Supporting Information for

Structure and Conformation of Zosteraphenols, Tetracyclic Diarylheptanoids from the Seagrass *Zostera marina*: an NMR and DFT Study

Laura Grauso,^{†,‡} Yan Li,^{§,‡} Silvia Scarpato,[⊥] Oleksandr Shulha,[§] Lucie Rárová,[∥] Miroslav Strnad,[∥] Roberta Teta,[⊥] Alfonso Mangoni,^{*,⊥} Christian Zidorn^{*,§}

⁺ Dipartimento di Agraria, Università degli Studi di Napoli Federico II, Via Università 100, 80055 Portici (NA), Italy

- [§] Pharmazeutisches Institut, Abteilung Pharmazeutische Biologie, Christian-Albrechts-Universität zu Kiel, Gutenbergstraße 76, 24118, Kiel, Germany
- ¹ Dipartimento di Farmacia, Università degli Studi di Napoli Federico II, Via D. Montesano 49, 80131 Napoli, Italy
- Laboratory of Growth Regulators, Faculty of Science, Palacký University, and Institute of Experimental Botany of the Czech Academy of Sciences, Šlechtitelů 27, CZ-78371 Olomouc, Czech Republic

Table of Contents

| Experimen | tal Section | S3 |
|------------|---------------------------------------------------------------------------------------------------------------------------|------------|
| Table S1. | ¹ H and ¹³ C NMR Data of zosteraphenol B (2) (700 MHz, 258 K, CDCl ₃) | S 7 |
| Table S2. | Cartesian coordinates of the lowest-energy conformations of the two rotamers of zosteraphenol A (1) , | |
| | optimized at the B3LYP/6-31+G(d,p) level. | S8 |
| Table S3. | Experimental chemical shifts, calculated isotropic shielding constants, and predicted chemical shifts of the | |
| | two rotamers of zosteraphenol A (1) . | S10 |
| Table S4. | Experimental and calculated chemical shift differences between the minor and the major rotamers of | |
| | zosteraphenol A. | S11 |
| Table S5. | Rotatory strengths (length formalism) calculated for rotamers 1a and 1b . | S11 |
| Table S6. | $Cytotoxicity(\mu M)inhumancelllinesandnormalfibroblastsafter72hoftreatmentofzosteraphenolsA(1)$ | |
| | and B (2) and isotedarene A (4) . | S12 |
| Figure S1. | Overlay of conformers of the two rotamers $1a$ and $1b$ of zosteraphenol A (1) generated by a 2000 K | |
| | molecular dynamics simulation. | S4 |
| Figure S2. | Predicted and experimental ECD spectra of $9R$ -zosteraphenol A (1). | S5 |
| Figure S3. | High-resolution ESI-MS spectrum of zosteraphenol A (1) (positive ion mode). | S13 |
| Figure S4. | Full ¹ H NMR spectrum (700 MHz, 258 K, CDCl ₃) of zosteraphenol A (1). | S13 |
| Figure S5. | Expansion of ¹ H NMR spectra (700 MHz, $CDCl_3$) of zosteraphenol A (1) at 298 K and 258 K. | S14 |
| Figure S6. | ¹³ C NMR Spectrum (175 MHz, 258 K, CDCl ₃) of zosteraphenol A (1). | S15 |
| Figure S7. | COSY spectrum (700 MHz, 258 K, CDCl ₃) of zosteraphenol A (1). | S16 |
| Figure S8. | NOESY/EXSY spectrum (700 MHz, 258 K, CDCl ₃) of zosteraphenol A (1). | S17 |
| Figure S9. | HSQC spectrum (700 MHz, 258 K, CDCl ₃) of zosteraphenol A (1). | S18 |
| Figure S10 | HMBC spectrum (700 MHz, 258 K, CDCl ₃) of zosteraphenol A (1). | S19 |
| Figure S11 | Expansion of the HMBC spectrum (700 MHz, 258 K, CDCl ₃) of zosteraphenol A (1). | S20 |
| Figure S12 | UV and ECD spectra (CH ₃ OH) of zosteraphenol A (1). | S21 |
| Figure S13 | High-resolution ESI-MS spectrum of zosteraphenol B (2) (positive ion mode). | S22 |
| Figure S14 | Expansion of ¹ H NMR spectra (700 MHz, CDCl ₃) of zosteraphenol B (2) at 298 K and 258 K. | S22 |
| Figure S15 | Full 'H NMR spectrum (700 MHz, 258 K, CDCl ₃) of zosteraphenol B (2). | S23 |
| Figure S16 | 13 C NMR Spectrum (175 MHz, 258 K, CDCl ₃) of zosteraphenol B (2). | S24 |
| Figure S17 | COSY spectrum (700 MHz, 258 K, CDCl ₃) of zosteraphenol B (2). | S25 |
| Figure S18 | NOESY/EXSY spectrum (700 MHz, 258 K, $CDCl_3$) of zosteraphenol B (2). | S26 |
| Figure S19 | HSQU spectrum (700 MHz, 258 K, CDCl ₃) of zosteraphenol B (2). | S27 |
| Figure S20 | HMBC spectrum ($/00$ MHz, 258 K, CDCl ₃) of zosteraphenol B (2). | S28 |
| Figure S21 | Expansion of the HMBC spectrum (700 MHz, 258 K, CDCl ₃) of zosteraphenol B (2). | S29 |
| Figure S22 | UV and ECD spectra (CH ₃ OH) of zosteraphenol B (2). | \$30 |

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco P-200 polarimeter at 589 nm using a 10 cm cell. UV spectra were recorded on a Jasco V-530 spectrophotometer. ECD spectra were recorded on a Jasco 715 spectropolarimeter. NMR spectra were determined on Varian Unity Inova spectrometers at 700 MHz; chemical shifts were referenced to the residual solvent signal (CDCl₃: δ_H 7.26, δ_C 77.0). The 2D NMR experiments (HSQC, HMBC, COSY, NOESY) were performed using standard Varian pulse sequences. High-resolution ESI mass spectra were measured on a Thermo LTQ Orbitrap XL mass spectrometer. Column chromatography separations were performed with Kieselgel Si 60 (Merck, Darmstadt, Germany).

Plant Material. *Zostera marina* L. (Zosteraceae), common eelgrass, is a perennial rhizomateous marine monocot of 30 to 100 cm length. Specimens of *Zostera marina* L. were collected at the coast close to the Olympiazentrum Schilksee, Kiel, Schleswig-Holstein, Germany in March 2018 (coordinates: N 54°25'39.0", E 10°10'17.5"; alt.: 0 m). The collected plants were unrooted plants freshly washed ashore. A voucher specimen is preserved in the Herbarium of the Institut für Botanik, Kiel University (voucher code: LP-20180326A-1; KIEL0005003).

Extraction and Isolation. Ground whole plants of *Z. marina* (1.25 kg) were successively extracted with *n*-hexane and acetone at room temperature for five times (each time with 3.5 L), yielding 6.75 g and 9.86 g of residue after evaporation of the solvent *in vacuo*, respectively. The crude hexane extract was further fractionated using medium pressure silica gel column chromatography, performed using a Büchi PrepChrom C-700 system with a silica gel column (Sepacore Silica 40–63 µm, 80 g, 194 × 31 mm) at a flow rate of 10.0 ml/min. The employed linear gradients started with 100% hexane to 100% CH₂Cl₂, and then continued from 100% CH₂Cl₂ to 100% MeOH; run time 2 hours, yielding a total of 22 fractions. Fraction A19 eluting with 45 % CH₂Cl₂ and 55% MeOH contained compound **1** and fraction A17 eluting with 52 % CH₂Cl₂ and 48% MeOH contained compound **2**. The fractions containing compound **1** (227 mg) and **2** (73.6 mg) were further purified by Sephadex LH-20 column chromatography (Sephadex LH-20, 2 cm × 100 cm, CH₂Cl₂/acetone (85:15 v:v) as eluant). Finally, partially purified compounds **1** (16.5 mg) and **2** (23.5 mg) were subjeted to semi-preparative reversed-phase HPLC to yield pure compounds **1** (7.7 mg) and **2** (10.5 mg) (Waters e2695; isocratic: 70% MeOH, 30% water; column: Nucleodur C₈, 1 cm × 25 cm; flow rate: 2.00 ml/min; detection wavelengths: 210 nm and 254 nm; collection times, compound **1**: 22.0-25.0 min and compound **2**: 40.0-43.5 min.).

Zosteraphenol A (1): colorless oil; $[a]_D^{20}$ –18 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (ϵ): 206 (20600), 254 (7700), 307 (3000) nm; ¹H NMR data, see Table 1; ECD (MeOH) λ_{max} ($\Delta \epsilon$): 195 (+13.0), 225 (–12.2), 252 (–1.4), 278 (–1.0), 317 (+2.5); ¹³C NMR data, see Table 1; HR-ESI-MS *m/z* 307.1332 [M+H]⁺ (calcd. for C₂₀H₁₉O₃ 307.1329).

Zosteraphenol B (**2**): colorless oil; $[\alpha]_D^{20}$ –16 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (ϵ): 206 (20000), 254 (7400), 306 (3000) nm; ECD (MeOH) λ_{max} ($\Delta\epsilon$): 197 (+22.2), 225 (–17.4), 276 (–1.0), 317 (+3.9); ¹H NMR data, see Table S1; ¹³C NMR data, see Table S1; HR-ESI-MS *m/z* 321.1488 [M+H]⁺ (calcd. for C₂₁H₂₁O₃ 321.1485).

Molecular Dynamics Simulations. MD calculations were performed using the CFF91 force field^{\$1} in the INSIGHT II/Discover package.^{\$2} The effect of the solvent was approximated by using a dielectric constant of 4.81 (chloroform). A series of 10-ns MD simulations were performed. The coordinates were saved every 50 ps, giving 200 structures for each simulation, which were minimized in the same force field. Because MD simulations must necessarily be very short, it is unlikely that a conformational change causing coalescence (i.e. when the average life of conformers is in the order of many milliseconds) can be observed during the duration of the simulation. If temperature is raised, however, the equilibrium is faster and the conformational change may eventually be observed. Therefore, simulations were performed at increasingly high temperature, namely 800 K, 1000 K, 1500 K, and 2000 K. The double bond at position 10 was restrained to *cis* during the simulations to avoid isomerization, ^{\$3} and the restraint was removed before minimization.

The 9*R* enantiomer of zosteraphenol A (9*R*-1) was arbitrarily chosen for calculations. No remarkable conformational change was observed for 1 in simulations run up to 1500 K. At 2000 K, inversion of the axial chirality of the molecule occurred several times during the simulation. After minimization, the 200 structures from MD simulation converged into a set of 11 conformations for each axial rotamer (hereafter called 1a and 1b, respectively), all within 3 kcal/mol from the lowest energy conformer (Figure S1). The conformers within each set showed the same conformation of the carbon skeleton, but differed in the dihedral angles about the C—O bonds. These were used as starting structures for DFT calculations.



Figure S1. Overlay of the conformers of the two rotamers **1a** and **1b** of zosteraphenol A (**1**) generated by a 2000 K molecular dynamics simulation, represented as stick (top) and wireframe (bottom) models.

Quantum-Mechanical Calculation of ¹H and ¹³C NMR Chemical Shifts and ECD Spectrum of Compound 1. Quantum-mechanical calculations were performed using density functional theory (DFT) in the program Gaussian 16.⁵⁴ The geometry of the two sets of conformers from MD was first optimized using the B3LYP/6-31G(d) level of theory to roughly estimate their energies and filter out high energy conformers. Optimization caused the 11 conformers of the rotamer 1a to converge into 6 conformers; in addition, all the conformers other than the lowest-energy conformer showed relative energies higher than 3 kcal/mol, and were therefore excluded from subsequent calculations. Likewise, the 11 conformers of the rotamer 1b converged into 6 conformers, and only the lowest-energy conformer was kept for subsequent calculations.

The lowest energy conformers of rotamers **1a** and **1b** (hereafter simply called **1a** and **1b**, respectively) were reoptimized at the B3LYP/6-31+G(d,p) level; vibrational frequency analysis revealed no imaginary frequencies, confirming **1a** and **1b** to be in true energy minima. The optimized rotamers are shown in Figure 4 in the main text, and their Cartesian coordinates are reported in Table S2. Finally, the ¹H and ¹³C NMR chemical shifts of **1a** and **1b** were calculated using the Gauge Invariant Atomic Orbitals (GIAO) method at the PBE0/6-311+G(2d,p) level of theory, using the PCM model for the solvent (chloroform). The scaling factors needed to convert the isotropic shielding constants calculated by Gaussian at this level of theory into chemical shifts have been pre-calculated and are available in the literature (¹H: slope -1.0958, intercept 31.7532; ¹³C: slope -1.0533, intercept: 187.3123).^{S5} Isotropic shielding constants and chemical shift calculated for **1a** and **1b** are reported in Table S3.

The theoretical ECD spectrum of zosteraphenol A (1) was calculated using time-dependent DFT (TDDFT), the same geometry as for NMR calculations, the B3LYP/6-311+G(2d,p) level of theory, and the PCM model for the solvent (methanol). The calculated rotational strengths of the two rotamers 1a and 1b are reported in Table S4. The Boltzmann-weighted mean of the two spectra was calculated using Boltzmann statistics (T = 298 K) on the basis of their population as estimated from NMR data. From this, the ECD curve of 1 (Figure S2 below) was obtained using the program SpecDis v. 1.71^{S6} and the adjustable parameters were optimized for the best fit between the theoretical and experimental spectra (the exponential half-width of the ECD bands, σ , was empirically set to 0.28 eV and the UV shift was set to -5 nm).



Figure S2. Predicted (red line) and experimental (blue line) ECD spectra of 9*R*-zosteraphenol A (1).

Cytotoxicity assay. Cytotoxicity of tested compounds in human acute lymphoblastic leukemia (CEM), human breast adenocarcinoma (MCF7), human cervical carcinoma (HeLa) cell lines, and normal fibroblasts (BJ) was determined by resazurin assay using manufacturer's protocol (Sigma Aldrich, St. Louis, MO, USA). The procedure was described earlier,⁵⁷ but it was performed using another dye (resazurin). The data shown are means ± standard deviation (SD) obtained from at least three independent experiments performed in triplicates.

Cell-Surface ELISA CD62E (E-Selectin, ELAM). Enzyme-linked activity assay (ELISA) was used to detect the levels of cell adhesion molecule ELAM on HUVECs after 30 min of incubation with tested compounds and 4 h of stimulation with $TNF\alpha$ as described earlier.⁵⁸ Experiments were repeated three times in triplicate.

Cytotoxicity Testing. Calcein AM (Molecular Probes, Invitrogen, Karlsruhe, Germany) cytotoxicity assays after 4 h of treatment in the HUVECs were used to measure the cytotoxicity of compounds for ELAM expression assay as described previously.⁵⁸ Triplicates of at least three independent experiments were used.

REFERENCES

- S1 Maple, J. R.; Dinur, U.; Hagler, A. T. Derivation of Force Fields for Molecular Mechanics and Dynamics from Ab Initio Energy Surfaces. *Proc. Natl. Acad. Sci.* **1988**, *85* (15), 5350–5354, DOI: 10.1073/pnas.85.15.5350.
- S2 BIOVIA, 5005 Wateridge Vista Drive, San Diego, CA 92121, USA.
- S3 Costantino, V.; Fattorusso, E.; Mangoni, A.; Perinu, C.; Teta, R.; Panza, E.; Ianaro, A. Tedarenes A and B: Structural and Stereochemical Analysis of Two New Strained Cyclic Diarylheptanoids from the Marine Sponge *Tedania ignis. J. Org. Chem.* 2012, 77(15), 6377–6383, DOI: 10.1021/jo300295j.
- Gaussian 16, Revision A.03, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.;
 Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; Li, X.; Caricato, M.; Marenich, A. V.; Bloino, J.; Janesko, B. G.;
 Gomperts, R.; Mennucci, B.; Hratchian, H. P.; Ortiz, J. V.; Izmaylov, A. F.; Sonnenberg, J. L.; Williams-Young, D.; Ding, F.;
 Lipparini, F.; Egidi, F.; Goings, J.; Peng, B.; Petrone, A.; Henderson, T.; Ranasinghe, D.; Zakrzewski, V. G.; Gao, J.; Rega, N.;
 Zheng, G.; Liang, W.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.;
 Nakai, H.; Vreven, T.; Throssell, K.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J. J.; Brothers, E. N.;
 Kudin, K. N.; Staroverov, V. N.; Keith, T. A.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar,
 S. S.; Tomasi, J.; Cossi, M.; Millam, J. M.; Klene, M.; Adamo, C.; Cammi, R.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.;
 Farkas, O.; Foresman, J. B.; Fox, D. J. Gaussian, Inc., Wallingford CT, 2016.
- S5 Lodewyk, M. W.; Siebert, M. R.; Tantillo, D. J. Computational Prediction of ¹H and ¹³C Chemical Shifts: A Useful Tool for Natural Product, Mechanistic, and Synthetic Organic Chemistry. *Chem. Rev.* 2012, *112* (3), 1839–1862, DOI: 10.1021/cr200106v.
- S6 Bruhn, T.; Schaumlöffel, A.; Hemberger, Y.; Pescitelli, G. SpecDis ver-sion 1.71, Berlin, Germany, 2017, http://specdissoftware.jimdo.com.

- S7 Rárová, L.; Steigerová, J.; Kvasnica, M.; Bartůněk, P.; Křížová, K.; Chodounská, H.; Kolář, Z.; Sedlák, D.; Oklestkova, J.; Strnad, M. Structure Activity Relationship Studies on Cytotoxicity and the Effects on Steroid Receptors of AB-Functionalized Cholestanes. *J. Steroid Biochem. Mol. Biol.* 2016, 159, 154–169.
- S8 Morrogh-Bernard, H. C.; Foitová, I.; Yeen, Z.; Wilkin, P.; de Martin, R.; Rárová, L.; Doležal, K.; Nurcahyo, W.; Olšanský, M. Self-Medication by Orang-Utans (Pongo Pygmaeus) Using Bioactive Properties of *Dracaena cantleyi. Sci. Rep.* 2017, 7(1), 16653.

| | | М | aior rotamer | Minor |
|-------|-------------------------|------|------------------------------------|---------|
| | | 101 | | rotamer |
| Pos. | δc | | δн, mult (J in Hz) | δн |
| 1 | 141.2 (C) | | - | - |
| 1-OMe | 62.5 (CH3) | | 3.87, s | 3.92 |
| 2 | 129.8 (C) | | - | - |
| 3 | 139.6 (C) | | - | - |
| 4 | 143.1 (C) | | - | - |
| 5 | 104.1 (CH) | | 6.98, s | 6.98 |
| 6 | 152.0 (C) | | - | - |
| 6-OMe | 56.0 (CH3) | | 3.97, s | 3.97 |
| 7 | 130.4 (CH) | | 6.75, dd (4.7, 1.7) | 6.67 |
| 8 | 140.0 (CH) | | 6.30, dd (4.7, 1.5) | 6.28 |
| 9 | 51.7 (CH) | | 4.80, br. d (11.2) | 3.60 |
| 10 | 126.8 (CH) | | 4.63, t (11.2) | 5.01 |
| 11 | 132.1 (CH) | | 5.16, ddd (11.8, 11.2, 4.8) | 5.78 |
| 12 | 27.5 (CH ₂) | proR | 2.99, dddd (13.6, 12.6, 11.8, 7.0) | 2.15 |
| | | proS | 2.43, ddd (13.6, 6.0, 4.8) | 2.43 |
| 13 | 34.2 (CH ₂) | proR | 2.65, ddd (14.4, 12.6, 6.0) | 2.61 |
| | | proS | 3.38, dd (14.4, 7.0) | 2.63 |
| 1' | 151.9 (C) | | - | - |
| 1'-OH | - | | 8.18, s | 7.46 |
| 2' | 123.0 (C) | | - | - |
| 3' | 134.9 (CH) | | 7.74, br. s | 8.44 |
| 4' | 132.0 (C) | | - | - |
| 5' | 129.9 (CH) | | 6.89, br. d (8.0) | 7.01 |
| 6' | 116.7 (CH) | | 6.85, d (8.0) | 6.85 |

Table S1. $^1\mathrm{H}$ and $^{13}\mathrm{C}$ NMR Data of zosteraphenol B (2) (700 MHz, 258 K, CDCl_3)



Table S2. Cartesian coordinates of the lowest-energy conformations of the two rotamers of zosteraphenol A (1), optimized at the B3LYP/6-31+G(d,p) level.

| Major rotamer (| (1a), E = -998.647341 | hartree |
|-----------------|-----------------------|---------|

| C | 3 236618 | 0 356271 | -0.006001 |
|--------|-----------|----------------|-----------|
| c | 3.015391 | 1 726151 | -0 123069 |
| C | 1 605605 | 2 178117 | 0.000070 |
| c | 0.594072 | 1 208/67 | -0.077770 |
| C | 0.807505 | 0.097126 | 0.113641 |
| C | 0.807303 | 0.522202 | 0.113041 |
| C | 2.130440 | -0.323292 | 0.123933 |
| C | 1 497990 | -1.047037 | 0.0112011 |
| C | -1.48/889 | -0.058570 | 0.8210// |
| C | -2./0559/ | -1.142192 | 0.552309 |
| C | -2.808599 | -2.218191 | -0.338061 |
| C | -1./29068 | -2./53441 | -0.936/9/ |
| C | -0.4/1923 | -2.160004 | -0./56942 |
| C | 1.2015// | 3.552355 | -0.20/346 |
| C | -0.141434 | 3.556776 | -0.1289/8 |
| С | -0.691307 | 2.154195 | 0.066221 |
| С | -3.957178 | -0.308655 | 0.982060 |
| Н | -3.092028 | 1.624854 | 1.516427 |
| С | -3.710210 | 1.216422 | 0.710590 |
| 0 | 4.513145 | -0.125858 | -0.021606 |
| Н | 3.857806 | 2.401128 | -0.233227 |
| 0 | 2.490656 | -1.881024 | 0.202404 |
| Η | -1.381820 | 0.161560 | 1.519434 |
| Η | -3.845117 | -2.624721 | -0.591135 |
| Η | -1.797056 | -3.595669 | -1.618106 |
| 0 | 0.548540 | -2.662697 | -1.508964 |
| Н | 1.400701 | -2.467225 | -1.074002 |
| Н | 1.841929 | 4.419980 | -0.324828 |
| Н | -0.789581 | 4.424570 | -0.173553 |
| С | -1.809475 | 1.840712 | -0.911368 |
| Н | -4.195557 | -0.430112 | 2.047244 |
| Н | -4.840988 | -0.642026 | 0.425821 |
| С | -3.079788 | 1.497538 | -0.635022 |
| H | -4.674955 | 1.735555 | 0.779551 |
| С | 2.356846 | -2.4772.32 | 1.513631 |
| Н | 4.457426 | -1.094617 | -0.047548 |
| Н | -3.734854 | 1.333125 | -1.491277 |
| н | -1 528146 | 1 944685 | -1 958624 |
| н | 3,001122 | -1 954727 | 2.228219 |
| н | 1 316853 | -2 437091 | 1 848018 |
| н ц | 2 675542 | 2.437091 | 1.0+0010 |
| и П | 1.006066 | 2 12/12/ | 1.408605 |
| 11 | -1.090000 | 2.124134 | 1.000907 |
| Min | r = -008 | 646161 hartroo | |
| C | 2202564 | 0.488204 | 0 120026 |
| C | 3.202304 | 0.400294 | -0.150920 |
| C | 2.911/4/ | 1.820330 | 0.160399 |
| C | 1.5/2160 | 2.181/46 | 0.312/32 |
| C | 0.51/1/2 | 1.255/64 | 0.140377 |
| С | 0.809109 | -0.106952 | -0.089326 |
| C | 2.163964 | -0.449861 | -0.242605 |
| C | -0.288321 | -1.114071 | -0.024017 |
| С | -1.475124 | -0.785139 | -0.686466 |
| С | -2.721692 | -1.287048 | -0.316426 |
| С | -2.739896 | -2.324996 | 0.626550 |
| С | -1.549190 | -2.816978 | 1.164818 |
| С | -0.321153 | -2.205424 | 0.875223 |
| С | 1.013417 | 3.448483 | 0.793877 |
| С | -0.318467 | 3.325876 | 0.948146 |
| | | | |

| С | -0.812788 | 1.971915 | 0.460242 |
|---|-----------|-----------|-----------|
| С | -3.954032 | -0.515705 | -0.738216 |
| Н | -3.612504 | 0.897615 | 0.883342 |
| С | -3.904420 | 0.947393 | -0.171390 |
| 0 | 4.502137 | 0.094851 | -0.266095 |
| Н | 3.722575 | 2.526194 | 0.308652 |
| 0 | 2.564059 | -1.781235 | -0.409483 |
| Н | -1.424949 | -0.006582 | -1.431725 |
| Η | -3.687115 | -2.748681 | 0.952485 |
| Η | -1.553003 | -3.642012 | 1.870261 |
| 0 | 0.778107 | -2.668296 | 1.539550 |
| Н | 1.580221 | -2.444393 | 1.032470 |
| Н | 1.607325 | 4.327357 | 1.020968 |
| Н | -1.000523 | 4.098854 | 1.283524 |
| С | -1.732611 | 2.257686 | -0.725534 |
| Н | -4.853518 | -1.010576 | -0.354949 |
| Η | -4.054019 | -0.473984 | -1.830610 |
| С | -3.001051 | 1.874729 | -0.953135 |
| Н | -4.921158 | 1.359915 | -0.199918 |
| С | 2.324948 | -2.342025 | -1.720349 |
| Н | 4.508381 | -0.874496 | -0.317507 |
| Η | -3.438857 | 2.234501 | -1.885891 |
| Н | -1.267943 | 2.896667 | -1.476687 |
| Η | 2.710096 | -3.362513 | -1.688679 |
| Н | 1.254819 | -2.353637 | -1.943554 |
| Н | 2.859143 | -1.760919 | -2.479431 |
| Н | -1.361402 | 1.430022 | 1.240411 |

| | major rotamer (1a) | | | mi | nor rotamer | (1b) | | | |
|----------------------|-----------------------------|-----------|-----------------------|------------------------|---------------------|-----------|-----------------------|-----------------------|--|
| | exp. δ _H | shielding | calcd. δ_{H^a} | $\Delta\delta_{\rm H}$ | exp. δ _H | shielding | calcd. δ_{H^a} | $\Delta\delta_{ m H}$ | |
| H-5 | 6.99 | 24.34 | 6.88 | -0.11 | 6.97 | 24.37 | 6.85 | -0.12 | |
| H-7 | 6.72 | 24.52 | 6.72 | 0.00 | 6.63 | 24.61 | 6.63 | 0.00 | |
| H-8 | 6.29 | 24.94 | 6.32 | 0.03 | 6.28 | 25.01 | 6.26 | -0.02 | |
| H-9 | 4.79 | 26.58 | 4.81 | 0.02 | 3.57 | 27.82 | 3.67 | 0.10 | |
| H-10 | 4.61 | 26.69 | 4.71 | 0.10 | 5.04 | 26.22 | 5.14 | 0.10 | |
| H-11 | 5.16 | 26.02 | 5.33 | 0.17 | 5.77 | 25.38 | 5.92 | 0.15 | |
| $H-12_{proR}$ | 2.99 | 28.52 | 3.02 | 0.03 | 2.15 | 29.37 | 2.24 | 0.09 | |
| H-12 _{proS} | 2.43 | 29.16 | 2.43 | 0.00 | 2.43 | 29.17 | 2.43 | 0.00 | |
| H-13proR | 2.65 | 28.90 | 2.67 | 0.02 | 2.65 | 28.91 | 2.67 | 0.02 | |
| H-13proS | 3.38 | 28.12 | 3.40 | 0.02 | 2.63 | 29.00 | 2.58 | -0.05 | |
| H-3' | 7.76 | 23.45 | 7.70 | -0.06 | 8.46 | 22.68 | 8.41 | -0.05 | |
| H-5' | 6.90 | 24.36 | 6.86 | -0.04 | 7.02 | 24.22 | 6.99 | -0.03 | |
| H-6' | 6.85 | 24.47 | 6.76 | -0.09 | 6.85 | 24.46 | 6.77 | -0.08 | |
| | | 28.07 | | | | 27.69 | | | |
| 1-OMe | 3.88 | 27.66 | 3.68 ^b | -0.20 | 3.97 | 27.55 | 3.75 ^b | -0.22 | |
| | | 27.71 | | | | 27.98 | | | |
| | | | RMSD ¹ H | 0.09 | | | RMSD ¹ H | 0.10 | |
| | exp. δ _C | shielding | calcd. δ_{C^c} | $\Delta\delta_{\rm C}$ | | | | | |
| C-1 | 139.7 | 42.52 | 138.2 | -1.5 | | | | | |
| C-2 | 128.3 | 51.27 | 129.8 | 1.5 | | | | | |
| C-3 | 139.5 | 41.35 | 139.3 | -0.2 | | | | | |
| C-4 | 143.6 | 36.76 | 143.7 | 0.1 | | | | | |
| C-5 | 107.7 | 76.37 | 105.8 | -1.9 | | | | | |
| C-6 | 147.9 | 31.74 | 148.5 | 0.6 | | | | | |

0.9

2.4

2.4

2.6

2.8

1.3

2.1

1.3

-0.2

0.0

-0.9

-0.5

-0.9

-3.3

1.7

Table S3. Experimental chemical shifts, calculated isotropic shielding constants, and predicted chemical shifts of the two rotamers of zosteraphenol A (1). Calculations were performed at the PBE0/6-311+G(2d,p)/PCM(CHCl₃)//B3LYP/6-31+G(d,p) level.

^a ¹H chemical shifts were obtained from the computed isotropic shielding constants according to ref. 5, using the equation: $\delta = (31.7532 - \text{shielding})/1.0958$

^b The average chemical shift of the three methyl protons is reported

C-7

C-8

C-9

C-10

C-11

C-12

C-13

C-1'

C-2'

C-3'

C-4'

C-5'

C-6'

C-OMe

130.4

140.0

51.4

127.0

132.0

27.5

34.2

151.6

122.4

134.8

132.3

130.1

116.5

62.8

49.70

38.05

130.63

51.42

46.00

156.77

148.96

27.15

59.15

46.01

49.53

51.42

66.06

124.63

131.3

142.4

53.8

129.6

134.8

28.8

36.3

152.9

122.2

134.8

131.4

129.6

115.6

59.5

RMSD ¹³C

 $^{c-13}$ C chemical shifts were obtained from the computed isotropic shielding constants according to ref. 5, using the equation: $\delta = (187.3123 - \text{shielding})/1.0533$

| | | $\delta(1b) - \delta(1a)$ | |
|----------|-------|---------------------------|-------|
| | exp. | calcd. | error |
| H-5 | -0.02 | -0.03 | -0.01 |
| H-7 | -0.09 | -0.09 | +0.00 |
| H-8 | -0.01 | -0.06 | -0.05 |
| H-9 | -1.22 | -1.14 | +0.08 |
| H-10 | +0.43 | +0.43 | +0.00 |
| H-11 | +0.61 | +0.59 | -0.02 |
| H-12proR | -0.84 | -0.78 | +0.06 |
| H-12proS | +0.00 | -0.01 | -0.01 |
| H-13proR | +0.00 | -0.01 | -0.01 |
| H-13proS | -0.75 | -0.82 | -0.07 |
| H-3' | +0.70 | +0.71 | +0.01 |
| H-5' | +0.12 | +0.13 | +0.01 |
| H-6' | +0.00 | +0.02 | +0.02 |
| OMe | +0.09 | +0.02 | -0.07 |

Table S4. Experimental and calculated chemical shift differences between the minor (1b) and the major (1a) rotamers of zosteraphenol A.

 Table S5. Rotatory strengths (length formalism) calculated for rotamers 1a and 1b.

| major rotamer (1a) | | minor rotamer (1a) | | |
|-----------------------------|-----------------------------------------------------|-----------------------------|-----------------------------------------------------|--|
| Wavelength | Rotatory strength | Wavelength | Rotatory strength | |
| (nm) | (10 ⁻⁴⁰ erg esu cm Gauss ⁻¹) | (nm) | (10 ⁻⁴⁰ erg esu cm Gauss ⁻¹) | |
| 174.3 | 2.526000 | 175.2 | 5.845800 | |
| 174.9 | 11.100900 | 175.4 | 10.798600 | |
| 175.3 | -8.707300 | 175.7 | 2.925100 | |
| 175.9 | 3.713700 | 175.9 | -37.875900 | |
| 176.1 | 9.015100 | 176.2 | -5.918100 | |
| 176.4 | -2.029500 | 176.9 | 4.093400 | |
| 176.8 | -2.784600 | 177.1 | 7.116600 | |
| 177.2 | 16.889600 | 177.8 | 9.999500 | |
| 177.4 | -22.305500 | 178.3 | 5.861500 | |
| 178.0 | 9.300700 | 178.7 | -5.385900 | |
| 178.2 | 12.834100 | 179.0 | -9.374400 | |
| 179.0 | -56.526700 | 179.3 | 50.183300 | |
| 179.5 | 45.076900 | 179.6 | 17.229700 | |
| 180.2 | -19.352000 | 180.0 | 8.109500 | |
| 180.7 | 34.096000 | 180.4 | 2.476300 | |
| 181.6 | 6.834000 | 180.8 | -6.145500 | |
| 182.0 | 12.124300 | 181.6 | 1.643900 | |
| 182.7 | -62.245700 | 181.8 | -38.750300 | |
| 183.4 | -0.942800 | 182.2 | 3.609900 | |
| 184.3 | 16.294400 | 183.2 | -19.427600 | |
| 184.9 | 2.420100 | 183.8 | -10.055800 | |
| 185.3 | 41.504500 | 184.9 | 0.666200 | |
| 185.6 | 10.734900 | 185.5 | -3.247700 | |
| 186.1 | -2.384000 | 185.7 | 1.013500 | |
| 186.9 | -12.282200 | 186.0 | 1.455800 | |
| 187.3 | -9.672800 | 186.8 | -0.874000 | |
| 187.9 | -16.267400 | 187.2 | -26.098200 | |
| 188.3 | 3.147600 | 187.4 | 11.149400 | |
| 188.8 | 6.762600 | 189.0 | 21.834800 | |
| 189.0 | -6.593100 | 189.4 | -70.338600 | |
| 190.1 | -44.721300 | 190.7 | -22.213300 | |
| 190.5 | 40.798200 | 190.9 | 11.796100 | |
| 191.5 | -25.100700 | 191.9 | -4.294700 | |
| 192.1 | 10.890600 | 193.0 | -32.158300 | |
| 193.0 | 27.666200 | 193.6 | -24.222000 | |
| | | | | |

| 193.6 | -8.476600 | 193.8 | -4.030400 |
|-------|------------|-------|-------------|
| 194.1 | -18.017100 | 194.3 | -40.140300 |
| 194.6 | 19.724300 | 194.8 | -66.379000 |
| 195.4 | -15.080700 | 195.1 | -8.110000 |
| 196.4 | 26.524000 | 196.6 | 70.683700 |
| 197.4 | -13.998700 | 197.3 | -12.739000 |
| 197.8 | -24.407200 | 197.9 | 25.611000 |
| 198.3 | 30.724700 | 198.0 | -17.786600 |
| 198.9 | 7.236700 | 198.8 | 11.932400 |
| 199.4 | -14.311500 | 200.6 | 35.600800 |
| 200.8 | -1.007300 | 200.9 | 3.176700 |
| 201.3 | -16.694500 | 201.4 | -0.576200 |
| 201.9 | 13.868700 | 202.9 | -74.876300 |
| 202.7 | 2.659500 | 203.7 | -17.119700 |
| 203.9 | 7.713500 | 205.1 | -0.385200 |
| 205.1 | 14.131400 | 206.2 | -29.953000 |
| 206.5 | 32.822500 | 207.6 | -14.451600 |
| 208.1 | -36.978400 | 208.4 | 3.164300 |
| 210.7 | 13.240300 | 208.7 | 22.589700 |
| 211.2 | 20.696600 | 211.4 | -23.361000 |
| 212.4 | -18.249200 | 212.2 | 70.924700 |
| 212.6 | 154.360500 | 212.5 | -106.583700 |
| 213.6 | 0.499900 | 212.9 | 16.831200 |
| 215.8 | 60.793700 | 215.1 | 17.916600 |
| 218.5 | -46.097100 | 217.4 | -28.223600 |
| 220.0 | -50.096900 | 217.6 | 18.293300 |
| 221.0 | -24.959500 | 221.4 | -23.792000 |
| 222.9 | -45.006600 | 221.9 | -25.812500 |
| 225.3 | -63.064600 | 223.9 | 5.690600 |
| 227.0 | 35.844500 | 226.1 | 136.279200 |
| 230.6 | -6.871200 | 232.0 | 6.096300 |
| 233.0 | -16.674900 | 232.5 | 44.209100 |
| 235.4 | 8.636100 | 235.0 | 14.964800 |
| 236.3 | -64.752600 | 238.4 | 29.264800 |
| 238.0 | 9.117700 | 239.6 | 19.702400 |
| 240.8 | -22.538700 | 242.0 | -1.432600 |
| 245.1 | -14.510100 | 246.5 | -87.201300 |
| 247.6 | 10.544300 | 249.1 | 35.901300 |
| 250.4 | -7.120400 | 250.9 | 38.139200 |
| 256.0 | -18.868600 | 259.2 | 11.015400 |
| 268.8 | 11.983200 | 264.7 | -45.935000 |
| 275.3 | 16.270700 | 273.4 | -24.125700 |
| 280.4 | -19.832600 | 279.9 | 24.051100 |
| 296.4 | -15.278000 | 294.4 | 46.060000 |
| 311.6 | 58.923700 | 316.7 | -46.777300 |

Table S6. Cytotoxicity (μ M) in human acute lymphoblastic leukemia (CEM), human breast adenocarcinoma (MCF7), human cervical carcinoma (HeLa) cells, and normal fibroblasts (BJ) after 72 h of treatment of zosteraphenols A (1) and B (2) and isotedarene A (4). Experiment was repeated three times in triplicates.

| | IC ₅₀ (μM) | | | | |
|----------|-----------------------|------|--------------|-----|--|
| Compound | CEM | MCF7 | HeLa | BJ | |
| 1 | >50 | >50 | >50 | >50 | |
| 2 | >50 | >50 | >50 | >50 | |
| 4 | 29.9 ± 3.0 | >50 | 27.7 ± 7.4 | >50 | |



Figure S3. High-resolution ESI-MS spectrum of zosteraphenol A (1) (positive ion mode).



Figure S4. Full ^{1}H NMR spectrum (700 MHz, 258 K, CDCl3) of zosteraphenol A (1).



Figure S5. Expansion of ¹H NMR spectra (700 MHz, CDCl₃) of zosteraphenol A (1) at 298 K (top spectrum) and 258 K (bottom spectrum).





Figure S6. Full (top) and expanded (bottom) ¹³C NMR Spectrum (175 MHz, 258 K, CDCl₃) of zosteraphenol A (1).



Figure S7. COSY spectrum (700 MHz, 258 K, $CDCl_3$) of zosteraphenol A (1).



Figure S8. NOESY/EXSY spectrum (700 MHz, 258 K, CDCl₃) of zosteraphenol A (1). In the positive NOE regime, exchange peaks (black) have the same phase as diagonal peaks and NOE peaks (red) have opposite phase.



Figure S9. HSQC spectrum (700 MHz, 258 K, $CDCl_3$) of zosteraphenol A (1).



Figure S10. HMBC spectrum (700 MHz, 258 K, $CDCl_3$) of zosteraphenol A (1).



Figure S11. Expansion of HMBC spectrum (700 MHz, 258 K, CDCl₃) of zosteraphenol A (1).



Figure S13. UV and ECD spectra (CH_3OH) of zosteraphenol A (1).



Figure S14. High-resolution ESI-MS spectrum of zosteraphenol B (2) (positive ion mode).



Figure S15. Full 1H NMR spectrum (700 MHz, 258 K, CDCl₃) of zosteraphenol B (2).



Figure S16. Expansion of ¹H NMR spectra (700 MHz, CDCl₃) of zosteraphenol B (2) at 298 K (top spectrum) and 258 K (bottom spectrum).



Figure S17. Full (top) and expanded (bottom) ¹³C NMR Spectrum (175 MHz, 258 K, CDCl₃) of zosteraphenol B (2).



Figure S18. COSY spectrum (700 MHz, 258 K, CDCl₃) of zosteraphenol B (2).



Figure S19. NOESY/EXSY spectrum (700 MHz, 258 K, CDCl₃) of zosteraphenol B (2). In the positive NOE regime, exchange peaks (black) have the same phase as diagonal peaks and NOE peaks (red) have opposite phase.



Figure S20. HSQC spectrum (700 MHz, 258 K, CDCl₃) of zosteraphenol B (2).



Figure S21. Expansion of the HMBC spectrum (700 MHz, 258 K, CDCl₃) of zosteraphenol B (2).



Figure S22. HMBC spectrum (700 MHz, 258 K, CDCl₃) of zosteraphenol B (2).



Figure S23. UV and ECD spectra (CH_3OH) of zosteraphenol B (2).