SUPPLEMENTAL MATERIALS

Interactions of 2,6-substituted purines with purine nucleoside phosphorylase from *Helicobacter pylori* in solution and in the crystal, and the effects of these compounds on cell cultures of this bacterium

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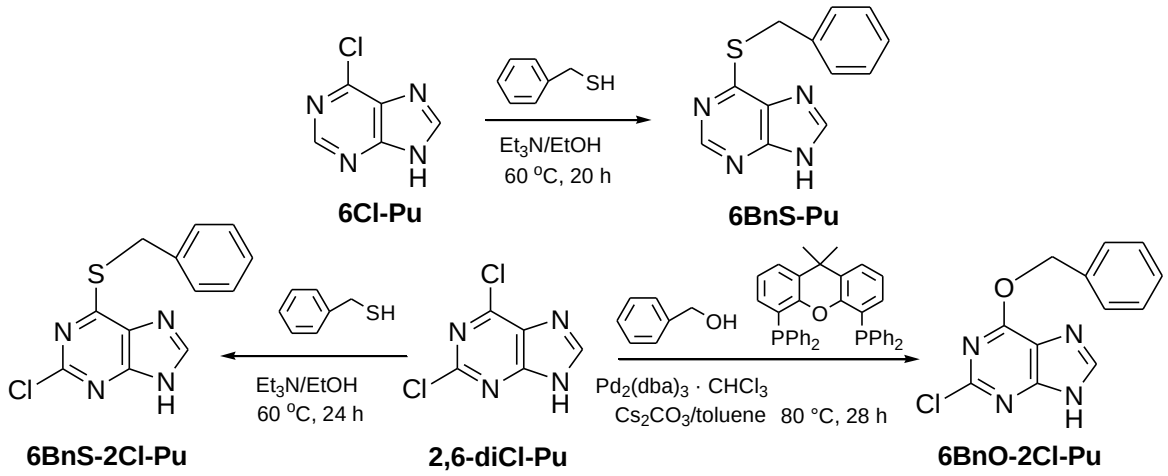
Chemical procedures

Purine bases modified in the C6 position and their derivatives possess a wide range of biological properties[1-4] and appear as clinically valuable molecules[5,6] which also participate in various cellular processes.[7,8]

Many substituents containing sulfur, oxygen, and nitrogen were introduced into the C6 position of purines by nucleophilic aromatic substitution (S_NAr) of 6-halopurine derivatives. The most commonly used synthesis of 6-benzylthio-substituted purines is a two-step reaction. In the first, 6-chloropurines are converted to 6-thiopurines, which are alkylated in the following step.[9-13] Another method for 2-chloro-6-benzylthio-substituted purine synthesis involves S_NAr displacement of 6-chlorine with benzylthio group in the presence of triethylamine as a base.[14] Synthesis of 6-oxobenzyl-substituted purines is based on the nucleophilic aromatic substitution of 6-chloropurine analogue with alkoxides in the presence of sodium metal[15] or with alcohols by the phase-transfer catalysis.[16,17]

The synthetic strategies adopted to synthesise our target compounds **6BnS-Pu**, **6BnS-2Cl-Pu**, and **6BnO-2Cl-Pu** are depicted in Scheme S1.

6BnS-Pu and **6BnS-2Cl-Pu** were obtained by one-pot synthesis through S_NAr reaction of the 6-chloropurines **6Cl-Pu** or **2,6-diCl-Pu** and benzyl mercaptan in the presence of triethylamine as a base. Recrystallization from the mixture of dichloromethane/hexane gave products **6BnS-Pu** and **6BnS-2Cl-Pu** in 35 % and 64 % yield, respectively.



Scheme S1. S_NAr displacement of 6-chlorine in **6Cl-Pu** and **2,6-diCl-Pu** with benzyl mercaptan and Pd-catalyzed coupling of **2,6-diCl-Pu** with benzyl alcohol.

When **2,6-diCl-Pu** was reacted with a large excess of benzyl alcohol in the presence of sodium metal, **6BnO-2Cl-Pu** was isolated in poor yield (~ 8 %) due to workup difficulties. The Vilarrasa method,[18] starting with protected 2,6-dichloropurine nucleoside and benzyl alcohol under Pd(0)-Xantphos catalysis, is known to give the corresponding 6-*O*-benzyl nucleoside in excellent yield. Using the same method, the reaction of **2,6-diCl-Pu** with benzyl alcohol in the presence of catalyst $Pd_2dba_3{\cdot}CHCl_3$, Xantphos as ligand, and Cs_2CO_3 in toluene gave **6BnO-2Cl-Pu** in 49 % yield.

General Information: Solvents were distilled from appropriate drying agents shortly before use. TLC was carried out on DC-plastikfolien Kieselgel 60 F254, and flash column chromatography was performed on silica gel Merck 0.040–0.063 mm. Melting points were determined on a Kofler hot-stage apparatus and were uncorrected. NMR spectra were recorded on AV600 and AV300 MHz spectrometers (Bruker BioSpin GmbH, Rheinstetten,

Germany), operating at 150.92 or 75.47 MHz for ^{13}C and 600.13 or 300.13 MHz for ^{1}H nuclei using DMSO-d_6 as the internal standard.

Synthesis:

6-(Benzylthio)-9*H*-purine, 6BnS-Pu

A mixture of **6Cl-Pu** (151 mg 0.98 mmol), benzyl mercaptan (138 µL, 1.18 mmol), and trimethylamine (164 µL, 1.18 mmol) in absolute ethanol (2.5 mL) was stirred at 60 °C for 20 h. After cooling, the reaction mixture was filtered, and the residue was washed with diethyl ether. Recrystallization from dichloromethane/hexane gave 82 mg (35 %) of product **6BnS-Pu** as a white solid: R_f = 0.61 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (DMSO-d_6) δ/ppm: 13.55 (s, 1H, NH), 8.73 (s, 1H, H-2), 8.43 (s, 1H, H-8), 7.58–7.04 (m, 5H, Ar), 4.65 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 151.4 (C-2), 143.0 (very broad signal, C-8), 137.8 (Cq, Ar), 128.9 (CH, Ar), 128.4 (CH, Ar), 127.1 (CH, Ar), 31.6 (CH_2). Signals for C-4, C-5, and C-6 are missing (Fig. S1). The proton and C-atom signals in the NMR spectra are consistent with the data in Ref.[10]

6-(Benzylthio)-2-chloro-9*H*-purine, 6BnS-2Cl-Pu

A mixture of **2,6-diCl-Pu** (137 mg 0.73 mmol), benzyl mercaptan (103 µL, 0.88 mmol), and trimethylamine (123 µL, 0.88 mmol) in absolute ethanol (2 mL) was stirred at 60 °C for 24 h. After cooling, the reaction mixture was filtered, and the residue was washed with diethyl ether. Recrystallization from dichloromethane/hexane gave 127 mg (64 %) of product **6BnS-2Cl-Pu** as a white solid: R_f = 0.7 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (DMSO-d_6) δ/ppm: 13.72 (s, 1H, NH), 8.48 (s, 1H, H-8), 7.58–7.12 (m, 5H, Ar), 4.60 (s, 2H, CH2); ^{13}C NMR (DMSO-d_6) δ/ppm: 151.8 (C-2), 144.6 (very broad signal, C-8), 137.3 (Cq, Ar), 129.1 (CH, Ar), 128.4 (CH, Ar), 127.3 (CH, Ar), 32.4 (CH_2). Signals for C-4, C-5, and C-6 are missing (Fig. S2).

6-(Benzyloxy)-2-chloro-9*H*-purine (5)

To a mixture of **2,6-diCl-Pu** (192 mg, 1.02 mmol), $Pd_2dba_3{\cdot}CHCl_3$ (53 mg, 51 µmol), Xantphos (91 mg, 153 µmol), and Cs_2CO_3 (470 mg, 1.43 mmol) in anhydrous toluene (5 mL), benzyl alcohol (112 µL, 1.08 mmol) was added. The flask was purged with argon, and the reaction mixture was stirred at 80 ºC for 28 h. Then the reaction mixture was diluted with CH_2Cl_2 (~60 mL), and filtered through a pad of Celite. The filtrate was concentrated under vacuum, and the residue was purified by flash chromatography (CH_2Cl_2/MeOH 9:1) to afford

6BnO-2Cl-Pu (122 mg, 49 %) as a white solid: R_f = 0.55 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (DMSO-d_6) δ/ppm: 13.65 (s, 1H, NH), 8.43 (s, 1H, H-8), 7.44 (m, 5H, Ar), 5.59 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 150.8 (C-2), 143.6 (very broad signal, C-8), 135.6 (Cq, Ar), 128.6 (CH, Ar), 128.4 (CH, Ar), 128.3 (CH, Ar), 68.8 (CH_2). Signals for C-4, C-5, and C-6 are missing (Fig. S3).

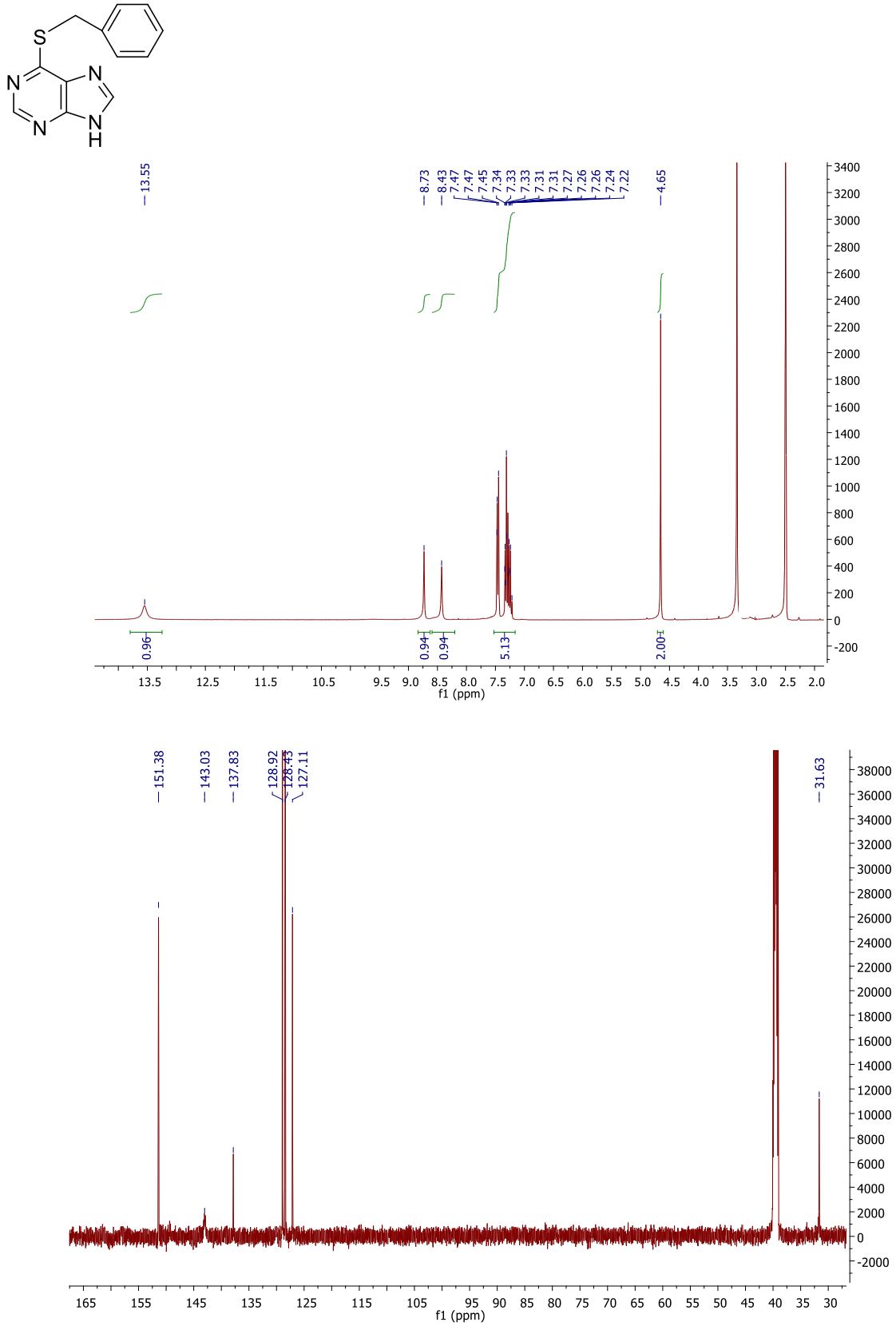


Figure S1. ^{1}H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (151 MHz, DMSO-d_6) spectra of **6BnS-Pu**.

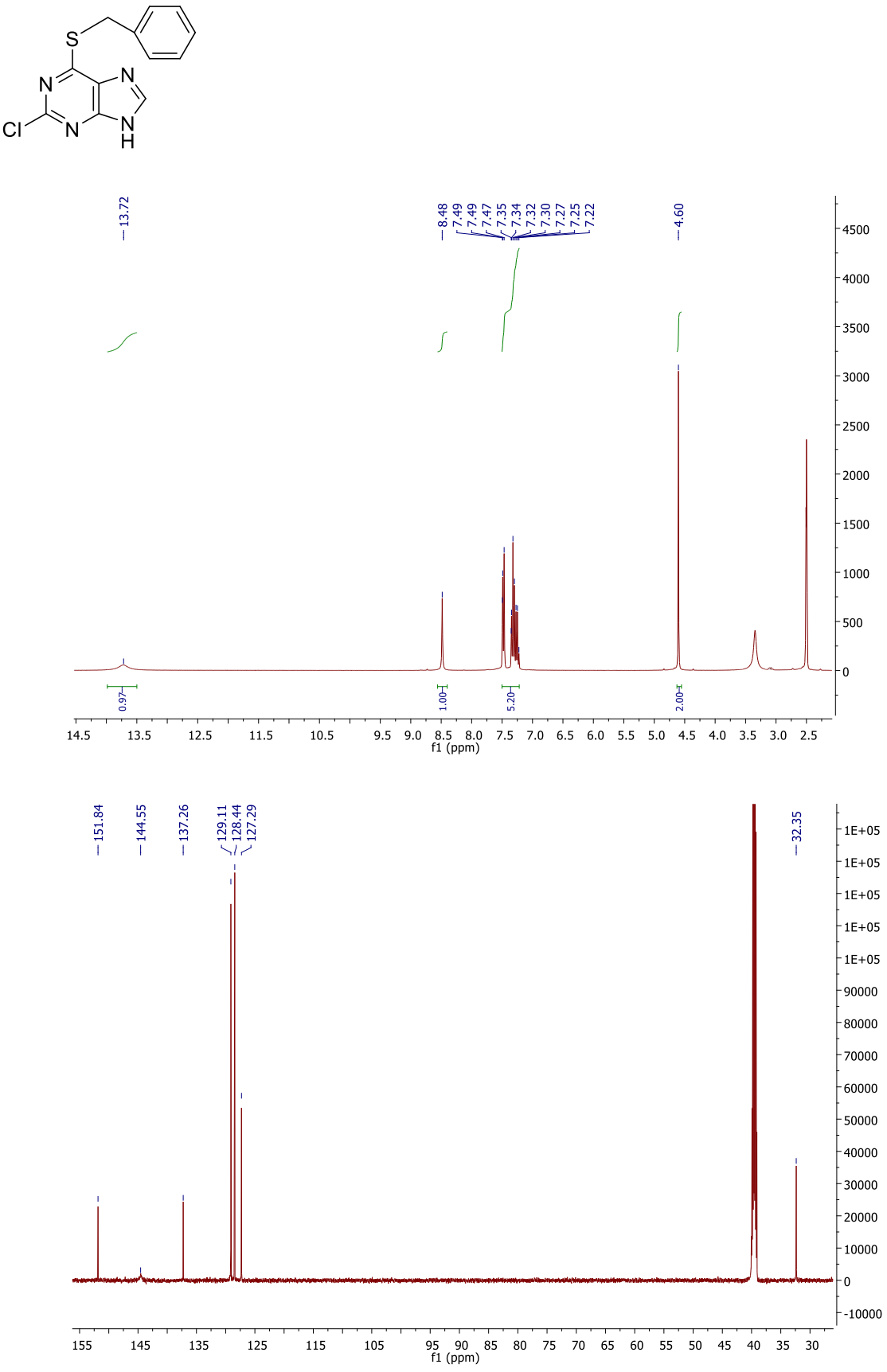


Figure S2. ^{1}H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (151 MHz, DMSO-d_6) spectra of **6BnS-2Cl-Pu**.

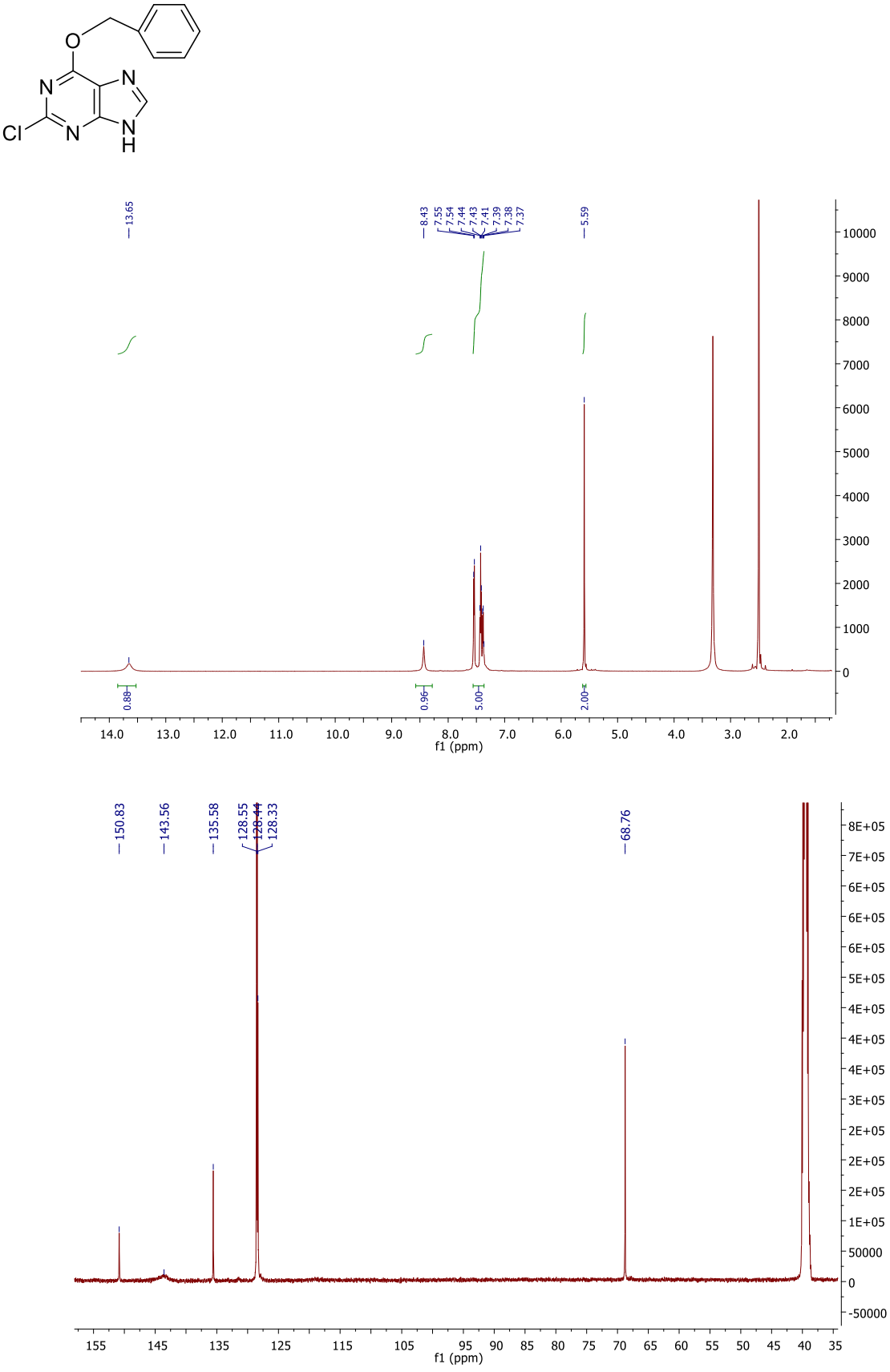


Figure S3. ^{1}H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (151 MHz, DMSO-d_6) spectra of **6BnO-2Cl-Pu**.

Inhibition of the *H. pylori* purine nucleoside phosphorylase

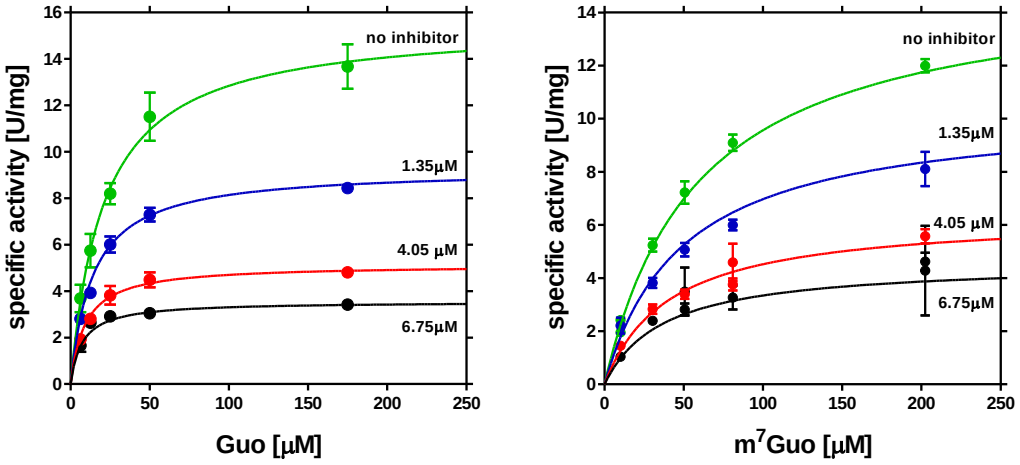


Figure S4. Inhibition of *H. pylori* PNP at 25°C, 50 mM Hepes/NaOH pH 7.0 by **6BnS-2Cl-Pu-9dr** with Guo (left panel) and m^7Guo (right panel) as variable substrates, in the presence of 50 mM phosphate as the second substrate. Initial velocity, v_o, *vs.* substrate concentration, with no inhibitor added (green circles) and with several various concentrations of the inhibitor; error bars represent SD; global fitting of mixed inhibition model (solid lines) is shown.

H. pylori cell culture inhibition

Table S1
Concentration of inhibitors in the solutions used to study their influence on the cell culture growth of *H. pylori* 26695 strain. In the experiment with the *H. pylori* cell culture inhibition, half of these concentrations were present (see Materials and methods).

Concentration in the diluted stocks [a] [mM]	Measured supernatant concentrations [b] after part of the inhibitor precipitated [mM]			
	6BnO-2Cl-Pu	**6BnS-2Cl-Pu**	**6BnS-2Cl-Pu-9-dr**	**6BnS-Pu**
0.70	0.190	0.080	-	-
0.60	0.180	0.057	-	-
0.40	0.120	0.050	0.0168	0.170
0.20	0.080	0.040	0.0208	0.138
0.14	-	0.034	-	-
0.10	-	-	0.0214	0.088
0.067	-	0.0165	-	-
0.05	-	-	0.0216	0.050
0.025	-	-	0.0144	0.025

[a]Based on weighted mass and the stock volume, intended to obtain, however part of the studied compound precipitated when 10 mM stock in DMSO was diluted with water. Nevertheless, the precipitated compound was present during the experiment

[b]Based on the UV spectrum of the stock, appropriate diluted with water to get absorbance at the maximum not exceeding 1, and using extinction coefficient 10,900 $M^{-1}cm^{-1}$ in the case of **6BnO-2Cl-Pu** and 18,000 $M^{-1}cm^{-1}$ in the case of 6BnS substituted inhibitors

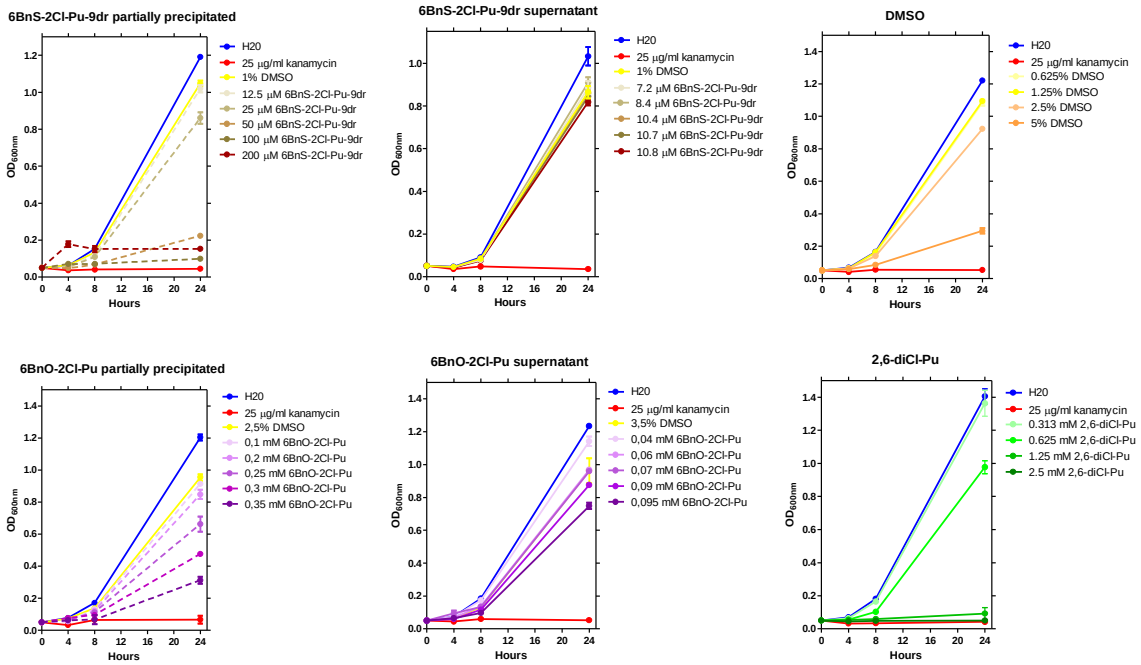
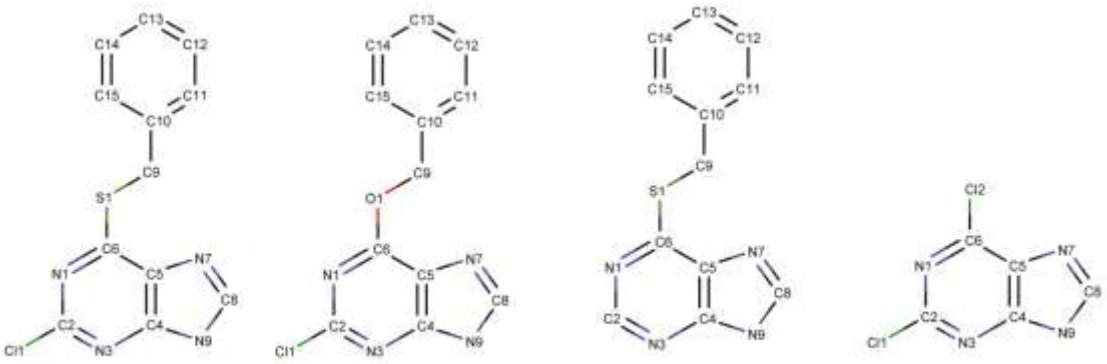


Figure S5. Growth curves of *H. pylori* 26695 strain in the presence of various concentrations of **6BnO-2Cl-Pu**, **6BnS-2Cl-Pu-9dr**, **2,6-diCl-Pu** and DMSO. For **6BnO-2Cl-Pu**, **6BnS-2Cl-Pu-9dr** two experiments are presented, showing the effects of the fully dissolved compound and of partially precipitated stocks of the same inhibitor (in which the actual concentration of dissolved inhibitor is lower). The stocks (supernatants) of the particular tested compound, and the respective filtered solution obtained from the respective stock are marked with the same color. Details are described in Materials and methods in the main manuscript.

X-**ray crystallography**



2-chloro-6-benzylthiopurine **6BnS-2Cl-Pu**

2-chloro-6-benzyloxypurine **6BnO-2Cl-Pu**

6-benzylthiopurine **6BnS-Pu**

2,6-dichloropurine **2,6-diCl-Pu**

Scheme S2. Atom numbering of ligands present in the X-ray structures obtained in this study.

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