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Design and synthesis of threading intercalators to target DNA

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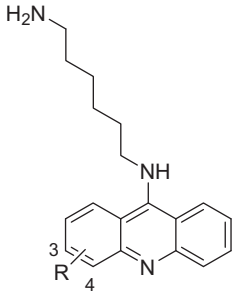
ABSTRACT

Threading intercalators are high affinity DNA binding agents that bind by inserting a chromophore into the duplex and locating one group in each groove. The first threading intercalators that can be conjugated to acids, sulfonic acids and peptides to target them to duplex DNA are described, based upon the well studied acridine-3- or 4-carboxamides. Cellular uptake of the parent acridine is rapid and it can be visualized in the nucleus of cells. Both the parent compounds and their conjugates maintain antitumor activity.

Directing compounds to duplex DNA through conjugation to high affinity DNA binders has potential to generate new compounds with novel biological activities.[1] Simply directing new fluorophores to the vicinity of nucleic acids can generate novel properties while the conjugation of peptides that can act as, for example, novel transactivation domains has been described.[2] In order to have simple and effective conjugates, a high affinity DNA binding molecule is required and much effort has been focused on developing sequence selective compounds based upon the natural products distamycin and netropsin.[3] Threading intercalators represent a class of DNA binding compound that has been largely neglected as a directing agent. We have previously used 9-aminoacridine-4-carboxamides to direct DNA cleaving moieties to DNA[4] whereas Bailly and co-workers generated a combilexin in which the threading intercalator was attached through the 4-carboxamide to a minor groove binder.[5] Beal and co-workers have described the incorporation of an acridine into a peptide structure to generate true threading peptides.[6] In this paper, we describe the synthesis of threading intercalators **1a** and **1b** (Fig. 1) containing a linker chain and a reactive moiety which allows conjugation to any species containing a complementary (in this case, carboxylic acid or sulfonic acid) functionality. The length of the chain means that the conjugation reaction should give a true hybrid in which the purpose of the acridine is to bind to the duplex and locate the attached group in the vicinity of DNA. Our previous work has shown that this is the optimum linker length for, for example, binding to a second strand of duplex DNA,[7] so should also be suitable for targeting DNA binding proteins. We describe the attachment of simple aromatic systems which do not bind well to DNA under normal circumstances[8] and the conjugation of a short peptide through solid phase methodology.

To synthesize the 9-(6-aminohexyl)aminoacridine carboxamides (**1a**, **1b**), 1,6-hexane diamine (**2**) was selectively mono-Boc protected using methods developed by Dardonville (Scheme 1).[9] A ratio of 1:5 in favor of the diamine **2** resulted in the selective protection of only one amine and compound **3** was isolated as a colorless oil in excellent yield (95%) without the need for purification.

The synthesis of the 9-chloroacridine-4/3-carboxamides (**4a**, **4b**) is described elsewhere.[10] The aminoacridine substrates were generated via an in situ reaction with the 9-phenoxyacridine, generated by, for example heating compound **4a**/**4b** in phenol for 15 min at 110 °C before reducing the temperature to 55 °C followed by the addition of 1.5 equiv of the mono-Boc protected amine. After a basic work up and column chromatography the Boc-protected acridines (**5a**, **5b**) were isolated in good yields



1a R = 4-CONH$(CH_2)_2N(CH_3)_2$
1b R = 3-CONH$(CH_2)_2N(CH_3)_2$

Figure 1. Structures of compounds **1a** and **1b**.

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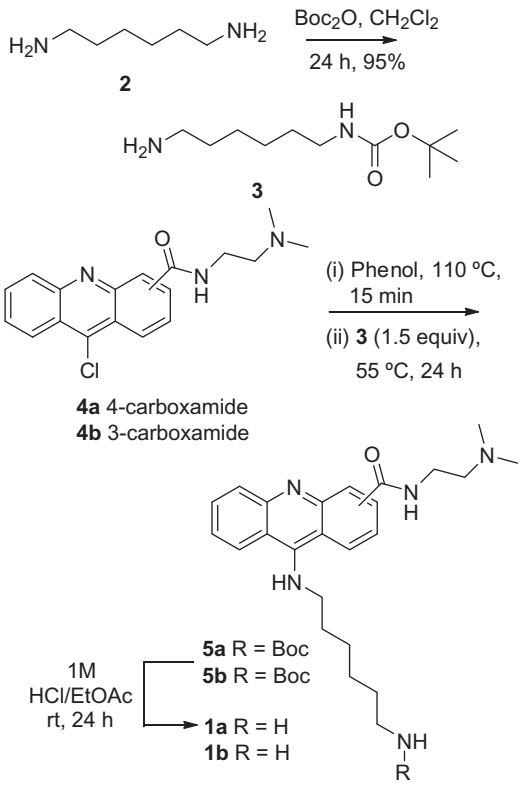


Scheme 1. Synthesis of compounds **1a** and **1b**.

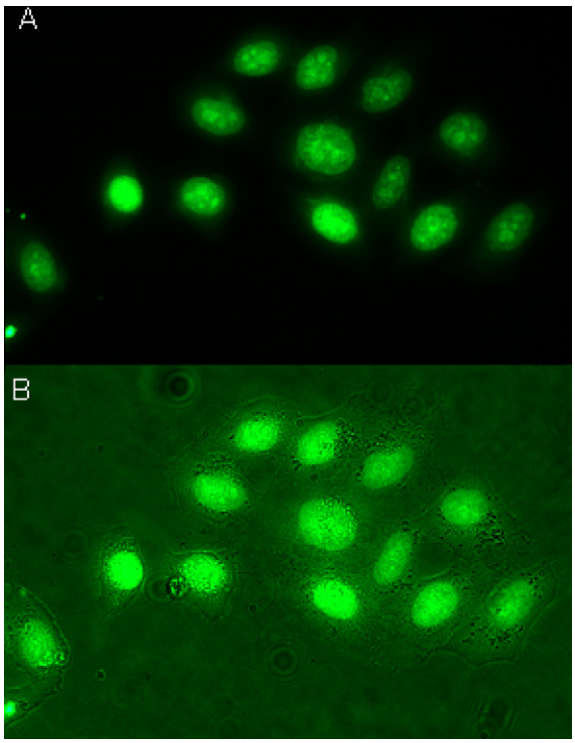


Figure 2. CHO cells were grown on coverslips overnight and then incubated with **1a** for 5 min at 37 °C. (A) Fluorescent labeling of the cell nuclei, (B) same cells in brightfield/fluorescence overlay. Pictures were taken using a Leica DMII inverted fluorescence microscope.

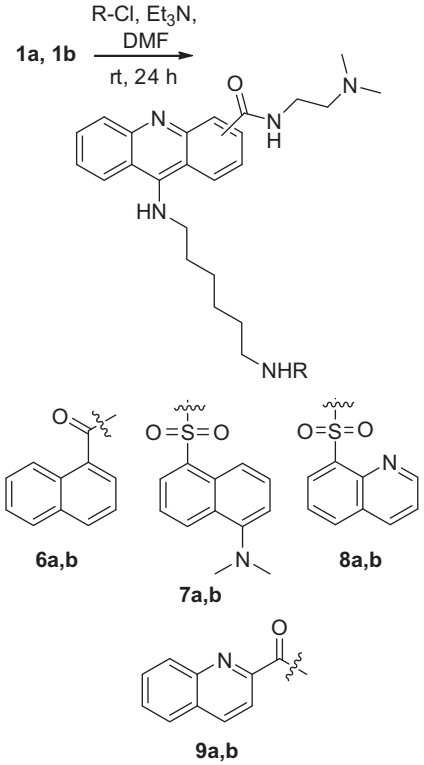
(**5a**—75%, **5b**—78%). Treatment with 1 M HCl/EtOAc removed the protecting group to yield the hydrochloride salts of the 9-(6-aminohexyl)aminoacridine carboxamides (**1a**, **1b**) in almost quantitative yields.[11] This is the first reported synthesis of the 9-(6-aminohexyl)aminoacridine-3/4-carboxamides.

The DNA binding ability of the two amines **1a** and **1b** was assessed using the ethidium bromide displacement assay and calf thymus (CT) DNA.[12] Both compounds had high affinities for duplex DNA with K_{app} values (**1a**, $3.10 \times 10^7\ M^{-1}$; **1b**, $9.83 \times 10^6\ M^{-1}$) 5–17-fold higher than that for 9-aminoacridine ($1.81 \times 10^6\ M^{-1}$), with the 4-carboxamide having a slightly higher affinity. This is consistent with our previous results with 3- versus 4-carboxamides[13] and is probably due to the 4-carboxamide having a better steric fit within the major groove compared to the regioisomer.

The antitumor activity of the compounds was assessed against the human leukaemia cell line HL60 and both compounds were found to have potent cytotoxic activity (IC_{50} **1a** 0.45 μM, **1b** 38.3 μM). We also briefly assessed the ability of the compounds to enter CHO cells (Fig. 2). After a 5 min incubation, **1a** is clearly visible in the nuclei of monolayer CHO cells. Counterstaining with DAPI confirmed that indeed **1a** was localized in the nuclei of the cells (see Supplementary data).

In order to assess the ability of the compounds to direct small aromatic compounds to DNA that would be expected to have poor DNA binding affinity in themselves (if any), a small library was synthesized by simple reaction with acid chlorides or sulfonyl chlorides (scheme 2). Compounds **1a** and **1b** were stirred in a basic solution of DMF followed by the addition of an excess of the commercially available naphthalene/quinoline substrates. After an appropriate workup the analogs were purified using normal phase column chromatography. Compounds **6**–**9a** and **6**–**9b** were isolated as hydrochloride salts in fair to good yields. The structures were confirmed by ^1H NMR, IR and high resolution mass spectrometry.

The DNA binding affinities and antitumor activities against the HL60 cell line for the conjugates are given in Table 1. All ten com-



Scheme 2. Synthesis of compounds **6–9**.

Table 1
Cytotoxicity and DNA binding affinity of compounds **6a–9a** and **6b–9b**

Compound	IC_{50}[a] (μM)	Log K_{app}[b]	K_{app} (M^{-1})
6a	3.5	6.72	5.24×10^6
6b	58.9	6.22	1.66×10^6
7a	2.1	7.23	1.70×10^7
7b	26.7	7.03	1.07×10^7
8a	16.0	6.61	4.07×10^6
8b	35.8	6.36	2.29×10^6
9a	22.6	6.68	4.79×10^6
9b	30.7	6.43	2.69×10^6
9-AA	NT[c]	6.26	1.80×10^6

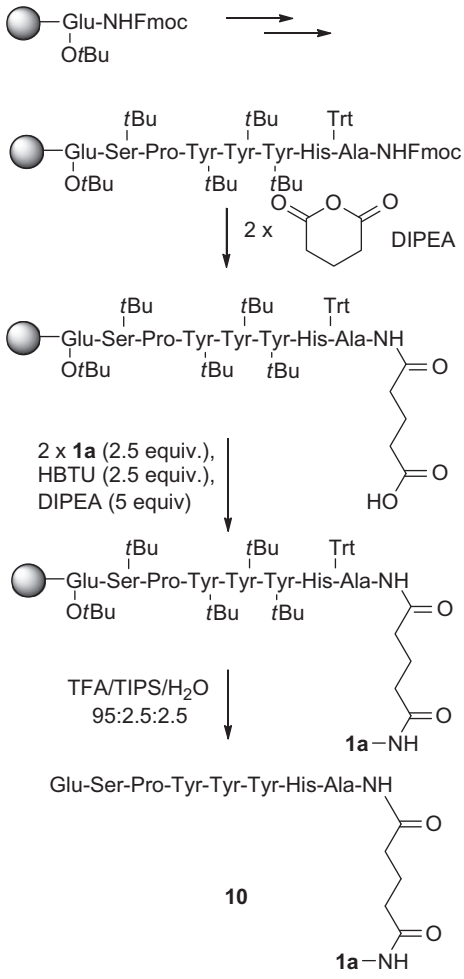
[a] HL60 cell line.
[b] Ethidium bromide competitive displacement assay.
[c] Not tested in this cell line. NCI 60 cell line screen gives a GI_{50} value of 2 μM for 9-aminoacridine against HL60 (listed as aminacrine, website (Sept 2010) dtp.nci.nih.gov/dtpstandard/cancerscreeningdata/index.jsp).

pounds maintained good DNA binding ability with the weakest binder (**6b**) having essentially the same DNA binding affinity as 9-aminoacridine. The high DNA binding affinity of the parent compounds probably comes in part from the electrostatic interaction possible with the protonated primary amine and there was some concern that removing this interaction would lead to a sizeable decrease in binding affinity, but this was not borne out by the addition of the hydrophobic naphthalene group. Clearly electrostatic interactions do play a part in binding as the dansyl group in **7a** and **7b** maintains the binding affinity of the parents. All the compounds also maintained the antitumor activity of the parents with the 4-carboxamides generally being more potent but with the 3-carboxamide analogs having enhanced or equivalent activity compared to the parent.

A more challenging proposition for targeting DNA was the conjugation of a non-DNA binding peptide that could then be directed to the duplex through a threading intercalation mode. Similarly, the incorporation of **1a** and/or **1b** into solid phase synthesis methodology would extend the potential utility of the compounds. We investigated the solid phase synthesis and conjugation of a short peptide (AHYYYPSE) identified by Mapp and co-workers as a potential artificial transcriptional activator in yeast.[14] The peptide was synthesized on solid phase using manual synthesis techniques with double couplings starting from pre-loaded Fmoc-Glu(O*t*Bu)-Wang Resin, with 2.5 equiv of each amino acid, 2.5 equiv HBTU and 5 equiv DIPEA using DMF as solvent (Scheme 3). At the end of the peptide synthesis, the resin was dried and divided into two. One half of the resin was cleaved at this point while the other half was resuspended in DMF, deprotected and treated with an excess of glutaric anhydride and DIPEA. Coupling of compound **1a** was then carried out using HBTU and DIPEA in DMF. The resulting conjugate was cleaved from the resin under standard conditions, dissolved in aqueous acetic acid, lyophilized and purified by preparative HPLC. The high resolution mass spectrum of the product confirmed the structure of the conjugate **10**.

The conjugate **10** was assessed for its binding affinity using the EtBr displacement assay. The unconjugated peptide had no measurable DNA binding affinity using this technique whereas the conjugate maintains the DNA binding affinity of the parent threading intercalator with a K_{app} of $3.68 \times 10^6\ M^{-1}$, a decrease of less than 10 fold in binding affinity even with the conjugation of the peptide. This decrease could arise from the presence of the C-terminal glutamic acid, which is likely to be deprotonated at pH 7.0, although binding would be enhanced by the protonated histidine.

In summary, we have described the synthesis of threading intercalators that can be conjugated to other molecules to direct them to duplex DNA. The high duplex DNA binding affinity of the parent compounds is maintained with only minor decreases even when conjugated to a small peptide.



Scheme 3. Synthesis of conjugate **10**.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.128.

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11. *Synthesis of* ***1a****: tert-butyl 6-aminohexylcarbamate (****3****)* 1,6-hexanediamine (**2**, 11.3 g, 100 mmol) was dissolved in CH_2Cl_2 (400 mL) and stirred in an ice bath

at 0 °C. A solution of Boc_2O (4.36 g, 10 mmol) in CH_2Cl_2 (40 mL) was added dropwise over 2 h. The reaction mixture was allowed to warm to room temperature and stirred over night. The solution was filtered to remove a white solid and the resulting filtrate was evaporated. The colourless oil was redissolved in EtOAc (100 mL) and washed with saturated aqueous brine (3 × 50 mL). The organic phase was dried over $MgSO_4$ and concentrated to yield a colourless oil (4.1 g, 95%). IR ν_{max} (neat)/cm^{-1} 3346 (NH_2) 3297 (NH) 2932 (CH_2), 1688 (C=O) ^1H NMR ($CDCl_3$, 400 MHz) δ_H ppm: 4.58 (1H, br s, N*H*), 3.06–3.04 (2H, m, CH_2NHBoc), 2.64 (2H, t, *J* = 7.0 Hz, CH_2NH_2), 2.22 (2H br s, NH_2), 1.42–1.27 (17H, m, $4CH_2 + 3CH_3$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ_H ppm: 166.99 (C=O), 68.91 (C(CH_3)), 41.75 (CH_2), 40.41 (CH_2), 33.08 (CH_2), 29.96 (CH_2), 28.37 ($3CH_3$), 26.52 (CH_2), 26.43 (CH_2). HRMS (ES+) calcd for $C_{11}H_{24}N_2O_2$ (M+H) 217.1911; found 217.1913. *tert-Butyl 6-(4-(2-(dimethylamino)ethylcarbamoyl)acridin-9-ylamino)hexylcarbamate* (***5a***) 9-chloro-*N*-(2-(dimethylamino)ethyl)acridine-4-carboxamide (**4a**) (500 mg, 1.5 mmol) and excess dry phenol (1.5 g) were heated and stirred at 110 °C for 15 min. On cooling to 55 °C *tert*-butyl 6-aminohexylcarbamate (**3**) (495 mg, 2.3 mmol) was added and the reaction stirred at 55 °C overnight. TLC confirmed the formation of the product. On cooling to rt, 2 M NaOH (20 mL) was added to the flask. A small amount of CH_2Cl_2 (10 mL) was added to a separating funnel and the reaction mixture poured on top. After separation of the organic phase, the aqueous phase was extracted successively with CH_2Cl_2 (3 × 15 mL). The organic extracts were combined, dried over $MgSO_4$ and filtered. The solvent was removed under vacuum to yield a dark yellow oil. Column chromatography (5% MeOH in EtOAc + 1% Et_3N) yielded the desired compound as a bright yellow oil (580 mg, 75%). IR ν_{max} (neat)/cm^{-1} 3329 (NH), 2930 (CH_2/CH_3), 2858 (CH_2/CH_3), 1691 (C=O). ^1H NMR ($CDCl_3$, 400 MHz) δ_H ppm: 8.79 (1H, br d, *J* = 5.2 Hz, Ar*H*(1)), 8.40 (1H, d, *J* = 8.4 Hz, Ar*H*(3)), 8.11 (1H, d, *J* = 8.4 Hz, Ar*H*(8)), 8.01 (1H, d, *J* = 8.4 Hz, Ar*H*(5)), 7.71–7.67 (1H, m, Ar*H*(6)), 7.42–7.35 (2H, m, Ar*H*(7+2)), 4.58 (1H, br s, N*H*Boc), 3.82 (2H, t, *J* = 7.0 Hz, $NHCH_2$), 3.76 (2H, q, *J* = 6.4 Hz, $CONHCH_2$), 3.09 (2H, q, *J* = 6.4 Hz, CH_2NHBoc), 2.71 (2H, t, *J* = 6.4 Hz, $CH_2N(CH_3)_2$), 2.42 (6H, s, $N(CH_3)_2$), 1.82–1.74 (2H, m, $NHCH_2CH_2$), 1.49–1.30 (15H, m, $3CH_2$, $C(CH_3)_3$). HRMS calcd for $C_{29}H_{42}N_5O_3$ (M+H) 508.3282; found 508.3275. *9-(6-Aminohexylamino)-N-(2-dimethylamino)ethyl)acridine-4-carboxamide hydrochloride* (**1a**) *tert*-butyl 6-(4-(2-(dimethylamino)ethylcarbamoyl)acridin-9-ylamino)hexylcarbamate (**5a**) (100 mg, 0.20 mmol) was stirred in a mixture of HCl/EtOAc (1 M, 5 mL) at room temperature overnight. The solvent was removed *in vacuo* to yield to bright yellow hygroscopic oil (84 mg, 96%). IR ν_{max} (neat)/cm^{-1} 3245 (NH), 2943 (CH_2/CH_3), 1622 (C=O). ^1H NMR (MeOD, 400 MHz) δ_H ppm: 8.75 (1H, d, *J* = 8.0 Hz, Ar*H*(1)), 8.59–8.54 (2H, m, 2Ar*H*(3+8)), 8.03–8.00 (1H, m, Ar*H*(6)), 7.93 (1H, dd, J_1 = 0.8 Hz, J_2 = 8.4 Hz, Ar*H*(5)), 7.68–7.60 (2H, m, 2Ar*H*(2+7)), 4.18 (2H, t, *J* = 7.6 Hz, CH_2NH_2), 3.91 (2H, t, *J* = 5.8 Hz, $CONHCH_2$), 3.52 (2H, t, *J* = 5.8 Hz, $CH_2N(CH_3)_2$), 3.05 (6H, s, $N(CH_3)_2$), 2.94 (2H, t, *J* = 7.6 Hz, $NHCH_2$), 2.08–2.01 (2H, m, $CH_2CH_2NH_2$), 1.74–1.67 (2H, m, $NHCH_2CH_2$), 1.57–1.47 (4H, m, $2CH_2$). HRMS (ES+) calcd for $C_{24}H_{34}N_5O$ (M+H) 408.2758; found 408.2759.

Synthesis of ***1b****: tert-butyl 6-(3-(2-(dimethylamino)ethylcarbamoyl)acridin-9-ylamino)hexylcarbamate* (***5b***) 9-chloro-*N*-(2-(dimethylamino)ethyl)acridine-3-carboxamide (**4b**) (100 mg, 0.31 mmol) and excess dry phenol (1 g) were heated and stirred at 110 °C for 15 min. On cooling to 55 °C *tert*-butyl 6-aminohexylcarbamate (**3**) (99 mg, 0.46 mmol) was added and the reaction stirred at 55 °C overnight. TLC confirmed the formation of the product. On cooling to rt, 2 M NaOH (10 mL) was added to the flask. A small amount of CH_2Cl_2 (5 mL) was added to a separating funnel and the reaction mixture poured on top. After separation of the organic phase, the aqueous phase was extracted successively with CH_2Cl_2 (3 × 10 mL). The organic extracts were combined, dried over $MgSO_4$ and filtered. The solvent was removed under vacuum to yield a dark yellow oil. Column chromatography (10% MeOH in EtOAc + 1% Et_3N) yielded the desired compound as a bright yellow oil (120 mg, 78%). IR ν_{max} (neat)/cm^{-1} 3310 (NH), 2925 (CH_2/CH_3), 2854 (CH_2/CH_3), 1684 (C=O). ^1H NMR ($CDCl_3$, 400 MHz) δ_H ppm: 8.35 (1H, d, *J* = 1.6 Hz, Ar*H*(4)), 8.16 (1H, d, *J* = 9.2 Hz, Ar*H*(1)), 8.12 (1H, d, *J* = 8.8 Hz, Ar*H*(8)), 7.99 (1H, d, *J* = 8.4 Hz, Ar*H*(5)), 7.78 (1H, dd, J_1 = 1.6 Hz, J_2 = 8.8 Hz, Ar*H*(2)), 7.66 (1H, m, Ar*H*(6)), 7.42 (1H, br s, CON*H*), 7.39–7.35 (1H, m, Ar*H*(7)), 4.58 (1H, br s, N*H*Boc), 3.87 (2H, t, *J* = 7.2 Hz, $NHCH_2$), 3.57 (2H, q, *J* = 5.6 Hz, $CONHCH_2$), 3.13–3.09 (2H, m, CH_2NHBoc), 2.56 (2H, t, *J* = 5.6 Hz, $CH_2N(CH_3)_2$), 2.30 (6H, s, $N(CH_3)2$), 1.87–1.80 (2H, m, $NHCH_2CH_2$), 1.50–1.35 (15H, m, $C(CH_3)3$, $3CH_2$). HRMS (ES+) calcd for $C_{29}H_{42}N_5O_3$ (M+H) 508.3282; found 508.3283. *9-(6-Aminohexylamino)-N-(2-dimethylamino)ethyl)acridine-3-carboxamide hydrochloride* (***1b***) *tert*-butyl 6-(3-(2-(dimethylamino)ethylcarbamoyl)acridin-9-ylamino)hexylcarbamate (**5b**) (2 g, 3.9 mmol) was stirred in a mixture of HCl/EtOAc (1 M, 30 mL)at room temperature overnight. The solvent was removed *in vacuo* to yield to bright yellow hygroscopic oil (1.7 g, 97%). IR ν_{max} (neat)/cm^{-1} 3238 (NH), 2927 (CH_2/CH_3), 1636 (C=O). ^1H NMR (MeOD, 400 MHz) δ_H ppm: 8.65 (1H, d, *J* = 8.4 Hz, Ar*H*(1)), 8.55 (1H, d, *J* = 8.4 Hz, Ar*H*(8)), 8.36 (1H, s, Ar*H*(4)), 8.03–7.99 (2H, m, 2Ar*H*(2+6)), 7.87 (1H, d, *J* = 8.4 Hz, Ar*H*(5)), 7.63–7.59 (1H, m, Ar*H*(7)), 4.20 (2H, t, *J* = 7.2 Hz, CH_2NH_2), 3.87 (2H, t, *J* = 5.2 Hz, $CONHCH_2$), 3.49 (2H, t, *J* = 5.6 Hz, $CH_2N(CH_3)_2$), 3.03 (6H, s, $N(CH_3)_2$), 2.94 (2H, t, *J* = 7.6 Hz, $NHCH_2$), 2.07–2.04 (2H, m, $CH_2CH_2NH_2$), 1.75–1.68 (2H, m, $NHCH_2CH_2$), 1.59–1.52 (4H, m, $2CH_2$). HRMS (ES+) calcd for $C_{24}H_{34}N_5O$ (M+H) 408.2758; found 408.2761.

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