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Synthesis and Conformational Analysis of Macrocyclic Dihydroxystilbenes Linked between the *para–para* Positions

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Abstract: A new family of diphenylethanes has been synthesized as conformationally restricted analogues of antimitotic combretastatins. The two phenyl rings are linked between the *para*-phenolic positions through a 3-oxapentamethylene or hexamethylene chain. The key macrocyclization step was achieved in moderate yields by using an intramolecular McMurry pinacol coupling of linked aromatic dialdehydes, except for the nitro-substituted compounds. The relative stereochemistry of the isomeric pinacols was determined by a combination of spectroscopic, chemical derivatization, and molecular-modeling approaches. The NMR spectra of these compounds (with a polyoxygenated crownophane skeleton) indicate severe conformational restrictions relative to their parent combretastatins; the rotation of the phenyl rings is hampered by interactions of their substituents and the linker and the conformational restrictions imposed by the substituted bridge.

Keywords: combretastatin analogues · conformational analysis · cyclophanes · macrocycles

Introduction

Conformational restriction of freely rotating moieties is a way to modify the properties of molecules, as is the case in the discovery and optimization of biological activities.[1] This approach, based on the benefit produced by the entropic factor derived from the absence of the required freezing of conformational freedom upon binding, has been applied to the investigation of different types of pharmacologically active families, for example, anticancer taxoids,[2] peptides,[3] and γ-aminobutyric acid (GABA) analogues[4] or histamine analogues.[5]

Combretastatins are very interesting diphenylethanoids of natural origin that have been thoroughly studied as a result of their high cytotoxic and antiangiogenic activities and their simple structures.[6] These compounds exert these activities through inhibition of microtubule polymerization by interaction at the colchicine site of tubulin.[7] Since their initial discovery in the early 1980s by Pettit et al.,[8] many structure–activity relationship (SAR) studies have been directed at the elucidation of the structural requirements for such small molecules to be cytotoxic. These SAR studies investigated the influence of the structure and substituents on the A,B rings and/or the structure of the bridge between them and showed that 3,4,5-trimethoxy- and 4-methoxy-3-X-substituted phenyl systems (X = H, OH, NH_2, and their amino acid, phosphate, or other derivatives for solubilizing purposes) in a close non-coplanar disposition and separated by 0–4-atom bridges are the common structural features of the active compounds.[9] These results are often summarized on the two-atom bridged structure of combretastatin A-4 (Scheme 1), one of the most potent inhibitors of tubulin polymerization, and colchicine binding to tubulin, which also shows highly potent cytotoxic and antiangiogenic activities.

In stilbenes, there is a low energetic barrier to the rotation of the phenyl rings,[10] but in combretastatins only the effects of the relative distance between the rings, coplanarity, and chirality on their biological activity have been investigated by the synthesis of many analogues. Towards this end, phenanthrene analogues,[11] the isomeric dioxolane analogues of CA-4,[12] and the isomeric pinacols derived from CA-4[13] and CA-1[14] have been synthesized and assayed. The *S,S* isomers showed stronger antitubulin activity and cyto-

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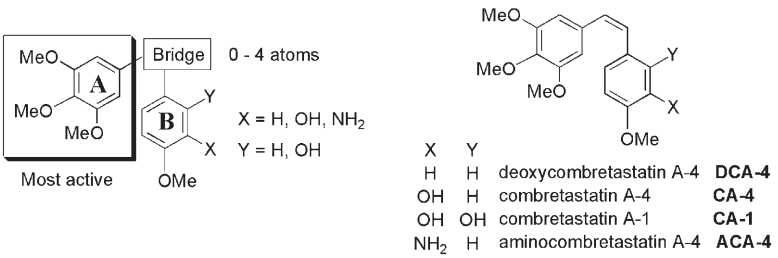
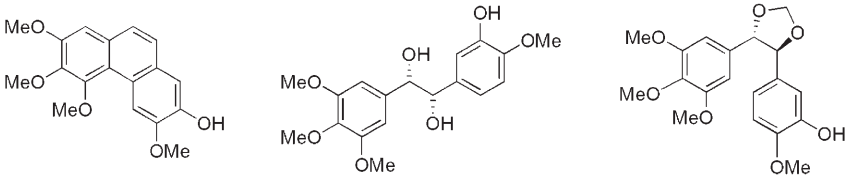


Scheme 1. General structure of combretastatins and related colchicine site ligands and the structures of relevant combretastatins.

toxic activity towards multidrug-resistant (MDR) cells (Scheme 2).

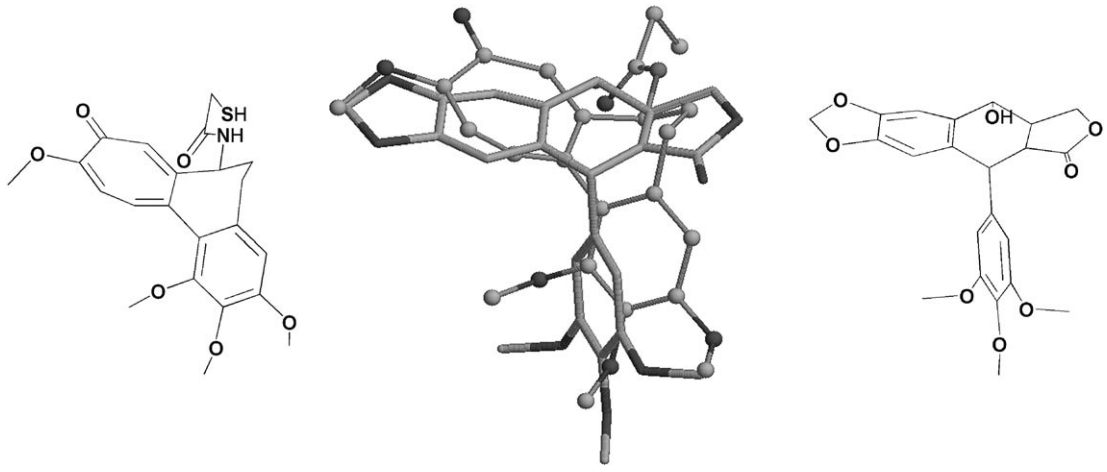


Scheme 2. Combretastatin analogues with different degrees of conformational restriction.

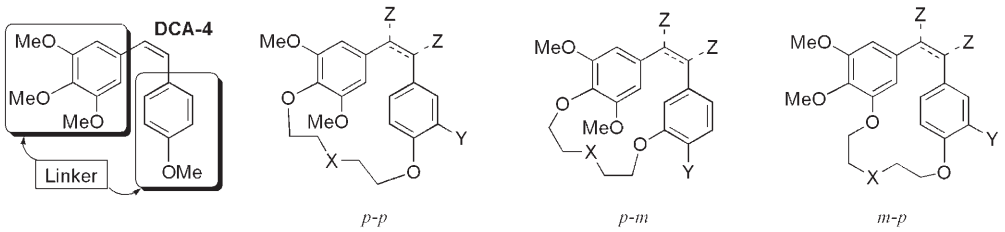
Recently, the structures of two colchicine binding site ligands, podophyllotoxin and *N*-deacetyl-*N*-(2-mercaptoacetyl)colchicine (DAMA-colchicine), complexed with tubulin have been disclosed.[15] The superposition of the bound ligands (Scheme 3) has shown that the protein adjusts to their shapes and allows a different disposition of the aromatic rings for the two ligands, in good agreement with the observed tolerance of bridges of different lengths between the two aromatic rings.

Taking into consideration that conformational restriction by macrocyclization has previously been intuited to enhance binding affinity and metabolic stability of the resulting products and that recently the effects of macrocyclization and stereochemistry (on both the precursors and the macro rings themselves) on the performance of small molecules in biological assays have been demonstrated,[16] we decided to investigate the effect of macrocyclization on the activity of several antimitotic agents. Accordingly, during our study directed at the synthesis and evaluation of new cytotoxic agents based on natural products,[17] we designed several stilbenophanes[18] and crownophanes[19] as macrocyclic derivatives of the antimitotic agents combretastatin A-4 and related compounds (Scheme 4).[20] Related paracyclophanes[21] have found application in supramolecular and materials chemistry[22] and, although much less frequently, as biologically active compounds.[23] The same design strategy was applied by Nelson and co-workers to bisindolemaleimides, whose conformations are controlled by the size of the macrocyclic ring in which they are constrained, thus allowing them to chemically compare and discriminate the adenosine 5′-triphosphate (ATP) binding sites of protein kinases in the absence of detailed structural information.[24]

Several possible macro ring sizes (depending on the linker size and bonding position on each ring) are attainable with the substitution pattern of the parent combretastatins for *p*–*p*, *p*–*m*, and *m*–*p* macrocyclic rings (Scheme 4). Herein, we disclose our results on the synthesis and the structural consequences of the cyclization through the *para* positions of



Scheme 3. Superimposition (center) and structures of DAMA-colchicine (left: ball and sticks in superimposition) and podophyllotoxin (right: thicker wireframe in superimposition) in complex with tubulin.



Scheme 4. Design of macrocyclic analogues of combretastatins and examples of structural possibilities (*p–p*, *p–m*, and *m–p* indicate the relative disposition between the bridge and the spacer in the tetrasubstituted–trisubstituted rings).

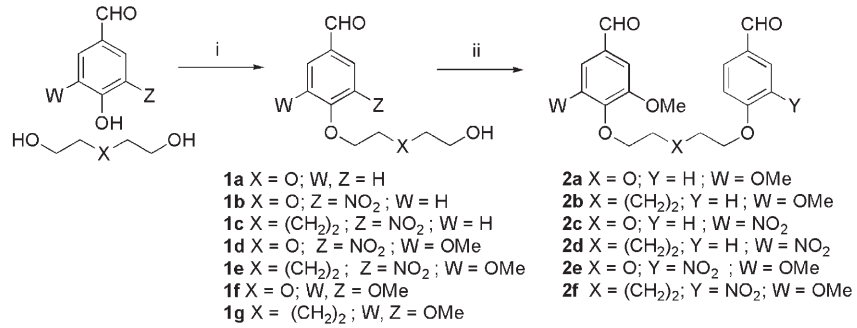
DCA4 that leads to conformationally restricted paracyclophane analogues of combretastatins.

The designed compounds are polyoxygenated macrocycles closed through a polymethylene or polyether chain that share, in part, the structure of crownophanes. The *para–para* macro-ring closure was selected in an attempt to modify by as little as possible the substitution pattern of DCA-4 and the superimposed models of CA-4, as it is taken into account that their calculated conformations are very close to those adopted by colchicine and podophyllotoxin when complexed with tubulin. The hexamethylene or the 3-oxapentamethylene linkers were selected as the smallest possible spacers able to connect the two *para* positions of the combretastatins without affecting bond angles and lengths. The synthesis of these derivatives and the study of their accessible conformations are of high interest to rationalize the effect that this modification could produce on their biological properties.

Results and Discussion

We planned the synthesis of the title compounds as depicted in Scheme 5. The formation of the bisbenzylic bond was selected for the key macrocyclization step for synthetic simplicity and versatility. Previous attempts of a related synthesis made us discard a strategy based upon ring-closing metathesis reactions.[20] We decided instead to attempt a McMurry pinacol reaction of cross-linked dialdehydes,[25] which could in turn be prepared from commercially available phenolic aldehydes through two consecutive Mitsunobu reactions.[26] The same synthetic sequence was considered suitable to access macrocyclic analogues of aminocombretastatin A-4 (ACA4) starting from appropriate nitro derivatives.

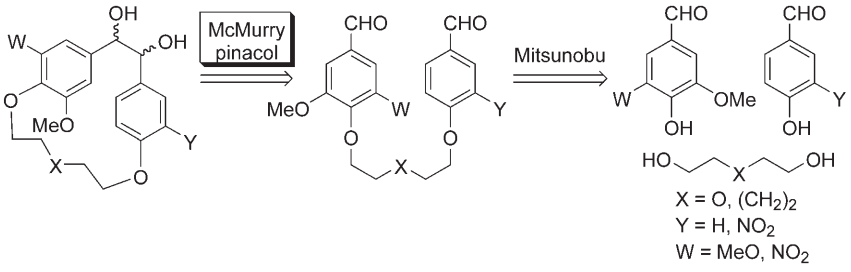
The first substitution was carried out using a four- to tenfold excess of either diethylene glycol or 1,6-hexanediol and the commercially available phenolic aldehydes under typical Mitsunobu conditions, thus yielding aldehydes **1a–g** (Scheme 6). The water-soluble starting-material diols were readily removed during aqueous work-up. Once the diols had been removed, the crude reaction products could be directly used in the second Mitsunobu reaction. The more sterically hindered *ortho*-disubstituted phenolic aldehydes were usually employed in the first instance, but similar yields were also achieved with the reverse order of the aldehydes. After the two Mitsunobu reactions, careful purification of dialdehydes **2a–f** is mandatory, as the reaction by-products interfere with the McMurry reaction. We finally decided to employ polymer-bound triphenylphosphine in the Mitsunobu



1a X = O; W, Z = H
1b X = O; Z = NO_2 ; W = H
1c X = $(CH_2)_2$; Z = NO_2 ; W = H
1d X = O; Z = NO_2 ; W = OMe
1e X = $(CH_2)_2$; Z = NO_2 ; W = OMe
1f X = O; W, Z = OMe
1g X = $(CH_2)_2$; W, Z = OMe

2a X = O; Y = H ; W = OMe
2b X = $(CH_2)_2$; Y = H ; W = OMe
2c X = O; Y = H ; W = NO_2
2d X = $(CH_2)_2$; Y = H ; W = NO_2
2e X = O; Y = NO_2 ; W = OMe
2f X = $(CH_2)_2$; Y = NO_2; W = OMe

Scheme 6. Synthesis of the dialdehydes. Reagents and conditions (1 equiv = 1 mol per mol): i) diethylene glycol or 1,6-hexanediol (4–10 equiv), DBAD or DIAD (1.5–2.0 equiv), Ph_3P resin (2 equiv), CH_2Cl_2 (dry), 48 h; ii) 3-substituted-4-hydroxybenzaldehyde (1.2 equiv), DBAD or DIAD (1.5–2.0 equiv), Ph_3P resin (2 equiv), 48–70 h (45–80 % over two steps).



Scheme 5. Retrosynthetic analysis of the macrocyclic analogues of combretastatins.

reactions, which increased the reaction times somewhat, but allowed ready removal of the reagent by filtration. The other excess reactants (diisopropyl diazodicarboxylate (DIAD) or di-*tert*-butylazodicarboxylate (DBAD)) or the reduced by-products were also detrimental for the coupling reactions, so we tried acid-labile derivatives in an attempt to simplify purification. However, the 3-oxapentamethylene bridge was unstable under the acidic conditions employed to remove DBAD, and also the semicarbazide and/or hydrazine released from these reagents reacted with the dialdehydes. Subsequent attempts to hydrolyze the semicarbazones or hydrazones did not yield the expected results, and we finally opted for a chromatographic purification of the dialdehydes before the McMurry reactions.

Under optimized McMurry pinacol coupling conditions, the macrocyclized products were obtained when no nitro groups were present (Scheme 7, right). In the presence of a nitro group, complex reaction mixtures are produced, from which no macrocyclized product could be isolated (Scheme 7, left). We planned chemoselective reductions of the nitro groups in the presence of the dialdehydes, but failed attempts of McMurry reactions on model aminobenzaldehydes discouraged us from pursuing this approach further.

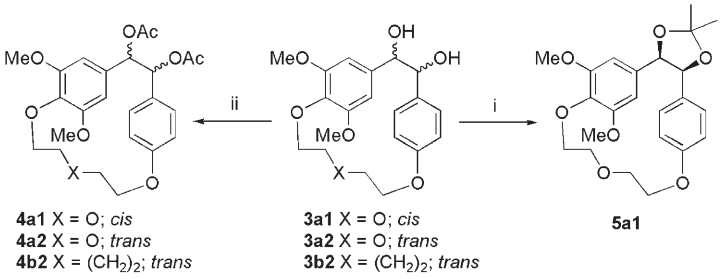
As described, when the McMurry reaction is carried under reflux, the diols are partly reduced to stilbenophanes.[20a] The olefins are not formed at room temperature and mixtures of isomeric glycols are produced in good yields, although they were strongly retained by the silica gel, thus lowering the yields of the isolated products. The bimolecular McMurry pinacol reaction is usually not very stereoselective[27] and produces diastereomeric mixtures of pinacols. Furthermore, when this reaction is carried out on linked dicarbonyl compounds, the product stereochemistry heavily depends on the precursors: *cis* diols usually result when small rings are formed, but the *trans* stereochemistry predominates for ring sizes of ten or above.[28] However, many exceptions to these rules are observed and are attributed to either substrate control or the reaction conditions.[29] In our case, the two possible diastereomers are formed for the 3-oxapentamethylene spacer and only one isomer was formed for the hexamethylene (Table 1).

Table 1. Summary of McMurry pinacol reaction results.

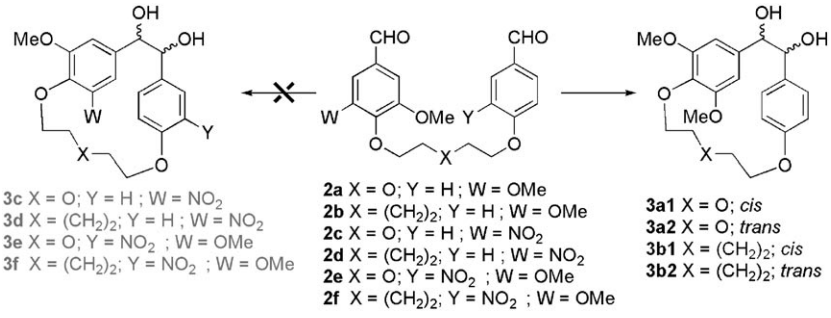
Dialdehyde	Reagent [mol mol^{-1}]	Product	Yield [%][a]
2a	[$TiCl_4$]–Zn (5:10)	**3a1**	11
		3a2	35
2b	[$TiCl_4$]–Zn (5:10)	**3b2**	23
2c–f	[$TiCl_4$]–Zn (5:10 or 11:22)	complex mixture	

[a] After column chromatography.

For the 3-oxapentamethylene series, which yielded both diastereomers, the relative stereochemistry was established by means of a combination of chemical-derivatization (Scheme 8), spectroscopic, and molecular-modeling techniques.[30] Treatment of the diol mixtures with acetic anhydride yields the two diacetates (**4a1** and **4a2**), which are more readily purified. To ascertain the relationship between the diols and the diacetates, they were hydrolyzed to their parent diols after isolation. Analogously, the mixture of diols was treated with 2,2-dimethoxypropane in the presence of catalytic trimethylsilyl chloride in an attempt to achieve the corresponding dioxolanes. Both diols reacted, but only one (**5a1**) of the two possible dioxolanes was formed. Examination of molecular models of the substrates suggests that the diol that forms the dioxolane is the *cis* isomer. To determine which diol was the precursor of the dioxolane, they were also formed from the isolated diols, and the minor isomer (**3a1**) was found to form the dioxolane. We attributed the reluctance of the putative *trans* diol to form the dioxolane to the conformational restriction imposed by macrocyclization, as both the *syn* and *anti* pinacols derived from combretastatin A4 form their corresponding dioxolanes.[12,31]



Scheme 8. Derivatization of macrocyclic pinacols. Conditions (1 equiv = 1 mol per mol): i) 2,2-dimethoxypropane (20 equiv), trimethylsilyl chloride (cat.), THF (dry), room temperature, 24 h; ii) Ac_2O (large excess), pyridine.

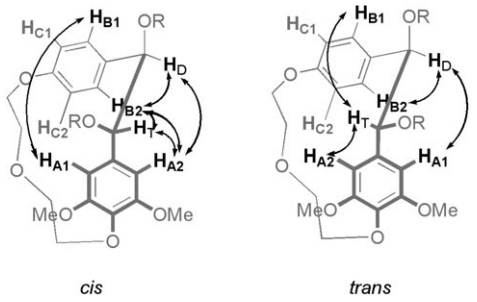


Scheme 7. Synthesis of the macrocyclic pinacols. Conditions (1 equiv = 1 mol per mol): 1) [$TiCl_4$]–Zn (5–10 equiv/10–22 equiv), THF (dry), room temperature, 30 min; 2) addition of **2** in THF dropwise, 3–5 h (5–55% of **3** after purification). Pale gray: not obtained/isolated compounds.

Spectroscopic studies: The ^1H and ^{13}C NMR spectra of the macrocyclized products show a distinct signal for each proton

or carbon atom of the chemically equivalent pairs. This behavior implies a decrease in the exchange rate of such pairs relative to the parent dialdehydes and the model nonmacrocyclic combretastatins, such that they slowly exchange on the NMR timescale. The two distinct signals for each of the equivalent aromatic proton pairs and the two methoxy groups in the macrocycles for the *cis* isomer can either be explained by two different slowly exchanging equally populated conformations (Scheme 9, option A) or by two different chemical environments at each side of each phenyl ring slowly exchanging by ring rotation on the NMR timescale, thus preventing signal averaging (Scheme 9, option B). The first hypothesis was discarded on the basis of the correlation spectra, which showed extensive cross-peaks between atoms that should otherwise be in different slowly exchanging conformations (e.g., the H_{A1}=H_{A2} signal should not correlate with the H_{A1}=$H_{A2'}$ signal according to Scheme 9, option A). Furthermore, two different signals should be observed for the bridge protons H_D and $H_{D'}$ and for H_T and $H_{T'}$ in option A of Scheme 9, which is not the case. Whereas small *para*-cyclophanes (e.g., [2.2]paracyclophane) are highly constrained molecules, the ring strain of [2.7]- or [2.8]paracyclophanes, such as those described herein, should be far less important. The interference of the methoxy groups on the aromatic ring with the linker and the conformational preferences of the pinacol subunit must both contribute to the increased rotational barrier of the phenyl rings, as the analogue stilbenophanes do not show such behavior.[20a] The same applies for the *trans* isomers.

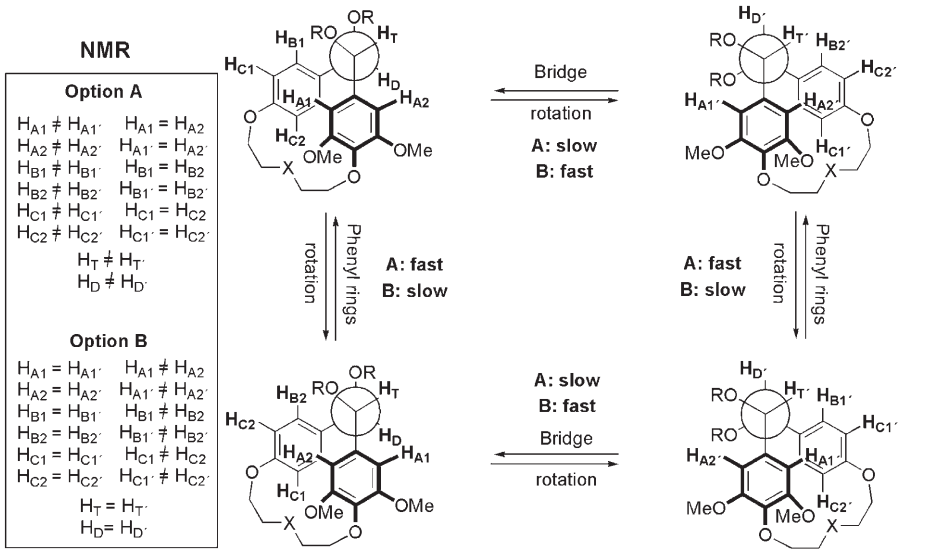
Each pinacol and its corresponding diacetate show very small differences in their NMR spectra (except for those expected for the replacement of a hydroxy by an acetate group), thus suggesting very similar structures and behavior for both derivatives. The stereochemical assignment for the isomeric pairs **3a1**–**3a2** and **4a1**–**4a2** and for the compounds **3b2**, **4b2**, and **5a1** was based on the observed NOE interactions between the hydrogen atoms on the bridge and the aromatic protons (Scheme 10) and the value of the coupling



Scheme 10. Characteristic NOE interactions for either *cis* or *trans* macrocycles.[32]

constants between the bridge protons (Table 2 and see Scheme 10 for proton identity). The observation that in one isomer the two hydrogen atoms on the bridge (H_T and H_D) have NOE interactions with the most upfield shifted *ortho* aromatic protons of each ring (H_{A2} and H_{B2}), whereas the most downfield shifted *ortho* protons of each aromatic ring (H_{A1} and H_{B1}) have NOE interactions with each other is only compatible with a *cis* isomer (Schemes 9 and 10 *cis* and Table 2: **3a1**, **4a1**, and **5a1**). The other isomer (Scheme 10 *trans*) showed NOE interactions between each benzylic proton on the bridge and its upfield shifted *ortho* aromatic proton (H_T–H_{A2} and H_D–H_{B2}) and the downfield shifted proton on the opposite aromatic ring (H_T–H_{B1} and H_D–H_{A1}); thus, this isomer was assigned as *trans*. These assignments were consistent with larger coupling constants for the benzylic protons in the *trans* isomers, with their chemical reactivity in the transketalation reaction with dimethoxyacetone, and with the preferential formation of *trans* isomers for rings larger than ten members in related pinacol reactions.[28]

The comparison of the NMR spectra of the 3-oxapentamethylene *cis* and *trans* glycols with the only hexamethylene-linked glycol formed (**3b2**) strongly suggests a *trans* relative stereochemistry for the latter one (Table 2). The ^{1}H and ^{13}C NMR spectra of **3b2** and its diacetate **4b2** show substantial line broadening of the signals that correspond to the disubstituted phenyl ring, thus precluding their assignment and reflecting a barrier to exchange of the disubstituted phenyl ring lower than for the 3-oxapentamethylene-



Scheme 9. Possibilities for the conformational equilibrium of **3a** or **4a** (*cis*). Option A: two bridge conformations in slow exchange with fast ring rotation; option B: two bridge conformations in fast exchange with slow ring rotation.

Table 2. Relevant NMR spectroscopic data and the deduced macrocycle relative stereochemistry.

Compound	**3a1**	**3a2**	**3b2**	**4a1**	**4a2**	**4b2**	**5a1**
H_{A1}	6.19	6.47	6.60	6.11	6.43	6.50	6.32
H_{A2}	5.67	5.37	5.68	5.90	5.62	6.04	5.80
H_{B1}	6.95	7.13	6.4–7.4	6.94	7.17	6.3–6.8	6.92
H_{B2}	6.36	6.08	6.4–7.4	6.55	6.36	6.3–6.8	6.61
H_{C1}	6.69	6.63	6.4–7.4	6.78	6.74	6.3–6.8	6.66
H_{C2}	6.59	6.51	6.4–7.4	6.70	6.66	6.3–6.8	6.61
H_T	4.75	4.40	4.55	6.05	5.74	5.93	5.36
H_D	4.83	4.52	4.61	6.08	5.84	6.00	5.44
J_{bridge} [Hz]	5.0	7.0	7.6	5.2	8.2	8.8	7.6
MeO	3.63	3.70	3.81	3.70	3.85	3.82	3.75
MeO	3.62	3.39	3.49	3.69	3.54	3.60	3.65
$\Delta\delta$(MeO) [ppm]	0.01	0.31	0.32	0.01	0.31	0.22	0.10
stereochemistry	*cis*	*trans*	*trans*	*cis*	*trans*	*trans*	*cis*
relevant	H_{A2}:H_D			H_{A2}:H_D	H_{A1}:H_D	H_{A1}:H_D	H_{A2}:H_D
NOE interactions	H_{A2}:H_T			H_{A2}:H_T	H_{A2}:H_T	H_{A2}:H_T	H_{A2}:H_T
	H_{A2}:H_{B2}			H_{A2}:H_{B2}	H_{A2}:H_{B1}	H_{B2}:H_D	H_{B2}:H_D
	H_{B2}:H_D			H_{B2}:H_D	H_{B2}:H_D	H_{B1}:H_T	H_{B2}:H_T
	H_{B2}:H_T			H_{B2}:H_T	H_{B1}:H_T		
				H_{A1}:H_{B1}			

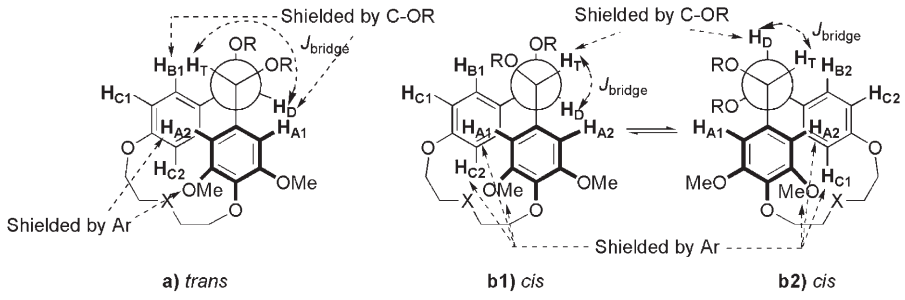
linked macrocycles. No improvement of the spectral quality was noted when the temperature was varied from −30 to 60 °C in $CDCl_3$. We attribute such a behavior to a more flexible situation that arises from the longer linker, thus allowing the less bulky disubstituted phenyl ring to populate several conformations of similar energy to lead to complex spectra at either lower or higher temperatures. The stereochemistry was thus assigned by comparison with the better resolved spectra of the 3-oxapentamethylene analogue. For a related system, we previously reported the formation of a single diastereomer with *trans* stereochemistry,[20b] which is expected for an 18-membered ring.[28]

Conformational analysis: Molecular-mechanics calculations showed a single low-energy conformation for the bridge of the *trans* isomers and two energetically very close conformations for the bridge for the *cis* isomers. (In Scheme 11 only the upper or the lower half of Scheme 9 has to be considered to account for the NMR spectra, as both halves would lead to identical spectra.) In every case, and forced by the length of the spacer, the two phenyl rings are *gauche* to each other, thus corresponding to synclinal conformations.[33] These results agree with experimental[34] and theoretical[35] results for uncyclized related compounds, such as dihydrobenzoins. These simple molecules show preference for both synclinal conformations for the *meso* form (corresponding to the *cis* diol) but prefer the synclinal conformation in which the two hydroxy groups are *gauche* to each other for the DL form (corresponding to the *trans* isomer). The shorter 3-oxapentamethylene bridge leads to somewhat more rigid structures than the longer hexamethylene bridge, which populates more conformations in the macrocycles, which is in good agreement with the NMR spectroscopic data. The models also explain the observed NOE interactions and coupling constants.

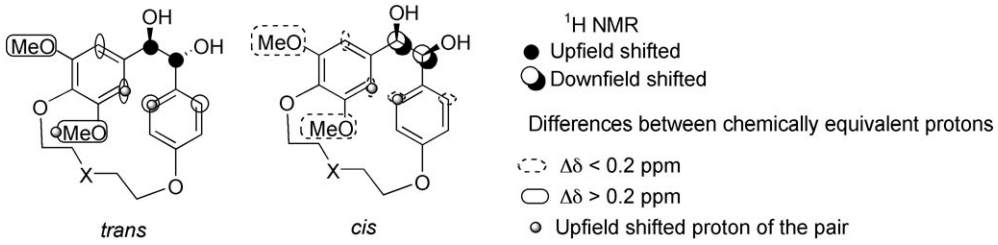
In the *trans* analogues, two distinct signals for each of the equivalent aromatic proton pairs (H_{A1}–H_{A2}, H_{B1}–H_{B2}, and H_{C1}–H_{C2}) and the two methoxy groups can be explained by the different chemical environments they are exposed to as a result of slow ring rotation on the NMR timescale that prevents signal averaging. The upfield shift of the protons or methoxy groups on one side of each aromatic ring (H_{A2} and its nearby methoxy group, and H_{B2} and less so, H_{C2}) are caused by the nearby aromatic ring. Two conformations are predicted by the models for the *cis* isomers, and they must exchange quickly on the NMR timescale to produce a single set of resonances.

The most remarkable differences between the *cis* and *trans* isomers are the differences in the proton chemical shifts between the two chemically equivalent methoxy groups (a very small difference for the *cis* isomers and a larger one of more than 0.2 ppm in the *trans* isomers; Scheme 12); between the chemically equivalent protons *ortho* to the bridge for each aromatic ring (same trend as before); and the upfield shift of the benzylic protons in the *trans* isomers with respect to the *cis* isomers (for both the pinacols and the diacetates). For the ^{13}C NMR spectra, the main differences in chemical shift between the isomers correspond to an expected upfield shift of benzylic positions of the *cis* isomers,[36] and a larger difference in chemical shift between the chemically equivalent aromatic carbon atoms.

These differences can be explained by the conformational equilibrium depicted in Scheme 11. Thus, the described



Scheme 11. The calculated most stable conformation for either a *trans* (a) or *cis* (b1 and b2) macrocycle (see text). For clarity, the H_B protons behind the phenyl rings in bold have been omitted (for labeling conventions see ref. [32].



Scheme 12. Most remarkable differences in chemical shift in the ^{1}H NMR spectra between the *cis* and *trans* isomers.

exchange process in the *cis* isomer places the aromatic protons in the shielding cone of the other ring in only one of the two conformations (e.g., H_{A1} is shielded in Scheme 11 b1 but not in Scheme 11 b2, and the opposite applies for its counterpart H_{A2}), thus decreasing the differences in chemical shift between the chemically equivalent proton pairs, relative to the *trans* analogues. The protons on the same side of the bridge oxygen atoms (e.g., H_{A1}, H_{B1}, and H_{C1}) are deshielded with respect to their counterparts on the opposite side (e.g., H_{A2}, H_{B2}, and H_{C2}; Scheme 11). Accordingly, the bridge protons (H_T and H_D) have NOE interactions with the more upfield shifted protons on the phenyl rings (which are on the same side, as described), instead of one unshielded and one shielded proton, as we would expect for a single conformation (e.g., H_T with H_{B2}, which in the single conformation depicted in Scheme 11 b2 should be unshielded, and H_{A2}, which should be shielded) as that observed for the *trans* isomers (H_{B1} and H_{A2} in Scheme 11 a). The shielding of the bridge protons in the *trans* isomer with respect to the *cis* isomer can be explained by the shielding effect of the *gauche* C−OR bond, which only occurs 50% of the time for the *cis* isomers (H_D is *anti* to the OR bond in Scheme 11 b1 and *gauche* in Scheme 11 b2).

To further investigate the conformational behavior of these pinacols, the *cis* and *trans* isomers with the 3-oxapentamethylene spacer and the *trans* hexamethylene-linked glycol were submitted to molecular dynamic (MD) simulations at virtual temperatures of 300, 400, 600, 1000, 1200, and 1500 K, with simulations lengths of 1–3 ns. The behavior of the bridges and the aromatic rings in the resulting trajectories were analyzed. At 300–600 K (Figure 1 A and B), the rotational barriers of the bridges are easily surmounted in both the *cis* and *trans* (*gauche* OH-C-C-OH conformation predominates) isomers but none of the aromatic rings rotate (Figure 1 C), in good agreement with the observed NMR spectra and the proposed conformational equilibrium. At higher temperatures (1200 K and above), the disubstituted phenyl ring can rotate in the hexamethylene-linked glycol (Figure 1 D), thus indicating a higher flexibility of the macrocycles with the longer spacer, but for the 3-oxapentamethylene series, no ring rotation was observed even at the highest virtual temperature tested (1500 K). The observed line broadening for the disubstituted phenyl ring of **3 b2** can thus be explained.

Conclusion

In conclusion, the synthesis of a new family of [2.7]- and [2.8]cyclophanes, conformationally restricted macrocyclic analogues of dihydroxydeoxycombretastatin A4, has been completed. The relatively small increase in molecular volume produced by the additional atoms between the rings makes them very similar in size to their parent compounds, and the restrictions imposed on both aromatic rings relative to related active dihydrocombretastatins turns them into suitable probes of the geometry of combretastatins when bound to tubulin. The spectroscopic analysis of these compounds shows the existence of severe restrictions to the rotation of their aromatic rings, thus allowing us to establish the conformational equilibria for *cis* and *trans* isomers. The results of molecular-dynamics simulations fully agree with the interpretation of the NMR evidence. The synthetic achievements and conformational knowledge obtained herein will allow us to design new members of this type of compound with different conformational bias, which are being pursued in our laboratory to test their biological properties.

Experimental Section

General: Reagents were used as purchased without further purification. Solvents (THF, *N,N*-dimethylformamide (DMF), CH_2Cl_2, and benzene) were dried and freshly distilled before use according to literature procedures. Chromatographic separations were performed by flash column chromatography on silica gel (Kieselgel 40, 0.040–0.063; Merck) or by gravity column chromatography (Kieselgel 60, 0.063–0.200 mm; Merck). TLC analysis was performed on precoated polyester plates of silica gel (thickness: 0.25 mm) with fluorescent indicator UV 254 (Polychrom SI F_{254}). Melting points were determined on a Buchi 510 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker WP 200-SY spectrometer at 200/50 MHz or on a Bruker SY spectrometer at 400/100 MHz. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane as the internal standard and the coupling constants (J values) are given in hertz. IR spectra were recorded on a Nicolet Impact 410 Spectrophotometer. GC–mass-spectrometric analysis was carried out in a Hewlett–Packard 5890 Serie II apparatus (70 eV). For fast-atom-bombardment high-resolution mass-spectrometry (FAB-HR-MS), a VG-TS250 apparatus (70 eV) was used. HPLC analysis was run on at least three different columns (5 mm, 4.6×150 mm): Waters X-Terra MS C_8 , Waters X-Terra MS C_{18}, and Waters X-Terra MS C_F on an Agilent HP series 1100 with at least two different solvent gradients (typically acetonitrile/water or methanol/water). Elemental analyses were recorded on a Perkin Elmer 2400 CHN apparatus.

General procedure for the Mitsunobu reactions yielding hydroxyaldehydes (1) and dialdehydes (2): A mixture of the phenolic aldehyde, the

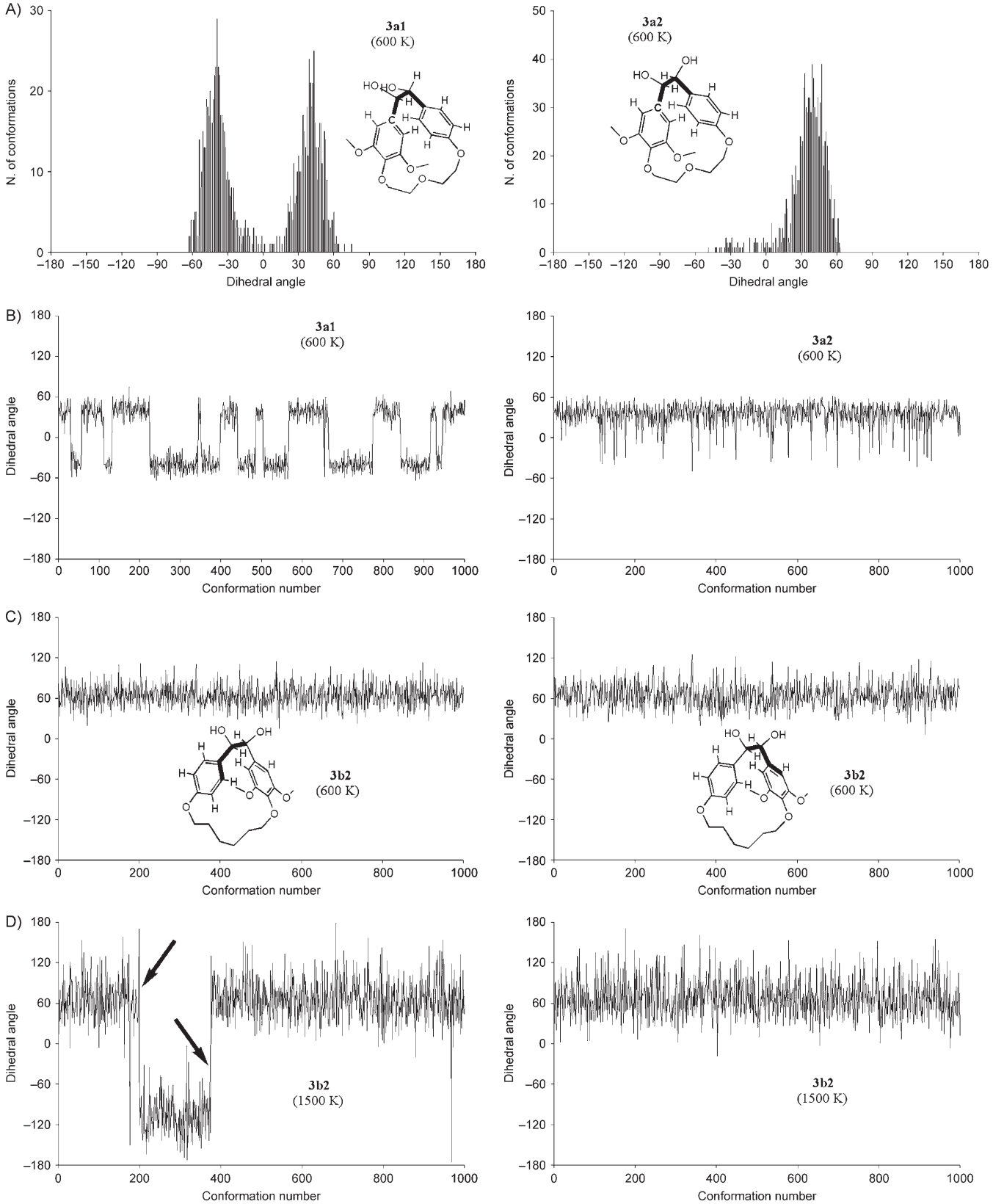


Figure 1. Graphical summary of the molecular dynamics simulation trajectories. A) Histograms and B) Trajectories of the bridge dihedral angles for *cis*-**3a1** (left) and *trans*-**3b2** (right) diols at 600 K. Trajectories at 600 (C) and 1500 K (D) of the dihedral angle between the disubstituted phenyl ring (left) and the tetrasubstituted ring (right) and the bridge for **3b2**. The ring flip is indicated by an arrow. The relevant dihedral angles are highlighted in the accompanying structures.

PPh_3 resin (1.0 mol per mol of aldehyde), and a large excess of the diol (5.0 mol per mol of aldehyde) in dry CH_2Cl_2 (10 mL per mmol of aldehyde) was stirred for 1 h before the slow addition of DBAD or DIAD (1.21 mol per mol of aldehyde) at room temperature. After 48 h, the reaction mixtures were filtered and the resin washed with ethyl acetate. The combined organic layers were evaporated, dissolved in ethyl acetate, and washed with NaOH (4%) and brine to neutrality. Once dried and evaporated, the crude reaction products were used in the next Mitsunobu reaction or purified by flash chromatography (hexane/ethyl acetate solvent mixtures) for characterization purposes. Using the same procedure used to prepare the hydroxyaldehydes (**1**), the (crude) reaction products were treated with 1.1 moles of phenolic aldehyde per mole of **1** to produce the dialdehydes (**2**).

4-[2-(2-Hydroxyethoxy)ethoxy]benzaldehyde (1a): An oily compound was obtained in 76% yield. ^{1}H NMR (400 MHz): δ = 3.60 (m, 2H), 3.68 (m, 2H), 3.80 (m, 2H), 4.12 (m, 2H), 6.90 (m, 2H), 7.73 (m, 2H), 9.76 (s, 1H) ppm; ^{13}C NMR (100.6 MHz): δ = 61.6 (CH_2), 67.7 (CH_2), 69.3 (CH_2), 72.8 (CH_2), 114.8 (2) (CH), 130.0 (C), 132.0 (2) (CH), 163.8 (C), 191.0 (CHO) ppm; FT-IR: $\tilde{\nu}$ = 3306, 1705 cm^{-1}; GC-MS: *m/z*: 210 ([M^+], 92%).

4-[2-(2-Hydroxyethoxy)ethoxy]-3-nitrobenzaldehyde (1b): Obtained as an oily compound in 64% yield. ^{1}H NMR (400 MHz): δ = 3.73 (m, 4H), 3.95 (t, J = 4.8 Hz, 2H), 4.38 (t, J = 4.8 Hz, 2H), 7.21 (d, J = 7.5 Hz, 1H), 8.08 (dd, J = 7.5, 1.8 Hz, 1H), 8.36 (d, J = 1.8 Hz, 1H), 9.92 (s, 1H) ppm; ^{13}C NMR (100.6 MHz): δ = 61.8 (CH_2), 68.9 (CH_2), 69.9 (CH_2), 72.9 (CH_2), 114.9 (CH), 127.5 (CH), 128.4 (C), 129.2 (C), 134.7 (CH), 156.4 (C), 188.8 (CHO) ppm; FT-IR: $\tilde{\nu}$ 3306, 1700 cm^{-1}.

4-(6-Hydroxyhexoxy)-3-nitrobenzaldehyde (1c): Obtained as an oily compound in 89% yield. ^{1}H NMR (400 MHz): δ = 1.50 (m, 4H), 1.85 (m, 4H), 3.63 (t, J = 6.0 Hz, 2H), 4.19 (t, J = 6.0 Hz, 2H), 7.19 (d, J = 7.8 Hz, 1H), 8.04 (dd, J = 2.2, 7.8 Hz, 1H), 8.30 (d, J = 2.2 Hz, 1H), 9.90 (s, 1H) ppm; ^{13}C NMR (100.6 MHz): δ = 25.3 (CH_2), 25.5 (CH_2), 28.6 (CH_2), 32.4 (CH_2), 62.3 (CH_2), 70.3 (CH_2), 114.7 (CH), 126.9 (CH), 128.6 (C), 135.1 (C), 139.7 (CH), 156.7 (C), 189.3 (CHO) ppm; FT-IR: $\tilde{\nu}$ = 3254, 1700 cm^{-1}.

4-[2-(2-Hydroxyethoxy)ethoxy]-3-methoxy-5-nitrobenzaldehyde (1d): Obtained as an oily compound in 82% yield. ^{1}H NMR (400 MHz): δ = 3.61 (m, 2H), 3.69 (m, 2H), 3.82 (t, J = 4.4 Hz, 2H), 3.99 (s, 3H), 4.43 (t, J = 4.4 Hz, 2H), 7.61 (d, J = 1.7 Hz, 1H), 7.83 (d, J = 1.7 Hz, 1H), 9.91 (s, 1H) ppm; ^{13}C NMR (100.6 MHz): δ = 56.7 (CH_3), 61.7 (CH_2), 70.0 (CH_2), 72.6 (CH_2), 73.9 (CH_2), 113.4 (CH), 119.6 (CH), 144.9 (C), 147.0 (C), 154.5 (C), 156.5 (C), 189.2 (CHO) ppm; FT-IR: $\tilde{\nu}$: 3258, 1691 cm^{-1}.

4-(6-Hydroxyhexoxy)-3-methoxy-5-nitrobenzaldehyde (1e): Obtained as an oily compound in 86% yield. ^{1}H NMR (400 MHz): δ = 1.49 (m, 4H), 1.60 (dd, J = 6.1, 7.0 Hz, 2H), 1.79 (m, 2H), 3.64 (t, J = 5.7 Hz, 2H), 4.00 (s, 3H), 4.24 (t, J = 6.6 Hz, 2H), 7.60 (d, J = 1.7 Hz, 1H), 7.81 (d, J = 1.7 Hz, 1H), 9.90 (s, 1H) ppm; ^{13}C NMR (100.6 MHz): δ = 25.4 (2) (CH_2), 29.9 (CH_2), 32.6 (CH_3), 56.7 (CH_2), 62.7 (CH_2), 75.2 (CH_2), 113.4 (CH), 119.6 (CH), 144.9 (C), 147.3 (C), 154.8 (C), 156.6 (C), 189.2 (CHO) ppm; FT-IR: $\tilde{\nu}$ = 3288, 1703 cm^{-1}.

4-[2-(2-Hydroxyethoxy)ethoxy]-3,5-dimethoxybenzaldehyde (1f):[20a] Obtained as an oily compound in 73% yield.

4-(6-Hydroxyhexoxy)-3,5-dimethoxybenzaldehyde (1g):[20a] Obtained as an oily compound in 70% yield.

4-{2-[2-(4-Formylphenoxy)ethoxy]ethoxy}-3,5-dimethoxybenzaldehyde (2a):[20a] Obtained as an oily compound in 80% yield.

4-[6-(4-Formylphenoxy)hexoxy]-3,5-dimethoxybenzaldehyde (2b):[20a] Obtained as an oily compound in 62% yield. M.p (hexane/Et_2O) 275°C.

4-{2-[2-(4-Formylphenoxy)ethoxy]ethoxy}-3-methoxy-5-nitrobenzaldehyde (2c): Obtained as an oily compound in 20% yield. ^{1}H NMR (400 MHz): δ = 3.92 (s, 3H), 4.18 (m, 4H), 4.30 (m, 2H), 4.42 (m, 2H), 6.96 (d, J = 8.0 Hz, 1H), 7.52 (d, J = 1.8 Hz, 1H), 7.78 (d, J = 8.0 Hz, 1H), 7.80 (d, J = 1.8 Hz, 1H), 9.81 (s, 1H), 9.83 (s, 1H) ppm.

4-[6-(4-Formylphenoxy)hexoxy]-3-methoxy-5-nitrobenzaldehyde (2d): Obtained in 25% yield. M.p. (hexane/Et_2O) 275°C; ^{1}H NMR (400 MHz): δ = 1.5–1.9 (m, 8H), 3.90 (s, 3H), 4.00 (t, J = 6.0 Hz, 2H), 4.24 (t, J = 6.0 Hz, 2H), 6.96 (d, J = 8.8 Hz, 1H), 7.58 (d, J = 1.7 Hz, 2H), 7.79 (d, J = 8.8 Hz, 1H), 7.79 (d, J = 1.7 Hz, 2H), 9.83 (s, 1H), 9.89 (s, 1H) ppm.

4-{2-[2-(4-Formyl-2-nitrophenoxy)ethoxy]ethoxy}-3,5-dimethoxybenzaldehyde (2e): Obtained as an oily compound 47% in yield. ^{1}H NMR (400 MHz): δ = 3.72 (s, 6H), 4.12 (m, 6H), 4.22 (m, 2H), 7.02 (s, 2H), 7.14 (d, J = 8.7 Hz, 1H), 7.87 (dd, J = 8.7, 1.8 Hz, 1H), 8.12 (d, J = 1.8 Hz, 1H), 9.65 (s, 1H), 9.73 (s, 1H) ppm; ^{13}C NMR (100.6 MHz): δ = 58.0 (2) (CH_3), 69.0 (CH_2), 69.5 (CH_2), 70.9 (CH_2), 72.4 (CH_2), 106.5 (2)(CH), 115.1 (CH), 126.9 (CH), 129.0 (C), 131.7 (C), 134.7 (CH), 139.8 (C), 142.4 (C), 154.0 (2)(C), 156.4 (C), 190.0 (CHO), 191.1 (CHO) ppm.

4-[6-(4-Formyl-2-nitrophenoxy)hexoxy]-3,5-dimethoxybenzaldehyde (2f): Obtained as an oily compound in 62% yield. ^{1}H NMR (400 MHz): δ = 1.59 (m, 4H), 1.85 (m, 4H), 3.91 (s, 6H), 4.12 (t, J = 7.0 Hz, 2H), 4.26 (t, J = 6.1 Hz, 2H), 7.13 (s, 2H) 7.29 (d, J = 8.7 Hz, 1H), 8.06 (dd, J = 8.7, 1.8 Hz, 1H), 8.30 (d, J = 1.8 Hz, 1H), 9.84 (s, 1H), 9.91 (s, 1H) ppm; ^{13}C NMR (100.6 MHz): δ = 25.3 (2)(CH_2), 28.6 (CH_2), 29.9 (CH_2), 56.0 (2)(CH_3), 70.2 (CH_2), 73.2 (CH_2), 106.5 (2)(CH), 114.6 (CH), 126.8 (CH), 128.6 (C), 131.5 (C), 134.7 (CH), 139.7 (C), 142.7 (C), 153.7 (2)(C), 156.5 (C), 189.0 (CHO), 191.0 (CHO) ppm; FT-IR: $\tilde{\nu}$ = 1686 cm^{-1}.

General procedure for McMurry reactions yielding pinacols 3: A mixture of [$TiCl_4$]·2THF (97%; 5 mol per mol of dialdehyde) and Zn (5 mol per mol of dialdehyde) in dry THF (30 mL per mmol of dialdehyde) was prepared at 0°C and heated to reflux for 30 min. A solution of dialdehydes **2a–f** in dry THF (5 mL per mmol of dialdehyde) was added and the reaction mixture maintained at either room temperature (no olefin formation) or heated to reflux for 3 h. The reaction was poured into a mixture of ethyl acetate and 2M HCl, the aqueous layer extracted, and the combined organic layers worked up. Products were separated by chromatography (SiO_2, hexane/ethyl acetate mixtures with 0.1% triethylamine).

(2*RS*,3*SR*)-6,20-Dimethoxy-8,11,14-trioxatricyclo[13.2.2.2^{4,7}]henicosa-1(17),4,6,15,18,20-hexaene-2,3-diol (3a1): Obtained as an oily compound in 11% yield. ^{1}H NMR (400 MHz): δ = 3.55 (m, 2H), 3.60 (m, 2H), 3.62 (s, 3H), 3.63 (s, 3H), 4.10 (m, 2H), 4.18 (m, 2H), 4.75 (d, J = 5.0 Hz, 1H), 4.83 (d, J = 5.0 Hz, 1H), 5.67 (bs, 1H), 6.19 (bs, 1H), 6.36 (dd, J = 8.4, 1.8 Hz, 1H), 6.59 (dd, J = 8.4, 2.5 Hz, 1H), 6.69 (dd, J = 8.6, J = 2.5 Hz, 1H), 6.95 (dd, J = 8.6, 1.8 Hz, 1H) ppm; ^{13}C NMR (100.6 MHz): δ = 55.5 (CH_3), 55.7 (CH_3), 66.8 (CH_2), 73.1 (CH_2), 73.4 (CH_2), 73.5 (CH_2), 75.6 (CH), 76.0 (CH), 102.2 (CH), 103.3 (CH), 113.5 (CH), 114.9 (CH), 126.0 (CH), 127.0 (CH), 131.1 (C), 135.6 (C), 136.7 (C), 151.8 (C), 152.7 (C), 157.9 (C) ppm; FT-IR: $\tilde{\nu}$ = 3453, 1608 cm^{-1}; HR-MS (EI): calcd ($C_{20}H_{24}O_7$): 376.1522; found: 376.1551.

(2*RS*,3*RS*)-6,20-Dimethoxy-8,11,14-trioxatricyclo[13.2.2.2^{4,7}]henicosa-1(17),4,6,15,18,20-hexaene-2,3-diol (3a2): Obtained as an oily compound in 35% yield. ^{1}H NMR (400 MHz): δ = 3.38 (m, 1H), 3.39 (s, 3H), 3.65 (m, 2H), 3.70 (s, 3H), 3.82 (m, 3H), 4.16 (m, 1H), 4.33 (m, 1H), 4.40 (d, J = 7.0 Hz, 1H), 4.52 (d, J = 7.0 Hz, 1H), 5.37 (bs, 1H), 6.08 (bd, J = 8.4 Hz, 1H), 6.47 (bs, 1H), 6.51 (bd, J = 8.4 Hz, 1H), 6.63 (bd, J = 8.0 Hz, 1H), 7.13 (bd, J = 8.0 Hz, 1H) ppm; ^{13}C NMR (100.6 MHz): δ = 55.1 (CH_3), 56.0 (CH_3), 66.7 (CH_2), 73.0 (CH_2), 73.4 (CH_2), 73.5 (CH_2), 81.3 (CH), 82.2 (CH), 101.5 (CH), 105.2 (CH), 112.5 (CH), 116.5 (CH), 125.4 (CH), 128.7 (CH), 131.9 (C), 136.3 (C), 136.7 (C), 152.2 (C), 152.4 (C), 157.8 (C) ppm; FT-IR: $\tilde{\nu}$ = 3403, 1608 cm^{-1}; HR-MS (EI): calcd ($C_{20}H_{24}O_7$): 376.1522; found: 376.1501.

(2*RS*,3*RS*)-6,21-Dimethoxy-8,15-dioxatricyclo[14.2.2.2^{4,7}]docosa-1(18),4,6,16,19,21-hexaene-2,3-diol (3b2): Obtained as an oily compound in 23% yield. ^{1}H NMR (400 MHz): δ = 1.1–1.6 (m, 8H), 3.8–4.2 (m, 4H), 3.49 (s, 3H), 3.81 (s, 3H), 4.55 (d, J = 7.6 Hz, 1H), 4.61 (d, J = 7.6 Hz, 1H), 5.68 (bs, 1H), 6.60 (bs, 1H), 6.4–7.2 (m, 4H) ppm; ^{13}C NMR (100.6 MHz): δ = 23.6 (CH_2), 23.7 (CH_2), 25.7 (CH_2), 27.0 (CH_2), 55.4 (CH_3), 56.2 (CH_3), 67.6 (CH_2), 71.5 (CH_2), 80.7 (CH), 81.5 (CH), 102.4 (CH), 106.0 (CH), 114.2 (2) (CH), 128.1 (2) (CH), 132.3 (C), 134.5 (C), 135.2 (C), 152.2 (C), 153.5 (C), 156.8 (C) ppm; FT-IR: $\tilde{\nu}$ = 3435, 1608 cm^{-1}; HR-MS (EI): calcd ($C_{22}H_{28}O_6$): 388.1886; found: 388.1897.

Acetylation reactions: Either a mixture of isomeric alcohols **3a1** and **3a2** or each one by itself and alcohol **3b2** were dissolved in pyridine (1 mL per mmol of alcohol) and treated with acetic anhydride (1 mL per mmol of alcohol). After 2 h, the mixture was diluted with ethyl acetate and extracted with 2M HCl, 5% $NaHCO_3$, and brine. The combined organic

layers were dried over anhydrous Na_2SO_4, filtered, and the solvent rotary evaporated. If necessary, the products were separated by chromatography (SiO_2, hexane/ethyl acetate mixtures with 0.1% triethylamine).

(2*RS*,3*SR*)-6,20-Dimethoxy-8,11,14-trioxatricyclo[13.2.2.2^{4,7}]henicosa-1(17), 4,6,15,18,20-hexaene-2,3-diyl diacetate (4a1): Obtained as an oily compound in 60% yield. ^{1}H NMR (400 MHz): δ=2.20 (s, 3H), 2.21 (s, 3H), 3.60 (m, 2H), 3.67 (m, 2H), 3.69 (s, 3H), 3.70 (s, 3H), 4.09 (ddd, J=2.8, 5.2, 13.6 Hz, 1H), 4.15 (ddd, J=2.8, 5.2, 13.6 Hz, 1H), 4.22 (ddd, J=2.5, 6, 14.0 Hz, 1H), 4.28 (ddd, J=2.8, 5.2, 14.0 Hz, 1H), 5.90 (d, J=1.7 Hz, 1H), 6.05 (d, J=5.2 Hz, 1H), 6.08 (d, J=5.2 Hz, 1H), 6.11 (d, J=1.7 Hz, 1H), 6.55 (dd, J=8.4, 2.0 Hz, 1H), 6.70 (dd, J=8.5, 2.7 Hz, 1H), 6.78 (dd, J=8.7, 2.7 Hz, 1H), 6.94 (dd, J=8.7, 2.2 Hz, 1H) ppm; ^{13}C NMR (100.6 MHz): δ=21.0 (2) (CH_3), 55.6 (CH_3), 55.8 (CH_3), 66.7 (CH_2), 73.3 (CH_2), 73.8 (CH_2), 73.9 (CH_2), 75.0 (CH), 75.1 (CH), 102.8 (CH), 103.9 (CH), 113.8 (CH), 115.2 (CH), 126.6 (CH), 127.0 (CH), 127.6 (C), 131.4 (C), 137.6 (C), 152.2 (C), 153.0 (C), 158.3 (C) 169.8 (2) (C) ppm; FT-IR: $\tilde{\nu}$=1747, 1610 cm^{-1}; GC-MS: m/z: 460 ([M^+], 22); HRMS (EI): calcd for $C_{24}H_{28}O_9$: 460.1733; found: 460.1734; elemental analysis (%) calcd: C 62.60, H 6.13; found: C 62.78, H 6.30.

(2*RS*,3*RS*)-6,20-Dimethoxy-8,11,14-trioxatricyclo[13.2.2.2^{4,7}]henicosa-1(17),4,6,15,18,20-hexaene-2,3-diyl diacetate (4a2): Obtained as an oily compound in 68% yield. ^{1}H NMR (400 MHz): δ=2.13 (s, 3H), 2.14 (s, 3H), 3.50 (m, 1H), 3.53 (m, 1H), 3.54 (s, 3H), 3.63 (m, 1H), 3.75 (ddd, J=11.2, 6.8, 2.3 Hz, 1H), 3.85 (s, 3H), 3.98 (ddd, J=13.3, 6.8, 2.2 Hz, 1H), 4.00 (ddd, J=13.9, 2.6, 1.6 Hz, 1H), 4.25 (ddd, J=2.3, 4.6, 13.3 Hz, 1H), 4.45 (bdd, J=8.0, 13.9 Hz, 1H), 5.62 (d, J=1.7 Hz, 1H), 5.74 (d, J=8.2 Hz, 1H), 5.84 (d, J=8.2 Hz, 1H), 6.36 (dd, J=2.2, 8.6 Hz, 1H), 6.43 (d, J=1.7 Hz, 1H), 6.66 (dd, J=8.6, 2.6 Hz, 1H), 6.74 (dd, J=8.5, 2.6 Hz, 1H), 7.17 (dd, J=8.5, 2.2 Hz, 1H) ppm; ^{13}C NMR (100.6 MHz): δ=21.1 (2) (CH_3), 55.4 (CH_3), 56.1 (CH_3), 66.9 (CH_2), 73.1 (CH_2), 73.4 (CH_2), 73.5 (CH_2), 78.6 (CH), 79.5 (CH), 101.9 (CH), 106.0 (CH), 113.0 (CH), 116.9 (CH), 125.8 (CH), 128.0 (C), 129.3 (CH), 132.1 (C), 137.6 (C), 152.6 (2) (C), 158.5 (C), 170.1 (C), 170.2 (C) ppm; FT-IR: $\tilde{\nu}$= 1744, 1610 cm^{-1}; GC-MS: m/z: 460 ([M^+], 23); HRMS (EI): calcd for $C_{24}H_{28}O_9$: 460.1733; found: 460.1756; elemental analysis (%) calcd: C 62.60, H 6.13; found: C 62.41, H 5.94.

(2*RS*,3*RS*)-6,21-Dimethoxy-8,15-dioxatricyclo[14.2.2.2^{4,7}]docosa-1(18),4,6,16,19,21-hexaene-2,3-diyl diacetate (4b2): Obtained as an oily compound in 57% yield. ^{1}H NMR (400 MHz): δ=1.1–1.8 (m, 8H), 2.10 (s, 3H), 2.11 (s, 3H), 3.60 (s, 3H), 3.8–4.2 (m, 4H), 3.82 (s, 3H), 5.93 (d, J=8.8 Hz, 1H), 6.00 (d, J=8.8 Hz, 1H), 6.04 (d, J=1.6 Hz, 1H), 6.50 (d, J=1.6 Hz, 1H), 6.4–7.4 (m, 4H) ppm; ^{13}C NMR (100.6 MHz): δ=21.1 (2) (CH_3), 23.4 (CH_2), 23.7 (CH_2), 25.8 (CH_2), 27.0 (CH_2), 55.7 (CH_3), 56.2 (CH_3), 67.7 (CH_2), 71.7 (CH_2), 77.9 (CH), 78.6 (CH), 103.1 (CH), 107.3 (CH), 128.5 (C), 131.3 (C), 135.2 (C), 152.4 (C), 153.5 (C), 157.3 (C), 170.3 (2) (C) ppm, four CH resonances not observed; FT-IR: $\tilde{\nu}$= 1744, 1610 cm^{-1}; HRMS (EI): calcd for $C_{26}H_{32}O_8$: 472.2097; found: 472.1990.

Transketalation reactions: Either a mixture of isomeric alcohols **3a1** and **3a2** or each one by itself were dissolved in acetone (5 mL per mmol of alcohol) and treated with 2,2-dimethoxypropane (50 mol per mol of glycol) and trimethylsilyl chloride (0.1 mol per mol of glycol). After 20 h at room temperature, the mixture was diluted with ethyl acetate and washed with 5% $NaHCO_3$, and brine. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and the solvent rotary evaporated. Products were separated by chromatography (SiO_2, hexane/ethyl acetate mixtures with 0.1% triethylamine).

(2*RS*,3*SR*)-2,3-Isopropylenedioxy-6,20-dimethoxy-8,11,14-trioxatricyclo[13.2.2.2^{4,7}]henicosa-1(17),4,6,15,18,20-hexaene (5a1): Obtained as an oily compound in 65% yield. ^{1}H NMR (400 MHz): δ=1.61 (s, 3H), 1.83 (s, 3H), 3.53 (m, 2H), 3.62 (m, 2H), 3.65 (s, 3H), 3.75 (s, 3H), 4.17 (m, 2H), 4.23 (m, 2H), 5.36 (d, J=7.6 Hz, 1H), 5.44 (d, J=7.6 Hz, 1H), 5.80 (d, J=1.2 Hz, 1H), 6.32 (d, J=1.2 Hz, 1H), 6.61 (m, 2H), 6.66 (dd, J=8.8, 2.0 Hz, 1H), 6.92 (d, J=8.8 Hz, 1H) ppm; ^{13}C NMR (100.6 MHz): δ=24.1 (CH_3), 26.6 (CH_3), 55.7 (CH_3), 55.8 (CH_3), 67.8 (CH_2), 71.3 (CH_2), 71.4 (CH_2), 72.0 (CH_2), 81.3 (CH), 81.6 (CH), 103.4 (CH), 104.1 (CH), 109.0 (C), 114.4 (CH), 114.9 (CH), 127.0 (CH), 127.4 (CH), 132.0 (C), 133.7 (C), 136.0 (C), 151.8 (C), 152.3 (C), 158.0 (C) ppm; FT-IR: $\tilde{\nu}$: 1610 cm^{-1}; GC-MS: m/z: 416 ([M^+], 100); HRMS (EI): calcd for $C_{22}H_{27}NaO_5$: 439.1733; found: 439.2538.

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