Design and Synthesis of Novel DNA Interstrand Cross-Linking Agents: C2-Linked Pyrrolo[2,1-*c*][1,4]benzodiazepine Polyamide Conjugates

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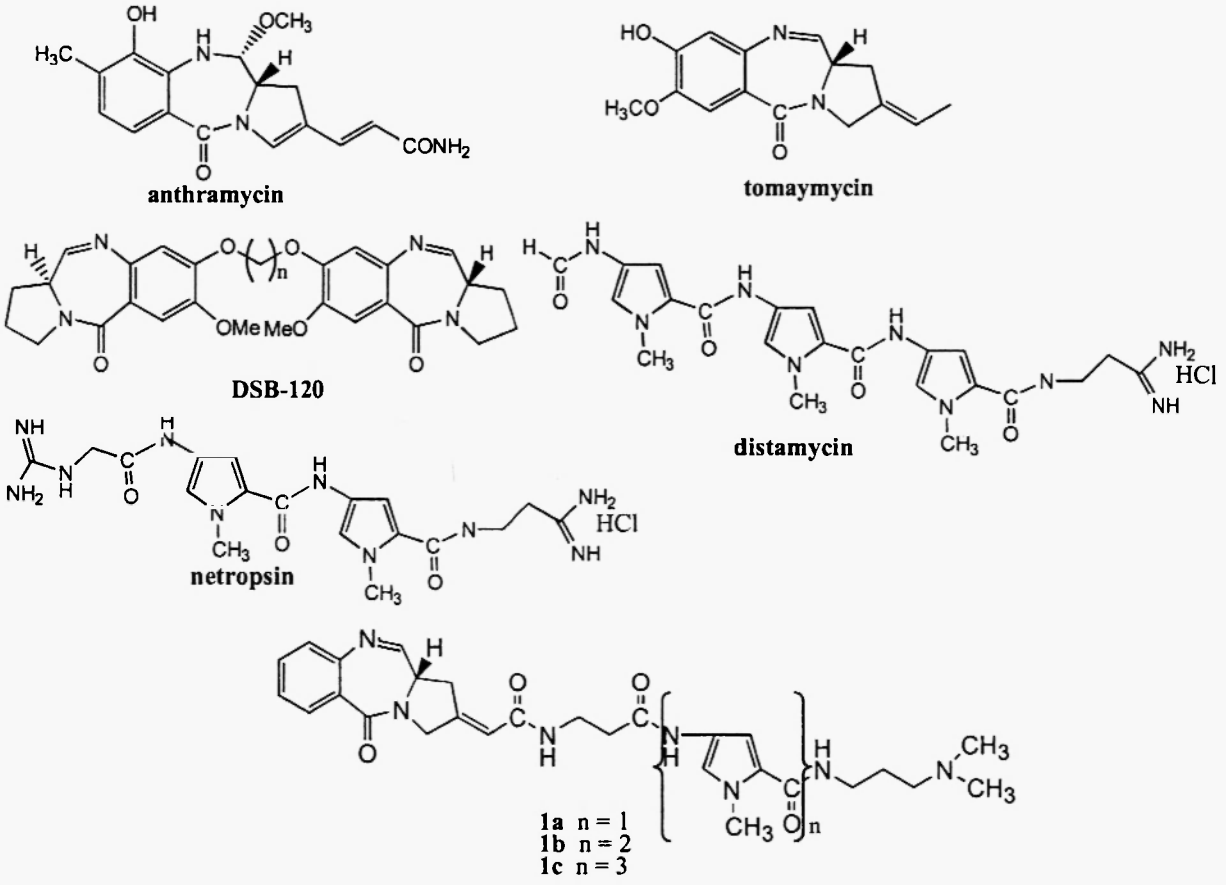
Abstract: The design and synthesis of C2- linked novel pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD)-polyamide conjugates (**1a - c**) as DNA minor groove binding agents are described.

Introduction

There is a growing interest in agents, such as the pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs), that can recognize and bond to specific sequence of DNA. They have potential as gene regulators with possible therapeutic application in the treatment of genetic disorders, including some cancers, as selective anti – infective agents, and as probes and tools for use in molecular biology[1]. PBDs are a group of naturally occurring antibiotics, examples of which include anthramycin, tomaymycin, neothramycins A and B, sibiromycin, mazethramycin, chicamycin, prothracarcin DC-81, and dextrochrysin[2]. The cytotoxicity and antitumor activity of these agents are attributed to their property of sequence – selective covalent binding to the 2-NH_2 group of guanine in the minor groove of duplex DNA via an acid labile aminal bond to the electrophilic imine at the N10-C11 position. The N10-C11 carbinolamine form may exist in the equivalent imine or carbinolamine methyl ether form depending on the precise structure of the compound and the method of isolation[3]. The (S)- stereochemistry at the C11a position provides a right – handed molecular twist, when viewed from the C–ring towards the A–ring, which enables the PBD to assume a snug fit in the minor groove of DNA[4]. Molecular modeling, solution NMR, fluorimetry and DNA footprinting experiments reveal that the PBDs recognize a 3 bp motif with a preference for 5'- PuGPu sequences[5].

In an earlier study, Wang et al.[6] showed that two tomaymycin molecules can be covalently bound to a 12 mer duplex DNA, where the drug molecules are on opposite strands six base pairs apart. Recently , a C8 - linked PBD dimer was prepared[7] which forms a symmetric interstrand cross-link with duplex DNA involving a four base-pair bonding site but spanning six DNA base-pairs overall[8]. Later Mountzouris et al.[9] demonstrated that DSB-120 forms a guanine-guanine interstrand cross link. They showed that the tomamycin

tail is close to the floor of the minor groove, while the five membered ring of DSB-120 is more shallowly immersed, perhaps due to strain from cross-linking with a very short linker unit.



A variety of polyamide conjugates of other cytotoxic agents which are minor groove binders, and with consequent improved selectivities, have been reported in a review[10] . Some efforts to date have been directed at different modifications on the PBD ring system with other well established DNA minor groove binders such as distamycin or netropsin. The latter agents bind to four or more consecutive A-T base-pairs[11]. In view of the commonly observed enhanced activity and selectivity of the parent drugs when conjugated with polyamides and the intrinsic activity of DSB-120, we recently reported the conjugation of selected polyamides (pyrrole, imidazole) with the PBD nucleus through the C-8 position with a variable linker [12,13] and also the synthesis of 22′ - PBD dimers[14]. We have also reported the cytotoxic activity of 22′ - PBD dimers and PBD- pyrrole polyamide conjugates against many human cancer cell lines[15]. We found that the PBD-pyrole polyamides have a wide spectrum of anticancer activity, which affect the cell growth of 17 cells in six cancer panels with *LC50* values lower than 9.0 μM. These cell lines include colon cancer, COLO 205, HCT-116 and

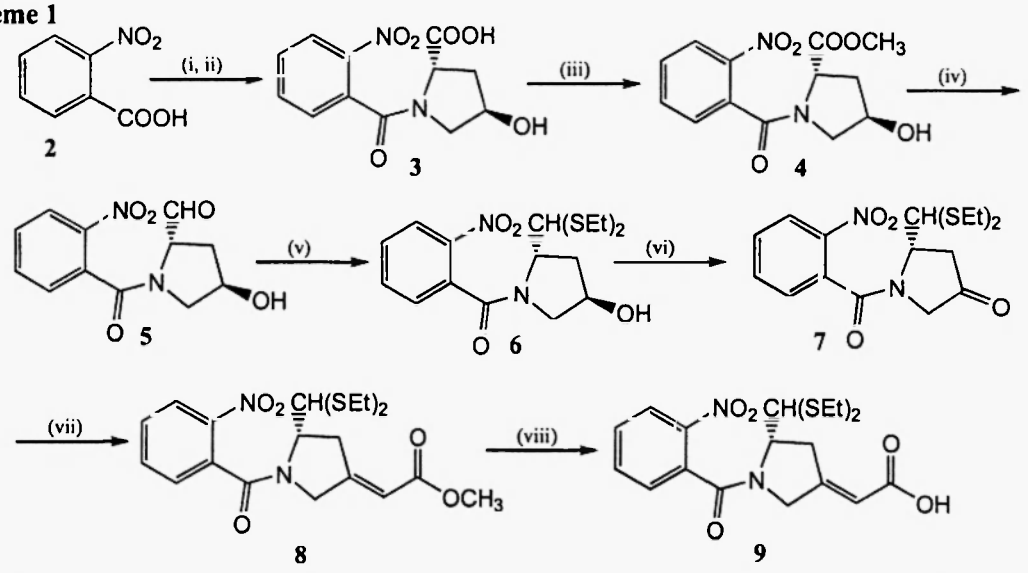
HCT-15 cell lines, melanoma cancer LOX IMVI, MALME-3M, M14 and UACC-257 cell lines, ovarian cancer OVCAR-8 cell line, renal cancer, ACHN, CAKI-1 RXF 393, SN 12C, TK-10 and UO-31 cell lines, breast cancer MDA-MB-435 and MDA-N cell lines. 22′ - PBD dimers exhibit lower cytotoxicity compared with the PBD- pyrrole polyamide conjugates. These result prompted us to design the synthesis of C2-PBD polyamide conjugates in order to examine their sequence selective binding affinity with DNA as well as their cytotoxicity against human cancer cells. We herein report the efficient synthesis of C2-linked PBD polyamide conjugates which contain different number of pyrrole units.

Results and discussion

The compounds **1a-c** were made according to the routes described in Schemes 1 and 2. The precursor of the PBD acid was synthesized according to Scheme 1. The coupling of 2-nitrobenzoic acid via its acid chloride with the trans-4-hydroxyproline gave (2S)-N- (2-nitrobenzoyl)-4-hydroxypyrrolidine-2-carboxylic acid 3 in 75% yield. This acid was converted into its methyl ester **4** with methanol and H_2SO_4. Reduction of nitro ester 4 with diisobutylaluminum hydride (DIBAL-H) to produce the corresponding aldehyde **5**, followed by the protection of the aldehydic group with ethanethiol afforded the corresponding thioacetal compound **6**. The C4 hydroxy group of compound **6** was oxidized into its corresponding oxo form **7** by using DMSO–Ac_2O reagent under mild conditions in 60% yield. Wittig reaction of the keto compound **7** with methyl (triphenylphosphoranylidene)acetate afforded the methyl ester **8** in 80% yield. In this reaction the (E) ester **8** was obtained exclusively, which upon hydrolysis with IN NaOH produced the corresponding acid compound **9** in 90% yield.

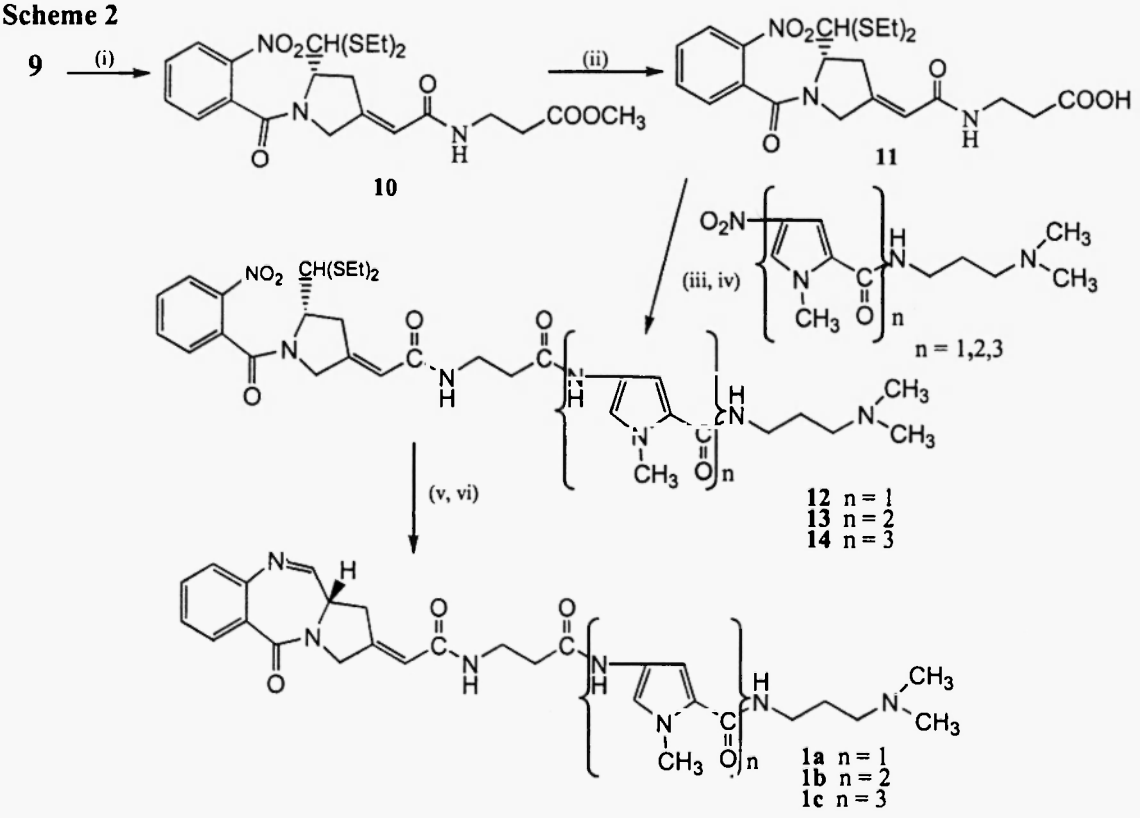
This acid **9** was then coupled with the β-alanine methyl ester hydrochloride under standard EDCI, HOBt coupling conditions[16] to afford the corresponding coupled ester **10** in 80% yield. Hydrolysis of **10** with 1 N NaOH produced the corresponding acid **11** in 90% yield. This latter compound was then coupled with the amine moiety of different pyrrole polyamides in the presence of EDCI in dry DMF to obtain the desired products **12-14** in 70 % yield. The corresponding amino compounds were then prepared by hydrogenation of the corresponding nitro polyamides. Hydrogenation of compounds **12-14** with H_2/Pd-C afforded 100% conversion of the nitro compounds to the corresponding amino compounds. The latter were then subjected to deprotective cyclization with $HgCl_2$/HgO in aqueous acetonitrile at room temperature affording the corresponding imines **1a-c** in 30 % yield. Since the conjugation of the polyamides with PBD resulted in highly polar imines, this necessitated the use of methanol in combination with DCM as eluent during the washing of the imines and, therefore, the product was obtained as a mixture of imine and its methyl ether in 1: 1 ratio. The presence of both the forms was confirmed by NMR and mass spectra. The final compounds were isolated in 25-30% yield.

Scheme 1



(i) $SOCl_2$, C_6H_6; (ii) 4-hydroxy-L-proline; (iii) MeOH, H_2SO_4; (iv) DIBAL-H; (v) EtSH, TMSCl; (vi) Ac_2O -DMSO; (vii) $Ph_3PCHCOOCH_3$, C_6H_6; (viii) 1 N NaOH

Scheme 2



(i) DMF, EDCI, HOBT, $NaHCO_3$, β-alanine methyl ester hydrochloride; (ii) 1N NaOH ; (iii) H_2,10%Pd-C, 50 psi, MeOH; (iv) DMF, EDCI, HOBT, RT; (v) H_2,10%Pd-C, 50 psi, MeOH ; (vi) $HgCl_2$, HgO, 75% aq CH_3CN

In conclusion, we described the synthesis of C2-PBD polyamide conjugates which contain pyrrole units bonded through the C2 position with a linker of two carbons. Their sequence –selective binding affinity with DNA as well as their cytotoxicity against human cancer cells will be reported in due course.

Experimental

Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. All the chemicals used were of reagent grade. Dimethylformamide (DMF) and methanol (MeOH) were of anhydrous grade procured from Aldrich Chemical Co. The ^{1}H NMR spectra were recorded on a Brucker (300MHz) spectrometer. Mass spectra were determined on an Associate Electrical Industries (AEI) MS-9 and MS-50 focussing high resolution mass spectrometers. Kieselgel 60 (230-400 mesh;E. Merck, Rahway, NJ) was used for flash column chromatography and precoated silica gel 60F-254 sheets (E. Merck) were used for thin layer chromatography. TLC plates were visualized by using UV light.

Methyl 3-[2-[(5S)-5-[Bis (ethylthio) methyl] -1- (2-nitrobenzoyl)–tetrahydro - 1*H* –3 -pyrrolyliden]-acetamido] propionate (10)

To a solution of acid **9** (3.0g, 7.31mmol) in dry DMF (20ml) were added EDCI (2.80g, 14.60mmol), HOBt (0.99g, 7.31mmol), $NaHCO_3$ (1.22g, 14.52mmol) and β-alanine methyl ester hydrochloride (0.90g, 8.77mole) under a nitrogen atmosphere and the mixture was stirred for 12 h. When TLC indicated the absence of starting material, DMF was removed under reduced pressure. The dark residue was purified by column chromatography on silica gel using 5% MeOH / dichloromethane as a eluent to afford the coupled ester **10** as a yellow oil in 70 % yield. ^{1}H NMR ($CDCl_3$) δ 1.29 (m, 6H), 2.50 (m, 2H), 2.70 (m, 4H), 3.18 (s, 2H), 3.48 (m, 2H), 3.56 (s, 3H), 3.87 (m, 1H), 4.15 (m, 1H), 4.81 (m, 1H), 5.40 (m, 1H), 5.92 (s, 1H), 6.50 (m, 1H), 7.44 (m, 1H), 7.61 (m, 1H), 7.75 (m, 1H), 8.17 (m, 1H). HR-MS m/z calcd. for $C_{22}H_{29}N_3O_6S_2$ 495.00, found 518.13 (M+Na).

3-[2-[(5S)-5 [Bis(ethylthio)methyl] -1- (2-nitrobenzoyl)–tetrahydro - 1*H* –3 -pyrrolyliden]-acetamido] propionic acid (11)

A mixture of ester **10** (3.0g, 6.06 mmol) in methanol and 10 ml of IN NaOH was placed in a flask fitted with a reflux condenser, then the reaction mixture was heated at 40–50 ^{0}C temperature until the ester completely disappeared as shown by TLC. The mixture was cooled in ice with stirring and treated with 0.5 N HCl slowly to pH 2. Then the mixture was extracted with ethyl acetate three times and dried over sodium sulfate and the solvent was removed under reduced pressure. The residue was purified by column chromatography; mp. 75-77 ^{0}C; ^{1}H NMR ($CDCl_3$) δ 1.25-1.45 (m, 6H), 2.50 (m, 2H), 2.70 (m, 4H), 3.14 (s, 2H), 3.48 (m, 2H), 3.90 (d, J= 6.0 Hz, 1H), 4.13 (d, J= 4.5 Hz, 1H), 4.81 (d, J= 2.0 Hz, 1H), 5.42 (brs, 1H),

5.95 (s, 1H), 6.50 (t, J= 4.5 Hz, 1H), 7.45 (d, J= 4.0 Hz, 1H), 7.57 (t, J= 4.0 Hz, 1H), 7.74 (t, J= 4.0 Hz, 1H), 8.19 (t, J= 4.2 Hz, 1H). HR-MS m/z calcd. for $C_{21}H_{27}N_3O_6S_2$ 481.00, found 504.12 (M+Na).

General procedure for the synthesis of compounds 12-14

A solution of the nitropolyamide in MeOH was hydrogenated over 10% Pd/C at 50 psi pressure for two hours and the catalyst was removed by filtration through a Celite pad. The filtrate was concentrated to dryness under reduced pressure (at RT) to afford the corresponding amine. Owing to the sensitivity of the amine to oxidation, it was used for the next reaction immediately. It was dissolved in dry DMF and a mixture of the acid **11**, hydroxybenzotriazole, and EDCI in DMF was added. This mixture was stirred at RT for 12 h and after completion of the reaction the solvent was removed under reduced pressure to afford a dark oil which was purified by flash column chromatography on silica gel by using methanol-dichloromethane as eluent to afford the coupled thioacetal **12-14** as a yellow crystalline solid in 60% yield.

Compound 12: ^{1}H NMR (DMSO-d_6, 300MHz) δ 1.25 (m, 6H), 1.71-1.95 (m, 3H), 2.44 (m, 9H), 3.03 (m, 6H), 3.18 (m, 2H), 3.40 (m, 2H), 3.81 (s, 3H), 3.90 (m, 1H), 4.13 (m, 1H), 4.65 (d, J = 2.2 Hz, 1H), 5.21 (m, 1H), 5.81 (s, 1H), 6.81 (d, J = 2.2 Hz, 1H), 7.10 (d, J = 2.2 Hz, 1H), 7.45 (m, 1H), 7.65 (m, 1H), 7.75 (m, 1H), 8.15 (m, 1H), 8.20 (m, 2H), 9.80 (s, 1H). HR-MS m/z calcd for $C_{32}H_{45}N_7O_6S_2$ 687.00, found 688.294 (M+1).

Compound 13: ^{1}H NMR (DMSO-d_6, 300MHz) δ 1.25 (m, 6H), 1.45 (m, 1H), 1.65 (m, 2H), 2.05- 2.51 (m, 9H), 2.70 (m, 4H), 2.97 (m, 2H), 3.14 (m, 2H), 3.48 (m, 2H), 3.81 (s, 3H), 3.86 (s, 3H), 3.85-3.95 (m, 1H), 4.12 (m, 1H), 4.65 (d, J = 2.2 Hz, 1H), 5.22 (m, 1H), 5.85 (s, 1H), 7.12 (d, J = 2.4 Hz, 1H), 7.18 (d, J = 2.4 Hz, 1H), 7.51 (d, J = 2.4 Hz, 1H), 7.55 (d, J = 2.4 Hz, 1H), 7.62 (m, 1H), 7.71 (m, 1H), 7.86 (m, 1H), 8.05 (m, 2H), 8.22 (m, 2H), 9.85 (s, 2H). HR-MS m/z calcd for $C_{38}H_{51}N_9O_7S_2$ 809.30, found 810.34 (M+1).

Compound 14: ^{1}H NMR (DMSO-d_6, 300MHz) δ 1.26 (m, 6H), 1.51-1.86 (m, 3H), 2.21- 2.70 (m, 9H), 2.75-3.10 (m, 6H), 3.14 (m, 2H), 3.35 (m, 2H), 3.80 (s, 3H), 3.82 (s, 3H), 3.83 (s, 3H), 3.90 (m, 1H), 4.12 (m, 1H), 4.66 (d, J = 2.4 Hz, 1H), 5.20 (s, 1H), 5.71 (s, 1H), 6.87 (d, J = 2.2 Hz, 1H), 6.90 (d, J = 2.2 Hz, 1H), 7.04 (d, J = 2.2 Hz, 1H), 7.15 (d, J = 2.2 Hz, 1H), 7.18 (d, J = 2.2 Hz, 1H), 7.23 (d, J = 2.2 Hz, 1H), 7.52 (m, 1H), 7.74 (m, 1H), 7.91 (m, 1H), 8.08 (t, J = 4.5 Hz, 1H), 8.15 (t, J = 4.6 Hz, 1H), 8.20 (m, 1H), 9.90 (s, 1H) 9.92 (s, 1H) 9.96 (s, 1H). HR-MS m/z calcd for $C_{44}H_{57}N_{11}O_8S_2$ 931.39, found 932.39 (M+1).

General procedure for the synthesis of compounds 1a-c

To a solution of polyamide coupled thioacetals **12-14** in methanol was added 10% Pd/C and the compound was hydrogenated in a Parr shaker at 50 psi pressure for 2 h. TLC indicated the complete consumption of starting material, and the reaction mixture was filtered on a small celite pad to remove Pd/C. The filtrate was concentrated under reduced pressure to remove methanol and dried under high vacuum to afford a yellow crystalline solid in almost 100% yield, the composition of which was confirmed by NMR and

mass spectral data. The resultant amino compound was dissolved in CH_3CN-H_2O (4:1) and $HgCl_2$ and HgO were added and the mixture was stirred slowly at RT for 48 h. When TLC ($CHCl_3$-MeOH-ammonia, 7:3:0.4) indicated the complete disappearance of the starting material, the mixture was filtered on a Buchner funnel to afford a yellow solid. This yellow solid was dissolved in a large amount of ethyl acetate to remove $HgCl_2$ and then again filtered. Evaporation afforded a yellow solid which was first washed with a large amount of dichloromethane and then 0.5 N HCl solution to remove the exces of HgO and finally washed with methanol. After complete removal of $HgCl_2$ and HgO the yellow solid was dried under high vacuum. NMR and mass spectra showed the product to be a mixture of the imine and the corresponding methyl ether in almost 1:1 ratio in 20-30%yield.

Compound 1a: ^{1}H NMR (DMSO-d_6, 300MHz) δ 1.68 (m, 2H), 1.81- 2.25 (m, 6H), 2.42-2.68 (m, 6H), 3.15-3.45 (m, 4H), 3.85 (s, 3H), 3.99 (s, 3H, methyl ether), 3.91-4.25 (m, 2H), 4.61-5.91 (m, 2H), 6.85-7.74 (m, 5H), 7.85 (d, J = 4.2 Hz, 1H, imine proton), 8.15 (m, 1H), 8.25 (m, 2H), 9.80 (s, 1H). HR-MS m/z calcd for $C_{28}H_{35}N_7O_4$ 533.00, found 534.30 (M+1) for its imine compound.

Compound 1b: ^{1}H NMR (DMSO-d_6, 300MHz) δ 1.65 (m, 2H), 1.80- 2.15 (m, 6H), 2.41-2.60 (m, 6H), 3.18-3.40 (m, 4H), 3.81-3.90 (m, 6H), 3.93 (s, 3H, methyl ether), 3.96-4.15 (m, 2H), 4.65-5.81 (m, 2H), 6.85-7.60 (m, 7H), 7.85 (d, J = 4.0 Hz, 1H, imine proton), 8.10 (m, 1H), 8.20 (m, 2H), 9.80 (m, 2H). HR-MS m/z calcd for $C_{34}H_{41}N_9O_5$ 655.33, found 656.33 (M+1) for its imine compound.

Compound 1c: ^{1}H NMR (DMSO-d_6, 300MHz) δ 1.68 (m, 2H), 1.82- 2.20 (m, 6H), 2.45-2.65 (m, 6H), 3.18-3.45 (m, 4H), 3.88 (m, 9H), 3.98-4.05 (m, 3H, methyl ether), 4.10-4.20 (m, 2H), 4.65-5.90 (m, 2H), 6.85-7.75 (m, 9H), 7.84 (d, J = 4.2 Hz, 1H, imine proton), 8.10 (m, 1H), 8.20 (m, 2H), 9.88 (m, 3H). HR-MS m/z calcd for $C_{40}H_{47}N_{11}O_6$ 777.30, found 778.31 (M+1) for its imine compound.

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