SUPPORTING INFORMATION

Characterization of DNA Minor Groove Binding Alkylating Agents

Prema Iyer, Ajay Srinivasan, Sreelekha K. Singh, Gerard P. Mascara, Elise Fouquerel, Sevara Zayitova, Brian Sidone, David Svilar, Robert W. Sobol, Michael S. Bobola, John R. Silber and Barry Gold

Chem. Res. Toxicol.

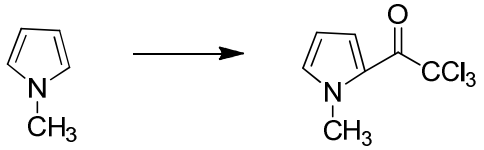
Details for the synthesis and characterization of all compounds (**Scheme S1.**).

Figure S1. Sample HPLC trace of control calf thymus DNA and DNA treated with compound **7.**

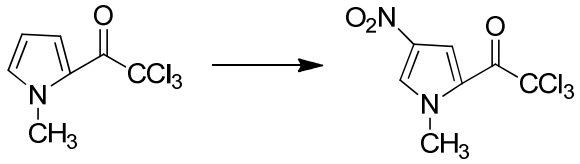
Figure S2. Temperature-dependent UV melts of calf thymus DNA in the absence and presence of the minor groove binding compounds.

Figure S3. Temperature-dependent UV melts of poly-d(A-T)·poly-d(A-T) DNA in the absence and presence of the minor groove binding compounds.

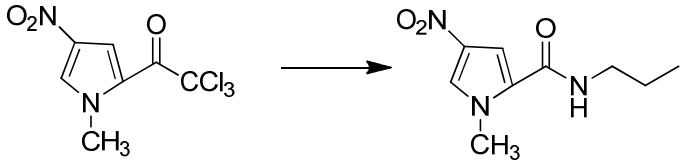
Synthesis of 1



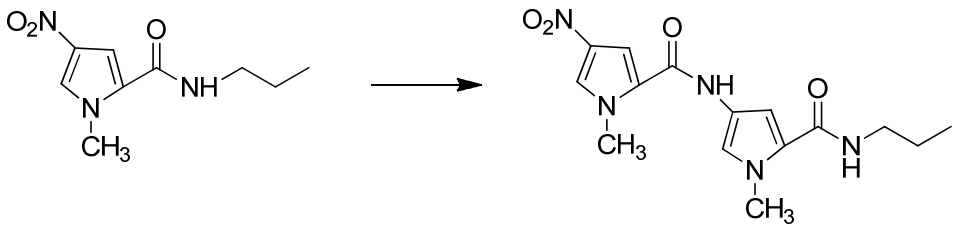
To a well stirred solution of trichloroacetyl chloride (100 mmol, 8.112 g) in 28 mL of anhydrous diethyl ether was added drop wwase a solution of *N*-methyl pyrrole (100 mmol, 8.9 mL) in 28 mL of anhydrous diethyl ether over a period of 30-40 min. The reaction mixture was stirred for another 1 h. The reaction mixture was quenched by the dropwise addition of a solution of K_2CO_3 .The organic layer was separated and the solvent evaporated to get a crystalline solid (18.26g, 80%). ^{1}HNMR (400 MHz, $CDCl_3$) δ 3.97 (s, 3H), 6.22 (apparent dd, 1H, J = 2.2 and 4.4), 6.97 (apparent t, 1H, J = 1.83), 7.50(dd, 1H, J = 1.47and 4.4).



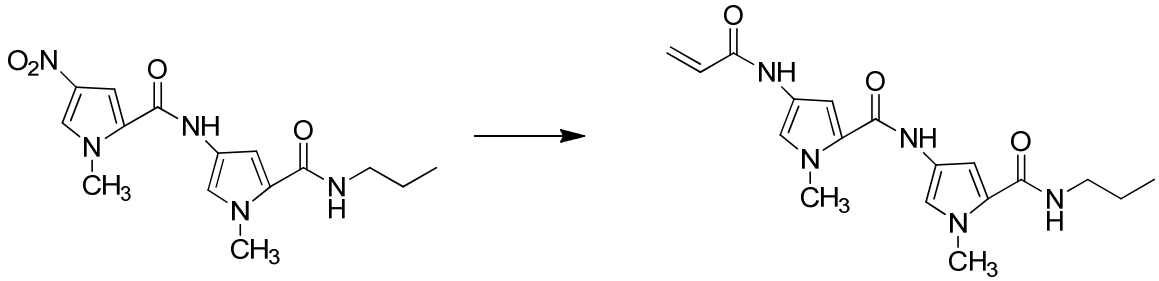
To a well stirred solution of 2, 2, 2-trichloro-1-(1-methyl-*1H*-pyrrol-2-yl)ethanone (22.076 mmole, 5 g) in acetic anhydride (50 mL) was added fuming $HN0_3$ (2.5 mL) at - 40º. The reaction mixture was maintained at -40 ºC for 45 min and the reaction mixture allowed to come to room temperature and then stirred for an additional 1 h. The solution was cooled in an ice bath and cold H_2O was added dropwise (30-40 mL). A pale yellow solid separated that was filtered and dried. The solid was then crystallized from EtOH to isolate the 4-nitro isomer (3.75 g, 69%) from the 3-nitro isomer. ^{1}HNMR (400 MHz, $CDCl_3$) δ 4.06 (s, 3H), 7.76 (apparent d, 1H, J = 0.73), 7.95 (d, 1H, J = 1.83).



To a solution of 2, 2, 2-trichloro-1-(1-methyl-4-nitro-*1H*-pyrrol-2-yl)ethanone (12.781 mmol, 3.47 g) in 96 mL of EtOAc was added propylamine (31.953 mmol, 2.6 mL) dropwise at 0 ºC. The reaction mixture was stirred at 0 ºC for an additional 5 min and the ice bath removed. The reaction mixture was then stirred for an additional 1h. The solvent was evaporated to dryness to yield product (2.64g, 98%). ^{1}HNMR (400 MHz, $CDCl_3$) δ 0.98 (t, 3H, J = 7.42), 1.57-1.66 (m, 2H), 3.32-3.367 (m, 2H), 3.98 (s, 3H), 6.00 (s, br, 1H), 7.05 (d, 1H, J = 1.56), 7.55 (d, 1H, J = 1.56).

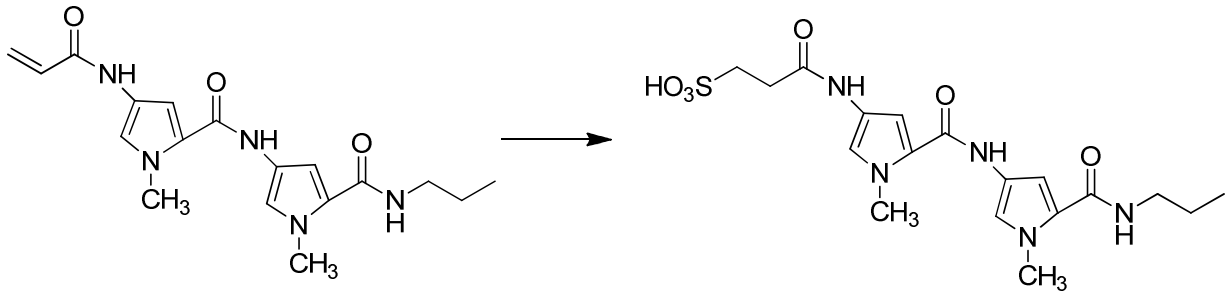


To a solution of 1-methyl-4-nitro-N-propyl-*1H*-pyrrole-2-carboxamide (4.734 mmole, 1 g) in EtOH (300 mL) was added 10% Pd-C (306 mg) and the reaction mixture shaken in a Parr apparatus at 50 psi till complete disappearance of starting material (based on TLC). The reaction mixture was filtered through a celite pad and the solvents evaporated to give 4-amino-1-methyl-N-propyl-*1H*-pyrrole-2-carboxamide (4.734 mmol). It was dissolved in EtOAc (6 mL) and the solution cooled to 0 ºC and to this was added 2, 2, 2-trichloro-1-(1-methyl-4-nitro-*1H*-pyrrol-2-yl)ethanone (6.154 mmol, 1.67 g) in EtOAc (15 mL) and the reaction mixture allowed to come to room temperature and stirred for 2 days. At end of 2 days, the solid that had precipitated was filtered off and washed with cold EtOAc and hexane, and dried thoroughly to give the product (1.074g, 73.66%). ^{1}HNMR (400 MHz, DMSO-d_6) δ 0.86 (t, 3H, *J* = 7.2), 1.44-1.53 (m, 2H), 3.09-3.15 (m, 2H), 3.80 (s, 3H), 3.95 (s, 3H), 6.84 (d, 1H, *J* = 1.96), 7.20 (d, 1H, *J* = 1.95), 7.58 (d, 1H, *J* = 1.95), 8.06 (t,1H, *J* = 5.47), 8.19 (d,1H,*J* = 1.95), 10.24 (s,1H).

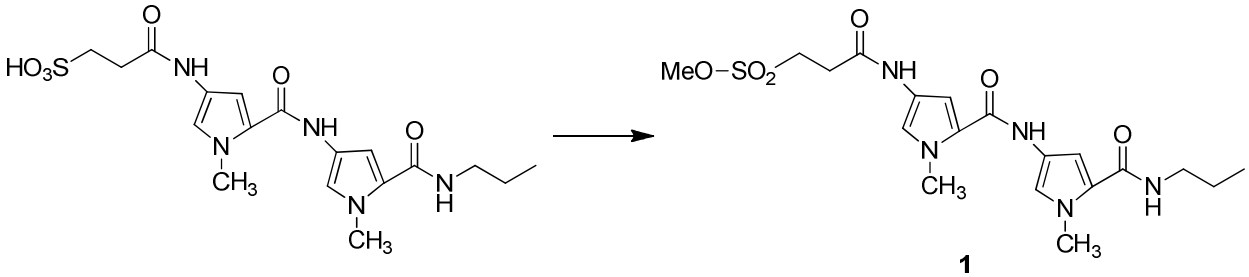


To a solution of 1-methyl-4-(1-methyl-4-nitro-*1H*-pyrrole-2-carboxamido)-N-propyl-*1H*-pyrrole-2-carboxamide (2.099 mole, 700 mg) in EtOH (380 mL) was added 10% Pd-C (350 mg) and the reaction mixture shaken in a Parr apparatus at 50 psi till complete disappearance of starting material (based on TLC). The reaction mixture was filtered through a celite pad and the solvents evaporated to give 4-amino-1-methyl-N-(1-methyl-5-(propylcarbamoyl)-*1H*-pyrrol-3-yl)-*1H*-pyrrole-2-carboxamide (2.099 mmol) that was dissolved in anhydrous THF (53 mL). To this was added di-isopropyl ethylamine (6.297 mmol, 1.1 mL). The reaction mixture was cooled to -78 ºC and acryloyl chloride (2.729 mmol, 222 µL) was added dropwise at -78 ºC. The reaction mixture was maintained at -78 ºC and in dark till disappearance of amine in the TLC plate and the reaction was slowly allowed to come to room temperature. THF was removed and H_2O added to the residue. The aqueous layer was extracted with CH_2Cl_2. The organic extracts were combined, dried over Na_2SO_4 and solvent evaporated to dryness. The compound was then isolated by silica gel column chromatography to yield the product which was yellow in color (510 mg, 68%).This yellow solid was then boiled in EtOAc to yield a white solid which was used for subsequent

syntheses. ^{1}HNMR (400MHz, DMSOd$_6$) δ 0.86(t, 3H, *J* = 7.42), 1.44-1.53 (m, 2H), 3.08-3.15 (m, 2H), 3.79 (s, 3H), 3.84 (s, 3H), 5.67(dd, 1H, *J* = 1.95 and 10.15), 6.18 (dd, 1H, *J* = 1.95 and 17.19), 6.37(dd, 1H, *J* = 10.15 and 17.18), 6.84(d, 1H, *J* = 1.56), 6.91 (d, 1H, *J* = 1.56), 7.18(d, 1H, *J* = 1.56), 7.27(d, 1H, *J* = 1.17), 8.01 (t, 1H, *J* = 5.47), 9.89 (s, 1H), 10.12 (s, 1H).



To a solution of 4-acrylamido-1-methyl-N-(1-methyl-5-(propylcarbamoyl)-*1H*-pyrrol-3-yl)-*1H*-pyrrole-2-carboxamide (1.259 mmol, 450 mg) in EtOH: H_2O (4:1, 25 mL) was added $NaHSO_3$ solution (2.518 mmol, 0.267 g) in 3.9 mL of H_2O. The pH of the reaction mixture was then adjusted to 8.0 using 5% NaOH solution. The reaction mixture was then refluxed until complete disappearance of alkene (based on TLC). The reaction mixture was cooled to 0 ºC and conc. HCl added dropwise so that the pH remained between 1 and 2. The solvents are evaporated to dryness and the solid residue treated with cold 1 N cold HCl and then centrifuged (4000 rpm, 10 min). The supernatant was decanted and the remaining solid was twice treated with 1 N cold HCl, centrifuged and the supernatant decanted. The white solid obtained was then dried thoroughly to give the product (510 mg, 92%). ^{1}HNMR (400 MHz, DMSO-d$_6$) δ 0.81 (t, 3H, *J* = 7.42), 1.44-1.53 (m, 2H), 2.54-2.56 (m, 2H), 2.65-2.69 (m, 2H), 3.08-3.14 (m, 2H), 3.78 (s, 3H), 3.81 (s, 3H), 6.84 (d, 1H, *J* = 1.95), 6.85 (d, 1H, *J* = 1.56), 7.15 (d, 1H, *J* = 1.95), 7.17 (d, 1H, *J* = 1.56), 8.00 (t, 1H, *J* = 5.47), 9.85 (s, 1H), 9.97 (s, 1H).

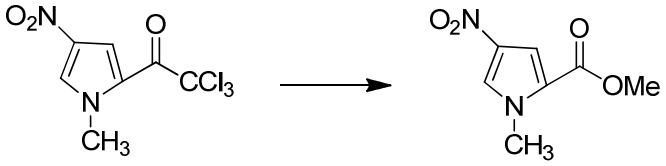


1

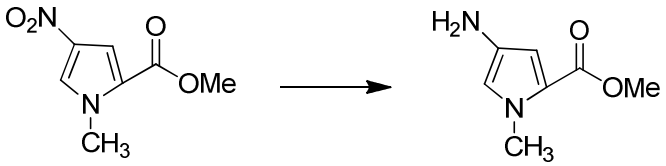
To a solution of 3-((1-methyl-5-((1-methyl-5-(propylcarbamoyl)-*1H*-pyrrol-3-yl) carbamoyl)-*1H*-pyrrol-3-yl) amino)-3-oxopropane-1-sulfonic acid (1.024 mmol, 450 mg) in dry dioxane (43mL) was added 3-methyl-1-*p*-tolyltriaz-1-ene (6.144 mmol, 917 mg). The reaction was heated in an oil bath at 80 ºC for about 1 h. The dioxane was evaporated to minimum volume and the residue loaded onto a silica gel column deactivated with Et_3N. The product was eluted with EtOAc (400 mg, 86%). ^{1}HNMR (400 MHz, DMS-d$_6$) δ 0.86 (t, 3H, *J* = 7.42), 1.44-1.53 (m, 2H), 2.73 (t, 2H, *J* = 7.42), 3.09-3.14 (m, 2H), 3.61 (t, 2H, *J* = 7.42), 3.79 (s, 3H), 3.83 (s, 3H), 3.86 (s, 3H), 6.84 (d, 1H, *J* = 1.96), 6.86 (d, 1H, *J* = 1.95), 7.17 (d, 2H, *J* = 1.57), 7.99 (t, 1H, *J* =

5.47), 9.86 (s, 1H), 10.06 (s, 1H). HRMS (ES+Na) m/z calcd for $C_{19}H_{27}N_5O_6NaS$ (476.1580) Found (476.1594).

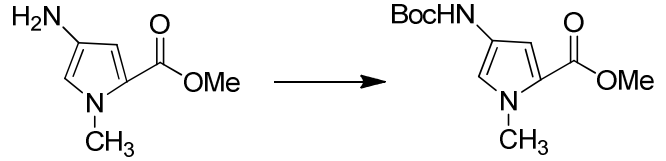
Synthesis of 2



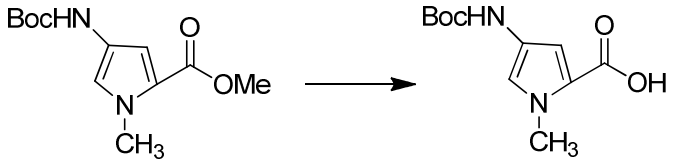
The synthesis of **2** followed previously reported method [Kumar, R., and Lown, J. W. (2003) Design, synthesis and *in vitro* cytotoxicity studies of novel pyrrolo[*2,1*][*1,4*] benzodiazepine-glycosylated pyrrole and imidazole polyamide conjugates. *Org. Biomol. Chem. 1*, 3327-3342.].



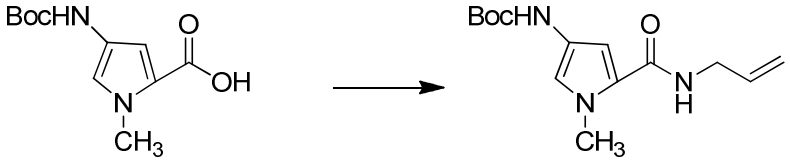
To a solution of methyl 1-methyl-4-nitro-*1H*-pyrrole-2-carboxylate (5.98 mmol, 1.1 g) in min amount of EtOAc (50 mL) and MeOH (350 mL) was added 10% Pd/C catalyst (389 mg) and the reaction mixture shaken in a Parr apparatus at 55 psi until complete disappearance of starting material (by TLC). The reaction mixture was filtered through a celite pad and solvents evaporated to dryness to afford the amine in quantitative yield (0.890 g, 96.73%). ^{1}HNMR (400 MHz, DMSO-d_6) δ 3.67 (s, 3H), 3.70 (s, 3H), 3.90 (br, 2H), 6.21 (d, 1H, J = 2.34), 6.41 (d, 1H, J = 2.34).



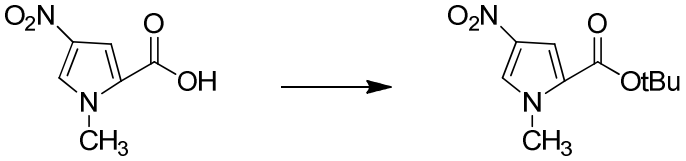
To a solution of methyl 4-amino-1-methyl-1H-pyrrole-2-carboxylate (5.778 mmol, 890 mg) in dry CH_2Cl_2 (40 mL) was added Et_3N (17.331 mmol, 2.4 mL). The reaction mixture was cooled to 0 ºC and Boc anhydride added (1.89 g, 8.67 mmol) in CH_2Cl_2 (15 mL). The reaction mixture was allowed to warm to room temperature and stirred for~ 18 h under N_2. The reaction mixture was diluted with CH_2Cl_2 and washed with H_2O. The aqueous layer was extracted thoroughly with EtOAc. The organic layers were combined and dried over Na_2SO_4 and solvents evaporated to dryness. The residue was purified by silica gel column chromatography to yield the product (1.065 g, 72.55%). ^{1}HNMR (400 MHz, DMSO-d_6) δ 1.44 (s, 9H), 3.70 (s, 3H), 3.78 (s, 3H), 6.61 (br, s, 1H), 7.10 (br, s, 1H), 9.12δ (s, br, 1H).



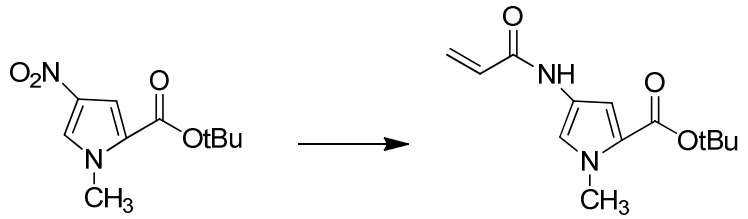
To a solution of methyl 4-((tert-butoxycarbonyl)amino)-1-methyl-*1H*-pyrrole-2-carboxylate (4.163 mmol,1.058 g) in MeOH (33 mL) was added solution of NaOH (826.54 mg in 33 mL of H_2O). The reaction mixture was refluxed at 65 ºC until complete disappearance of starting material (based on TLC). The MeOH was evaporated to minimum volume and the reaction mixture was cooled to 0 ºC with ice and acidified with Dowex 50WX4 ion exchange resin to pH 5-6. The resin beads were filtered and washed thoroughly with MeOH. The solvents were evaporated to dryness and the solid material obtained was then dried (908 mg, 90.8%). ^{1}HNMR (400 MHz, DMSO-d_6) δ 1.43 (s, 9H), 3.76 (s, 3H), 6.24 (br, s, 1H), 6.70 (br, s, 1H), 8.80 (s, br, 1H).



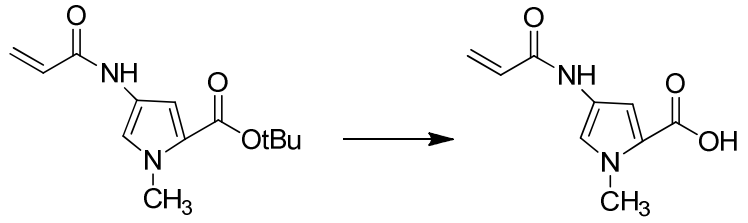
To a solution of 4-((tert-butoxycarbonyl)amino)-1-methyl-*1H*-pyrrole-2-carboxylic acid (3.721 mmol, 894 mg) and HOBT (5.582 mmol, 754 mg) in CH_2Cl_2 (20 mL) was added Et_3N (14.884 mmol, 2 mL). This was followed by the addition of EDC (7.442 mmol, 1.43 g) in CH_2Cl_2 (25 mL). The reaction mixture was stirred under N_2 at room temperature for 1 h and then cooled to 0 ºC and allyl amine (7.442 mmol, 557 µL) was added. The reaction mixture was allowed to come to room temperature and stirred for ~ 18 h. The white solid that precipitated was filtered off and the filtrate diluted with CH_2Cl_2 and washed with H_2O. The aqueous layer was extracted thoroughly with EtOAc. The combined organic layers were dried over Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel column chromatography to yield the product (854 mg, 82%). ^{1}HNMR (400 MHz, DMSO-d_6) δ 1.44 (s, 9H), 3.75 (s, 3H), 3.76-3.78 (m, 2H), 5.04 (dd, 1H, J = 10.16 and 1.95), 5.11 (dd, 1H, J = 17.18 and 1.95), 5.79-5.87 (m, 1H), 6.66 (br, s, 1H), 6.84 (br, s, 1H), 8.14 (t, 1H, J = 5.47), 9.04 (s, br, 1H).



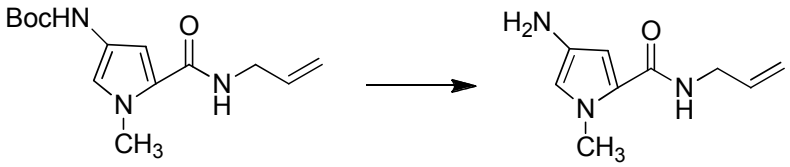
Synthesis followed previously published procedure [Wurtz, N. R., Turner, J. M., Baird, E. E., and Dervan, P. B. (2001) Fmoc solid phase synthesis of polyamides containing pyrrole and imidazole amino acids. *Org.Lett. 3*, 1201-1203.].



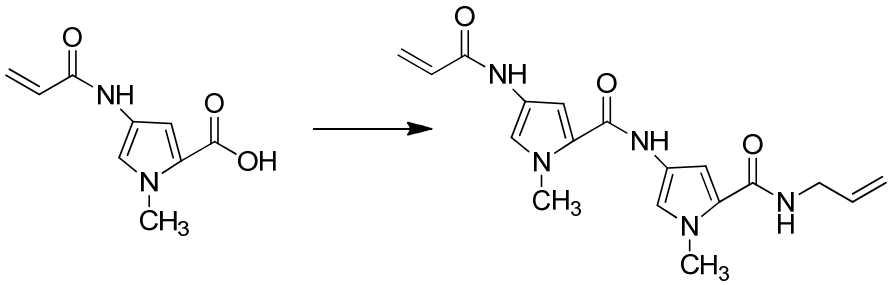
To a solution of tert-butyl 1-methyl-4-nitro-*1H*-pyrrole-2-carboxylate (6.188 mmol, 1.4 g) in MeOH (350 mL) was added 10% Pd-C (1 g) and the reaction mixture shaken in a Parr apparatus at 50 psi until complete disappearance of starting material (based on TLC). The reaction mixture was filtered through a celite pad and the solvents evaporated to give the amine (5.401 mmol, 1.06 g) that was dissolved in anhydrous THF (70 mL). To this was added diisopropylethylamine (16.203 mmol, 2.8 mL). The reaction mixture was cooled to -78 ºC and acryloyl chloride (8.102 mmol, 658 µL) was added dropwise at -78 ºC. The reaction mixture was maintained at -78 ºC in the dark until the disappearance of amine (based on TLC) and the reaction was then slowly allowed to warm to room temperature. THF was evaporated and H_2O added to the residue. The aqueous layer was extracted with CH_2Cl_2. The organic extracts are combined, dried over Na_2SO_4 and solvent evaporated to dryness. The compound was then isolated by silica gel column chromatography to yield the product (1.0388 g, 77%). ^{1}HNMR (400 MHz, DMSO-d_6) δ 1.49 (s, 9H), 3.79 (s, 3H), 5.67 (dd, 1H, *J* = 10.16 and 2.34), 6.16 (dd, 1H, *J* = 17.19 and 2.34), 6.27-6.34 (m,1H), 6.71 (d,1H, *J* = 1.95), 7.34 (d, 1H, *J* = 1.95), 10.08 (br, s, 1H,-N*H*)



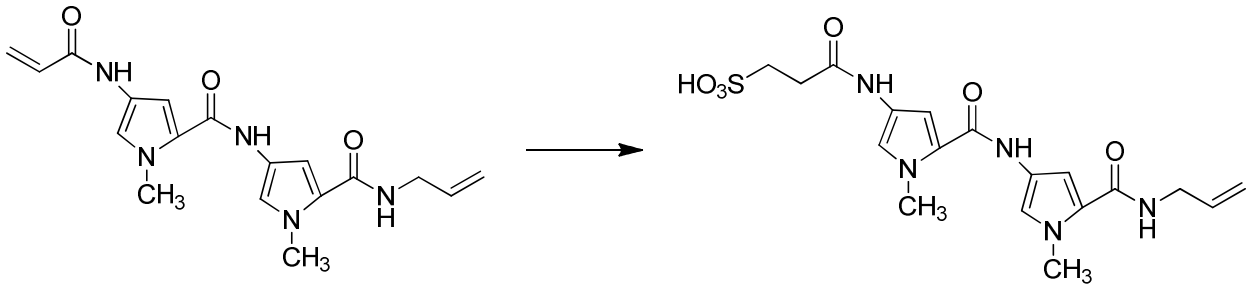
To a solution of tert-butyl 4-acrylamido-1-methyl-*1H*-pyrrole-2-carboxylate (4.12 mmol, 1.032 g) in CH_2Cl_2 (30 mL) was added 1M $TiCl_4$ solution (10.31 mmol, 10 mL) at 0 ºC. The reaction mixture was stirred at 0 ºC and slowly allowed to come to room temperature until complete disappearance of starting material. The reaction mixture was diluted with CH_2Cl_2 and washed with H_2O. The aqueous layer was extracted thoroughly with EtOAc (4 x 50 mL). The organic solvents was dried over Na_2SO_4 and evaporated to dryness to give the product (782 mg, 97.62%). ^{1}HNMR (400 MHz, DMSO-d_6) δ 3.81 (s, 3H), 5.67 (dd, 1H, *J* = 9.77 and 1.96), 6.17 (dd, 1H, *J* = 17.18 and 1.96), 6.29-6.35 (m, 1H), 6.71 (d, 1H, *J* = 1.95), 7.38 (d, 1H, *J* = 1.95), 10.09 (br, s),12.22 (br, s, 1H).



To a solution of tert-butyl (5-(allylcarbamoyl)-1-methyl-*1H*-pyrrol-3-yl)carbamate (2.585 mmol, 722 mg,) in anhydrous CH_2Cl_2 (13 mL) was added 50% TFA:CH_2Cl_2 (38 mL) at 0 ºC such that the final concentration of TFA was around 40%. The reaction mixture was stirred under N_2 at 0 ºC until complete disappearance of starting material. The solvents were evaporated to dryness to give the amine, N-allyl-4-amino-1-methyl-*1H*-pyrrole-2-carboxamide in quantitative yields. ^{1}HNMR (400 MHz, DMSO-d_6) δ 3.80-3.83 (m, 2H), 3.84 (s, 3H), 5.07 (dd, 1H, *J* = 10.16 and 1.95), 5.13 (dd, 1H, *J* = 17.18 and 1.95), 5.81-5.91 (m, 1H), 6.81 (d, 1H, *J* = 1.95), 7.05 (d, 1H, *J* = 1.95), 8.357 (t, 1H, *J* = 5.7), 9.64 (s, br).

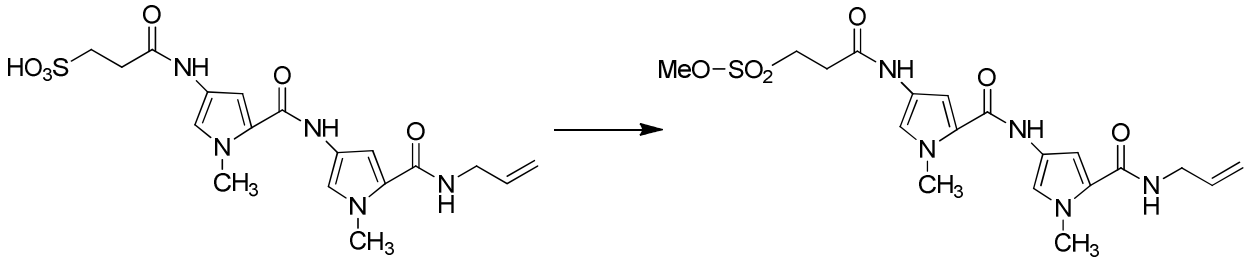


To a solution of 4-acrylamido-1-methyl-*1H*-pyrrole-2-carboxylic acid (1.293 mmol, 251 mg) and HOBT (1.94 mmol, 262 mg) in anhydrous CH_2Cl_2 (30 mL) was added Et_3N (5.172 mmol, 721 µL). This was followed by the addition of EDC (2.59 mmol, 496 mg) in CH_2Cl_2 (25 mL). The reaction mixture was stirred under N_2 at room temperature for 1 h and then cooled to 0 ºC and N-allyl-4-amino-1-methyl-*1H*-pyrrole-2-carboxamide (2.586 mmol, 0.496 g) added in CH_2Cl_2 (13 mL) containing Et_3N (0.6 mL). The reaction mixture was stirred under N_2 and at room temperature for 18 h and the reaction mixture diluted with CH_2Cl_2 and washed with H_2O. The aqueous layer was extracted thoroughly with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and solvents evaporated to dryness. The residue was purified by silica gel column chromatography to get the product (444mg, 96%). ^{1}HNMR (400 MHz, DMSO-d_6) δ 3.78-3.81 (m, 2H), 3.79 (s, 3H), 3.84 (s, 3H), 5.06 (dd, 1H, *J* = 10.16 and 1.95), 5.13 (dd, 1H, *J* = 17.18 and 1.95), 5.67 (dd, 1H, *J* = 9.76 and 1.95), 5.82-5.91 (m, 1H), 6.18 (dd, 1H, *J* = 16.79 and 1.95), 6.34-6.41 (m, 1H), 6.89 (d, 1H, *J* = 1.95), 6.92 (d, 1H, *J* = 1.95), 7.21 (d, 1H, *J* = 1.95), 7.27 (d, 1H, *J* = 1.95), 8.21 (t, 1H, *J* = 6.25), 9.91 (s, 1H), 10.11 (s, 1H).



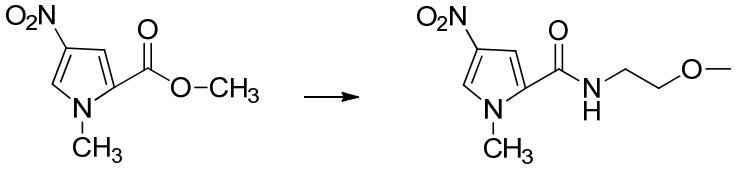
To a solution of 4-acrylamido-N-(5-(allylcarbamoyl)-*1H*-pyrrol-3-yl)-1-methyl-*1H*-pyrrole-2-carboxamide (0.416 mmol, 148 mg) in EtOH: H_2O (4:1,10 mL) was added $NaHSO_3$ solution (0.833 mmol, 88 mg) in 1.2 mL of H_2O. The pH of the reaction mixture was then adjusted to 8.0 using a 5% NaOH solution. The reaction mixture was then heated to 80 ºC until complete disappearance of starting material (based on TLC). The reaction mixture was cooled to 0 ºC and

conc, HCl added dropwise so that the pH was between 1 and 2. The solvents were evaporated to dryness and the solid obtained was treated with 1 N cold HCl solution and then centrifuged (4000 rpm, 10 min). The supernatant was decanted and the solid twice treated with 1 N cold HCl, centrifuged and the supernatant decanted. The solid obtained was then dried thoroughly to give the product (111 mg, 60%). ^{1}HNMR (400 MHz, DMSO-d_6) δ 2.66-2.68 (m, 2H), 3.76-3.84 (m, 10H), 5.05 (dd, 1H, *J* = 10.55 and 1.56), 5.13 (dd, 1H, *J* = 17.18 and 1.56), 5.81-5.91 (m, 1H), 6.85 (d, 1H, *J* = 1.56), 6.88 (d, 1H, *J* = 1.95), 7.15 (d, 1H, *J* = 1.56), 7.20 (d, 1H, *J* = 1.95), 8.20 (t, 1H, *J* = 5.46), 9.87 (s, 1H), 9.97 (s, 1H).



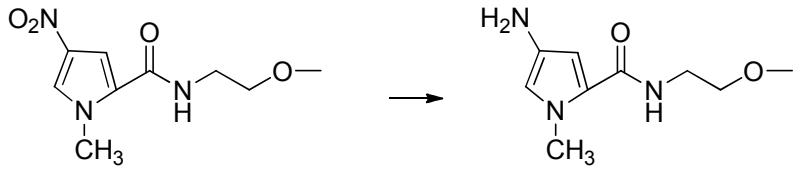
To a solution of 3-((5-((5-(allylcarbamoyl)-1-methyl-*1H*-pyrrol-3-yl)carbamoyl)-1-methyl-*1H*-pyrrol-3-yl)amino)-3-oxopropane-1-sulfonic acid (0.254 mmol, 111 mg) in dry dioxane (15 mL) was added 3-methyl-1-*p*-tolyltriaz-1-ene (1.522 mmol, 227 mg). The reaction was heated in an oil bath at 80 ºC till disappearance of starting material. The dioxane was evaporated to minimum volume and the residue was loaded onto a silica gel column deactivated with Et_3N. The product was eluted with EtOAc (88 mg, 76%). ^{1}HNMR (400 MHz, DMSO-d_6) δ 2.73 (t, 2H, J = 7.03), 3.61 (t, 2H, J = 7.03), 3.77-3.82 (m, 5H), 3.83 (s, 3H), 3.86 (s, 3H), 5.06 (dd, 1H, *J* = 10.16 and 1.56), 5.13 (dd, 1H, *J* = 17.18 and 1.56), 5.83-5.91 (m, 1H), 6.87 (d, 1H, J = 1.95), 6.88 (d, 1H, J = 1.95), 7.17 (d, 1H, J = 1.95), 7.20 (d, 1H, J = 1.95), 8.20 (t, 1H, *J* = 5.47), 9.89 (s, 1H), 10.06 (s, 1H).
^{13}CNMR (150 MHz, DMSO-d_6) 29.85, 36.45, 36.62, 41.17, 44.50, 60.22, 104.32, 104.75, 115.14, 118.51, 118.63, 122.10, 122.47, 123.20, 123.32, 136.43, 158.77, 161.50, 166.03.
HRMS (ES+H) m/z calcd for $C_{19}H_{26}N_5O_6S$ (452.1604) Found (452.1591).

Synthesis of 3

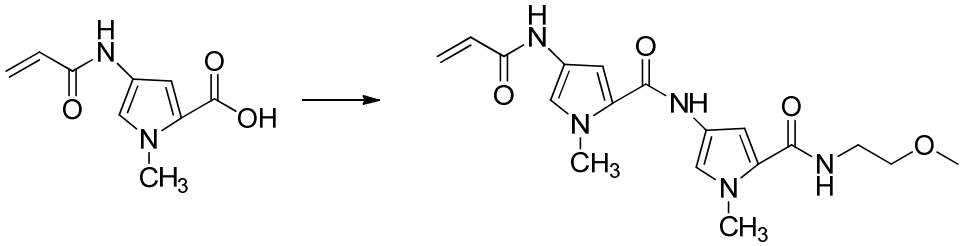


Synthesis followed previously reported method [Sabot, C., Kumar, K. A., Meunier, S., and Mioskowski, C. (2007) A convenient aminolysis of esters catalyzed by 1,5,7-triazabicyclo [4.4.0]dec-5-ene (TBD) under solvent-free conditions. *Tet. Lett. 48*, 3863-3866.].To methyl 1-methyl-4-nitro-*1H*-pyrrole-2-carboxylate (5.433 mmol, 1 g) was added 1,5,7-triazabicyclo-[*4.4.0*]dec-5-ene (TBD) (225 mg, 30 mol%) followed by 2-methoxyethylamine (10.866 mmol, 949 µL) under N_2 atmosphere. The reaction mixture was slowly warmed up to 75 ºC and stirred for 12 h. The reaction mixture was then cooled to room temperature and purified by silica gel

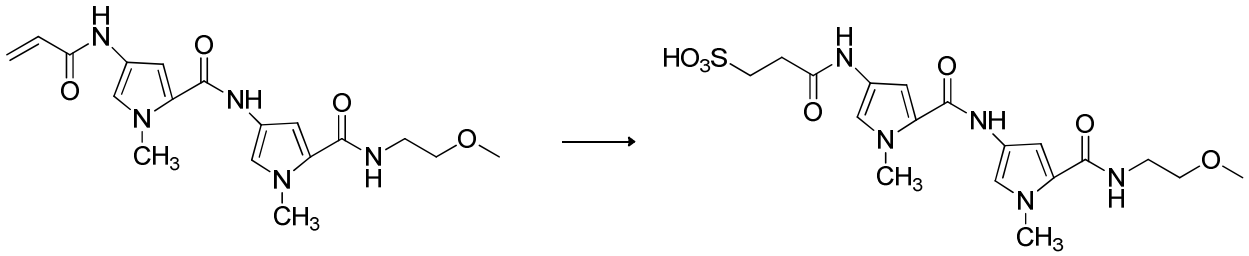
column chromatography to yield the product (1.168g, 94.6%). ^{1}HNMR (600 MHz, $CDCl_3$) δ 3.41 (s, 3H), 3.53-3.55 (m, 2H), 3.57-3.60 (m, 2H), 3.99 (s, 3H), 6.326 (br, 1H), 7.09 (d, 1H, *J* = 1.8), 7.56 (d, 1H, *J* = 1.8).



To a solution of N-(2-methoxyethyl)-1-methyl-4-nitro-*1H*-pyrrole-2-carboxamide (5.109 mmol,1.16 g) in MeOH (320 mL) was added 10% Pd/C catalyst (330 mg) and the reaction mixture stirred in an atmosphere of H_2 at room temperature until complete disappearance of starting material (based on TLC). The reaction mixture was filtered through a celite pad and solvents evaporated to dryness to get the desired amine, 4-amino-N-(2-methoxyethyl)-1-methyl-*1H*-pyrrole-2-carboxamide in quantitative yield. ^{1}HNMR (600 MHz, DMSO-d_6) δ 3.24 (s, 3H), 3.26-3.29 (m, 2H), 3.36-3.38 (m, 2H), 3.67 (s, 3H), 4.09-4.126 (br, 2H), 6.18-6.19 (m, 2H), 7.72 (t, 1H, *J* = 6).



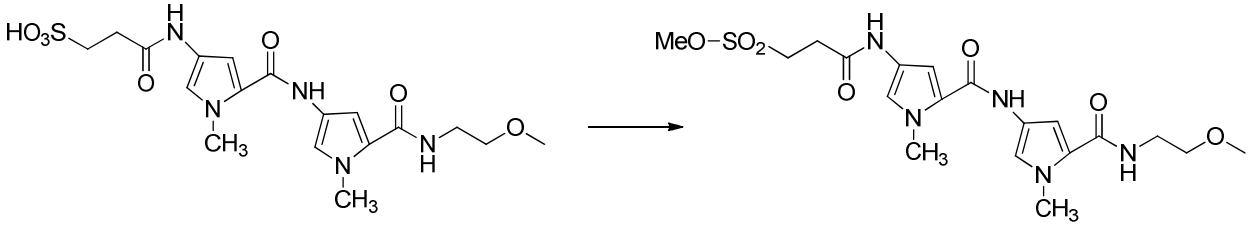
To a solution of 4-acrylamido-1-methyl-*1H*-pyrrole-2-carboxylic acid (1.416 mmol, 275 mg) and HOBT (2.124 mmol, 287 mg) under N_2 in CH_2Cl_2 (10mL) was added Et_3N (5.665 mmol, 789 µL). This was followed by the addition of EDC (2.832 mmol, 543 mg) in CH_2Cl_2 (20 mL). The reaction mixture was stirred under N_2 at room temperature for 1 h and then cooled to 0 ºC and 4-amino-N-(2-methoxyethyl)-1-methyl-*1H*-pyrrole-2-carboxamide (1.814 mmol, 385 mg) was added in CH_2Cl_2 (15 mL). The reaction mixture was stirred for about 18 h under N_2 and allowed to come to room temperature, diluted with CH_2Cl_2 and washed with H_2O. The aqueous layer was extracted thoroughly with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4 and solvents evaporated to dryness. The residue was purified by silica gel column chromatography to get the product (365mg, 68.9%). ^{1}HNMR (600 MHz, $CDCl_3$) δ 3.38 (s, 3H), 3.55-3.56 (m, 2H), 3.58-3.61 (m, 2H), 3.87 (s, 3H), 3.90 (s, 3H), 5.75 (dd, 1H, *J* = 10.8 and 1.2), 6.27-6.32 (m, 1H), 6.42 (dd, 1H, *J* = 16.2 and 1.2), 6.53-6.57 (m, 2H), 7.14 (d, 1H, *J* = 1.2), 7.24 (d, 1H, *J* = 1.2), 7.36 (s, 1H, br), 7.87 (s, 1H, br).



To a solution of 4-acrylamido-N-(5-((2-methoxyethyl)carbamoyl)-1-methyl-*1H*-pyrrol-3-yl)-1-methyl-*1H*-pyrrole-2-carboxamide (0.956 mmol, 357 mg) in EtOH:H_2O (4:1, 19 mL) was added $NaHSO_3$ solution (1.912 mmol, 203mg) in 2.9 mL of H_2O. The pH of the reaction mixture was then adjusted to 8.0 using 5% NaOH solution. The reaction mixture was then heated to 80 ºC until complete disappearance of starting material (based on TLC). The reaction mixture was cooled to 0 ºC and conc. HCl added dropwise so that the pH was between 1 and 2. The solvents were evaporated to dryness and the solid obtained treated with 1 N cold HCl solution and then centrifuged (4000 rpm, 10 min). The supernatant was decanted and the solid was twice treated with 1 N cold HCl, centrifuged and the supernatant decanted. The solid obtained was then dried thoroughly to give the product (75 mg, 17%)

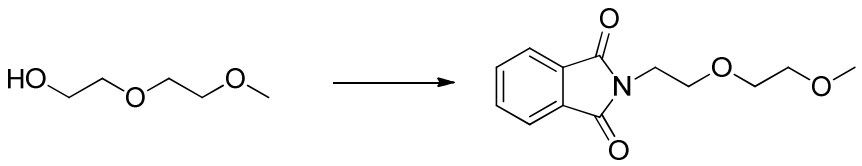
(**Since the yields were very poor we suspected that the compound might have been soluble in the HCl supernatant. So the supernatant was evaporated to get about 400 mg of material).

^{1}HNMR (600 MHz, DMSO-d_6) δ 2.52-2.55 (m, 2H), 2.66-2.68 (m, 2H), 3.26 (s, 3H), 3.30-3.33 (m, 2H), 3.39-3.42 (m, 2H), 3.79 (s, 3H), 3.81 (s, 3H), 6.84-6.85 (m, 2H), 7.15 (d, 1H, J = 1.8), 7.19 (d, 1H, J = 1.2), 8.02 (t, 1H, J = 6), 9.86 (s, 1H), 9.97 (s, 1H).

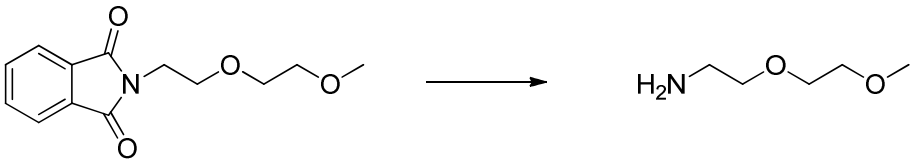


To a solution of 3-((5-((5-((2-methoxyethyl)carbamoyl)-1-methyl-*1H*-pyrrol-3-yl)carbamoyl)-1-methyl-*1H*-pyrrol-3-yl)amino)-3-oxopropane-1-sulfonic acid (0.147 mmol, 67 mg) in dry dioxane (10 mL) was added 3-methyl-1-*p*-tolyltriaz-1-ene (1.522 mmol, 132 mg).The reaction was heated in an oil bath at 80 ºC until disappearance of starting material. The dioxane was evaporated to minimum volume and the residue was loaded onto a silica gel column deactivated with Et_3N. The product was eluted with sequential EtOAc and then with 2%MeOH:EtOAc to yield the product (34 mg, 49%). ^{1}HNMR (600 MHz, DMSO-d_6) δ 2.73 (t, 2H, J = 7.2), 3.26 (s, 3H), 3.31-3.34 (m, 2H), 3.39-3.42 (m, 2H), 3.62 (t, 2H, J = 7.2), 3.79 (s, 3H), 3.83 (s, 3H), 3.86 (s, 3H), 6.85-6.86 (m, 2H), 7.17-7.19 (m, 2H), 8.02 (t, 1H, J = 5.4), 9.88 (s, 1H), 10.06 (s, 1H). HRMS (ES+Na) m/z calcd for $C_{19}H_{27}N_5O_7NaS$ (492.1529) Found (492.1537).

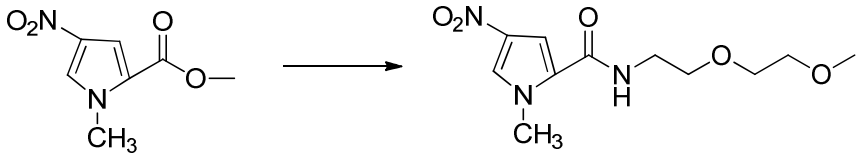
Synthesis of 4



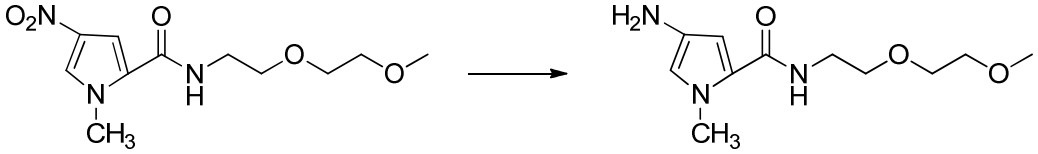
Prepared as previously reported [Dombi, K. L., Griesang, N., and Richert, C. (2002) Oligonucleotide arrays from aldehyde-bearing glass with coated background. *Synthesis 6*, 816–824.]. To a mixture of triphenyl phosphine (24 mmol, 6.3 g) and phthalimide (24 mmol, 3.53 g) in THF (100 mL) was added 2-(2-methoxyethoxy)ethanol (20 mmol, 2.35 mL). The reaction mixture was stirred at room temperature for 15 min and DIAD (24 mmol, 4.7 mL) was added dropwise. The reaction mixture was allowed to stir at room temperature under N_2 overnight. The reaction mixture was quenched with EtOH (40 mL) and solvents evaporated to dryness. The solid obtained was treated with 1:1 petroleum ether: EtOAc and stirred at 40 ºC for 1 h. The white residue was filtered off and washed with 1:1 petroleum ether: EtOAc (10 mL) and the filtrate evaporated to dryness. The residue obtained was purified by silica gel column chromatography to yield product (2.47 g, 49%) ^{1}HNMR (600 MHz, $CDCl_3$) δ 3.32 (s, 3H), 3.49-3.51 (m, 2H), 3.65-3.66 (m, 2H), 3.76 (t, 2H, J = 5.4), 3.92 (t, 2H, J = 5.4), 7.71-7.73 (m, 2H), 7.85-7.86 (m, 2H).



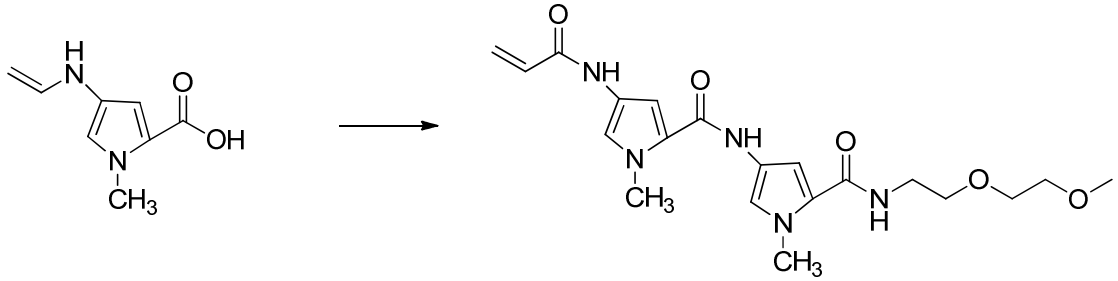
Compound was prepared as previously described [Dombi, K. L., Griesang, N., and Richert, C. (2002) Oligonucleotide arrays from aldehyde-bearing glass with coated background. *Synthesis 6*, 816–824.]. To a solution of 2-(2-(2-methoxyethoxy)ethyl)isoindoline-1,3-dione (9.49 mmol, 2.37 g) in EtOH (26 mL) was added hydrazine monohydrate (10.44 mmol, 506 µL). The resulting mixture was refluxed at 100 ºC for 5 h when a white precipitate formed. The slurry was allowed to cool and treated with conc. HCl (2.3 mL), followed by refluxing again for 1 h. The slurry was allowed to cool to room temperature and the white solid was filtered off. The filtrate was evaporated and the residue was taken up in H_2O (14 mL) and the pH of the solution adjusted to 11.0 with 1 N NaOH. The aqueous phase was saturated with NaCl and extracted with CH_2Cl_2. The organic extracts are dried over $MgSO_4$ and solvents evaporated. The residue was then distilled (Kugelrohr distillation) (0.5 Torr, heating chamber 190-195 ºC, compound distilled at 130 ºC) to yield product (0.682 g, 60%). ^{1}HNMR (600 MHz, $CDCl_3$) δ 2.88 (t, 2H, J = 5.4), 3.39 (s, 3H), 3.52 (t, 2H, J = 5.4), 3.55-3.57 (m, 2H), 3.61-3.63 (m, 2H).



To methyl 1-methyl-4-nitro-*1H*-pyrrole-2-carboxylate (2.861 mmol, 0.527 g) was added 1,5,7-triazabicyclo[*4.4.0*]dec-5-ene (118 mg, 30 mol%) followed by 2-(2-methoxyethoxy)ethanamine (5.723 mmol, 628 mg) under N_2 atmosphere. The reaction mixture was slowly warmed up to 75 ºC and stirred for 12 h. The reaction mixture was cooled to room temperature and purified by silica gel column chromatography to yield product (0.430 g, 55%). ^{1}HNMR (600 MHz, $CDCl_3$) δ 3.45 (s, 3H), 3.58-3.61 (m, 4H), 3.66-3.69 (m, 4H), 3.99 (s, 3H), 6.71 (s, br), 7.13 (d, 1H, *J* = 1.2), 7.55 (d, 1H, *J* = 1.2).

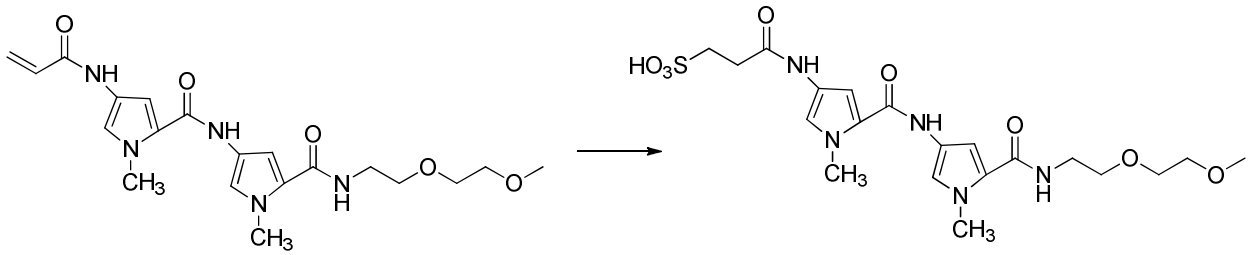


To a solution of N-(2-(2-methoxyethoxy)ethyl)-1-methyl-4-nitro-*1H*-pyrrole-2-carboxamide (1.452 mmol, 0.394 g) in MeOH (3 8mL) was added 10% Pd/C catalyst (67 mg) and the reaction mixture stirred in an atmosphere of H_2 at room temperature until complete disappearance of starting material (based on TLC). The reaction mixture was filtered through a celite pad and solvents evaporated to dryness to afford 4-amino-N-(2-(2-methoxyethoxy)ethyl)-1-methyl-*1H*-pyrrole-2-carboxamide in quantitative yield. ^{1}HNMR (600 MHz, $CDCl_3$) δ 3.41 (s, 3H), 3.55-3.58 (m, 4H), 3.61-3.65 (m, 4H), 3.83 (s, 3H), 6.13 (d, 1H, *J* = 2.4), 6.29-6.32 (m, 2H).

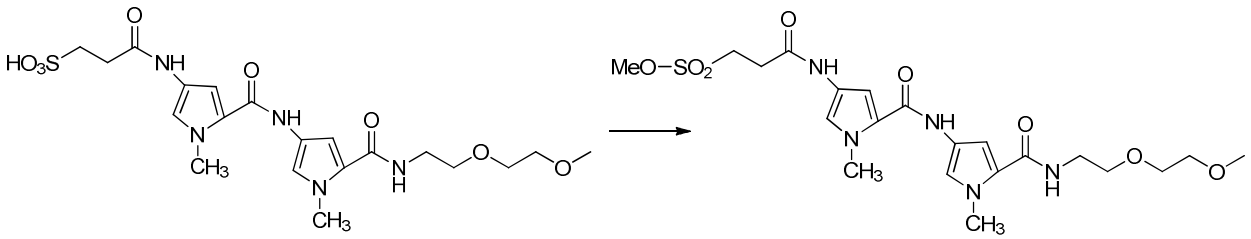


To a solution of 4-acrylamido-1-methyl-*1H*-pyrrole-2-carboxylic acid (1.128 mmol, 219 mg) and HOBT (1.692 mmol, 229 mg) under N_2 in CH_2Cl_2 (20 mL) was added Et_3N (4.511 mmol, 629 µL), followed by the addition of EDC (2.256 mmol, 432 mg) in CH_2Cl_2 (20 mL). The reaction mixture was stirred under N_2 at room temperature for 1 h and then cooled to 0 ºC and 4-amino-N-(2-(2-methoxyethoxy)ethyl)-1-methyl-*1H*-pyrrole-2-carboxamide (1.450 mmol, 350 mg) added in CH_2Cl_2 (15 mL). The reaction mixture was stirred for about 18 h under N_2, allowed to come to room temperature and then diluted with CH_2Cl_2 and washed with H_2O. The aqueous layer was extracted thoroughly with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and solvents evaporated to dryness. The residue was purified by silica gel column chromatography to give product (339 mg, 72%). ^{1}HNMR (600 MHz, DMSO-d_6) δ 3.29-3.32 (m, 2H), 3.33 (s, 3H), 3.43-3.45 (m, 2H), 3.48 (t, 2H, *J* = 6), 3.52-3.54 (m, 2H), 3.79 (s, 3H), 3.83 (s, 3H), 5.67 (dd, 1H

J = 10.2 and 1.8), 6.18 (dd, 1H, *J* = 16.8 and 1.8), 6.35-6.39 (m, 1H), 6.85 (d, 1H, *J* = 1.8), 6.91 (d, 1H, *J* = 1.8), 7.19 (d, 1H, *J* = 1.8), 7.27 (d, 1H, *J* = 1.8), 8.02 (t, 1H, *J* = 6), 9.91 (s, 1H), 10.11 (s, 1H).

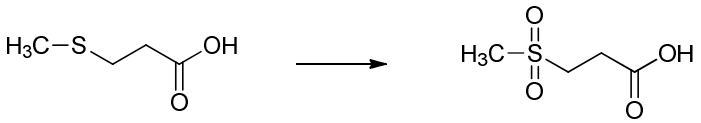


To a solution of 4-acrylamido-N-(5-((2-(2-methoxyethoxy)ethyl)carbamoyl)-1-methyl-*1H*-pyrrol-3-yl)-1-methyl-*1H*-pyrrole-2-carboxamide (0.099 mmol, 41.7 mg) in EtOH:H_2O (4:1, 1.9 mL) was added $NaHSO_3$ solution (0.199 mmol, 21.1 mg) in 0.3 mL of H_2O. The pH of the reaction mixture was then adjusted to 8.0 with using 5% NaOH solution. The reaction mixture was then heated to 80 ºC until complete disappearance of starting material (based on TLC). The reaction mixture was cooled to 0 ºC and conc. HCl added dropwise so that the pH remained between 1 and 2. The solvents were evaporated to dryness to yield the product (44 mg, 88%). ^{1}HNMR (600 MHz, DMSO-d_6) δ 2.53-2.56 (m, 2H), 2.67-2.69 (m, 2H), 3.25 (s, 3H), 3.29-3.32 (m, 2H), 3.43-3.46 (m, 2H), 3.46-3.49 (m, 2H), 3.52-3.53 (m, 2H), 3.79 (s, 3H), 3.81 (s, 3H), 6.84-6.86 (m, 2H), 7.15 (s, 1H), 7.19 (s, 1H), 8.01 (t, 1H, *J* = 5.4), 9.87 (s, 1H), 9.98 (s, 1H).

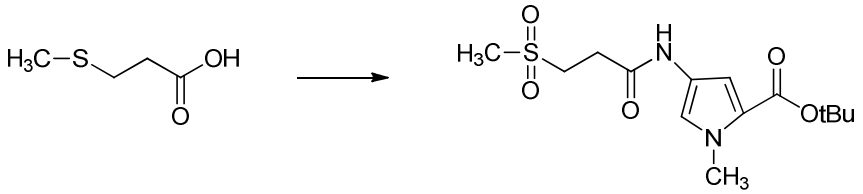


To a solution of 3-((5-((5-((2-(2-methoxyethoxy)ethyl)carbamoyl)-1-methyl-*1H*-pyrrol-3-yl)carbamoyl)-1-methyl-*1H*-pyrrol-3-yl)amino)-3-oxopropane-1-sulfonic (0.0372 mmol, 18.6 mg) in dry dioxane (5 mL) was added 3-methyl-1-*p*-tolyltriaz-1-ene (0.149 mmol, 23 mg).The reaction was heated in an oil bath at 80 ºC for 1 h, then another 20 mg of 3-methyl-1-*p*-tolyltriaz-1-ene was added and the reaction heated for another 30 min. The dioxane was evaporated to minimum volume and the residue was loaded onto a silica gel column deactivated with Et_3N. The product was sequentially eluted with EtOAc and then 5% MeOH:EtOAc to yield product (15mg, 78%). ^{1}HNMR (600 MHz, DMSO-d_6) δ 2.50-2.53 (m, 2H), 2.73 (t, 2H, *J* = 7.2), 3.24 (s, 3H), 3.43-3.4 (m, 2H), 3.47 (t, 2H, *J* = 6.6), 3.52-3.54 (m, 2H), 3.62 (t, 2H, *J* = 7.2), 3.79 (s, 3H), 3.81 (s, 3H), 3.86 (s, 3H), 6.85 (d, 1H, *J* = 1.8), 6.87 (d, 1H, *J* = 1.8), 7.17 (d, 1H, *J* = 1.8), 7.19 (d, 1H, *J* = 1.8), 8.01 (t, 1H, *J* = 5.4), 9.88 (s, 1H), 10.06 (s, 1H). HRMS (ES+Na) m/z calcd for $C_{21}H_{31}N_5O_8NaS$ (536.1791) Found (536.1765).

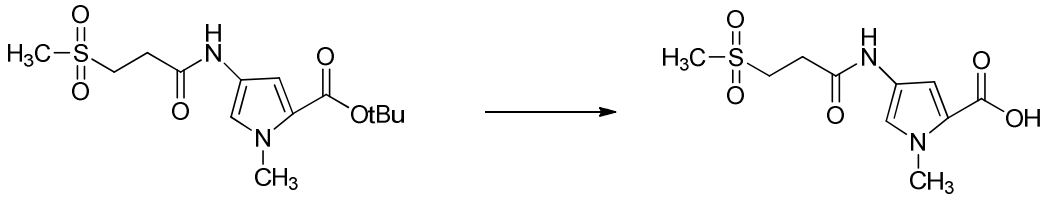
Synthesis of 5



Prepared as previously described [Truce, W. E., and Knospe, R. H. (1955) The preparation of 0-keto sulfones by the Claisen condensation. *J. Am. Chem. Soc. 77*, 5063-5067.]. To a solution of 3-(methylthio)propionic acid (6.241 mmol, 0.65 mL) in a 1:1 mixture of AcOH:Ac_2O (6 mL) was added 35% H_2O_2 (3 mL) and the reaction was stirred at room temperature for 48 h and the excess of peroxide quenched by addition of a trace amount of MnO_2 under N_2. The solvent was removed by distillation to give a white solid in quantitative yield. ^{1}HNMR (600 MHz, DMSO-d_6) δ 2.68 (t, 2H, *J* = 7.8) 3.00 (s, 3H), 3.33 (t, 2H, *J* = 7.8), 12.56 (s, br, 1H).

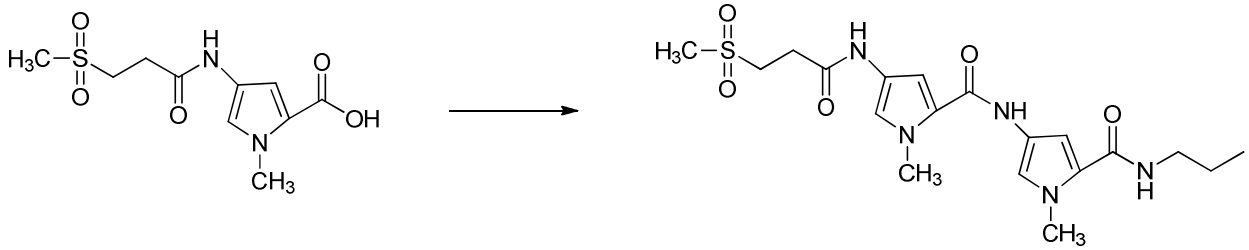


To a solution of 3-(methylthio)propanoic acid (1.045 mmol, 159 mg) and HOBT (1.484 mmol, 200 mg) in CH_2Cl_2 (15 mL) was added Et_3N (3.956 mmol, 0.551 mL), followed by the addition of EDC (1.978 mmol, 0.379 g) in CH_2Cl_2 (15 mL). The reaction mixture was stirred under N_2 at room temperature for 1 h and then cooled to 0 ºC and tert-butyl 4-amino-1-methyl-*1H*-pyrrole-2-carboxylate (1.484 mmol) added. The reaction mixture was allowed to warm to room temperature and stirred for about 18 h and then diluted with CH_2Cl_2 and washed with H_2O. The aqueous layer was extracted thoroughly with CH_2Cl_2 and the organic layer dried over Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel column chromatography to yield product (282 mg, 81.7%). ^{1}HNMR (400 MHz, DMSO-d_6) δ 1.49 (s, 9H), 2.69 (t, 2H, *J* = 7.8), 3.00 (s, 3H), 3.39 (t, 2H, *J* = 7.8), 3.78 (s, 3H), 6.65 (d, 1H, *J* = 1.96), 7.23 (d, 1H, *J* = 1.96), 10.03 (s, br, 1H).



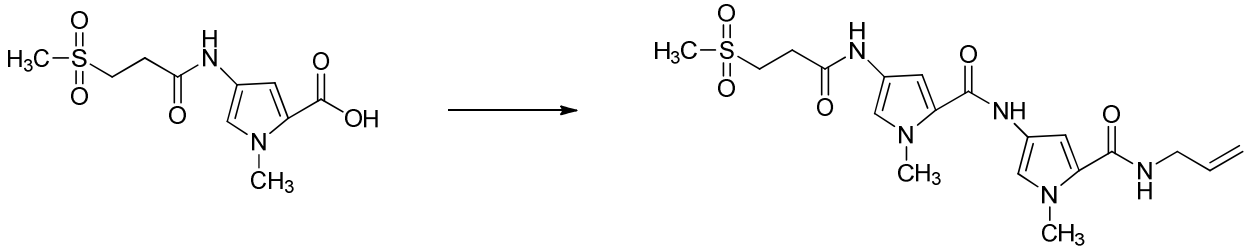
To a solution of tert-butyl 1-methyl-4-(3-(methylsulfonyl)propanamido)-*1H*-pyrrole-2-carboxylate (0.847 mmol, 0.280 g) in CH_2Cl_2 (27 mL) was added 1M $TiCl_4$ solution (1.694 mmol, 1.7 mL) at 0 ºC. The reaction mixture was stirred at 0 ºC and slowly allowed to come to room temperature until complete disappearance of starting material. The reaction mixture was diluted with CH_2Cl_2 and washed with H_2O. The aqueous layer was extracted thoroughly with

EtOAc (4 x 50 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated to give a quantitative yield of the acid product. ^{1}HNMR (400 MHz, DMSO-d_6) δ 2.70 (t, 2H, *J* = 7.8), 3.0 (s, 3H), 3.39 (t, 2H, *J* = 7.8), 3.79 (s, 3H), 6.66 (d, 1H *J* = 1.95), 7.28 (d, 1H, *J* = 1.95), 10.08 (s, br, 1H).



To a solution of 1-methyl-4-(3-(methylsulfonyl)propanamido)-*1H*-pyrrole-2-carboxylic acid (0.551 mmol, 151 mg) and HOBT (0.765 mmol, 103 mg) in anhydrous DMF (2 mL) was added Et_3N (2.042 mmol, 0.285 mL) followed by the addition of EDC (1.02 mmol, 0.196 g) in CH_2Cl_2 (15 mL). The reaction mixture was stirred under N_2 at room temperature for 1 h and the reaction mixture cooled to 0 ºC and 4-amino-1-methyl-N-propyl-*1H*-pyrrole-2-carboxamide (0.765 mmol) was added in CH_2Cl_2 (10 mL) and Et_3N (100 µL). The reaction mixture was allowed to come to room temperature and stirred for about 18 h and the solvent removed. The residue was purified by silica gel column chromatography to yield product (111 mg, 46%). ^{1}HNMR (400 MHz, DMSO-d_6) δ 0.863 (t, 3H, *J* = 7.42), 1.44-1.51 (m, 2H), 2.73 (t, 2H, *J* = 7.8), 3.01 (s, 3H), 3.09-3.14 (m, 2H) 3.40 (t, 2H, *J* = 7.8), 3.79 (s, 3H), 3.83 (s, 3H), 6.84 (d, 1H, *J* = 1.95), 6.86 (d, 1H, *J* = 1.95), 7.16-7.17 (m, 2H,), 8.0 (t, 1H, *J* = 6.25), 9.86 (s, br, 1H), 10.06 (s, br, 1H). ^{13}CNMR (150MHz, DMSOd_6) δ 11.91, 23.05, 28.68, 36.37, 36.61, 40.65, 40.91, 50.24, 104.31, 104.56, 118.20, 118.62, 122.15, 122.42, 123.33, 123.56, 158.76, 161.68, 166.55. HRMS (ES+Na) m/z calcd for $C_{19}H_{27}N_5O_5NaS$ (460.163) Found (460.162).

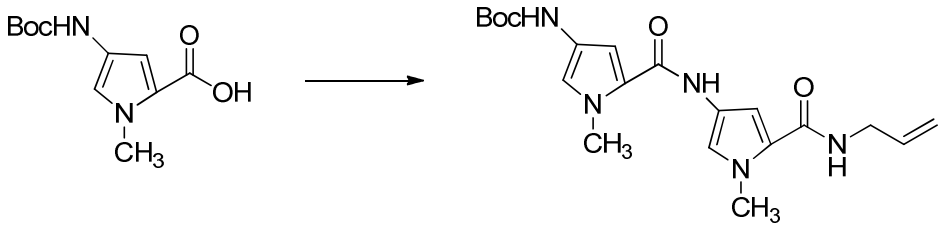
Synthesis of 6



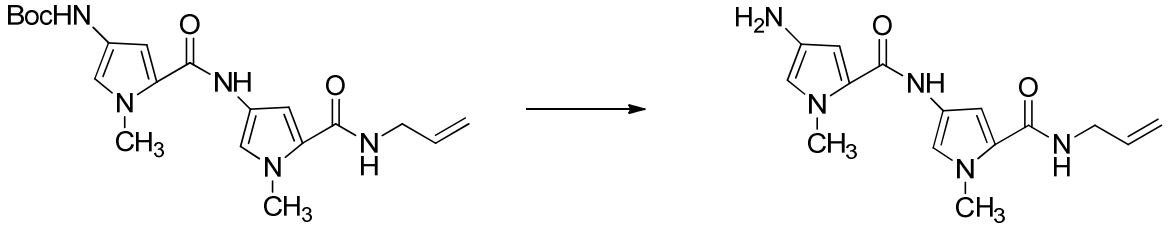
To a solution of 1-methyl-4-(3-(methylsulfonyl)propanamido)-*1H*-pyrrole-2-carboxylic acid (0.193 mmol, 53 mg) and HOBT (0.289 mmol, 39 mg) in anhydrous CH_2Cl_2 (10 mL) was added Et_3N (0.773 mmol, 0.108 mL) followed by the addition of EDC (0.386 mmol, 74 mg) in CH_2Cl_2 (15 mL). The reaction mixture was stirred under N_2 at room temperature for 1 h and the reaction mixture cooled to 0 ºC and N-allyl-4-amino-1-methyl-*1H*-pyrrole-2-carboxamide (0.289 mmol) added in CH_2Cl_2 (10 mL) and Et_3N (100 µL). The reaction mixture was allowed to come to room temperature and stirred for about 18 h and diluted with H_2O and extracted with CH_2Cl_2. The

organic solvents were dried over Na_2SO_4, the solvent evaporated and the residue purified by silica gel column chromatography to yield product (26 mg, 31%). ^{1}HNMR (400 MHz, DMSO-d_6) δ 2.73 (t, 2H, *J* = 7.80), 3.01 (s, 3H), 3.38-3.42 (m, 2H), 3.76-3.80 (m, 5H), 3.82 (s, 3H), 5.05 (dd, 1H, *J* = 10.15 and 1.56), 5.13 (dd, 1H, *J* = 17.18 and 1.56), 5.81-5.90 (m, 1H) 6.86 (d, 1H, *J* = 1.95), 6.88 (d, 1H, *J* = 1.95), 7.16 (d, 1H, *J* = 1.95), 7.20 (d, 1H, *J* = 1.95), 8.20 (t, 1H, *J* = 7.68), 9.88 (s, br, 1H), 10.06 (s, br, 1H). ^{13}CNMR (150 MHz, DMSO-d_6) δ 28.68, 36.45, 36.60, 40.91, 41.18, 50.24, 104.32, 104.74, 115.15, 118.52, 118.63, 122.15, 122.48, 123.21, 123.31δ, 136.44, 158.78, 161.50, 166.55. HRMS (ES+Na) m/z calcd for $C_{19}H_{25}N_5O_5NaS$ (458.147) Found (458.145).

Synthesis of 8

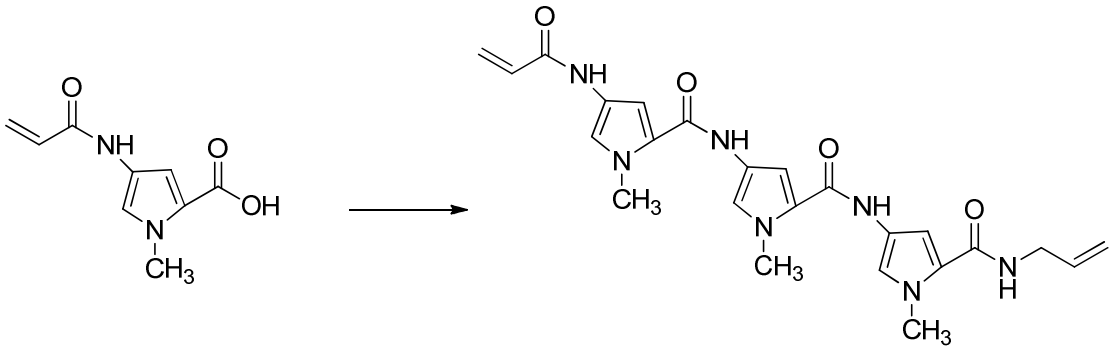


To a solution of 4-((tert-butoxycarbonyl) amino)-1-methyl-*1H*-pyrrole-2-carboxylic acid (2.805 mmol, 674 mg) and HOBT (4.208 mmol, 569 mg) in anhydrous CH_2Cl_2 (20 mL) was added Et_3N (11.221 mmol, 1.56 mL) followed by the addition of EDC (5.61 mmol, 1.075 g) in CH_2Cl_2 (25 mL). The reaction mixture was stirred under N_2 at room temperature for 1 h and then cooled to 0 ºC and N-allyl-4-amino-1-methyl-*1H*-pyrrole-2-carboxamide (5.61 mmol added in CH_2Cl_2 (15 mL) and Et_3N (1 mL). The reaction mixture was allowed to come to room temperature and stirred for about 18 h and then the reaction was diluted with H_2O and extracted with CH_2Cl_2. The combined organic solvents were dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography to yield product (1.011 g, 89%). ^{1}HNMR (400 MHz, DMSO-d_6) δ 1.45 (s, 9H), 3.74-3.84 (m, 8H), 5.05 (dd, 1H, *J* = 8.59 and 1.56), 5.13 (dd, 1H, *J* = 15.23 and 1.56), 5.81-5.91 (m, 1H), 6.82 (br, s, 1H), 6.86-6.92 (m, 2H), 7.18 (d, 1H, *J* = 1.95), 8.18 (t, 1H, *J* = 6.25), 9.09 (s, 1H), 9.83 (s, 1H).

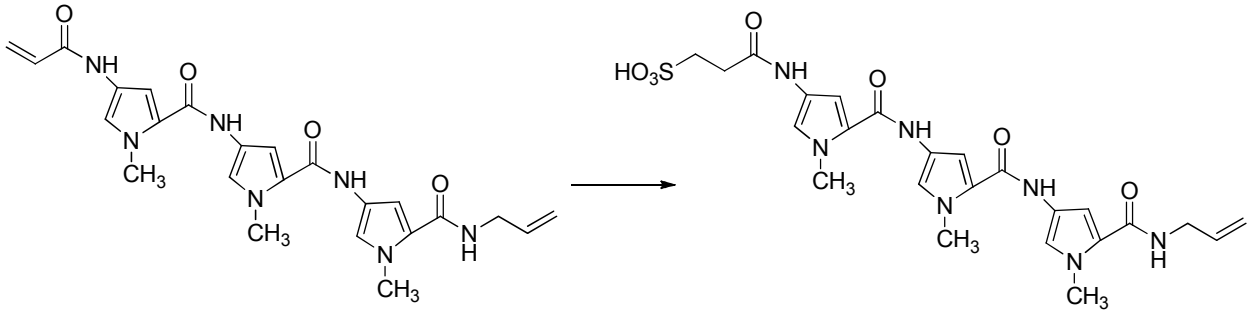


To a solution of tert-butyl (5-((5-(allylcarbamoyl)-1-methyl-*1H*-pyrrol-3-yl)-carbamoyl)-1-methyl-1H-pyrrol-3-yl) carbamate (2.391 mmol, 960 mg,) in anhydrous CH_2Cl_2 (12 mL) was added 50% TFA: dichloromethane (34mL) at 0 ºC such that the final concentration of TFA was ~40%. The reaction mixture was stirred under N_2 and at 0 ºC until complete disappearance of starting material. The solvents were evaporated to dryness to give the N-allyl-4-(4-amino-1-

methyl-*1H*-pyrrole-2-carboxamido)-1-methyl-*1H*-pyrrole-2-carboxamide product in quantitative yield. ^{1}HNMR (400 MHz, DMSO-d_6) δ 3.78-3.82 (m, 5H), 3.89 (s, 3H), 5.06 (dd, 1H, *J* = 10.16 and 1.95), 5.13 (dd, 1H, *J* = 15.23 and 1.95), 5.82-5.91 (m, 1H), 6.88 (d, 1H, *J* = 1.95), 6.94 (d, 1H, *J* = 1.95), 7.11 (d, 1H, *J* = 1.95), 7.21 (d, 1H, *J* = 1.95), 8.22 (t, 1H, *J* = 6.25), 9.71 (s, br, 2H), 10.00 (s,1H).

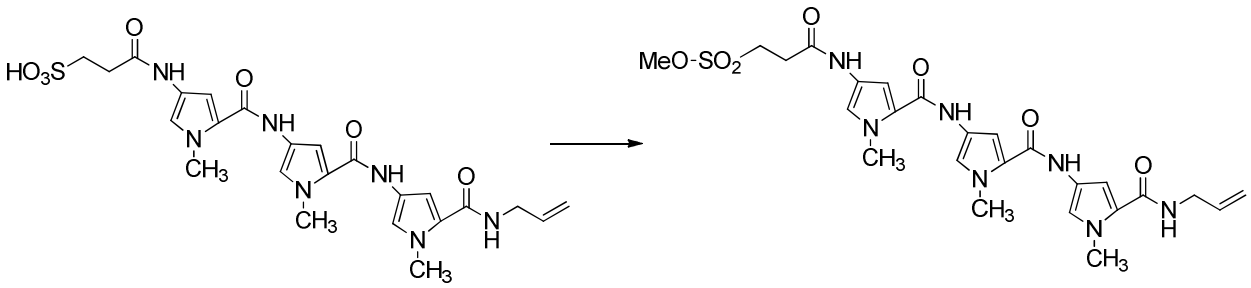


To a solution of 4-acrylamido-1-methyl-*1H*-pyrrole-2-carboxylic acid (1.545 mmol, 300 mg) and HOBT (2.318 mmol, 313 mg) in anhydrous CH_2Cl_2 (25 mL) was added Et_3N (6.18 mmol, 0.861 mL) followed by the addition of EDC (3.09 mmol, 0.592 g) in CH_2Cl_2 (20 mL). The reaction mixture was stirred under N_2 at room temperature for 1 h, cooled to 0 ºC and N-allyl-4-(4-amino-1-methyl-*1H*-pyrrole-2-carboxamido)-1-methyl-*1H*-pyrrole-2-carboxamide (2.39 mmol) added in CH_2Cl_2 (10 mL) and Et_3N (1 mL). The reaction mixture was allowed to come to room temperature, stirred for 18 h and then diluted with H_2O and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated and the residue purified by silica gel column chromatography to give product (0.555 g, 75%). ^{1}HNMR (400 MHz, DMSO-d_6) δ 3.77-3.82 (m, 5H), 3.847 (s, 3H), 3.854 (s, 3H), 5.06 (dd, 1H, *J* = 10.16 and 1.56Hz), 5.13 (dd, 1H, *J* = 15.23 and 1.56), 5.67 (dd, 1H, *J* = 10.16 and 1.95), 5.82-5.91 (m, 1H), 6.19 (dd, 1H, *J* = 17.19 and 1.95), 6.34-6.41 (m, 1H), 6.90 (d, 1H, *J* = 1.56), 6.94 (d, 1H, *J* = 1.95), 7.04 (d, 1H, *J* = 1.95), 7.21 (d, 1H, *J* = 1.56), 7.25 (d, 1H, *J* = 1.95), 7.28 (d, 1H, *J* = 1.95), 8.21 (t, 1H, *J* = 6.25), 9.91 (s, 1H), 9.96 (s, 1H), 10.117 (s, 1H).



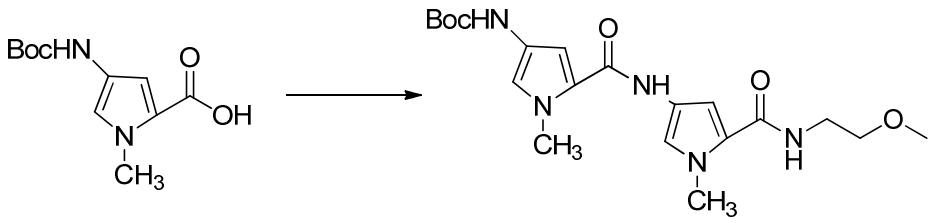
To a solution of 4-acrylamido-N-(5-((5-(allylcarbamoyl)-1-methyl-*1H*-pyrrol-3-yl)carbamoyl)-1-methyl-*1H*-pyrrol-3-yl)-1-methyl-*1H*-pyrrole-2-carboxamide (1.114 mmol, 532 mg) in

EtOH:H_2O (4:1, 24 mL) was added $NaHSO_3$ solution (2.228 mmol, 236 mg) in 3.2 mL of H_2O. The pH of the reaction mixture was then adjusted to 8.0 using 5% NaOH solution. The reaction mixture was then heated at 80 ºC until complete disappearance of starting material (based on TLC). The reaction mixture was cooled to 0 ºC and conc. HCl added dropwise so that the pH remained between 1 and 2. The solvent were evaporated to dryness and the solid residue treated with 1 N cold HCl solution and then centrifuged (4000 rpm, 10 min).The supernatant was decanted and the solid was twice treated with 1 N cold HCl, centrifuged and the supernatant decanted. The solid obtained was then dried thoroughly to give product (550 mg, 88%). ^{1}HNMR (400 MHz, DMSO-d_6) δ 2.53-2.57 (m, 2H), 2.66-2.70 (m, 2H), 3.75-3.87(m, 11H), 5.06 (dd, 1H, *J* = 10.16 and 1.56), 5.13 (dd, 1H, *J* = 17.18 and 1.56), 5.82-5.91 (m, 1H), 6.87 (d, 1H, *J* = 1.56), 6.90 (d, 1H, *J* = 1.56), 7.03 (d, 1H, *J* = 1.56), 7.15 (d, 1H, *J* = 1.56), 7.21 (d, 1H, *J* = 1.56), 7.25 (d, 1H, *J* = 1.56) 8.20 (t, 1H, *J* = 6.25), 9.91 (s, 2H), 9.97 (s, 1H).

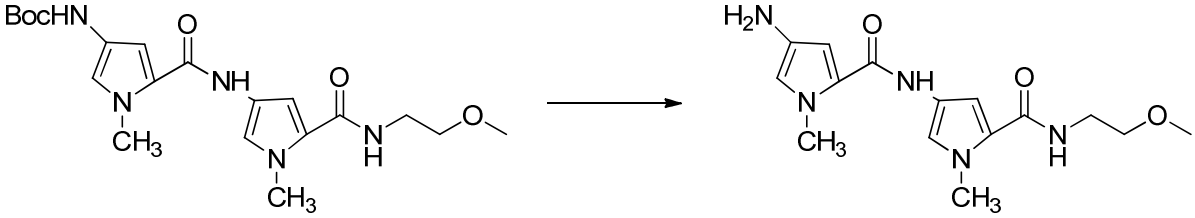


To a solution of 3-((5-((5-((5-(allylcarbamoyl)-1-methyl-*1H*-pyrrol-3-yl)carbamoyl)-1-methyl-*1H*-pyrrol-3-yl)carbamoyl)-1-methyl-*1H*-pyrrol-3-yl)amino)-3-oxopropane-1-sulfonic acid (0.947 mmol, 530 mg) in dry dioxane (53 mL) was added 3-methyl-1-*p*-tolyltriaz-1-ene (5.683 mmol, 848 mg).The reaction was heated in an oil bath at 80 ºC for about 1 h and then the dioxane evaporated to minimum volume and the residue loaded onto a silica gel column deactivated with Et_3N. The product was eluted in EtOAc (159 mg, 29%). ^{1}HNMR (400 MHz, DMSO-d_6) δ 2.74 (t, 2H, J = 7.03), 3.62 (t, 2H, J = 7.03), 3.77-3.82 (m, 5H), 3.83-3.86 (s, br, 6H), 3.87 (s, 3H), 5.06 (dd, 1H, *J* = 10.16 and 1.56), 5.13 (dd, 1H, *J* = 17.18 and 1.56), 5.82-5.91 (m, 1H), 6.89 (d, 1H, J = 1.95), 6.90 (d, 1H, J = 1.56), 7.04 (d, 1H, J = 1.95), 7.18 (d, 1H, J = 1.56), 7.21 (d, 1H, J = 1.95), 7.25 (d, 1H, J = 1.95), 8.20 (t, 1H, *J* = 6.25), 9.91 (s, 1H), 9.93 (s, 1H), 10.07 (s, 1H). ^{13}CNMR (150 MHz, DMSO-d_6) δ 29.84, 36.44, 36.57, 36.63, 41.18, 44.50, 57.38, 104.37, 104.80, 105.15, 115.16, 118.45, 118.65, 118.94, 122.12, 122.53, 123.62, 123.20, 123.23, 123.33, 136.45, 158, 158.93, 161.53, 166.05. HRMS (ES+H) m/z calcd for $C_{25}H_{32}N_7O_7S$ (574.2084) Found (574.2072).

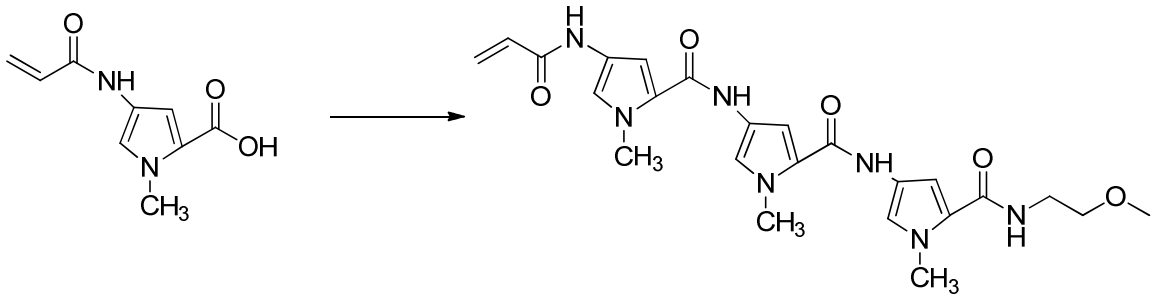
Synthesis of 9



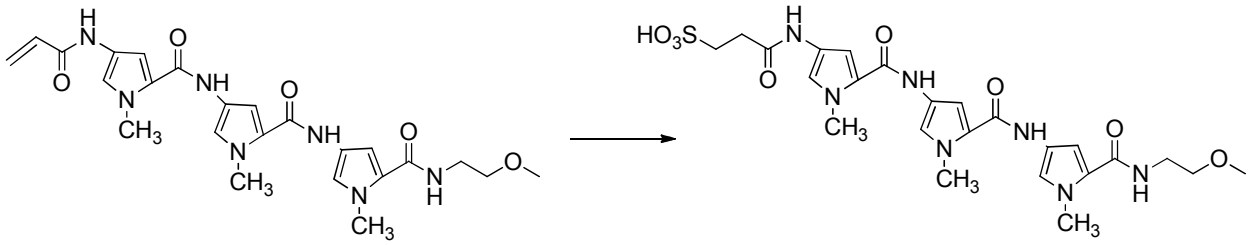
To a solution of 4-((tert-butoxycarbonyl)amino)-1-methyl-*1H*-pyrrole-2-carboxylic acid (3.90 mmol, 937 mg) and HOBT (5.85 mmol, 790 mg) in anhydrous CH_2Cl_2 (25mL) was added Et_3N (15.60 mmol, 2.17 mL) followed by the addition of EDC (7.80 mmol, 1.495 g) in CH_2Cl_2 (25mL). The reaction mixture was stirred under N_2 at room temperature for 1 h and then cooled to 0 ºC and 4-amino-N-(2-methoxyethyl)-1-methyl-*1H*-pyrrole-2-carboxamide (5.07 mmol) added in CH_2Cl_2 (30 mL). The reaction mixture was allowed to warm to room temperature and stirred for 18h, diluted with H_2O and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated, and the residue purified by silica gel column chromatography to yield product (1.141 g, 69%). ^{1}HNMR (600 MHz, DMSO-d_6) δ 1.45 (s, 9H), 3.26 (s, 3H), 3.30-3.32 (m, 2H), 3.39-3.42 (m, 2H,), 3.78 (s,3H), 3.79δ (s, 3H), 6.81 (s, 1H), 6.86 (d, 1H, *J* = 1.26Hz), 6.88 (s, 1H), 7.16 (d, 1H, *J* = 1.2), 7.99 (t, 1H, *J* = 5.4), 9.09 (s, 1H), 9.80 (s, 1H).



To a solution of tert-butyl (5-((5-((2-methoxyethyl) carbamoyl)-1-methyl-*1H*-pyrrol-3-yl) carbamoyl)-1-methyl-*1H*-pyrrol-3-yl) carbamate (2.625 mmol, 1.101 g,) in anhydrous CH_2Cl_2 (13 mL) was added 50%TFA:CH_2Cl_2 (40 mL) at 0 ºC such that the final concentration of TFA was around 40%. The reaction mixture was stirred under N_2 at 0 ºC until complete disappearance of starting material. The solvent was removed to give 4-amino-N-(5-((2-methoxyethyl)carbamoyl)-1-methyl-*1H*-pyrrol-3-yl)-1-methyl-*1H*-pyrrole-2-carboxamide in quantitative yield. ^{1}HNMR (600 MHz, DMSO-d_6) δ 3.26 (s, 3H), 3.31-3.34 (m, 2H), 3.39-3.42 (m, 2H), 3.80 (s,3H), 3.89 (s,3H), 6.86 (d, 1H, *J* = 1.8), 6.94 (d, 1H, *J* = 1.8), 7.11 (d, 1H, *J* = 1.8), 7.19 (d, 1H, *J* = 1.8), 8.04 (t, 1H, *J* = 5.4), 9.73 (s, br ,2H), 9.99 (s, 1H).

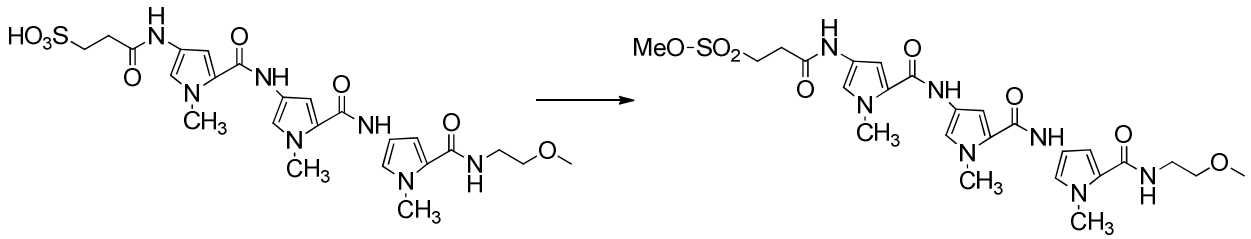


To a solution of 4-acrylamido-1-methyl-*1H*-pyrrole-2-carboxylic acid (2.059 mmol, 400 mg) and HOBT (3.086 mmol, 417 mg) in anhydrous CH_2Cl_2 (40 mL) was added Et_3N (8.24 mmol, 1.15 mL) followed by the addition of EDC (4.12 mmol, 0.789 g) in CH_2Cl_2 (30 mL). The reaction mixture was stirred under N_2 at room temperature for 1 h, cooled to 0 ºC and 4-amino-N-(5-((2-methoxyethyl)carbamoyl)-1-methyl-*1H*-pyrrol-3-yl)-1-methyl-*1H*-pyrrole-2-carboxamide (2.68 mmol) added in CH_2Cl_2 (30 mL) and Et_3N (0.5 mL). The reaction mixture was allowed to come to room temperature and stirred for about 18h, diluted with H_2O and sequentially extracted with CH_2Cl_2, EtOAc and MeOH. The organic extracts were dried over Na_2SO_4, evaporated and the residue purified by silica gel column chromatography to yield yellow product (0.720 g, 70%) that was washed with hot EtOH to give white product. ^{1}HNMR (600 MHz, DMSO-d_6) δ 3.26 (s, 3H), 3.31-3.34 (m, 2H), 3.40-3.42 (m, 2H), 3.80 (s, 3H), 3.846 (s, 3H), 3.85 (s, 3H), 5.67 (dd,1H, *J* = 10.2 and 1.8), 6.19 (dd,1H, *J* = 17.4 and 1.8), 6.35-6.39 (m,1H), 6.88 (d, 1H, *J* = 1.2), 6.94 (d, 1H, *J* = 1.8), 7.04 (d, 1H, *J* = 1.8), 7.19 (d, 1H, *J* = 1.8), 7.25 (d, 1H, *J* = 1.8), 7.28 (d, 1H, *J* = 1.8), 8.02 (t, 1H, *J* = 5.4), 9.91 (s, 1H), 9.95 (s, 1H), 10.11 (s, 1H).



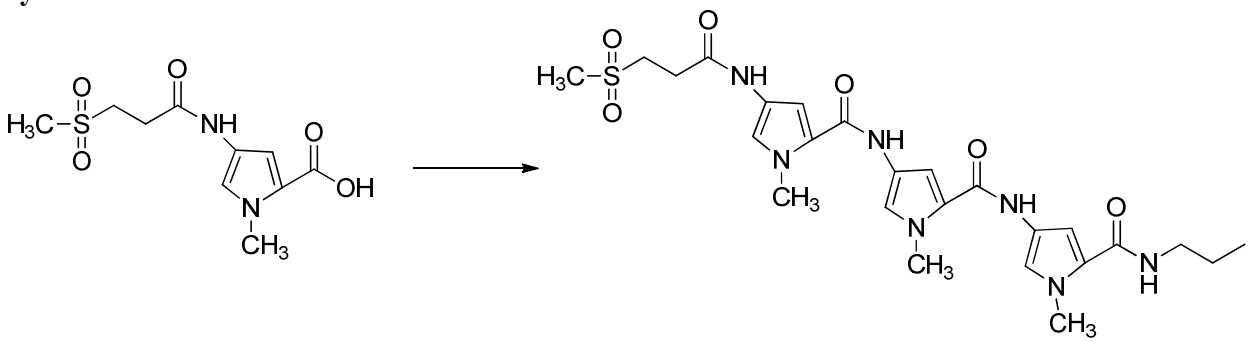
To a solution of 4-acrylamido-N-(5-((5-((2-methoxyethyl)carbamoyl)-1-methyl-*1H*-pyrrol-3-yl)carbamoyl)-1-methyl-*1H*-pyrrol-3-yl)-1-methyl-*1H*-pyrrole-2-carboxamide (0.0787 mmol, 39 mg) in EtOH:H_2O (4:1, 2 mL) was added $NaHSO_3$ solution (0.157 mmol, 16.7 mg) in 0.242 mL of H_2O. The pH of the reaction mixture was then adjusted to 8.0 using 5% NaOH solution. The reaction mixture was then heat at 80 ºC until complete disappearance of starting material (based on TLC). The reaction mixture was cooled to 0 ºC and conc. HCl was added dropwise so that the pH was between 1 and 2. The solvent was then evaporated to dryness and the solid residue was then treated with 1 N cold HCl solution and then centrifuged (4000 rpm, 10 min).The supernatant was decanted and the solid was twice treated with 1 N cold HCl, centrifuged and the supernatant decanted. The solid obtained was then dried thoroughly to give product (38mg, 83%). ^{1}HNMR (600 MHz, DMSO-d_6) δ 2.52-2.54 (m, 2H), 2.65-2.68 (m, 2H), 3.26 (s, 3H), 3.31-3.34 (m, 2H), 3.41 (t, 2H, *J* = 6), 3.80 (s, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 6.87 (d, 2H, *J* = 1.2), 7.02 (d, 1H, *J* = 1.2), 7.15 (d, 1H, *J* = 1.2), 7.19 (d, 1H, *J* = 1.8), 7.24(d, 1H, *J* = 1.8), 8.02

(t, 1H, J = 5.4), 9.90 (s, 1H), 9.91 (s, 1H), 9.98 (s, 1H). HRMS (EI^+) m/z calcd for $C_{24}H_{31}N_7O_8S$ (577.61) Found (577.20).



To a solution of 3-((5-((5-((5-((2-methoxyethyl)carbamoyl)-1-methyl-*1H*-pyrrol-3-yl)carbamoyl)-1-methyl-*1H*-pyrrol-3-yl)carbamoyl)-1-methyl-*1H*-pyrrol-3-yl)amino)-3-oxopropane-1-sulfonic acid (0.0658 mmol, 38 mg) in dry dioxane (5 mL) was added 3-methyl-1-*p*-tolyltriaz-1-ene (0.263 mmol, 39.2 mg).The reaction was heated in an oil bath at 80 ºC for about 1h and the dioxane reduced to a minimum volume and then loaded onto a silica gel column deactivated with Et_3N. The product was sequentially eluted in EtOAc to 5% MeOH:EtOAc (4 mg, 10%). ^{1}HNMR (600 MHz, DMSO-d_6) δ 2.52-2.56 (m, 2H), 2.74 (t, 2H, J = 7.2), 3.27 (s, 3H), 3.39-3.43 (m, 2H), 3.62 (t, 2H, J = 7.2), 3.78 (s, 3H), 3.841 (s, 3H), 3.843(s, 3H), 3.87(s,3H), 6.87 (d, 2H, J = 1.8), 6.89(d,1H, J = 1.8), 7.03 (d, 1H, J = 1.8), 7.17 (d, 1H, J = 1.2), 7.19 (d, 1H, J = 1.8), 7.24(d, 1H, J = 1.8), 8.02 (t, 1H, J = 5.4), 9.89 (s, 1H), 9.92 (s, 1H), 10.07 (s, 1H).

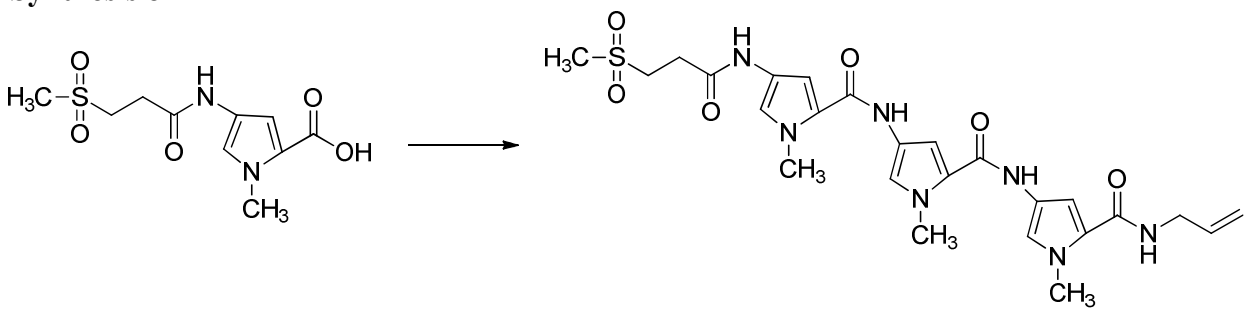
Synthesis of 10



To a solution of 1-methyl-4-(3-(methylsulfonyl)propanamido)-*1H*-pyrrole-2-carboxylic acid (0.397 mmol, 109 mg) and HOBT (0.548 mmol, 74 mg) in anhydrous DMF (2.5 mL) was added Et_3N (1.46 mmol, 0.203 mL). This was followed by the addition of EDC (0.73 mmol, 0.140 g) in CH_2Cl_2 (15 mL). The reaction mixture was stirred under N_2 at room temperature for 1 h, cooled to 0 ºC and amine, **f** (0.548 mmol) was added in CH_2Cl_2 (10 mL) and Et_3N (0.1 mL). The reaction mixture was allowed to come to room temperature and stirred for about 18 h, solvent removed and the residue purified by silica gel column chromatography to yield product (66 mg, 30%). The product had a yellow color and was treated with hot EtOH to yield a white solid ethanol when a white solid product. ^{1}HNMR (400 MHz, DMSO-d_6) δ 0.867 (t, 3H, J = 7.42), 1.442-1.532 (m, 2H) 2.73 (t, 2H, J = 7.8), 3.01(s, 3H), 3.10-3.15 (m, 2H) 3.41 (t, 2H, J = 7.8), 3.79 (s, 3H), 3.84 (s, br 6H), 6.86 (d, 1H, J = 1.95), 6.89 (d, 1H, J = 1.95), 7.03 (d, 1H, J =

1.95), 7.16-7.18 (m, 2H) 7.24 (d, 1H, *J* = 1.97), 8.0 (t, 1H, *J* = 5.86), 9.89 (s, br, 1H), 9.93 (s, br, 1H), 10.07 (s, br, 1H). ^{13}CNMR (150 MHz, DMSO-d_6) δ 11.92, 23.07, 28.69, 36.36, 36.57, 36.61, 40.66, 40.91, 50.24, 104.39, 104.58, 105.13, 118.15, 118.67, 118.93, 122.17, 122.56, 123.25, 123.33, 123.56, 158.83, 158.91, 161.71, 166.57. HRMS (ES+H) m/z calcd for $C_{25}H_{34}N_7O_6S$ (560.229) Found (560.224).

Synthesis of 11



To a solution of 1-methyl-4-(3-(methylsulfonyl)propanamido)-*1H*-pyrrole-2-carboxylic acid (0.390 mmol, 107 mg) and HOBT (0.518 mmol, 70 mg) in anhydrous DMF (2 mL) was added Et_3N (1.38 mmol, 0.192 mL) followed by the addition of EDC (0.69 mmol, 133 mg) in CH_2Cl_2 (10 mL). The reaction mixture was stirred under N_2 at room temperature for 1 h, cooled to 0 ºC and N-allyl-4-(4-amino-1-methyl-*1H*-pyrrole-2-carboxamido)-1-methyl-*1H*-pyrrole-2-carboxamide (0.518 mmol) added in CH_2Cl_2 (10 mL) and Et_3N (0.10 mL). The reaction mixture was allowed to come to room temperature, stirred for about 18 h and the solvent evaporated. The residue was purified by silica gel column chromatography to yield product (19 mg, 8.7%). ^{1}HNMR (600 MHz, DMSO-d_6) δ 2.73 (t, 2H, *J* = 7.8), 3.01 (s, 3H), 3.41 (t, 2H, *J* = 7.8), 3.78-3.82 (m, 5H), 3.84 (s, 3H), 3.85(s, 3H), 5.06 (dd, 1H, *J* = 10.80 and 1.80), 5.14 (dd, 1H, *J* = 17.40 and 1.80), 5.84-5.90 (m, 1H) 6.89(d, 1H, *J* = 1.2), 6.90 (d, 1H, *J* = 1.2), 7.04 (d, 1H, *J* = 1.2), 7.17 (d, 1H, *J* = 1.2), 7.21 (d, 1H, *J* = 1.2), 7.24 (d, 1H, *J* = 1.2), 8.19(t, 1H, *J* = 5.4), 9.90(s, br, 1H), 9.92 (s, br, 1H), 10.05 (s, br, 1H). ^{13}CNMR (150 MHz, DMSO-d_6) δ 28.69, 36.45, 36.57, 36.62, 40.91, 41.19, 50.24, 104.39, 104.78, 105.16, 115.16, 118.47, 118.67, 118.94, 122.17, 122.54, 122.62, 123.21, 123.24, 123.32, 136.45, 158.83, 158.94, 161.54, 166.57. HRMS (ES+H) m/z calcd for $C_{25}H_{32}N_7O_6S$ (558.213) Found (558.214).

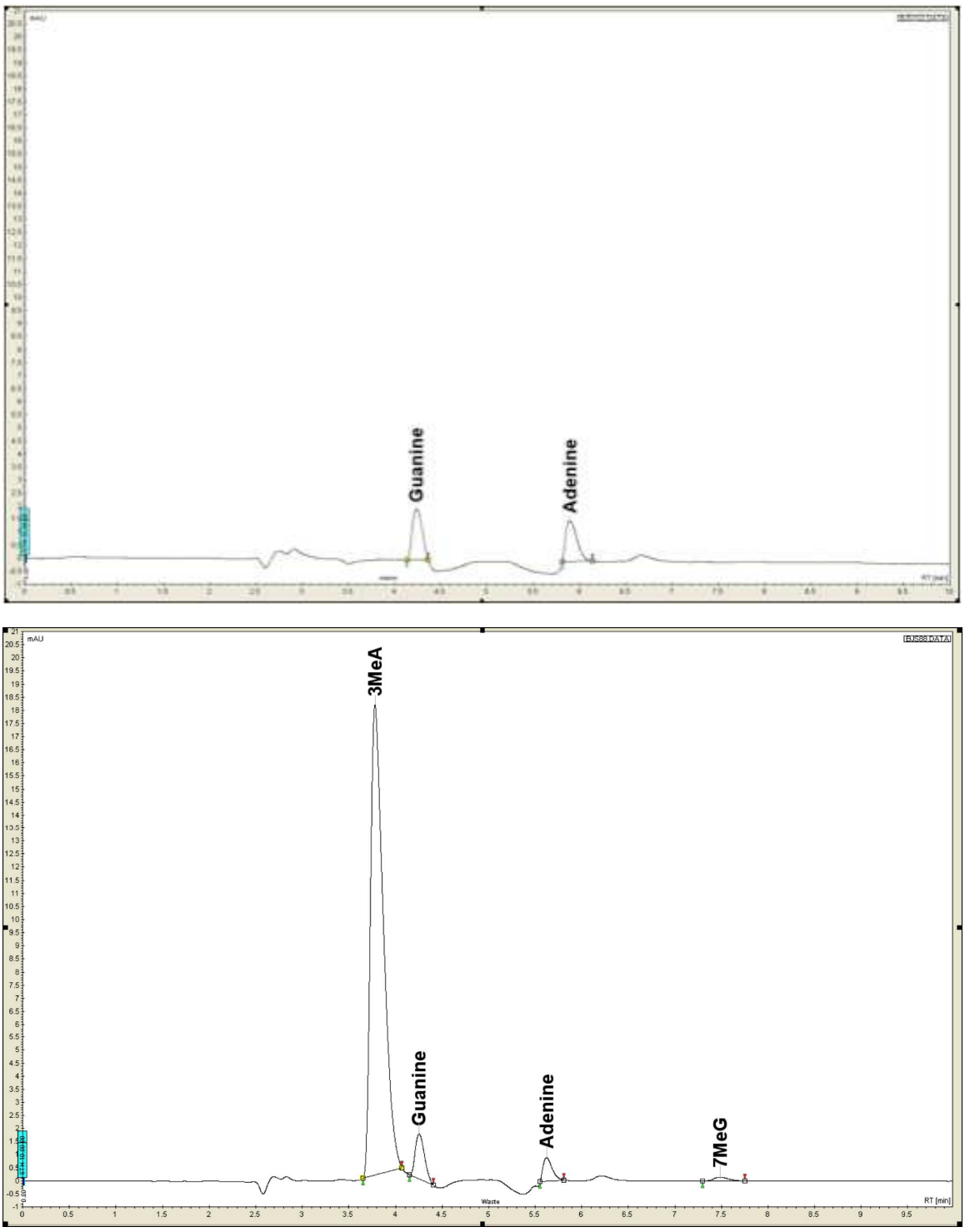


Figure S1. HPLC trace of untreated calf thymus DNA (top) and DNA treated with compound **7**.

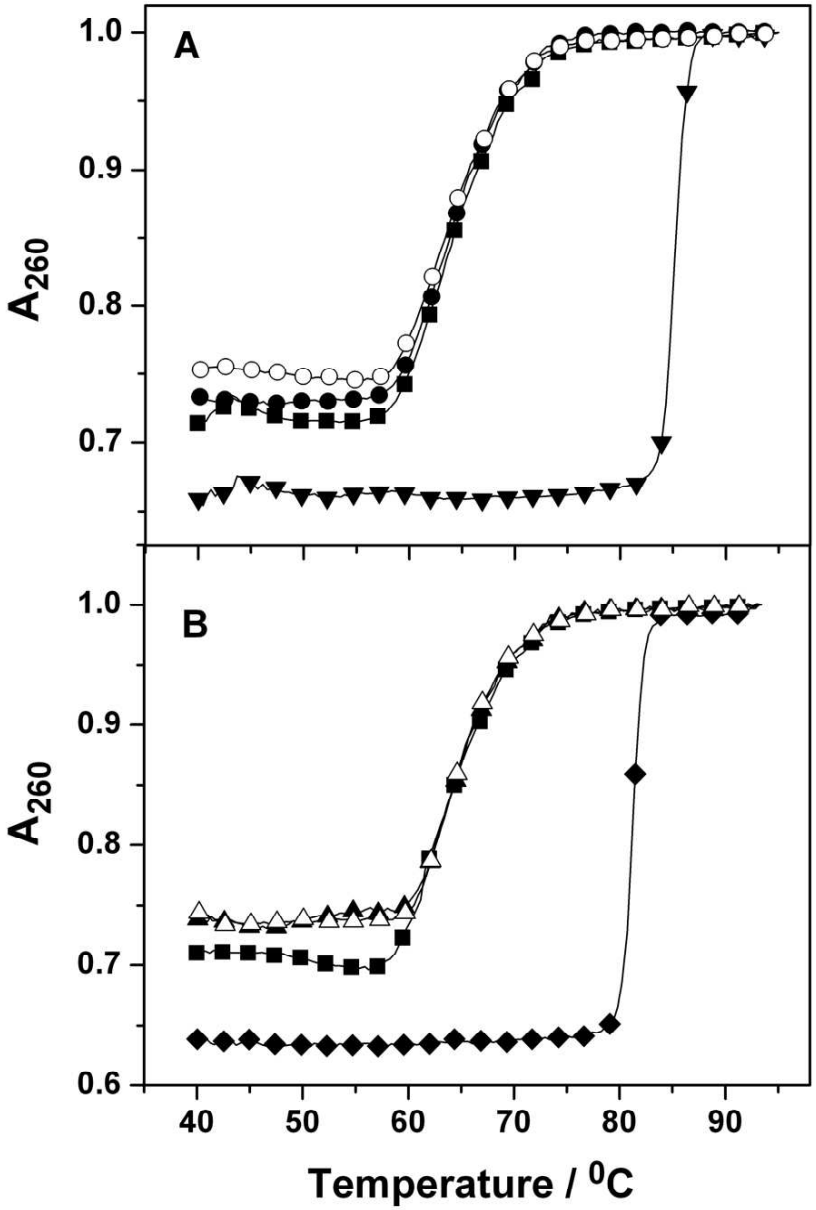


Figure S2. UV melting curves of calf thymus DNA (~ 76 μM) in the absence (■) and presence of 1:1 complex of (**A**) compound **5** (●), compound **6** (o) and netropsin (▼) and (**B**) compound **10** (▲), compound **11** (Δ) and distamycin (◆) in 10% ethanol-phosphate buffer, pH 7 at 260 nm.

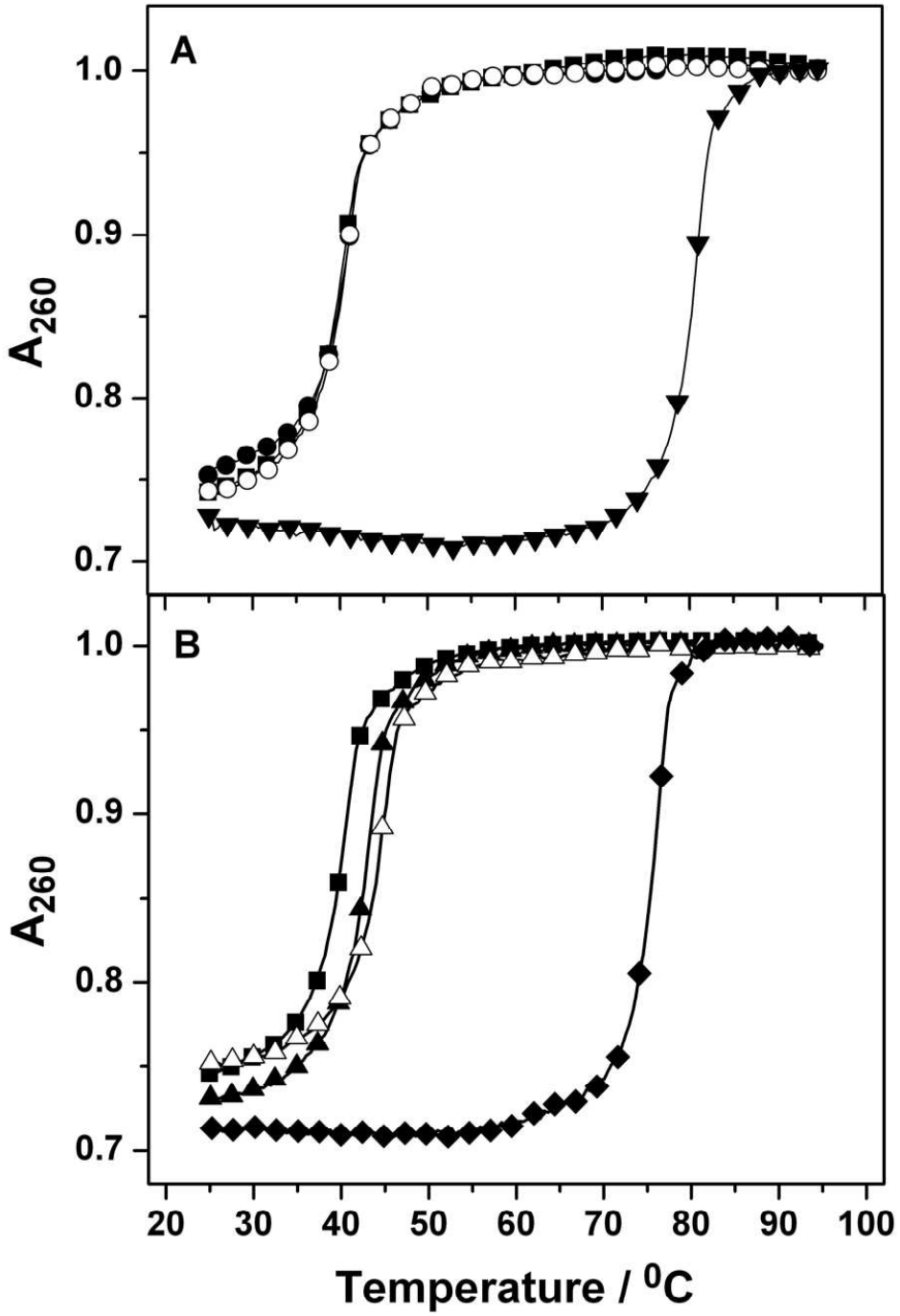


Figure S3. UV melting curves of poly-d(A-T) · poly-d(A-T) ~ 76 μM, in the absence (■) and presence of 1:1 complex of (**A**) compound 1 (●), compound 2 (o) and netropsin (▼) and (**B**) compound 3 (▲), compound 4 (Δ) and distamycin (◆) in 10% EtOH-phosphate buffer, pH 7 at 260 nm.