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Sphaeropsidin A C15-C16 Cross-Metathesis Analogues with Potent Anticancer Activity

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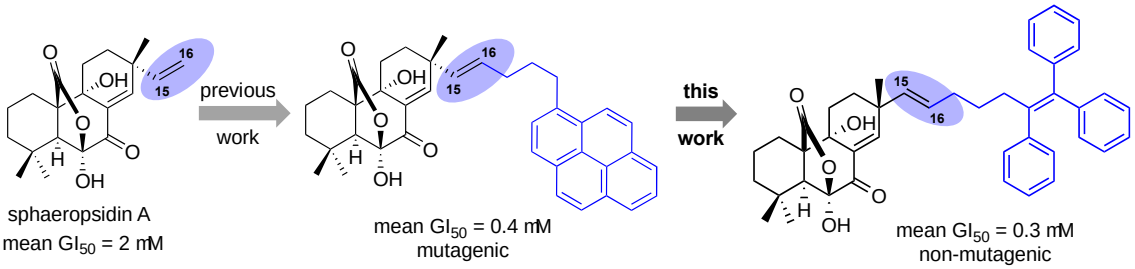

Abstract: We recently discovered that sphaeropsidin A (SphA), a fungal metabolite from *Diplodia cupressi*, overcomes apoptosis resistance in cancer cells by inducing cellular shrinkage by impairing regulatory volume increase. Previously, we prepared a pyrene-conjugated derivative of SphA by a cross-metathesis reaction involving the phytotoxin's C15,C16-alkene. This derivative's evaluation in a cancer cell panel revealed a significant increase in potency, with the IC_{50} values 5–10× lower than those displayed by the original natural product. Herein, we describe the preparation and anticancer evaluation of fifteen novel C15,C16-alkene cross-metathesis analogues in which the pyrene moiety was replaced with other aromatic or non-aromatic hydrophobic groups. The idea for this replacement was to prepare a family of compounds that would not be predicted to be mutagenic compared with the original pyrene analogue. We predict several of our new compounds to be non-mutagenic, while retaining the high potency of the original pyrene-containing analogues. Examples of these potential lead compounds included those containing pentamethylphenyl and triphenylethylene pendant groups. As an additional feature of the current investigation, we prepared several deuterated pyrene-containing compounds to overcome intellectual property issues associated with non-patentability of the original pyrene derivative.

Introduction

Alterations in ion channels and transporters play an integral part in cancer[1,2] and are often important mechanisms by which cancer cells develop resistance to apoptosis.[3] One such mechanism that precedes the cytochrome C release, caspase-3 activation or DNA laddering,[4,5] involves impairment of the reduction in cell volume, an important hallmark of apoptosis.[6] Indeed, a broad-spectrum caspase inhibitor has been shown to block these biochemical apoptotic events, but failed to block cell shrinkage, indicating that it is an early prerequisite to

apoptotic cell death.[4] Cells develop apoptosis resistance through various mechanisms, including regulatory volume increase (RVI), whereby the volume of shrunken cells is restored.[7-9]

Our team recently discovered that sphaeropsidin A (SphA (**1**), for structure see Fig. 1), a fungal metabolite from *Diplodia cupressi*, overcomes apoptosis resistance in cancer cells by inducing a marked and rapid cellular shrinkage through the impairment of RVI.[10] Cell shrinkage was determined to be the cause, not the consequence of apoptosis, and was related to the rapid loss of intracellular Cl^-.[10] Our results suggest that the prolonged effect of SphA (**1**) on [Cl^-] leads to sustained cellular shrinkage, which triggers apoptosis directly, thus bypassing the classical signaling pathways and overcoming apoptosis resistance.

Against the NCI panel of 60 cell lines, SphA (**1**) registered GI_{50} in the range 0.3−6.3 μM, with an average value of 1.6 μM.[10] The lack of close correlations of the differential cellular sensitivities with 763,000 compounds in the NCI database suggested a possibly novel mechanism of action. Multidrug resistant cell sublines, characterized by ABCB1, ABCG2, ABCC1 and MVP-mediated resistance mechanisms, showed similar or even higher sensitivity to SphA (**1**) than the parent cell lines. Normal cells, such as melanocytes, were an order of magnitude less sensitive (GI_{50} = 13 μM).[10]

We recently prepared pyrene-conjugated derivative **2** of SphA (**1**) by a cross-metathesis reaction involving the C15,C16-alkene (Fig. 1).[11] Its evaluation for *in vitro* anticancer effects revealed a significant increase in potency with the IC_{50} values 5 to 10 times lower than those displayed by natural product **1**. We found that **2** triggered severe ER swelling associated with strong proteasomal inhibition and consequent cell death, a feature not observed in the natural product's mode of action. Furthermore, analysis of the NCI 60 cell line testing did not reveal any correlations between the pyrene derivative **2** and any other compound in the database (including SphA), except at high concentrations (LC_{50}). Importantly, 1-(pent-4-en-1-yl)pyrene (**3**) was determined to be inactive when administered alone, and its efficacy in combination with **1** (as a 1:1 mixture) was equivalent to **1** alone. This suggested that the SphA and pyrene fragments in **2** do not engage two separate targets in cancer cells.

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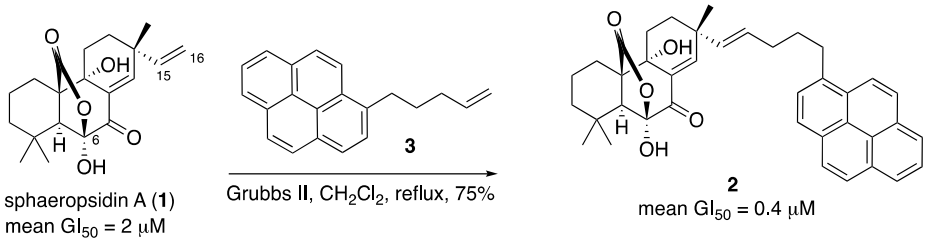

Figure 1. Synthesis of pyrene-conjugated SphA derivative **2**.

A recent study looked at the mutagenic groups in drug scaffolds to assist medicinal chemists in preventing the development of potentially mutagenic therapeutic agents in early drug discovery.[12] This study identified several core structural features in drugs contributing to their mutagenicity using a scaffold analysis, and pyrene was identified as one of four major mutagenic groups, along with acridine, phenanthrene and quinoxaline. Indeed, pyrene was considered the most important of the four, as all 39 pyrene-containing compounds analyzed were mutagenic. Apparently, any compound with pyrene as part of its structure should be avoided when selecting a drug candidate.[12] Thus, we wondered whether the pyrene moiety was necessary for the high potencies of the C15,C16-alkene conjugates and whether non-pyrene moieties could be used in derivatives of this type. Furthermore, a student thesis describing the pyrene conjugate **2** was released to the public domain as its embargo had expired and we were not in a position to secure it in the form of intellectual property. To remedy the situation, deuterated analogues of **2** were prepared and we were able to protect them with a patent application. Preparation and biological evaluation of such deuterated analogues is described herein as well.

Results and discussion

Synthetic Chemistry

We started by developing methods to synthesize pentenylarenes analogous to 1-(pentenyl)pyrene (**3**) in Figure 1 as precursors for cross-metathesis. Figure 2 shows two sets of conditions for the preparation of pentenylarenes **4–11**. Of interest, Grignard reagents derived from bromides leading to pentenylarenes **4–7** underwent smooth coupling with 5-bromo-1-pentene without any need for a copper catalyst (conditions A, Fig. 2). In contrast, in synthetic preparation of pentenylarenes **8–11** copper catalysis was found to be beneficial (conditions B, Fig. 2).

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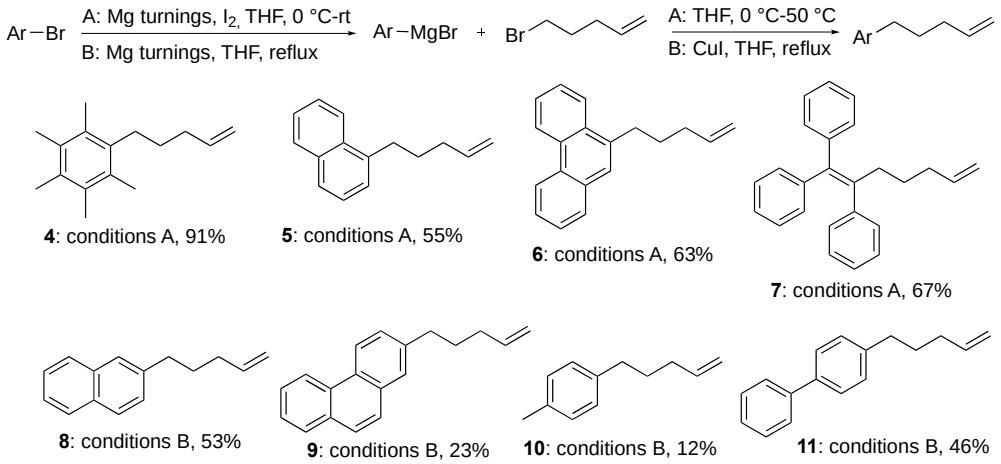

Figure 2. Two sets of conditions (**A** and **B**) for the preparation of pentenylarenes **4–11**.

Pentenylanthracene (**12**) and pentenylfluorene (**13**) cross-metathesis precursors were synthesized using lithiation procedures shown in Figure 3. Coupling with 5-bromo-1-pentene did not require a copper catalyst and proceeded smoothly with the organolithium compounds.

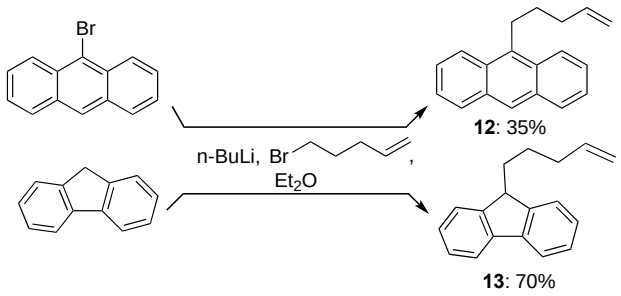

Figure 3. Lithiation procedures used for the synthesis of pentenyl compounds **12** and **13**.

To synthesize regioisomeric cross-coupling partners based on substituted pyrene **3** (Fig. 1), 2-pentenylpyrene (**17**) (Fig. 4), we followed a reported procedure involving a regiospecific borylation of pyrene with an iridium-based catalyst prepared *in situ* from [{Ir(μ-OMe)cod}$_2$] with 4,4′-di-*tert*-butyl-2,2′-bipyridine to give 2-(Bpin)pyrene (**14**) in a satisfactory yield of 72%.[13] Reaction of the latter with three equivalents of $CuBr_2$ in MeOH/H_2O (1:1) gave 2-bromopyrene (**15**), which underwent a smooth Suzuki–Miyaura coupling reaction with 4-pentenylboronic acid pinacol ester (**16**) using a catalytic combination of $Pd(OAc)_2$ and RuPhos[14] to give the desired 2-(pent-4-en-1-yl)pyrene (**17**) in a reasonable yield of 64%.

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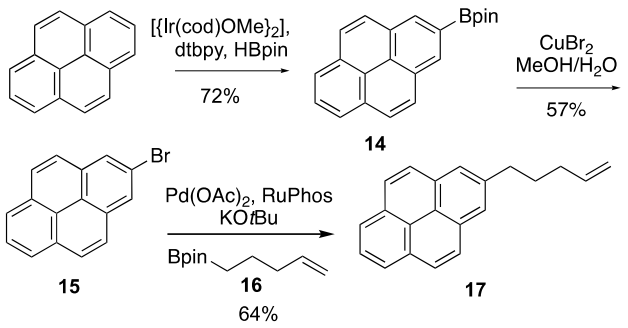

Figure 4. Synthesis of regioisomeric 2-(pent-4-en-1-yl)pyrene (**17**).

Next, we initiated the synthesis of deuterated pentenylpyrene cross-metathesis precursors. Using a reported procedure for perdeuteration of pyrene,[15] involving deprotonation/reprotonation in a mixture of potassium *tert*-butoxide in DMF-d_7, we obtained pyrene-d_{10} (**18**) with >95% deuteration (Fig. 5). Regioselective bromination[16] of this material with NBS in CH_2Cl_2 afforded 1-bromopyrene-d_9 (**19**), which underwent Suzuki–Miyaura coupling with 4-pentenylboronic acid pinacol ester (**16**) using our standard conditions[14] to give the desired 1-(pent-4-en-1-yl)pyrene-2,3,4,5,6,7,8,9,10-d_9 (**20**) in 67% yield.

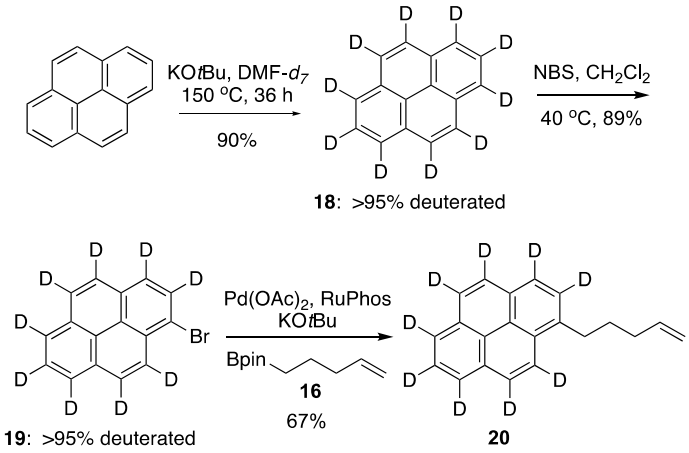

Figure 5. Synthesis of 1-(pent-4-en-1-yl)pyrene-2,3,4,5,6,7,8,9,10-d_9 (**20**)

To synthesize a monodeuterated pyrene cross-metathesis precursor with the pentenyl group at position 1, pinacol boronate **14** was regioselectively brominated at C-1 to give **21** in an acceptable yield of 51% (Fig. 6). The latter was deuterated using a procedure adapted from literature[17] to give monodeuterated bromide **22** in 79% yield. Finally, a Suzuki–Miyaura coupling with 4-pentenylboronic acid pinacol ester **16** at the site of bromide

location on the pyrene ring applying our established procedure[14] provided the desired 1-(pent-4-en-1-yl)pyrene-7-*d* (**23**).

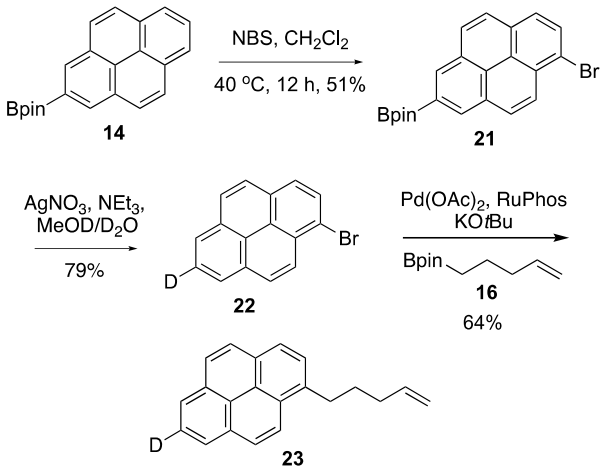

Figure 6. Synthesis of 1-(pent-4-en-1-yl)pyrene-7-*d* (**23**).

Finally, in order to synthesize a monodeuterated pyrene cross-metathesis precursor with the pentenyl group at position 2 of the pyrene, we again followed the reported procedure involving a regiospecific C-2 borylation of pyrene using [{Ir(μ-OMe)cod}$_2$] and 4,4′-di-*tert*-butyl-2,2′-bipyridine.[13] Application of this approach to 2-bromopyrene (**15**) provided pinacol boronate **24** in an admittedly low yield of 27% (Fig. 7). Deuteration of the latter gave monodeuterated 2-bromopyrene **25**, which underwent a Suzuki–Miyaura coupling as before[14] at the brominated site on the pyrene to give the desired 2-(pent-4-en-1-yl)pyrene-7-*d* (**26**).

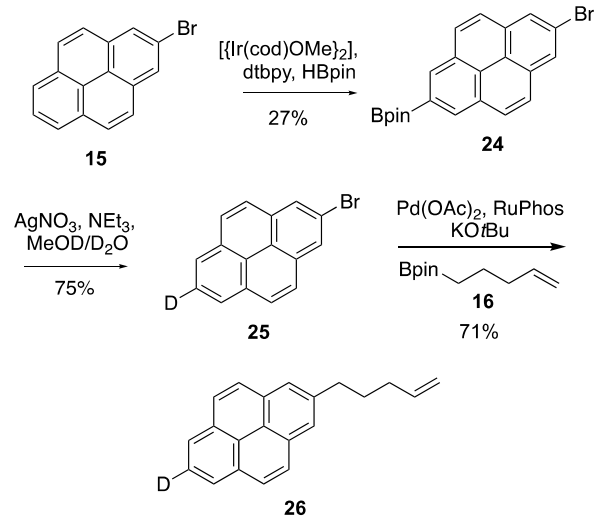

Figure 7. Synthesis of 2-(pent-4-en-1-yl)pyrene-7-*d* (**26**)

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We then employed the synthesized pentenylarenes **4–11**, **17**, **20**, **23** and **26** as substrates in the cross-metathesis reaction with SphA (**1**) using the Grubbs II complex as a catalyst. As previously observed, we found that SphA (**1**) does not homo-couple when the cross-metathesis was performed in refluxing CH_2Cl_2, most likely due to steric hindrance. To achieve good cross-metathesis yields, we added pentenylarenes in three portions to the refluxing mixture of SphA (**1**) and Grubbs II catalyst to lower the probability of the pentenylarene homo-coupling. The reactions were generally complete within 2 h, and gave solely *E*-alkenes as established by *J*-coupling. The structures of the synthesized C15-C16 cross-metathesis analogues and their yields are provided in Figure 8.

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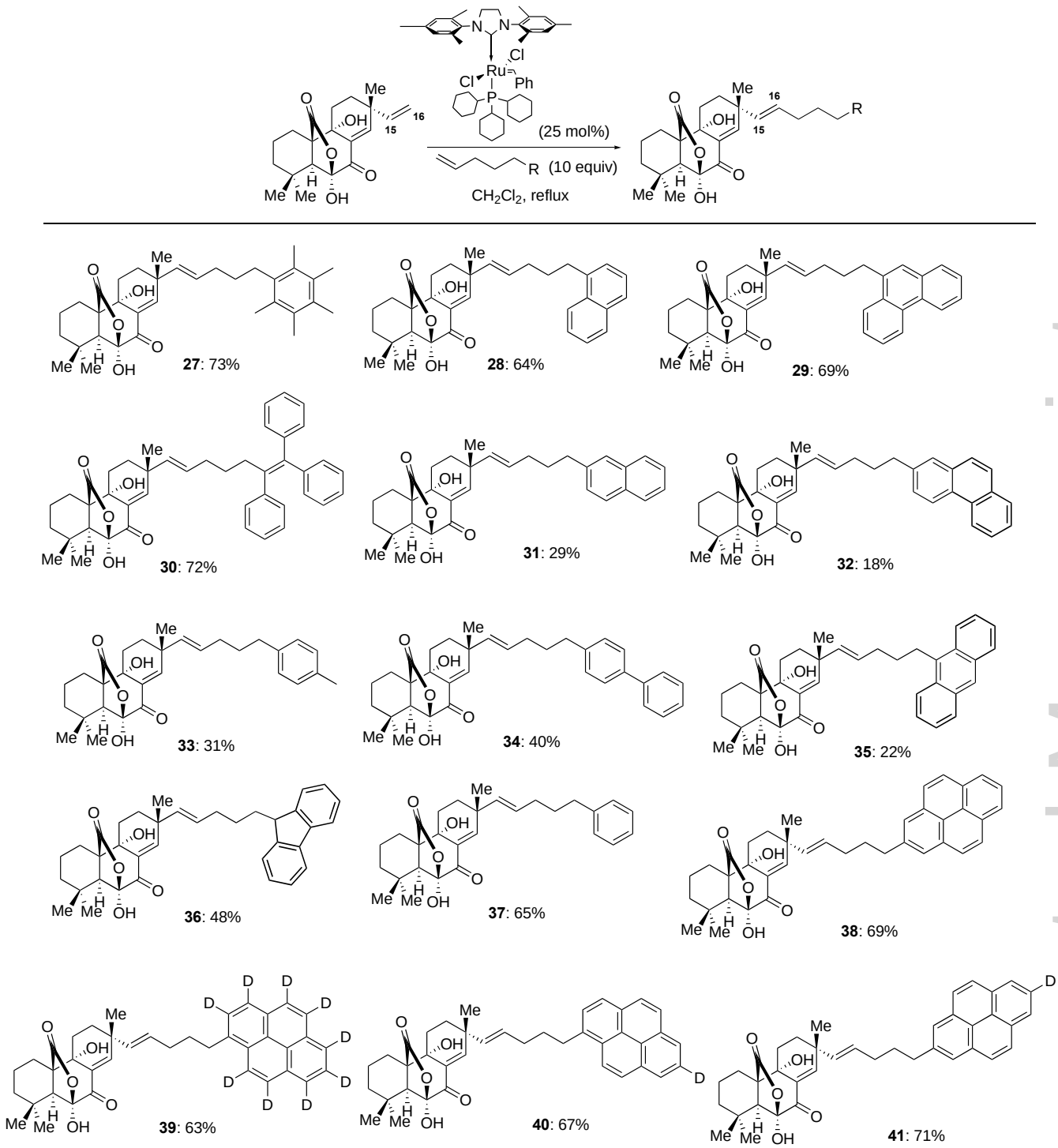


Figure 8. Cross-metathesis reaction between SphA (**1**) and pentenylarenes, whose syntheses are provided in Figures 2–7. Shown are the structures of the product C15-C16-alkene cross-metathesis analogues and their yields.

Biochemical Experiments

The synthesized cross-metathesis SphA analogues **2**, **27**–**41** as well as SphA (**1**) were evaluated for *in vitro* growth inhibition using the MTT colorimetric assay against a panel of five cancer cell lines. This included cells resistant to a number of pro-apoptotic stimuli, such as human A549 non-small cell lung cancer (NSCLC),[18] human glioblastoma U373,[19.20] and human SKMEL-28 melanoma,[21] as well as tumor models, which are largely susceptible to apoptosis-inducing stimuli, such as human Hs683 anaplastic oligodendroglioma[20] and mouse B16F10 melanoma[21] (Table 1).

Table 1. IC_{50} determined by MTT assays. Mean concentration in μM required to reduce the viability of cells by 50% after a 72 h treatment relative to the control. Data are expressed as mean ± SEM of the six replicates of one experiment. [a] Data are from ref. 11. [b] NA = not available.

Analogue	Resistant to apoptosis			Sensitive to apoptosis		
	A549	U373	SKMEL-28	Hs683	B16F10	mean
1[a]	1.71 ± 0.26	2.13 ± 0.17	2.32 ± 0.15	2.31 ± 0.23	1.55 ± 0.27	2.00 ± 0.16
2[a]	0.38 ± 0.03	0.57 ± 0.08	0.31 ± 0.01	0.35 ± 0.05	0.29 ± 0.01	0.38 ± 0.05
27	0.38 ± 0.02	0.39 ± 0.01	0.31 ± 0.01	0.34 ± 0.02	0.25 ± 0.02	0.33 ± 0.03
28	0.86 ± 0.01	1.17 ± 0.07	0.75 ± 0.02	0.90 ± 0.01	0.68 ± 0.02	0.87 ± 0.08
29	0.39 ± 0.01	0.43 ± 0.01	0.32 ± 0.02	0.35 ± 0.01	0.30 ± 0.01	0.36 ± 0.02
30	0.29 ± 0.02	0.40 ± 0.02	0.35 ± 0.01	0.31 ± 0.02	0.23 ± 0.01	0.32 ± 0.03
31	1.70 ± 0.09	1.21 ± 0.10	0.82 ± 0.03	2.07 ± 0.12	0.68 ± 0.01	1.30 ± 0.26
32	NA[b]	0.53 ± 0.01	0.34 ± 0.01	0.51 ± 0.01	0.29 ± 0.01	0.42 ± 0.06
33	2.05 ± 0.09	1.14 ± 0.10	2.11 ± 0.07	1.93 ± 0.05	0.89 ± 0.10	1.62 ± 0.25
34	0.79 ± 0.02	1.46 ± 0.22	0.63 ± 0.02	0.74 ± 0.01	0.40 ± 0.02	0.80 ± 0.18
35	NA	0.42 ± 0.01	0.32 ± 0.01	0.32 ± 0.01	0.24 ± 0.01	0.33 ± 0.04
36	NA	1.30 ± 0.08	0.74 ± 0.01	0.81 ± 0.02	0.56 ± 0.01	0.85 ± 0.16
37	2.57 ± 0.11	2.31 ± 0.23	2.81 ± 0.09	2.17 ± 0.21	1.75 ± 0.08	2.32 ± 0.18
38	0.37 ± 0.02	0.39 ± 0.02	0.33 ± 0.01	0.31 ± 0.03	0.33 ± 0.01	0.34 ± 0.01
39	NA	0.56 ± 0.02	0.35 ± 0.01	0.56 ± 0.01	0.31 ± 0.01	0.45 ± 0.07
40	NA	0.61 ± 0.03	0.36 ± 0.01	0.63 ± 0.03	0.33 ± 0.01	0.48 ± 0.08
41	NA	0.32 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	0.14 ± 0.01	0.27 ± 0.04

As expected, the deuterium analogues of the pyrene-conjugated derivative **2** with the pyrene attached at position 1, namely compounds **39** and **40**, exhibited similar antiproliferative properties to the original compound **2**. The regioisomeric attachment of the pyrene through position 2, present in analogues **38** and **41**, did not have a significant effect on activity. Here, again the deuterium analogue **41** showed similar antiproliferative properties to the hydrogen counterpart **38**. The rest of the data indicated that analogues containing small aromatics, such as α-naphthyl (**28**), β-naphthyl (**31**), *p*-methylphenyl (**33**), biphenyl (**34**) and phenyl (**37**), all showed lower potencies compared with analogues containing larger hydrophobic groups, such as pentamethylphenyl (**27**), 9-phenanthrene (**29**), triphenylethylene (**30**), 2-phenanthrene (**32**) and 9-anthracene (**35**).

As mentioned in the introduction, pyrene moieties are not an ideal in a drug candidate because they are potentially mutagenic. Therefore, we evaluated the mutagenic properties of synthesized molecules, containing alternative hydrophobic groups in this position. Normally, a battery of in vitro and/or in vivo assays is used to evaluate mutagenicity. Within this battery, the bacterial reverse gene mutation assay (also referred to as 'AMES assay') plays a central role. However, performing the Ames requires large amounts of test compound, that is often not available in the drug discovery stage. For this reason, in silico models for mutagenicity have been developed. These models rely on large datasets (in this case Ames mutagenicity data) to predict the mutagenic potenital of compounds solely based on their structure.[22] Herein, we used VEGA software[23] to perform a consensus assessment based on the predictions of four mutagenicity models (CAESAR, SARpy, ISS and KNN). For each prediction class (i.e. mutagenic or non-mutagenic) a score is calculated as the sum of the weights for each model and the calculated score is normalized, so that it is in the range 0–1. The consensus score is calculated separately for the two outcomes – mutagenic consensus score (CS_M) and non-mutagenic consensus score (CS_{NM}) and the compound is assigned to the positive class if $CS_M \geq CS_{NM}$. Based on these considerations, Table 2 provides mutagenic versus non-mutagenic predictions for the synthesized cross-metathesis SphA analogues. The results indicate that the mutagenic analogues include compounds with large aromatic

residues capable of DNA intercalation, such as compounds containing the pyrene (**2**), phenanthrene (**29** and **32**) and anthracene (**35**) residues. In contrast, non-mutagenic analogues include compounds with small aromatic residues, such as α-naphthyl (**28**), β-naphthyl (**31**), methylphenyl (**33**) and phenyl (**37**) or large non-intercalating moieties, such as pentamethylphenyl (**27**), triphenylethylene (**30**) and biphenyl (**34**). Of interest is the mutagenicity of the natural product (**1**) itself, whose activity likely stems from the electrophilicity of the enone functionality in SphA (**1**). Once the hydrophobic groups are added through C15,C16 cross-metathesis, for example resulting in potent non-intercalating pentamethylphenyl (**27**), triphenylethylene (**30**) analogues, these moieties decrease the importance/reactivity of individual toxicophores, such as the enone, by increasing the size of the molecule.

Table 2. Computational assessment of mutagenicity using VEGA.[23]

analogue	# models	CS_M	CS_{NM}	prediction
1	4	0.15	0.15	mutagenic
2	4	0.15	0.15	mutagenic
27	4	0	0.65	non-mutagenic
28	4	0.05	0.35	non-mutagenic
29	4	0.15	0.15	mutagenic
30	4	0	0.4	non-mutagenic
31	4	0	0.5	non-mutagenic
32	4	0.15	0.15	mutagenic
33	4	0	0.5	non-mutagenic
34	4	0	0.5	non-mutagenic
35	4	0.15	0.15	mutagenic
36	4	0.05	0.35	non-mutagenic
37	4	0	0.5	non-mutagenic
pyrene	3	1	0	mutagenic
anthracene	2	1	0	mutagenic
phenathrene	2	1	0	mutagenic

triphenylethylene	4	0.15	0.35	non-mutagenic
hexamethylbenzene	4	0	0.75	non-mutagenic

Since the mutagenicity data indicated that DNA intercalation might be responsible for this activity, whether it is part of its biological mechanism of action or an off-target effect, we used a DNA intercalation assay involving displacement of ethidium bromide (EtBr).[24] The fluorescence of EtBr increases 20-fold when it is intercalated into DNA. Monitoring its fluorescence decrease will thus be proportional to its displacement from DNA by another intercalator. Here, we used positive control proflavine and negative vehicle DMSO control (Fig. 9). For the assay, we chose as representative species non-mutagenic **37**, containing a small phenyl moiety, and mutagenic **2**, **32** and **35**, containing large aromatic pyrene, phenanthrene and anthracene groups, respectively. Our data were consistent with phenanthrene- and anthracene-containing analogues **32** and **35** being moderate intercalators, while pyrene-containing analogue **2** is stronger yet and the phenyl analogue **37** does not intercalate. These data are consistent with our structural understanding of intercalation (large flat aromatic moieties are required) and the mutagenicity data in Table 2.

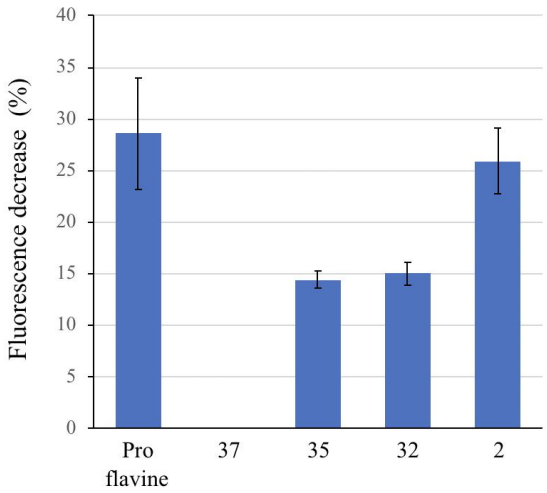


Figure 9. DNA intercalation assessed using the ethidium bromide (EtBr) displacement method. Proflavine, control DNA intercalating agent.

Conclusions

Our previous studies revealed that incorporation of a pyrene moiety into fungal metabolite sphaeropsidin A (**1**) though cross-metathesis at the C15,C16 alkene gave an analogue (**2**) with a significant increase in potency against cancer cell lines, which likely worked through a distinct mechanism involving severe ER swelling associated with strong proteasomal inhibition and consequently cell death. The goal of the present investigation was twofold. In one, we successfully prepared a regioisomeric pyrene counterpart (**38**) attached through C-2 as opposed to the original C-1 as well as deuterated analogues (**39**, **40** and **41**), allowing us to resolve intellectual property issues stemming from public disclosure and non-patentability of the original pyrene-containing compound (**2**). All these compounds had similar potencies and thus this part of the investigation was met with success. Further, we addressed the issue of potential mutagenicity of pyrene-containing compounds through the preparation of C15,C16-alkene cross-metathesis analogues containing alternative aromatic and some non-aromatic hydrophobic moieties. In the present study we found that intercalation properties of the pyrene were not required for the potent *in vitro* anti-cancer effects and mutagenicity appeared linked to DNA intercalation properties of the analogue. Thus, the challenge was to obtain non-intercalating and non-pyrene compounds that maintained high *in vitro* anti-cancer potencies, a feature absent from our previous study.[11] Here, we discovered compounds such as pentamethylphenyl (**27**) and triphenylethylene (**30**) analogues with characteristically high *in vitro* anti-cancer potencies exceeding 5-fold those of SphA (**1**). These new compounds are our next lead compounds in the advancement of this promising series of SphA derivatives.

Experimental Section

General: All the reagents, solvents, and catalysts were purchased from commercial sources (Thermo Fisher Scientific and Sigma-Aldrich) and used without purification. All air- and moisture-sensitive reactions were performed in oven-dried glassware with a Teflon-coated stirrer bar and dry septum under nitrogen and monitored by thin-layer chromatography (TLC) on precoated with (250 μm) silica gel XHL glass-backed plates (Sorbent Technologies Inc.). Visualization was accomplished with UV light. Flash and Gravity column chromatography was performed on silica gel (32–63 μm, 60 Å pore size). ^{1}H and ^{13}C NMR spectra were taken on Bruker 400 and 500 MHz spectrometers. Chemical shifts (δ) were recorded in ppm with reference to internal standard tetramethylsilane (TMS). $CDCl_3$ was referenced to 7.26 ppm in ^{1}H NMR and 77.16 ppm in ^{13}C NMR spectra. The following abbreviations were used to describe the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet (quintet), dd = doublet of doublets, td = triplet of doublets, ddd = doublet of doublet of doublets, m = multiplet, m_c = centered multiplet, *J* = coupling constants (Hz). HRMS analyses were performed using a Waters Synapt XS spectrometer. Sphaeropsidin A **1** was obtained by purification of the organic extract of *Diplodia cupressi* culture filtrates as reported previously.[25] Pent-4-en-1-ylbenzene used in the cross-metathesis reaction to prepare conjugate **34** was synthesized according to the literature procedure.[26] Grubbs II generation [1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(phenylmethylene)(tricyclohexylphosphine)ruthenium was used for the olefin cross metathesis reactions.

Preparation of pentylarenes 4-11.

Method A:

In a 25 mL oven-dried flask, Mg turnings (37.8 mg, 1.43 mmol, 1.3 equiv.) were added and activated using propane torch under vacuum. After cooling to rt, the flask was refilled with N_2 and 5 mL anhydrous THF. The solution was then cooled to 0 °C and aryl bromide (e.g., 1-bromo-2,3,4,5,6-

pentamethylbenzene, 250 mg, 1.10 mmol, 1.0 equiv.) in THF was then added, followed by iodine (13.9 mg, 0.055 mmol, 5 mol%). The reaction was then warmed to rt and stirred for 2 h. The resulting pentamethyl magnesium bromide was transferred (using a cannula) to another flask containing 5-bromopent-1-ene (197 mg, 1.32 mmol, 1.2 equiv.) in 5 mL THF at 0 °C. After 20 min, the reaction solution was allowed to stir at room temperature. The reaction progress was monitored using TLC (reaction was heated to 50 °C, if starting material is not consumed in 4 h). After complete conversion, the reaction was quenched using saturated aqueous NH_4Cl and extracted with diethyl ether (3 × 20 mL). The organic layer was then combined, washed with brine, dried using Na_2SO_4 and concentrated on rotary evaporator with a cold bath. The residue was purified using column chromatography on silica gel (eluted with 5–15% diethyl ether in hexane) to afford the desired product 1,2,3,4,5-pentamethyl-6-(pent-4-en-1-yl)benzene **4** (217 mg, 1.0 mmol) in 91% yield. . Furthermore, compounds **5**–**7** were obtained in a similar manner and their percentage yields and characterization are described below

Compound 4 (91%): ^{1}H NMR (500 MHz, chloroform-*d*) δ 5.91 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H), 5.09 (dd, *J* = 17.2, 1.9 Hz, 1H), 5.02 (dd, *J* = 10.2, 1.7 Hz, 1H), 2.73 – 2.64 (m, 2H), 2.30 – 2.20 (m, 15H), 2.24 – 2.17 (m, 2H), 1.63 – 1.51 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.7, 136.5, 132.6, 132.6, 131.7, 114.8, 34.4, 30.3, 29.1, 17.0, 16.9, 16.5.

Compound 5 (55%): ^{1}H NMR (500 MHz, chloroform-*d*) δ 8.08 – 8.02 (m, 1H), 7.90 – 7.82 (m, 1H), 7.73 (d, *J* = 8.2 Hz, 1H), 7.56 – 7.45 (m, 2H), 7.41 (dd, *J* = 8.2, 7.0 Hz, 1H), 7.34 (dd, *J* = 7.0, 1.2 Hz, 1H), 6.01 – 5.81 (m, 1H), 5.09 (dd, *J* = 17.1, 1.8 Hz, 1H), 5.03 (dd, *J* = 10.2, 1.2 Hz, 1H), 3.11 (t, *J* = 7.9 Hz, 2H), 2.27 – 2.16 (m, 2H), 1.95 – 1.81 (m, 2H);^{13}C NMR (126 MHz, $CDCl_3$) δ 138.6, 134.0, 132.0, 128.9, 128.0, 126.6, 126.1, 125.8, 125.6, 125.5, 124.0, 115.0, 33.8, 32.5, 30.0.

Compound 6 (63%): ^{1}H NMR (500 MHz, chloroform-*d*) δ 8.79 – 8.69 (m, 1H), 8.70 – 8.59 (m, 1H), 8.18 – 8.06 (m, 1H), 7.88 – 7.77 (m, 1H), 7.69 – 7.61 (m, 2H), 7.61 – 7.54 (m, 3H), 6.00 – 5.83 (m, 1H), 5.10

(dd, *J* = 17.1, 2.0 Hz, 1H), 5.05 – 5.02 (m, 1H), 3.14 (t, *J* = 7.4 Hz, 2H), 2.34 – 2.14 (m, 2H), 2.02 – 1.88 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.6, 136.7, 132.0, 131.4, 130.9, 129.8, 128.1, 126.7, 126.6, 1126.2, 126.2, 126.0, 124.5, 123.3, 122.5, 115.1, 33.9, 32.9, 29.5.

Compound 7 (67%): ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.35 (dd, *J* = 7.5 Hz, 2H), 7.30 – 7.23 (m, 3H), 7.19 – 7.13 (m, 2H), 7.13 – 7.09 (m, 3H), 7.04 – 6.96 (m, 3H), 6.89 (dd, *J* = 8.0, 1.8 Hz, 2H), 5.76 – 5.57 (m, 1H), 4.95 – 4.83 (m, 2H), 2.54 – 2.39 (m, 2H), 1.96 (m, 2H), 1.50 – 1.38 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 143.5, 143.1, 142.5, 140.9, 139.5, 138.6, 130.8, 129.7, 129.6, 128.2, 127.9, 127.5, 126.7, 126.3, 125.8, 114.6, 35.5, 33.9, 28.3.

Method B:

In this reaction, we used an oven-dried 25 mL round bottom flask equipped with a condenser. A mixture of magnesium turnings (e.g., 45.8 mg, 1.88 mmol, 1.3 equiv.) and aryl bromide (e.g., 2-bromonaphthalene, 300 mg, 1.45 mmol, 1.0 equiv.) in THF (10 mL) was refluxed for 1.5 h under a nitrogen atmosphere. The resulting mixture was treated with CuI (e.g., 27.6 mg, 0.144 mmol, 10 mol%) and 5-bromo-1-pentene (259.1 mg, 1.74 mmol, 1.2 equiv.) at 0 °C. The reaction mixture was heated to reflux again, and the reflux continued for 20 h. After the completion of the reaction, the solvent was removed under reduced pressure, and hexane was used to extract the crude mixture. The crude mixture was filtered through the short plug of silica gel, which was washed with hexane (3 × 10 mL). The crude mixture was purified using gravity column chromatography eluting with hexane. The desired product (e.g., 2-(pent-4-en-1-yl)naphthalene **8**, 151 mg, 0.769 mmol) was obtained in 53% yield. Furthermore, compounds **9**–**11** were obtained in a similar manner and their percentage yields and characterization are described below.

Compound 8 (53%): ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.89 (ddd, *J* = 14.3, 7.9, 3.0 Hz, 3H), 7.72 (s, 1H), 7.59 – 7.49 (m, 2H), 7.43 (dd, *J* = 8.4, 1.7 Hz, 1H), 5.98 (m, 1H), 5.25 – 5.01 (m, 2H), 3.00 – 2.76 (m, 2H), 2.25 (m, 2H), 2.02 – 1.79 (m, 2H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 140.1, 138.7, 133.8, 132.1, 128.0, 127.7, 127.6, 127.5, 126.6, 126.0, 125.2, 114.9, 35.6, 33.5, 30.6.

Compound 9 (23%): ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.66 (d, *J* = 8.2 Hz, 1H), 8.61 (d, *J* = 8.5 Hz, 1H), 7.88 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.74 – 7.70 (m, 2H), 7.68 (s, 1H), 7.66 – 7.62 (m, 1H), 7.59 – 7.55 (m, 1H), 7.50 (dd, *J* = 8.5, 1.9 Hz, 1H), 5.88 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H), 5.12 – 4.94 (m, 2H), 2.88 – 2.82 (m, 2H), 2.20 – 2.11 (m, 2H), 1.86 (p, *J* = 7.4 Hz, 2H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 140.9, 138.6, 132.2, 131.8, 130.4, 128.5, 128.5, 127.7, 127.7, 126.9, 126.8, 126.5, 126.2, 122.7, 122.5, 114.8, 35.2, 33.4, 30.6.

Compound 10 (12%): ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.21 – 6.92 (m, 4H), 5.84 (ddt, *J* = 16.9, 10.1, 6.6 Hz, 1H), 5.07 – 4.84 (m, 2H), 2.59 (t, 2H), 2.33 (s, 3H), 2.15 – 1.99 (m, 2H), 1.81 – 1.58 (m, 2H). ^{13}C NMR (101 MHz, chloroform-d) δ 139.5, 138.8, 135.2, 129.1, 128.5, 114.8, 35.0, 33.5, 30.9, 21.1.

Compound 11 (46%): ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.67 – 7.62 (m, 2H), 7.57 (d, *J* = 8.1 Hz, 2H), 7.48 (dd, *J* = 7.6 Hz, 2H), 7.40 – 7.34 (m, 1H), 7.31 (d, *J* = 7.9 Hz, 2H), 5.92 (ddt, *J* = 16.9, 10.0, 6.6 Hz, 1H), 5.23 – 4.95 (m, 2H), 2.81 – 2.62 (m, 2H), 2.19 (m, 2H), 1.82 (p, *J* = 7.7 Hz, 2H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 141.7, 141.2, 138.8, 138.6, 128.9, 128.8, 127.1, 127.1, 114.9, 35.0, 33.4, 30.7.

Compound 12: In this reaction, we used an oven-dried 25 mL round bottom flask equipped with a condenser. The solution of 9-bromoanthracene (300 mg, 1.17 mmol) in diethyl ether (12 mL) was treated with *n*BuLi (700 µL, 1.75 mmol) and 5-bromo-1-pentene (552 µL, 4.67 mmol) sequentially. The resulting mixture was heated to reflux, and refluxing was continued for 2 h. After the completion of the reaction, which was monitored by TLC in pentane, the reaction mixture was quenched with cold deionized water and extracted with diethyl ether (3 × 10 mL). The organic extract was washed with brine (10 mL), dried over $MgSO_4$ and concentrated under reduced pressure to get the crude product. The desired product was purified using gravity column chromatography by employing hexane as eluent. This resulted in a 35% yield of 9-(4-penten-1-yl)anthracene (**12**). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.36 (s, 1H), 8.30 (d, *J* = 8.5 Hz, 2H), 8.03 (d, *J* = 8.3 Hz, 2H), 7.59 – 7.45 (m, 4H), 6.00 (m, 1H), 5.31 – 5.03 (m, 2H), 3.72 – 3.58 (m, 2H), 2.38 (m, 2H), 1.98 (p, *J* = 7.3 Hz, 2H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 138.4, 135.0, 131.7, 129.6, 129.2, 125.7, 125.4, 124.8, 124.5, 115.2, 34.3, 30.4, 27.4.

Compound 13: In this reaction, we used an oven-dried 50 mL two-neck round bottom flask. The solution of fluorene (500 mg, 3.01 mmol) in anhydrous diethyl ether (15 mL) was treated with *n*BuLi (1.20 mL, 3.01 mmol) at room temperature under a nitrogen atmosphere. After 4 h of stirring, 5-bromo-1-pentene (356 µL, 3.01 mmol) was added slowly to the reaction mixture at −78 °C. Subsequently, the reaction was kept at stirring overnight before quenching with cold deionized water. The reaction mixture was extracted with diethyl ether (3 × 10 mL) and the organic extract was then washed with brine (10 mL). The resulting mixture was dried over $MgSO_4$ and concentrated under reduced pressure to get the crude product. The desired product was purified using flash column chromatography by employing hexane as eluent. This resulted in a 70% yield of 9-(4-penten-1-yl)-9*H*-fluorene (**13**). ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.51 (d, *J* = 7.4 Hz, 2H), 7.37 (dd, *J* = 7.5 Hz, 2H), 7.30 (d, *J* = 7.4 Hz, 2H), 5.72 (m, 1H), 5.07 – 4.86 (m, 2H), 3.99 (t, *J* = 5.8 Hz, 1H), 2.09 – 1.97 (m, 4H), 1.34 – 1.22 (m, 2H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 147.4, 141.2, 138.6, 126.9, 126.8, 124.3, 119.8, 114.6, 47.3, 34.0, 32.5, 24.8.

Compound 14: [{Ir(cod)OMe}$_2$] (166 mg, 250 µmol, 2.50 mol %) and dtbpy (134 mg, 500 µmol, 5.00 mol %) were dissolved in THF (10.0 mL, 1.0 M) and stirred for 10 min. Then, HBpin (1.54 mL, 1.28 g, 10.0 mmol, 1.00 equiv.) was added in one portion and resulting solution was stirred for 10 min. Next, pyrene (6.06 g, 30.0 mmol, 3.00 equiv.) was added and the reaction mixture was heated at 80 °C for 12 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography (SiO_2, *n*-pentane/Et_2O = 100:1-10:1). The title product **14** was obtained as a amorphous yellow solid (2.36 g, 7.20 mmol, 72%). R_f (hexanes/Et_2O) = 0.27 (UV, $KMnO_4$). ^{1}H NMR (500 MHz, chloroform-*d*): δ 8.62 (s, 2H, C*H*), 8.17 (d, *J* = 8.2 Hz, 2H, C*H*), 8.11 (d, *J* = 9.0 Hz, 1H, C*H*), 8.06 (d, *J* = 9.1 Hz, 2H, C*H*), 8.02 (d, *J* = 8.2, 8.2 Hz, 1H, C*H*), 1.47 (s, 12H) ppm. ^{13}C NMR (126 MHz, chloroform-*d*, no ^{13}C signal detected for *C*B): δ 132.0 (2C), 131.6, 130.7 (2C), 128.3 (2C), 127.5 (2C), 126.8 (2C), 126.4, 125.3 (2C), 125.1 (2C), 88.0 (2C), 24.7 (4C) ppm. ^{11}B NMR (160.55 MHz, $CDCl_3$): δ = 31.4 (1B) ppm. HRMS ($C_{22}H_{22}{}^{11}BO_2$; $[M+H]^+$, pos. APCI): calcd: 329.1713, found: 329.1713.

Compound 15: Compound **14** (656 mg, 2.00 mmol, 1.00 equiv.) and $CuBr_2$ (1.34 g, 6.00 mmol, 3.00 equiv.) were suspended in MeOH/H_2O (1:1, 20 mL, 0.1 M) and heated under air at 90 °C for 18 h. The reaction mixture was then diluted with H_2O (25 mL) and extracted with CH_2Cl_2 (3 x 40 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, *n*-pentane). The title product **15** was obtained as a colorless solid (323 mg, 1.15 mmol, 57%). R_f (hexanes) = 0.39 (UV, $KMnO_4$). mp: 135 °C. ^{1}H NMR (500 MHz, chloroform-*d*): δ 8.29 (s, 2H, C*H*), 8.10 (d, *J* = 9.0 Hz, 2H, C*H*), 8.03 (dd, *J* = 7.9, 7.3 Hz, 1H, C*H*), 7.98 (d, *J* = 9.0 Hz, 2H, C*H*) ppm. ^{13}C NMR (126 MHz, chloroform-*d*): δ = 132.9 (2C), 131.0 (2C), 128.8 (2C), 127.2 (2C), 126.4 (2C), 126.4, 125.9 (2C), 124.5, 123.3, 120.1 ppm. HRMS ($C_{16}H_9Br$; $[M]^+$, pos. ESI): calcd: 279.9882, found: 279.9882.

Compound 17: $Pd(OAc)_2$ (11.2 mg, 50.0 µmol, 10.0 mol.-%) and RuPhos (46.7 mg, 100 µmol, 20.0 mol.-%) were dissolved in toluene/H_2O (10:1, 1.1 mL, 0.5 M) at rt and stirred for 10 min. The resulting solution was then treated subsequently with KO*t*Bu (168 mg, 1.50 mmol, 3.00 equiv.), 2-bromopyrene (141 mg, 0.500 mmol, 1.00 equiv.) and 4,4,5,5-tetramethyl-2-(pent-4-en-1-yl)-1,3,2-dioxaborolane (**16**)[27] (245 mg, 1.25 mmol, 2.50 equiv.). The resulting mixture was heated at 80 °C for 24 h. It was then concentrated under reduced pressure and the residue purified by flash column chromatography (SiO_2, *n*-pentane). The title product **17** was obtained as a colorless gum (93.0 mg, 344 µmol, 69%). R_f (hexanes) = 0.44 (UV, $KMnO_4$). ^{1}H NMR (500 MHz, chloroform-*d*): δ 8.16 (d, *J* = 7.6 Hz, 2H), 8.06 (d, *J* = 9.0 Hz, 2H), 8.03 (d, *J* = 9.0 Hz, 2H), 8.01 (s, 2H), 7.98 (t, *J* = 7.6 Hz, 1H), 5.91 (ddt, *J* = 17.1, *J* = 10.2, *J* = 6.7 Hz, 1H), 5.09 (ddt, *J* = 17.1, *J* = 2.0, *J* = 1.4 Hz, 1H), 5.03 (ddt, *J* = 10.2, *J* = 2.0, *J* = 1.1 Hz, 1H), 3.08 (t, *J* = 7.7 Hz, 2H), 2.21 (m_c, 2H), 1.97 (m_c, 2H) ppm. ^{13}C NMR (126 MHz, chloroform-*d*): δ 140.5, 138.7, 131.4, 131.0, 127.5 (2C), 127.3 (3C), 125.6 (2C), 125.3 (2C), 125.0 (2C), 124.8, 123.3, 115.0, 36.1, 33.6, 31.4 ppm. HRMS ($C_{21}H_{19}$; $[M+H]^+$, pos. APCI): calcd: 271.1481, found: 271.1479.

Compound 18: Pyrene (607 mg, 3.00 mmol, 1.00 equiv.) and KO*t*Bu (6.73 g, 60.0 mmol, 20.0 equiv.) were dissolved in DMF-d_7 (6.0 ml, 0.5 M) and subsequently heated at 150 °C for 36 h. The reaction mixture was then quenched with H_2O (10 mL) and extracted with CH_2Cl_2 (10 mL). The combined organic phases were washed with $LiCl_{aq}$ (sat., 2 × 30 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (SiO_2, hexane). The title product **18** was obtained as a colorless solid (575 mg, 2.71 mmol, 90%). R_f (hexanes) = 0.51 (UV, $KMnO_4$). mp: 148 °C. ^{1}H NMR spectroscopic analysis with 1,3,5-trimethoxybenzene as an internal standard showed >95:5 deuterium incorporation. ^{13}C NMR (126 MHz, chloroform-*d*): 130.9 (s, 2C), 126.8 (t, *J* = 24.0 Hz, 4C), 125.3 (t, *J* = 24.5 Hz, 2C), 124.6 (s, 2C), 124.4 (t, *J* = 24.0 Hz, 4C) ppm.

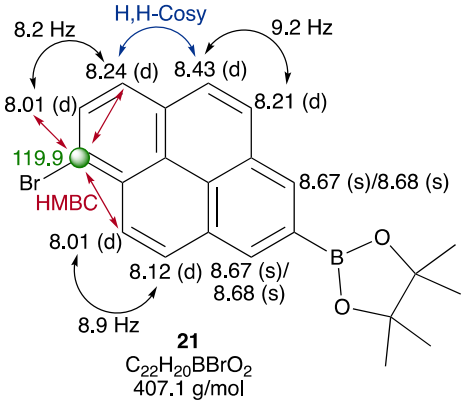
HRMS ($C_{16}D_{10}$; $[M]^+$, pos. ESI): calcd: 212.1410, found: 212.1410. Deuterium incorporation: 82% d_{10} : 16% d_9 : 2% d_8.

Compound 19: Pyrene-d_{10} (**18**) (212 mg, 1.00 mmol, 1.00 equiv.) was dissolved in CH_2Cl_2 (2.0 mL, 0.5 M) at rt and treated with NBS (187 mg, 1.05 mmol, 1.05 equiv.) at once. The resulting solution was stirred at 40 °C for 12 h. The solvent was then removed under reduced pressure and the residue was purified by flash column chromatography (SiO_2, hexane). The title product **19** was obtained as an amorphous colorless solid (259 mg, 0.892 mmol, 89%). R_f (hexanes) = 0.39 (UV, $KMnO_4$). mp: 91 °C. ^{1}H NMR spectroscopic analysis with 1,3,5-trimethoxybenzene as an internal standard showed >95:5 deuterium incorporation. ^{13}C NMR spectroscopic analysis was hardly significant due to the overlapping of all, split pyrene signals. HRMS ($C_{16}D_9Br$; $[M]^+$, pos. ESI): calcd: 289.0453, found: 289.0452.

Compound 20: $Pd(OAc)_2$ (11.2 mg, 50.0 µmol, 10.0 mol.-%) and RuPhos (46.7 mg, 100 µmol, 20.0 mol.%) were dissolved in toluene/H_2O (10:1, 1.1 mL, 0.5 M) at rt and stirred for 10 min. The resulting solution was then treated subsequently with KO*t*Bu (168 mg, 1.50 mmol, 3.0 equiv.), 1-bromopyrene-2,3,4,5,6,7,8,9,10-d_9 (**19**) (141 mg, 0.50 mmol, 1.0 equiv.) and 4,4,5,5-tetramethyl-2-(pent-4-en-1-yl)-1,3,2-dioxaborolane (**16**)[27] (245 mg, 1.25 mmol, 2.5 equiv.). The resulting mixture was heated at 80 °C for 24 h. It was then concentrated under reduced pressure and the residue purified by flash column chromatography (SiO_2, *n*-pentane). The title product **20** was obtained as a colorless gum (93.6 mg, 335 µmol, 67%). R_f (hexanes) = 0.44 (UV, $KMnO_4$). ^{1}H NMR (500 MHz, chloroform-*d*): δ 5.93 (ddt, *J*= 17.0, *J* = 10.3, *J* = 6.7 Hz, 1H, C*H*), 5.10 (ddt, *J*= 17.1, *J* = 2.0, *J* = 1.4 Hz, 1H), 5.04 (ddt, *J* = 10.2, *J* = 2.1, *J* = 1.1 Hz, 1H), 3.37 (m_c, 2H), 2.25 (m_c, 2H), 1.98 (m_c, 2H) ppm. ^{13}C NMR (126 MHz, chloroform-*d*): δ 138.7, 136.9, 131.4, 130.9, 129.8, 128.7, 123.0–127.5 (11C), 115.1, 33.9, 33.0, 31.1 ppm. HRMS ($C_{21}H_9D_9$; $[M]^+$, pos. APCI): calcd: 279.1971, found: 279.1968.

Compound 21. 4,4,5,5-Tetramethyl-2-(pyren-2-yl)-1,3,2-dioxaborolane (**14**) (164 mg, 0.500 mmol, 1.00 equiv.) was dissolved in CH_2Cl_2 (2.5 mL, 0.2 M) at rt. NBS (93.5 mg, 0.525 mmol, 1.05 equiv.) was then added at once and the reaction mixture was heated at 40 °C for 12 h. The solvent was then evaporated under reduced pressure and the residue was purified by flash column chromatography (SiO_2, *n*-pentane/Et_2O = 20:1–10:1). The title product **21** was obtained as an amorphous yellow solid (104 mg, 255 µmol, 51%). R_f (hexanes/Et_2O = 10:1) = 0.32 (UV, $KMnO_4$). ^{1}H NMR (500 MHz, chloroform-*d*): δ 8.68 (s, 1H),), 8.67 (s, 1H), 8.43 (d, *J* = 9.2 Hz, 1H), 8.24 (d, *J* = 8.2 Hz, 1H), 8.21 (d, *J* = 9.2 Hz, 1H), 8.12 (d, *J* = 8.9 Hz, 1H), 8.01 (d, *J* = 8.9 Hz, 1H), 8.01 (d, *J* = 8.2 Hz 1H), 1.47 (s, 12H) ppm. ^{13}C NMR (126 MHz, chloroform-*d*): δ 132.2, 132.1, 131.8, 131.3, 130.6, 130.6, 130.4, 130.3, 129.5, 128.2, 127.1, 126.0, 125.9, 125.6, 119.9, 84.4 (2C), 25.2 (4C) ppm. ^{11}B NMR (161 MHz, chloroform-*d*): δ 31.9 (1B) ppm. HR-MS ($C_{22}H_{21}O_2BBr$; $[M+H]^+$, pos. APCI): calcd: 407.0812, found: 407.0814.

2D-NMR spectroscopic couplings (H,H-COSY, HMBC):



Compound 22: Compound **21** (96.0 mg, 236 µmol, 1.0 equiv.), $AgNO_3$ (4.01 mg, 23.6 µmol, 10.0 mol.-%) and NEt_3 (32.7 µL, 23.9 mg, 236 µmol, 1.0 equiv.) were dissolved in MeOD/D_2O (1:1, 0.25 M). The reaction mixture was heated at 80 °C for 12 h. The solvents were then removed under reduced pressure

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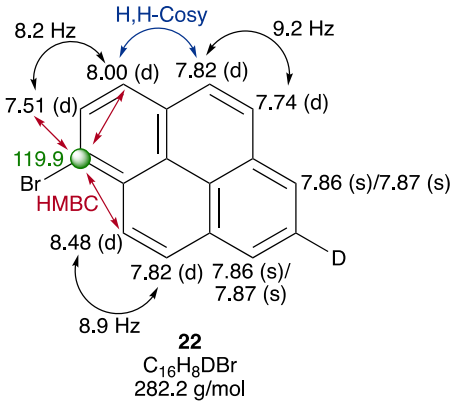
and the crude residue was purified by flash column chromatography (SiO_2, *n*-pentane). The title product **22** was obtained as a colorless solid (52.5 mg, 186 µmol, 79%). R_f (hexanes) = 0.30 (UV, $KMnO_4$). mp: 97 °C. ^{1}H NMR (500 MHz, d_6-benzene): δ 8.48 (d, J = 9.2 Hz, 1H), 8.00 (d, J = 8.2 Hz, 1H), 7.87 (s, 1H), 7.86 (s, 1H), 7.82 (d, J = 9.2 Hz, 1H), 7.74 (d, J = 8.9 Hz, 1H), 7.63 (d, J = 8.9 Hz, 1H), 7.51 (d, J = 8.2 Hz, 1H) ppm. ^{13}C NMR (126 MHz, d_6-benzene, ^{13}C signal for *C*D was undetectable): δ 131.2, 131.0, 130.6, 129.9, 129.8, 128.7, 128.0, 127.8, 127.6, 126.3, 125.9, 125.6, 125.4, 124.2, 119.9 ppm. HRMS ($C_{16}H_8DBr$; $[M+H]^+$, pos. APCI): calcd: 282.0023, found: 282.0028.

2D-NMR spectroscopic couplings (H,H-COSY, HMBC):



Compound 23: $Pd(OAc)_2$ (5.6 mg, 50 µmol, 10 mol.-%) and RuPhos (23.4 mg, 50.0 µmol, 20.0 mol%) were dissolved in toluene/H_2O (10:1, 0.6 mL, 0.5 M) at rt and stirred for 10 min. The resulting solution was then treated subsequently with KO*t*Bu (79.0 mg, 0.750 mmol, 3.0 equiv.), 1-bromopyrene-7-*d* (**22**) (141 mg, 0.25 mmol, 1.0 equiv.) and 4,4,5,5-tetramethyl-2-(pent-4-en-1-yl)-1,3,2-dioxaborolane (**16**)[27] (123 mg, 0.625 mmol, 2.50 equiv.). The resulting mixture was heated at 80 °C for 24 h. It was then concentrated under reduced pressure and the residue purified by flash column chromatography (SiO_2, *n*-pentane). The title product **23** was obtained as a colorless gum (44.0 mg, 162 µmol, 64%). R_f (hexanes) = 0.41 (UV, $KMnO_4$). ^{1}H NMR (500 MHz, chloroform-*d*): δ 8.28 (d, J = 9.3 Hz, 1H), 8.16 (m_c, 2H), 8.11 (m_c, 2H), 8.03 (s, 1H), 8.02 (s, 1H), 7.88 (d, J = 7.8 Hz, 1H), 5.92 (ddt, J= 17.0, J = 10.3, J = 6.7 Hz, 1H), 5.10 (ddt, J= 17.1, J = 2.1, J = 1.4 Hz, 1H), 5.04 (ddt, J = 10.2, J = 2.2, J = 1.1 Hz, 1H), 3.36 (t, J = 7.8 Hz, 2H), 2.25 (m_c, 2H), 1.97 (m_c, 2H) ppm. ^{13}C NMR (126 MHz, chloroform-*d*, ^{13}C signal for *C*D was undetectable): δ 138.7, 137.0, 131.6, 131.1, 129.9, 128.8, 127.7, 127.4, 127.3, 126.7, 125.9, 125.3, 124.9, 124.9, 124.7, 123.6, 115.2, 33.9, 33.1, 31.2 ppm. HRMS ($C_{21}H_{17}D$; $[M]^+$, pos. APCI): calcd: 271.1544, found: 271.1541.

Compound 24: [{Ir(cod)OMe}$_2$] (8.3 mg, 13 µmol, 2.50 mol%) and dtbpy (6.7 mg, 25 µmol, 5.0 mol%) were dissolved in THF (1.0 mL, 0.5 M) and stirred for 10 min. Then, HBpin (72.7 µL, 64.0 mg, 0.50 mmol, 1.0 equiv.) was added in one portion and resulting solution was stirred for 10 min. Next, 2-bromopyrene (**15**) (141 mg, 0.50 mmol, 1.0 equiv.) was added and the reaction mixture was heated at 80 °C for 12 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography (SiO_2, *n*-pentane/CH_2Cl_2 = 100:1-5:1). The title product **24** was obtained as an amorphous yellow solid (55.8 mg, 137 µmol, 27%). R_f (hexanes/CH_2Cl_2 = 20:1) = 0.23 (UV, $KMnO_4$). ^{1}H NMR (500 MHz, chloroform-*d*): δ 8.65 (s, 2H), 8.26 (s, 2H), 8.11 (d, *J* = 9.0 Hz, 2H), 7.95 (d, *J* = 9.0 Hz, 2H), 1.47 (s, 12H) ppm. ^{13}C NMR (126 MHz, chloroform-*d*, for *C*B no signal was detectable): δ 133.3 (2C), 130.2 (2C), 129.2 (2C), 127.1 (2C), 126.3 (2C), 126.1, 123.3, 120.6, 84.4 (2C), 25.2 (4C) ppm. ^{11}B NMR (161 MHz, chloroform-*d*): δ = 31.6 (1B) ppm. HRMS ($C_{22}H_{21}O_2BBr$; $[M+H]^+$, pos. APCI): calcd: 407.0812, found: 407.0810.

Compound 25: Compound **24** (84.7 mg, 300 µmol, 1.00 equiv.), $AgNO_3$ (5.10 mg, 30.0 µmol, 10.0 mol.-%) and NEt_3 (41.6 µL, 30.4 mg, 300 µmol, 1.00 equiv.) were dissolved in MeOD/D_2O (1:1, 0.25 M). The reaction mixture was heated at 80 °C for 12 h. The solvents were then removed under reduced pressure and the crude residue was purified by flash column chromatography (SiO_2, *n*-pentane). The title product **25** was obtained as a colorless solid (61.4 mg, 218 µmol, 73%). R_f (hexanes) = 0.40 (UV, $KMnO_4$). mp: 133 °C. ^{1}H NMR (500 MHz, d_6-benzene): δ 8.29 (s, 2H), 8.21 (s, 2H), 8.10 (d, *J* = 9.0 Hz, 2H), 7.99 (d, *J* = 9.0 Hz, 2H) ppm. ^{13}C NMR (126 MHz, d_6-benzene, ^{13}C signal for *C*D was undetectable): δ 132.9 (2C), 131.0 (2C), 128.8 (2C), 127.2 (2C), 126.4 (2C), 125.8 (2C), 124.5, 123.4, 120.1 ppm. HRMS ($C_{16}H_8DBr$; $[M+H]^+$, pos. APCI): calcd: 282.0023, found: 282.0025.

Compound 26: $Pd(OAc)_2$ (4.5 mg, 20 μmol, 10 mol%) and RuPhos (18.7 mg, 40.0 μmol, 20.0 mol%) were dissolved in toluene/H_2O (10:1, 0.4 mL, 0.5 M) at rt and stirred for 10 min. The resulting solution was then treated subsequently with KO*t*Bu (67.3 mg, 0.60 mmol, 3.0 equiv.), 2-bromopyrene-7-*d* (**25**) (56.4 mg, 0.20 mmol, 1.0 equiv.) and 4,4,5,5-tetramethyl-2-(pent-4-en-1-yl)-1,3,2-dioxaborolane (**16**)[27] (98.1 mg, 0.50 mmol, 2.5 equiv.). The resulting mixture was heated at 80 °C for 24 h. It was then concentrated under reduced pressure and the residue purified by flash column chromatography (SiO_2, *n*-pentane). The title product **26** was obtained as a colorless gum (38.8 mg, 143 μmol, 71%). R_f (hexanes) = 0.37 (UV, $KMnO_4$). ^{1}H NMR (500 MHz, chloroform-*d*): δ 8.16 (s, 2H, C*H*), 8.06 (d, *J* = 8.9Hz, 2H), 8.03 (d, *J* = 9.0 Hz, 2H), 8.01 (s, 2H), 5.90 (ddt, *J*= 17.0, *J* = 10.3, *J* = 6.7 Hz, 1H), 5.08 (ddt, *J*= 17.1, *J* = 2.0, *J* = 1.5 Hz, 1H), 5.02 (ddt, *J* = 10.2, *J* = 2.1, *J* = 1.1 Hz, 1H), 3.08 (t, *J* = 7.7 Hz, 2H), 2.20 (m_c, 2H), 1.97 (m_c, 2H) ppm. ^{13}C NMR (126 MHz, chloroform-*d*, ^{13}C signal for CD was undetectable): δ 140.5, 138.7, 131.4 (2C), 131.0 (2C), 127.5 (4C), 127.3 (2C), 125.3 (2C), 124.9 (2C), 115.0, 36.0, 33.6, 31.4, ppm. HRMS ($C_{21}H_{17}D$; $[M]^+$, pos. APCI): calcd: 271.1544, found: 271.1545.

General Procedure for the Cross-Metathesis Reaction: SphA (**1**, 10 mg, 0.028 mmol) and Grubbs second generation catalyst ([1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(phenylmethylene)(tricyclohexylphosphine)ruthenium, 7.4 mg, 0.008 mmol) were dissolved in CH_2Cl_2 in a round-bottom flask, a condenser applied, placed under N_2 and brought to reflux. The desired alkene (0.28 mmol) was added in three portions over 2 h intervals. The reaction mixture was allowed to reflux for 12 – 72 h with TLC monitoring. The solvent was evaporated under reduced pressure and the residue was purified by gravity column chromatography on silica gel (hexane/EtOAc, 3:1) or reversed phase column chromatography (C_{18}-SiO_2, H_2O/MeCN = 95:5-0:100) to give the products as amorphous solids.

Compound 27 (73%): ^{1}H NMR (500 MHz, chloroform-*d*) δ 6.84 (d, *J* = 1.8 Hz, 1H), 5.60 – 5.52 (m, 1H), 5.48 (d, *J* = 15.8 Hz, 1H), 5.20 (s, 1H), 2.71 (s, 1H), 2.68 – 2.62 (m, 2H), 2.34 (d, *J* = 5.0 Hz, 1H), 2.25 – 2.20 (m, 15H), 2.19 – 2.12 (m, 2H), 1.91 – 1.79 (m, 3H), 1.70 – 1.48 (m, 7H), 1.41 – 1.32 (m, 1H), 1.20 (d, *J* = 2.3 Hz, 6H), 1.08 (s, 3H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 191.8, 174.7, 153.7, 136.9, 136.3, 132.7, 132.7, 131.7, 129.4, 103.7, 71.2, 57.2, 51.4, 40.5, 38.7, 33.1, 32.7, 32.4, 30.4, 30.2, 29.6, 27.1, 24.9, 23.0, 22.5, 18.1, 17.0, 17.0, 16.6; HRMS (ESI) *m/z* calcd for $C_{34}H_{46}O_5$ $[M+Na]^+$ 557.3237, found 557.3225.

Compound 28: (64%): ^{1}H NMR (500 MHz, chloroform-*d*) δ 8.04 (d, *J* = 8.8 Hz, 1H), 7.88 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.73 (d, *J* = 8.1 Hz, 1H), 7.58 – 7.45 (m, 2H), 7.45 – 7.38 (m, 1H), 7.33 (dd, *J* = 7.0, 1.2 Hz, 1H), 6.85 (d, *J* = 1.7 Hz, 1H), 5.59 – 5.50 (m, 1H), 5.47 (d, *J* = 15.7 Hz, 1H), 5.21 (s, 1H), 3.10 (t, 2H), 2.73 (s, 1H), 2.29 – 2.21 (m, 1H), 2.18 (q, *J* = 6.9 Hz, 2H), 1.92 – 1.80 (m, 5H), 1.69 – 1.54 (m, 5H), 1.41 – 1.35 (m, 1H), 1.22 (d, *J* = 2.0 Hz, 6H), 1.08 (s, 3H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 191.8, 174.7, 153.6, 138.5, 136.9, 134.1, 132.6, 132.0, 129.2, 129.0, 126.8, 126.2, 125.9, 125.7, 125.6, 123.9, 103.7, 71.2, 57.2, 51.4, 40.5, 38.7, 32.7, 32.7, 32.6, 32.3, 30.4, 30.3, 27.1, 24.9, 23.0, 22.5, 18.1; HRMS (ESI) *m/z* calcd for $C_{33}H_{38}O_5$ $[M+Na]^+$ 537.2611, found 537.2599.

Compound 29 (69%): ^{1}H NMR (500 MHz, chloroform-*d*) δ 8.76 – 8.72 (m, 1H), 8.66 (dd, *J* = 8.2, 1.3 Hz, 1H), 8.08 (dd, *J* = 7.7, 1.9 Hz, 1H), 7.82 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.69 – 7.62 (m, 2H), 7.62 – 7.55 (m, 3H), 6.84 (d, *J* = 1.7 Hz, 1H), 5.58 – 5.50 (m, 1H), 5.46 (d, *J* = 15.7 Hz, 1H), 5.19 (s, 1H), 3.12 (t, 2H), 2.71 (s, 1H), 2.25 – 2.18 (m, 3H), 1.97 – 1.76 (m, 5H), 1.67 – 1.56 (m, 5H), 1.41 – 1.31 (m, 1H), 1.19 (d, *J* = 1.9 Hz, 6H), 1.06 (s, 3H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 191.8, 174.7, 153.6, 137.0, 136.4, 132.7, 132.0, 131.4, 130.9, 129.8, 129.2, 128.2, 126.8, 126.7, 126.4, 126.3, 126.1, 124.5, 123.4, 122.6, 103.7, 71.2, 57.2, 51.4, 40.5, 38.7, 33.0, 32.8, 32.7, 32.4, 30.3, 29.9, 27.1, 24.9, 23.0, 22.5, 18.1; HRMS (ESI) *m/z* calcd for $C_{37}H_{40}O_5$ $[M+Na]^+$ 587.2768, found 587.2757.

Compound 30 (72%): ^{1}H NMR (500 MHz, chloroform-*d*) δ 7.38 – 7.32 (m, 2H), 7.29 – 7.21 (m, 3H), 7.19 – 7.14 (m, 2H), 7.13 – 7.08 (m, 3H), 7.04 – 6.95 (m, 3H), 6.88 (dd, *J* = 8.0, 1.7 Hz, 2H), 6.75 (d, *J* = 1.7 Hz, 1H), 5.33 – 5.25 (m, 2H), 5.20 (s, 1H), 2.71 (s, 1H), 2.48 – 2.34 (m, 2H), 2.28 – 2.16 (m, 1H), 1.97 – 1.70 (m, 5H), 1.66 – 1.52 (m, 5H), 1.47 – 1.30 (m, 3H), 1.20 (d, *J* = 3.9 Hz, 6H), 0.98 (s, 3H). ^{13}C NMR (126 MHz, chloroform-*d*) δ 191.8, 174.7, 153.7, 143.6, 143.0, 142.5, 140.6, 139.7, 136.6, 132.6, 130.8, 129.7, 129.6, 129.1, 128.4, 128.0, 127.5, 126.8, 126.4, 125.9, 103.7, 71.1, 57.1, 51.4, 40.5, 38.6, 35.5, 32.8, 32.7, 32.4, 30.3, 28.6, 27.0, 24.9, 23.0, 22.5, 18.1; HRMS (ESI) *m/z* calcd for $C_{43}H_{46}O_5$ $[M+Na]^+$ 665.3237, found 665.3219.

Compound 31 (29%): ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.82 – 7.76 (m, 3H), 7.60 (s, 1H), 7.47 – 7.39 (m, 2H), 7.33 – 7.30 (m, 1H), 6.83 (s, 1H), 5.53 – 5.39 (m, 2H), 5.19 (s, 1H), 2.78 (t, *J* = 7.5 Hz, 2H), 2.70 (s, 1H), 2.22 (d, *J* = 6.7 Hz, 1H), 2.10 (q, *J* = 6.9 Hz, 3H), 1.88 – 1.76 (m, 7H), 1.66 – 1.57 (m, 5H), 1.36 (d, *J* = 14.2 Hz, 2H), 1.20 (s, 6H), 1.05 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 191.6, 174.5, 153.6, 139.7, 136.7, 133.6, 132.5, 132.0, 129.1, 127.9, 127.6, 127.4, 127.3, 126.5, 125.9, 125.1, 103.5, 71.0, 57.0, 51.2, 40.3, 38.6, 35.5, 32.5, 32.3, 32.2, 30.7, 30.1, 26.9, 24.8, 22.9, 22.4, 18.0. HRMS *m/z* calcd for $C_{33}H_{38}O_5$ $(M+H^+)^+$ 515.2792, found 515.2777.

Compound 32 (18%): ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.63 (dd, *J* = 19.8, 8.2 Hz, 2H), 7.87 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.74 – 7.65 (m, 3H), 7.63 (dd, *J* = 8.3, 1.5 Hz, 1H), 7.57 (ddd, *J* = 8.1, 7.0, 1.3 Hz, 1H), 7.49 (dd, *J* = 8.5, 1.9 Hz, 1H), 6.83 (s, 1H), 5.56 – 5.37 (m, 2H), 5.19 (s, 1H), 2.83 (t, *J* = 7.6 Hz, 2H), 2.69 (s, 1H), 2.21 (d, *J* = 8.4 Hz, 1H), 2.16 – 2.09 (m, 2H), 1.87 – 1.74 (m, 5H), 1.67 – 1.59 (m, 5H), 1.39 – 1.32 (m, 1H), 1.19 (s, 6H), 1.06 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 191.6, 174.5, 153.5, 140.6, 136.7, 132.5, 132.2, 131.8, 130.3, 129.1, 128.5, 127.7, 127.6, 127.0, 126.7, 126.6, 126.2, 122.7, 122.5, 103.5, 71.0, 57.0, 51.2, 40.3, 38.6, 35.3, 32.5, 32.3, 32.2, 30.9, 30.1, 26.9, 24.8, 22.9, 22.4, 18.0. HRMS (ESI) *m/z* calcd for $C_{37}H_{40}O_5$ $[M+Na]^+$ 587.2773, found 587.2785.

Compound 33 (31%): ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.10 – 7.04 (m, 4H), 6.83 (s, 1H), 5.52 – 5.38 (m, 2H), 5.19 (s, 1H), 2.71 (s, 1H), 2.57 (t, *J* = 7.7 Hz, 2H), 2.32 (s, 3H), 2.23 (d, *J* = 7.3 Hz, 1H), 2.06 (q, *J* = 6.7 Hz, 3H), 1.89 – 1.77 (m, 5H), 1.72 – 1.65 (m, 4H), 1.65 – 1.59 (m, 5H), 1.39 – 1.33 (m, 2H), 1.19 (d, *J* = 1.8 Hz, 6H), 1.05 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 191.5, 174.4, 153.4, 139.0, 136.3, 132.3, 129.0, 128.8, 128.1, 103.4, 70.8, 56.8, 51.1, 40.2, 38.4, 34.7, 32.4, 32.0, 30.8, 30.0, 26.8, 24.6, 22.7, 22.18, 20.8, 17.8. HRMS *m/z* calcd for $C_{30}H_{38}O_5$ $(M+H^+)^+$ 479.2792, found 465.2787.

Compound 34 (40%): ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.60 – 7.56 (m, 2H), 7.52 (d, *J* = 8.2 Hz, 2H), 7.46 – 7.40 (m, 2H), 7.35 – 7.30 (m, 1H), 7.24 (d, *J* = 8.2 Hz, 2H), 6.84 (s, 1H), 5.54 – 5.40 (m, 2H), 5.19 (s, 1H), 2.71 (s, 1H), 2.65 (d, *J* = 7.7 Hz, 2H), 2.22 (d, *J* = 6.8 Hz, 1H), 2.10 (q, *J* = 6.8 Hz, 2H), 1.90 – 1.82 (m, 3H), 1.78 (dd, *J* = 9.6, 7.7 Hz, 3H), 1.74 – 1.66 (m, 2H), 1.66 – 1.56 (m, 6H), 1.38 (s, 1H), 1.19 (s, 6H), 1.06 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 191.7, 174.5, 153.6, 141.4, 141.1, 138.8, 136.7, 132.5, 129.1, 128.9, 128.7, 127.1, 127.0, 103.5, 71.0, 57.0, 51.2, 40.3, 38.6, 35.0, 32.5, 32.3, 32.2, 30.9, 30.2, 26.9, 24.8, 22.6, 22.4, 18.0. HRMS *m/z* calcd for $C_{35}H_{40}O_5$ $(M+H^+)^+$ 541.2949, found 541.2947.

Compound 35 (22%): ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.34 (d, *J* = 2.5 Hz, 1H), 8.23 (dd, *J* = 8.8, 2.4 Hz, 2H), 8.01 (d, *J* = 8.3 Hz, 2H), 7.49 (dddd, *J* = 16.8, 8.6, 5.0, 2.0 Hz, 4H), 6.85 (s, 1H), 5.63 – 5.46 (m, 2H), 5.19 (s, 1H), 3.65 – 3.56 (m, 2H), 2.71 (s, 1H), 2.32 – 2.25 (m, 2H), 2.22 (d, *J* = 6.4 Hz, 1H), 2.05 (s, 1H), 1.95 – 1.78 (m, 6H), 1.63 (d, *J* = 18.4 Hz, 4H), 1.36 (d, *J* = 14.0 Hz, 2H), 1.19 (s, 6H), 1.09 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 191.6, 174.5, 153.5, 137.0, 134.7, 132.6, 131.7, 129.6, 129.3, 129.1, 125.7, 125.5, 124.8, 124.3, 103.6, 71.0, 57.0, 51.2, 40.3, 38.6, 32.5, 32.2, 30.8, 30.2, 27.3, 27.0, 24.8, 22.9, 22.4, 18.0. HRMS (ESI) *m/z* calcd for $C_{37}H_{40}O_5$ $[M+Na]^+$ 587.2773, found 587.2785.

Compound 36 (48%): ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.75 (d, *J* = 4.5 Hz, 2H), 7.49 (d, *J* = 4.1 Hz, 2H), 7.39 – 7.28 (m, 4H), 6.78 (s, 1H), 5.33 (d, *J* = 4.2 Hz, 2H), 5.19 (s, 1H), 3.99 (q, *J* = 5.1 Hz, 1H),

2.70 (s, 1H), 2.20 (s, 1H), 2.08 – 1.98 (m, 3H), 1.98 – 1.78 (m, 6H), 1.78 – 1.71 (m, 2H), 1.64 – 1.55 (m, 5H), 1.35 (d, *J* = 13.3 Hz, 2H), 1.18 (s, 8H), 1.02 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 191.6, 174.5, 153.6, 147.2, 141.2, 136.4, 129.1, 127.0, 126.9, 124.2, 119.9, 103.5, 71.0, 57.0, 51.2, 47.2, 40.3, 38.5, 33.0, 32.5, 32.3, 32.2, 30.1, 26.9, 24.9, 24.8, 22.9, 22.4, 18.0. HRMS (ESI) *m/z* calcd for $C_{36}H_{40}O_5$ $[M+K]^+$ 591.2513, found 591.2526.

Compound 37 (65%): ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.31 – 7.26 (m, 2H), 7.18 (t, *J* = 8.0 Hz, 3H), 6.82 (s, 1H), 5.52 – 5.38 (m, 2H), 5.19 (s, 1H), 2.71 (s, 1H), 2.64 – 2.58 (m, 2H), 2.22 (d, *J* = 7.6 Hz, 1H), 2.10 – 2.03 (m, 2H), 1.87 – 1.77 (m, 3H), 1.73 – 1.68 (m, 2H), 1.67 – 1.59 (m, 4H), 1.39 – 1.33 (m, 1H), 1.20 (s, 6H), 1.06 (s, 3H). ^{13}C NMR (101 MHz, chloroform-*d*) δ 191.5, 174.4, 153.4, 142.1, 136.4, 132.3, 128.9, 128.3, 128.2, 125.6, 103.4, 70.8, 56.8, 51.1, 40.2, 38.9, 35.2, 32.4, 32.0,30.7, 20.0, 26.8, 24.6, 22.7, 22.2, 17.8. HRMS *m/z* calcd for $C_{29}H_{36}O_5$ $(M+H^+)^+$ 465.2636, found 465.2625.

Compound 38 (69%): R_f (hexanes/AcOEt = 2:1) = 0.31 (UV, $KMnO_4$). ^{1}H NMR (500 MHz, chloroform-*d*): δ 8.16 (d, *J* = 7.6 Hz, 2H), 7.90-8.07 (m, 7H), 6.84 (d, *J* = 1.8 Hz, 1H), 5.52 (d, *J* = 15.7, 6.5 Hz, 1H), 5.44 (d, *J* = 15.7 Hz, 1H), 3.07 (t, *J* = 7.6 Hz, 2H), 2.70 (s, 1H), 2.17 (m_c, 2H), 1.95 (m_c, 2H), 1.74-1.87 (m, 4H), 1.52-1.64 (m, 6H), 1.19 (s, 3H), 1.19 (s, 3H), 1.06 (s, 3H) ppm. ^{13}C NMR (126 MHz, chloroform-*d*): δ 191.7, 174.7, 153.6, 140.2, 136.9, 132.6, 131.4, 131.0, 129.2, 127.3 (2C), 125.7 (2C), 125.3 (2C), 125.1 (2C), 71.1, 57.1, 51.4, 40.5, 38.7, 36.1, 32.7, 32.5, 32.4, 31.6, 30.3, 29.9, 27.1, 24.9, 23.0, 22.5, 18.1 ppm. HRMS ($C_{39}H_{41}O_5$; $[M+H]^+$, pos. APCI): calcd: 589.2949, found: 589.2949.

Compound 39 (63%): R_f (hexanes/AcOEt = 2:1) = 0.20 (UV, $KMnO_4$). ^{1}H NMR (500 MHz, chloroform-*d*): δ 6.83 (d, *J* = 1.8 Hz, 1H), 5.53 (dt, *J* =15.7, 6.4 Hz, 1H), 5.45 (d, *J* = 16.8 Hz, 1H), 5.19 (s, 1H), 3.35 (t, *J* = 7.7 Hz), 2.70 (s, 1H), 2.20 (m_c, 2H), 1.96 (m_c, 2H), 1.76-1.87 (m, 3H), 1.57-1.64 (m, 3H), 1.36 (m, 1H), 1.19 (s, 3H), 1.19 (s, 3H), 1.06 (s, 3H) ppm. ^{13}C NMR (126 MHz, chloroform-d_3, ^{13}C signals for *C*D

were undetectable): δ 191.7, 174.6, 153.6, 137.0, 132.6, 129.2, 103.7, 71.1, 57.1, 51.4, 40.5, 38.7, 33.0, 32.7, 32.7, 32.4, 31.5, 30.3, 29.9, 27.1, 24.9, 23.0, 22.5, 18.1 ppm. HRMS ($C_{39}H_{32}D_9O_5$; $[M+H]^+$, pos. APCI): calcd: 598.3513, found: 598.3511.

Compound 40 (67%): R_f (hexanes/AcOEt = 2:1) = 0.32 (UV, $KMnO_4$). ^{1}H NMR (500 MHz, chloroform-*d*): δ 8.25 (d, *J* = 9.3 Hz, 1H), 8.17 (m_c, 2H), 8.12 (s, 1H), 8.10 (s, 1H), 8.03 (m_c, 2H), 7.86 (d, *J* = 7.8 Hz, 1H), 6.83 (d, *J* = 1.8 Hz, 1H), 5.53 (dt, *J* = 15.7, 6.2 Hz, 1H), 5.46 (d, *J* = 15.7 Hz, 1H), 3.35 (t, *J* = 7.6 Hz, 2H), 2.61 (s, 2H), 2.20 (m_c, 2H), 1.96 (m_c, 2H), 1.75-1.87 (m, 3H), 1.57-1.64 (m, 4H), 1.33-1.37 (m, 2H), 1.19 (s, 3H), 1.19 (s, 3H), 1.06 (s, 3H) ppm. ^{13}C NMR (101 MHz, chloroform-*d*, ^{13}C signal for *C*D was undetectable): δ 191.7, 174.6, 153.6, 137.0, 136.7, 132.6, 131.6, 131.1., 130.0, 129.2, 128.8, 127.4, 127.4, 126.8, 124.9, 124.8, 123.5, 103.7, 71.1, 57.1, 51.4, 41.2, 40.5, 38.7, 33.1, 32.7, 32.7, 32.4, 31.5, 30.3, 29.9, 27.1, 24.9, 23.0, 22.5, 18.1 ppm. HRMS ($C_{39}H_{40}DO_5$; $[M+H]^+$, pos. APCI): calcd: 590.3011, found: 590.3011.

Compound 41 (71%): R_f (hexanes/AcOEt = 2:1) = 0.33 (UV, $KMnO_4$). ^{1}H NMR (500 MHz, chloroform-*d*): δ 8.15-8.21 (m_c, 2H), 7.99-8.07 (m, 7H), 6.83 (d, *J* = 1.8 Hz, 1H), 5.54 (d, *J* = 15.6, 6.5 Hz, 1H), 5.46 (d, *J* = 15.6 Hz, 1H), 5.19 (s, 1H, O*H*), 3.06 (t, *J* = 7.6 Hz, 2H), 2.69 (s, 1H), 2.19 (m_c, 2H), 1.94 (m_c, 2H), 1.74-1.87 (m, 4H), 1.62-165 (m, 6H), 1.19 (s, 3H), 1.19 (s, 3H), 1.06 (s, 3H) ppm. ^{13}C NMR (101 MHz, chloroform-*d*, ^{13}C signal for *C*D was undetectable): δ 191.8, 174.6, 153.7, 140.2, 136.9, 132.6, 131.4, 131.0, 129.2, 127.6 (2C), 127.3 (2C), 125.3 (2C), 125.0 (2C), 71.1, 57.1, 51.4, 40.5, 38.7, 36.1, 32.7, 32.5, 32.3, 31.6, 30.3, 29.9, 27.1, 24.9, 23.0, 22.5, 18.1 ppm. HRMS ($C_{39}H_{40}DO_5$; $[M+H]^+$, pos. APCI): calcd: 590.3011, found: 590.3011.

Cell lines

Cancer cell lines were obtained from either the European Collection of Cell Cultures (ECACC; Salisbury, UK) or the American Type Culture Collection (ATCC; Manassas, VA). The two glioma cell lines were the U373 (ECACC code 08061901) and Hs683 (ATCC HTB-138) cell lines. The carcinoma cell line was the A549 non-small cell carcinoma cell line (ATCC code CCL 185). The melanoma cell lines were the mouse B16F10 cells (ATCC code CRL-6475) and the human SKMEL-28 cells (ATCC code HTB-72). The correct origin of all cell models was confirmed by STR during performance of the experiments. All cell lines were cultured in RPMI 1640 culture medium supplemented with 10% heat-inactivated fetal bovine serum, 2% L-glutamine (0.6 mg/mL), 2% penicillin/streptomycin (200 IU/mL and 200 μg/mL) and 0.1 mg/mL gentamicin.

MTT Assay

The overall growth level of human cancer cell lines was determined using the colorimetric MTT (3-[4,5-dimethylthiazol-2yl]-diphenyl tetrazolium bromide, Sigma, Belgium) assay.[28] Briefly, the cell lines were incubated for 24 h in 96-microwell plates (at a concentration of 10,000 to 40,000 cells/mL culture medium depending on the cell type) to ensure adequate plating prior to cell growth determination. The assessment of cell population growth by means of the MTT colorimetric assay is based on the capability of living cells to reduce the yellow product MTT (3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to a blue product, formazan, by a reduction reaction occurring in the mitochondria. The number of living cells after 72 h of culture in the presence (or absence: control) of the various compounds is directly proportional to the intensity of the blue, which is quantitatively measured by spectrophotometry, in our case using a Biorad Model 680XR (Biorad, Nazareth, Belgium) at a 570 nm wavelength (with a reference of 630 nm). One set of experimental conditions included six replicates. Each experiment was carried out twice.

DNA Intercalation Assay

The DNA intercalation ability of SphA analogues was assessed by displacement of ethidium bromide from salmon genomic DNA. 100 µL assays were conducted with five replicates in 96-well microtiter plates. Each assay contained salmon DNA (15 µg/mL), 1.5 µM ethidium bromide, 10 µM of compounds and 2 µM sodium phosphate buffer. DMSO and **37** were used as negative controls and proflavine, as a positive control. Compounds **32**, **35** and **2** were tested. Fluorescence was measured using excitation wavelength at 320 nm and emission wavelength at 600 nm.

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