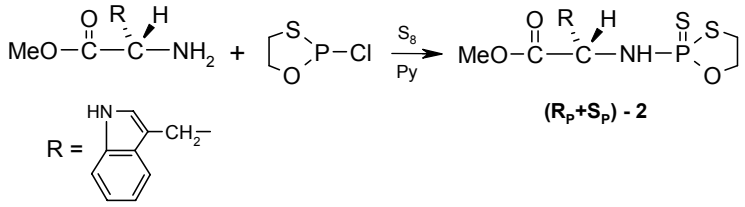
Supplementary Information

Stereochemistry of rHint1 Hydrolase Assisted Cleavage of P-N Bond in Nucleoside 5'-*O*-phosphoramidothioates

Agnieszka Krakowiak[†], Renata Kaczmarek[†], Janina Baraniak[†], Michał Wieczorek[‡] and Wojciech J. Stec[†]*.

[†]Department of Bioorganic Chemistry, Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, 112 Sienkiewicza Str., 90-363 Łódź, [‡] Institute of Chemistry and Environmental Protection, Pedagogical University of Częstochowa, Al. Armii Krajowej 13/15, 42-200 Częstochowa, Poland



Synthesis of N-(2-Thiono-1,3,2-oxathiaphospholanyl)-L-tryptophan Methyl Ester (2)[1]

Elemental sulfur (512 mg, 2 mmol) was added to a solution of L-tryptophan methyl ester hydrochloride (254 mg, 1 mmol) in of dry pyridine (5 mL). 2-Chloro-1,3,2-oxathiaphospholane[2] (142 mg, 1 mmol) was then added dropwise. The reaction mixture was stirred at rt for 12 h. The solvent was then removed under reduced pressure and acetonitrile (10 mL) was added and an excess of sulfur was filtered off. After evaporation of the solvent the residue was dissolved in chloroform (2-3 mL) and applied to a silica gel column (200-300 mesh, 2.5 x 18 cm). The column was eluted with chloroform and appropriate fractions were combined and evaporated under reduced pressure to give the desired product as a mixture of diastereoisomers **2** (267 mg, 75%); ^{31}P NMR (121MHz, $CDCl_3$) δppm 96.54 and 95.52. FAB-MS m/z 355 (M-1); ^{1}H NMR (300MHz, $CDCl_3$) δppm: 8.09 (bs, 1H), 7.57 (d, 1H), 7.39- 7.35 (m, 1H), 7.24- 7.06 (m, 3H), 4.38- 4.06 (m, 4H), 3.67 (s, 3H), 3.44- 3.26 (m, 4H).

The diastereoisomers of **2** were separated by fractional crystallization from chloroform to give the less-soluble isomer ***R*$_P$-2,** resonating at lower field [δ_P (121MHz, $CDCl_3$) 96.54ppm]. Crystals of this diastereoisomer were suitable for X-ray analysis which proved the R-configuration at phosphorus. The mother liquors were concentrated and the solid residue was crystallised from chloroform-diethyl ether (10:1)to give the pure diastereomer of **S$_P$-2** resonating at higher field [δ_P (121MHz, $CDCl_3$) 95.52 ppm].

Synthesis of Adenosine 5'-*O*-[*N*-(*L*-tryptophanylamide)]phosphoramidothioates (1).

The individual diastereoisomers of *N*-(2-thiono-1,3,2-oxathiaphospholanyl)-L-tryptophan methyl ester (**2,** 356 mg**,** 1mmol) were separately dissolved in dry acetonitrile (5 mL) and into this solution was dropped a solution of N^6,$O^{2'}$,$O^{3'}$-tribenzoyladenosine (579 mg, 1 mmol) and DBU (167 mg, 1.1 mmol) in dry acetonitrile (6 mL). The reaction mixture was stirred at rt for 12 h then concentrated under reduced pressure. The residual solid was suspended in aqueous ammonia (10 ml, 20% v/v) and left for 24 h at rt in a tightly closed vial. Ammonia was then evaporated and the residue dissolved in water and purified on a Sephadex A-25 column (3 x 20 cm): product **1** being eluted with a linear gradient of triethylammonium bicarbonate buffer (pH 7.5) from 0.05 to 0.4 M. The appropriate fractions were combined and evaporated to yield the oily product, which was dissolved in water and passed through a Dowex 50Wx2 (Na^+) column (1.5 x10 cm). Fractions containing the product were combined and lyophilized to provide **1** as a colorless solid.

S_P-1 (prepared from R_P-2)

(389 mg, 71.0%); ^{31}P NMR (121MHz, D_2O,) δppm 55.65; ^{1}H NMR (300MHz, D_2O,) δppm: 8.20 (1H, s), 8.08 (1H, s), 7.32 (1H, d), 7.12 (1H, d), 7.03 (1H, s), 6.84 (1H, t), 6.67 (1H, t), 5.82 (1H, d), 4.44 (1H, m), 4.12 (2H, m), 3.80 (2H, m), 3.71 (1H, m), 2.97 (2H, m); HRFAB-MS m/z calcd for $(M-H)^-$ 547.1954, found 547.1942.

R_P-1 (obtained from S_P-2)

(408 mg, 74.5%); ^{31}P NMR (121 MHz, D_2O,) δppm 57.96; ^{1}H NMR (300 MHZ, D_2O,) δppm: 8.28 (1H, s), 8.06 (1H, s), 7.32 (1H, d), 7.13 (1H, d), 7.02 (1H, s), 6.85 (1H, t), 6.69 (1H, t), 5.82 (1H, d), 4.50 (1H, m), 4.16 (2H, m), 3.94 (1H, m), 3.70 (2H, m), 2.97 (2H, m); HRFAB-MS m/z calcd for $(M-H)^-$ 547.1954, found 547.1940.

Crystallographic analysis of 2

The crystal and molecular structure of *N*-(2-thiono-1,3,2-oxathiaphospholanyl)-L-tryptophan methyl ester **2,** (δ_P 96.54 isomer) was determined using data collected at low temperature on a KUMA (Oxford Instruments KM4) diffractometer[3] with graphite monochromatized MoKα radiation. Compound **R_P-2** crystallises in monoclinic system in space group $P2_1$ with 2 molecules per unit cell. Crystal data and experimental details are shown in Table 1. The lattice constants were refined by least-squares fit of 5000 reflections in the θ range 3.53°-28.53°.

A total of 3476 independent reflections with $I \geq 0$ were used to solve the structure by direct methods[4,5] and to refine it by full matrix least-squares using F^2. Hydrogen atoms were found on difference Fourier map and refined isotropicaly. Anisotropic thermal parameters were refined for all non-hydrogen atoms. The final refinement of converged to R = 0.0204 for 268 refined parameters and 3417 observed reflections with $I \geq 2\sigma(I)$. The absolute configurations at the chiral atoms were established as R_{P1}, S_{C3}. The absolute structure was determined by the Flack method[6] with result $\chi = 0.03(4)$. Data processing was carried out with the KM4CCD software[3,7] structure solution SHELXS[4] structure refinement SHELXL[5].

The authors have deposited all crystallographic data for this structure with the Cambridge Crystallographic Data Centre[8].

Table 1. Crystal data for **2**

Molecular formula	$C_{14}H_{17}N_2O_3S_2$
Formula weight	356.39
Temperature (K)	173(1)
Wavelength (Å)	0.71073
Crystallographic system	monoclinic
Space group	$P2_1$
a (Å)	8.4537(6)
b (Å)	9.5906(7)
c (Å)	10.0814(8)
α (°)	90.00
β (°)	104.847(6)
γ(°)	90.00
V (Å^3)	790.07(10)
Z	2
D_c (g/cm^3)	1.498

μ [cm^{-1}]		4.51
Crystal dimensions (mm)		0.20x0.55x0.65
Maximum 2θ(°)		57.06
Scan mode		ω
hkl ranges:	*h* =	-11 10
	k =	-12 12
	l =	-9 13
No. of reflections: unique		3476
with $I>0\sigma(I)$		3476
obs. with $I>2\sigma(I)$		3417
No. of parameters refined		268
Largest diff. peak ($eÅ^{-3}$)		0.205
Largest diff. hole ($eÅ^{-3}$)		-0.227
shift/esd max		0.001
R_{obs}		0.0204
wR_{obs}		0.0530
S_{obs}		1.046
weighting coeff.*	m	0.0319
	n	0.1732
extinction coef.**	k	0.0208(22)
R_{int}		0.0230
$T_{meas.}$		110(2)
F(000)		372
Absolute structure		S_{P1}, S_{C3}
Flack parameter χ		0.03(4)

* weighting scheme $w=[\sigma^2(Fo^2)+(mP)^2+nP]^{-1}$
where $P=(Fo^2+2Fc^2)/3$

** extinction method SHELXL,
extinction expression $Fc^*=kFc[1+0.001xFc^2\lambda^3/\sin(2\theta)]^{-1/4}$

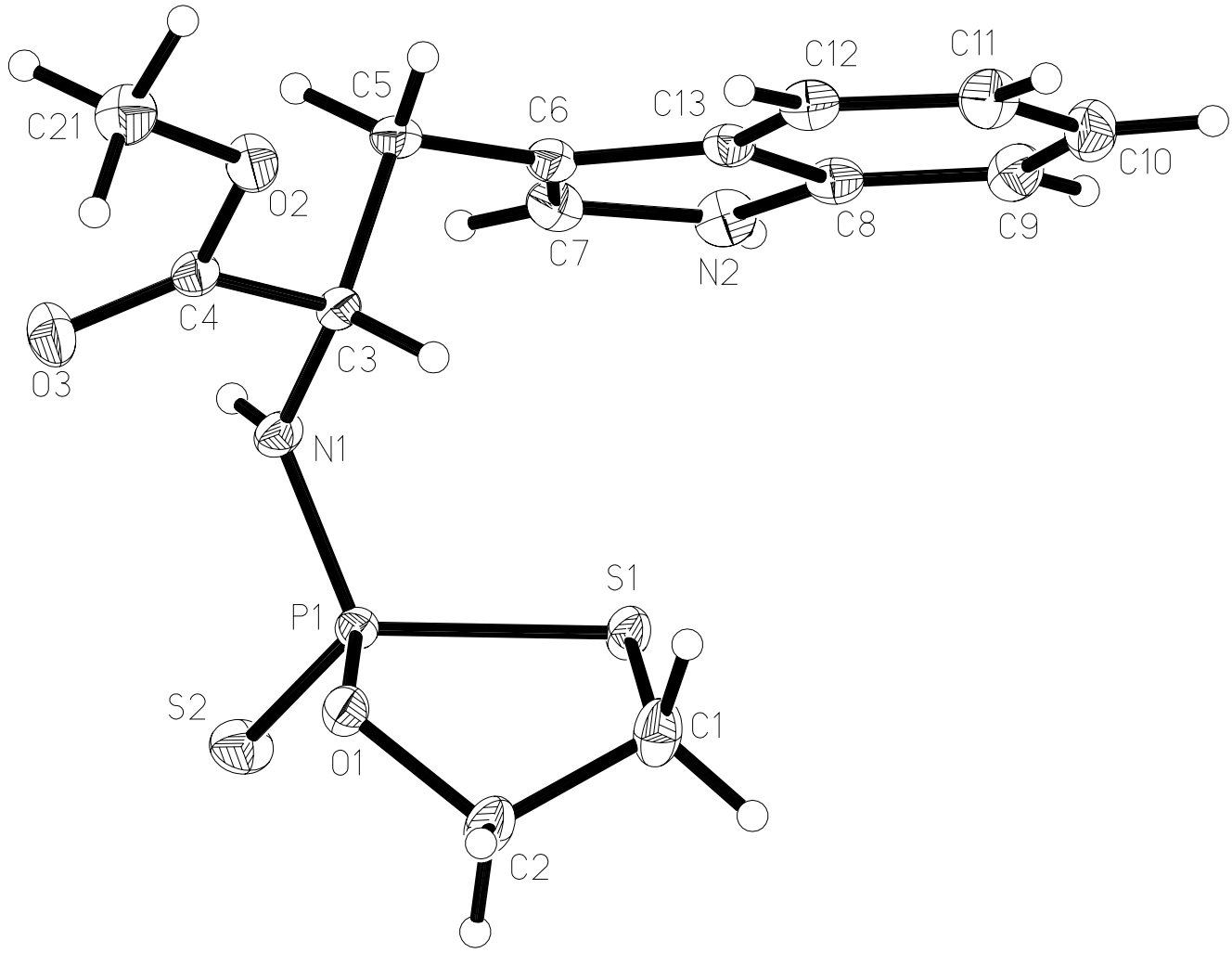


Figure 1. Thermal ellipsoidal view with the atom numbering scheme for R_P-**2**. Ellipsoids are shown with 50% probability.

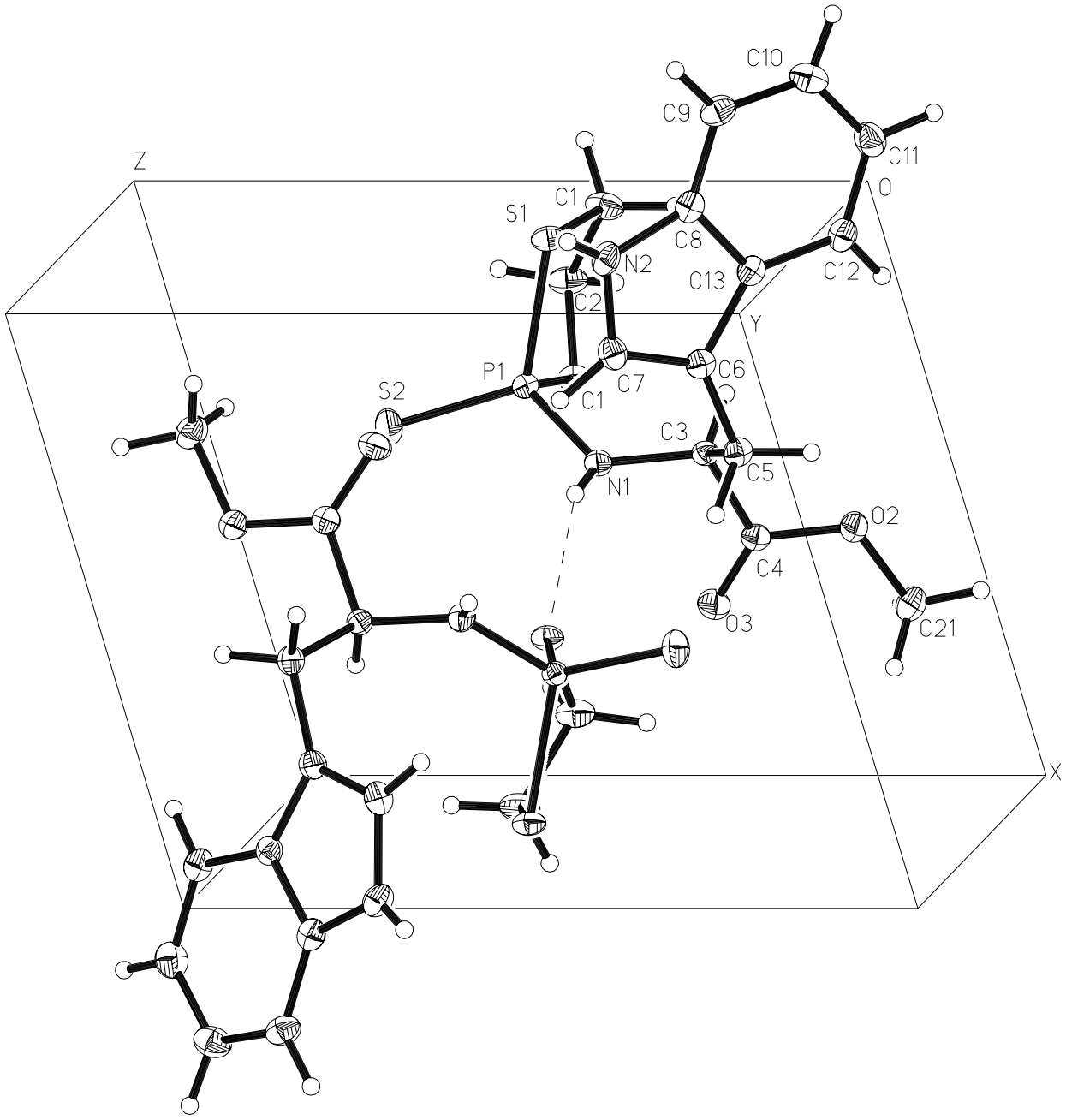


Figure 2. Crystal packing diagram for R_P-**2**. Ellipsoids are shown with 50% probability.

References

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(7) KM4CCD software. Data Reduction Program. CrysAlis RED. Version 1.163, **2000**, UNILIC & KUMA Diffraction Instruments GmbH

(8) Deposition number: CCDC 614931, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, (UK).