SUPPORTING INFORMATION

Structure-based design of a bisphosphonate 5'(3')-deoxyribonucleotidase inhibitor

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SUPPORTING INFORMATION

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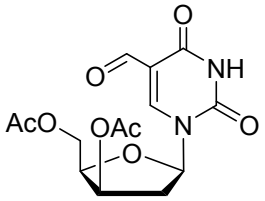
Chemistry Experimental NMR Validation Spectra

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). 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 (^1H 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 ^1H and 39.7 ppm for ^{13}C) or to the 1,4-dioxane signal (3.75 ppm for ^1H 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 ^1H 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.

5-Formyl-1-(3,5-di-*O*-acetyl-2-deoxy-β-D-threo-pentofuranosyl)uracil (5)

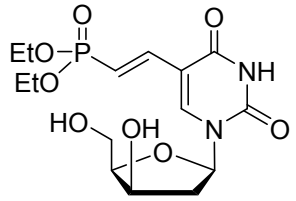


5

Starting nucleoside **4** (5.79 g, 17.8 mmol) was dissolved in CH_3CN (73 mL) and 2,6-lutidine (7.8 mL) was added. Separately, $Na_2S_2O_8$ (8.76 g, 35.6 mmol) and $CuSO_4.5H_2O$ (1.81 g, 7.12 mmol) was dissolved in H_2O (73 mL). The solutions were mixed and warm to 65 °C for 3 hours. The progress of reaction was monitored by TLC (5% EtOH/$CHCl_3$). When finished, reaction was dissolved in $CHCl_3$ (500 mL) and organic layer was extracted by aqueous 5% EDTA and finally with brine. Organic layer was dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel column with a linear gradient of EtOH in $CHCl_3$ (0→10%) to give aldehyde **5** (3.69 g, 61 %) as colorless solid. HRMS $(M+Na)^-$ for $C_{14}H_{16}O_8N_2Na$: calcd *m/z* 363.07989, obs. 363.07983; IR ($CHCl_3$, cm^{-1}) 3381, 3190, 3070, 2966, 2917, 2860, 1748, 1730, 1706, 1693, 1602, 1472, 1436, 1409, 1375, 1277, 1240, 1056, 612, 603. ^1H NMR (d_6DMSO) δ 11.80 (br s, NH), 9.83 (s, CH=O), 8.44 (s, H6), 6.14 (dd, *J* =

7.8 and 1.7 Hz, H1´), 5.33 (ddd, *J* = 5.1, 3.0 and 0.9 Hz, H3´), 4.37 (m, H5´a), 4.36 (m, H4´), 4.27 (m, H5´b), 2.78 (ddd, *J* = 15.4, 7.8 and 5.1 Hz, H2´´), 2.25 (ddd, *J* = 15.4, 1.7 and 0.9 Hz, H2´), 2.07 (s, 5'-OAc), 1.95 (s, 3'-OAc); ^{13}C NMR (d_6DMSO) δ 186.68 (CH=O), 170.41 (5'-O-CO-CH_3), 169.54 (3'-O-CO-CH_3), 162.19 (C4), 149.83 (C2), 146.02 (C6), 110.63 (C5), 85.89 (C1´), 80.68 (C4´), 72.30 (C3´), 61.61 (C5´), 38.52 (C2´), 20.76 (5'-O-CO-CH_3), 20.74 (3'-O-CO-CH_3).

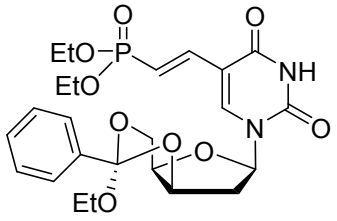
Diethyl 2-[1-(2-deoxy-β-D-threo-pentofuranosyl)uracil-5-yl]vinylphosphonate (6)



6

Tetraethyl methylendiphosphonate (0.729 mL, 2.94 mmol) was dissolved in dry THF (15 mL) and the solution was cooled under argon atmosphere to -20 °C. Then 60% NaH suspension in mineral oil (118 mg, 2.94 mmol) was added and the reaction mixture was slowly warmed to rt. After 1 hour the reaction mixture was cooled to -78 °C and the solution of aldehyde **5** (0.5 g, 1.47 mmol) in dry THF (5 mL) (before reaction was dried by co-evaporation with dry THF) was added. The resulting mixture was slowly warmed to rt and then stirred for 24 hours. The progress of reaction was monitored by TLC (10% EtOH/$CHCl_3$). When finished, the reaction mixture was poured on silica gel column and chromatographed with a linear gradient of EtOH in $CHCl_3$ (0→10%) to give a mixture of partially deacetylated products. This mixture was treated with 0.1M MeONa in dry MeOH (20 mL) under stirring overnight and then neutralized by the addition of solid DOWEX 50 in H^+ cycle. Filtration and evaporation of the solution provided desired vinylphosphonate **6** (373 mg, 65 %). HRMS $(M-H)^-$ for $C_{15}H_{22}O_8N_2P$: calcd *m/z* 389.11193, obs. 389.11191; IR ($CHCl_3$, cm^{-1}) 3387, 3207, 1713, 1693, 1622, 1466, 1436, 1394, 1369, 1313, 1283, 1240, 1164, 1131, 1091, 1053, 1029, 971, 546. ^{1}H NMR ($CDCl_3$) δ 9.29 (br s, NH), 8.51 (s, H6), 7.10 (dd, *J* = 24.5 and 17.3 Hz, C5-CH=), 6.79 (dd, *J* = 21.0 and 17.3 Hz, P-CH=), 6.18 (dd, *J* = 7.7 and 1.7 Hz, H1´), 4.56 (ddd, *J* = 5.0, 3.2 and 0.9 Hz, H3´), 4.14 (dd, *J* = 12.0 and 5.6 Hz, H5´a), 4.08 (dd, *J* = 12.0 and 3.8 Hz, H5´b), 4.06 (m, 4H, 2x P-O-CH_2), 4.02 (ddd, *J* = 5.6, 3.8 and 3.2 Hz, H4´), 2.60 (ddd, *J* = 15.0, 7.7 and 5.0 Hz, H2´´), 2.30 (ddd, *J* = 15.0, 1.7 and 0.9 Hz, H2´), 1.33 (t, *J* = 7.0 Hz, -CH_3), 1.32 (t, *J* = 7.0 Hz, -CH_3); ^{13}C NMR (d_6DMSO) δ 161.78 (C4), 149.32 (C2), 144.72 (C6), 142.05 (d, *J*(P,C) = 8.3 Hz, C5-CH=), 112.54 (d, *J*(P,C) = 188.7 Hz, P-CH=), 108.73 (d, *J*(P,C) = 24.0 Hz, C5), 85.88 (C1´), 84.47 (C4´), 70.65 (C3´), 62.29 (d, *J*(P,C) = 5.6 Hz, P-O-CH_2), 62.26 (d, *J*(P,C) = 5.6 Hz, P-O-CH_2), 60.47 (C5´), 41.59 (C2´), 16.27 (d, *J*(P,C) = 6.4 Hz, CH_3), 16.25 (d, *J*(P,C) = 6.6 Hz, CH_3).

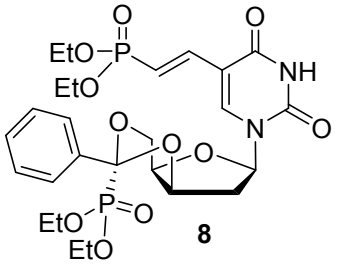
Diethyl 2-[1-(2-deoxy-3,5-*O*-methoxybenzylidene-β-D-threo-pentofuranosyl)uracil-5-yl]vinylphosphonate (7)



7

Vinylphosphonate **6** (373 mg, 0.96 mmol) was dried by co-evaporation with DMF, then dissolved in DMF (10 mL) under argon. Triethyl orthobenzoate (0.436 mL, 1.92 mmol) was added, the pH of reaction was adjusted to 3 (on wet pH paper) with dry HCl in Et_2O, and the reaction mixture was stirred at rt overnight. Progress of reaction was monitored by TLC (10% EtOH/$CHCl_3$). When finished, the reaction mixture was neutralized by Amberlyst 21, filtered, the solution was evaporated and the solid residue was chromatographed on silica gel column with a linear gradient of EtOH in $CHCl_3$ (0→10%) to give orthoester **7** (340 mg, 68 %) as colorless solid. HRMS $(M+H)^+$ for $C_{24}H_{32}O_9N_2P$: calcd *m/z* 523.18399, obs. 523.18407; IR ($CHCl_3$, cm^{-1}) 3388, 3183, 3064, 3028, 1711, 1690, 1621, 1604, 1580, 1495, 1464, 1453, 1434, 1393, 1382, 1368, 1360, 1240, 1162, 1132, 1110, 1099, 1058, 1031, 983, 965, 702, 611. ^{1}H NMR (d_6DMSO) δ 11.50 (br s, NH), 8.00 (s, H6), 7.43 (m, 2H, *ortho*-ArH), 7.36 (m, 2H, *meta*-ArH), 7.30 (m, 1H, *para*-ArH), 6.52 (dd, *J* = 20.9 and 17.2 Hz, C5-CH=), 6.46 (dd, *J* = 23.9 and 17.2 Hz, P-CH=), 5.98 (dd, *J* = 7.7 and 1.3 Hz, H1´), 4.70 (bdd, *J* = 4.6, 2.6 and <1 Hz, H3´), 4.40 (dd, *J* = 13.1 and 2.3 Hz, H5´a), 4.28 (dd, *J* = 13.1 and 1.0 Hz, H5´b), 4.17 (ddd, *J* = 2.6, 2.3 and 1.0 Hz, H4´), 3.88 (m, 4H, 2x P-O-CH_2), 3.15 (m, 2H, O-CH_2), 2.70 (ddd, *J* = 15.0, 7.7 and 4.6 Hz, H2´´), 2.24 (bdd, *J* = 15.0, 1.3 and <1 Hz, H2´), 1.23 (t, *J* = 7.1 Hz, -CH_3), 1.20 (t, *J* = 7.1 Hz, -CH_3), 1.07 (t, *J* = 7.1 Hz, -CH_3); ^{13}C NMR (d_6DMSO) δ 162.08 (C4), 149.29 (C2), 143.45 (C6), 141.12 (d, *J*(P,C) = 7.9 Hz, C5-CH=), 137.49 (*ipso*-ArC), 129.20 (*para*-ArC), 128.29 (2C, *meta*-ArC), 125.88 (2C, *ortho*-ArC), 112.79 (d, *J*(P,C) = 187.5 Hz, P-CH=), 109.91 (C6'), 107.27 (d, *J*(P,C) = 22.9 Hz, C5), 85.84 (C1´), 75.31 (C4´), 69.05 (C3´), 61.12 (d, *J*(P,C) = 5.5 Hz, P-O-CH_2), 61.06 (d, *J*(P,C) = 5.4 Hz, P-O-CH_2), 59.97 (C5´), 58.89 (O-CH_2), 39.50 (C2´), 16.47 (d, *J*(P,C) = 5.9 Hz, CH_3), 16.44 (d, *J*(P,C) = 5.8 Hz, CH_3), 14.76 (CH_3).

(*S*)-1-[2-Deoxy-3,5-*O*-(4-diethylphosphonobenzylidene)-β-D-threo-pentofuranosyl]-5-[(*E*)-2-(diethoxyphosphoryl)vinyl]uracil (8)

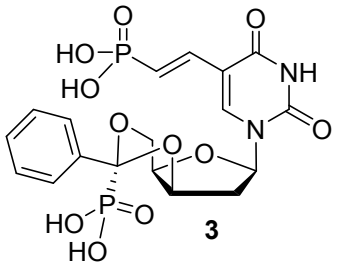


8

Orthoester **7** (340 mg, 0.65 mmol) was dried by co-evaporation with dry CH_3CN, then dissolved in CH_3CN (10 mL) under an argon atmosphere, and the solution was cooled to -40 °C. The diethyl chlorophosphite (187 μL, 1.3 mmol) was added and the solution was slowly warm to rt. Progress of reaction was monitored by TLC (10% EtOH/$CHCl_3$). When finished, the reaction mixture was cooled to -40 °C and quenched with 1M TEAB in 50% EtOH (1 mL). The mixture was evaporated, the residue was co-distilled with EtOH and chromatographed on silica gel column with a linear gradient of EtOH in $CHCl_3$ (0→10%) to give bisphosphonate **8** (316 mg, 79 %) as colorless solid. HRMS $(M+H)^+$ for $C_{26}H_{37}O_{11}N_2P_2$: calcd *m/z* 615.18671, obs. 615.18675; IR ($CHCl_3$, cm^{-1}) 3389, 3170, 3063, 2993, 1710, 1690, 1621, 1603, 1494, 1464, 1452, 1433, 1393, 1369, 1316, 1300, 1281, 1239, 1163, 1133, 1097, 1080, 1053, 1035, 990, 969, 913, 698, 617. ^{1}H NMR (d_6DMSO) δ 11.56 (br s, NH), 7.75 (s, H6), 7.43 (m, 2H, *ortho*-ArH), 7.33 (m, 2H, *meta*-ArH), 7.23 (m, 1H, *para*-ArH), 6.41 (dd, *J* = 20.8 and 17.2 Hz, C5-CH=), 6.18 (dd, *J* = 24.0 and 17.2 Hz, P-CH=), 5.98 (dd, *J* = 7.9 and 1.7 Hz, H1´), 5.20 (ddd, *J* = 4.8, 2.5 and 2.0 Hz, H3´), 4.83 (ddd, *J* = 13.2, 3.3 and 2.0 Hz, H5´a), 4.25 (m, H4' and H5´b), 3.95 (m, 8H, 4x P-O-CH_2), 2.77 (ddd, *J* = 15.2, 7.9 and 4.8 Hz, H2´´), 2.43 (dd, *J* = 15.2 and 1.7 Hz, H2´), 1.27 (t, *J* = 7.2 Hz, -CH_3), 1.22 (t, *J* = 7.2 Hz, -

CH_3), 1.17 (t, *J* = 7.2 Hz, -CH_3), 1.12 (t, *J* = 7.2 Hz, -CH_3); ^{13}C NMR (d_6DMSO) δ 161.92 (C4), 149.35 (C2), 143.61 (C6), 140.82 (d, *J*(P,C) = 8.4 Hz, C5-CH=), 137.69 (d, *J*(P,C) = 8.8 Hz, *ipso*-ArC), 128.94 (*para*-ArC), 127.81 (2C, *meta*-ArC), 127.07 (2C, *ortho*-ArC), 112.84 (d, *J*(P,C) = 187.7 Hz, P-CH=), 107.27 (d, *J*(P,C) = 23.1 Hz, C5), 97.11 (d, *J*(P,C) = 190.7 Hz,C6'), 85.76 (C1´), 75.94 (C4´), 71.71 (C3´), 63.08 (d, *J*(P,C) = 6.8 Hz, P-O-CH_2), 63.02 (C5´), 63.00 (d, *J*(P,C) = 6.8 Hz, P-O-CH_2), 61.06 (d, *J*(P,C) = 5.5 Hz, P-O-CH_2), 61.01 (d, *J*(P,C) = 5.4 Hz, P-O-CH_2), 39.47 (C2´), 16.51 (d, *J*(P,C) = 6.3 Hz, CH_3), 16.47 (d, *J*(P,C) = 6.7 Hz, CH_3), 16.43 (d, *J*(P,C) = 6.6 Hz, CH_3), 16.39 (d, *J*(P,C) = 5.7 Hz, CH_3).

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



3

Tetraethyl bisphosphonate **8** (316 mg, 0.51 mmol) dried by co-evaporation with CH_3CN was treated under an argon atmosphere with bromotrimethylsilane (673 μl, 5.1 mmol) and 2,6-lutidine (239 μl, 2 mmol) in dry MeCN (10 mL) at rt overnight (TLC isopropanol-25% ammonia-water 7:1:2). When finished, the reaction mixture was concentrated, 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 (0→100%). Yield 87 mg (32 %). The product was converted into the sodium salt on Dowex 50 in Na^+ form. HRMS $(M-H)^-$ for $C_{18}H_{19}O_{11}N_2P_2$: calcd *m/z* 501.04696, found 501.04727; IR (KBr, cm^{-1}) 3433, 3244, 3065, 1695, 1624, 1472, 1449, 1413, 1315, 1286, 1137, 1065, 987, 921, 765, 703. 617. ^{1}H NMR (D_2O) δ 7.75 (s, H6), 7.45 (m, 2H, *ortho*-ArH), 7.30 (m, 3H, *meta*-ArH and *para*-ArH), 6.19 (dd, *J* = 17.7 and 17.5 Hz, P-CH=), 6.13 (dd, *J* = 21.8 and 17.5 Hz, C5-CH=), 5.98 (dd, *J* =5.7 and 2.6 Hz, H1´), 5.30 (m, H3´), 4.94 (ddd, *J* = 13.2, 2.9 and 1.2 Hz, H5´a), 4.34 (ddd, *J* = 2.9, 2.8 and 2.0 Hz, m, H4'), 4.27 (dd, *J* = 13.2 and 2.0 Hz, and H5´b), 2.72 (m, H-2' and H2´´); ^{13}C NMR (D_2O) δ 165.96 (C4), 153.49 (C2), 144.38 (C6), 142.68 (d, *J*(P,C) = 8.0 Hz, *ipso*-ArC), 135.40 (d, *J*(P,C) = 7.1 Hz, C5-CH=), 131.17 (*para*-ArC), 130.52 (2C, *meta*-ArC), 128.66 (2C, *ortho*-ArC), 124.40 (d, *J*(P,C) = 178.9 Hz, P-CH=), 112.23 (d, *J*(P,C) = 22.8 Hz, C5), 101.30 (d, *J*(P,C) = 179.4 Hz,C6'), 90.19 (C1´), 80.58 (C4´), 73.31 (C3´), 65.25 (C5´), 42.36 (C2´); ^{31}P NMR (D_2O) 12.72 (dd, *J* = 11.0 and 7.5 Hz, P-CH=CH-), 10.82 (s, P-Ph).

Biochemistry Experimental

Protein preparation. Both enzymes were prepared according to published protocols [1, 2], already modified in [3].

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 °C 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.

Inhibition assay. To determine the K_i values, a series of activity assays were performed in the presence of compounds **1** and **3**. 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 [4, 5]. The true K_i value was then calculated presuming the competitive type of inhibition.

X-ray crystallography experimental

Crystallization. For crystallization of mdN, the conditions successfully used by others were utilized [2, 3, 6]. The crystals of mdN were obtained using the hanging drop vapour diffusion technique with a precipitating solution containing 20 mM KH_2PO_4, PEG 8000 8% (w/v), glycerol 10% (v/v) whereas nuclei from similar drop were transferred using a streak seeding technique with the cat whisker [7]. The crystals grew to a final size within three days. The crystals were soaked for 12h in 1 μL crystallization drop supplemented with 10 mM compound **3** and 90 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.

Successful crystallization of cdN worked based on previously used procedure [2, 3, 6]. Crystals of cdN were obtained using the hanging drop vapor diffusion technique at 291 K by mixing 1 μl of protein (11.8 $mg.ml^{-1}$) with 1 μl of reservoir solution containing 40 mM KH_2PO_4, pH 4.5, 5% (w/v) PEG 8000, and 20% (v/v) glycerol. Crystals of final size were transferred to 5 μl drop of precipitating solution with PEG 8000 concentration raised to 16% (w/v). Lately 0.5 μl of solution with 31 mM compound **3** was slowly added. After 48 hours soaking with final concentration of compound **3** equal 2.8 mM, crystals were fished out, cryoprotected by soaking for 10 s in a reservoir solution supplemented with 30% (v/v) glycerol and flash-cooled by plunging into liquid nitrogen. Until the X-ray diffraction experiments crystals were stored in liquid nitrogen.

Data collection and structure determination. The diffraction data for crystal of mdN soaked with compound **3** were collected at 100 K at the MX14.1 beamline of the BESSY, Berlin, Germany[8] at a wavelength of 0.915 Å. The diffraction data to 1.50 Å resolution were

integrated and reduced using using XDS.[9] Crystal 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 1.

The diffraction data for crystal of cdN soaked with compounds **3** were collected at 100 K at the MX14.2 beamline of the BESSY, Berlin, Germany at a wavelength of 0.918 Å. The diffraction data to resolution were integrated and reduced using XDS [9] and its graphical interface XDSAPP [10]. Crystal exhibited the symmetry of space group *P*1 and contained two molecules in the asymmetric unit with. Crystal parameters and data collection statistics are summarized in Table 1.

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 [11] Program Coot [12] was used for model building. The final refinement steps included translation-libration-screw (TLS) refinement [13, 14] using 4 TLS groups. The refinement statistics are given in Table 1.

The structure of cdN was determined by molecular replacement using program MOLREP [15] with coordinates from the structure of the identical protein (PDB code 4L57). Refinement was carried out using the program REFMAC 5.5 [11] Program Coot [12] was used for model building. The final refinement steps included translation-libration-screw (TLS) refinement [13, 14] using 8 TLS groups. The refinement statistics are given in Table 1.

The quality of the final models was validated with Molprobity [16]. All figures showing structural representations were prepared with the program PyMOL [17]. The atomic coordinates and experimental structure factors have been deposited with the Protein Data Bank under the accession codes 4YIH and 4YIK

Table S1. Crystal parameters, data collection and refinement statistics

Data collection statistics		
Compound	**mdN**	**cdN**
PDB code	4YIK	4YIH
Space group	$P4_32_12$	$P1$
Cell parameters (Å, º)	a = b = 73.5 c = 105.4 α = β = γ= 90	a =38.8 b =46.5 c = 61.7 α = 111.8 β = 88.2 γ= 104.7
Number of molecules in AU	1	2
Wavelength (Å)	0.915	0.918
Resolution (Å)	28.05-1.48 (1.56-1.48)	31.42-1.82 (1.93-1.82)
Number of unique reflections	49,438 (7,108)	33,500 (5,331)
Multiplicity	8.0 (7.6)	2.2 (2.2)
Completeness (%)	100 (100)	95.8 (94.1)
R_{meas}[a]	7.3 (85.7)	6.2 (62.5)
Average $I/\sigma(I)$	15.3 (2.5)	12.3 (2.1)
Wilson B (Å^2)[b]	25.3	35.7
Refinement statistics		
Resolution range (Å)	28.05-1.48	31.42-1.82
No. of reflections in working set	46,824	31,786
No. of reflections in test set	2,497	1,672
R value (%)[b]	15.3	18.5
R_{free} value (%)[c]	17.6	22.8
RMSD bond length (Å)	0.017	0.020
RMSD angle (º)	1.935	2.083
Number of atoms in AU	2,040	3350
Number of protein atoms in AU	1680	3069
Number of water molecules in AU	268	197
Mean B value (Å^2)	22.6	36.9

Ramachandran plot statistics[d]:		
Residues in favored regions (%)	96.8	97.0
Residues in allowed regions (%)	3.2	3.0

[a] R_{meas} defined in ref. [18]. [b] Wilson B by Sfcheck program from CCP4 suite [19, 20]. [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 [21]. [d] as determined by MolProbity [16].

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