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Design and synthesis of new thiazolated cross-linked DNA binding polyamides for altered sequence recognition

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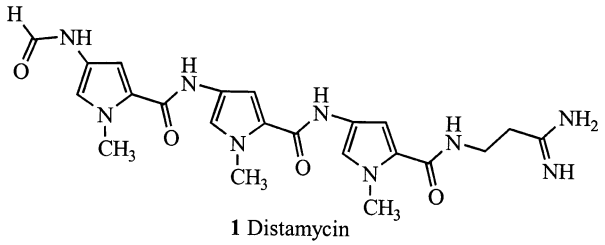
Abstract—The design and synthesis are described for six new thiazolated versions of cross-linked polyamides (**9a**–**f**), in which the Th–Py–Py array of heterocycles is tethered from the central pyrrole by C5 and C7 methylene chains. The thiazole ring in the different cross-linked polyamides (**9a**–**f**) bears three different functional groups (viz. H, NHCHO and NH_2). These six cross-linked polyamides were analyzed for their gyrase inhibition properties, which showed that amTh–Py–Py (**9b**), tethered by a C7 linker, with an inhibitory concentration against gyrase of (IC_{50}=0.01 μM) is the most potent inhibitor among all the thiazolated cross-linked polyamides analyzed so far.

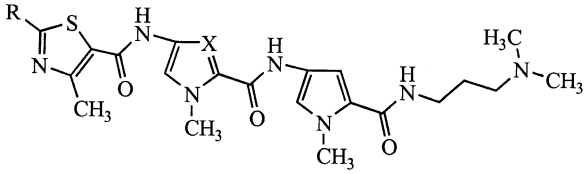
1. Introduction

Over the past two decades significant advances have been made in understanding the interactions of double helical DNA with small molecules important in antitumor, antibiotic and antiviral chemotherapy. An understanding of drug–DNA interactions at the molecular level is crucial in facilitating the design of new drugs and probes that can recognize specific DNA sequences.[1–5] Netropsin and distamycin are two well studied members of the pyrrolocarboxamidine class of naturally occurring antibiotic oligopeptides. The discovery of Pelton and Wemmer[6] of stable side by side minor groove binding led to the design of hairpin, cross-linked (stapled) and bispolyamides for extended recognition of the minor groove of DNA.[7] One legitimate aspect of molecular design in the context of polyamides is the introduction of heterocyclic moieties capable of specific DNA recognition by hydrogen bond acceptance and donation. The natural product distamycin (**1**) was first modified for a change in sequence recognition from an AT site to a GC site by Lown and Dickerson[8] independently by introducing an imidazole ring in place of the pyrrole ring in the natural product. Since then, Lown's and Dervan's group have done extensive work on the introduction of new heterocycles in the polyamide system to alter sequence specification and recognition as compared with the natural product. One such modification performed by our group is the introduction of the thiazole ring in three ring systems **2** and **3** (Fig. 1) and also to the cross-linked system. Recently Dervan's group has incorporated this ring modification into a hairpin system.[9]

We have studied the binding affinities of a series of thiazole–imidazole–pyrrole (TIP) monomers **3** and cross-linked dimers, and evaluated the effect on selectivity and binding affinity by introducing different N-terminal head groups attached to the leading thiazole ring and different lengths of the crosslinking methylene chain.[10,11] In continuation of our ongoing research to optimize the binding of these cross-linked polyamides in 2:1 stoichometry, we wish to report the synthesis of cross-linked thiazolated polyamides in which the linking is carried out with the polymethylene chain in the center with the 1*H* pyrrole flanked by the pyrrole with the side chain of dimethylaminopropylamine and the thiazole linkage with different active functionalities on the other side of the structure



1 Distamycin



R = NH_2 or NHCHO or H

2 X = CH (TPP)

3 X = N(TIP)

Figure 1.

Keywords: heterocycle; DNA; polyamides; thiazoles.

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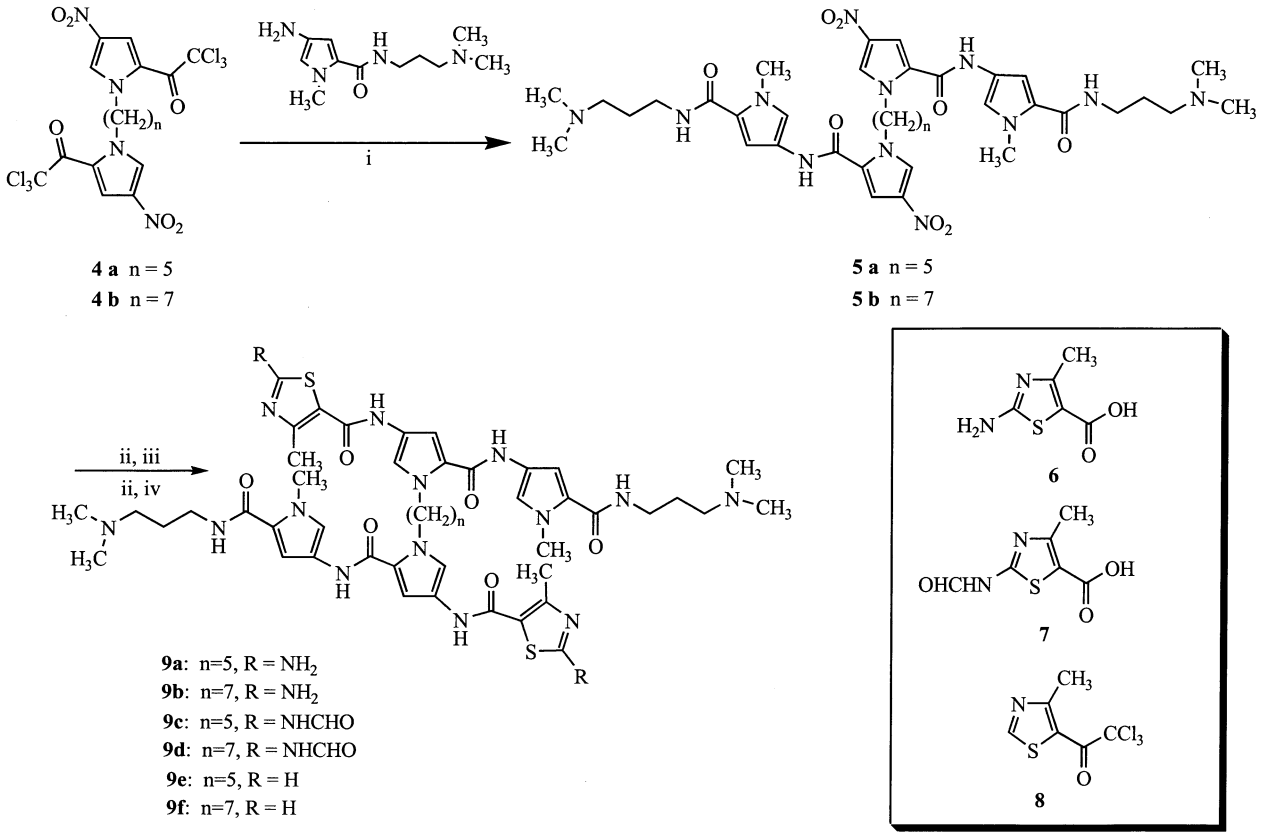

Scheme 1. *Reagent and conditions*: (i) DMF/THF (1:1), RT, 5 h, 72%, (ii) H_2/Pd–C, MeOH, 50 psi, 2 h, (iii) **6** or **7**, DMF, DCC, HOBt, RT, 24 h, 34, 35%, (iv) **8**, DMF/THF (1:1), RT, 5 h, 42%.

(TPP). These cross-linked polyamides were evaluated for their gyrase inhibition activity. These activities were compared with the previous thiazolated cross-linked polyamides (Th–Im–Py) (**3** tethered by methylene linker from the central imidazole ring), which shows that the (Th–Py–Py) (**2** tethered by methylene linker from the central pyrrole ring) are more potent inhibitors than the earlier reported compounds.

2. Results and discussion

The synthesis of cross-linked polyamides (**9a**–**f**) started with the synthesis of the specifically functionalized central bispyrrole unit (**4a**,**b**) (Scheme 1). The choice of polymethylene linkers $(CH_2)_5$ and $(CH_2)_7$ was based upon previous studies conducted in our group.[11] The alkane linkers $(CH_2)_5$ and $(CH_2)_7$ were introduced by the alkylation of the pyrrole with 1,5-dibromopentane and 1,7-dibromoheptane to the potassium salt of pyrrole followed by the trichloroacetylation and nitration with trichloroacetylchloride and fuming nitric acid in acetic anhydride at the 2 and 4 positions of the bis-linked pyrrole to give compounds (**4a**,**b**) respectively. The synthesis of cross-linked tetrapyrrole unit (**5a**,**b**) involved the catalytic reduction of *N*-[3-(dimethylamino)propyl]-1-methyl-4-nitropyrrole-2-carboxamide[12] followed by the in situ coupling of the amine to **4a** in DMF/THF (1:1). The reaction was completed after stirring the reaction mixture at room temperature for 5 h. The tetrapyrrole crosslinked polyamide **5a** was purified on a silica gel column. Compound **5b** was synthesized in a similar manner using **4b** as the starting material. The nitro group of **5a** was catalytically reduced to an amine which was subsequently coupled with 2-amino-4-methyl thiazole-5-carboxylic acid **6** catalyzed by 1,3-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole which afforded the target cross-linked polyamides **9a** in 34% yield. Compound **9b** was synthesized in a similar manner starting from **5b**. Attempts to obtain formylated analogues of **9a**,**b** by direct formylation with acetic anhydride/formic acid or by the protection of amino function with di-*tert*-butyldicarbonate and then deblocking it with excess of formic acid to incorporate formyl group were unsuccessful. Instead introduction of the N-formyl group in the cross-linked polyamides was achieved by coupling the reduced amine of (**5a**,**b**) with 2-(formylamino)-4-methyl thiazole-5-carboxylic acid **7** as previously described[11] to afford (**9c**,**d**) in 35% yield. Attempted deaminations of **9a** or **b** using the reported procedures[13] were unsuccessful. The deaminated cross-linked polyamides **9e** were obtained by coupling the reduced amine of **5a** with 4-methyl-5-trichloroacetyl thiazole **8** in 42% yield. Similarly **9f** was obtained by coupling the reduced amine of **5b** with 4-methyl-5-trichloroacetyl thiazole **8**.

These cross-linked polyamides of the type (thiazole–pyrrole–pyrrole) were analyzed for their DNA sequence specific recognition. Studies have shown that the $(CH_2)_7$ polymethylene linker with amino functional group on the thiazole ring **9b** strongly binds to the target DNA sequence whereas rest of the compounds **9a** and **c**–**f** showed poor DNA binding specificity[15] as indicated by our earlier

studies.[14] Therefore compound **9b** alone was studied for the gyrase inhibition by cleavage reaction (which is most specific, because a restriction fragment with a single strong gyrase site was used).[16] The ^{32}P-labelled 162 basepair fragment from pBR 322 was used as substrate in buffer. Cleavage occurs on the gyrase recognition site T GGCC A and is detected in presence of ciprofloxacin. In this reaction two fragments are produced (seen in the gel). In the presence of the polyamide **9b** the formation of the two fragments is inhibited, which was quantitatively detected using the reported procedure.[16] Comparative in vitro studies on the influence on gyrase (from S. Noursei) catalyzed cleavage of the DNA studies showed that the $(CH_2)_7$ cross-linked polyamide (**9b**) is the most potent inhibitor with an IC_{50} value 0.01 μM as compared to that of (amTh–Im–Py)[14a] $(CH_2)_7$ IC_{50} is 0.17 μM,[14a] and that of the monomers **2** and **3**, and of natural product **1** is 1.0 μM. The sequence around a single gyrase site, which is contained in the 162 bp fragment (using BgII/Sau3A-162 bp fragment from pBR322) was used as substrate in the cleavage assay, can reasonably explain the pronounced antigyrase effect of the dimer. A section with gyrase cleavage site (bold) has a high affinity site (underlined) for the polyamide dimer located near the former.

CGATGGCCTTCCCCATTAT
CCTA**CCGG**AAGGGGTAATA

It seems possible that binding of the dimer along the AT sequence containing a T.A base pair step induces bending of DNA and hence efficiently blocks the enzyme mediated cleavage on the upstream located GGCC site.[17] Unlike the dimer the monomers and distamycin showed no or rather weak inhibitory effects at low concentrations comparable to that of the dimer since much higher drug concentrations would be required to produce bending. Thus **9b** is the most potent inhibitor of gyrase mediated cleavage of all the thiazolated cross-linked polyamides tested to date.

3. Experimental

3.1. General

All chemicals used were of reagent grade. The reactions were carried out in anhydrous solvents. Anhydrous *N*,*N*′-dimethylformamide (DMF), methanol (MeOH), *N*,*N*-diisopropylethylamine (DIEA), triethylamine (TEA), 1,3-dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBt), and 4-methylthiazole were purchased from Aldrich Chemical Co. and were used without any purification. The reactions were monitored by analytical thin layer chromatography (TLC) using silica gel (60F-254, Merck) coated aluminum backed plates. ^{1}H NMR spectra were recorded on a 300 MHz spectrometer and the chemical shifts are reported in δ ppm with respect to tetramethylsilane as an internal standard. The values for the coupling constant (*J*) are expressed in hertz. Mass spectra and high resolution mass spectra (HRMS) were done by positive-mode electrospray ionization with Micromass ZapSpec Hybrid Sector-TOF. Melting points were determined on an electrothermal melting point apparatus and are uncorrected.

Compound **4**–**8** were synthesized by the reported method.[7c,11]

3.1.1. 1,1′-(1,5-Pentamethylene)bis[*N*-[5-[[(3,3-dimethylamino)propyl]carbonyl]-1-methyl-4-pyrrol-3-yl]-4-[[[2-amino-4-methylthiazole-5-yl]carbonyl]amino]-pyrrole]-2-carboxamide (9a). Palladium charcoal (10%, 500 mg) was added to a solution of **5a** (1.00 g, 1.25 mmol) in anhydrous DMF/MeOH (1:1 v/v; 100 mL). Degassing of the solution was done by argon, and then the mixture was hydrogenated in a Parr shaker for 2 h at 50 psi. The catalyst was then removed by filtration and the filtrate was washed with methanol. The combined filtrate was evaporated under high vacuum to remove traces of methanol. The residual reduced product was redissolved in anhydrous DMF (50 mL), and compound **6** (396 mg, 2.51 mmol) and HOBt (474 mg, 3.51 mmol) were added to it with constant stirring. A solution of DCC (723 mg, 3.51 mmol) in anhydrous DMF (3 mL) was added slowly to the above stirred solution, and the stirring is continued at room temperature for an additional 18 h. The reaction mixture was filtered and the solvent was removed in vacuo. The impure product was purified on silica gel column chromatography. The pure compound **9a** was eluted with CH_2Cl_2/MeOH/NH_4OH (80:20:0.4, v/v/v) as a yellow powder in 34% yield (430 mg), mp 162–164°C. ^{1}H NMR (DMSO-d_6): δ 1.25 (m, 2H), 1.58 (m, 2H), 1.76 (q, 4H), 2.21 (s, 12H), 2.37 (t, *J*=7.0 Hz, 4H), 2.48 (s, 6H), 3.25 (dt, *J*=6.0 Hz, 4H), 3.91 (s, 6H), 4.41 (t, 4H), 6.83, 6.94 (2d, *J*=1.6 Hz, 2H each), 7.38 (s, 4H), 7.79 (d, *J*=1.6 Hz, 2H), 8.00 (t, *J*=6.0 Hz, 2H, D_2O exchangeable), 8.25 (d, *J*=1.7 Hz, 2H), 9.95, 10.25 (2s, 2H each, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 180.25, 168.13, 161.37, 158.51, 155.45, 135.69, 133.86, 130.32, 122.88, 121.11, 118.21, 111.45, 104.40, 54.53, 47.18, 42.01, 38.58, 36.04, 35.50, 30.79, 28.13, 25.85, 24.46, 15.59. HRMS calcd for $C_{47}H_{65}N_{16}O_6S_2$ 1013.471, found 1013.471 (M^++H, 100%).

3.1.2. 1,1′-(1,7-Heptamethylene)bis[*N*-[5-[[(3,3-dimethylamino)propyl]carbonyl]-1-methyl-4-pyrrol-3-yl]-4-[[[2-amino-4-methylthiazole-5-yl]carbonyl]amino]-pyrrole]-2-carboxamide (9b). The title compound was synthesized in a similar way as described above from **5b** and **6** as a yellow powder in 34% yield (440 mg), mp 158–163°C. ^{1}H NMR (DMSO-d_6): δ 1.25 (m, 6H), 1.60 (m, 8H), 2.15 (s, 12H), 2.25 (t, *J*=7.0 Hz, 4H), 2.38 (s, 6H), 3.20 (dt, *J*=6.0 Hz, 4H), 3.80 (s, 6H), 4.25 (t, 4H), 6.80, 6.94, 7.15, 7.20 (4d, *J*=1.6 Hz, 2H each), 7.40 (s, 4H, D_2O exchangeable), 8.08 (t, *J*=6.0 Hz, 2H, D_2O exchangeable), 9.44, 9.86 (2S, 2H, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 180.05, 166.42, 160.41, 158.51, 155.45, 136.23, 131.42, 130.32, 122.88, 120.15, 118.21, 114.73, 104.40, 57.38, 47.19, 42.01, 38.37, 36.15, 35.53, 30.79, 27.24, 25.85, 23.66, 21.57, 20.07, 15.89. HRMS calcd for $C_{49}H_{69}N_{16}O_6S_2$ 1041.531, found 1041.532 (M^++H, 100%).

3.1.3. 1,1′-(1,5-Pentamethylene)bis[*N*-[5-[[(3,3-dimethylamino)propyl]carbonyl]-1-methyl-4-pyrrol-3-yl]-4-[[[2-formylamino-4-methylthiazole-5-yl]carbonyl]amino]-pyrrole]-2-carboxamide (9c). Compound **5a** (1.00 g, 1.25 mmol) was reduced by palladium charcoal (500 mg) in a similar manner as described above. The reduced product was redissolved in anhydrous DMF (50 mL) and **7**

(465 mg, 2.50 mmol) and HOBt (472 mg, 3.50 mmol) were added to it at room temperature under nitrogen with constant stirring. A solution of DCC (721 mg, 3.50 mmol) in anhydrous DMF (3 mL) was added to this stirred solution, and the stirring was continued at room temperature for additional 18 h. The reaction mixture was filtered and the solvent was removed in vacuo. The impure product was purified on silica gel column chromatography. The pure compound **9c** was eluted with CH_2Cl_2/MeOH/NH_4OH (80:20:0.3, v/v/v) as a yellow powder in 35% yield (460 mg), mp 170–173°C. ^{1}H NMR (DMSO-d_6): δ 1.25 (m, 2H), 1.54 (m, 2H), 1.67 (q, 4H), 2.20 (s, 12H), 2.37 (t, J=7.0 Hz, 4H), 2.45 (s, 6H), 3.15 (dt, J=6.0 Hz, 4H), 3.95 (s, 6H), 4.35 (t, 4H), 6.86, 6.74 (2d, J=1.6 Hz, 2H each), 7.59 (s, 2H, D_2O exchangeable), 7.92, (d, J=1.6 Hz, 2H), 8.00 (t, J=6.0 Hz, 2H, D_2O exchangeable), 8.15 (d, J=1.7 Hz, 2H), 8.55 (s, 2H), 9.62, 9.75 (2s, 2H each, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 181.33, 167.23, 161.37, 159.60, 156.23, 155.45, 135.69, 132.86, 130.32, 122.88, 122.11, 118.21, 110.63, 102.20, 57.81, 47.18, 42.15, 36.58, 36.14, 35.50, 30.79, 28.22, 25.07, 24.32, 14.73. HRMS calcd for $C_{49}H_{65}N_{16}O_8S_2$ 1069.461, found 1069.460 (M^++H, 100%).

3.1.4. 1,1′-(1,7-Heptamethylene)bis[*N*-[5-[[(3,3-dimethylamino)propyl]carbonyl]-1-methyl-4-pyrrol-3-yl]-4-[[[2-formylamino-4-methylthiazole-5-yl]carbonyl]amino]-pyrrole]-2-carboxamide (9d). The title compound was synthesized in a similar manner from **5b** and **7** as a yellow powder in 37% yield (490 mg), mp 174–178°C. ^{1}H NMR (DMSO-d_6): δ 1.25 (m, 6H), 1.65 (m, 8H), 2.15 (s, 12H), 2.30 (t, J=7.0 Hz, 4H), 2.40 (s, 6H), 3.29 (dt, J=6.0 Hz, 4H), 3.65 (s, 6H), 4.20 (t, 4H), 6.82, 6.98, 7.15, 7.20 (4d, J=1.6 Hz, 2H each), 7.72 (s, 2H), 8.57 (s, 2H), 8.00 (t, J=6.0 Hz, 2H, D_2O exchangeable), 9.50, 9.68 (2S, 2H, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 181.05, 165.41, 161.23, 160.33, 156.54, 155.45, 136.43, 130.42, 130.32, 127.89, 120.25, 118.24, 114.43, 104.43, 57.34, 48.29, 42.11, 37.77, 36.23, 35.50, 30.74, 27.34, 25.85, 23.66, 21.43, 20.03, 15.75. HRMS calcd for $C_{51}H_{69}N_{16}O_8S_2$ 1097.492, found 1097.489 (M^++H, 100%).

3.1.5. 1,1′-(1,5-Pentamethylene)bis[*N*-[5-[[(3,3-dimethylamino)propyl]carbonyl]-1-methyl-4-pyrrol-3-yl]-4-[[[4-methylthiazole-5-yl]carbonyl]amino]-pyrrole]-2-carboxamide (9e). Compound **5a** (1.20 g, 1.00 mmol) was reduced by palladium charcoal (600 mg) in a similar way as described above. The reduced product was redissolved in anhydrous DMF (50 mL), **8** (490 mg, 2.00 mmol) in DMF/THF (1:1, v/v 20 mL) was added to the above reduced product dropwise at room temperature under nitrogen over a period of 30 min and the stirring was continued at room temperature for additional 18 h. The solvent was removed in vacuo. The impure product was purified on silica gel column chromatography. The pure compound **9e** was eluted with CH_2Cl_2/MeOH/NH_4OH (80:20:0.4, v/v/v) as a yellow powder in 42% yield (415 mg), mp 163–166°C. ^{1}H NMR (DMSO-d_6): δ 1.25 (m, 2H), 1.50 (m, 2H), 1.62 (q, 4H), 2.20 (s, 12H), 2.30 (t, J=7.0 Hz, 4H), 2.45 (s, 6H), 3.10 (dt, J=6.0 Hz, 4H), 3.85 (s, 6H), 4.40 (t, 4H), 6.70, 6.77, (2d, J=1.6 Hz, 2H each), 7.60 (s, 2H), 7.55 (s, 2H), 7.82 (d, J=1.7 Hz, 2H), 8.05 (t, J=6.0 Hz, 2H, D_2O exchangeable), 8.10 (d, J=1.7 Hz, 2H), 9.79, 9.80 (2s, 2H each, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 179.27, 167.24, 161.34, 158.52, 155.45, 141.24, 136.71, 133.86, 131.23, 122.77, 121.14, 118.23, 112.32, 104.41, 54.63, 48.28, 42.11, 38.59, 36.14, 35.50, 30.70, 28.14, 25.63, 24.46, 15.72. HRMS calcd for $C_{47}H_{63}N_{14}O_6S_2$ 983.449, found 983.452 (M^++H, 100%).

3.1.6. 1,1′-(1,7-Heptamethylene)bis[*N*-[5-[[(3,3-dimethylamino)propyl]carbonyl]-1-methyl-4-pyrrol-3-yl]-4-[[[4-methylthiazole-5-yl]carbonyl]amino]-pyrrole]-2-carboxamide (9f). The title compound was synthesized in a similar manner as described above from **5b** and **8** as a yellow powder in 45% yield (450 mg), mp 168–172°C. ^{1}H NMR (DMSO-d_6): δ 1.20 (m, 6H), 1.70 (m, 8H), 2.10 (s, 12H), 2.35 (t, J=7.0 Hz, 4H), 2.42 (s, 6H), 3.30 (dt, J=6.0 Hz, 4H), 3.65 (s, 6H), 4.20 (t, 4H), 6.80, 6.92, 7.10, 7.24 (4d, J=1.6 Hz, 2H each), 7.60 (s, 2H), 8.15 (t, J=6.0 Hz, 2H, D_2O exchangeable), 8.56 (s, 2H), 9.56, 9.83 (2S, 2H, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 179.35, 166.42, 161.47, 158.51, 155.54, 142.34, 137.27, 131.42, 130.32, 123.76, 120.18, 118.29, 114.73, 104.43, 57.38, 47.29, 42.01, 38.37, 36.23, 35.73, 30.79, 27.24, 25.75, 23.66, 21.57, 20.17, 15.89. HRMS calcd for $C_{49}H_{67}N_{14}O_6S_2$ 1011.480, found 1011.480 (M^++H, 100%).

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