Phenol quaternary ammonium derivatives: charge and linker effect on their DNA photo-inducible cross-linking abilities

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We report here that phenol derivatives with two and four quaternary ammoniums were synthesized and their abilities to bind and cross-link DNA were investigated. Thermal denaturizing studies indicated that derivatives possess similar DNA binding abilities and gel electrophoresis revealed that more charges (series B) and electronic donation substitute linkers (like –S–) dramatically increase the DNA cross-linking abilities.

Introduction

The DNA double helical structure contains the richest source of information within a living organism. Its sequence codes for protein synthesis, *via* the process of translation, and for RNA synthesis.[1] Therefore, DNA remains one of the major targets for cytotoxic anticancer drugs. DNA interstrand cross-links (ISC) induced by chemical agents play a very important role in antitumor treatment.[2] Bifunctional alkylating agents may cause different lesions, show the most toxic of all alkylation events and, ultimately, inhibit the processes of translation and replication. Several important clinical drugs (*e.g.* cisplatin, chlorambucil, and mitomycin C) are known to induce DNA ISC formation which can disrupt cell maintenance and replication.[2] The design of novel dimeric agents targeting DNA has been developed by several groups.[2,3] Photochemical cross-linking agents are of particular interest because such agents could be selectively activated both in time and space, and then be released precisely when and where a desired biological effect is wanted.[4] The psoralens are the only class of compounds known to induce ISC on photolysis.[5] They have been employed in clinics for the treatment of psoriasis, vitiligo, and cutaneous T-cell lymphoma.[6] Among these anti-tumor agents, one acting mechanism was involved in *o*-quinone methide (*o*-QM, Fig. 1a) intermediate. *o*-QM is a derivative of quinone methide which has played important roles in organic syntheses and as well as in chemical and biological processes.[7] *o*-QMs are significant for their nucleic acid bases and DNA alkylation.[8] More recently, Wan's group,[9] Freccero's group[10] and Kresge's group[11] have reported significant findings on the generation of *o*-QMs by photochemical and thermal activation in aqueous solution.

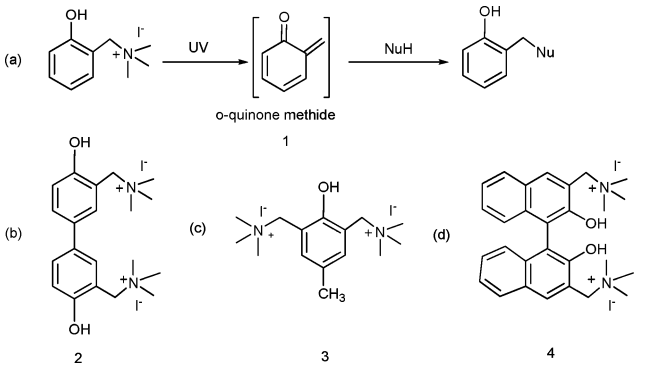

Fig. 1 Structures of compound **2**, **3**, **4** and *o*-quinone methide intermediate **1**.

In our previous study, we initially reported on a potent, water-soluble and photo inducible DNA cross-linking agent (Fig. 1b).[12] We found that cross-linking was achieved by mixing DNA with the quaternary ammonium salt derivatives upon illumination. In succession to our study, Freccero's group had found compound **4** as an important precursor to cross-link DNA after photoactivation. However, the structure relationship of the quaternary ammonium derivatives' binding to DNA has not yet been established.

To gain a better understanding of the quaternary ammonium derivative cross-linking of DNA under the prototype of compound **2**, we synthesized two series of quaternary ammonium phenol derivatives, and investigated the effect of different charges and linkers on the DNA binding profile and cross-linking activity of these new compounds.

Results and discussion

Synthesis of phenol derivatives with two quaternary ammonium salts (series A)

Different biphenol derivatives were mixed with formaldehyde and dimethylamine *via* a Mannich reaction (Scheme 1, series A) and purification could be finished by silica gel chromatography

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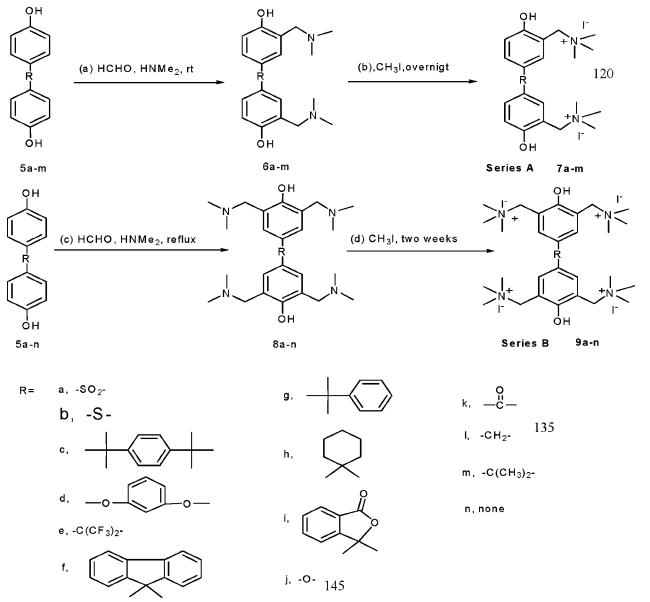

Scheme 1 Synthesis of two and four quaternary ammonium derivatives.

using ethanol solvent; the appropriate white or buff powder was obtained. Quaternary ammonium compounds **7a**–**m** were prepared by adding methyl iodide in a solvent of CH_3CN and then recrystallized from ether. All methylated compounds were obtained in good yields.

Synthesis of phenol derivatives with four quaternary ammonium salts (series B)

Compound **2** has two quaternary ammonium arms on different phenol rings, and compound **3** has two quaternary ammonium salts on the same phenol ring. They both have the ability to cross-link DNA after photoactivation, *via* the intermediate of *o*-quinone methide. Therefore, adding more quaternary ammonium by increasing the number of positive charges might favour their interaction with DNA and increase their ability to cross-link DNA. Based on this ideas, we designed and synthesized a series of compounds with four quaternary ammonium salts on the two phenol rings, each ring has two quaternary ammonium arms (Scheme 1, series B). This strategy has three advantages: (1) increase the solubility of compound in water; (2) increase the ability to bind the DNA backbone; (3) increase the yields to produce *o*-quinone methide. Series B was synthesized by improving the reaction conditions. Precursors were treated with more formaldehyde and dimethylamine (>10 equiv.), and the Mannich reaction occurred on the both *ortho*-positions of hydroxyl to obtain the compounds **8a**–**n**.

Due to their prodigious polarity, 2,5,2′,5′-tetra(trimethylamine methylene)-biphenol derivatives can only be separated in a co-solvent of ethanol and triethylamine (10 : 1). The methylation step was rather time-consuming, for the sake of gaining pure final product; all the reactions were carried out over two weeks.

All the new compounds were fully characterized by ^{1}H NMR, ^{13}C NMR, HRESIMS or EI.

Table 1 X-ray crystallographic data for **7k**

	7k
Empirical formula	$C_{21}H_{30}I_2N_2O_3\cdot 2.5H_2O$
Fw	657.31
Space group	Orthorhombic, Fdd2
a/Å	21.868 (4)
b/Å	25.240 (4)
c/Å	9.6164 (16)
deg	90
V/D^3	5307.8 (15)
Z	8
T/K	293 (2)
Wavelength	0.71073 (Å)
Final R indices	$R1 = 0.0468$, $wR2 = 0.1192$

X-Ray analysis of compound 7k

The crystal data and structure of compound **7k** are shown in Table 1 and Fig. 2.† The angle between C(2)–C(1)–C(2A) (which makes the whole molecule look like a claw) is around 119° and is mainly due to the lone electron pair on the carbonyl between two phenyl rings. The dihedral angle between two phenyl rings plane is around 55°, this twist is owed to the steric effect of the two phenol rings, and makes the molecule fit better with the double stranded DNA backbone. Two quaternary ammonium salt groups are seated in the opposite direction. The distance between N (1) to N (1A), O (2) to O (2A) are 11.458 and 10.105 Å, respectively. From the crystal structure of compound **7k**, we suggest that the distance between two quaternary ammonium salt groups is long enough to access the DNA base on the different strand. Also, there is little possibility to block **7k** interacting with DNA.

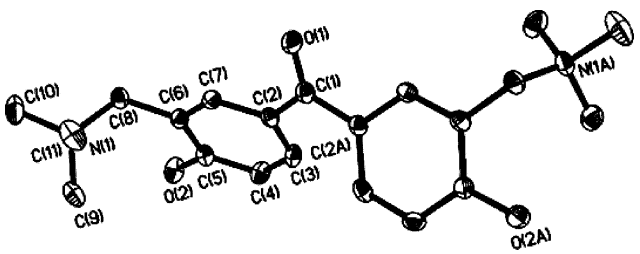

Fig. 2 ORTEP representation of **7k** with hydrogen atoms omitted for clarity. Thermal ellipsoids are drawn at the 30% probability level.

DNA cross-linking assay

The DNA cross-linking abilities of different compounds are illustrated in Fig. 3 and 4. In our previous paper,[12] compound **2** could cross-link DNA at a 1 μM level. In this paper, we designed a series of compounds **7a**–**m**, which have different linkers between the phenol rings, DNA cross-links could be induced upon illumination. Even at the concentration of 1 mM, compounds **7a**–**m** had poor DNA cross-linking abilities. Only **7b**, **7d** (line 3 and 5) could cross-link linear DNA above 50% under these conditions, no cross-linked DNA was observed in the case of compounds **7a**, **7c**, **7e**, **7f**, **7g**, **7i** and **7l** (line 2, 4, 6, 7, 8, 10 and 13) (Fig. 3). Compounds **9a**–**n** had been further modified with four quaternary ammonium

† CCDC reference number 230785. For crystallographic data in CIF format see DOI: 10.1039/b604552a

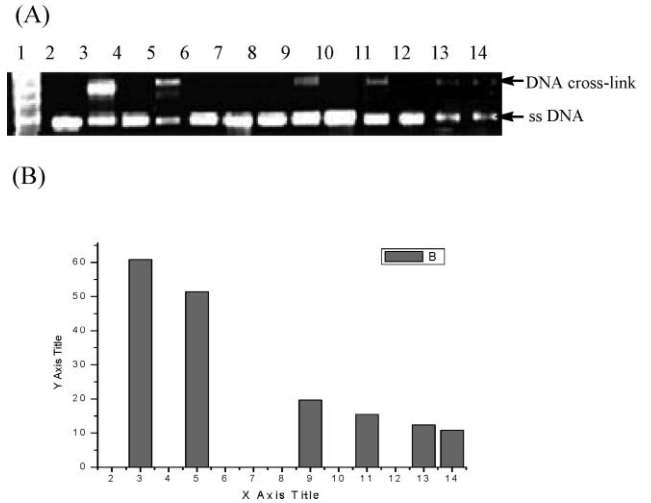

Fig. 3 Agarose gel electrophoresis of two biphenol quaternary ammonium salts derivatives; (A) lane 1 1.5 μg lambda DNA/Hind III (molecular weight standard); lane 2–14 1.0 μg pBR322 + 1 mM **7a**–**m** + *hv*, 30 min. (B) Cross-linking percents of different compounds (columns).

salts based on the structure of compounds **7a**–**m** and **2**. Apparently, our design for compounds **9a**–**n** with four positive charges could increase the ability to cross-link DNA (Fig. 4). Compared to **2**, compound **9n** slightly increased the DNA cross-linking ability from 64.7 to 75.0%. Excepting compound **9a** (line 4, 0%), all the four quaternary ammonium salts dramatically increased the DNA cross-linking ability compared to the derivatives with two quaternary ammonium salts, respectively, even at a much lower concentration (10 μM). Compound **9b** induced 98.2% cross-linking efficiently upon exposure to light for 30 min (lane 5). From Fig. 4c, we can see that in most cases the compounds with electron donating linkers have higher DNA cross-linking abilities than those with electron withdrawing linkers, which suggests that high electronic density might enhance the formation of *o*-quinone methide formation. Among these factors, the number of charges is the main factor to induce DNA cross-linking. Most of the compounds with four quaternary ammonium salts can induce more than 50% cross-linking at 10 μM, while most of the two quaternary ammonium salts induced less than 50% cross-linking, even at 1 mM. These results suggested that increasing the number of quaternary ammonium salts could increase the chance to generate *o*-QM, which is beneficial to DNA cross-linking ability. From Fig. 4c, we can see that electron withdrawing substitute linkers reduced the cross-linking efficiency. Compounds **9a**, **9i** and **9k** only induced 0, 1.1 and 0.8% cross-linking, respectively. Electron donating linkers might have two advantages, one is to favour the quaternary ammonium salts removal to generate *o*-QM; the other might be to stabilize *o*-QM intermediate.[16]

Thermal denaturizing study

The DNA binding abilities for these quaternary ammonium salts derivatives have been determined by thermal denaturation studies using calf thymus DNA (CT-DNA).[13] Interestingly, in this assay, all the compounds elevate the helix melting temperature of CT-DNA up to about 10 °C after incubation with drugs for 1 h. (Table 2). The result indicated that all compounds fit DNA

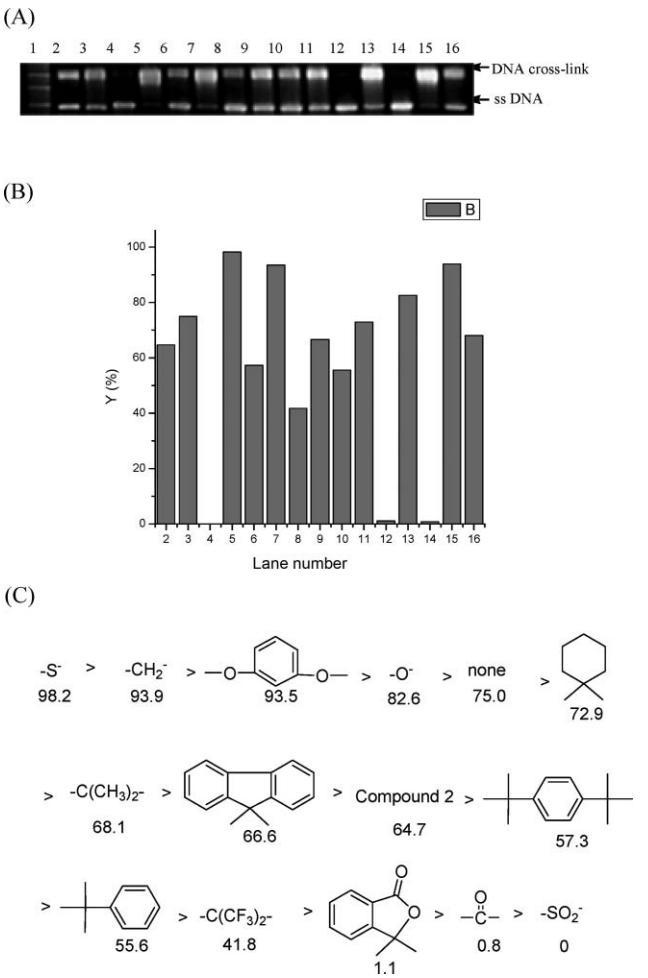

Fig. 4 Agarose gel electrophoresis of compound **2** and four biphenol quaternary ammonium derivatives; (A) lane 1 1.5 μg lambda DNA/Hind III (molecular weight standard); lane 2–16 1.0 μg pBR322 + 10 μM **2**, **9n**, **9a**–**9m** + *hv*, 30 min. (B) Cross-linking percents of different compounds (columns). (C) The sequence of cross-linking ability of compound **2** and different linker four biphenol quaternary ammonium salts in decreasing order.

phosphate backbone quite well, and all compounds possess similar DNA binding abilities.

Conclusion

Two series of different biphenol quaternary ammonium derivatives with different linkers between the two phenyl rings have been designed and prepared. We found that derivatives with four quaternary ammonium salts, derivatives **9a**–**n**, could efficiently cross-link DNA compared to the compounds with two quaternary ammoniums, derivatives **7a**–**m**. DNA cross-linking by quaternary ammonium salts can be obtained in two steps; first, compound binding to DNA because of the electrostatic attraction between quaternary ammonium ion and phosphate groups of the DNA backbone, the second step is that the compound generates *o*-QM intermediates by photo-irradiation and induces DNA cross-linking. Thermal denaturation studies suggested that these quaternary ammonium derivatives might possess similar DNA binding abilities. In other words, the binding step contribution or

Table 2 Thermal denaturizing data of series A and series B

Series A	ΔT_m[a,b]	Series B	ΔT_m
7a	10.3	**9a**	10.4
7b	7.4	**9b**	8.9
7c	8.4	**9c**	10.5
7d	10.6	**9d**	10.4
7e	7.8	**9e**	9.3
7f	8.6	**9f**	8.4
7g	7.4	**9g**	8.2
7h	6.2	**9h**	8.1
7i	8.2	**9i**	8.5
7j	8.8	**9j**	8.1
7k	6.4	**9k**	6.2
7l	7.5	**9l**	6.6
7m	6.9	**9m**	6.4
2	11.5	**9n**	11.3

[a] For CT-DNA alone at pH 7.40 ± 0.01, T_m = 72.9 ± 0. 1, sodium phosphate buffer [10 mM sodium phosphate + 1 mM EDTA, pH = 7.40 ± 0.01]. [b] For a 1 : 10 molar ratio of [drug] : [DNA], where CT-DNA concentration = 10 μM and drug concentration = 1 μM in aqueous sodium phosphate buffer [10 mM sodium phosphate +1 mM EDTA, pH = 7.40 ± 0.01].

structure–activity relationship study in these types of compounds is small. The structure of compounds **9a** and **9b** were alike, and **9a** held the higher ΔT_m. **9b** could cross-link 98.2% DNA, but **9a** could not induce DNA cross-linking at all. This result suggests that the structure of the linker is less important than the electronic effect. Crystal analysis of **7k** predicted a good interaction potential, which was not verified by experiment. This confirms that the electron effect might play the major role in the formation of *o*-quinone methide. Our results indicated that the number of quaternary ammonium salts and the linkers are important factors to influence the DNA cross-linking efficiency. These studies might raise new opportunities to design and search more potent anti-tumor drugs based on their abilities to DNA cross-link.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury 300 and 600 spectrometers. Chemical shifts were reported as values relative to internal standards as $CHCl_3$ or D_2O. Mass spectra were determined using Bruker Daltonics APEXII 47e. Elemental analysis was recorded on PE-240 C. Super coiled pBR322 and CT DNA was purchased from Dafeng Co. (China). All the drugs and DNA were performed in 0.1 M phosphate buffer (pH = 7.7). 4,4′-Dihydroxyldipenyl and other derivatives were purchased from Fischer Co. and TCI. All other chemicals and solvents were commercial available.

General procedure 1: synthesis of two Mannich base phenol derivatives 6a–m

Appropriate 4,4′-dihydroxydiphenol was dissolved in ethanol in a round bottom flask and aqueous solutions of dimethylamine (3.0 equiv., 33%) and formaldehyde (4.0 equiv., 37%) were added. The reaction mixture was stirred overnight at room temperature. The solvents were then evaporated under reduced pressure and the crude product was subjected to silica gel chromatography with ethanol. After evaporation of the solvents, the appropriate white or buff powder was obtained.

2,2′-Dimethylaminemethylene-4,4′-sulfonyldiphenol (6a). Yield: 125 mg, 34%; R_f = 0.2 (ethanol) (the ratio of the distance of migration by a substance compared with the solvent (ethanol here) front); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.68 (d, J = 9.0 Hz, 2 H, ArH), 7.53 (s, 2 H, ArH), 6.84 (d, J = 8.4 Hz, 2 H, ArH), 3.67 (s, 4 H, –CH_2–), 2.33 (s, 12 H, –CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 162.53, 131.86, 128.61, 127.53, 121.93, 116.58, 62.22, 44.35; HRESIMS: m/z 365.1525 (calcd for $C_{18}H_{24}O_4N_2S$ + H, 365.1535).

2,2′-Dimethylaminemethylenebis(4-hydroxyphenyl)sulfide (6b). Yield: 240 mg, 14%; R_f = 0.2 (ethanol); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.12 (d, J = 4.2 Hz, 2 H, ArH), 6.96 (s, 2 H, ArH), 6.74 (d, J = 4.2 Hz, 2 H, ArH), 3.57 (s, 4 H, –CH_2–), 2.31 (s,12 H, –CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 157.84, 132.26, 131.63, 125.48, 122.93, 117.16, 62.71, 44.68; HRESIMS: m/z 333.1625 (calcd for $C_{18}H_{24}O_2N_2S$ + H, 333.1637).

2,2′-Dimethylaminemethylene-α,α′-bis(4-hydroxyphenyl)-1,4-diisopropylbenzene (6c). Yield: 310 mg, 13%; R_f = 0.2 (ethanol); ^{1}H NMR($CDCl_3$, 300 MHz) δ 7.07 (s, 4 H, ArH), 7.02 (d, J = 8.7 Hz, 2 H, ArH), 6.81 (s, 2 H, ArH), 6.70 (d, J = 8.7 Hz, 2 H, ArH), 3.57 (s, 4 H, –CH_2–), 2.30 (s, 12 H, –CH_3),1.60 (s, 12 H, –CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 155.63, 147.96, 141.12, 127.11, 126.49, 126.12, 121.01, 115.27, 63.13, 44.53, 41.71, 30.94; HRESIMS: m/z 461.3160 (calcd for $C_{30}H_{40}O_2N_2$ + H, 461.3168).

2, 2′ - Dimethylaminemethylene - 1,3 - bis(4 - hydroxyphenyloxyl)-benzene (6d). Yield: 202 mg, 50%; R_f = 0.2 (ethanol); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.15 (t, J = 8.1 Hz, 1 H, ArH), 6.88–6.84 (q, 2 H, ArH), 6.78 (d, J = 9.0 Hz, 2 H, ArH), 6.69 (d, J = 2.4 Hz, 2 H, ArH), 6.56–6.53 (q, 2 H, ArH), 6.49 (t, J = 2.4 Hz,1 H, ArH), 3.60 (s, 4 H, –CH_2–), 2.33 (s,12 H, –CH_3), ^{13}C NMR ($CDCl_3$, 150 MHz) δ 160.27, 154.69, 148.14, 130.24, 123.00, 120.68, 120.48, 117.16, 110.95, 106.62, 62.68, 44.67; HRESI-MS: m/z 409.2124 (calcd for $C_{24}H_{28}O_4N_2$ + H, 409.2127).

2,2′ - Dimethylaminemethylene - 2,2 - bis(4 - hydroxyphenyl)hexafluoropropane (6e). Yield: 120 mg, 14%; R_f = 0.2 (ethanol); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.14 (d, J = 8.1 Hz, 2 H, ArH), 6.96 (s, 2 H, ArH), 6.77 (d, J = 8.4 Hz, 2 H, ArH), 3.61 (s, 4 H, –CH_2–), 2.32 (s, 12 H, –CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 158.46, 130.80, 129.93, 125.42, 123.77, 121.36, 115.59, 114.53, 62.88, 44.43; HRESIMS: m/z 451.1818 (calcd for $C_{21}H_{24}O_2N_2F_6$ + H, 451.1820).

2,2′-Dimethylaminemethylene-9,9-bis(4-hydroxyphenyl)fluorene (6f). Yield: 464 mg, 50%; R_f = 0.2 (ethanol); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.73 (d, J = 7.2 Hz, 2 H, ArH), 7.36–7.30 (m, 4 H, ArH), 7.26–7.22 (m, 2 H, ArH), 7.04–6.92 (m, 2 H, ArH), 6.72 (s, 2 H, ArH), 6.65 (d, J = 8.7 Hz, 2 H, ArH), 3.48 (s, 4 H, –CH_2–), 2.25 (s, 12 H, –CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 156.80, 152.20, 140.12, 136.79, 128.81, 128.11, 127.77, 127.35, 126.27, 121.43, 120.22, 115.87, 64.33, 63.01, 44.65; HRESIMS: m/z 465.2537 (calcd for $C_{31}H_{32}O_2N_2$ + H, 465.2542).

2,2′ - Dimethylaminemethylene - 4,4′ - (1 - α - methylbenzylidene)-bisphenol (6g). Yield: 250 mg, 21%; R_f = 0.2 (ethanol); ^{1}HNMR($CDCl_3$, 300 MHz) δ 7.27–7.18 (m, 3 H, ArH), 7.09–7.06

(dd, $J = 1.5$, 7.5 Hz, 2 H, ArH), 6.85–6.83 (t, 2 H, ArH), 6.72 (s, 2 H, ArH), 6.69 (d, 2 H, ArH), 3.55 (s, 4 H, $-CH_2-$), 2.31 (s, 12 H, $-CH_3$), 2.11 (s, 3 H, $-CH_3$); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 156.13, 150.23, 140.19, 129.23, 128.88, 128.65, 127.94, 125.94, 121.22, 115.31, 63.30, 51.28, 44.73, 30.95; HRESIMS: m/z 405.2530 (calcd for $C_{26}H_{32}O_2N_2$ + H, 405.2542).

2,2′-Dimethylaminemethylene-1,1-bis(4-hydroxyphenyl)cyclohexane (6h). Yield: 203 mg; 13%, $R_f = 0.2$ (ethanol); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.04 (d, $J = 8.4$ Hz, 2 H, ArH), 6.79 (s, 2 H, ArH), 6.72 (d, $J = 8.4$ Hz, 2 H, ArH), 3.57 (s, 4 H, $-CH_2-$), 2.29 (s, 12 H, $-CH_3$), 2.16 (s, 4 H, $-CH_2-$), 1.49 (s, 6 H, $-CH_2-$); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 155.31, 139.46, 127.36, 127.08, 121.26, 115.41, 63.16, 44.95, 44.47, 37.52, 26.48, 22.96; HRESIMS: m/z 383.2689 (calcd for $C_{24}H_{34}O_2N_2$ + H, 383.2698).

2,2′-Dimethylaminemethylenephenolphthalein (6i). Yield: 301 mg, 35%; $R_f = 0.2$ (ethanol); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.89 (d, $J = 7.8$ Hz, 1 H, ArH), 7.65 (m, 1 H, ArH), 7.53–7.46 (m, 2 H, ArH), 7.04–7.00 (m, 2 H, ArH), 6.91 (s, 2 H, ArH), 6.71 (d, $J = 4.5$ Hz, 2 H, ArH), 3.55 (s, 4 H, $-CH_2-$), 2.28 (s, 12 H, $-CH_3$); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.20, 158.34, 152.86, 134.04, 131.30, 129.03, 127.82, 127.18, 125.83, 125.48, 124.04, 121.71, 115.71, 92.10, 62.67, 44.44; HRESIMS: m/z 433.2126 (calcd for $C_{26}H_{28}O_4N_2$ + H, 433.2127).

2,2′-Dimethylaminemethylene-4,4′-dihydroxydiphenylether (6j). Yield: 149 mg, 24%; $R_f = 0.2$ (ethanol); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.12 (d, $J = 6.6$ Hz, 2 H, ArH), 6.74 (d, $J = 6.6$ Hz, 2 H, ArH), 6.60 (s, 2 H, ArH), 3.57 (s, 4 H, $-CH_2-$), 2.31 (s, 12 H, $-CH_3$); ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm) 153.62, 150.20, 122.98, 118.76, 118.74, 116.72, 63.12, 44.96. HRESIMS: m/z 317.1864 (calcd for $C_{18}H_{24}O_3N_2$ + H, 317.1860).

2,2′-Dimethylaminemethylene-4,4′-dihydroxybenzophenone (6k). Yield: 279 mg, 42%; $R_f = 0.2$ (ethanol); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.58 (d, $J = 8.4$ Hz, 2 H, ArH), 7.51 (s, 2 H, ArH), 6.83 (d, $J = 8.1$ Hz, 2 H, ArH), 3.72 (s, 4 H, $-CH_2-$), 2.38 (s, 12 H, $-CH_3$). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 161.99, 131.86, 130.53, 129.13, 121.65, 115.42, 63.09, 45.16, 30.55. HRESIMS: m/z 329.1867 (calcd for $C_{19}H_{24}O_3N_2$ + H, 329.1860).

2,2′-Dimethylamine methylene-4,4′-dihydroxydiphenylmethane (6l). Yield: 197 mg, 31%; $R_f = 0.2$ (ethanol); ^1H NMR ($CDCl_3$, 300 MHz) δ 6.96 (d, $J = 8.1$ Hz, 2 H, ArH), 6.76–6.69 (q, 4 H, ArH), 3.77 (s, 2 H, ArH), 3.59 (s, 4 H, $-CH_2-$), 2.32 (s, 12 H, $-CH_3$). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 155.94, 131.91, 128.92, 128.68 121.82, 115.92, 63.49, 45.27, 40.92. HRESIMS: m/z 315.2069 (calcd for $C_{19}H_{26}O_2N_2$ + H, 315.2067).

2,2′-Dimethylaminemethylene-4,4′-isopropylidenediphenol (6m). Yield: 82 mg, 12%; $R_f = 0.2$ (ethanol); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.02–6.98 (m, 2 H, ArH), 6.74 (d, $J = 8.1$ Hz, 2 H, ArH), 6.70 (d, $J = 8.1$ Hz, 2 H, ArH), 3.55 (s, 4 H, $-CH_2-$), 2.29 (s, 12 H, $-CH_3$), 1.58 (s, 6 H, $-CH_3$). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 155.62, 141.71, 126.97, 126.83, 121.25, 115.32, 63.49, 44.89, 41.89, 31.64. HRESIMS: m/z 343.2371 (calcd for ($C_{21}H_{30}O_2N_2$ + H), 343.2380).

General procedure 2: synthesis of 2,2′-bis(trimethylammoniummethylene)-4,4′-diphenol iodide derivatives 7a–m

6a–m was dissolved in CH_3CN and CH_3I (0.5 mL, 8.0 mmol) added to the mixture. The reaction mixture was stirred in the dark for one night. After adding 40 mL of absolute ether and the product was precipitated and crude product was obtained. Finally, pure product was obtained by crystallizing from ether.

2,2′-Bis(trimethyl-ammoniummethylene)-4,4′-sulfonyldiphenol iodide (7a). Yield: 98 mg, 76%; ^1H NMR (D_2O, 300 MHz) δ 7.82 (s, 2 H, ArH), 7.73 (d, $J = 9.0$ Hz, 2 H, ArH), 6.95(d, $J = 9.0$ Hz, 2 H, ArH), 4.34 (s, 4 H, $-CH_2-$),2.91 (s, 18 H, $-CH_3$); ^{13}C NMR (D_2O, 150 MHz) δ 162.11, 134.05,131.97, 130.81,117.62,115.78, 63.26, 52.69; HRESIMS: m/z 521.0967 (calcd for $C_{20}H_{30}O_4N_2SI_2-I$, 521.0971).

2,2′-Bis(trimethylammoniummethylene)bis(4-hydroxyphenyl)-sulfide iodide (7b). Yield: 75 mg, 82%; ^1H NMR (D_2O, 300 MHz) δ 7.26 (s, 2 H, ArH), 7.23 (d, $J = 8.4$ Hz, 2 H, ArH), 6.80 (d, $J = 8.4$ Hz, 2 H, ArH), 4.25 (s, 4 H, $-CH_2-$), 2.88 (s, 18 H, $-CH_3$); ^{13}C NMR (D_2O, 150 MHz) δ 156.48, 137.55, 135.84, 126.36, 117.62, 115.80, 63.74, 52.60; HRESIMS: m/z 489.1065 (calcd for $C_{20}H_{30}O_2N_2SI_2-I$, 489.1073).

2,2′-Bis(trimethylammoniummethylene)-α,α′-bis(4-hydroxyphenyl)-1,4-diisopropylbenzene iodide (7c). Yield: 88 mg, 78%; ^1H NMR (D_2O, 300 MHz) δ 7.00 (d, $J = 7.5$ Hz, 4 H, ArH), 6.89 (s, 4 H, ArH) 6.66 (d, $J = 7.2$ Hz, 2 H, ArH) 4.16 (s, 4 H, $-CH_2-$), 2.80 (s, 18 H, $-CH_3$), 1.35 (s, 12 H, $-CH_3$), ^{13}C NMR (D_2O, 150 MHz) δ 154.20, 148.31, 142.89, 132.93, 130.92, 126.53, 115.99, 113.89, 64.22, 52.46, 41.47,29.85; HRESIMS: m/z 617.2595 (calcd for $C_{32}H_{46}O_2N_2I_2-I$, 617.2604).

2,2′-Bis(trimethylammoniummethylene)-1,3-bis(4-hydroxyphenyloxyl) benzene iodide (7d). Yield: 28 mg, 67%; ^1H NMR (D_2O, 300 MHz) δ 7.10 (t, $J = 9.0$ Hz, 1 H, ArH) 6.98 (d, $J = 6.9$ Hz, 4 H, ArH), 6.81 (d, $J = 9.6$ Hz, 2 H, ArH), 6.52 (d, $J = 6.0$ Hz, 2 H, ArH), 6.30 (s, 1 H, ArH), 4.23 (s, 4 H, $-CH_2-$), 2.87 (s, 18 H, $-CH_3$); ^{13}C NMR (D_2O, 150 MHz) δ 159.44, 153.38, 148.34, 125.70, 124.54, 117.71, 115.56, 112.70, 111.74, 106.38, 63.79, 52.54; HRESIMS: m/z 565.1553 (calcd for $C_{26}H_{34}O_4N_2I_2-I$, 565.1563).

2,2′-Bis(trimethylammoniummethylene)-2,2-bis(4-hydroxyphenyl)hexafluoropropane iodide (7e). Yield: 86 mg, 88%; ^1H NMR (D_2O, 300 MHz) δ 7.27 (d, $J = 9.0$ Hz, 2 H, ArH) 7.21 (s, 2 H, ArH), 6.83 (d, $J = 8.7$ Hz, 2 H, ArH), 4.26 (s, 4 H, $-CH_2-$), 2.85 (s, 18 H, $-CH_3$); ^{13}C NMR (D_2O, 150 MHz) δ 155.51, 134.17, 132.19, 122.99, 122.28, 121.10, 114.37, 112.63, 61.74, 50.54; HRESIMS: m/z 607.1249 (calcd for $C_{23}H_{30}O_2N_2F_6I_2-I$, 607.1256).

2,2′-Bis(trimethylammoniummethylene)-9,9-bis(4-hydroxyphenyl)fluorene iodide (7f). Yield: 63 mg, 65%; ^1H NMR (D_2O, 300 MHz) δ 7.73 (d, $J = 7.5$ Hz, 2 H, ArH), 7.30–7.25 (m, 4 H, ArH), 7.19–7.12 (m, 4 H, ArH), 6.84 (s, 2 H, ArH), 6.71 (d, $J = 8.4$ Hz, 2 H, ArH), 4.06 (s, 4 H, $-CH_2-$), 2.78 (s, 18 H, $-CH_3$); ^{13}C NMR (D_2O, 150 MHz) δ 155.49, 150.86, 139.56, 137.26, 133.36, 132.32, 128.37, 125.77, 120.79, 116.46, 114.12, 63.83, 63.37, 52.37; HRESIMS: m/z 621.1966 (calcd for $C_{33}H_{38}O_2N_2I_2-I$, 621.1978).

2,2′-Bis(trimethylammoniummethylene)-4,4′-(1-α-methylbenzylidene)bisphenol iodide (7g). Yield: 197 mg, 89%; ^{1}H NMR (D_2O, 300 MHz) δ 7.09–7.05 (m, 3 H, ArH), 7.03–6.69 (m, 4 H, ArH), 6.82 (s, 2 H, ArH), 6.70 (d, $J = 8.7$ Hz, 2 H, ArH), 4.13 (s, 4 H, –CH_2–), 2.75 (s, 18 H, –CH_3), 1.88 (s, 3 H, –CH_3); ^{13}C NMR (D_2O, 150 MHz) δ 153.81, 148.20, 140.07, 133.69, 131.65, 127.56, 127.44, 125.60, 115.07, 113.01, 63.24, 51.51, 49.98, 28.58; HRESIMS: m/z 561.1967 (calcd for $C_{28}H_{38}O_2N_2I_2$–I, 561.1978).

2,2′-Bis(trimethylammoniummethylene)-1,1-bis(4-hydroxyphenyl)cyclohexane iodide (7h). Yield: 159 mg, 91%; ^{1}H NMR (D_2O, 300 MHz) δ 7.18 (d, $J = 8.4$ Hz, 2 H, ArH), 7.10 (s, 2 H, ArH), 6.72 (d, $J = 8.4$ Hz, 2 H, ArH), 4.20 (s, 4 H, –CH_2–), 2.82 (s, 18 H, –CH_3), 2.05 (s, 4 H, –CH_2–), 1.27 (s, 6 H, –CH_2–);^{13}C NMR (D_2O, 150 MHz) δ 154.23, 132.92, 131.22, 116.34, 114.31, 64.28, 52.36, 44.40, 36.21, 25.74, 22.53; HRESIMS: m/z 539.2184 (calcd for $C_{26}H_{40}O_2N_2I_2$–I, 539.2135).

2,2′-Bis(trimethylammoniummethylene)phenolphthalein iodide (7i). Yield: 65 mg, 78%; ^{1}H NMR (D_2O, 300 MHz) δ 7.83 (d, $J = 3.6$ Hz, 1 H, ArH), 7.72–7.68 (m, 1 H, ArH), 7.57–7.51 (m, 2 H, ArH), 7.24–7.21 (m, 2 H, ArH), 7.16 (d, $J = 2.4$ Hz, 2 H, ArH), 6.84 (d, $J = 8.4$ Hz, 2 H, ArH), 4.26 (s, 4 H, –CH_2–),2.88 (s, 18 H, –CH_3); ^{13}C NMR (D_2O, 150 MHz) δ 157.91, 151.99, 135.78, 133.21, 131.64, 131.23, 130.19, 126.13, 124.02, 116.87, 114.75, 92.51, 63.84, 55.22, 52.49, 35.81; HRESIMS: m/z 589.1558 (calcd for $C_{28}H_{34}O_4N_2I_2$–I, 589.1563).

2,2′-Bis(trimethylammoniummethylene)-4,4′-dihydroxydiphenylether iodide (7j). Yield: 78 mg, 90%; ^{1}H NMR (D_2O, 300 MHz) δ 6.85–6.78 (m, 4 H, ArH), 6.78 (s, 2 H, ArH), 4.22 (s, 4 H, –CH_2–), 2.87 (s, 18 H, –CH_3); ^{13}C NMR (D_2O, 150 MHz) δ 152.60, 150.19, 124.12, 122.96, 117.87, 115.74, 64.11, 52.91. HRESIMS: m/z 173.1119 (calcd for ($C_{20}H_{30}O_3N_2I_2 - 2I$)/2), 173.1123).

2,2′-Bis(trimethylammoniummethylene)-4,4′-dihydroxylbenzophenone iodide (7k). Yield: 349 mg, 62%; ^{1}H NMR (D_2O, 300 MHz) δ 7.73 (s, 2 H, ArH), 7.68 (d, $J = 8.7$ Hz, 2 H, ArH), 6.99 (d, $J = 8.1$ Hz, 2 H, ArH), 4.43 (s, 4 H, –CH_2–), 3.01 (s, 18 H, –CH_3); ^{13}C NMR (D_2O, 150 MHz) δ 161.16, 137.08, 135.12, 128.85, 116.19, 114.88, 64.13, 53.19. HRESIMS: m/z 179.1120 (calcd for ($C_{21}H_{30}O_3N_2I_2 - 2I$)/2), 179.1123).

2,2′-Bis(trimethylammoniummethylene)-4,4′-dihydroxydiphenylmethane iodide (7l). Yield: 69 mg, 71%; ^{1}H NMR (D_2O, 300 MHz) δ 7.13 (s, 2 H, ArH), 7.08 (m, 2 H, ArH), 6.78 (d, $J =$ 8.7 Hz, 2 H, ArH), 4.22 (s, 4H, –CH_2–), 3.64 (s, 2 H, –CH_2–), 2.90 (s, 18H, –CH_3). ^{13}C NMR (D_2O, 150 MHz) δ 154.90, 134.65, 133.98, 132.97, 116.89, 114.69, 64.24, 52.77, 39.02. HRESIMS: m/z 172.1222 (calcd for ($C_{21}H_{32}O_2N_2I_2 - 2I$)/2), 172.1226).

2,2′-Bis(trimethylammoniummethylene)-4,4′-isopropylidenediphenol iodide (7m). Yield: 67 mg, 70%; ^{1}H NMR (D_2O, 300 MHz) δ 7.18 (d, $J = 8.1$ Hz, 2 H, ArH), 7.11(s, 2 H, ArH), 6.78 (d, $J = 8.7$ Hz, 2 H, ArH), 4.31 (s, 4 H, –CH_2–), 2.93 (s, 18 H, –CH_3), 1.56 (s, 6 H, –CH_3); ^{13}C NMR (D_2O, 150 MHz) δ 154.45, 143.09, 132.77, 131.20, 116.26, 114.23, 64.43, 52.63, 41.36, 29.93. HRESIMS: m/z 172.1222 (calcd for ($C_{23}H_{36}O_2N_2I_2 - 2I$)/2), 172.1226).

General procedure 3: synthesis of four Mannich base phenol derivatives 8a–n

Appropriate 4,4′-dihydroxydiphenyl was dissolved in a solvent of ethanol in a round bottom flask, and two kinds of aqueous solutions of dimethylamine (10.0 equiv., 33%) and formalaldehyde (10.0 equiv., 37%) were added, respectively. The reaction mixture was kept at reflux for about 3 h and reaction was monitored by TLC. After finished reaction, the solvents were evaporated under reduced pressure and the crude product was subjected to silica gel chromatography with ethanol and triethylamine (10 : 1). After evaporated the solvent, the appropriate white or buff powder was obtained.

2,5,2′,5′-Tetra(trimethylaminemethylene)-4,4′-sulfonyldiphenol (8a). Yield: 370 mg, 77%; $R_f = 0.15$ (ethanol–triethylamine = 10 : 1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.58 (s, 4 H, ArH), 3.55 (s, 8 H, –CH_2–), 2.28 (s, 24 H, –CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 161.73, 131.57, 128.45, 124.14, 60.26, 45.04; HRESIMS: m/z 479.2686 (calcd for $C_{24}H_{38}O_4N_4S$ + H, 479.2692).

2,5,2′,5′-Tetra(trimethylaminemethylene)bis(4-hydroxyphenyl)-sulfide (8b). Yield: 512 mg, 57%; R_f = 0.15 (ethanol–triethylamine = 10 : 1); ^{1}H NMR($CDCl_3$, 300 MHz) δ 6.96 (s, 4 H, ArH), 3.43 (s, 8 H, –CH_2–), 2.22 (s, 24 H, –CH_3); ^{13}CNMR ($CDCl_3$, 150 MHz) δ 156.55, 132.10, 124.76, 124.33, 60.44, 45.05; HRESIMS: m/z 447.2792 (calcd for $C_{24}H_{38}O_2N_4S$ + H, 447.2794).

2,5,2′,5′-Tetra(trimethylaminemethylene)-α,α′-bis(4-hydroxyphenyl)-1,4-diisopropylbenzene (8c). Yield: 210 mg, 63% R_f = 0.15 (ethanol–triethylamine = 10 : 1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.05 (s, 4 H, ArH), 6.92 (s, 4 H, ArH), 3.61 (s, 8 H, –CH_2–), 2.33 (s, 24 H, –CH_3),1.62 (s, 12 H, –CH_3);^{13}CNMR ($CDCl_3$, 150 MHz) δ 154.82, 148.23, 140.77, 128.09, 126.33, 121.55, 60.40, 44.64, 41.93, 31.11; HRESIMS: m/z 575.4323 (calcd for $C_{36}H_{54}O_2N_4$ + H, 575.4325).

2,5,2′,5′-Tetra(trimethylaminemethylene)-1,3-bis(4-hydroxyphenyloxyl)benzene (8d). Yield: 170 mg, 88%; $R_f = 0.15$ (ethanol–triethylamine = 10 : 1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.13 (t, $J = 8.7$ Hz, 1 H, ArH), 6.78 (s, 4 H, ArH), 6.53 (d, $J = 1.2$ Hz, 2 H, ArH) 6.50 (s, 1 H, ArH), 3.55 (s, 8 H, –CH_2–), 2.31 (s, 24 H, –CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 160.28, 153.55, 147.61, 130.22, 123.92, 121.12, 110.89, 106.77, 60.14, 44.85; HRESIMS: m/z 523.3274 (calcd for $C_{30}H_{42}O_4N_4$ + H, 523.3284).

2,5,2′,5′-Tetra(trimethylaminemethylene)-2,2-Bis(4-hydroxyphenyl)hexafluoropropane (8e). Yield: 300 mg, 72%; R_f = 0.15 (ethanol–triethylamine = 10 : 1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.01 (s, 4 H, ArH), 3.56 (s, 8 H, –CH_2–), 2.30 (s, 24 H, –CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 157.62, 131.13, 125.62, 123.72, 123.06, 122.68, 60.57, 44.87; HRESIMS: m/z 565.2977 (calcd for $C_{27}H_{38}O_2N_4F_6$ + H, 565.2977).

2,5,2′,5′-Tetra(trimethylaminemethylene)-9,9-bis(4-hydroxyphenyl)fluorene (8f). Yield: 653 mg, 57%; R_f = 0.15 (ethanol–triethylamine = 10 : 1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.74 (d, $J = 8.4$ Hz, 2 H, ArH), 7.34 (t, $J = 8.1$ Hz, 4 H, ArH), 7.24 (t, $J = 7.2$ Hz, 4 H, ArH), 6.78 (s, 4 H, ArH), 3.43 (s, 8 H, –CH_2–), 2.23 (s, 24 H, –CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 155.65, 152.02, 139.89, 135.71, 129.00, 127.53, 127.12, 126.00, 121.97,

120.01, 63.97, 60.31, 44.59; HRESIMS: m/z 579.3697 (calcd for $C_{37}H_{46}O_2N_4$ + H, 579.3699).

2,5,2′,5′-Tetra(trimethylaminemethylene)-4,4′-(1-α-methylbenzylidene)bisphenol (8g). Yield: 620 mg, 60%; $R_f = 0.15$ (ethanol–triethylamine = 10 : 1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.26–7.18 (m, 3 H, ArH), 7.05 (d, $J = 7.2$ Hz, 2 H, ArH), 6.66 (s, 4 H, ArH), 3.42 (s, 8 H, –CH_2–), 2.22 (s, 24 H, –CH_3), 2.09 (s, 3 H, –CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 155.00, 150.29, 139.32, 129.61, 128.85, 127.87, 125.86, 122.22, 60.90, 51.21, 44.99, 30.90; HRESIMS: m/z 519.3692 (calcd for $C_{32}H_{46}O_2N_4$ + H, 519.3699).

2,5,2′,5′-Tetra(trimethylaminemethylene)-1,1-bis(4-hydroxyphenyl)cyclohexane (8h). Yield: 743 mg, 75%; $R_f = 0.15$ (ethanol–triethylamine = 10 : 1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.78 (s, 4 H, ArH), 3.41 (s, 8 H, –CH_2–), 2.18 (s, 24 H, –CH_3), 2.11 (s, 4 H, –CH_2–), 1.42 (s, 6 H, –CH_2–); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 154.31, 138.88, 127.98, 122.62, 61.03, 45.04, 37.58, 26.71, 23.25; HRESIMS: m/z 497.3853 (calcd for $C_{30}H_{48}O_2N_4$ + H, 497.3856).

2,5,2′,5′-Tetra(trimethylaminemethylenephenolphthalein (8i). Yield: 460 mg, 45%; $R_f = 0.15$ (ethanol–triethylamine = 10 : 1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.90 (d, $J = 7.8$ Hz, 1 H, ArH), 7.66 (t, $J = 7.5$ Hz, 1 H, ArH), 7.52 (t, $J = 6.6$ Hz, 2 H, ArH), 6.90 (s, 4 H, ArH), 3.44 (s, 8 H, –CH_2–), 2.22 (s, 24 H, –CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.49, 157.26, 153.20, 134.25, 130.72, 129.18, 128.05, 126.03, 125.67, 124.22, 123.27, 92.47, 60.75, 45.21; HRESIMS: m/z 547.3284 (calcd for $C_{32}H_{42}O_4N_4$ + H, 547.3284).

2,5,2′,5′-Tetra(trimethylaminemethylene)-4,4′-dihydroxydiphenylether (8j). Yield: 282 mg, 88%; $R_f = 0.15$ (ethanol–triethylamine = 10 : 1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.71 (s, 4 H, ArH), 3.58 (s, 8 H, –CH_2–), 2.34 (s, 24 H, –CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 152.61, 149.69, 123.22, 119.61, 59.89, 44.58; HRESIMS: m/z 431.3025 (calcd for $C_{24}H_{38}O_3N_4$ + H, 431.3022).

2,5,2′,5′-Tetra(trimethylaminemethylene)-4,4′-dihydroxybenzophenone (8k). Yield: 371 mg, 72%; $R_f = 0.15$ (ethanol–triethylamine = 10 : 1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.51 (s, 4 H, ArH), 3.57 (s, 8 H, –CH_2–), 2.30 (s, 24 H, –CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 195.13, 161.40, 131.78, 128.76, 123.09, 60.49, 45.09; HRESIMS: m/z 443.3012 (calcd for $C_{25}H_{38}O_3N_4$ + H, 443.3022).

2,5,2′,5′-Tetra(trimethylaminemethylene)-4,4′-dihydroxydiphenylmethane (8l). Yield: 118 mg, 69%; $R_f = 0.15$ (ethanol–triethylamine = 10 : 1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.79 (s, 4 H, ArH), 3.74 (s, 2 H, –CH_2–), 3.48 (s, 8 H, –CH_2–) 2.26 (s, 24 H, –CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 154.77, 131.44, 129.22, 122.94, 60.34, 44.80, 40.14; HRESIMS: m/z 429.3228 (calcd for $C_{25}H_{40}O_2N_4$ + H, 429.3229).

2,5,2′,5′-Tetra(trimethylaminemethylene)-4,4′-isopropylidenediphenol (8m). Yield: 230 mg, 77%; $R_f = 0.15$ (ethanol–triethylamine = 10 : 1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.81 (s, 4 H, ArH), 3.46 (s, 8 H, –CH_2–), 2.24 (s, 24 H, –CH_3), 1.59 (s, 6 H, –CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 154.53, 141.09, 127.59, 122.39, 60.86, 45.00, 41.63, 31.38; HRESIMS: m/z 457.3530 (calcd for $C_{27}H_{44}O_2N_4$ + H, 457.3543).

2,5,2′,5′-Tetra(trimethylaminemethylene)-4,4′-dihydroxydiphenyl (8n). Yield: 331 mg, 24%; $R_f = 0.15$ (ethanol–triethylamine = 10 : 1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.26 (s, 4 H, ArH), 3.69 (s, 8 H, –CH_2–), 2.37 (s, 24 H, –CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 156.00, 131.21, 127.66, 122.13, 59.65, 44.215; HRESIMS: m/z 415.3062 (calcd for $C_{24}H_{38}O_2N_4$ + H, 415.3073).

General procedure 4: synthesis of 2,5,2′,5′ - tetra (trimethylammoniummethylene)-4,4′-dihydroxydiphenyl iodide derivatives 9a–n

8a–**n** was dissolved in CH_3CN and CH_3I (5.0 mL, 80 mmol) was added to the mixture. The reaction mixture was stirred in the dark for about two weeks. After removed the solvent by reduced pressure evaporated and crude product was obtained. Finally, pure product was obtained by crystallizing from ether.

2,5,2′,5′ - Tetra (trimethylammoniummethylene) - 4,4′-sulfonyldiphenol iodide (9a). Yield: 86 mg, 82%; ^{1}H NMR (D_2O, 300 MHz) δ 7.97 (s, 4 H, ArH), 4.47 (s, 2 H, –CH_2–), 4.40 (s, 6 H, –CH_2–), 3.01 (s, 9 H, –CH_3), 2.95 (s, 27 H, –CH_3); ^{13}CNMR (D_2O, 150 MHz) δ 137.29, 136.54, 127.34, 125.08, 119.32, 64.12, 63.31, 53.07, 52.74; anal. found: C, 31.39; H, 4.74; N, 5.18; calcd for $C_{28}H_{50}O_4N_4I_4S{\cdot}H_2O$: C, 31.59; H, 4.92; N, 5.26%.

2,5,2′,5′-Tetra(trimethylammoniummethylene)bis(4-hydroxyphenyl)sulfide iodide (9b). Yield: 123 mg, 90%; ^{1}H NMR (D_2O, 300 MHz) δ 7.47 (s, 4 H, ArH), 4.34 (s, 8 H, –CH_2–), 2.88 (s, 36 H, –CH_3); ^{13}C NMR (D_2O, 150 MHz) δ 157.36, 140.50, 140.11, 119.64, 63.74, 52.77; anal. found: C, 31.56; H, 5.18; N, 5.36; calcd for $C_{28}H_{50}O_2N_4I_4S{\cdot}3H_2O$: C, 31.47; H, 5.28; N, 5.24%.

2,5,2′,5′-Tetra(trimethylammoniummethylene)-α,α′-bis(4-hydroxyphenyl)-1,4-diisopropylbenzene iodide (9c). Yield: 96 mg, 87%; ^{1}H NMR (D_2O, 300 MHz) δ 7.20 (s, 4 H, ArH), 7.01 (s, 4 H, ArH), 4.28 (s, 8 H, –CH_2–), 2.80 (s, 36 H, –CH_3), 1.42 (s, 12 H, –CH_3);^{13}C NMR (D_2O, 150 MHz) δ 157.61, 148.32, 136.07, 126.85, 117.95, 64.42, 52.64, 41.80, 29.68; anal. found: C, 39.62; H, 6.37; N, 5.20; calcd for $C_{40}H_{66}O_2N_4I_4.4H_2O{\cdot}0.5CH_3CN$: C, 39.87; H, 6.16; N, 5.10%.

2,5,2′,5′-Tetra(trimethylammoniummethylene)-1,3-bis(4-hydroxyphenyloxyl)benzene iodide (9d). Yield: 55 mg, 88.5%; ^{1}H NMR (D_2O, 300 MHz) δ 7.03 (s, 4 H, ArH), 6.96 (t, $J = 8.7$ Hz, 1 H, ArH), 6.52 (s, 1 H, ArH), 6.39 (d, $J = 7.5$ Hz, 2 H, ArH), 4.19 (s, 8 H, –CH_2–), 2.74 (s, 36 H, –CH_3), ^{13}C NMR (D_2O, 150 MHz) δ 158.56, 152.81, 149.94, 131.24, 128.07, 120.06, 113.17, 109.03, 63.80, 52.75; anal. found: C, 36.35; H, 5.31; N, 4.64; calcd for $C_{34}H_{54}O_4N_4I_4{\cdot}3H_2O{\cdot}0.5C_2H_5OC_2H_5$: C, 36.59; H, 5.54; N, 4.74%.

2,5,2′,5′-Tetra(trimethylammoniummethylene)-2,2-Bis(4-hydroxyphenyl)hexafluoropropane iodide (9e). Yield: 45 mg, 74%; ^{1}H NMR (D_2O, 300 MHz) δ 7.51 (s, 4 H, ArH), 4.42 (s, 8 H, –CH_2–), 2.88 (s, 36 H, –CH_3);^{13}C NMR (D_2O, 150 MHz) δ 161.35, 141.82, 139.67, 127.28, 121.47, 121.16, 66.29, 55.57; anal. found: C, 31.71; H, 4.61; N, 4.82; calcd for $C_{31}H_{50}O_2N_4I_4F_6{\cdot}2H_2O$: C, 31.86; H, 4.66; N, 4.79%.

2,5,2′,5′-Tetra(trimethylammoniummethylene)-9,9-bis(4-hydroxyphenyl)fluorene iodide (9f). Yield: 64 mg, 81%; ^{1}H NMR (D_2O, 300 MHz) δ 7.74 (d, $J = 7.5$ Hz, 2 H, ArH), 7.36–7.25 (m, 8 H, ArH), 7.15 (m, 2 H, ArH), 4.26 (s, 8 H, –CH_2–), 2.83 (s, 36 H, –CH_3); ^{13}C NMR (D_2O, 150 MHz) δ 155.89, 150.11, 139.72, 138.55, 137.01, 128.78, 128.63, 125.55, 121.17, 118.66,

63.67, 63.26, 52.79; anal. found: C, 40.07; H, 5.80; N, 4.60; calcd for $C_{41}H_{58}O_2N_4I_4 \cdot 5H_2O$: C, 39.82; H, 5.54; N, 4.53%.

2,5,2′,5′-Tetra(trimethylammoniummethylene)-4,4′-(1-α-methylbenzylidene)bisphenol iodide (9g). Yield: 111 mg, 66%; ^{1}H NMR (D_2O, 300 MHz) δ 7.18 (s, 4 H, ArH), 7.14–7.07 (m, 5 H, ArH), 4.32 (s, 8 H, –CH_2–), 2.83 (s, 36 H, –CH_3), 2.00 (s, 3 H, –CH_3); ^{13}C NMR (D_2O, 150 MHz) δ 155.09, 148.00, 142.02, 137.65, 128.58, 128.40, 126.93, 118.01, 64.06, 52.67, 51.16, 29.49; anal. found: C, 37.63; H, 5.49; N, 4.81; calcd for $C_{36}H_{58}O_2N_4I_4 \cdot 3.5H_2O$: C, 37.61; H, 5.69; N, 4.87%.

2,5,2′,5′-Tetra(trimethylammoniummethylene)-1,1-bis(4-hydroxyphenyl)cyclohexane iodide (9h). Yield: 98 mg, 91%; ^{1}H NMR (D_2O, 300 MHz) δ 7.34 (s, 4 H, ArH), 4.34 (s, 8 H, –CH_2–), 2.84 (s, 36 H, –CH_3), 2.10 (s, 4 H, –CH_2–), 1.28 (s, 6 H, –CH_2–);^{13}C NMR (D_2O, 150 MHz) δ 154.37, 141.89, 136.49, 118.32, 64.19, 52.65, 44.93, 36.37, 25.61, 22.52; anal. found: C, 36.68; H, 5.71; N, 4.99; calcd for $C_{34}H_{60}O_2N_4I_4 \cdot 3H_2O$: C, 36.51; H, 5.94; N, 5.00%.

2,5,2′,5′-Tetra(trimethylammoniummethylene)phenolphthalein iodide (9i). Yield: 46 mg, 53%; ^{1}H NMR (D_2O, 300 MHz) δ 7.79 (d, $J = 3.6$ Hz, 2 H, ArH) 7.64 (d, $J = 3.6$ Hz, 2 H, ArH), 7.49–7.44 (m, 4 H, ArH), 4.34 (s, 8 H, –CH_2–), 2.84 (s, 36 H, –CH_3); ^{13}C NMR (D_2O, 150 MHz) δ 171.85,157.66, 151.37, 136.50, 136.18, 132.92, 130.67, 126.56, 124.13, 123.66, 118.76, 90.89, 63.52, 52.80; anal. found: C, 35.52; H, 5.50; N, 4.59; calcd for $C_{36}H_{54}O_4N_4I_4 \cdot 6H_2O$: C, 35.37; H, 5.44; N, 4.58%.

2,5,2′,5′-Tetra(trimethylammoniummethylene)-4,4′-dihydroxydiphenylether iodide (9j). Yield: 311 mg, 89%; ^{1}H NMR (D_2O, 300 MHz) δ 7.53 (s, 4 H, ArH), 4.42 (s, 8 H, –CH_2–), 2.95 (s, 36 H, –CH_3); ^{13}C NMR (D_2O, 150 MHz) δ 152.93, 150.73, 127.67, 120.46, 63.94, 53.10; anal. found: C, 31.80; H, 5.51; N, 5.16; calcd for $C_{28}H_{50}O_3N_4I_4 \cdot 3.5H_2O$: C, 31.68; H, 5.54; N, 5.28%.

2,5,2′,5′-Tetra(trimethylammoniummethylene)-4,4′-dihydroxybenzophenone iodide (9k). Yield: 356 mg, 78%; ^{1}H NMR (D_2O, 300 MHz) δ 7.95 (s, 4 H, ArH), 4.54 (s, 8 H, –CH_2–), 3.03 (s, 36 H, –CH_3); ^{13}C NMR (D_2O, 150 MHz) δ 195.58, 162.17, 139.84, 129.89, 118.42, 63.92, 53.03; anal. found: C, 32.56; H, 5.40; N, 5.01; calcd for $C_{29}H_{50}O_3N_4I_4 \cdot 3.5H_2O$: C, 32.45; H, 5.35; N, 5.22%.

2,5,2′,5′-Tetra(trimethylammoniummethylene)-4,4′-dihydroxydiphenylmethane iodide (9l). Yield: 160 mg, 86%; ^{1}H NMR (D_2O, 300 MHz) δ 7.38 (s, 4 H, ArH), 4.44 (s, 8 H, –CH_2–), 3.94 (s, 2 H, –CH_2–), 2.97 (s, 36 H, –CH_3); ^{13}C NMR (D_2O, 150 MHz) δ 154.97, 137.97, 134.95, 118.76, 64.17, 52.83; anal. found: C, 35.02; H, 6.04; N, 4.87; calcd for $C_{29}H_{52}O_2N_4I_4 \cdot 3H_2O \cdot C_2H_5OC_2H_5$: C, 35.25; H, 6.09; N, 4.98%.

2,5,2′,5′-Tetra(trimethylammoniummethylene)-4,4′-isopropylidenediphenol iodide (9m). Yield: 207 mg, 77%; ^{1}H NMR (D_2O, 300 MHz) δ 7.38 (s, 4 H, ArH), 4.45 (s, 8 H, –CH_2–), 3.035–2.955 (d, 36 H, –CH_3), 1.58 (s, 6 H, –CH_3); ^{13}C NMR (D_2O, 150 MHz) δ 154.58, 144.04, 136.20, 118.26, 64.20, 52.83, 41.86, 30.03; anal. found: C, 34.36; H, 6.11; N, 4.90; calcd for $C_{31}H_{56}O_2N_4I_4 \cdot 4H_2O \cdot 0.25C_2H_5OC_2H_5$: C, 34.47; H, 6.01; N, 5.02%.

2,5,2′,5′-Tetra(trimethylammoniummethylene)-4,4′-dihydroxydiphenyl iodide (9n). Yield: 76 mg, 71%; ^{1}H NMR (D_2O, 300 MHz) δ 7.59 (s, 4 H, ArH), 4.44 (s, 8 H, –CH_2–), 2.93 (s, 36 H, –CH_3); ^{13}C NMR (D_2O, 150 MHz) δ 156.28, 136.17, 132.98, 119.05, 64.18, 53.03; anal. found: C, 32.94; H, 5.41; N, 5.42; calcd for $C_{28}H_{50}O_2N_4I_4 \cdot 2H_2O$: C, 33.02; H, 5.34; N, 5.50%.

Crystallographic data collection

Compound crystals were grown up by slow evaporation of methanol for one month. A single crystal was picked up for X-ray analysis. Data for the X-ray structures was recorded using a Bruker Smart APEX equipped with a CCD detector in the *c* range of 2.36 to 26.00°. The structure was solved by the direct method in conjunction with standard difference Fourier techniques. Hydrogen atoms were placed in calculated positions using a standard riding model and were refined isotropically.

Alkaline agarose gel electrophoresis assay

To test DNA–DNA cross-linking ability of these compounds, we used linear plasmid DNA by denaturing alkaline agarose gel electrophoresis reported by Cech[14] and William.[15] The duplex DNA linearized by restriction endonuclease digestion with *Eco*R I. DNA cross-linking experiments were carried out in phosphate buffer (pH = 7.7). Samples were exposed to a 50 W high pressure mercury lamp placed 20 cm away at 25 °C. The crude reaction mixtures were loaded onto a denaturing 0.9% alkaline agarose gel. Lambda Hind III was employed as a molecular weight marker. The gel was subsequently stained in an ethidium bromide solution (100 μL of a 10 mg mL^{-1} ethidium bromide solution in 1 L of 1 M Tris–1.5 M NaCl buffer at pH = 7.5) for 1 h. Gels were visualized by UV and photographed using Vilber Lourmat video system.

Thermal denaturation study

The concentrations of CT DNA were determined spectrophotometrically from appropriate molar absorptivity values and calculated using a molar absorptivity of 6600 mol L^{-1} cm^{-1} at 260 nm. In order to test the interaction between drug molecules with DNA, the melting curves were recorded at different compounds to CT DNA by following the absorption change at 260 nm as a function of temperature with a heating rate of 1.0 °C min^{-1}. T_m values were determined from the maximum of the first derivative or from the graphs at the mid point of the transition curves. ΔT_m values were calculated as usual[13] by subtracting the T_m of the free nucleic acid from the T_m of the complex.

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