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Design, synthesis and cytotoxic activity of water-soluble quinones with dibromo-*p*-benzoquinone cores and amino oligo(ethylene glycol) side chains against MCF-7 breast cancer cells†

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A series of novel quinones was synthesized by reacting tetrabromo-*p*-benzoquinone with amino oligo(ethylene glycol) dendrons of generation numbers $g = 0$ – 2 . According to the performed shake-flask experiments, their aqueous solubility ($S = 18 \text{ mg l}^{-1}$ – 1.6 g ml^{-1}) and partition coefficients ($\log P_{\text{oct/wat}} = 2.53$ – 0.21) can be tuned in a wide range as a function of g . *In vitro* cytotoxicity assays of tetrabromo-*p*-benzoquinone and its derivatives against MCF-7 human breast cancer cells showed a concentration- and generation-specific biological activity with IC_{50} -values as low as $0.8 \text{ }\mu\text{M}$. Further investigations revealed a considerable selectivity against cancer cells, as indicated by a weak cytotoxicity against human skin fibroblast cells ($>80\%$ survival) within the studied range of concentrations. The results demonstrate that these novel amino oligo(ethylene glycol) dendrons depict versatile tools to ameliorate physical and pharmacological characteristics of extremely hydrophobic molecules and make them susceptible to biological applications.

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Introduction

Cancer is a serious global health problem and a leading cause of death in developed countries.^{1,2} Exceeded only by lung cancer, breast cancer is the second most common of all cancers and by far the most frequent cancer amongst women, who bear a lifetime risk of developing breast cancer of roughly one in eight.³

Although substances used as chemotherapeutics encompass a wide range of molecular structures, a significant number of these agents contain quinones, which are broadly considered to be among the most powerful pharmacophores available today.⁴ The molecular mechanisms of quinone cytotoxicity have been extensively reviewed,^{5–8} with oxygen activation by redox cycling and alkylation of essential macromole-

cules likely being the two main modes of action by which quinones induce their cytotoxicity. However, many representatives exhibit merely poor bioavailability and low solubility in aqueous systems, which hampers their systemic administration *in vivo* and reduces their therapeutic value.⁹ As new drug development imposes significant costs¹⁰ and bears an uncertainty of success, increasing efforts have been devoted to enhancing the bioavailability of established hydrophobic drugs through novel formulations.^{11–13}

The concept of polymer prodrug conjugates was initially introduced by Ringsdorf¹⁴ and Kopeček¹⁵ and has gained a pivotal role in modern pharmacology ever since.¹⁶ Important characteristics of polymer drug delivery systems arguably include water solubility, lack of both toxicity and immunogenicity, low polydispersity, ease of excretion from living organisms, as well as the presence of multiple and highly accessible functional handles for drug attachment.¹⁷ However, the inherent polydispersity and statistical drug distribution in ill-defined polymeric carriers generally do not favor systemic applications as they may lead to irreproducible drug release kinetics and transient peaks in drug concentration, which may result in locally unacceptable toxicities.

Owing to their stepwise mode of synthesis, dendrimers offer a level of structural control not attainable with most linear polymers.¹⁸ This way, several key molecular parameters of dendrimers, such as the number and chemistry of peripheral

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functional groups and the overall molecular weight, allow for tailoring their biocompatibility and pharmacokinetics to specific demands. Unsurprisingly, dendrimers have been considered to be promising candidates for a variety of biomedical applications ranging from biological imaging¹⁹ and artificial enzyme mimics²⁰ to nanocarriers for targeted drug delivery.²¹ In the latter case, the drug molecules are either non-covalently encapsulated in the interior of dendrimers or covalently conjugated to form macromolecular prodrugs. Conjugation of hydrophilic, dendritic moieties to poorly water-soluble drugs has been demonstrated to be a valuable strategy for endowing such compounds with sufficient water solubility for systemic administration and to improve their pharmacological and/or pharmacokinetic properties.^{22–25}

In the course of a recent study on thermoresponsive dendrimers derived from water-insoluble 2,3,5,6-tetrabromohydroquinone (TBHQ) and hydrophilizing oligo(ethylene glycol)-containing dendrons, we found that such dendrimers show *in vitro* cytotoxicity against MCF-7 breast cancer cells with IC₅₀ values as low as 0.6 μM.²⁶ Motivated by the promising characteristics of these hydroquinones, we extended our investigations to the effects of chemical structure, dendron generation number and mode of dendron attachment on the biological properties of tetrabromo-*p*-benzoquinone (bromanil, TBBQ).

We herein report on the design and synthesis of three amino oligo(ethylene glycol)s (amino-OEGs) of generation numbers *g* = 0–2, which were subsequently attached to bromanil in order to obtain novel water-soluble dibromo-*p*-benzoquinones (DB-OEG conjugates). These molecules differ only in the number of appendices around the quinone core, which makes it possible to tailor their hydrophilic–lipophilic balance and elucidate structure–toxicity relationships. More specifically, their water solubility and partitioning between water and octan-1-ol was determined by the “shake-flask” method and the *in vitro* cytotoxicity of these compounds against MCF-7 breast cancer cells and human skin fibroblast cells was investigated using the WST-1 cell viability assay.

Results and discussion

Bromanil is only sparsely soluble in water (*vide infra*), which limits its applicability in biological applications. However, its reactivity towards amines is well established,^{27,28} which offers the possibility for conjugation with water-solubilizing moieties. The herein chosen oligo(ethylene glycol)-derived linear and dendritic appendices possess certain desirable features, including solubility in a large variety of solvents and non-charged chemical structures under a wide range of pH conditions. Due to the multitude of readily available hydroxyl-terminated oligo(ethylene glycol)s, the amines reported herein were derived from their corresponding alcohols *via* their respective azide intermediates.

All synthetic procedures are located in the experimental section and further characterization data is provided in the ESI.†

Synthesis of amino-OEG dendrons

Linear amine 2 was prepared in two steps from commercially available diethylene glycol monomethyl ether (DEGME) (Scheme 1).²⁹ The novel first- and second-generation amino oligo(ethylene glycol) dendrons 5 and 9 were derived from their hydroxyl analogues 3 and 7,³⁰ whereby the synthetic procedures reported herein feature several improvements in terms of efficacy and scale. The synthetic route towards the first-generation dendron 5 is delineated in Scheme 2. Alcohol 3 was readily obtained from 2-methoxyethanol (EGME) and epichlorohydrin (ECH) under aqueous, basic conditions after separation from excess EGME and higher-generation homologues by fractionation under reduced pressure (55–68% yield). Subsequently, mesylation of alcohol 3 and nucleophilic displacement by sodium azide afforded the corresponding azide 4 in a two-step reaction (86% yield). Finally, amine 5 was obtained quantitatively by reduction of azide 4 under an atmosphere of hydrogen in the presence of palladium on carbon (10 wt% loading). The synthetic route towards the second-generation dendron 9 is delineated in Scheme 3. Initial attempts to prepare alcohol 7 from ECH and 3 analogously to the procedure applied in the synthesis of the first-generation dendron gave rise to various by-products. Partial and complete generations were obtained despite employing a two-step procedure under anhydrous conditions involving first the nucleophilic displacement of the chloride in ECH by a stoichiometric amount of alcohol 3, followed by ring opening of the epoxide by a second equivalent of 3. However, the yields could be significantly improved by exploiting the double bond in 3-chloro-2-chloromethyl-1-propene (MDC), which prevents the formation of higher generations. In addition, the isolation of compound 6 from the crude product mixture could be facilitated by removing excess starting alcohol 3 *in vacuo* first and subjecting the remainder to simple column chromatography (67% yield). Ozonolysis of the unsaturated compound 6 followed by reductive workup using sodium borohydride afforded the secondary alcohol 7 in quantitative yield. Azide 8 and amine 9 were subsequently obtained in analogy to compounds 4 and 5 and in high yields (90% and 73%, respectively).

Synthesis of DB-OEG conjugates

Bromanil³¹ was reacted with amines 2, 5 and 9 as delineated in Scheme 4. Reactions of this type have been shown to proceed *via* 1,4-addition of the amine to the quinone in a Michael fashion, followed by rearrangement to the substituted hydroquinone and oxidation by a second quinone molecule.^{27,28} However, the use of excess quinone can be avoided (while preserving the quinoid structure in the final product)

Scheme 1 Reagents and conditions: a) 1. MsCl, Et₃N, CH₂Cl₂, RT; 2. NaN₃, DMF, 120 °C; b) Ph₃P, THF, RT; 2. H₂O, RT.

Scheme 2 Reagents and conditions: a) NaOH, 80 °C; b) 1. MsCl, Et₃N, CH₂Cl₂, RT; 2. NaN₃, DMF, 120 °C; c) H₂, Pd/C, MeOH, RT.

Scheme 3 Reagents and conditions: a) NaH, KI, THF, 65 °C; b) 1. O₃, -78 °C, CH₂Cl₂/MeOH; 2. NaBH₄, RT; c) MsCl, Et₃N, CH₂Cl₂, RT; 2. NaN₃, DMF, 120 °C; d) H₂, Pd/C, MeOH, RT.

by performing the reaction in the presence of oxygen.³² The claimed regioselectivity is further substantiated by the ¹³C NMR spectra of compounds **D0–D2**, which feature three signals at approximately 172, 146, and 89 ppm each, corresponding to three chemically non-equivalent carbon atoms in the quinone ring (*cf.* Fig. S16, S18, and S20 of the ESI†). In this regard, amination adjacent to an electron-donating substituent such as an amino group to give the 1,2-substituted product is unfavorable. Taking our own experimental observations and the literature^{27,28,32} together, it is reasonable to assume that the regioselectivity of **D0–D2** is as given in Scheme 4. Compound **D0** has two linear appendices ($g = 0$), whereas compounds **D1** and **D2** can be considered as dendrimers of generation numbers $g = 1$ and $g = 2$, respectively. All DB-OEG conjugates are stable for at least 5 months when stored at -20 °C under an atmosphere of nitrogen and soluble in water as well as in a plethora of organic solvents including dimethyl sulfoxide, *N,N*-dimethylformamide, 1,4-dioxane, chloroform and methylene

Scheme 4 Reagents and conditions: a) Et₃N, EtOH, 65 °C.

chloride. A key structural feature of these molecules is that their hydrophilic–lipophilic balance can be systematically tuned by the generation of the oligo(ethylene glycol) appendices (*vide infra*). The physical appearance of the synthesized DB-OEG conjugates ranges from an amorphous powder (**D0**) and a sticky solid (**D1**) to a viscous liquid (**D2**). In all cases, the synthetic protocols described herein gave products with purities exceeding 97% (**D0**: 98.4%; **D1**: 97.4%; **D2**: 98.7%) as established by a combination of TLC, elemental analysis and NMR spectroscopy.

Water solubility and partitioning of DB-OEG conjugates

In order to assess the bio-intake of the synthesized drugs, their water solubility and lipophilicity was determined by the well-established “shake-flask” method and UV-vis spectrophotometric quantification.³³ The UV-vis spectra of **D0–D2** and bromanil in water (Fig. S1) as well as the calibration curves used for their quantification (Fig. S2–S5) are provided in the ESI† Detailed experimental procedures are given in the experimental section and the obtained results are summarized in Table 1. Compared to unfunctionalized bromanil ($\lambda_{\text{max}} = 309$ nm), the corresponding absorption maxima of **D0–D2** are red-shifted by approximately 50 nm due to the electron-donating influence of the amino-OEG dendrons, which are directly conjugated to the quinoid system. The influence of the dendron generation on the location of the corresponding absorption maxima is small albeit noticeable, with an additional red-shift of 5 nm upon the transition from $g = 0$ in **D0** ($\lambda_{\text{max}} = 359$ nm) to $g = 2$ in **D2** ($\lambda_{\text{max}} = 364$ nm). This is likely to originate from an increased inductive effect and/or electrostatic interactions between the quinone moiety

Table 1 Cytotoxicity, water solubility and partition coefficients of bromanil and amino-OEG derivatives. The reported quantities are apparent average values and standard errors from replicate determinations ($N = 3$) are indicated

Entry	Compound	IC ₅₀ ^a [μM]	UV-vis		Aqueous solubility ^b		Partition coefficients	
			ϵ [L mol ⁻¹ cm ⁻¹]	$\lambda_{\max}$ [nm]	[mg L ⁻¹]	[mol L ⁻¹]	log P_{calc} ^c	log P_{exp} ^d
1	TBBQ	>100	3069.9	309	18 ± 13	5.43×10^{-5}	2.57	2.53
2	D0	11.8	18 007.4	359	220 ± 25	4.40×10^{-4}	1.27	1.38
3	D1	0.8	23 553.7	363	1720 ± 90	2.28×10^{-3}	0.62	0.67
4	D2	>100	24 655.2	364	$>1.6 \times 10^5$	>1.33	-1.40	0.21

^a Determined using the WST-1 assay. ^b Determined by the shake-flask method at 25 °C and UV-vis spectrophotometric quantification.

^c Predictions from ChemDraw Molecular Networks plugin. ^d Determined by partitioning between octan-1-ol/water at 25 °C and UV-vis spectrophotometric quantification.

and backfolded appendices creating a more polar microenvironment.

The solubility of bromanil, D0 and D1 in water was determined from saturated solutions at 25 °C. In the case of D2, a saturated solution could not be prepared due to the full miscibility of this viscous liquid in water. Whereas bromanil is only sparsely soluble in water (*ca.* 18 mg l⁻¹), the solubility of D0–D2 is significantly enhanced by increasing the dendron generation from $g = 0$ in D0 (*ca.* 220 mg l⁻¹) and $g = 1$ in D1 (*ca.* 1.7 g l⁻¹) to $g = 2$ in D2 (>1.6 g ml⁻¹).

The lipophilicity of the quinones was determined from their distribution ratio between layers of octan-1-ol and water ($P_{\text{oct/wat}} = c_{\text{oct}}/c_{\text{wat}}$). Following the procedure described in detail in the experimental section, the relative lipophilicity order calculated *in silico* is in agreement with the results obtained from the experiments (log P TBBQ > D0 > D1 > D2). Except for D2, the log P values obtained by both methods are comparable and mostly within a narrow range. Interestingly, doubling the number of ethylene glycol chains around the core effects a bisection of the experimentally determined log P values in a first approximation. The higher than predicted log P value of D2 may be explained by the pronounced amphiphilic character of this compound and a dendritic effect, which could potentially lead to the formation of micro-emulsions and underestimation of substance in the aqueous phase. Based on the estimate that drug-like log P values range between $0.5 < \log P < 3.5$,³⁴ the experimentally determined partition coefficients of the three DB-OEG conjugates suggest that both D0 and D1 might qualify as potential drug candidates due to their favorable hydrophilic–lipophilic balance, whereas the cellular uptake of bromanil (log $P = 2.53$) and D2 (log $P = 0.21$) may be compromised by their poor aqueous solubility and enhanced hydrophilicity, respectively (*vide infra*).

Cytotoxicity of DB-OEG conjugates

Chloranil and *p*-benzoquinone have been shown to induce DNA damage by Michael adduct formation and oxidative stress.^{35–37} In light of the high degree of structural similarity between these particular quinones and the synthesized DB-OEG conjugates, it is reasonable to assume a shared mode of action. Hence, a dedicated investigation of the underlying mechanism was outside the scope of the present study. Nev-

ertheless, we attempt to rationalize the observed structure–toxicity relationships based on their chemical structures, experimentally determined water solubility and partitioning behavior.

Fig. 1(a) illustrates the MCF-7 cell viabilities after 24 h exposure to different drug concentrations and a drug-free recovery time of 72 h (dose–response curves), which were determined by using the WST-1 colorimetric assay.³⁸ In the case of bromanil, a slight decrease in MCF-7 cell viability of up to

Fig. 1 (a) Dose–response curve of compounds bromanil, D0, D1 and D2 against MCF-7 breast cancer solid tumor cells. (b) Dose–response curve of compounds bromanil and D0 against human skin fibroblast cells.

20% is observable at low drug concentrations (0.1–0.5 μM) but higher concentrations do not reduce the cell viability further. In contrast, two of the tested DB-OEGs show significant cytotoxic effects, *i.e.* **D1** features the lowest IC_{50} value (most potent) of 0.8 μM and a resistant fraction of 32.2% (adj. $R^2 = 0.99$, $p = 0$) and **D0** exhibits an IC_{50} of 11.8 μM and a resistant fraction of 31.6% (adj. $R^2 = 0.92$, $p = 1.9 \times 10^{-4}$). Compound **D2** is significantly less potent against MCF-7 cells with an $\text{IC}_{50} > 100 \mu\text{M}$ and a resistant fraction of 49.7%. To put these values into perspective, under comparable conditions, *i.e.* using MCF-7 cells *in vitro* and the same durations of drug exposure and recovery, the IC_{50} of **D1** represents a seven-fold improvement in potency relative to the well-known cytotoxic compound cisplatin ($\text{IC}_{50} = 5.8 \mu\text{M}$).³⁹ On the other hand, doxorubicin (Adriamycin®), which is one of the primary anthracycline chemotherapeutics used for the treatment of breast cancer, reportedly exhibits an even higher activity against MCF-7 cells ($\text{IC}_{50} = 0.1 \mu\text{M}$) after just 2 h of incubation.⁴⁰ Compared to the previously reported hydroquinone-derived 1,4-dialkoxybenzene dendrimers,²⁶ the herein presented quinones require three-fold shorter drug exposure times in order to arrive at similar half maximal inhibitory concentrations. This could be attributed to the much larger sizes and higher molecular weights of the previously used dendrimers, which presumably slowed down their passive diffusion through the membrane and restricted their biological activity. Also, these compounds need to undergo extensive enzymatic metabolism, *i.e.* O-dealkylation and oxidative activation, in order to generate cytotoxic species.⁴¹

In light of several comprehensive studies addressing the influence of dendrimer end groups on cell toxicity in general^{42–44} and the known biocompatibility of dendritic polyglycerols in particular,⁴⁵ it is reasonable to assume that the present oligo(ethylene glycol) appendices are well-tolerated and neither disrupt nor impact membrane permeability. Hence, the transport of these relatively small molecules across biological membranes proceeds mostly *via* simple or facilitated diffusion down the concentration gradient and at a rate related to their lipophilicity.⁴⁶ Seminal work by Lipinski and coworkers established that permeability within a series diminishes with increasing polarity.⁴⁷ Following this line of argument, the second-generation OEG-dendrons surrounding the quinone core in compound **D2** apparently provide enough hydrophilicity to prevent efficient cellular uptake. In addition, the red-shifted absorption maximum suggests steric shielding of the quinone core, which may impede the interaction and covalent binding to important macromolecules such as proteins or DNA in the cell. On the other hand, compound **D0** bearing only two linear appendices features the lowest water solubility (highest lipophilicity) of the series, which hampers its bioavailability as well. Evidently, compound **D1** exhibits the highest cytotoxic performance due to the accessibility of its quinone core and the most favorable hydrophilic–lipophilic balance of the compounds investigated. The seemingly paradoxical data obtained with bromanil, *i.e.* an arguably higher reactivity due

to its sterically less encumbered structure and higher electrophilicity yet lower cytotoxicity, could either result from the low bioavailability of this compound or its rapid detoxification with a greater variety of nucleophiles, including those abundant in the cell culture medium (FBS) and the well-known cellular antioxidant glutathione (GSH).³⁸

Since one major goal of cancer therapy is the selective targeting of cancer cells over normal cells, bromanil and **D0** were exemplarily used in further studies with human skin fibroblast cells in order to investigate a potential selectivity of these compounds for cancer cells. As apparent from the dose–response curves shown in Fig. 1(b), both bromanil and **D0** show only weak cytotoxicity over the studied range of concentrations. Whereas the low activity of bromanil is in line with the results obtained with the MCF-7 cells, the significantly reduced cytotoxicity of **D0** against fibroblast cells is remarkable as the bioavailability and potency of this compound against MCF-7 cells has been clearly demonstrated (*cf.* Fig. 1(a)). Although a more detailed investigation of the cellular targets of the herein presented DB-OEG conjugates remains subject to future studies, a potential explanation for the observed selectivity could be based on the frequently observed differences in enzyme expression patterns between normal tissues and solid tumors.⁴⁸ By way of example, the enzyme NAD(P)H:quinone oxidoreductase 1 (NQO1) is typically sparsely expressed in normal tissue but it has been found to be greatly overexpressed in most solid tumors, including human breast carcinoma.⁴⁹ NQO1 is a 2-electron reductase responsible for the detoxification of a variety of xenobiotics, including quinones to give (more) stable hydroquinones.⁴⁸ However, certain hydroquinones are not stable and undergo further chemistry such as redox cycling, *i.e.* rapid reaction with molecular oxygen in the cell to give two moles of superoxide and regenerate the quinone. Such bio-reductive agents, which are activated upon enzymatic reduction and are capable of rapidly and catalytically generating large quantities of toxic ROS in the corresponding enzyme-overexpressing cells only, hold considerable potential as antitumor drugs with improved therapeutic indices.⁴⁸

Conclusions

In summary, this study describes the design, synthesis and biological evaluation of three novel tetrabromo-*p*-benzoquinone-derived compounds (**D0–D2**) bearing amino oligo(ethylene glycol) appendices of generation numbers $g = 0–2$. The amino-OEGs are prepared from their corresponding and readily accessible alcohols *via* azide intermediates and obtained in high yields. Owing to their efficient synthesis and ample reactivity, the herein presented amino-OEGs depict viable and predictable means to endow highly hydrophobic molecules with sufficient hydrophilicity for use in aqueous applications and, at the same time, tailor their hydrophilic–lipophilic balance. *In vitro* cytotoxicity studies using **D0–D2** against MCF-7 human breast cancer cells reveal a concentration- and generation-specific activity, which can

be ascribed to the differences in bioavailability and steric hindrance between the respective compounds. Notably, the minor cytotoxicity determined against normal human skin fibroblast cells suggests that D0–D2 might be bio-reductive antitumor agents capable of selectively targeting cancer cells while leaving healthy cells intact. Given their synthetic accessibility, tunable pharmacokinetic properties and biological activity, they may hold considerable potential for future developments in the field of personalized medicine.

Experimental section

Materials and methods

All starting materials were purchased from commercial sources and used as received: hydroquinone (Sigma-Aldrich, >99%), 2-methoxyethanol (EGME) (Sigma-Aldrich, >99%), palladium on carbon (Sigma-Aldrich, 10 wt%), triphenylphosphine (ABCR, 99%), bromine (Acros Organics, >99%), methanesulfonyl chloride (Acros Organics, >99%), sodium hydride (Acros Organics, 60% dispersion in mineral oil), 2-(2-methoxyethoxy)ethanol (DEGME) (Acros Organics, 99%), sodium hydroxide (Fisher Chemicals, pellets), nitric acid (Fisher Chemicals, 65%), glacial acetic acid (Fisher Chemicals, 99%), 3-chloro-2-chloromethyl-1-propene (MDC) (TCI, >98%), potassium iodide (VWR International, 99%), sodium borohydride (Merck Millipore, 99%), sodium azide (Fluka, 99%) and (±)-epichlorohydrin (ECH) (Fluka, 99%), octan-1-ol (Sigma-Aldrich, >99%). Milli-Q water (Merck Millipore, resistivity 18.2 MΩ cm) was used in the experimental determination of water solubility and partition coefficients. Ozone was generated with a MP-8000 multi-purpose ozone generator from A2Z Ozone. Unless stated otherwise, all reactions were carried out in dried Schlenk glassware, in anhydrous solvents and under nitrogen atmosphere. Anhydrous solvents were of analytical grade and obtained from a LC Technology Solutions solvent purification system. Chromatography solvents were purchased as technical grade and distilled once prior to use. The reactions were monitored by thin-layer chromatography (TLC) carried out on pre-coated aluminum sheets (silica gel 60G/UV254, 0.20 mm, Macherey-Nagel). UV-light (254 nm) or VSS staining was used for detection. Column chromatography was conducted on silica gel (60 Å, 230–400 mesh particle size, Fluka) as the stationary phase. ¹H and ¹³C NMR spectra were recorded on a Bruker Avance 300 spectrometer at 300 MHz and 76 MHz, respectively. Chemical shifts are reported as δ values (ppm) relative to residual solvents as an internal standard (CDCl₃: 7.26 ppm for ¹H and 77.0 ppm for ¹³C; [D₆]DMSO: 2.50 ppm for ¹H and 40.0 ppm for ¹³C). ¹H multiplicities are designated by the following abbreviations: s = singlet, d = doublet, t = triplet, m = multiplet, b = broad. UV-vis spectroscopy was performed on a JASCO J670 spectrophotometer equipped with a thermally regulated cell holding quartz cuvettes with path lengths of 1 cm. Fourier transform infrared (FT-IR) spectra were recorded on a Thermo Fisher Scientific Nicolet 6700 spectrometer by using the KBr pellet technique, at Assiut University. High-

resolution ESI-Qq-TOF-MS (Bruker maXis; solvent, DCM/MeOH; ion polarity, positive; set capillary, 4500.0 V) and ESI/MALDI-FTICR-MS (Bruker solariX 94; matrix, THA; solvent, DCM/MeOH); ion polarity, positive; set capillary, 4500.0 V) analyses were performed by the Service for Mass Spectrometry of the Laboratory of Organic Chemistry (LOC) at ETH Zurich. Elemental analysis was carried out on a Perkin-Elmer EA 240 by the Micro-Laboratory of the LOC at ETH Zurich after drying of samples in high vacuum (HV) to constant weight. Compound purities were calculated by comparing the theoretically expected and experimentally determined CHN contents first and averaging the purities obtained for each element afterwards.

Prediction of molecular lipophilicity

Depending on the particular log *P* of a compound, the *V*_{oct}/*V*_{wat}-ratio in the shake-flask procedure has to be chosen carefully in order to ensure that an adequate amount of analyte remains in the aqueous phase for spectrophotometric quantification.⁵⁰ To this end, computational estimates of log *P* were obtained for bromanil and the synthesized drugs using ChemBio3D Ultra, version 14.0 (CambridgeSoft, PerkinElmer Inc.). The chemical structures were drawn using ChemDraw and exported to the ChemBio3D environment, where energy minimizations using the MM2 Force Field method were performed. Properties were subsequently predicted using ChemDraw's Molecular Networks plugin (Molecular Networks GmbH, Germany), since the log *P* value for bromanil derived this way is very similar to its experimentally determined and published value.⁵¹

Shake-flask procedures for the determination of water solubility and partition coefficients (log *P*)

All shake-flask experiments were performed in glass vials sealed with Teflon caps on a thermally regulated mechanical shaker at 25 °C. Spectrophotometric measurements were conducted using quartz cuvettes and in a thermally regulated cell at 25 °C. The experiments were run in triplicate and the given error bars refer to the standard deviation of the measurements. Calibration curves were constructed by dissolving a defined drug amount in water, followed by incremental dilution and spectrophotometric determination of the resulting absorption values at the absorption maxima of the respective quinones (TBBQ, A₃₃₀; D0, A₃₅₉; D1, A₃₆₃; D2, A₃₆₄).

The water solubility of the compounds was experimentally determined by placing approximately 3 mL of Milli-Q water in a glass vial, followed by addition of a surplus of sample. The resulting suspension was placed sideways on a mechanical shaker and agitated at 25 °C for 48 h, followed by sedimentation for 12 h. Saturated solutions were obtained by filtration of the suspensions through 0.2 μm syringe filters prior to adequate dilution (1:50–1:200, v/v) for UV-vis spectroscopy. The concentrations of the saturated solutions were counted back from the measured concentrations and the applied dilution factors.

The $\log P$ values were experimentally determined in partitioning experiments using octan-1-ol and Milli-Q water. Since the chemical structures of bromanil and D0–2 do not readily form ionized species under neutral pH conditions (which would be detrimental to the accuracy of the method) all UV-vis spectrophotometric quantifications and the partitioning experiments were carried out without adding any buffer to the aqueous phase. Although the solubility of octan-1-ol in water is very low (7.5×10^{-5} mole fraction), the one of water in octan-1-ol is fairly high (0.2705 mole fraction), which makes it necessary to mutually saturate both phases prior to their use in the partitioning experiments.^{52,53} Therefore, both solvents were heavily shaken in a separating funnel and the layers were allowed to separate at room temperature (25 °C) overnight. The experiments were performed using octan-1-ol/water-ratios of 1:1 (v/v) and sample concentrations of approximately 0.1 mM in order to arrive at absorbance values in the aqueous phases ranging from 0.1 to 0.9 after partitioning. In the case of D2, an octan-1-ol/water-ratio of 10:1 (v/v) was chosen to account for the high hydrophilicity of this compound, as predicted by the respective calculation. A typical partitioning experiment included the preparation of an aqueous sample solution ($c \approx 0.1$ mM) and the spectrophotometric determination of its precise concentration (c_0). 3 mL of sample solution (0.3 mL in the case of D2) and 3 mL of water-saturated octan-1-ol were pipetted into a glass vial that was sealed with a Teflon cap. The vial was placed sideways on a mechanical shaker and the layers were mixed for 30 min. Thereafter, centrifugation at 1500 rpm for 30 min afforded complete phase separation and the octanol layer was removed using a syringe. Given that the volume and volume-ratio of both phases and the total amount of substance in the flask remain constant throughout the experiment, the analyte's concentration before and after partitioning can be determined spectrophotometrically in the aqueous phase only (c_{wat}) and the concentration in the octanol phase is obtained by difference ($c_{\text{oct}} = c_0 - c_{\text{wat}}$).^{54,55} Thus, the partition coefficient ($P_{\text{oct/wat}}$) was calculated according to eqn (1):⁵⁴

$$P_{\text{oct/wat}} = \frac{C_0 - C_{\text{wat}}}{C_{\text{wat}}} \cdot \frac{V_{\text{wat}}}{V_{\text{oct}}} \quad (1)$$

Cell culture and cytotoxicity assays

Human breast adenocarcinoma cell line MCF-7 was purchased from the American Type Culture Collection (ATCC, Germany) and cultured in RPMI 1640 medium supplemented with 10% FBS, streptomycin (100 $\mu\text{g mL}^{-1}$), penicillin (100 U mL^{-1}). Cell cultures were incubated in a humidified incubator of 5% CO_2 at 37 °C and the culture medium was changed every 2–3 days. Exponentially growing cells were seeded in 96-well plates at a density of 2×10^4 cells per well and allowed to settle overnight in 5% CO_2 at 37 °C. The medium was

discarded and was replaced with a fresh one containing different final concentrations (0.1, 1, 10, 25, 50, 75 and 100 μM) of bromanil, D0, D1, or D2. Three wells were used for each concentration of every compound, and incubation was continued for 24 h. A control group incubated in fresh medium was included. At the end of the incubation period, the cells were rinsed with PBS and treated with 10 μL of the cell proliferation assay reagent WST-1 (Sigma-Aldrich) for 2 h. Cleavage of the stable tetrazolium salt WST-1 into a formazan is mediated by the mitochondrial succinate-tetrazolium reductase enzyme, which is mostly dependent on the glycolytic NAD(P)H production of viable cells.³⁸ The amount of formazan dye formed is directly proportional to the number of metabolically active cells in the culture and was followed spectrophotometrically by monitoring the decrease of absorption at $\lambda = 450$ nm using a FLUOstar Omega microplate reader (BMG Labtech, Germany) at Zewail City of Science and Technology, Giza, Egypt.

Determination of IC_{50} values

For each drug, the half-maximal inhibitory concentration (IC_{50}) was calculated by plotting the concentration against the percentage inhibition and fitting the dose-response curve in OriginPro v8 (OriginLab, MA, USA) using non-linear curve fitting with variable hill slope according to eqn (2):

$$Y = A_1 + \frac{A_2 + A_1}{1 + 10^{(\log x_0 - x)S}} \quad (2)$$

where A_1 and A_2 are the bottom and top asymptotes (A_2 fixed at 100%), x is the logarithm of dose and S is the hill slope. $\log x_0$ is the center of the curve that is used to calculate the concentration for half response according to eqn (3):

$$\text{IC}_{50} = 10^{\log x_0} \quad (3)$$

The resistant fraction was calculated based on the computed A_1 value following dose-response curve fitting.

Monomers syntheses

General procedure A for the synthesis of azides from alcohols. Caution: The stability of organic azides is strongly dependent on the carbon-to-nitrogen ratio in the substrate;^{56,57} although no explosions were encountered during the work described below, great care should be taken when carrying out similar reactions.

The alcohol and Et_3N (1.1 equiv.) were dissolved in Et_2O or toluene and the solution was cooled to -10 °C. Methanesulfonyl chloride (1.1 equiv.) was added drop wise to the solution in the cold before the reaction was allowed to reach room temperature. After TLC confirmed the consumption of the alcohol, the mixture was filtered and concentrated *in vacuo* at 40 °C. The residue was re-dissolved in DCM, washed once with water, dried over MgSO_4 , and concentrated *in vacuo* at 40 °C. The mesylated crude product was obtained

quantitatively and used in the following reaction step without further purification. The mesylate was dissolved in DMF and sodium azide (2.5–5.0 equiv.) was added at room temperature. The mixture was stirred at 120 °C for 3–14 h. After cooling to room temperature, the resulting precipitate was filtered off, and the volatiles were removed *in vacuo* at 40 °C. The crude product was purified by column chromatography.

General procedure B for the hydrogenation of secondary azides to amines. A solution of azide in methanol ($c = 0.18 \text{ mol l}^{-1}$) was treated with a catalytic amount of Pd/C (10 wt% on activated carbon). The mixture was stirred under an atmosphere of hydrogen for 12–40 h at room temperature. The catalyst was removed by filtration through a short pad of Celite. The volatiles were removed *in vacuo* at 40 °C. The amine was obtained quantitatively with no further purification required.

Tetrabromo-*p*-benzoquinone (bromanil). Hydroquinone (11.0 g, 100 mmol) was dissolved in a solvent mixture of glacial acetic acid (100 ml) and methanol (25 ml). Liquid bromine (50 g) was added drop wise over a period of 90 min. Excess bromine was liberated by slight warming in a water bath in the presence of 10 ml nitric acid. The resulting yellow precipitate was filtered off and washed several times with water. Recrystallization from 1,4-dioxane gave the title compound (88%) as yellow plates: mp: 298 °C.

1-Azido-2-(2-methoxyethoxy)ethane (1). Following general procedure A, 2-(2-methoxyethoxy)ethanol (49.4 ml, 415 mmol) and Et₃N (60.4 ml, 436 mmol) were dissolved in Et₂O (500 ml). Methanesulfonyl chloride (33.7 ml, 436 mmol) in Et₂O (50 ml) was added and the reaction was stirred for 1 h. Mesylate: ¹H NMR (300 MHz, CDCl₃) $\delta = 4.41\text{--}4.31$ (m, 2H, CH₂), 3.79–3.69 (m, 2H, CH₂), 3.69–3.59 (m, 2H, CH₂), 3.57–3.47 (m, 2H, CH₂), 3.35 (s, 3H, CH₃), 3.04 (s, 3H, CH₃); ¹³C NMR (76 MHz, CDCl₃) $\delta = 71.7, 70.5, 69.1, 69.0, 59.0, 37.6$; ESI-TOF m/z [M + H]⁺ calcd for C₆H₁₅O₅S: 199.0635, found: 199.0636. The mesylate (24.2 g, 0.12 mol) and sodium azide (19.9 g, 0.31 mol) in DMF (200 ml) were stirred at 120 °C for 3 h. Column chromatography on silica gel (hexane/EtOAc 1 : 2) gave the title compound (15.0 g, 85%) as a colorless liquid: $R_f = 0.73$ (hexane/EtOAc 1 : 2); ¹H NMR (300 MHz, CDCl₃) $\delta = 3.67\text{--}3.61$ (m, 4H, CH₂), 3.56–3.52 (m, 2H, CH₂), 3.37 (m, 5H, CH₂, CH₃); ¹³C NMR (76 MHz, CDCl₃) $\delta = 71.9, 70.5, 69.9, 59.0, 50.6$; ESI-TOF m/z [M + H]⁺ calcd for C₅H₁₂N₃O₂: 146.0924, found: 146.0925.

2-(2-Methoxyethoxy)ethanamine (2). Azide 1 (10.0 g, 68.9 mmol) was dissolved in THF (80 ml). Ph₃P (19.4 g, 73.8 mmol) was added and the mixture was vigorously stirred overnight. Water (110 ml) was added to the colorless solution and stirring was continued for 24 h. The resulting turbid white mixture was washed with toluene (1 × 200 ml) and CH₂Cl₂ (1 × 200 ml). The aqueous phase was taken to dryness to give the title compound (5.38 g, 66%) as a colorless liquid, which was used in the next step without further purification: ¹H NMR (300 MHz, CDCl₃) $\delta = 3.59\text{--}3.57$ (m, 2H, CH₂), 3.56–3.49 (m, 2H, CH₂), 3.47 (t, $J = 5.2 \text{ Hz}$, 2H, CH₂), 3.35 (s, 3H, CH₃), 2.84 (t, $J = 5.2 \text{ Hz}$, 2H, CH₂), 1.81 (s, 2H, NH₂); ¹³C

NMR (76 MHz, CDCl₃) $\delta = 73.2, 71.8, 70.1, 58.9, 41.6$; ESI-TOF m/z [M + H]⁺ calcd for C₅H₁₄NO₂: 120.1019, found: 120.1019.

2,5,9,12-Tetraoxatridecan-7-ol (3). Epichlorohydrin (79 ml, 1.0 mol) and 2-methoxyethanol (315 ml, 4.0 mol) were mixed in a 3-necked flask equipped with a stirrer, a dropping funnel and a reflux condenser and the mixture was warmed to 50 °C. 50% aqueous NaOH solution (31 ml, 1.0 mol) was added drop wise to the stirred reaction mixture over a period of 90 min, followed by stirring at 80 °C overnight. After cooling to room temperature, the reaction mixture was filtered, neutralized with conc. aqueous HCl, extracted with CH₂Cl₂ (5 × 150 ml), followed by removal of the volatiles at a rotary evaporator (60 °C, 4 mbar). The residue was subjected to fractionation *in vacuo* and the desired product was collected at 118 °C under an absolute pressure of 1.4×10^{-1} mbar as a colorless oil (128 g, 61%); $R_f = 0.31$ (hexane/EtOAc 1 : 1, 10% MeOH); ¹H NMR (300 MHz, CDCl₃) $\delta = 3.99$ (m, 1H, CH), 3.66–3.63 (m, 4H, CH₂), 3.59–3.46 (m, 8H, CH₂), 3.36 (s, 6H, CH₃), 2.86 (s, 1H, OH); ¹³C NMR (76 MHz, CDCl₃) $\delta = 72.5, 71.9, 70.7, 69.4, 59.0$; MALDI-TOF (3-HPA) m/z [M + Na]⁺ calcd for C₉H₂₀O₅Na: 231.1203, found: 231.1203; anal. calcd for C₉H₂₀O₅: C 51.91, H 9.68, O 38.41, found: C 51.04, H 9.93, O 39.15.

7-Azido-2,5,9,12-tetraoxatridecane (4). Following general procedure A, alcohol 3 (10.7 g, 51.6 mmol) and Et₃N (7.88 ml, 56.8 mmol) were dissolved in toluene (100 ml). Methanesulfonyl chloride (4.40 ml, 56.8 mmol) in toluene (30 ml) was added and the reaction was stirred for 3 h. The mesylate (14.7 g, 51.6 mol) and sodium azide (8.39 g, 129.1 mmol) in DMF (120 ml) were stirred at 120 °C for 10 h. Column chromatography on silica gel (hexane/EtOAc 1 : 2) gave the title compound (10.4 g, 86%) as a pale yellow oil: $R_f = 0.62$ (hexane/EtOAc 1 : 2); ¹H NMR (300 MHz, CDCl₃) $\delta = 3.77\text{--}3.71$ (m, 1H, CH), 3.64–3.50 (m, 12H, CH₂), 3.35 (s, 6H, CH₃); ¹³C NMR (76 MHz, CDCl₃) $\delta = 71.8, 70.9, 70.8, 60.5, 59.0$; ESI-TOF m/z [M + H]⁺ calcd for C₉H₂₀N₃O₄: 234.1448, found: 234.1455.

2,5,9,12-Tetraoxatridecan-7-amine (5). Following general procedure B, azide 4 (8.74 g, 37.5 mmol) and Pd/C (2.00 g) were dissolved in methanol (210 ml). The title compound (7.38 g, 96%) was obtained after 40 h as a colorless oil, which was used in the next step without further purification: ¹H NMR (300 MHz, CDCl₃) $\delta = 3.59\text{--}3.51$ (m, 4H, CH₂), 3.50–3.41 (m, 6H, CH₂), 3.31–3.27 (m, 8H, CH₂, CH₃), 3.22–3.10 (m, 1H, CH), 1.77 (br, 2H, NH₂); ¹³C NMR (76 MHz, CDCl₃) $\delta = 73.6, 71.7, 70.5, 58.9, 50.6$; ESI-TOF m/z [M + H]⁺ calcd for C₉H₂₂NO₄: 208.1543, found 208.1546.

2,5,8,12,15,18-Hexaoxononadecane (6). NaH (12.7 g, 317 mmol), KI (1.8 g, 10.6 mmol) and MDC (12.2 ml, 106 mmol) were suspended in THF (150 ml) and the mixture was cooled to –10 °C. Alcohol 3 (110 g, 528 mmol) was dissolved in THF (150 ml) and added drop wise in the cold over a period of 1 h. The mixture was slowly warmed to room temperature and then heated to 65 °C with stirring for 4 d. After cooling to room temperature, the reaction was quenched with H₂O (300 ml) and extracted with CH₂Cl₂ (3 × 300 ml). The combined organic layers were dried over MgSO₄, filtered and concentrated

in vacuo. The excess alcohol was distilled off (35.8 g, 172 mmol, 54% recovered). The residue was purified by column chromatography on silica gel (gradient hexane/EtOAc 3:1, 5% MeOH to 1:1, 5% MeOH) to yield the title compound (33.1 g, 67%) as a pale yellow oil: R_f = 0.49 (hexane/EtOAc 2:1, 5% MeOH); ^1H NMR (300 MHz, CDCl_3) δ = 5.17 (s, 2H, $\text{C}=\text{CH}_2$), 4.14 (s, 4H, CH_2), 3.69–3.50 (m, 26H, CH, CH_2), 3.36 (s, 12H, CH_3); ^{13}C NMR (76 MHz, CDCl_3) δ = 143.3, 113.9, 77.1, 71.9, 71.2, 70.8, 59.0; ESI-TOF m/z $[\text{M} + \text{NH}_4]^+$ calcd for $\text{C}_{22}\text{H}_{48}\text{NO}_{10}$: 486.3273, found: 486.3271.

7,13-Bis((2-methoxyethoxy)methyl)-2,5,8,12,15,18-hexaoxa-nonadecan-10-ol (7). Unsaturated compound **6** (25.6 g, 54.6 mmol) was dissolved in a solvent mixture of dry CH_2Cl_2 (280 ml) and dry MeOH (40 ml) and cooled to -78°C . O_3 was bubbled through the solution with good stirring until the appearance of a light blue color. Ozonolysis was stopped and excess O_3 was removed from the reaction by purging with N_2 until the solution turned colorless. NaBH_4 (3.11 g, 81.9 mmol) was added at -78°C and the mixture was then allowed to slowly warm to room temperature with stirring overnight. After quenching with saturated aqueous NH_4Cl solution (8 ml) and extraction with CH_2Cl_2 (4 $\times$ 150 ml), the combined organic layers were dried over MgSO_4 , filtered and concentrated *in vacuo*. Column chromatography on silica gel (hexane/EtOAc 1:1, 10% MeOH) gave the title compound (25.8 g, 99%) as a colorless oil: R_f = 0.32 (hexane/EtOAc 1:1, 10% MeOH); ^1H NMR (300 MHz, CDCl_3) δ = 3.92–3.83 (m, 1H, CH), 3.70–3.45 (m, 31H, OH, CH, CH_2), 3.33 (s, 12H, CH_3); ^{13}C NMR (76 MHz, CDCl_3) δ = 78.6, 72.0, 71.8, 71.8, 71.4, 71.3, 70.7, 70.6, 69.6, 58.9; ESI-TOF m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{21}\text{H}_{45}\text{O}_{11}$: 473.2956, found 473.2956.

10-Azido-7,13-bis((2-methoxyethoxy)methyl)-2,5,8,12,15,18-hexaoxa-nonadecane (8). Following general procedure A, alcohol **7** (17.5 g, 37.0 mmol) and Et_3N (5.66 ml, 40.7 mmol) were dissolved in toluene (75 ml). Methanesulfonyl chloride (3.15 ml, 40.7 mmol) in toluene (20 ml) was added and the reaction was stirred for 3 h. The mesylate (20.4 g, 37.0 mmol) and sodium azide (12.0 g, 185.4 mmol) in DMF (100 ml) were stirred at 120°C for 14 h. Column chromatography on silica gel (hexane/EtOAc 1:6) gave the title compound (16.6 g, 90%) as a pale yellow oil: R_f = 0.60 (hexane/EtOAc 1:6); ^1H NMR (300 MHz, CDCl_3) δ = 3.77–3.49 (m, 31H, CH, CH_2), 3.36 (s, 12H, CH_3); ^{13}C NMR (76 MHz, CDCl_3) δ = 78.8, 71.9, 71.4, 70.7, 70.3, 61.2, 59.0; ESI-TOF m/z $[\text{M} + \text{NH}_4]^+$ calcd for $\text{C}_{21}\text{H}_{47}\text{N}_4\text{O}_{10}$: 515.3287, found: 515.3284.

7,13-Bis((2-methoxyethoxy)methyl)-2,5,8,12,15,18-hexaoxa-nonadecan-10-amine (9). Following general procedure B, azide **8** (16.4 g, 32.9 mmol) and Pd/C (1.75 g, 10 wt% Pd) were suspended in methanol (170 ml). The title compound (11.0 g, 73%) was obtained after 40 h as a colorless liquid, which was used in the next step without further purification: ^1H NMR (300 MHz, CDCl_3) δ = 3.66–3.44 (m, 30H, CH, CH_2), 3.34 (s, 12H, CH_3), 3.11 (m, 1H, CH), 1.73 (br, 2H, NH_2); ^{13}C NMR (76 MHz, CDCl_3) δ = 78.3, 72.7, 71.9, 71.2, 70.7, 58.9, 51.3; ESI-TOF m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{21}\text{H}_{46}\text{NO}_{10}$: 472.3116, found 472.3114.

DB-OEG syntheses

2,5-Dibromo-3,6-bis((2-(2-methoxyethoxy)ethyl)amino)-cyclohexa-2,5-diene-1,4-dione (D0). Bromanil (2.85 g, 6.73 mmol) was suspended in EtOH (60 ml) and amine **2** (1.60 g, 13.5 mmol) and Et_3N (1.89 ml, 13.5 mmol) were added drop wise at room temperature. The resulting mixture was heated to 70°C under ambient atmosphere for 4 h. The thus obtained violet solution was allowed to cool to room temperature and stirring was continued overnight. The resulting, brown precipitate was collected by filtration. Column chromatography on silica gel (hexane/EtOAc 1:2) gave the title compound (1.87 g, 56%) as a red-brown solid: R_f = 0.30 (hexane/EtOAc 1:2); mp: $74\text{--}75^\circ\text{C}$; ^1H NMR (300 MHz, CDCl_3) δ = 7.50 (s, 2H, NH), 4.10 (q, J = 5.5 Hz, 4H, CH_2), 3.70 (t, J = 5.2 Hz, 4H, CH_2), 3.63 (dt, J = 4.7, 3.1 Hz, 4H, CH_2), 3.54 (dt, J = 4.2, 2.8 Hz, 4H, CH_2), 3.37 (s, 6H, CH_3); ^{13}C NMR (76 MHz, CDCl_3) δ = 172.0, 146.7, 88.2, 71.8, 70.4, 69.4, 59.0, 44.6; FTIR (KBr, cm^{-1}): 3226 (NH str. vib.), 1663 ($\text{C}=\text{O}$ quinone), 1091 (C–O–C ether); MALDI-TOF m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{16}\text{H}_{25}\text{Br}_2\text{N}_2\text{O}_6$: 499.0074, found: 499.0073; anal. calcd for $\text{C}_{16}\text{H}_{24}\text{Br}_2\text{N}_2\text{O}_6$: C 38.42, H 4.84, N 5.60, Br 31.95, found: C 38.37, H 4.79, N 5.39, Br 32.10.

2,5-Bis(2,5,9,12-tetraoxatridecan-7-ylamino)-3,6-dibromocyclohexa-2,5-diene-1,4-dione (D1). Bromanil (2.16 g, 5.10 mmol) was suspended in EtOH (60 ml) and amine **5** (2.22 g, 10.7 mmol) and Et_3N (1.57 ml, 11.2 mmol) were added drop wise to the vigorously stirred mixture at room temperature. The resulting mixture was heated to 70°C under ambient atmosphere for 4 h. The thus obtained violet solution was allowed to cool to room temperature and stirring was continued overnight. After removal of the volatiles *in vacuo*, purification by column chromatography on silica gel (hexane/EtOAc 1:2) gave the title compound (1.52 g, 44%) as a red-brown sticky solid: R_f = 0.22 (hexane/EtOAc 1:2); ^1H NMR (300 MHz, CDCl_3) δ = 7.54 (br, 2H, NH), 5.04 (p, J = 9.5, 4.6 Hz, 2H, CH), 3.79–3.71 (m, 4H, CH_2), 3.67–3.58 (m, 12H, CH_2), 3.56–3.50 (m, 8H, CH_2), 3.36 (s, 12H, CH_3); ^{13}C NMR (76 MHz, CDCl_3) δ = 172.2, 146.1, 88.7, 71.7, 70.7, 69.5, 59.0, 52.6; FTIR (KBr, cm^{-1}): 3263 (NH str. vib), 1659 ($\text{C}=\text{O}$ quinone), 1103 (C–O–C ether). MALDI-TOF m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{41}\text{Br}_2\text{N}_2\text{O}_{10}$: 675.1122, found: 675.1124; anal. calcd for $\text{C}_{24}\text{H}_{40}\text{Br}_2\text{N}_2\text{O}_{10}$: C 42.62, H 5.96, Br 23.63, N 4.14, found: C 43.06, H 6.27, Br 22.82, N 4.07.

2,5-Bis(7,13-bis((2-methoxyethoxy)methyl)-2,5,8,12,15,18-hexaoxa-nonadecan-10-ylamino)-3,6-dibromocyclohexa-2,5-diene-1,4-dione (D2). Bromanil (1.09 g, 2.59 mmol) was suspended in EtOH (30 ml) and amine **9** (2.57 g, 5.44 mmol) and Et_3N (0.80 ml, 5.70 mmol) were added drop wise to the vigorously stirred mixture at room temperature. The resulting mixture was stirred for 2 d under ambient atmosphere, followed by heating to 70°C for 2 h and subsequent cooling to room temperature. After removal of the volatiles *in vacuo*, purification by column chromatography on silica gel (EtOAc, 5% MeOH) gave the title compound (2.07 g, 66%) as a deep red oil: R_f = 0.46 (EtOAc, 5% MeOH); ^1H NMR (300 MHz,

CDCl_3) δ = 7.35 (br, 2H, NH), 5.08–4.94 (m, 2H, CH), 3.95–3.84 (m, 4H, CH_2), 3.81–3.65 (m, 8H, CH, CH_2), 3.62–3.45 (m, 48H, CH_2), 3.36 (s, 24H, CH_3); ^{13}C NMR (76 MHz, CDCl_3) δ = 172.1, 146.1, 88.8, 78.7, 71.8, 71.9, 71.3, 70.8, 68.8, 59.0, 53.0; FTIR (KBr, cm^{-1}): 3272 (NH str. vib), 1656 (C=O quinone), 1110 (C–O–C ether); MALDI-TOF m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{48}\text{H}_{89}\text{Br}_2\text{N}_2\text{O}_{22}$: 1203.4268, found: 1203.4262; anal. calcd for $\text{C}_{48}\text{H}_{89}\text{Br}_2\text{N}_2\text{O}_{22}$: C 47.84, H 7.36, Br 13.26, N 2.32, found: C 47.46, H 7.42, Br 12.60, N 2.27.

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References

- 1 L. A. Torre, F. Bray, R. L. Siegel, J. Ferlay, J. Lortet-Tieulent and A. Jemal, *Ca-Cancer J. Clin.*, 2015, **65**, 87–108.
- 2 L. Rahib, B. D. Smith, R. Aizenberg, A. B. Rosenzweig, J. M. Fleshman and L. M. Matrisian, *Cancer Res.*, 2014, **74**, 2913–2921.
- 3 J. Ferlay, H.-R. Shin, F. Bray, D. Forman, C. Mathers and D. M. Parkin, *Int. J. Cancer*, 2010, **127**, 2893–2917.
- 4 N. R. Bachur, S. L. Gordon and M. V. Gee, *Cancer Res.*, 1978, **38**, 1745–1750.
- 5 J. W. Lown, *Mol. Cell. Biochem.*, 1983, **55**, 17–40.
- 6 P. J. O'Brien, *Chem.-Biol. Interact.*, 1991, **80**, 1–41.
- 7 J. L. Bolton, M. A. Trush, T. M. Penning, G. Dryhurst and T. J. Monks, *Chem. Res. Toxicol.*, 2000, **13**, 135–160.
- 8 T. J. Monks and D. C. Jones, *Curr. Drug Metab.*, 2002, **3**, 425–438.
- 9 W. Ma and Y.-T. Long, *Chem. Soc. Rev.*, 2014, **43**, 30–41.
- 10 J. A. DiMasi, R. W. Hansen and H. G. Grabowski, *J. Health Econ.*, 2003, **22**, 151–185.
- 11 R. Haag and F. Kratz, *Angew. Chem., Int. Ed.*, 2006, **45**, 1198–1215.
- 12 F. Kratz, I. A. Müller, C. Ryppa and A. Warnecke, *ChemMedChem*, 2008, **3**, 20–53.
- 13 S. R. MacEwan, D. J. Callahan and A. Chilkoti, *Nanomedicine*, 2010, **5**, 793–806.
- 14 H.-G. Batz, G. Franzmann and H. Ringsdorf, *Angew. Chem., Int. Ed. Engl.*, 1972, **11**, 1103–1104.
- 15 J. Kopeček and K. Ulbrich, *Prog. Polym. Sci.*, 1983, **9**, 1–58.
- 16 K. Ulbrich, K. Holá, V. Šubr, A. Bakandritsos, J. Tuček and R. Zbořil, *Chem. Rev.*, 2016, **116**, 5338–5431.
- 17 T. M. Allen and P. R. Cullis, *Science*, 2004, **303**, 1818–1822.
- 18 D. Astruc, E. Boisselier and C. Ornelas, *Chem. Rev.*, 2010, **110**, 1857–1959.
- 19 M. C. Parrott, S. R. Benhabbour, C. Saab, J. A. Lemon, S. Parker, J. F. Valliant and A. Adronov, *J. Am. Chem. Soc.*, 2009, **131**, 2906–2916.
- 20 J. Kofoed and J.-L. Reymond, *Curr. Opin. Chem. Biol.*, 2005, **9**, 656–664.
- 21 J. B. Wolinsky and M. W. Grinstaff, *Adv. Drug Delivery Rev.*, 2008, **160**, 1037–1055.
- 22 H. Nemoto, J. G. Wilson, H. Nakamura and Y. Yamamoto, *J. Org. Chem.*, 1992, **57**, 435.
- 23 H. Nemoto, T. Araki, M. Kamiya, T. Kawamura and T. Hino, *Eur. J. Org. Chem.*, 2007, **18**, 3003–3011.
- 24 H. Nemoto, A. Katagiri, M. Kamiya, T. Kawamura, T. Matsushita, K. Matsumura, T. Itou, H. Hattori, M. Tamaki, K. Ishizawa, L. Miyamoto, S. Abe and K. Tsuchiya, *Bioorg. Med. Chem.*, 2012, **20**, 5559–5567.
- 25 L. Miyamoto, M. Watanabe, C. Taoka, M. Kono, Y. Tomida, T. Matsushita, M. Kamiya, H. Hattori, K. Ishizawa, S. Abe, H. Nemoto and K. Tsuchiya, *Mol. Pharmaceutics*, 2013, **10**, 2723–2729.
- 26 M. A. Abdel-Rahman and A. M. Al-Abd, *Eur. J. Med. Chem.*, 2013, **69**, 848–854.
- 27 A. E. Mathew, R. K. Y. Zee-Cheng and C. C. Cheng, *J. Med. Chem.*, 1986, **29**, 1792–1795.
- 28 L. S. Hegedus, T. A. Mulhern and A. Mori, *J. Org. Chem.*, 1985, **50**, 4282–4288.
- 29 J. Sun, G. M. Stone, N. P. Balsara and R. N. Zuckermann, *Macromolecules*, 2012, **45**, 5151–5156.
- 30 X. Sun, J.-P. Lindner, B. Bruchmann and A. D. Schlüter, *Macromolecules*, 2014, **47**, 7337–7346.
- 31 H. A. Torrey and W. H. Hunter, *J. Am. Chem. Soc.*, 1912, **34**, 702–716.
- 32 C. J. Cavallito, A. E. Soria and J. O. Hoppe, *J. Am. Chem. Soc.*, 1950, **72**, 2661–2665.
- 33 J. C. Dearden and G. M. Bresnen, *Quant. Struct.-Act. Relat.*, 1988, **7**, 133–144.
- 34 T. Kenakin, *Pharmacology in Drug Discovery*, Academic Press, 2011.
- 35 K. Pongracz, S. Kaur, A. L. Burlingame and W. J. Bodell, *Carcinogenesis*, 1990, **11**, 1469–1472.
- 36 P. H. Lin, J. Nakamura, S. Yamaguchi, P. B. Upton, D. K. La and J. A. Swenberg, *Carcinogenesis*, 2001, **22**, 627–634.
- 37 X. Wang, B. Thomas, R. Sachdeva, L. Arterburn, L. Frye, P. G. Hatcher, D. G. Cornwell and J. Ma, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 3604–3609.
- 38 M. Ishiyama, M. Shiga, K. Sasamoto, M. Mizoguchi and P. He, *Chem. Pharm. Bull.*, 1993, **41**, 1118–1122.
- 39 J. O. Suberu, I. Romero-Canelón, N. Sullivan, A. A. Lapkin and G. C. Barker, *ChemMedChem*, 2014, **9**, 2791–2797.
- 40 F. A. Fornari, J. K. Randolph, J. C. Yolowich, M. K. Ritke and D. A. Gewirtz, *Mol. Pharmacol.*, 1994, **45**, 649–656.
- 41 M. R. Eno, B. E.-D. M. El-Gendy and M. D. Cameron, *Chem. Res. Toxicol.*, 2016, **29**, 784–796.
- 42 H.-T. Chen, M. F. Neerman, A. R. Parrish and E. E. Simanek, *J. Am. Chem. Soc.*, 2004, **126**, 10044–10048.
- 43 S. Fuchs, T. Kapp, H. Otto, T. Schöneberg, P. Franke, R. Gust and A. D. Schlüter, *Chem. – Eur. J.*, 2004, **10**, 1167–1192.
- 44 S. Hong, P. R. Leroueil, E. K. Janus, J. L. Peters, M.-M. Kober, M. T. Islam, B. G. Orr, J. R. Baker and M. M. Banaszak Holl, *Bioconjugate Chem.*, 2006, **17**, 728–734.
- 45 H. Frey and R. Haag, *Rev. Mol. Biotechnol.*, 2002, **90**, 257–267.

- 46 P. D. Dobson and D. B. Kell, *Nat. Rev. Drug Discovery*, 2008, **7**, 205–220.
- 47 C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeney, *Adv. Drug Delivery Rev.*, 1997, **23**, 3–25.
- 48 E. I. Parkinson and P. J. Hergenrother, *Acc. Chem. Res.*, 2015, **48**, 2715–2723.
- 49 D. Siegel and D. Ross, *Free Radical Biol. Med.*, 2000, **29**, 246–253.
- 50 A. Andrés, M. Rosés, C. Ràfols, E. Bosch, S. Espinosa and V. Segarra, *Eur. J. Pharm. Sci.*, 2015, **76**, 181–191.
- 51 F. E. Dayan, *J. Chem. Res., Synop.*, 2002, **10**, 518–519.
- 52 B. E. Lang, *J. Chem. Eng. Data*, 2012, **57**, 2221–2226.
- 53 Y. J. Marcus, *J. Solution Chem.*, 1990, **19**, 507–517.
- 54 M. Medić-Šarić, A. Mornar, T. Badovinac-Črnjević and I. Jasprica, *Croat. Chem. Acta*, 2004, **77**, 367–370.
- 55 A. Leo, C. Hansch and D. Elkins, *Chem. Rev.*, 1971, **71**, 525–616.
- 56 H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004–2021.
- 57 S. Bräse, C. Gil, K. Knepper and V. Zimmermann, *Angew. Chem., Int. Ed.*, 2005, **44**, 5188–5240.