Pseudo-complementary PNA actuators as reversible switches in dynamic DNA nanotechnology

Damian Ackermann, Michael Famulok*

LIMES Institute, Chemical Biology and Medicinal Chemistry Unit, c/o Kekulé Institute of Organic Chemistry and Biochemistry, University of Bonn, Gerhard-Domagk-Strasse 1, 53121 Bonn, Germany

CONTENT.....PAGE

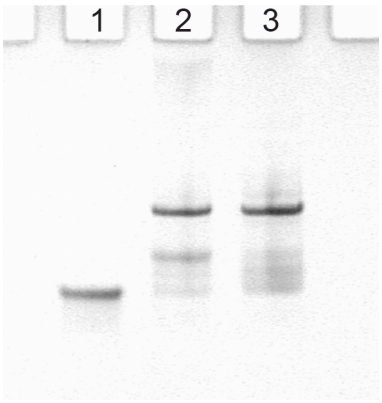
Supplementary Table T1 – **Oligonucleotides used in this study**

name	sequence
spherical stopper	
Ring1 r (56)	5'phos-CGCGCTTTTTGCGCGTTTTTTCCGTCTTTTTGGCACTTTTTTCTCGCTTTTTAGAT
RingSE a (54)	5'phos-GACGGAAAAACTGTCAAAAAATGTTCAAAAAGTGCAGCACCTCACGTCTCATGG
RingSE b (52)	5'phos-TCGTGCCATGAGACGTGAGGTGCTGCTGGAAAAAATATCTAAAAAGCGAGAA
GE-1 (39)	5'phos-AAGCGAGAAAAAAGTGCCAAAAAGACGGAAAAAACGCGC
GE-2 (42)	5'phos-AAAAAGCGCGAAAAAAGGGTCTTTTTGGCACTTTTTTCTCGC
GE-3 (45)	5'phos-TTTTTAGATATTTTTTCCAACTTTTTGAACATTTTTTGACAGTTT
GE-4 (42)	5'phos-TTCCGTCTTTTTTGCGTCAAAAAGTTGGAAAAAATATCTAAA
HJalpha b (53)	5'phos-AAAAGTGCCAAAAAGACCAAAAACTGTCAAAAAACGGAGAAAAATGTTCAAAA
HJalpha c (61)	5'phos-TTTCCATATTTTTTGAACATTTTTCTCCGTTTTTTGACAGTTTTTGGCGGAAAAAACGCGC
HJalpha d (33)	5'phos-CGCGCTTTTTGCGCGTTTTTTCCGCCCTTTTTT
Bogen f (47)	5'phos-AAAAAGCGCGAAAAAAGGGCCAAAAACTGTCAAAAAACGGAGAAAAA
Bogen r (47)	5'phos-TTTTTGAACATTTTTCTCCGTTTTTTGACAGTTTTTGGCCCTTTTTT
HJbeta a (56)	5'phos-AATATGGAAAAACGCGCAAAAAAGACGGAAAAACTGTCAAAAAATGTCGCAAAAAA
HJbeta kc (32)	5'phos-ATTTTTTCCAACTTTTTGAGCTTTTTCCATAT
HJbeta kd (60)	5'phos-TGTTCAAAAAATATGGAAAAAGCACATTTTTTGACAGTTTTTCCGTCTTTTTTGCGCGTT
PGR126	
AGRalpha f (57)	5'-AAATAATGAAAAAAGTGCCAAAAAGACGGAAAAAACGCGCAAAAAGCGCGAAAAAAG
AGRalpha r (47)	5'phos-TTTGGCCCTTTTTTCGCGCTTTTTGCGCGTTTTTTCCGTCTTTTTGG
AGRbeta f (55)	5'phos-GGCCAAAAACTGGCAAAAAAGACGGAAAAACTGTCAAAAAATGTTCAAAAAGCGG
AGRbeta r (35)	5'phos-ATTTTTTGACAGTTTTTCCGTCTTTTTTGCCAGTT
PGRgamma r (44)	5'phos-CACTTTTTTCATTATTTTCCGAGTTACAACTCCGCTTTTTGAAC
PGR-RO p (14)	5'phos-AGTTGTAACTCGGA
pcRod	
EFCT-2 (25)	5'phos-CACGATCCAGGTACAGTAACTGTCA
EFCT-1 (20)	5'-TTAGTTCACAGGGATAACAG
rodA-DNA (19)	5'phos-CACGACTGTTATCCCTGTG
SD5-DNA (20)	5'phos-AACTAASGSDDCSCTGACTG
rodC-DNA (14)	5'phos-TTGCTGTACCTGGA
pc-anti-RO (14)	5'TCDDCDSSGDGCCT
PNA	
psiPNA (14)	NH_2-Lys-TCDDCDSSGDGCCT-Lys-H

S: 2-thiouracil (for PNA); 2-thiothymine (for DNA). D: 2,6-diaminopurin.

PNA: -NH_2 = carboxy end (carboxamide); -H = amino end.

Figure S1
PAGE of pcRod assembly



10% PAGE of pcRod assembly. Lane 1: mixture of unligated ODNs; lane 2: product of ligation, main band corresponds to pcRod; lane 3: reference DNA rod (EFCT-1 and EFCT-2 hybridized with a corresponding unmodified 53-mer DNA strand).

Figure S2
Secondary structure of PGR gap-ring

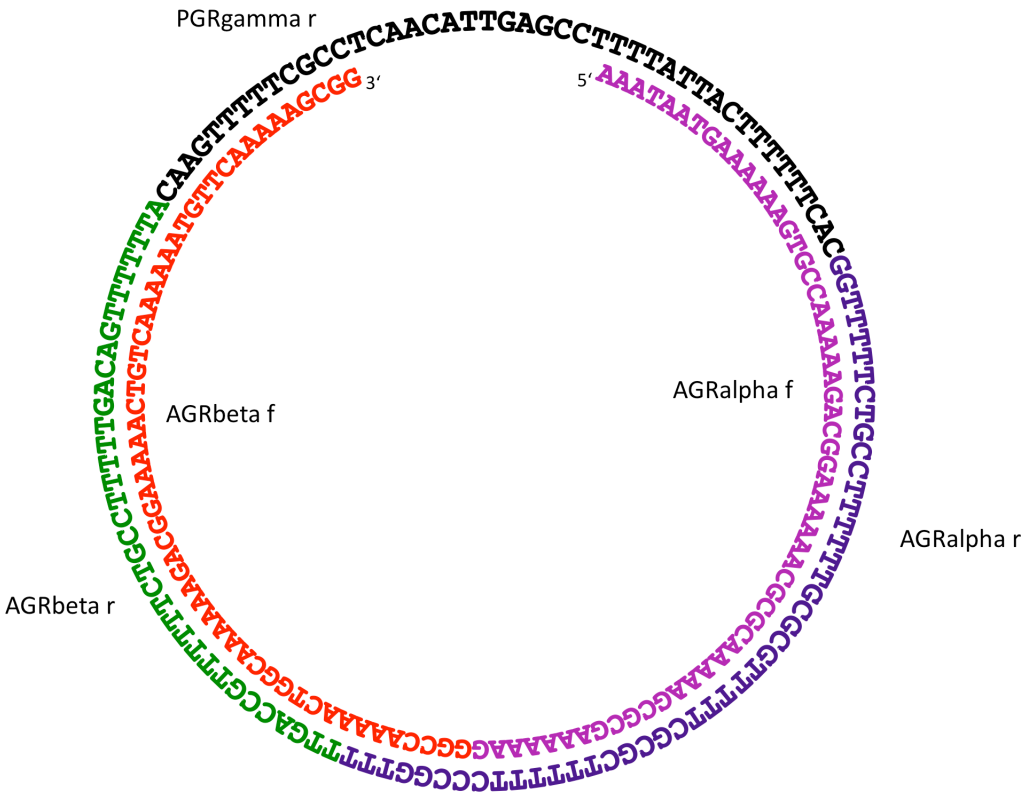


Figure S3
Secondary structure of spherical stoppers

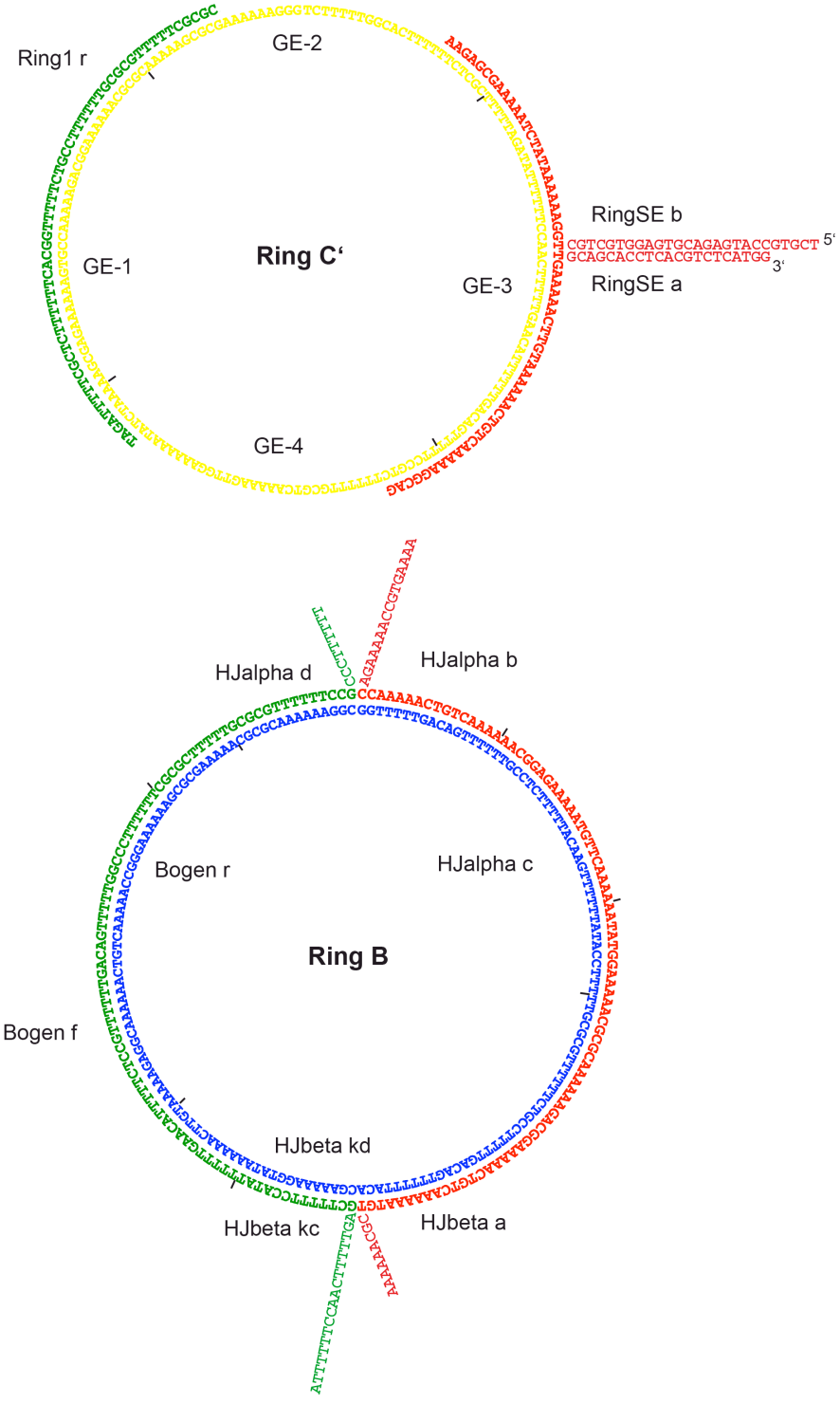


Figure S4
Crystal structure of 2-thiothymidine (10)
Measured on a Nonius KappaCCD.

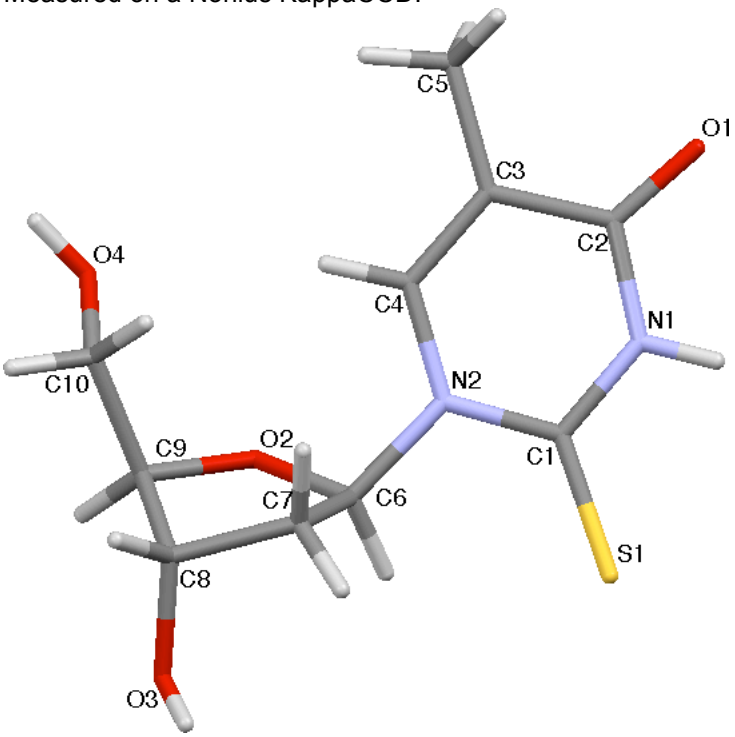
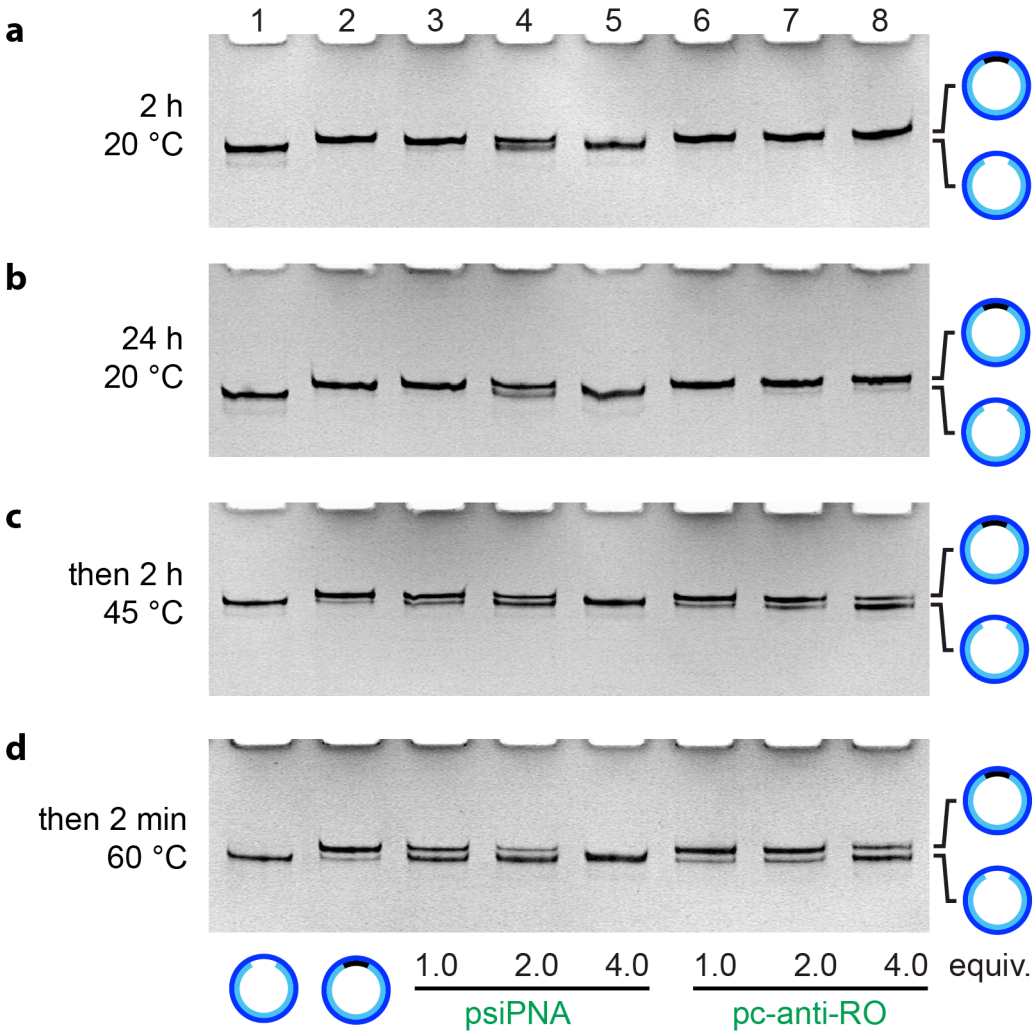


Table of atomic coordinates (x 10^4) for **10**.

	x	y	z
C(1)	10826	1030	3923
C(2)	13446	477	1605
C(3)	13953	1245	990
C(4)	12860	1842	1793
C(5)	15682	1345	-538
C(6)	10134	2434	4029
C(7)	12137	2820	5795
C(8)	11347	3668	5498
C(9)	10539	3740	3185
C(10)	12976	3943	2356
N(1)	11898	431	3059
N(2)	11289	1742	3212
O(1)	14251	-129	974
O(2)	9423	2987	2449
O(3)	8980	3834	6272
O(4)	12288	3871	197
S(1)	9102	865	5715

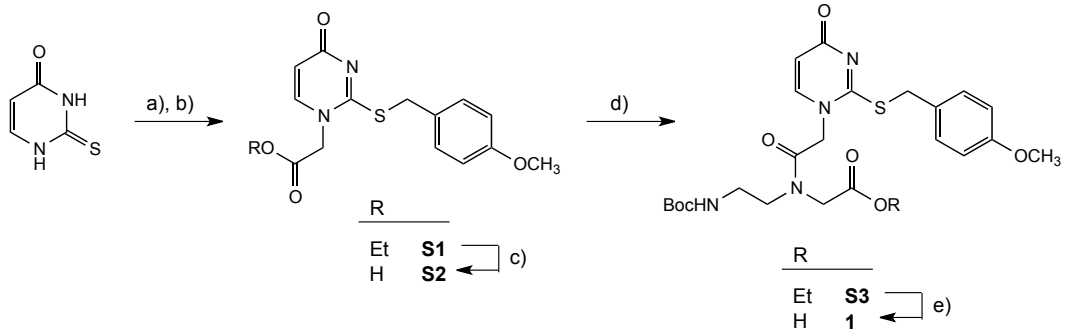
Figure S5
PAGE of macrocycle invasion studies



Polyacrylamide gels (10 %) of the macrocycle invasion studies after incubation at indicated conditions. Lane 1: PGR (reference). Lane 2: PGR·PGR-RO nanoring. Lane 3-5: dsDNA nanoring incubated with 1.0, 2.0, and 4.0 equiv. of psiPNA. Lane 6-8: dsDNA nanoring incubated with 1.0, 2.0, and 4.0 equiv. of pc-anti-RO.
At 20 °C the displacement occurs only in the presence of psiPNA but not pc-anti-RO (a). Even after 24 h the pc-anti-RO does not displace the oligo from the macrocycle (b). When the sample is warmed up to 45 °C for 2 h, partial displacement occurs also in case of pc-anti-RO (c). In PAGE (d) the samples were warmed up to 60 °C for only 2 min. At this temperature the PGR-RO and the macrocycle are completely dissociated so that all involved components re-hybridize “statistically” in the thermodynamically favoured state. This is reflected by the band intensities given for free gap-ring and the full macrocycle. In PAGE (c) the PGR-RO partially dissociates from the macrocycle and represents an intermediary event between (a) and (d).

Supplementary Methods

Synthesis of 2-thiouracil PNA monomer (1)



Scheme S1. a) NaOH, 4-methoxybenzyl chloride, EtOH/H_2O (1:1), 45°, 60 min, 77%. b) NaH, ethyl bromoacetate, CH_3CN, 0° → rt, 2 h, 40%. c) 2.0 M LiOH, THF/MeOH (1:1), rt, 10 min, 51%. d) Ethyl *N*-(Boc-aminoethyl)glycinate, HBTU, DMF, EtN^iPr_2, rt, 2h, 80%. e) 2.0 M LiOH, THF, 0°, 6 min, 77%.

***N*-1-(Ethyloxycarboxymethyl)-*S*-(4-methoxybenzyl)-2-thiouracil (S1)**. A solution of 2-thiouracil (19.4 g, 151 mmol) in EtOH (90 ml) was mixed with a solution of NaOH (7.2 g, 166 mmol) in H_2O (90 ml) and warmed to 45 °C. 4-Methoxybenzyl chloride (22.7 ml, 180 mmol) was added during 5 min and the suspension stirred for 60 min at 45 °C. After removing EtOH under reduced pressure, the mixture was neutralized by the addition of sat. aq. $NaHCO_3$ soln. The product was filtered, washed 2 x with H_2O and 3 x with Et_2O to give the Pmb-protected 2-thiouracil (28.9 g, 77%) as white solid. This intermediate (13.4 g, 54 mmol) was suspended in CH_3CN (170 ml), cooled to 4 °C, and slowly treated with freshly activated NaH (from NaH 60% dispersion in mineral oil, 2.27 g, 56.7 mmol) and then allowed to warm to room temperature. Ethyl bromoacetate (9.0 ml, 81 mmol) was drop wise added during 10 min. After stirring for 120 min the reaction was stopped by neutralization with 1.0 M aq. phosphate buffer soln. (200 ml, pH 7.0). The mixture was filtered and, after removal of CH_3CN under reduced pressure, partitioned between CH_2Cl_2/^{i}PrOH 9:1 (200 ml) and the aq. phosphate buffer. The organic layer was dried with $MgSO_4$, evaporated under reduced pressure and the crude was adsorbed on SiO_2 (30 g). CC (silica gel (150 g), CH_2Cl_2 → CH_2Cl_2/MeOH 94:6) gave **S1** (7.1 g, 40%) as yellow oil. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.72 (d, *J* = 7.5 Hz, 1H, H-C(6)), 7.47 - 7.30 (m, 2H, arom. H), 7.00 - 6.84 (m, 2H, arom. H), 5.99 (d, *J* = 7.5 Hz, 1H, H-C(5)), 4.84 (s, 2H, NCH_2), 4.41 (s, 2H, $BnCH_2$), 4.22 (q, *J* = 7.1 Hz, 2H, OCH_2CH_3), 3.78 (s, 3H, OCH_3), 1.23 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3). ^{13}C-NMR (75 MHz, DMSO-d_6) δ 167.41, 166.79, 162.72, 159.12, 145.97, 130.85, 128.35, 114.33, 109.11, 62.14, 55.52, 52.87, 40.86, 34.79, 14.34. ESI-MS (pos.) 335.1 ($[M+H]^+$, $C_{16}H_{19}N_2O_4S^+$; calc. 335.1).

***N*-1-(Carboxymethyl)-*S*-(4-methoxybenzyl)-2-thiouracil (S2)**. A solution of **S1** (7.1 g, 21.2 mmol) in THF/MeOH 1:1 (200 ml) was treated with 2.0 M aq. LiOH soln. (23 ml) for 10 min. at room temperature. After addition of 1.0 M aq. HCl (46 ml) the organic solvents were slowly removed under reduced pressure whereby the product precipitated. The solid was filtered, washed 2 x with H_2O and 3 x with Et_2O to give **S2** (3.30 g, 51%) as white powder. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 13.6 (br. s, 1H, COOH), 7.67 (d, *J* = 7.5 Hz, 1H), 7.39 - 7.26 (m, 2H, arom. H), 6.95 - 6.79 (m, 2H, arom. H), 5.92 (d, *J* = 7.5 Hz, 1H, H-C(5)), 4.66 (s, 2H, $CH_2N(1)$), 4.34 (d, *J* = 5.4 Hz, 2H, $BnCH_2$), 3.72 (s, 3H, OCH_3). ^{13}C-NMR (101 MHz, DMSO) δ 168.37, 166.58, 162.37, 158.70, 145.71, 130.46, 127.95, 113.94, 108.55, 55.11, 52.61, 34.36. ESI-MS (pos.) 307.0 ($[M+H]^+$, $C_{14}H_{15}N_2O_4S^+$; calc. 307.1).

Ethyl-*N*-(2-(*t*-butyloxycarbonylamino)ethyl)-*N*-(*S*-(4-methoxybenzyl)-2-thiouracil-1-ylacetyl)glycinate (S3). A solution of **S2** (3.30 g, 10.7 mmol) and EtN^iPr_2 (1.67 g, 12.9 mmol) in DMF (30 ml) was treated with HBTU (4.9 g, 12.9 mmol) for 5 min at room temperature. Then a solution of ethyl *N*-(Boc-aminoethyl)glycinate (3.18 g, 12.9 mmol) in DMF (10 ml) was added and the mixture stirred at room temperature for 18 h. The mixture was partitioned between CH_2Cl_2 and sat. aq. $NaHCO_3$ soln., the organic layer washed with 10% aq. citric acid soln., dried with $MgSO_4$ and evaporated under reduced pressure. The crude was adsorbed on SiO_2 (20 g). CC (silica gel (75 g), CH_2Cl_2 → CH_2Cl_2/MeOH 98:2) gave **S3** (4.63 g, 80%) as yellow foam. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.48 and 7.46 [d (maj/min), *J* = 7.5 Hz, 1H, H-C(6)] 7.34 - 7.30 (m, 2H, arom. H), 6.90 and 6.71 [t (maj/min), *J* = 5.9 Hz, 1H, NHBoc], 6.88 - 6.84 (m,

2H, arom. H), 5.91 and 5.89 [d (maj/min), *J* = 7.5 Hz, 1H, H-C(5)], 4.96 and 4.76 [s (maj/min), 2H, $CH_2N(1)$], 4.34, and 4.26 [s (maj/min), 2H, $BnCH_2$], 4.12 [q (min), *J* = 7.1 Hz, 0.66H, OCH_2CH_3], 4.08 - 4.02 [m, 3.33H, CH_2COOEt, (maj) OCH_2CH_3], 3.72 (s, 3H, OCH_3), 3.39 - 3.29 (overlayed with H_2O signal), and 3.17 - 3.14 and 3.02 - 3.00 (2m, 4H, NCH_2CH_2N), 1.35 and 1.32 [s (min/maj), 9H, $C(CH_3)_3$], 1.17 and 1.15 [t (min/maj), *J* = 7.1 Hz, 3H, OCH_2CH_3]. ^{13}C-NMR (101 MHz, DMSO-d_6) δ 169.55 and 169.13 (min/maj), 167.03 and 166.99 (maj/min), 166.58 and 166.29 (min/maj), 163.10, 159.09, 156.16, 146.20 and 146.15 (min/maj), 130.85, 128.33, 114.34 and 114.31 (maj/min), 108.94 and 108.90 (maj/min), 78.51 and 78.21 (maj/min), 61.67 and 61.00 (min/maj), 55.51 and 55.33 (maj/min), 53.06 and 52.67 (min/maj), 49.44, 48.37, 47.44, 38.57 and 37.96 (maj/min), 34.91, 28.62 and 28.55 (min/maj), 14.42 and 14.39 (maj/min). ESI-MS (pos.) 535.4 ($[M+H]^+$, $C_{25}H_{35}N_4O_7S^+$; calc. 535.2).

***N*-(2-(*t*-butyloxycarbonylamino)ethyl)-*N*-(*S*-(4-methoxybenzyl)-2-thiouracil-1-ylacetyl)glycine (1)**. **S3** (2.79 g, 5.2 mmol) was dissolved in THF/H_2O 2:1 (65 ml), cooled to 4 °C and treated with 2.0 M aq. LiOH soln. (7.8 ml). After stirring for 10 min at 4 °C the reaction was neutralized by the addition of 1.0 M aq. HCl soln. (15.6 ml) and the mixture evaporated to dryness. The crude was suspended in CH_2Cl_2/^{i}PrOH 9:1 (50 ml) by sonication. After addition of EtOAc (100 ml) the solid was filtered, washed 3 x with H_2O and 3 x with Et_2O to give the **1** (2.04 g, 77%) as white powder. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 12.7 (br. s, 1H, COOH), 7.49 and 7.45 [d (maj/min), *J* = 7.6 Hz, 1H, H-C(6)], 7.35 - 7.31 (m, 2H, arom. H), 6.91 and 6.72 [t (maj/min), *J* = 5.9 Hz, 1H, NHBoc], 6.88 - 6.84 (m, 2H, arom. H), 5.91 and 5.89 [d (maj/min), *J* = 7.6 Hz, 1H, H-C(5)], 4.95 and 4.75 [s (maj/min), 2H, $CH_2N(1)$], 4.34, and 4.32 [s (maj/min), 2H, $BnCH_2$], 4.13 and 3.97 [s (min/maj), 2H, CH_2COO], 3.72 (s, 3H, OCH_3), 3.36 and 3.30 [t (maj/min), *J* = 6.6 Hz, 2H, NCH_2CH_2NH], 3.18 – 3.13 and 3.03 – 2.99 [m (maj/min), 2H, NCH_2CH_2NH], 1.35 and 1.32 [s (min/maj), 9H, $C(CH_3)_3$]. ^{13}C-NMR (101 MHz, DMSO-d_6) δ 170.71 and 170.25 (min/maj), 166.67 and 166.63 (min/maj), 166.16 and 165.72 (maj/min), 162.76 and 162.72 (min/maj), 163.10 and 159.09 (maj/min), 156.76 and 155.57 (maj/min), 145.84 and 145.80 (min/maj), 130.45 and 130.43 (maj/min), 128.04 and 127.94 (maj/min), 113.94 and 113.92 (maj/min), 108.52 and 108.45 (maj/min), 78.08, and 77.78 (maj/min), 55.10, 52.64 and 52.30 (min/maj), 49.22 and 47.68 (min/maj), 47.06 and 46.91 (min/maj), 38.10 and 37.52 (maj/min), 34.54, 34.49 (min/maj), 28.23 and 28.16 (min/maj). ESI-MS (pos.) 507.3 ($[M+H]^+$, $C_{23}H_{31}N_4O_7S^+$; calc. 507.2).