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Bifunctional Phenol Quinone Methide Precursors: Synthesis and Biological Activity

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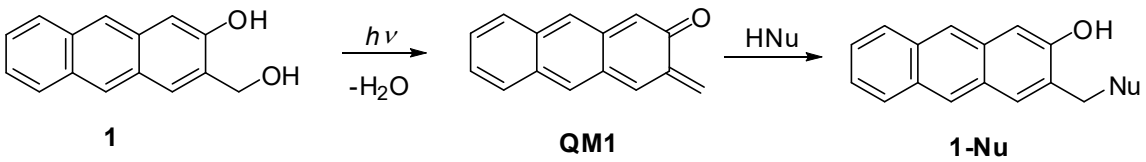
Abstract: New bifunctional quinone methide (QM) precursors, bisphenols **2a–2e**, and monofunctional QM precursor **7** were synthesized. Upon treatment with fluoride, desilylation triggers formation of reactive intermediates, QMs, which was demonstrated by trapping QM with azide or methanol. The ability of QMs to alkylate and cross-link DNA was assayed by investigation of the effects of QMs to DNA denaturing, but without conclusive evidence. Furthermore, treatment of a plasmid DNA with compounds **2a**–**2e** and KF, followed by the analysis by alkaline denaturing gel electrophoresis, did not provide evidence for the DNA cross-linking. MTT test performed on two human cancer cell lines (MCF7 breast adenocarcinoma and SUM159 pleomorphic breast carcinoma), with and without fluoride, indicated that **2a**–**2e** or the corresponding QMs did not exhibit cytotoxic activity, in line with the lack of ability to cross-link DNA. The lack of reactivity with DNA and biological activity were explained by sequential formation of QMs where bifunctional cytotoxic reagent is probably never produced. Instead, the sequential generation of monofunctional QM followed by a faster hydrolysis leads to the destruction of biologically active reagent. The findings described here are particularly important for the rational design of new generation of QM precursor molecules that will attain desirable DNA reactivity and cytotoxicity.

Keywords: antiproliferative activity, DNA alkylation, Grignard reaction, nucleic acid, quinone methide, structure-based drug design.

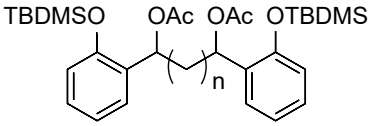
INTRODUCTION

QUINONE methides (QMs) are important intermediates in chemistry and photochemistry of phenols,[1] owing to their applications in organic synthesis,[2] and biological activity.[3] It has been demonstrated that QMs react with amino acids[4] and proteins,[5] and inhibition of some enzymes has been reported including hydroxylases,[6] β-lactamase,[7] β-glucosidases,[8] phosphatase[9] or ribonuclease-A.[10] However, biological action of QMs has mostly been related to their reactivity with nucleosides[11] and alkylation of DNA,[12] since some anticancer antibiotics such as mitomycin[13] exert their antiproliferative action on metabolic formation of QMs that alkylate DNA. In particular, S. Rokita et al. demonstrated reversible alkylation ability of QMs leading to "immortalization of QM" by DNA as a nucleophile,[14] whereas Freccero et al. investigated ability of QMs to alkylate G4 regions of DNA.[15]

QMs are very reactive species that cannot be stored, they have to be generated *in situ*. Some thermal reactions to generate QMs include oxidation of phenols,[16] dehydration from hydroxybenzyl alcohols,[17] elimination of nitriles from 1,2-benzoxazines,[18] and the most extensively used, fluoride induced desilylation.[12b,c] On the other hand, photochemical methods offer much milder approach to QMs, particularly for biological systems.[19] The most common reactions to generate QMs in photochemical reactions are photodehydration[20] and photodeamination from the suitably substituted phenols.[4,21] An on-going interest in our group is the photochemical generation of quinone methides (QM) from suitable precursors, and investigation of their biological effects.[22] In particular, we have recently demonstrated that photogenerated QM



Scheme 1. Photochemical dehydration on anthrol **1** and formation of **QM1** that exhibits cytotoxicity on cancer stem cells.



2a n = 4
2b n = 5
2c n = 6
2d n = 7
2e n = 8

Figure 1. Structures of bifunctional QM precursors.

formed from anthrol **1** (Scheme 1) exhibit higher cytotoxicity on cancer stem cell lines then on normal cancer cells.[23] Interestingly, our results indicate that enhanced antiproliferative effects probably does not originate from the reaction of QMs with DNA, but rather with proteins, since we have shown that compounds do not enter cell nucleus.[22d,23] However, all molecules that we studied to date were monofunctional, so in principle, they could only alkylate DNA. On the other hand, DNA cross-linking by bi-functional molecules is known to be the most cytotoxic event leading to the cell death.[24]

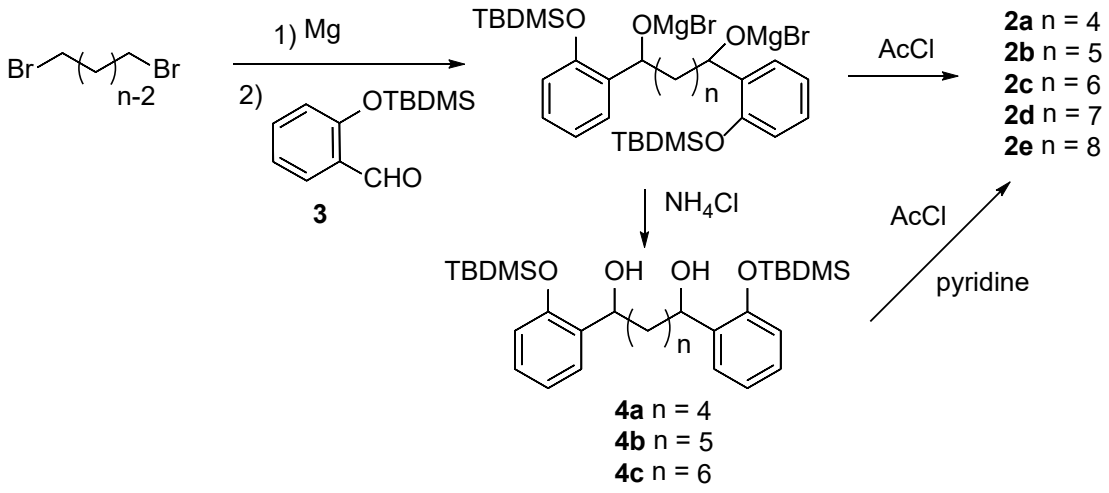
Herein we report an investigation of F^- induced desilylation and generation of bi-functional QMs that are anticipated to enable DNA cross-linking (Figure 1). Bisphenol derivatives **2** are bi-functional derivatives of phenols that are substituted by TBDMS at the phenolic oxygen and by acetyl at the benzyl alcohol. According to literature precedent, F^- induced desilylation should induce the cleavage of the silyl group, elimination of the acetyl and formation of QMs.[12b,c] The QM precursors are separated by alkyl spacer of different lengths to probe for the effect of molecular structure to the efficiency of DNA cross-linking.

Formation of QMs was probed by F^- induced reactions and trapping with nucleophiles. Antiproliferative activity of bisphenols was investigated *in vitro* against two human cancer cell lines: MCF-7 (breast adenocarcinoma) and SUM159 (pleomorphic breast carcinoma). The idea was to link the antiproliferative activity to the ability of QMs to cross-link DNA molecules. Interestingly, treatment of plasmid DNA in the presence of bisphenols and analysis by alkaline agarose electrophoresis, or measurement of DNA denaturation show that QMs do not cross-link plasmid DNA, and the reasons for the lack of reactivity were disclosed.

RESULTS

Synthesis

Synthesis of bis-QM precursors was based on the one-pot double Grignard reaction with the appropriately protected



Scheme 2. Synthesis of bisphenol QM precursors.

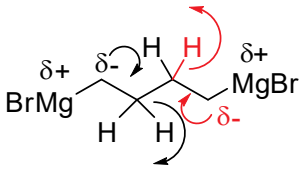


Figure 2. Structure of C-4 di-Grignard reagent that is prone to β-hydride transfer.

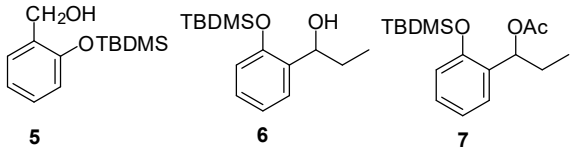


Figure 3. Structure of products **5**–**7**.

carbaldehyde. Salicylaldehyde component was TBDMS-protected **3**[25] (for the preparation see the SI) that reacted with double Grignard reagent formed from 1,*n*-dibromoalkane. The reaction gave alkoxide intermediates that can be quenched by H_2O and isolated as alcohols **4**, or acetylated in one-pot (Scheme 2). An attempt to quench the alkoxide with acetic anhydride failed, but the treatment with acetyl chloride was successful giving acetyl esters. The one pot procedure gave better yields and one purification step less was needed. It should be noted that the double Grignard reaction created two stereogenic centers in **4** or **2**, and therefore, a mixture of two enantiomers (*RR*, *SS*) and the *meso*-compound (*SR*) were obtained, which were inseparable by achiral chromatography.

On attempts to prepare bisphenol containing alkyl spacer with less than 6 C-atoms, in the Grignard reaction with 1,4-dibromobutane or 1,3-dibromopropane, a reduction of the TBDMS-protected aldehyde took place giving benzyl alcohol **5**. Such β-hydride transfer reactions are known to compete with Grignard reactions,[26] particularly with sterically congested carbonyl groups.[27] In our case, the di-Grignard reagent formed from 1,4-dibromobutane contained reactive H-atoms at the β-position (Figure 2), allowing the facile β-hydride transfer and preventing the addition to the carbonyl group. Furthermore, based on literature precedent, formation of di-Grignard reagent from 1,3-dibromopropane is proble-matic since the di-Grignard reagent is very unstable.[28] Therefore, only bisphenols containing more than 6 C atoms in the linker were synthesized. In addition to bisphenol derivatives, we have synthesized also model compounds **6** and **7** (Figure 3), that contain only one phenol moiety, and which were needed in the biological investigations.

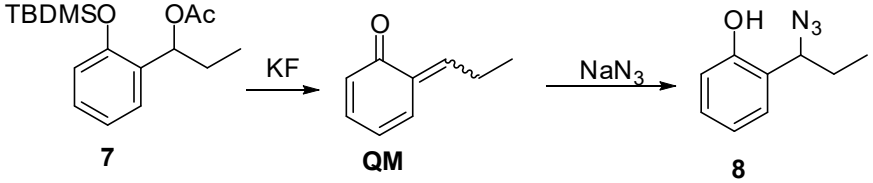
QM Formation by F^- Induced Desilylation

Formation of QM in the desilylation reaction was first probed on model compound **7**. The compound was dissolved in DMSO and diluted with PBS buffer (pH 7.4). Formation of QM was initiated by addition of KF (incubation 1 h). The formation of QMs was proven by quenching with nucleophiles, NaN_3, the ubiquitous QM quencher[19] whereupon adduct **8** was isolated by preparative HPLC (Scheme 3). Furthermore, we have shown that adducts were formed practically immediately after the addition of KF, whereas in the absence of KF adducts were not formed over 24 h.

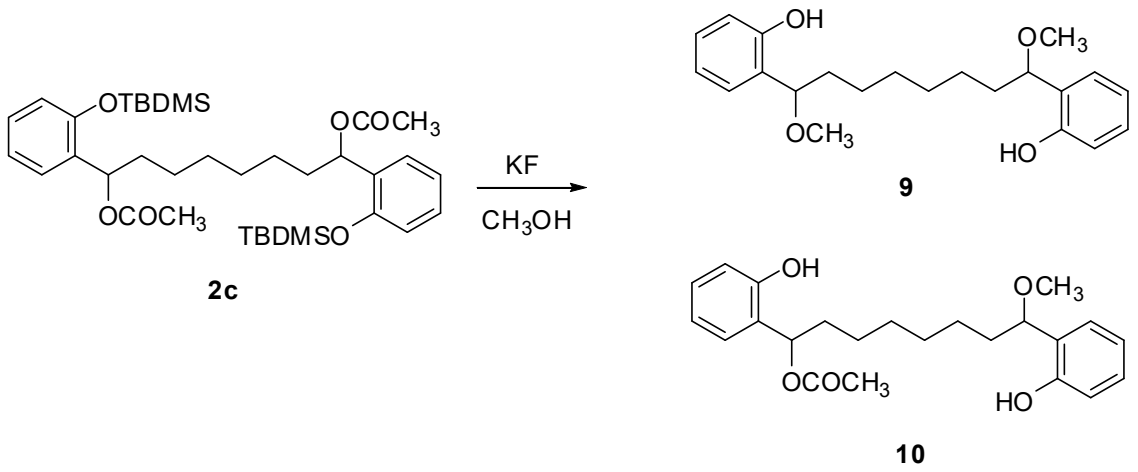
To show that bisphenols can also undergo F^- desilylation giving QMs we have treated **2c** with KF in methanolic solution. The reaction gave two products **9** and **10** (Scheme 4) that were isolated and characterized. Formation of **10**, where only one benzyl alcohol is substituted by methoxy group indicates that formation of QMs and methanolysis probably take place sequentially. That is, upon formation of a QM, the reaction of solvolysis probably takes place more rapidly than formation of the second QM in the same molecule. Thus, it is not probable that one molecule contains two QM centers at the same time.

Investigation of DNA Cross-linking Ability of Compounds

After demonstrating that F^- induces formation of QMs, DNA cross-linking ability of bisphenols **2** was assayed. Plasmid pCMVbeta DNA (2 μg) in PBS buffer was mixed with **2a**–**2e** and **7** (1 mM), and treated with KF (200 mM). After 18 h incubation, the alkaline agarose gel electrophoresis was performed, as described in the Experimental section. As a positive control, plasmid was irradiated in the presence of psoralene derivative that is known to cross-link DNA.[12d] Interstrand cross-linking activities of psoralen were evident as X-band of circular form (X-CC). However, no X-bands and no difference in migratory ability compared to control DNA were observed in the presence of **2** (Figure 4).



Scheme 3. F^- induced formation of QM and reaction with NaN_3.



2c **9** **10**

Scheme 4. F^- induced methanolysis from bisphenol **2c**.

Investigation of the DNA Cross-linking by Thermal Denaturation

Compounds that bind to DNA cause stabilization of the duplex and increase of the DNA melting temperature (ΔT_m). Similarly, we anticipated that the covalent binding, and particularly, cross-linking of DNA should affect the ΔT_m. Therefore, the ability of compounds **2b**–**2e** to form bifunctional QMs and cross-link DNA was assayed also by their effects on ΔT_m (Figure 5 and Figures S1 and S2 in the supporting information). The problem in the measurement represented poor solubility of **2** under these conditions. Therefore, the measurement was conducted in H_2O-DMSO solvent mixture (9:1, or 8:2 for **2c**), containing sodium cacodylate buffer (c = 0.05 M, pH = 7.0). Under such conditions, ct-DNA revealed T_m = 75.1±0.5 °C. Addition of compounds alone or in combination with KF did not affect the T_m significantly, changes being within the error of the method (±0.5 °C, Figure 5), suggesting that the eventual alkylation and cross-linking was either of very low efficiency (leaving large amount of free DNA available for denaturation) or did not take place at all. However, the thermal denaturation experiment could not give the unambiguous conclusion whether QMs from **2** were able to alkylate and cross-link at least some of DNA, since even low cross-linking efficiency could have measurable biological effect. Therefore, we decided to assay these systems on human cancer cell lines.

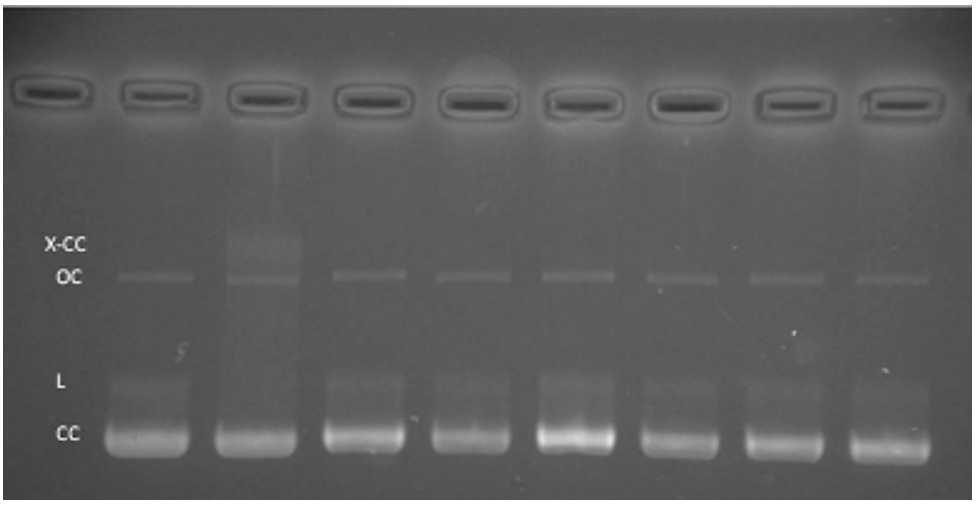


Figure 4. Alkaline agarose gel electrophoresis: pCMVbeta plasmid DNA (2 μg) in PBS buffer (pH = 7.4) was mixed with **2a**–**2e** or **7** (1 mM), and KF (200 mM). After 18 h incubation, the samples were loaded and the gel was running for 5 h. Columns from left to right: 1) plasmid DNA, 2) psoralen (PS) (20 μM) and irradiation 5 min at 300 nm, 3) **2a** 4) **2b**, 5) **2c**, 6) **2d**, 7) **2e**, 8) **7**. CC-closed circular DNA, OC-open circular DNA, L-linear DNA, X- cross-linked DNA.

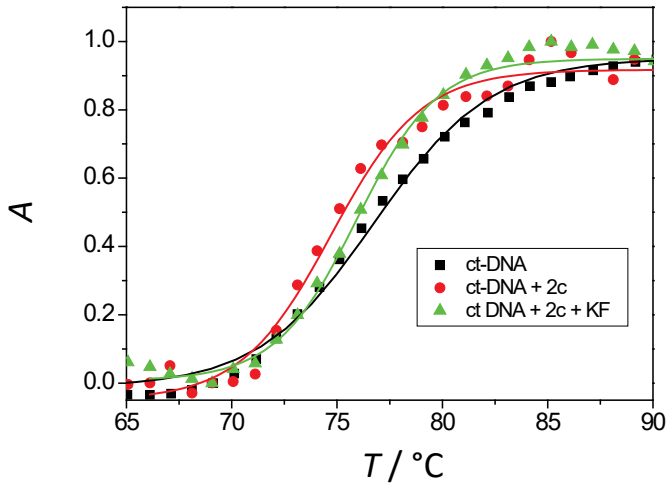


Figure 5. Normalized dependence of the ct-DNA (1.76×10^{-5} M) absorbance at 260 nm, in H_2O-DMSO (8:2) containing sodium cacodylate buffer (c = 0.05 M, pH = 7.0), neat or in the presence of **2c** (1.6×10^{-3} M), or **2c** (1.6×10^{-3} M) and KF (0.2 M) after incubation for 3 h.

Antiproliferative Activity

Antiproliferative effect of the F^- generated QMs on two cancer cell lines, MCF7 (breast adenocarcinoma) and SUM159 (pleomorphic breast carcinoma) was investigated. Cells were divided into two groups; cells treated with compounds and KF, and cells that were treated only with compounds. KF was added two hours after the compounds in order to allow bisphenols to permeate the cell's membrane. Cells were incubated with all of the compounds for three days. The activities expressed as IC_{50} (concentration that causes 50 % inhibition of the cell growth) are compiled in Table 1. In general, all of the compounds exhibited low antiproliferative activity. Compound **7** had moderate activity toward MCF7 cells. We observed enhancement of cytotoxicity in SUM159 cells upon activation of compound **7** with KF, but this effect could also be attributed to toxicity of KF toward SUM159 cell line (Figure S4). Small improvement of cytotoxicity was also observed in MCF7 cells after activation of compound **2d**.

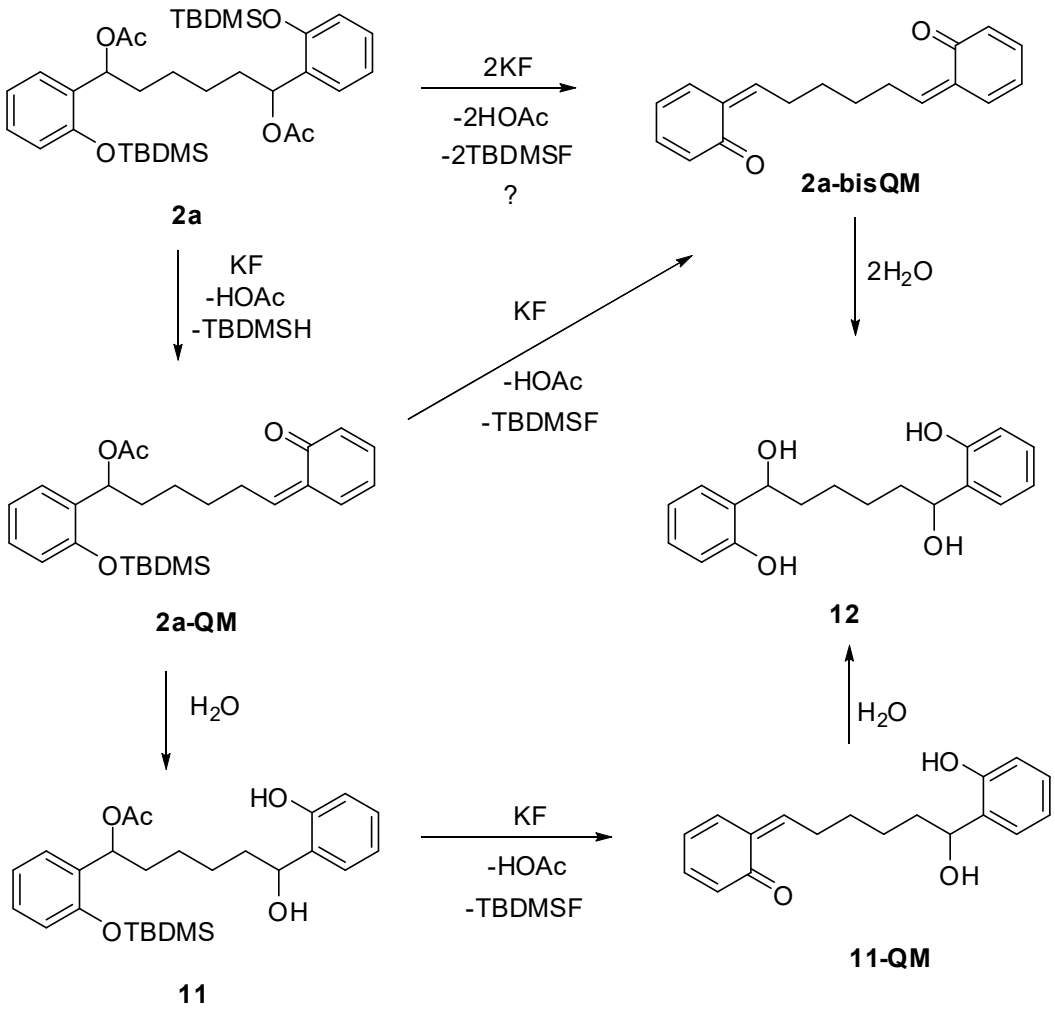
DISCUSSION

The treatment of monofunctional QM precursor **7** with KF in the presence of azide whereupon adduct **8** was isolated clearly indicated that F^- induced formation of QMs, in accord with literature precedent.[12b,c] Furthermore, KF induced methanolysis of bisphenol **2c**, indicating that formation of QMs takes place upon treatment of **2a**-**2e** with KF. However, we do not have any evidence if the formation of bis-QM takes place (such as **2a-bisQM**, Scheme 5). Namely, it is plausible that F^- induced formation of QM takes place stepwise giving **2a-QM**. In principle, this monofunctional QM molecule can undergo two competitive reactions, hydrolysis to **11** and secondary elimination to

Table 1. IC_{50} values (in μM)[(a)] induced with compounds **2** and **7**

	SUM159		MCF7	
Comp.	No F^-	F^- (1 mM)	No F^-	F^- (1 mM)
2a	>100	>100	>100	>100
2b	>100	>100	>100	>100
2c	>100	>100	>100	>100
2d	>100	>100	>100	77 ± 27
2e	>100	>100	>100	>100
7	>100	63.5 ± 0.6	28 ± 4	24 ± 2

[(a)] Concentration that causes 50 % inhibition of tumor cell growth.



Scheme 5. Plausible pathways for the formation of QM and bisQM from **2a** and their hydrolysis.

2a-bisQM. The concentration of **2a-bisQM** is therefore determined by a ratio of the corresponding reaction rate constants and concentrations [F^-] vs [H_2O]. Hydrolyzed product **11** can also undergo F^- induced elimination giving **11-QM**. Eventually, both reactive sides in **2a** are used and transformed to the bis-hydroxymethylphenol **12**, which is not reactive.

Pathways presented in Scheme 5 may account for the lack of DNA cross-linking with compounds **2**. Namely, the cross-linking is only feasible if **2a-bisQM** is formed, or if **2a-QM** reacts faster with DNA then with H_2O. Although, the hydrolyzed product **11** forms **11-QM**, it can only alkylate DNA and cannot induce DNA cross-linking. This situation is different to bifunctional phenols bearing leaving groups at the position 2 and 6, such as **13**. We have demonstrated by transient spectroscopy that **13** undergoes photodeamination giving **13-QMa**. The attack of a nucleophile to **13-QMa** at the methylene 2 position gives new QM with a methylene at the 6 position, **13-QMb** (Figure 6).[21b] This specific reactivity of bifunctional QMs leads to long-lived transient species and enable reversible reactions of the QM along a DNA chain as if the QM "walks on the DNA".[29]

Attempts to induce DNA cross-linking by **2a**–**2e** and **7**, and probe the process by the alkaline agarose gel electrophoresis or thermal denaturation failed, or gave no unambiguous conclusion. Furthermore, we have shown that compounds in the presence of KF do not exhibit cytotoxicity. These findings indicate that bisphenols **2** probably do not form bifunctional QMs. Consequently, they cannot cross-link DNA and do not show significant cytotoxicity.

CONCLUSION

Monofunctional and bifunctional quinone methide (QM) precursors were synthesized and their ability to form QMs in the reaction with fluoride was demonstrated. The ability

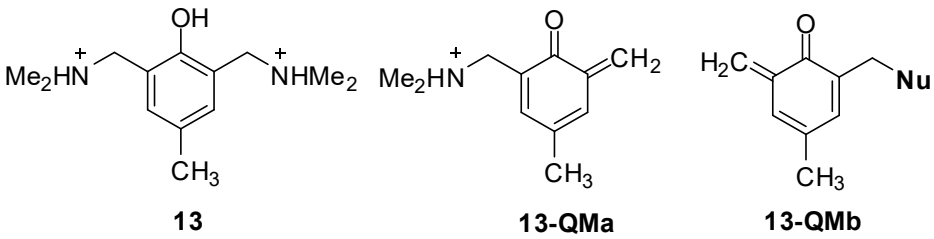


Figure 6. Structure of bifunctional QM precursor **13** and the corresponding QMs.

of QMs to alkylate and cross-link DNA was assayed by investigation of the effects of QMs to DNA denaturing and the treatment of a plasmid DNA with compounds **2a**–**2e** and **7**, and KF, followed by the analysis by alkaline denaturing gel electrophoresis. Both methods did not provide evidence for the DNA cross-linking, in agreement with the MTT test performed on two human cancer cell lines (MCF7 breast adenocarcinoma and SUM159 pleomorphic breast carcinoma), which indicated that **2a**–**2e** or the corresponding QMs did not exhibit significant cytotoxic activity. The lack of DNA reactivity was rationalized by sequential formation of QMs where bifunctional cytotoxic reagent is probably never produced. Instead, the sequential generation of monofunctional QM followed by a faster hydrolysis leads to the destruction of biologically active reagent. The findings described here are particularly important for the rational design of new generation of QM precursor molecules that should have structures that enable long-lived QM species such as 2,6-bifunctional phenols.

EXPERIMENTAL

General

Chemicals for the synthesis were purchased from the usual suppliers, whereas solvents for the synthesis and chromatographic separations were purified by distillation, or used as received (p.a. grade). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV- 300, 400 or 600 MHz. The NMR spectra were taken in $CDCl_3$ or DMSO-d_6 at rt using TMS as a reference. HRMS were obtained on an Applied Biosystems 4800 Plus MALDI TOF/TOF instrument (AB, Foster City, CA). For the sample analysis a Shimadzu HPLC equipped with a Diode-Array detector and a Phenomenex Luna 3u C18(2) column was used. Mobile phase was CH_3OH-H_2O (20 %). For the chromatographic separations silica gel (Merck 0.05-0.2mm) was used. Analytical thin layer chromatography was performed on Polygram® SILG/UV_{254} (Machery-Nagel) plates. In the irradiation experiments, CH_3CN and MeOH were HPLC grade pure, and mQ-H_2O (Millipore) was used. ct-DNA was purchased from Aldrich. Preparation of known precursor molecule **5** is fully described in the ESI.

1-[2-(*tert*-Butyldimethylsilyloxy)phenyl]propane-1-ol (6)

In a two neck flask (25 mL), equipped with a condenser and septum, under inert N_2 atmosphere, Mg (0.12 g, 4.94 mmol), dry THF (2 mL) and a crystal of iodine were added. A small quantity of the solution of bromoethane (0.36 mL, 4.83 mmol) in THF (5 mL) was added dropwise through the septum via a syringe. When the reaction was initiated by heating, the remaining bromoethane solution was added at rt. After the addition was complete, the reaction mixture was heated at the temperature of reflux 1 h, cooled to rt, and a solution of the protected salicylaldehyde (**3**, 0.95 g, 4.01 mmol) in THF (5 mL) was added dropwise. The reaction mixture was stirred at rt 2 h, and then a saturated aqueous solution of NH_4Cl (5 mL) was added, and the mixture was stirred 15 min. The mixture was transferred to a separatory funnel, the layers were separated and the aqueous layer was extracted with ethyl acetate (3×15 mL). The organic extracts were dried over anhydrous $MgSO_4$, filtered, and the solvent was removed on a rotary evaporator. The residue was chromatographed on a column of silica gel using hexane/ethyl acetate (7:3) as eluent to afford the pure product (0.89 g, 84 %) in the form of colorless viscous oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm: 7.36 (dd, 1H, J = 7.6 Hz, J = 1.7 Hz), 7.13 (td, 1H, J = 7.7 Hz, J = 1.9 Hz), 6.95 (td, 1H, J = 7.4 Hz, J = 0.8 Hz), 6.79 (dd, 1H, J = 8.1 Hz, J = 0.9 Hz), 4.95-4.85 (m, 1H), 2.12 (d, 1H, J = 4.5 Hz), 1.84-1.68 (m, 2H), 1.02 (s, 9H), 0.3 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm: 152.5, 134.8, 127.8, 126.8, 121.1, 118.1, 71.0, 30.4, 25.8, 18.2, 10.3, −4.02, −4.1.

1-[2-(*tert*-Butyldimethylsilyloxy)phenyl]prop-1-yl-acetate (7)

A two neck flask (5 mL), equipped with a condenser and septum, under N_2 inert atmosphere, was charged with **6** (0.14 g, 0.52 mmol), ether (2 mL) and pyridine (0.10 mL, 1.24 mmol). The reaction mixture was heated at reflux for 5 min and then cooled to rt. Acetyl chloride (0.09 mL, 1.27 mmol) was added and the mixture was heated at reflux for 3 h. The solvent was removed on a rotary evaporator and the residue was chromatographed on a column of silica gel using hexane/diethyl ether (9:1) as eluent to afford the pure product (0.055 g, 35 %) in the form of colorless viscous oil.

^{1}H NMR ($CDCl_3$, 600 MHz) δ/ppm: 7.28 (dd, 1H, J = 7.6 Hz, J = 1.6 Hz), 7.13 (td, 1H, J = 8.0 Hz, J = 1.5 Hz), 6.93 (td, 1H, J = 7.5 Hz, J = 0.8 Hz), 6.79 (dd, 1H, J = 8.1 Hz, J = 0.9 Hz), 6.09 (t, 1H, J = 6.4 Hz), 2.08 (s, 3H), 1.84–1.77 (m, 2H), 1.03 (s, 9H), 0.89 (t, 3H, J = 7.4 Hz), 0.27 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ/ppm: 170.1, 152.4, 131.6, 128.1, 126.6, 120.9, 118.2, 71.9, 28.5, 25.7, 21.1, 19.4, 9.7, −4.1. HRMS (MALDI TOF/TOF): calcd. for $C_{17}H_{28}O_3Si$ $[M+H]^+$ 309.1886; found 309.1880.

One-pot Synthesis of 7

In a two neck flask (25 mL), equipped with a condenser and septum, under inert N_2 atmosphere, Mg (0.060 g, 2.47 mmol), dry THF (2mL) and a crystal of iodine were added. A small quantity of the solution of bromoethane (0.18 mL, 2.41 mmol) in THF (5 mL) was added dropwise through the septum via a syringe. When the reaction was initiated by heating, the remaining bromoethane solution was added at rt. After the addition was complete, the reaction mixture was heated at the temperature of reflux for 1 h, cooled to rt, and a solution of the protected salicylaldehyde (**3**, 0.47 g, 2.00 mmol,) in THF (3 mL) was added dropwise. The reaction mixture was stirred at rt for 1 h, and then acetyl chloride (0.18 mL, 2.53 mmol) was added and the stirring was continued over 2 h. The solvent was removed on a rotary evaporator and the residue was purified on a column of silica gel using hexane/diethyl ether (9:1) as eluent to afford the pure product (0.36 g, 58 %) in the form of colorless viscous oil.

Preparation of Bisphenol Derivatives 4 - General Procedure

In a two neck flask (50 mL), equipped with a condenser and septum, under inert N_2 atmosphere, Mg (1.5 mmol), dry diethyl ether (1 mL / 1 mmol Mg) and a crystal of iodine were added. A small quantity of the solution of 1,*n*-dibromoalkane (0.6 mmol) in ether (5 mL / 1 mmol) was added dropwise through the septum via a syringe. When the reaction was initiated by heating, the remaining dibromoalkane solution was added at rt or with a moderate heating. After the addition was complete, the reaction mixture was heated at the temperature of reflux for 1 h, cooled to rt, and a solution of the protected salicylaldehyde (**3**, 1 mmol) in dry ether (2.5 mL) was added dropwise. The reaction mixture was stirred at rt for 2 h, and then a saturated aqueous solution of NH_4Cl (5 mL) was added and the mixture was stirred for 15 min. The mixture was transferred to a separatory funnel, the layers were separated and the aqueous layer was extracted with ethyl acetate (3×15 mL). The organic extracts were dried over anhydrous $MgSO_4$, fil-tered, and the solvent was removed on a rotary evaporator. The residue was chromatographed on a column of silica gel using hexane/ethyl acetate (7:3) as eluent.

1,6-Bis[2-(*tert*-butyldimethylsilyloxy)phenyl]hexane-1,6-diol (4a)

According to the general procedure, the Grignard reagent was prepared from 1,4-dibromobutane (0.395 mL, 3.30 mmol) and Mg (0.218 g, 8.97 mmol) in dry ether. In the reaction with the protected aldehyde (**5**, 1.30 g, 5.52 mmol) the product (0.26 g, 48 %) was obtained in the form of colorless viscous oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm: 7.33 (dd, 2H, J = 7.6 Hz, J = 1.4 Hz), 7.12 (td, 2H, J = 8.0 Hz, J = 1.7 Hz), 6.95 (td, 2H, J = 7.5 Hz, J = 0.8 Hz), 6.78 (dd, 2H, J = 8.0 Hz, J = 0.7 Hz), 4.99-4.89 (m, 2H), 2.15 (br s, 2H), 1.80-1.69 (m, 4H), 1.45-1.31 (m, 4H), 1.00 (s, 18H), 0.26 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ / ppm: 152.4, 134.9, 127.9, 126.79, 126.78, 121.2, 118.1, 69.86, 69.81, 37.5, 37.4, 26.2, 26.1, 25.8, 18.2, −4.1; HRMS (MALDI TOF/TOF): calcd for $C_{30}H_{50}O_4Si_2$ $[M+Na]^+$ 553.3145; found 553.3140.

1,7-Bis[2-(*tert*-butyldimethylsilyloxy)phenyl]heptane-1,7-diol (4b)

According to the general procedure, the Grignard reagent was prepared from 1,5-dibromopentane (0.45 mL, 3.33 mmol) and Mg (0.22 g, 8.97 mmol) in dry ether. In the reaction with the protected aldehyde (**5**, 1.30 g, 5.52 mmol) the product (0.26 g, 48 %) was obtained in the form of colorless viscous oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm: 7.34 (dd, 2H, J = 7.5 Hz, J = 1.5 Hz), 7.11 (td, 2H, J = 7.8 Hz, J = 1.7 Hz), 6.94 (dd (t), 2H, J = 7.5 Hz), 6.78 (d, 2H, J = 7.8 Hz), 4.98–4.90 (m, 2H), 2.13-2.11 (m, 2H), 1.76–1.69 (m, 4H), 1.53–1.48 (m, 2H), 1.39–1.29 (m, 4H), 1.01 (s, 18H), 0.26 (s, 12H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ / ppm: 152.4, 134.9, 127.8, 126.7, 121.1, 118.1, 69.7, 37.4, 29.7, 26.0, 25.7, 18.1, −4.1; HRMS (MALDI TOF/TOF): calcd. for $C_{31}H_{55}O_4Si_2$ $[M+Na]^+$ 567.3302; found 567.3309.

1,8-Bis[2-(*tert*-butyldimethylsilyloxy)phenyl]octane-1,8-diol (4c)

According to the general procedure, the Grignard reagent was prepared from 1,6-dibromohexane (0.52 mL, 2.74 mmol) and Mg (0.22 g, 8.97 mmol) in dry ether. In the reaction with the protected aldehyde (**5**, 1.30 g, 5.52 mmol) the product (0.26 g, 48 %) was obtained in the form of colorless viscous oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm: 7.34 (dd, 2H, J = 7.5 Hz, J = 1.6 Hz), 7.12 (td, 2H, J = 8.0 Hz, J = 1.8 Hz), 6.95 (td, 2H, J = 7.5 Hz, J = 0.8 Hz), 6.78 (dd, 2H, J = 8.0 Hz, J = 0.8 Hz), 4.98–4.90 (m, 2H), 2.11 (d, 2H, J = 4.8 Hz), 1.76-1.69 (m, 4H), 1.57–1.47 (m, 2H), 1.36–1.26 (m, 6H), 1.01 (s, 18H), 0.26 (s, 12H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ/ppm: 152.4, 135.0, 127.8, 126.7, 121.1, 118.1, 69.7, 37.5, 29.6, 25.9, 25.7, 18.2, −4.1; HRMS (MALDI TOF/TOF): calcd for $C_{32}H_{54}O_4Si_2$ $[M+Na]^+$ 581.3458; found 581.3469.

Acetylation of 4 - General Procedure

A two neck flask (20 mL), equipped with a condenser and septum, under N_2 inert atmosphere, was charged with **4** (1 mmol), ether (10 mL) and pyridine (4.4 mmol). The reaction mixture was heated at reflux for 5 min and then cooled to rt. Acetyl chloride (4.5 mmol) was added and the mixture was heated at reflux for 30 min. The solvent was removed on a rotary evaporator and the residue was chromatographed on a column of silica gel using hexane/diethyl ether (9:1) as eluent to afford the pure product.

1,6-Bis[2-(*tert*-butyldimethylsilyloxy)phenyl]hexane-1,6-diacetate (2a)

According to the general procedure, from **4a** (0.27 g, 0.50 mmol), acetyl chloride (0.16 g, 2.25 mmol) and pyridine the reaction furnished the product (0.27 g, 87 %) in the form of colorless viscous oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm: 7.24 (dd, 2H, J = 7.3 Hz, J = 1.5 Hz), 7.11 (td, 2H, J = 7.5 Hz, J = 1.6 Hz), 6.91 (td, 2H, J = 6.8 Hz, J = 0.8 Hz), 6.76 (dd, 2H, J = 8.1 Hz, J = 0.6 Hz), 6.09 (t, 2H, J = 6.7 Hz), 2.04 (s, 6H), 1.80-1.69 (m, 4H), 1.42-1.18 (m, 4H), 1.01 (s, 18H), 0.25 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ/ppm: 169.9, 152.3, 131.7, 128.1, 126.5, 120.9, 118.3, 70.9, 35.5, 25.7, 25.5, 21.1, 18.1, −4.1; HRMS (MALDI TOF/TOF): calcd. for $C_{34}H_{54}O_6Si_2$ $[M]^+$ 614.3459; found 614.3429.

1,7-Bis[2-(*tert*-butyldimethylsilyloxy)phenyl]heptane-1,7-diacetate (2b)

According to the general procedure, from **4b** (0.27 g, 0.50 mmol), acetyl chloride (0.16 g, 2.25 mmol) and pyridine the reaction furnished the product (0.16 g, 50 %) in the form of colorless viscous oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm: 7.26 (dd, 2H, J = 7.3 Hz, J = 1.4 Hz), 7.11 (td, 2H, J = 7.9 Hz, J = 1.4 Hz), 6.92 (t, 2H, J = 7.3 Hz), 6.77 (d, 2H, J = 8.1 Hz), 6.09 (t, 2H, J = 6.5 Hz), 2.05 (s, 6H), 1.80-1.68 (m, 4H), 1.37–1.16 (m, 6H), 1.02 (s, 18H), 0.26 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ / ppm: 170.0, 152.3, 131.8, 128.0, 126.5, 120.9, 118.3, 70.9, 35.5, 29.4, 25.7, 25.4, 21.1, 18.1, −4.3; HRMS (MALDI TOF/TOF): calcd. for $C_{35}H_{56}O_6Si_2$ $[M]^+$ 628.3615; found 628.3606.

1,8-Bis[2-(*tert*-butyldimethylsilyloxy)phenyl]octane-1,8-diacetate (2c)

According to the general procedure, from **4c** (0.28 g, 0.50 mmol) and acetyl chloride (0.16 g, 2.25 mmol) and pyridine the reaction furnished the product (0.21 g, 65 %) in the form of colorless viscous oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ / ppm: 7.26 (dd, 2H, J = 7.3 Hz, J = 1.4 Hz), 7.11 (td, 2H, J = 7.9 Hz, J = 1.4 Hz), 6.92 (t, 2H, J = 7.4 Hz), 6.77 (dd, 2H, J = 8.0 Hz, J = 0.5 Hz), 6.10 (t, 2H, J = 6.4 Hz), 2.05 (s, 6H), 1.82–1.68 (m, 4H), 1.31–1.18 (m, 8H), 1.02 (s, 18H), 0.26 (s, 12H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ/ppm: 170.1, 152.3, 131.8, 128.0, 126.5, 120.9, 118.2, 70.9, 35.6, 29.4, 25.7, 25.4, 21.1, 18.1, −4.1; HRMS (MALDI TOF/TOF): calcd. for $C_{36}H_{58}O_6Si_2$ $[M-H]^-$ 641.3694, found 641.3690.

One-pot synthesis of 2 - general procedure

In a two neck flask (50 mL), equipped with a condenser and septum, under inert N_2 atmosphere, Mg (1.3 mmol), dry ether (2mL) and a crystal of iodine were added. A small quantity of the solution of the 1,*n*-dibromoalkane (0.5 mmol) in ether (5 mL) was added dropwise through the septum via a syringe. When the reaction was initiated by heating, the remaining dibromoalkane solution was added at rt. After the addition was complete, the reaction mixture was heated at the temperature of reflux for 1 h, cooled to rt, and a solution of the protected salicylaldehyde (**3**, 1 mmol) in ether (3 mL) was added dropwise. The reaction mixture was stirred at rt for 2 h, and then acetyl chloride (2.2 mmol) was added and the stirring was continued over 2 h. The solvent was removed on a rotary evaporator and the residue was purified on a column of silica gel using hexane/diethyl ether (9:1) as eluent to afford the pure product in the form of colorless viscous oil.

1,8-Bis[2-(*tert*-butyldimethylsilyloxy)phenyl]octane-1,8-diacetate (2c)

According to the general procedure, the Grignard reagent was prepared from 1,6-dibromohexane (0.30 mL, 1.43 mmol) and Mg (0.11 g, 4.48 mmol) in dry ether. In the reaction with the protected aldehyde (**5**, 0.65 g, 2.76 mmol), and subsequently acetyl chloride (0.46 mL, 6.47 mmol) the product (0.43 g, 48 %) was obtained in the form of colorless viscous oil.

1,9-Bis[2-(*tert*-butyldimethylsilyloxy)phenyl]nonane-1,9-diacetate (2d)

According to the general procedure, the Grignard reagent was prepared from 1,7-dibromoheptane (0.08 mL, 0.47 mmol) and Mg (0.04 g, 1.65 mmol) in dry ether. In the reaction with the protected aldehyde (**5**, 0.22 g, 0.93 mmol), and subsequently acetyl chloride (0.13 mL, 1.83 mmol) the product (60 mg, 22 %) was obtained in the form of colorless viscous oil.

^{1}H NMR ($CDCl_3$, 600 MHz) δ/ppm: 7.26 (dd, 2H, J = 7.7 Hz, J = 1.5 Hz), 7.11 (td, 2H, J = 7.6 Hz, J = 1.6 Hz), 6.92 (t, 2H, J = 7.6 Hz), 6.78 (d, 2H, J = 8.1 Hz), 6.11 (t, 2H, J = 6.2 Hz), 2.06 (s, 6H), 1.81-1.69 (m, 4H), 1.37-1.28 (m, 2H), 1.26-1.19 (m, 8H), 1.02 (s, 18H), 0.26 (s, 12H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ / ppm: 170.0, 152.3, 131.9, 128.0, 126.6, 120.9, 118.3 71.0, 35.6, 29.4, 29.3, 25.7, 25.4, 21.1, 18.1, −4.1; HRMS (MALDI TOF/TOF): calcd. for $C_{37}H_{60}O_6Si_2$ $[M]^+$ 656.3928; found 656.3950.

1,10-Bis[2-(*tert*-butyldimethylsilyloxy)phenyl]decane-1,10-diacetate (2e)

According to the general procedure, the Grignard reagent was prepared from 1,8-dibromooctane (0.09 mL, 0.48 mmol) and Mg (0.04 g, 1.72 mmol) in dry ether. In the reaction with the protected aldehyde (**5**, 0.22 g, 0.93 mmol), and subsequently acetyl chloride (0.13 mL, 1.83 mmol) the product (33 mg, 11 %) was obtained in the form of colorless viscous oil.

^{1}H NMR ($CDCl_3$, 600 MHz) δ/ppm: 7.27 (dd, 2H, J = 7.7 Hz, J = 1.6 Hz), 7.11 (td, 2H, J = 7.6 Hz, J = 1.6 Hz), 6.92 (td, 2H, J = 7.4 Hz, J = 0.6 Hz), 6.78 (dd, 2H, J = 8.1 Hz, J = 0.8 Hz), 6.11 (t, 2H, J = 6.6 Hz), 2.06 (s, 6H), 1.82-1.69 (m, 4H), 1.27–1.18 (m, 10H), 1.03 (s, 18H), 0.26 (s, 12H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ/ppm: 170.0, 152.3, 131.9, 128.0, 126.6, 120.9, 118.3, 71.0, 35.6, 29.3, 25.7, 25.4, 21.1, 18.2, −4.3; HRMS (MALDI TOF/TOF): calcd. for $C_{38}H_{62}O_6Si_2$ $[M+Na-H]^-$ 692.3904; found 692.3893.

2-(1-Azido-1-propyl)phenol (8)

A flask (5 mL) was charged with **7** (30 mg, 0.09 mmol), sodium azide (19 mg, 0.29 mmol), acetonitrile (0.6 mL) and H_2O (0.3 mL). To the reaction mixture, a solution of KF (12 mg, 0.21 mmol) in H_2O (0.3 mL) was added dropwise. The reaction mixture was stirred at rt for 12 h, diluted with H_2O (10 mL), and extracted with ethyl acetate (3 × 10 mL). The organic extracts were dried over anhydrous $MgSO_4$, filtered, and the solvent was removed on a rotary evaporator. The residue was chromatographed on a column of silica gel by use of CH_2Cl_2/CH_3OH (9:1) as eluent to afford the pure product (8 mg, 47 %) in the form of colorless viscous oil.

^{1}H NMR ($CDCl_3$, 600 MHz) δ/ppm: 7.19 (td, 1H, J = 8.0 Hz, J = 1.2 Hz), 7.18 (dd, 1H, J = 7.6 Hz, J = 1.3 Hz), 6.91 (td, 1H, J = 7.4 Hz, J = 0.9 Hz), 6.85 (dd, 1H, J = 8.0 Hz, J = 0.9 Hz), 4.62 (t, 1H, J = 6.9 Hz), 1.94–1.81 (m, 2H), 0.97 (t, 3H, J = 7.3 Hz).

Methanolysis of 2c

Bisphenol **2c** (30 mg, 0.05 mmol) was dissolved in methanol (2.0 mL), and a solution of KF (19 mg, 0.33 mmol) in H_2O (0.3 mL) was added dropwise. The mixture was stirred at rt for 1 h, transferred to a separatory funnel, diluted with H_2O (10 mL) and extracted with diethyl ether (3 × 5 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and the solvent was removed on a rotary evaporator. The residue was purified on a column of silica gel using cyclohexane/EtOAc (7:1) as eluent to afford bisphenol **9** (8 mg, 49 %) and **10** (6 mg, 33 %) in the form of colorless viscous oils.

2,2'-(1,8-Dimethoxyoctane-1,8-diyl)diphenol (9): ^{1}H NMR ($CDCl_3$, 600 MHz) δ/ppm: 7.91 (s, 2H), 7.20-7.16 (m, 2H), 6.93-6.90 (m, 2H), 6.87-6.85 (m, 2H), 6.84-6.81 (m, 2H), 4.22 (t, 2H, J = 6.9 Hz), 3.37 (s, 6H), 1.92-1.84 (m, 2H), 1.72-1.63 (m, 2H), 1.43-1.35 (m, 2H), 1.31-1.19 (m, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ/ppm: 155.3 (s, 2C), 128.9 (d, 2C), 128.4 (d, 2C), 125.0 (s, 2C), 119.5 (d, 2C), 116.7 (d, 2C), 86.0 (d, 2C), 57.2 (q, 2C), 35.8 (t, 2C), 29.2 (t, 2C), 25.6 (t, 2C); HRMS (MALDI TOF/TOF): calcd. for $C_{22}H_{30}O_4$ $[M+H]^+$ 359.2222; found 359.2235.

1,8-Bis(2-hydroxyphenyl)-8-methoxyoctyl acetate (10): ^{1}H NMR ($CDCl_3$, 600 MHz) δ/ppm: 7.91 (d, 1H, J = 1.4 Hz), 7.30 (br. s, 1H), 7.27–7.25 (m, 1H), 7.23-7.19 (m, 1H), 7.19-7.15 (m, 1H), 6.94–6.90 (m, 3H), 6.86 (dd, 1H, J = 8.1 Hz, J = 1.0 Hz), 6.82 (tt, 1H, J = 6.3 Hz, J = 1.0 Hz), 5.80 (t, 1H, J = 7.0 Hz), 4.22 (dd (t), 1H, J = 6.9 Hz), 3.37 (d, 3H, J = 0.8 Hz), 2.07 (d, 3H, J = 1.2 Hz), 2.07-2.01 (m, 1H), 1.91-1.81 (m, 2H), 1.72-1.65 (m, 1H), 1.34–1.16 (m, 8H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ/ppm: 172.6 (s), 155.3 (s), 154.6 (s), 129.9 (d), 128.9 (d), 128.4 (d), 127.4 (d), 125.6 (s), 125.0 (s), 120.8 (d), 119.6 (d), 118.0 (d), 116.7 (d), 86.0 (d), 72.1 (d), 57.2 (q), 35.8 (t), 33.5 (t), 29.1 (t), 29.0 (t), 25.8 (t), 25.6 (t), 21.1 (q); HRMS (MALDI TOF/TOF): calcd. for $C_{23}H_{30}O_5$ $[M+H]^+$ 387.2171; found 387.2160.

Alkaline Agarose Gel Assay

Plasmid CMVbeta DNA (2 μg) was mixed with bisphenols **2a–2e** (1 mM) and KF (200 mM) in PBS buffer (pH = 7.4). After 18 h incubation, the samples were added to the alkaline agarose gel loading buffer [50 mM NaOH, 1 mM ethylenediaminetetraacetic acid (EDTA), 3 % Ficoll, and 0.02 % bromophenol blue] and loaded on a 1 % agarose gel. Prior to the loading, the gel was soaked in 50 mM NaOH and 1 mM EDTA for 1h, and the same solution was used as the running buffer. Gels were run at 30 V constant voltage in horizontal electrophoresis system (BIO-RAD, USA) for 5 h. After the run, gels were neutralized with 0.5 M Tris (pH 7) for 30 min, and stained with ethidium bromide (1 μg/mL) for 30 min. Resulting products were visualized and documented with UV light at 254 nm (Image Master VDS, Pharmacia Biotech, Sweden). As a positive control, instead of **2** and KF, the plasmid was treated with psoralene (20 μM) and irradiated in a Luzchem reactor 5 min with 6 lamps (1 lamp 8 W) at 300 nm.

DNA Denaturation Experiments

For the DNA denaturation experiments, a stock solution of compound **2** was prepared in DMSO (c = 2×10^{-2} M), whereas a stock solution of ct-DNA (c = 1.1×10^{-3} M) and a stock solution of KF (c = 0.2 M) were prepared in H_2O-DMSO (8:2), containing sodium cacodylate (c = 0.05 M, pH = 7.0). The cross-linking was attempted by a treatment of DNA solution (20 μL) with compound **2** (10 μL) and KF (20 μL) which were incubated 3 h. This solution (40 μL) was then diluted [(ct-DNA (c = 1.76×10^{-5} M), in the presence of **2** (c = 1.6×10^{-4} M), and KF (0.2 M)] and the dependence of the absorbance at 260 nm as a function of temperature was

measured. The melting temperature T_m values are the mid-points of the transition curves, determined from the maximum of the first derivative. ΔT_m values were calculated by subtracting T_m of the free nucleic acid from that of the respective complex with ΔT_m values are the average of at least two independent measurements and the error in ΔT_m is ca. ± 0.5 °C.

Antiproliferative Investigation

MTT test was conducted on 2 human tumour cell lines, MCF7 (breast adenocarcinoma) and SUM159 (pleomorphic breast carcinoma). MCF7 cells were cultured as monolayers and maintained in DMEM medium, supplemented with 10 % fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin in a humidified atmosphere with 5 % CO_2 at 37 °C. The cell line SUM159 was maintained using the same conditions, but in different cell media: Ham's F12 medium supplemented with 5 % fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin, 5 µg/mL insulin, 1 µg/mL hydrocortisone. Cell lines were inoculated in parallel onto a series of standard 96-well microtiter plates on day 0. The cells were seeded at 2 × 10^4 cells/mL for SUM159 and 3 × 10^4 cells/mL for MCF7, depending on the doubling times of specific cell line. Test agents were then added in five 10-fold dilutions (10^{-8} to 10^{-4} M) and incubated for a further 72 h. For each cell line one of the plates was treated with 1mM KF, concentration that has low impact on cells (see supporting information) while the other was treated with the cell culture medium, 2 h after the addition of the compounds. Suitable concentration of KF was determined in advance by titration of KF using the same cell lines (see the Supporting material, Figure S3). Working dilutions of compounds were freshly prepared on the day of testing. After 72 h of incubation, the cell growth rate was evaluated by performing the MTT assay, as described previously.[22,23] The results were expressed as IC_{50}, a concentration necessary for 50 % of inhibition. The IC_{50} values for each compound were calculated from concentration-response curves using linear regression analysis. Each result is an average value from at least two separate experiments.

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Supplementary Information. Synthetic procedures for the preparation of known intermediates, Investigation of the DNA cross-linking by thermal denaturation, Antiproliferative activity and KF cytotoxicity data, ^{1}H and ^{13}C NMR spectra of all new compounds. Supporting information to the paper is attached to the electronic version of the article at: http://doi.org/10.5562/cca3455.

PDF files with attached documents are best viewed with Adobe Acrobat Reader which is free and can be downloaded from Adobe's web site.

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