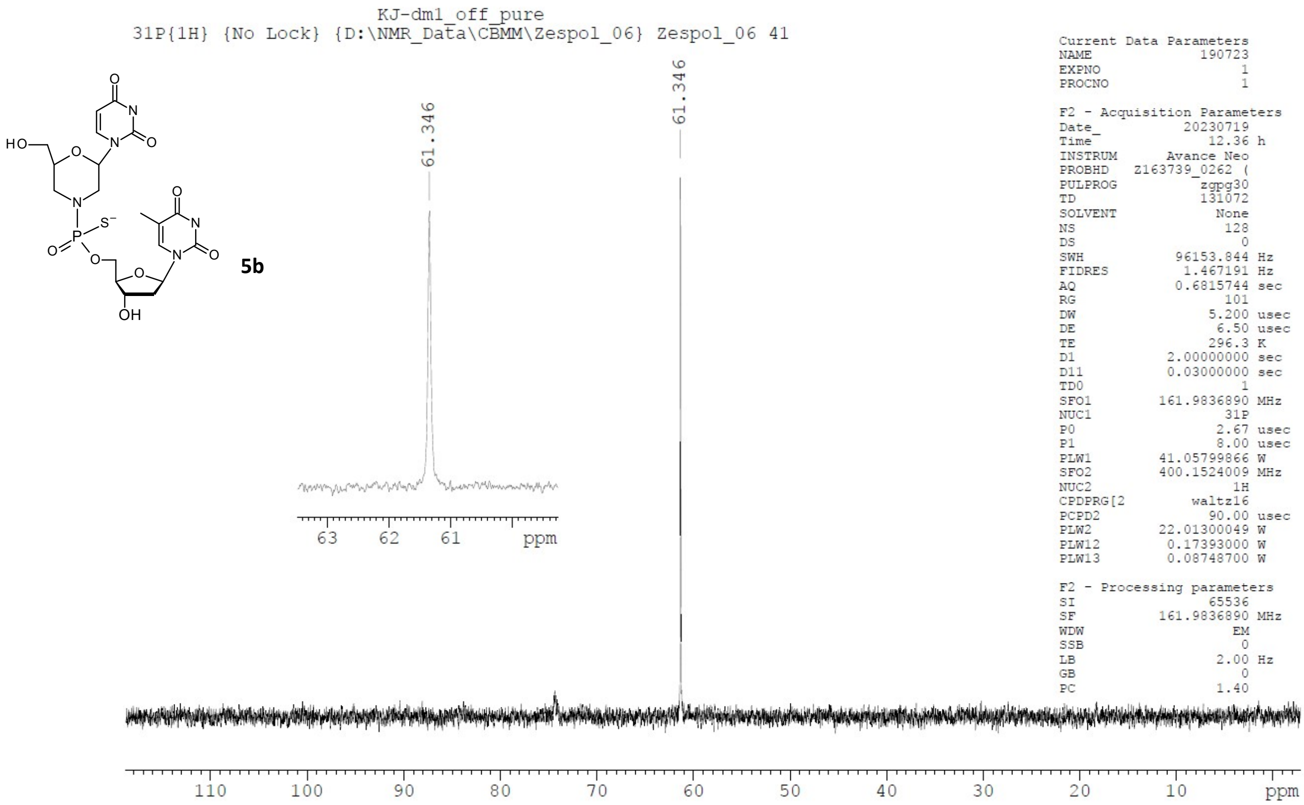

Figure S24. ^{31}P NMR spectrum of purified dinucleotide *m*U_{PS}T **5b** (in non-deuterium acetonitrile)

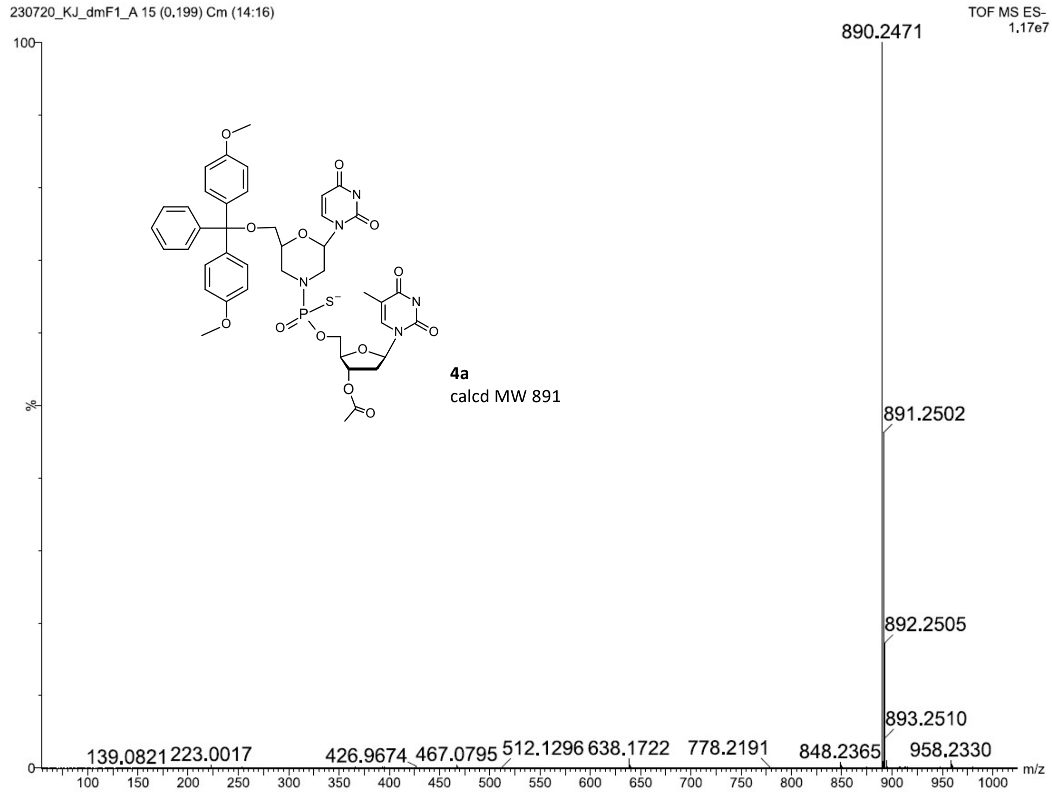

Figure S25. HR MS spectra for dinucleotide *m*U_{PS}T **4a** obtained from *fast*-eluting P-diastereomer

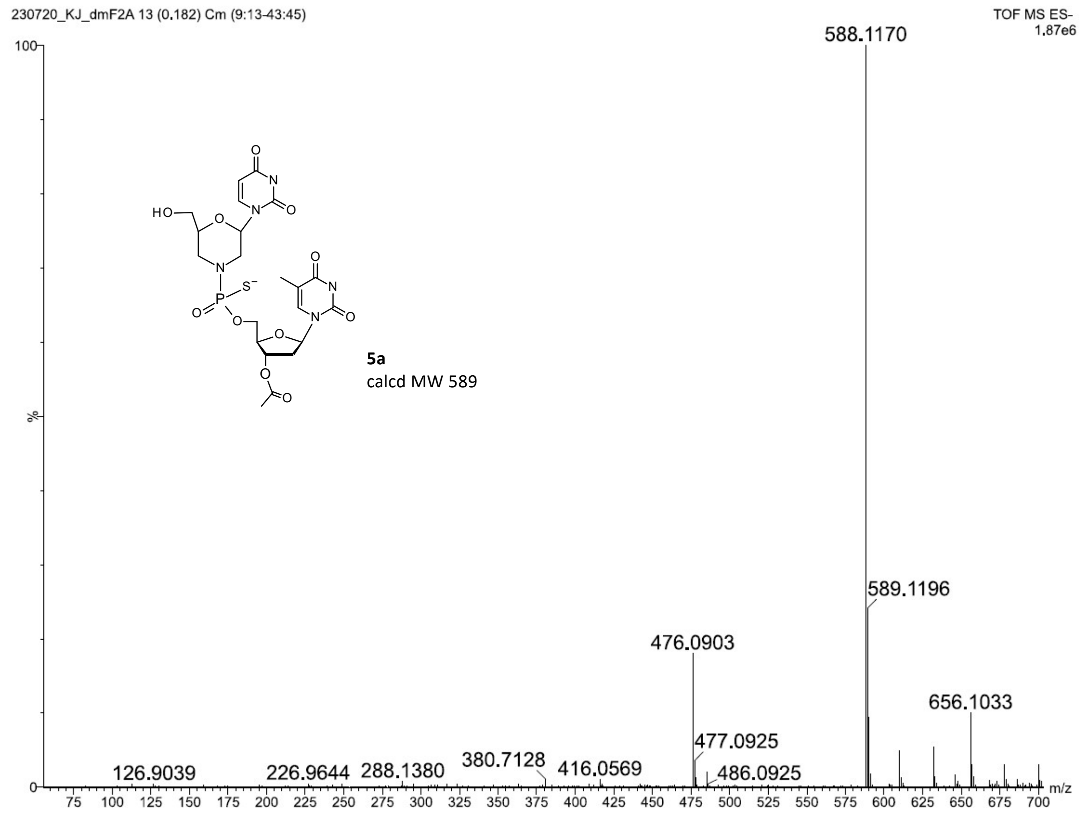

Figure S26. HR MS spectra for dinucleotide *m*U_{PS}T **5a** (before NH_4OH)

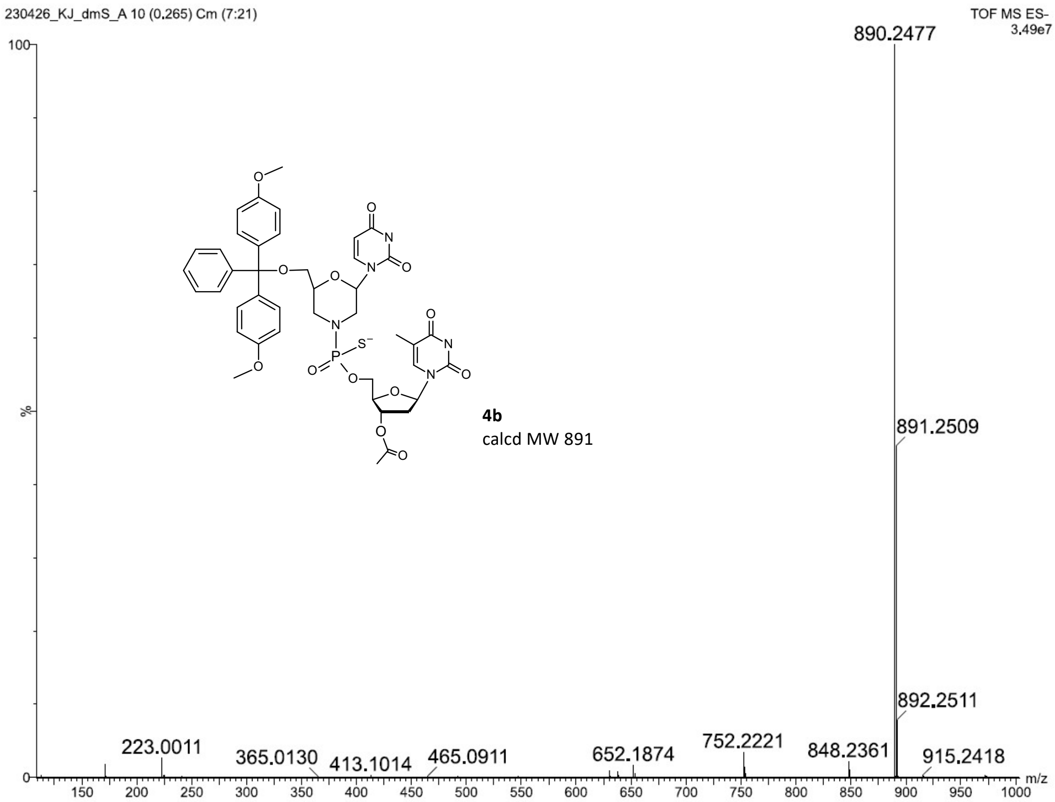

Figure S27. HR MS spectra for dinucleotide *m*U_{PS}T **4b** obtained from *slow*-eluting P-diastereomer

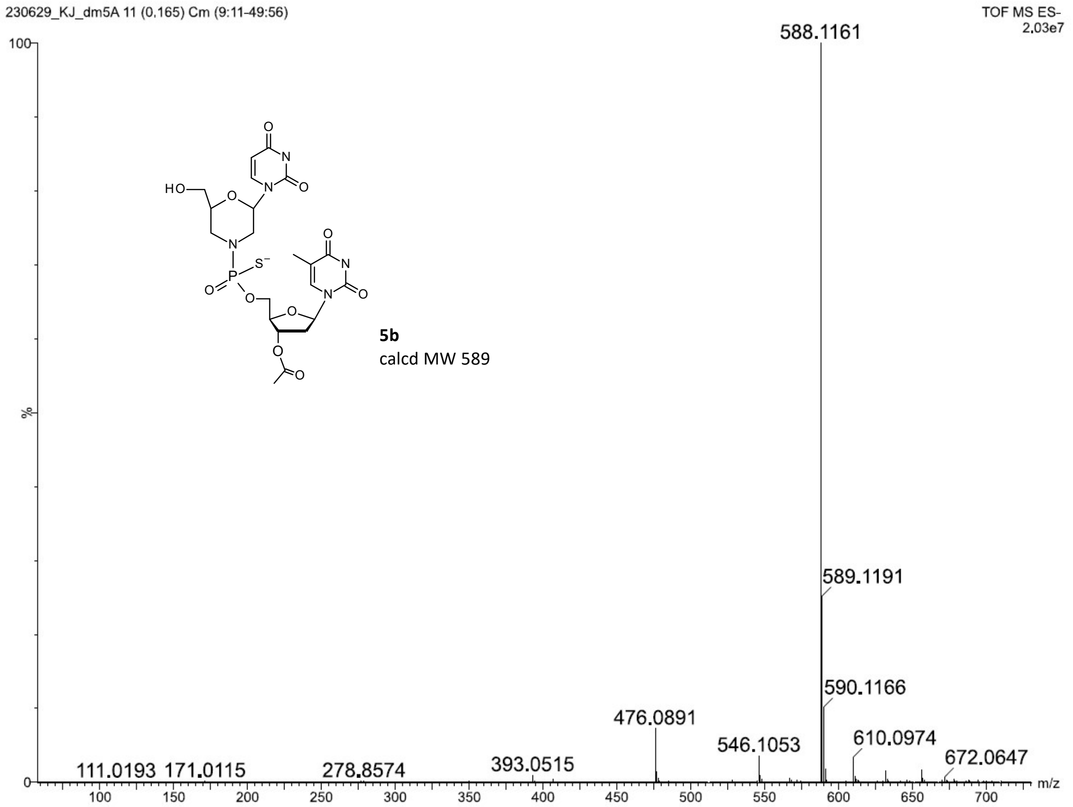

Figure S28. HR MS spectra for dinucleotide *m*U_{PS}T **5b** (before NH_4OH)

X-ray data collection, processing, structure solution, refinement and analysis

The data collection strategy was calculated within *CrysAlisPRO*[2] to ensure desired data redundancy and percent completeness. Data were processed, integrated and scaled using *CrysAlis PRO* and AIMLESS[3]. Data acquisition and processing statistics are shown in Table S7.
Molecular replacement was conducted with RNase A coordinates (PDB ID: 6f60[4]) using *MOLREP*software[5]. Modeling and molecular visualization were performed in *COOT*[6]. Ligand restraints were calculated using *Phenix*[7] and refinement was performed using *REFMAC5*[8]. All refinement steps were monitored with R_{cryst} and R_{free} values. The stereochemical quality of the resulting models was assessed using the program *MOLPROBITY*[9] and the validation tools implemented in *COOT*. The values of the mean temperature factors for protein main and side chains, ligands and water molecules were calculated using the program BAVERAGE from CCP4 suite[10]. The refinement statistics of the described structures are listed in Table S8.

Table S7. Crystallographic parameters and data collection statistics for dimer **5b**.

PDB ID	8R5V
Crystallization conditions	22 % w/v PEG4000, 0.1 M sodiumcitratepH5.5
Crystal size (μm)	200 × 70 × 30
Ligand	[Sp-PS]mU-dT
Ligand code	LWC
Soaking time (min.)	15
X-ray source	Rigaku XtaLAB Synergy-S
Wavelength (Å)	1.54184
Detector	HyPix-6000HE
Detector distance (mm)	32
Oscillation width (°)	0.25
Temperature (K)	100
No. of frames	712
Space group	*C*2
Unit-cell parameters	
a (Å)	100.32
a (Å)	32.71
c (Å)	72.80
α (°)	90.00
β (°)	90.07
γ (°)	90.00
Total no. of	68071 (3579)

reflections	
Unique reflections	22015 (1248)
Completeness (%)	98.8 (95.8)
Resolution (Å)	17.43-1.80 (1.84-1.80)
R_{merge}[a]	0.078 (0.243)
$R_{p.i.m}$	0.051 (0.161)
Multiplicity	3.1 (2.9)
Mosaicity	1.03
Wilson *B* factor	9.4
Mean *I*/sd(*I*)	9.6 (3.4)
CC(1/2)	0.994 (0.930)

Table S8. Refinement statistics for dimer **5b**.

PDB ID	8R5V
No. of reflections used in refinement	20874
No. of reflections used to R_{free}	1122
R_{cryst} (R_{free})	0.164 (0.233)
No. of non H-atoms	
Protein	1995
Solvent	381
Ligands	54
R.m.s.d.s from ideal values	
Bond lengths (Å)	0.007
Bond angles (°)	1.530
Ramachandran plot	
Favoured [%]	96.7
Allowed [%]	3.3
Outliers [%]	0

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