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Synthesis of C8–C8/C2–C8-linked triazolo pyrrolobenzodiazepine dimers by employing 'click' chemistry and their DNA-binding affinity

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Abstract

A series of 1,2,3-triazole-containing pyrrolo[2,1-*c*][1,4]benzodiazepine dimers have been prepared efficiently by employing a 'click' chemistry protocol. This method involves 1,3-dipolar cycloaddition of terminal alkynes with organic azides using a Cu(I)-catalyst. Further, these molecules exhibited interesting DNA-binding affinity profiles.

Keywords: Pyrrolo[2,1-*c*][1,4]benzodiazepines; 1,2,3-Triazoles; DNA-binding affinity; 'Click' chemistry

DNA interstrand cross-linking agents have attracted the attention of many researchers because of their potent anticancer activity as exhibited in most compounds with a pyrrolobenzodiazepine (PBD) ring system.[1] There has been considerable interest in the past few years in the design and synthesis of symmetrical and unsymmetrical cross-linking agents, particularly those based on PBDs.[2] In the literature, a number of PBD dimers have been designed and synthesized that exhibit varying degrees of cytotoxicity and DNA cross-linking activity.[3] The PBD antitumor antibiotics are produced by various *Streptomyces* species and are generally referred to as the anthramycin family, which comprise of representative members including DC-81 (**1**), anthramycin, tomaymycin, and chicamycin. These PBD dimers are linked through different positions such as *A*-C7/*A*-C7′, *A*-C8/*A*-C8′, and *C*-C2/*C*-C2′, among these *A*-C8/*A*-C8-linked PBD dimers have shown promising cytotoxicity and efficient cross-linking properties. Further, extensive studies have been carried out on both the solution[4] and solid-phase[5] synthesis of PBDs, and a sound understanding of structure–activity relationships within the family has been developed.[6] In a recent development, Thurston and co-workers[7] tethered two DC-81 units at the C8-positions[8] by using different alkane spacers to give bisfunctional–alkylating agents capable of cross-linking DNA. One of these dimers, DSB-120 **2a**, forms an irreversible interstrand cross-link between two guanine bases within the minor groove of DNA via their exocyclic N2 atoms and spans six base pairs, thereby actively recognizing a central 5′-GATC sequence.[9] Moreover, in this laboratory, mixed imine–amide PBD dimers such as **2b** have been designed and synthesized which shows efficient DNA binding ability with significant anticancer activity in a number of human cancer cell lines.[10] In continuation of these efforts toward the design and synthesis of nitrogen-rich PBD dimer analogues, as well as the development of new synthetic strategies,[11] we became interested in exploring the DNA-binding ability of C8–C8/C2–C8-linked 1,2,3-triazole-containing PBD dimers (Fig. 1).

1,2,3-Triazoles are heterocycles with a wide range of applications that are receiving growing attention in biological activity studies and are employed widely as pharmaceuticals and agrochemicals.[12] Earlier studies have revealed that the azole group due to the aromaticity and lone-pair electrons provides great potential for several

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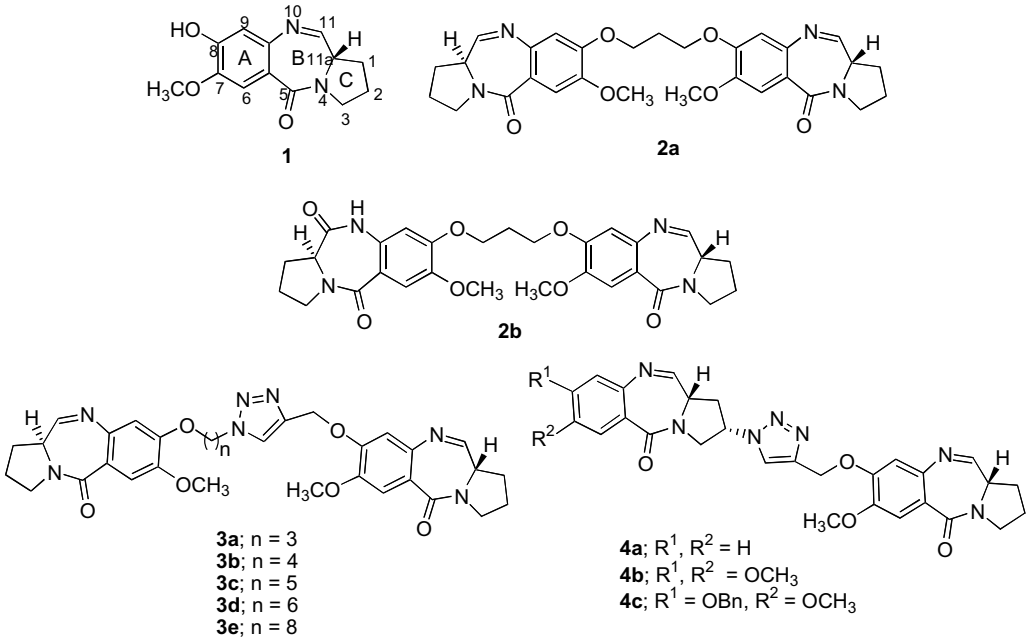


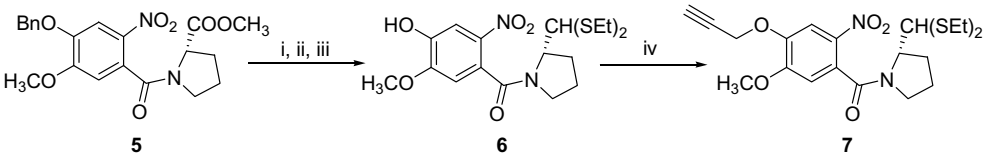
Fig. 1. Chemical structures of DC-81 (**1**), DSB-120 (**2a**), imine-amide PBD dimer (**2b**), and 1,2,3-triazole-PBD dimer analogues (**3a**–**e** and **4a**–**c**).

applications. Interestingly, the in situ generated triazole unit is revealed as a very active pharmacophore instead of a passive linker.[13a] Furthermore, dimerization of biologically useful molecules having a triazole moiety can take place easily.[13b] In recent years, alkylating agents have been studied extensively with regard to cancer chemotherapy, and this has led to the development of many new and more selective alkylating agents including molecules that are based on the triazole moiety.[14] The conventional route to 1,2,3-triazoles is the Huisgen dipolar cycloaddition of alkynes with organic azides.[15] This process provides biologically diverse molecules in a single-step and is also an example of 'click' chemistry. In this connection, we herein report a new class of C8–C8/C2–C8-linked pyrrolo[2,1-*c*]-[1,4]benzodiazepine dimers that are linked through 1,2,3-triazoles units, which are prepared by employing the 'click' reaction. Moreover, one of compound **3d** has shown enhanced DNA binding ability compared to the previously reported PBD dimer, DSB-120.

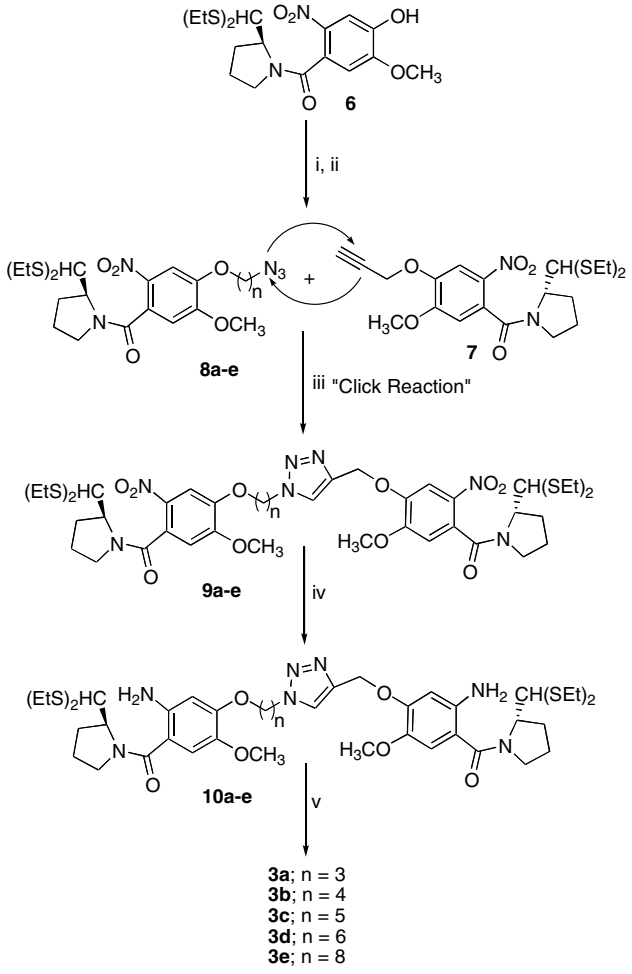
The synthesis of these 1,2,3-triazole-containing C8–C8-linked PBD dimers **3a**–**e** was carried out by employing the (2*S*)-*N*-[4-benzyloxy-5-methoxy-2-nitrobenzoyl]proline methyl ester (**5**), which was obtained according to the literature method starting from vanillin.[16] This, upon selective reduction employing DIBAL-H and protection with TMSCl/EtSH followed by deprotection using $BF_3{\cdot}OEt_2$/EtSH gave sulfide **6**. The etherification of **6** with propargyl bromide in the presence of K_2CO_3 afforded the required intermediate **7** in excellent yield (93%) as shown in Scheme 1.

The synthesis of the other starting substrates **8a**–**e** was accomplished using the similar procedures. Etherification of compound **6** with various dibromoalkanes followed by azidation with NaN_3 gave azides **8a**–**e**. Alkyne **7** and azides **8a**–**e** underwent the 'click' reaction in the presence of Cu(I) catalyst (1 mol %) and sodium ascorbate (5 mol %) to afford 1,2,3-triazole-containing PBD intermediates **9a**–**e**. Next, **9a**–**e** were reduced with $SnCl_2{\cdot}2H_2O$ followed by deprotection with $HgCl_2/CaCO_3$ to give the target molecules **3a**–**e**[17] in good yields as depicted in Scheme 2.

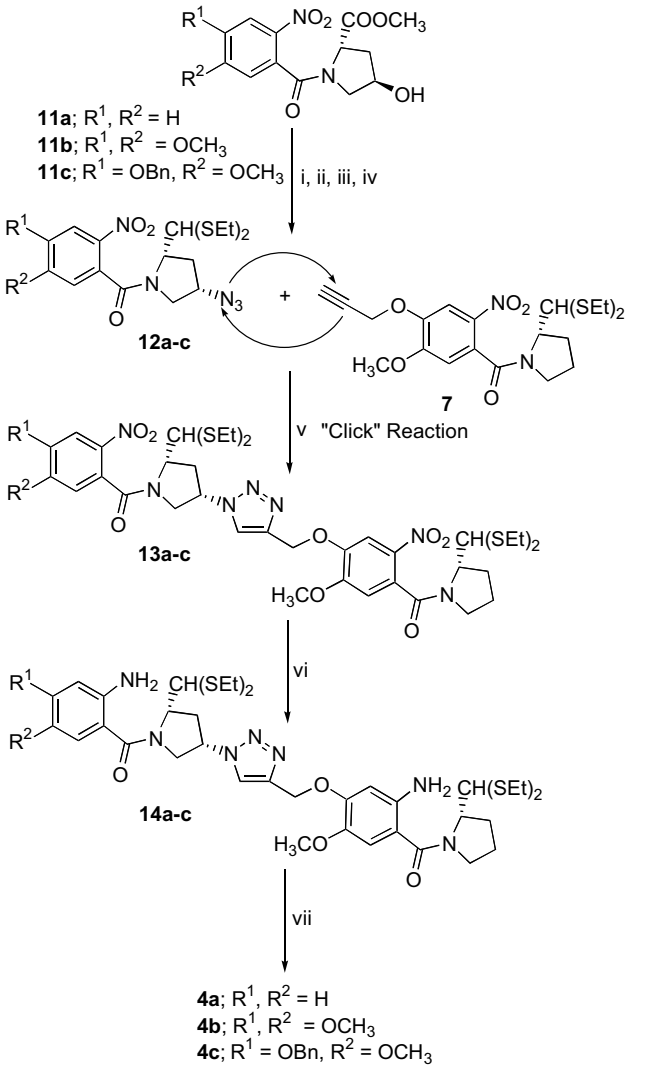
We next turned our attention to the exploration of the diversity at the C2-position. The C2–C8-linked triazole-containing PBD dimers **4a**–**c** were thus prepared by employing substrates **11a**–**c** , which were prepared using a reported method.[18] Thus, mesylation of the C2-hydroxy group of **11a**–**c** followed by azidation via S_N2 reaction with NaN_3 afforded azides. These upon selective reduction with DIBAL-H followed by protection with TMSCl/EtSH gave



Scheme 1. Reagents and conditions: (i) DIBAL-H, dry CH_2Cl_2, −78 °C, 1 h, 72%; (ii) TMSCl/EtSH, dry CH_2Cl_2, rt, 12 h, 92%; (iii) $BF_3{\cdot}OEt_2$/EtSH, dry CH_2Cl_2, rt, 12 h, 85%; (iv) K_2CO_3, propargyl bromide, dry DMF, rt, 12 h, 93%.



Scheme 2. Reagents and conditions: (i) K_2CO_3, dibromo alkane spacers, dry DMF, rt, 12 h, 88%; (ii) NaN_3 (0.5 M in DMSO), 80 °C, 4 h, 83–90%; (iii) $CuSO_4{\cdot}5H_2O$ (1 mol %), sodium ascorbate (5 mol %), *t*-BuOH/H_2O (1:1), rt, 12 h, 82–88%; (iv) $SnCl_2{\cdot}2H_2O$, MeOH, reflux, 6 h, 80%; (v) $HgCl_2$–$CaCO_3$, CH_3CN–H_2O (4:1), rt, 12 h.



Scheme 3. Reagents and conditions: (i) mesyl chloride, Et_3N, CH_2Cl_2, 0 °C–rt, 6 h, 88%; (ii) NaN_3, dry DMF, 50–60 °C, 6 h, 82–90%; (iii) DIBAL-H, dry CH_2Cl_2, −78 °C, 1 h, 78%; (iv) TMSCl/EtSH, dry CH_2Cl_2, rt, 12 h, 92%; (v) $CuSO_4{\cdot}5H_2O$ (1 mol %), sodium ascorbate (5 mol %), *t*-BuOH/H_2O (1:1), rt, 12 h, 78–85%; (vi) $SnCl_2{\cdot}2H_2O$, MeOH, reflux, 6 h, 78%; (vii) $HgCl_2$–$CaCO_3$, CH_3CN–H_2O (4:1), rt, 12 h.

12a–**c** , which were subjected to cycloaddition with **7** by employing the 'click' chemistry protocol to produce triazoles **13a**–**c** . The reduction of **13a**–**c** followed by deprotective cyclization gave the required dimers **4a**–**c**[17] in good yields (72–78%) as illustrated in Scheme 3.

The DNA-binding ability of C8–C8/C2–C8-linked 1,2,3-triazole-PBD dimers **3a**–**e** and **4a**–**c** was examined by thermal denaturation studies[19] using calf thymus (CT) DNA at pH 7.0, with incubation at 37 °C, and DNA/ligand molar ratios of 5:1. The increase in the helix melting temperature (ΔT_m) for each compound was examined after 0 and 18 h of incubation at 37 °C. Further, the data for compounds **1** and **2** are included in Table 1 for comparison. 1,2,3-Triazole-containing PBD dimers showed ΔT_m values ranging from 0.9 to 18.7 °C (Table 1). Interestingly, compound **3d** elevates the helix melting temperature of CT-DNA to 18.7 °C after incubation at 37 °C for 18 h. Naturally occurring DC-81 (**1**) shows a ΔT_m of 0.7 °C, whereas synthetic DC-81 dimer **2** (DSB-120) exhibits a ΔT_m of 15.1 °C under similar experimental conditions. This result illustrates the significant effect of introducing the 1,2,3-triazole moiety between the two DC-81 PBD subunits through different alkane spacers. Further, it was interesting to observe that as the linker chain increased from three to four carbons, there was a decrease in the DNA-binding ability, while on further increase from five to six carbons in the chain, DNA stabilization was enhanced, as in the case of **3d**. Further, increase in the chain length up to eight carbons led to a decline in the DNA-binding ability, as in the case of **3e**. These results indicate that the incorporation of a 1,2,3-triazole moiety in the alkane spacer enhances the DNA-binding ability, particularly in the case of **3d** (Table 1). The length of the linker and the triazole moiety may play an important role in the DNA-binding ability. Furthermore, the C2–C8-linked 1,2,3-triazole PBD dimer of compound **4b** showed a noticeable ΔT_m, that is, 3.9 °C at

Table 1
Thermal denaturation data for C8–C8/C2–C8-linked 1,2,3-triazole-containing PBD dimers (**3a**–**e** and **4a**–**c**) with calf thymus CT-DNA

PBD dimers	[PBD]/[DNA] molar ratio[a]	ΔT_m (°C)[b] After incubation at 37 °C for	
		0 h	18 h
3a	1:5	5.5	11.3
3b	1:5	2.0	3.8
3c	1:5	4.8	9.8
3d	1:5	11.9	18.7
3e	1:5	7.1	13.9
4a	1:5	0.9	2.5
4b	1:5	3.9	5.9
4c	1:5	1.5	4.8
DC-81	1:5	0.3	0.7
DSB-120	1:5	10.2	15.1

[a] For CT-DNA alone at pH 7.00 ± 0.01, T_m = 69.6 °C ± 0.01 (mean value from 10 separate determinations), all T_m values are ±0.05–0.1 °C.
[b] For a 1:5 molar ratio of [PBD]/[DNA], where CT-DNA concentration = 100 μM and ligand concentration = 20 μM in aqueous sodium phosphate buffer [10 mM sodium phosphate + 1 mM EDTA, pH 7.00 ± 0.01].

0 h while the melting temperature increased to 5.9 °C upon incubation for 18 h at 37 °C. The C2–C8-linked triazole PBD dimers have shown appreciable affinity; however, the binding ability is not that significant, probably due to the structural rigidity at C2 of the PBD subunit.

In conclusion, the synthesis of a new class of C8–C8/C2–C8-linked 1,2,3-triazole-containing pyrrolo[2,1-*c*][1,4]benzodiazepine dimers has been demonstrated through 'click' chemistry. These compounds have been evaluated for their DNA-binding affinity using DNA thermal denaturation studies. Representative dimer **3d** has shown significant DNA-binding ability. Detailed studies on the mechanistic aspects, and anticancer activity and molecular modeling investigations on these dimers are in progress.

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17. *Spectral data*: Compound **3a**: Yield: 72%; FT-IR: cm^{-1} 2928.3; 1655.3; 1613.1; 1505.5; 1463.1; 1431.5; 1260.7; 1220.2; 1134.7; 1020.7; 871.5; 786.4. ^1H NMR (200 MHz, $CDCl_3$): δ 7.60 (d, 2H, $J = 3.47$ Hz); 7.45 (s, 2H); 6.90 (s, 1H); 6.69 (s, 1H); 5.22 (s, 2H); 4.51–4.57 (m, 4H); 3.91–4.06 (m, 2H); 3.87 (s, 6H); 3.47–3.80 (m, 6H); 2.35–2.43 (m, 2H); 2.20–2.26 (m, 3H); 1.88–2.05 (m, 4H); FABMS: *m/z* 613 [M^++H]. HRMS calcd For $C_{32}H_{35}N_7O_6$ [M^++H] 613.3042, found 613.3036. Compound **3b**: Yield: 68%; FT-IR: cm^{-1} 2927.3; 1655.3; 1613.1; 1505.5; 1463.1; 1431.5; 1260.7; 1220.2; 1134.7; 1020.7; 871.5; 786.4. ^1H NMR (400 MHz, $CDCl_3$): δ 7.68 (d, 2H, $J = 4.50$ Hz); 7.51 (s, 2H); 7.00 (s, 1H); 6.80 (s, 2H); 5.31 (s, 2H); 4.46–4.56 (m, 2H); 4.04–4.16 (m, 2H); 3.92 (s, 6H); 3.79–3.88 (m, 2H); 3.69–3.74 (m, 4H); 3.54–3.60 (m, 2H); 2.32 (m, 4H); 2.04–2.17 (m, 4H); 1.85–1.90 (m, 1H); 1.59–1.67 (m, 1H). FABMS: *m/z* 628 [M^+ +H]. HRMS calcd For $C_{33}H_{37}N_7O_6$ [M^+ +H] 628.3034, found 628.3040. Compound **3c**: Yield: 75%; FT-IR: cm^{-1} 2926.9; 1655.3; 1608.2; 1505.5; 1463.1; 1431.5; 1260.7; 1220.2; 1134.7; 1020.7; 871.7; 787.4. ^1H NMR (300 MHz, $CDCl_3$): δ 7.68 (t, 1H, $J = 4.26$ Hz); 7.51 (d, 2H, $J = 4.62$ Hz); 6.99 (s, 2H); 6.78 (s, 2H); 5.31 (s, 2H); 4.37–4.41 (m, 2H); 4.00–4.11 (m, 2H); 3.93 (s, 6H); 3.78–3.86 (m, 2H); 3.70–3.75 (m, 2H); 3.53–3.62 (m, 4H); 2.29–2.34 (m, 4H); 1.89–2.10 (m, 6H); 1.52–1.60 (m, 2H). FABMS: *m/z* 642 [M^++H]; HRMS calcd for $C_{34}H_{39}N_7O_6$ [M^+ +H] 642.3040, found 642.3034. Compound **3d**: Yield: 73%; FT-IR: cm^{-1} 2928.3; 1655.3; 1613.1; 1505.5; 1463.1; 1431.5; 1260.7; 1220.2; 1134.7; 1020.7; 871.5; 786.4. ^1H NMR (400 MHz, $CDCl_3$): δ 7.67–7.74 (d, 2H, $J = 6.34$ Hz); 7.39–7.41 (m, 3H); 6.49–6.54 (m, 2H); 5.32 (s, 2H); 4.34–4.49 (m, 2H); 4.05 (d, 2H $J = 5.39$ Hz); 3.88 (s, 6H); 3.72–3.82 (m, 4H); 3.56–3.63 (m, 4H); 2.73 (m, 2H); 2.28–2.35 (m, 2H); 1.93–2.01 (m, 8H); 1.77–1.83 (m, 2H). FABMS: *m/z* 656 [M^++H]; HRMS calcd for $C_{35}H_{31}N_7O_6$ [M^++H] 656.3040, found 656.3034. Compound **3e**: Yield: 78%; FT-IR: cm^{-1} 2928.3; 1655.3; 1613.1; 1505.5; 1463.1; 1431.5; 1260.7; 1220.2; 1134.7; 1020.7; 871.5; 786.4. ^1H NMR (200 MHz, $CDCl_3$): δ 7.66 (dd, 2H, $J = 1.16$, 2.57 Hz); 7.50 (d, 2H, $J = 3.49$ Hz); 6.98 (s, 1H); 6.79 (s, 2H); 5.30 (s, 2H); 4.30–4.37 (t, 2H, $J = 7.18$ Hz); 3.99–4.09 (m, 2H); 3.92 (s, 6H); 3.70–3.83 (m, 4H); 3.50–3.63 (m, 2H); 2.27–2.37 (m, 4H); 2.01–2.11(m, 2H); 1.82–1.88 (m, 4H); 1.25–1.35 (m, 10H); ^{13}C NMR (150 MHz, $CDCl_3$): 164.6; 164.4; 162.5; 162.4; 159.2; 150.7; 143.2; 140.5; 140.4; 123.1; 123.0; 119.9; 111.5; 111.3; 110.2; 68.8; 62.8; 62.2; 56.3; 56.2; 50.4; 46.9; 46.8; 33.3; 32.2; 30.1; 29.6; 29.5; 29.3; 29.0; 28.8; 28.7; 26.3; 25.7. FABMS: *m/z* 684 [M^+ +H]; HRMS calcd For $C_{37}H_{45}N_7O_6$ [M^+ +H] 684.3044, found 684.3036. Compound **4a**: Yield: 78%; FT-IR: cm^{-1} 2998.5; 1693.1; 1636.1; 1505.5; 1463.1; 1431.5; 1260.7; 1220.2; 1134.7; 1020.7; 871.5; 756.4. ^1H NMR (300 MHz, $CDCl_3$): δ 7.63–7.70 (m, 3H); 7.52 (d, 2H, $J = 2.80$ Hz); 7.34 (d, 2H, $J = 3.15$ Hz); 6.92–7.00 (m, 2H); 5.31 (s, 2H); 5.10–5.29 (m, 2H); 4.23–4.30 (m, 1H); 3.91 (s, 3H); 3.77–3.88 (m, 2H); 3.70–3.74 (m, 2H); 3.52–3.61 (m, 2H); 2.62–2.83 (m, 2H); 2.30–2.32 (m, 2H). FABMS: *m/z* 526 [M^++H]; HRMS calcd for $C_{28}H_{28}N_7O_4$ [M^++H] 526.2202, found 526.2212. Compound **4b**: Yield: 76%; FT-IR: cm^{-1} 2998.3; 1693.3; 1636.1; 1505.5; 1463.1; 1431.5; 1260.7; 1220.2; 1134.7; 1020.7; 871.5; 786.4; 647.5. ^1H NMR (300 MHz, $CDCl_3$): δ 7.63–7.69 (dd, 2H, $J = 4.38$, 4.11 Hz); 7.53 (d, 1H, $J = 5.11$ Hz); 6.99 (s, 2H); 6.84 (s, 2H); 5.33 (s, 2H); 4.27–4.31 (m, 2H); 4.00 (d, 1H, $J = 4.03$ Hz); 3.96 (s, 3H); 3.94 (s, 3H); 3.92 (s, 3H); 3.83–3.86 (m, 2H); 2.29–2.36 (m, 4H); 2.01–2.11 (m, 4H). FABMS: *m/z* 586 [M^++H]; HRMS calcd for $C_{30}H_{32}N_7O_6$ [M^+ +H] 586.2414, found 586.2438. Compound **4c**: Yield: 72%; FT-IR: cm^{-1} 2928.3; 1655.3; 1613.1; 1505.5; 1463.1; 1431.5; 1260.7; 1220.2; 1134.7; 1020.7; 871.5; 770.4; 690.5. ^1H NMR (400 MHz, $CDCl_3$): δ 7.67 (d, 2H, $J = 4.61$ Hz); 7.51–7.58 (m, 7H); 7.37 (s, 1H); 6.92 (s, 2H); 5.32 (s, 2H); 5.11–5.29 (m, 2H); 4.21–4.29 (m, 1H); 3.92 (s, 3H); 3.89 (s, 3H); 3.74–3.85 (m, 2H); 3.70–3.74 (m, 2H); 3.49–3.58 (m, 2H); 2.62–2.78 (m, 2H); 2.33–2.01 (m, 4H). FABMS: *m/z* 662 [M^++H]. HRMS calcd for $C_{36}H_{35}N_7O_6$ [M^++H] 662.2422, found 586.2434.
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