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Thio-ether functionalized glycolipid amphiphilic compounds reveal a potent activator of SK3 channel with vasorelaxation effect†

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The modulation of SK3 ion channels can be efficiently and selectively achieved by using the amphiphilic compound Ohmline (a glyco-glycero-ether-lipid). We report herein a series of Ohmline analogues featuring the replacement of one ether function by a thioether function located at the same position or shifted close to its initial position. The variation of the lipid chain length and the preparation of two analogues featuring either one sulfoxide or one sulfone moiety complete this series. Patch clamp measurements indicate that the presence of the thioether function (compounds **7** and **17a**) produces strong activators of SK3 channels, whereas the introduction of a sulfoxide or a sulfone function at the same place produces amphiphiles devoid of an effect on SK3 channels. Compounds **7** and **17a** are the first amphiphilic compounds featuring strong activation of SK3 channels (close to 200% activation). The cytosolic calcium concentration determined from fluorescence at 3 different times for compound **7b** (13 min, 1 h, 24 h) revealed that the effect is different suggesting that the compound could be metabolized over time. This compound could be used as a strong SK3 activator for a short time. The capacity of **7b** to activate SK3 was then used to induce vasorelaxation *via* an endothelium-derived hyperpolarization (EDH) pathway. For the first time, we report that an amphiphilic compound can affect the endothelium dependent vasorelaxation.

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Introduction

Among the wide diversity of potassium channels,[1] SKCa channels are activated by and sensitive to cytosolic calcium concentration. This family of ion channels is composed of 3 channel isoforms (SK1, SK2, and SK3 also identified as KCa2.1, KCa2.2, and KCa2.3 with the genes *KCNN1*, *KCNN2*, and *KCNN3*). These channels are involved in different physiological roles (*e.g.*, regulation of neuronal excitability and sinoatrial node[2] and implication in blood pressure regulation[3]) and pathological situations (*e.g.* neuronal disease,[4] cancer,[5] and neuropathy[6]). Modulators of SKCa channels (activation or inhibition) were developed to assess the role of SKCa channels and for potent medical applications.

Briefly, activation of SKCa channels was used concomitantly with *N*-methyl-D-aspartate receptor antagonists to produce antinociceptive effects.[7] Other works have shown that the activation of SK2/SK3 channels improved the mobility of transgenic SCA2 mice,[8] and the activation of SK3 reduced the inflammatory response activated by microglia.[9] In addition, activators of SK3 channels may be used in pathologies in which SK3 induced hyperpolarization control excitation–contraction coupling such as to prevent preterm labor,[10–12] to prevent overactive bladder[13] or to treat systemic hypertension by activating the endothelium-derived hyperpolarizing factor (EDHF) and nitric oxide-mediated vasodilation.[14] On the other hand, to mention a few examples of the effect of the inhibition of the SK3 channel, it is worth mentioning the possibility to reduce migration/invasion of microglia;[15] an SK3 blocker could reduce taxane-induced peripheral neuropathy.[6] It is noteworthy that in some cancer cells the expression of the SK3 channel is modified or abnormally expressed (not expressed in some epithelial cells compared to cancer epithelial cells). For

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instance, in cancer cells (*e.g.* MDA-MB-435s), the SK3 channel is expressed, whereas in non-tumor cells, it is not. Interestingly, biopsies of cancer cells have shown that SK3 channels are also expressed in melanoma,[16] breast, prostate,[17] and colon cancer.[18] When the SK3 channel is expressed in cancer cells, it promotes at least cell migration.[19] These results suggested developing inhibitors of SK3 channels with the aim to reduce cancer cell migration. As detailed in a recent review,[5] the first strategy to modulate SK3 currents involved the use of peptides (*e.g.* apamin[20,21]) or heterocyclic compounds featuring cationic moieties (*e.g.* UCL1684[22] and dequalinium[23]). The inhibition can be also achieved by other classes of heterocyclic compounds that modified the sensitivity of cytosolic calcium concentration $[Ca]_{cyt.}$ (*e.g.* NS8593 [24]). This action likely occurs by interfering with the gating mechanism.[25–27] On the other hand, other heterocyclic compounds were identified as activators of SK3 channels (*e.g.* CyPPA [28] and NS13001 [8]).

More recently, we found that some amphiphilic compounds inhibit SK3 channel activity with an interesting selectivity.[29,30] Ohmline (Fig. 1) appeared as an efficient and selective inhibitor of SK3 channels. This compound inhibited SK3 currents by 73% (effect observed 2 minutes after adding Ohmline at 10 μM and measured by the patch clamp technique) and inhibited SK3-dependent cancer cell migration (corresponding to 50% of MDA-MB-435s cell migration) at a lower concentration (300 nM) for 24 hours.[17] Interestingly this compound is one of the most selective inhibitors of SK3 since it has a weak effect on SK1 (−20%) and no effect on both SK2 and IKCa (intermediate conductance calcium activated potassium channel). This compound is neither a pore blocker (cork effect; observed for tetraethylammonium[31]) nor a modulator of the sensitivity of cytosolic calcium concentration.[24] The amphiphilic nature of Ohmline that belongs to the class of ether lipids[32] suggests a different mechanism of action when compared to the other modulators of SK3 channels. The alkyl chain acts as a membrane anchor and the glycerol moiety and the lactose unit interact with the polar groups of the constituents of the plasma membranes (*e.g.* phospholipid, cholesterol) as recently shown by ^{2}H NMR and molecular dynamic simulation.[33] On this basis, Ohmline could modulate SK3 channel function by generating a modification of the biophysical properties of the membrane and/or by a direct interaction with the SK3 protein within the lipid bilayer. Its action could be viewed as an illustration of the concept of membrane lipid therapy.[34] Ohmline is also efficient *in vivo* to prevent the occurrence of SK3+ bone metastases and to reduce lung metastasis in a murine model of metastatic breast cancer.[17] In this case, Ohmline induced the dissociation of a protein–protein complex constituted by two ion channels (Orai 1 and SK3) that are located in the plasma membrane. This compound is also the first amphiphilic compound that reduces SOCE-dependent cell migration and modifies anti-EGFR monoclonal antibody effects.[18]

With the aim to identify new Ohmline analogues, we have previously modulated the structure of the polar head group,[35,36] integrated a phosphate moiety[37] and reported a fluorescent analogue of Ohmline.[38] In the continuity of these studies, we report herein the synthesis and properties of new Ohmline analogues that feature a sulfur-based function (thioether, sulfoxide, and sulfone) in replacement of one ether function. We also report the replacement of the methoxy group by an ethoxy group (compounds **17a** and **17b**, Scheme 1). All these structural changes aim to modulate steric

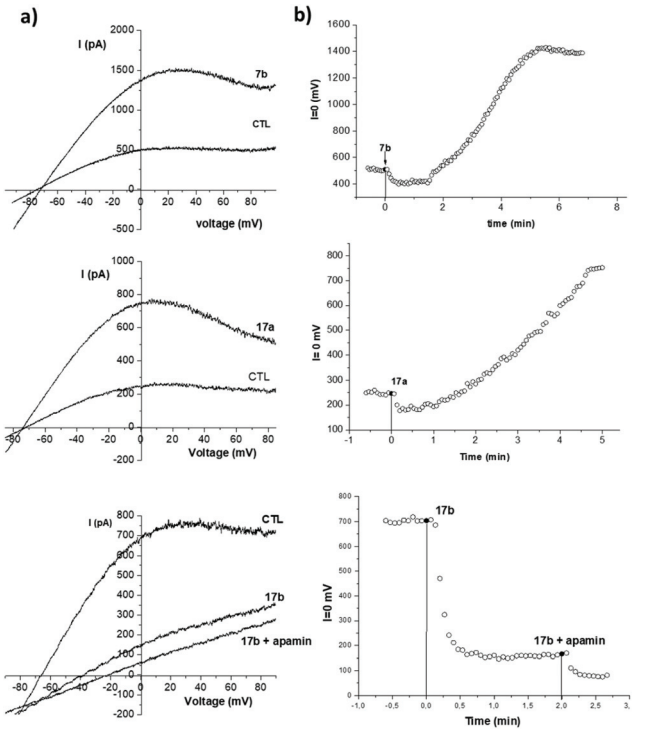
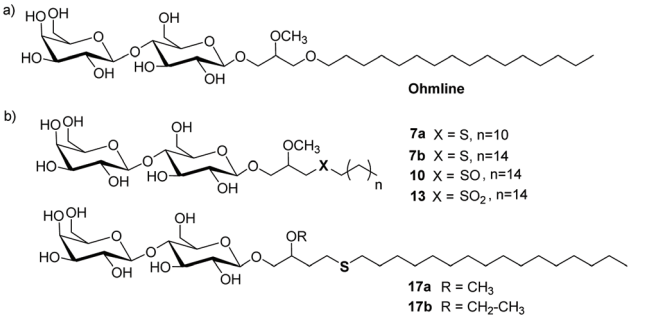


Fig. 1 Representative SK3 whole-cell currents recorded in HEK293T expressing SK3 channels before and after application of Ohmline analogues. Whole-cell currents were generated using the ramp protocol from −90 to 90 mV in 500 ms from a constant holding of 0 mV with a pCa 6. (a) Recording of currents under control conditions (vehicle application) and after exposition of compounds **7b** (top), **17a** (middle) and **17b** (bottom) at 10 μM. An addition of 100 nM apamin after exposition to **17b** is used to inhibit residual SK currents. (b) Kinetics of modulation of SK3 currents measured at 0 mV with **7b** (top), **17a** (middle) and **17b** (bottom). Cells were perfused with the vehicle and the moment when the vehicle is substituted by the analogue is indicated by a black round figure.



Scheme 1 (a) Chemical structure of Ohmline. (b) Chemical structure of the new Ohmline analogues reported herein.

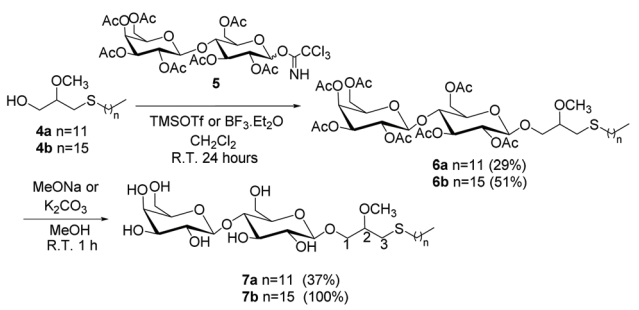
hindrance and electronic demand. As reported below, some of the new compounds are strong activators or strong inhibitors of SK3 channels. One of the activators (**7b**) is the first amphiphilic compound that affects the endothelium dependent vasorelaxation.

Results and discussion

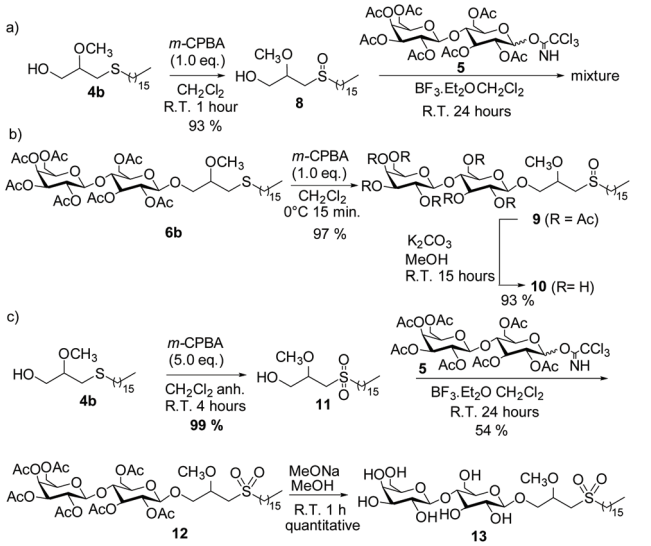
The replacement of the glycerol moiety present in Ohmline by a thioglycerol unit was the starting point of this study. We hypothesized that the replacement of the ether group by a thioether would induce geometrical modifications similar to those observed when we compare diethylether and diethylthioether (diethyl ether features C–O–C angles of 112° and C–O bonds are close to 1.43 Å (ref. 39), whereas diethylthioether features a C–S–C bond angle of 100° and a C–S bond length close to 1.81 Å (ref. 40)). As a consequence, these modifications could influence the interaction of Ohmline analogues with the other amphiphiles present in the plasma membranes and, then, impact SK3 channel function. It is noteworthy that it was previously evidenced that the incorporation of the thioether function within lipid chains of ionic liquids,[41] bolaamphiphiles[42] or cationic amphiphiles, influenced their physicochemical properties.[43,44] The other differences induced by the replacement of one ether moiety by a thioether function are: (i) the difference of polarity due to the respective electronegativity of the oxygen and sulfur atoms; (ii) the size of the sulfur atom, which is larger than the oxygen atom and more polarizable; and (iii) the possibility of the thioether derivatives to be oxidized in sulfoxide[45] and subsequently in sulfone.[46,47]

The synthesis of the thioether analogues of Ohmline uses thioglycerol as a starting material (see the ESI† for full details of the synthesis), which is converted into the thioether **4a** or **4b** in four synthesis steps (overall yield: 40%).[48–50] Compound **4a** or **4b** that possesses either a C12 or C16 lipid chain (Scheme 2) was then engaged in a glycosylation reaction with the lactose acetate **5** activated at its anomeric position with a trichloroacetimidate moiety to produce compound **6a** or **6b**. The deprotection of the seven alcohol functions, achieved by transesterification with potassium carbonate in methanol (for **7a**) or with sodium methanolate (for **7b**), produced **7a** and **7b** in 37% and 100% yields, respectively. It is important to emphasize that the precursors **4a** and **4b** are racemic because we have previously shown that the configuration at the sn-2 position of the glycerol unit of Ohmline has no effect on the modulation of SK3 function.[29]

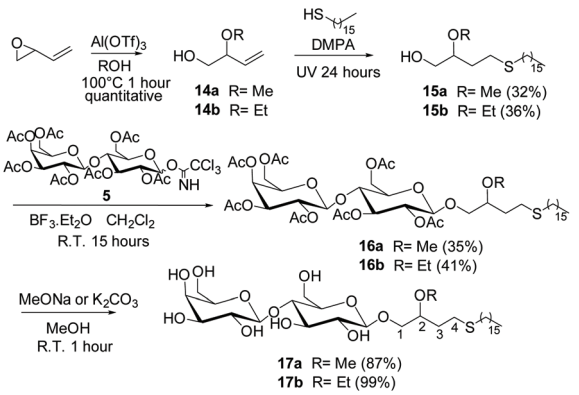
Then, we prepared analogues of compound **7b** in which the thioether function was oxidized in sulfoxide or sulfone (Scheme 3). The synthesis of the sulfoxide starts with **4b** that was oxidized with mCPBA (1 eq. at room temperature) as reported by Ayers *et al.*[51] The sulfoxide **8** was glycosylated with lactose-acetate trichloroacetimidate **5**. This reaction, which is catalyzed by BF_3, produced a mixture of compounds that was impossible to separate. The presence of the sulfoxide that can also interact with BF_3 is likely the reason for the side reaction (Scheme 3a). To overcome these difficulties, we investigated a second approach for achieving the oxidation of the thioether after the glycosylation step. Accordingly, the addition of one equivalent of mCPBA to the thioether **6b** yields the expected sulfoxide **9** as a mixture of diastereoisomers. The deprotection of the alcohol functions present on the lactose moiety produces the expected compound **10** (Ohmline-4-SO) in 93% yield (Scheme 3b). The preparation of the sulfone was achieved by using the strategy initially attempted for the sulfoxide (Scheme 3c). The oxidation of compound **4b** with an excess of mCPBA (5 equivalents) for a longer reaction time (4 h) produced almost quantitatively the sulfone **11**. Its glycosylation with the activated lactose **5** produced compound **12** in 54% yield. Finally, the deprotection of alcohol functions yielded compound **13** (Ohmline 4-SO_2) in quantitative yield. A fourth analogue of Ohmline was prepared with the aim to shift the thioether moiety away from the polar head group by one methylene unit (Scheme 4). The synthesis scheme is completely different and starts with the synthesis of the vinyl alcohol



Scheme 2 Synthesis of thio-ether analogues of Ohmline **7a** and **7b**.



Scheme 3 (a) Attempts to synthesize compound **10** by a glycosylation reaction involving the sulfoxide **8**. (b) Synthesis of the sulfoxide analogue of Ohmline **10** (Ohmline-4-SO) by oxidation of the thioether **6b**. (c) Synthesis of the sulfone **13** from **4b**. Synthesis of thio-ether analogues of Ohmline **7a** and **7b**.

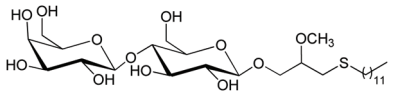
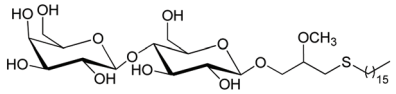
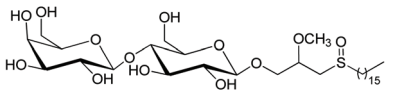
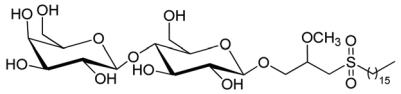
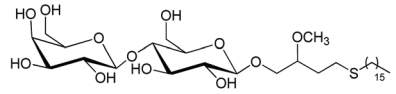
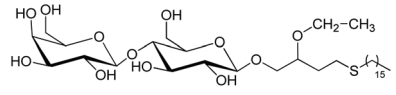


Scheme 4 Synthesis of the thioether analogue of Ohmline **17a-b**.

by a regioselective opening of the vinyl epoxide in acidic media by adopting a reported procedure.[52] Then, we applied a photo-click thiol–ene reaction[53] that engaged compounds **14a** and **14b** with hexadecanethiol. For this reaction, the conditions were inspired from a procedure we have previously optimized to prepare other types of amphiphilic compounds.[54,55] Then, the intermediate **15a** or **15b** was engaged in a glycosylation reaction with **5** in the presence of BF_3–Et_2O to produce **16a** or **16b** in 35% and 41% yield, respectively. The deprotection of the alcohol function with sodium methanolate (**17a**) or potassium carbonate (**17b**) produced compound **17a** or **17b** in 87% and 99% yield, respectively.

Then, we tested the capacity of the molecules **7a**, **7b**, **10**, **13**, **17a** and **17b** (Table 1 and Fig. 1) to change the activity of SK3

Table 1 Effect of Ohmline's analogues on SK3 currents

	Structure	SK3 current[a]
7a		−9.7% ± 5.2 (N = 4)
7b		+177% ± 67 (N = 4)
10		+29% ± 8.6 (N = 5)
13		−19.1% ± 1.8 (N = 5)
17a		+203% ± 59 (N = 5)
17b		−91.9% ± 1.8 (N = 4)

[a] Acute effect of 10 μM Ohmline analogues in SK3 current recorded at 0 mV in HEK293 cells expressing the recombinant SK3 channel. Results are normalized to control conditions (vehicle) and expressed as mean ± S.E.M. N corresponds to the number of cells tested (independent experiments).

currents using the whole cell configuration of the patch clamp technique. For these acute tests, we used a concentration of 10 μM for each of the new amphiphilic compounds as already performed with Ohmline (Ohmline reduces SK3 current by −73%).[29] The results of the patch clamp measurements are summarized in Table 1. First, we can observe that the replacement of the ether link by a thioether function produces a strong activator of the SK3 channel (**7b**) but this effect depends on the length of the lipid chain. Indeed, the comparison of **7a** and **7b** indicates that the reduction of the length of the lipid chain from 16 carbon atoms (same as Ohmline) to 12 carbon atoms abolished the effect on SK3. We hypothesize that a short lipid chain reduces the membrane anchor properties of the amphiphilic compounds, thus abolishing the inhibition of SK3.

Then, when the position of the sulfur atom was changed, the compound retained its activation capacity (comparison of compounds **7b** and **17a**). Fig. 1a shows typical SK3 currents recorded at membrane potentials varying from −90 to +90 mV for 500 ms under control conditions (vehicle) and after the application of compounds **7b** and **17a**. We observed that the amplitude of outward currents strongly increased after the application of compounds **7b** and **17a**. SK3 currents were analysed at 0 mV to minimize chloride currents and they showed an increase close to 200% (Table 1). The entire time course of these experiments is depicted in Fig. 1b and we can observe a relatively slow effect of these compounds. Indeed, we found that the maximum of the activation was reached 5 minutes after the application of either compound **7b** or **17a** (Fig. 1a and b), whereas the maximum inhibitory effect of Ohmline is observed only 2 minutes after application.[29] It can be speculated that compounds **7b** and **17a** are incorporated more slowly than Ohmline within the plasma membrane. Then, we investigated the consequence of the presence of a sulfoxide or sulfone function. Interestingly, the introduction of a sulfoxide or sulfone function at the same place where the thioether function was present in compound **7b** produced amphiphiles with reduced or no action on SK3 currents (comparison of compound **7b** with **10** and **13**). The oxidation of thioether produced more polar functions (sulfoxide or sulfone) that likely influenced the position of the amphiphilic compound within the plasma membrane. Moreover, steric hindrance constraints and supramolecular interactions are completely different from a thioether to a sulfoxide or a sulfone.[56] Nevertheless, these results show for the first time that the incorporation of thioether in place of an ether function produced the first amphiphilic activators of SK3, whereas the oxidation of this thioether function produced almost non-active compounds.

We next tested compound **17b** that differed from compound **17a** in the presence of an ethoxy group at the sn-2 position in place of a methoxy group present in **17a**. We have previously shown that the methoxy group at the sn-2 position has an important effect on the modulation capacity of SK3. Indeed, LysoPAF which is an ether lipid possessing a phosphocholine polar head group and an unsubstituted hydroxy group at the sn-2 position has no effect on SK3.[29] The result was

unexpected because the replacement of the methoxy group by an ethoxy group converts the activator **17a** in a new and original inhibitor of the SK3 channel **17b** that reduced SK3 currents by −91.9 ± 1.8% (Fig. 1). The kinetics of the inhibition is even quicker than that of Ohmline because the maximum inhibitory effect is reached after 30 seconds (Fig. 1a, right).

We have previously reported that the modulation of SK3 channel activity influences the migration of various cancer cells. The underlying mechanism implies a variation of cytosolic calcium concentration through calcium entries from the extracellular space by calcium channels. More precisely, when the activity of SK3 is increased, it leads to plasma membrane hyperpolarization which increases the driving force for calcium entries through calcium channels. In contrast, a reduction of SK3 activity induces a depolarization of the plasma membrane that subsequently reduces the driving force for calcium entries. In this respect, SK3 channels control calcium entries that occur following two mechanisms: 1 – constitutive entries: the SK3 channel forms a complex with the calcium channel Orai1 that is constitutively open leading to a constitutive calcium influx.[59] 2 – Store Operated Calcium Entries (SOCE): the SK3 channel forms a complex with the calcium channel Orai1 and TRPC1 that is induced by the reticular STIM1 protein leading to SOCE (a variation of SOCE reduces or promotes colon and prostate cancer cell migration in the case of Ohmline and CYPPA, respectively[18,57,58]). It is noteworthy that these two types of calcium entries have different relative importance in cell migration depending on the cell line. For instance, the MDA-MB-435s cell line features mostly SK3-dependent constitutive entries, whereas PC3 and HCT116 feature mostly SK3-dependent calcium entries *via* SOCE.

Then, we assess the effect of the strong SK3 activators (**7b** and **17a**) and inhibitor (**17b**) on the migration of MDA-MB-435s. Before this, it must be noted that these compounds were not toxic according to MTT assays (Fig. ESI6-1†). For the migration assays, we applied the same protocol we used previously that involved observing the effect of these compounds (1 μM) on cell migration after 24 h. As shown in Fig. ESI3-1,† none of these compounds have an effect on the migration of MDA-MB-435s (under similar conditions, Ohmline reduces MDA-MB-435s cell migration by 50%).[29] Then, we focused on the SK3 activators (**7b** and **17a**) and tested their effect on the migration of PC3 at 1 μM and 10 μM. Under these conditions, neither **7b** nor **17a** had an impact on the migration of PC3 (Fig. ESI3-1†). The discrepancy between the strong effect of **7b**, **17a**, and **17b** on SK3 activity and the absence of an effect on cell migration raises questions on the effect of these compounds on calcium entries.

Accordingly, the cytosolic calcium concentration induced by SOCE was measured on two SK3+ cell lines (colon HCT116 and prostate PC3 cancer cell lines) at a short time of 13 min (recording the data at a shorter time was difficult due to experimental constraints). It is observed that **7b** and **17a** (10 μM) induced an increase of cytosolic calcium concentration (by 18% and 15%, respectively, on HCT116). Under similar conditions, Ohmline reduces cytosolic calcium by 26% (ref. 18)) as expected for an inhibitor of the SK3 channel (Fig. 2).[59] On PC3, **7b** and **17a** increased the calcium concentration by +24% and +70%, respectively. Then, we focused our investigation on **7b**, which is a direct thio-analogue of Ohmline. The same experiment was repeated for **7b** after 24 h because this time corresponds to the migration assay that was negative for **7b**. Surprisingly, we found that after 24 h, SOCE were significantly reduced as shown in Fig. 2E and F. This result likely explains the absence of the increase of PC3 cell migration after 24 h (Fig. ESI3-1†). Altogether, these results suggest that **7b** should

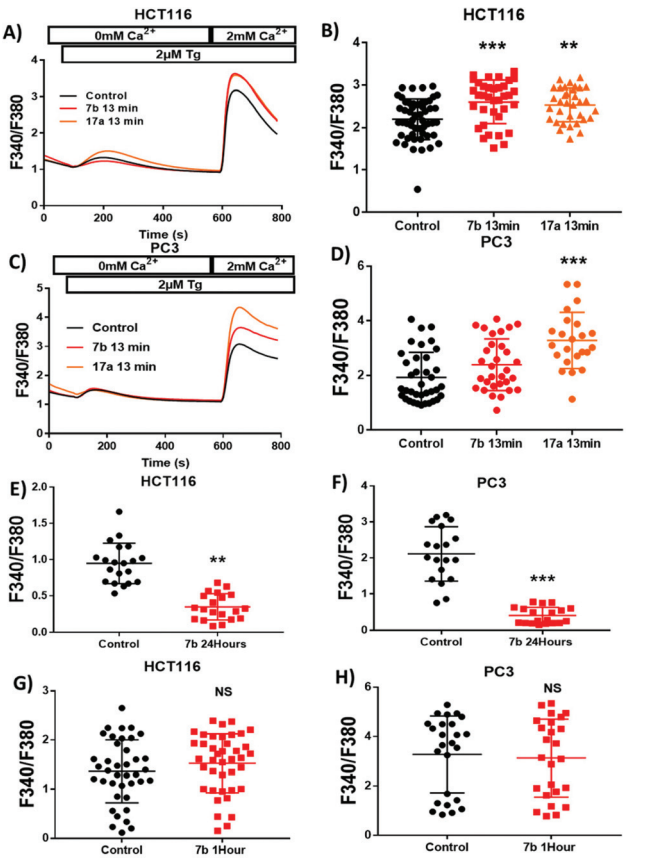


Fig. 2 Effect of compounds **7b** and **17a** at 10 μM on cytosolic calcium concentration of the HCT116 colon cancer cell line and PC3 prostate cancer cell line. Mean traces representing Ca^{2+} signals (indicated by the F340/F380 fluorescence ratio after addition of 2 mM Ca^{2+} and normalized to control conditions) triggered by adding 2 μM thapsigargin (Tg) to HCT116 (A) and PC3 cells (C) with or without pre-treatment of the chosen drugs (shown in various coloured lines, each at 10 μM dose with different times). (B–D) Scatter plots showing the peak SOCE levels triggered by Tg in control cells and cells treated with a 10 μM dose of each drug after a short period of exposure (13 minutes). Scatter plots showing the peak SOCE levels triggered by Tg in control cells and cells pre-treated with each drug at a 10 μM dose over 24 h (E and F) or 1 h (G and H). Each value (mean ± SD) was derived from 3–5 individual experiments and a total of 20–52 wells. The statistical comparison among the values for all conditions was performed using one-way ANOVA followed by Dunnett's test or Mann–Whitney test. NS, $^{**}P < 0.01$, $^{***}P < 0.001$.

activate cell migration after 13 min application and then should weakly inhibit cell migration after 24 h application. To evaluate the effect of **7b** on SOCE at an intermediate time, we recorded calcium entries in PC3 and HCT116 1 h after the addition of **7b** (10 μM). Interestingly, after 1 h application, compound **7b** has no effect on SOCE (Fig. 2G and H). Thus, this compound time dependently switches from an activator (13 min) to a non-active compound (1 h) and to a weak inhibitor of SOCE (24 h) suggesting a time-dependent metabolization. It must be noted that Ohmline in a similar experiment and after 24 h reduces SOCE[18] suggesting a limited metabolization over 24 h. The only difference between Ohmline and **7b** is the replacement of an ether function by a thioether function. It is likely that the metabolization of **7b** comes from this difference. The more likely possibility would be the oxidation of the thioether function to a sulfoxide (**10**) and then to a sulfone (**13**). **10** and **13** are a weak activator and a weak inhibitor, respectively, of SK3 (Table 1). In conclusion, the effects of **10** and **13** on SK3 activity support the hypothesis that **7b** could be oxidized over time leading to its conversion to a weak activator and then to a weak inhibitor. This variation of the effect over time could explain the absence of an effect on cell migration after 24 h. Nevertheless, a dedicated study, which is beyond the scope of this work, would be necessary to confirm this hypothesis.

Then, we studied **17b**, which is a strong inhibitor of SK3 (Table 1). **17b** has no effect on cell migration (1 μM; 24 h) as shown on Fig. ESI3-1.† It has also no effect on SOCE after a short time (13 min) as shown in Fig. ESI4-1.† 13 min is likely too short for its metabolization. Therefore, this result suggests that its effect on SK3 activity and subsequently on calcium entries is compensated by a concomitant action on other partners (*e.g.* ion channels and receptors) that have not been identified so far. Our conclusion is that **17b** has a strong inhibition effect on the SK3 channel but this effect is likely not selective leading to the absence of a variation of calcium entries and subsequently to the absence of an effect on cell migration.

Then, we selected compound **7b**, because it is the direct thio-analogue of Ohmline, to assess its effect on vasorelaxation. Indeed, the activation of the SK3 channel, found to be expressed in endothelial cells of small mesenteric arteries, is supposed to induce vasorelaxation *via* the EDHF pathway.[3] Moreover, a vasorelaxation effect is usually observed after a short exposure time to a potent drug (maximal effect usually observed before 30 min). This feature was compatible with the properties of **7b**, which is a strong activator for a short time. Thus, we tested the capacity of the SK3 activator **7b** to induce vasorelaxation by modulating excitation–contraction coupling of mesenteric smooth muscle. The activator **7b** was tested on isolated rat mesenteric arteries precontracted with the thromboxane analog U46619. As expected, acute exposure to the compound **7b** at 1 μM induced an endothelium-dependent vasorelaxant effect (−34%+/−9%, n = 8) which reached a maximum value after about 20 min of incubation (Fig. 3).

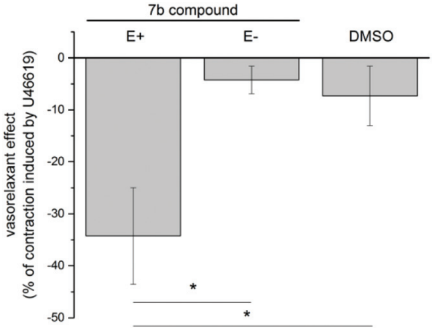


Fig. 3 Relaxant effect of **7b** (1 μM) on isolated rat mesenteric artery rings pre-contracted with U46619 (3 μM) in endothelium-intact (*E*+) and endothelium denuded (*E*−) rings. * $p < 0.05$ (Student t-test).

Conclusions

We report herein the synthesis of Ohmline analogues in which the ether function linked to the lipid chain is replaced by a thioether. We also prepared one sulfoxide and one sulfone analogues. The evaluation of these compounds by patch clamp measurements indicated that only the thioether containing compound **7b** exhibited a strong activation effect on SK3 (+177% ± 67). The presence of a shorter lipid chain (12 carbon atoms instead of 16 atoms in **7b**) produced a compound devoid of any action on SK3, thus emphasizing the importance of a long lipid chain. Then, we report the synthesis of one analogue of compound **7b** featuring a thioether function shifted by one methylene unit away from the polar head group. We found that this compound **17a** also features a strong and rapid activation effect (+203% ± 59) on SK3 channels at 10 μM. If **7b** and **17a** are strong activators of SK3, unlike Ohmline, they have no effect on cancer cell migration after 24 h. This behaviour raised questions about their possible metabolization over time. Then, we recorded the SK3-dependent calcium entries at different times (13 min, 1 h and 24 h) triggered by **7b** by using fluorescence techniques. We report for the first time that **7b** is a strong and rapid SK3 activator, which is correlated with the calcium entry SOCE as shown by cytosolic calcium measurements. However, after 24 h a strong decrease of SOCE was measured, indicating that **7b** has a time dependent effect: first, it increases SOCE, then has no effect (1 h) and after 24 h it reduces SOCE. Its biochemical transformation, possibly by oxidation, is one hypothesis but the non-selective effect *via* its action on other ion channels (*e.g.* inhibition of the calcium channel) is also hypothesized and will require further investigations. The last modulation dealt with the replacement of the methoxy group present in compound **17a** by an ethoxy group to produce compound **17b**. Surprisingly, **17b** has a strong inhibitory effect on SK3 at 10 μM (−91.9% ± 1.8) pointing out the great influence of the substituent present on the central oxygen atom. However, **17b** has no effect on cancer cell migration after 24 h, suggesting non-specific action. Finally, **7b**, which is a strong activator for a short time, is the first amphiphilic compound that induces vasorelaxation *via* an

endothelium-derived hyperpolarization, thus opening a new field of investigation for synthetic amphiphilic compounds.

Experimental

General information

Commercially available chemicals were used without additional purification. The products were characterized by ^{1}H and ^{13}C NMR spectroscopy (Bruker AC 300, Avance DRX 400 and Avance DRX 500 spectrometers) and mass spectrometry (BrukerAutoflex MALDI TOF-TOF III, LRF200CID and Waters Synapt XS). For NMR, scalar coupling constants J are given in hertz. The following abbreviations are used: s for singlet, d for doublet, t for triplet, q for quadruplet, qt for quintuplet and m for multiplet. The purity of all final compounds was ≥95% as determined by TLC (one spot after staining with I_2 and/or H_2SO_4). The description of the synthesis of compounds **1–4** is reported in ESI-1.†

Synthesis

1-*S*-Dodecyl-2-*O*-methyl-rac-thioglycer-3-yl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1–4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (6a). A solution of lactose trichloroacetimidate **5** [60] (850 mg, 1.08 mmol, 1.0 eq.) and **4a** (300 mg, 1.03 mmol, 0.95 eq.) in dry CH_2Cl_2 (4 mL) is stirred with molecular sieves 4 Å for one hour under an inert atmosphere. At 0 °C, $BF_3{\cdot}Et_2O$ in dry CH_2Cl_2 (2 mL) (196 µL, 1.08 mmol, 1 eq.) is added dropwise and the mixture is stirred for 4 hours at 0 °C under an inert atmosphere. Triethylamine is added at 0 °C and the mixture is stirred for 15 minutes. The mixture is concentrated and the compound is diluted in ethyl acetate (20 mL), filtered on Celite and concentrated to give the crude compound **6a**, which is purified on a column of silica gel (eluent: hexane/ethyl acetate: 9/1 to 8/2) to give **6a** (270 mg) in 29% yield. R_f (hexane/acetone (6/4)): 0.8; ^{1}H NMR ($CDCl_3$, 500.253): 5.33 (d, 1 H, $H_{4'}$); 5.2 (t, 1H, $H_{2'}$); 5.1 (m, 1 H, $H_{3'}$); 5.0–4.85 (m, 2H, H_1 + $H_{1'}$); 4.6–4.4 (m, 3H, H_{6b} + H_2 + $H_{1'}$); 4.2–4.0 (m, 3 H, H_{6b} + H_{6a} + $H_{5'}$); 3.7–3.4 (m, 1 H, $H_{5'}$ + H_{6a}); 3.7–3.55 (m, 2 H, CH_2 sn-3); 3.5–3.4 (m, 1 H, C*H* sn-2); 3.4 (2s, 3 H, OCH_3 two diastereoisomers); 2.7–2.6 (m, 2 H, CH_2 sn-1); 2.52 (t, 2H, J_{HH} = 7 Hz, CH_2 α fatty chain); 2.0–2.2 (m, 21H, CH_3 acetyl); 1.55 (qt, 2 H, J_{HH} = 7 Hz, CH_2 β fatty chain); 1.35 (m, 2H, CH_2 fatty chain); 1.3–1.2 (m, 26 H, CH_2 fatty chain); 0.88 (t, 3 H, J_{HH} = 7 Hz, CH_3 fatty chain); ^{13}C NMR ($CDCl_3$, 125.803): 170.4 (s, *C*=O); 170.2 (s, *C*=O); 170.1 (s, *C*=O); 169.8 (s, *C*=O); 169.7 (s, *C*=O); 169.1 (s, *C*=O); 101.1 (s, $C_{1'}$); 101.0–100.9 (C_1 two diastereoisomers); 79.8 (*C*H sn-2, two diastereoisomers); 76.2 (s, C_4); 72.6 (s, C_5); 71.6 (s, $C_{5'}$); 71.0 (CH_2 fatty chain); 70.9 (CH_2 sn-1); 70.7 (C_2); 69.5 (CH_2 sn-3); 69.1 (s, $C_{2'}$); 66.6 (s, $C_{4'}$); 62.0 (s, C_6); 60.8 (s, $C_{6'}$); 58.0–57.7 (OCH_3, two diastereoisomers); 33.2 (CH_2 fatty chain); 33.1 (CH_2 α fatty chain); 32.8 (s, CH_2 fatty chain); 32.7 (s, CH_2 fatty chain); 31.9 (s, CH_2 fatty chain); 32.7 (s, CH_2 fatty chain); 31.9 (s, CH_2 fatty chain); 29.8 (s, CH_2 fatty chain); 29.7 (s, CH_2 fatty chain); 29.6 (s, CH_2 fatty chain); 29.5 (s, CH_2 fatty chain); 29.4 (s, CH_2 fatty chain); 29.2 (s, CH_2 fatty chain); 28.9 (s, CH_2 fatty chain); 22.7 (s, CH_2 fatty chain); 20.8 (s, OCH_3); 14.1 (s, CH_3 fatty chain).

1-*S*-Hexadecyl-2-*O*-methyl-rac-thioglycer-3-yl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1–4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (6b). A solution of lactose trichloroacetimidate **5** (442 mg, 0.57 mmol, 1.0 eq.) and **4b** [49] (200 mg, 0.58 mmol, 1.02 eq.) in dry CH_2Cl_2 (15 mL) is stirred with molecular sieves 4 Å for one hour under N_2. At 0 °C, $BF_3{\cdot}Et_2O$ (28 µL, 0.23 mmol, 0.4 eq.) is added dropwise and the mixture is stirred for 24 hours at room temperature. The mixture is quenched by the addition of water (10 mL). The organic layer is washed twice with aqueous saturated $NaHCO_3$ solution (2 × 10 mL) and an aqueous saturated NaCl solution (10 mL). The organic layer is dried with $MgSO_4$, filtered and concentrated to give the crude compound **6b**. The product is purified by chromatography on silica gel (eluent: petroleum spirit/ethyl acetate: 8/2 to 7/3) to give **6b** (284 mg) in 51% yield (estimated purity: 95%). R_f (petroleum spirit/ethyl acetate (7 : 3)): 0.28; ^{1}H NMR ($CDCl_3$, 399.972): 5.33 (d, 1 H, J_{HH} = 3.2 Hz, $H_{4'}$); 5.18 (t, 1 H, J_{HH} = 10.0 Hz, H_3); 5.09 (dd, 1 H, J_{HH} = 10.0 Hz, J_{HH} = 7.9 Hz, $H_{2'}$); 4.95–4.88 (m, 2 H, H_2 + $H_{3'}$); 4.53–4.45 (m, 3 H, H_1 + H_{6a} + $H_{1'}$); 4.11–4.06 (m, 3 H, H_{6b} + $H_{6'a}$ + $H_{6'b}$); 3.98–3.85 (m, 2 H, $H_{5'}$ + H_a CH_2 sn-3); 3.78 (t, 1 H, J_{HH} = 10.0 Hz, H_4); 3.67–3.54 (m, 2 H, H_5 + H_b CH_2 sn-3); 3.48–3.42 (m, 1 H, CH sn-2); 3.39–3.38 (2 s, 3 H, OCH_3 two diastereoisomers); 2.63–2.59 (m, 2 H, CH_2 sn-1); 2.49 (t, 2 H, J_{HH} = J_{HH} = 7.4 Hz, CH_2 α fatty chain); 2.13 (s, 3 H, CH_3 acetyl); 2.10 (s, 3 H, CH_3 acetyl); 2.04 (s, 3 H, CH_3 acetyl); 2.03 (s, 9 H, CH_3 acetyl); 1.95 (s, 3 H, CH_3 acetyl); 1.54 (qt, 2 H, J_{HH} = 7.6 Hz, CH_2 β fatty chain); 1.34–1.22 (m, 26 H, CH_2 fatty chain); 0.86 (t, 3 H, J_{HH} = 6.8 Hz, CH_3 fatty chain); ^{13}C NMR ($CDCl_3$, 125.804): 170.4 (s, C=O); 170.2 (s, C=O); 170.1 (s, C=O); 169.8 (s, C=O); 169.7 (s, C=O); 169.1 (s, C=O); 101.1 (s, $C_{1'}$); 101.0–100.9 (C_1 two diastereoisomers); 79.9–79.7 (CH sn-2, two diastereoisomers); 76.2 (s, C_4); 72.7 (s, C_3); 72.6 (s, C_5); 71.6 (s, C_2); 71.0 ($C_{3'}$); 70.6 ($C_{5'}$); 69.5 (CH_2 sn-3); 69.0 (s, $C_{2'}$); 66.6 (s, $C_{4'}$); 62.0 (s, C_6); 60.8 (s, $C_{6'}$); 58.1–57.7 (OCH_3, two diastereoisomers); 33.2–32.8(CH_2 sn-1, two diastereoisomers); 32.6 (CH_2 α fatty chain); 31.9 (s, CH_2 fatty chain); 29.7 (s, CH_2 fatty chain); 29.6 (s, CH_2 fatty chain); 29.5 (s, CH_2 fatty chain); 29.4 (s, CH_2 fatty chain); 29.3 (s, CH_2 fatty chain); 28.9 (s, CH_2 fatty chain); 22.7 (s, CH_2 fatty chain); 20.9 (s, OCH_3); 20.8 (s, OCH_3); 20.7 (s, OCH_3); 20.5 (s, OCH_3); 14.1 (s, CH_3 fatty chain); mass (MALDI-TOF; matrix dithranol: 100 mg mL^{-1}): *m*/*z* calcd for $C_{46}H_{76}O_{19}SNa$ $[M + Na]^+$: 987.4599, found 987.4651.

1-*S*-Dodecyl-2-*O*-methyl-rac-thioglycer-3-yl-β-D-galactopyranosyl-(1–4)-β-D-glucopyranoside (7a – Ohmline-4S). K_2CO_3 (182 mg, 1.32 mmol, 6 eq.) is added to a solution of **6a** (200 mg, 0.22 mmol, 1.0 eq.) in MeOH (5 mL). The mixture is stirred at room temperature for 2 hours. After filtration on resin DOWEX, the filtrate is concentrated to give the crude compound **7a**. The crude compound is purified using a column of silica gel ($CHCl_3$/MeOH: 80/20) to give **7a** (50 mg) in 37% yield (estimated purity: 95%). ^{1}H NMR (DMSO-d_6, 500.252): 5.10 (brs, 2 H, 2 O*H*); 4.8 (brs, 1 H, O*H*); 4.66 (brs, 2 H, 2 O*H*); 4.51 (brs, 2 H, 2 O*H*); 4.21–4.18 (m, 2 H, H_1 + $H_{1'}$);

3.9–3.72 (m, 3 H, H_{6a} + CH_2 sn-3); 3.58–3.54 (m, 3 H, H_5 + H_{6b} + $H_{4'}$); 3.53–3.42 (m, 5 H, $H_{5'}$ + $H_{6'a}$ + $H_{6'b}$ + H_{6b} + C*H* sn-2); 3.42–3.27 (m, 7 H, H_3 + H_4 + $H_{2'}$ + $H_{3'}$ + OCH_3); 2.99 (m, 1 H, H_2); 2.70–2.57 (m, 2 H, CH_2 sn-1); 2.60–2.50 (m, 4 H, CH_2 α fatty chain + DMSO); 1.51–1.48 (m, 1 H, CH_2 β fatty chain); 1.32–1.28 (m, 2 H, CH_2 fatty chain); 1.23 (s, 24 H, CH_2 fatty chain); 0.87–0.83 (t, 3 H, J_{HH} = 7 Hz, CH_3 fatty chain); ^{13}C NMR (DMSO-d_6, 125.803): 103.9 (s, $C_{1'}$); 103.0–102.9 (C_1 diastereoisomers); 80.8 (C_4) 79.6–79.5 (*C*H sn-2, diastereoisomers); 75.5 (s, $C_{5'}$); 75.0 (s, C_3); 74.9 (s, C_5); 73.3 (s, C_2); 73.1 (s, $C_{3'}$); 70.6 ($C_{2'}$); 69.5–69.3 (*C*H_2 sn-3, diastereoisomers); 68.1 ($C_{4'}$); 60.5–60.4 (2s, C_6 + $C_{6'}$); 57.0–56.9 (O*C*H_3, diastereoisomers); 32.4–32.2 (*C*H_2 sn-1, diastereoisomers); 32.1 (*C*H_2 α fatty chain); 31.4 (s, *C*H_2 fatty chain); 29.3 (s, *C*H_2 fatty chain); 29.1 (s, *C*H_2 fatty chain); 28.8 (s, *C*H_2 fatty chain); 28.7 (s, *C*H_2 fatty chain); 28.3 (s, *C*H_2 fatty chain); 22.2 (s, *C*H_2 fatty chain); 14.0 (s, *C*H_3 fatty chain); mass (MALDI-TOF; matrix dithranol: 100 mg mL^{-1}): *m*/*z* calcd for $C_{28}H_{54}O_{12}SK$ $[M + K]^+$: 653.297, found: 653.412.

1-*S*-Hexadecyl-2-*O*-methyl-rac-thioglycer-3-yl-β-D-galactopyranosyl-(1–4)-β-D-glucopyranoside (7b – Ohmline-4S). MeONa in methanol (2.8 mL, 0.02 mmol, 0.5 eq.) is added to a solution of **6b** (40 mg, 4.14×10^{-5} mol, 1.0 eq.) in MeOH (5 mL). The mixture is stirred at room temperature for 15 hours. Amberlyst IR-120 (H+) is added and the mixture is stirred for 30 minutes at room temperature. The reaction mixture is warmed (reflux), quickly filtered and concentrated to give the crude compound **7b** (28 mg) in a quantitative yield (estimated purity: >95%). ^{1}H NMR (DMSO-d_6, 500.133): 5.12–5.10 (m, 2 H, 2 O*H*); 4.80 (brs, 1 H, O*H*); 4.68–65 (m, 2 H, 2 O*H*); 4.52–4.50 (m, 2 H, 2 O*H*); 4.22–4.20 (m, 2 H, H_1 + $H_{1'}$); 3.83–3.72 (m, 3 H, H_{6a} + CH_2 sn-3); 3.62–3.60 (m, 3 H, H_5 + H_{6b} + $H_{4'}$); 3.53–3.43 (m, 4 H, $H_{5'}$ + $H_{6'a}$ + $H_{6'b}$ + C*H* sn-2); 3.36–3.27 (m, 7 H, H_3 + H_4 + $H_{2'}$ + $H_{3'}$ + OCH_3); 3.00–2.98 (m, 1 H, H_2); 2.70–2.64 (m, 1 H, H_a CH_2 sn-1); 2.60–2.50 (m, 3 H, CH_2 α fatty chain + H_b CH_2 sn-1); 1.52–1.47 (m, 2 H, β CH_2 fatty chain); 1.32–1.23 (m, 26 H, CH_2 fatty chain); 0.85 (t, 3 H, J_{HH} = 6.8 Hz, CH_3 fatty chain); ^{13}C NMR (DMSO-d_6, 125.803): 103.9 (s, $C_{1'}$); 103.0–102.9 (C_1 diastereoisomers); 80.8 (C_4) 79.6–79.5 (*C*H sn-2, diastereoisomers); 75.5 (s, $C_{5'}$); 75.0 (s, C_3); 74.9 (s, C_5); 73.3 (s, C_2); 73.1 (s, $C_{3'}$); 70.6 ($C_{2'}$); 69.5–69.3 (*C*H_2 sn-3, diastereoisomers); 68.1 ($C_{4'}$); 60.5–60.4 (2s, C_6 + $C_{6'}$); 57.0–56.9 (O*C*H_3, diastereoisomers); 32.4–32.2 (*C*H_2 sn-1, diastereoisomers); 32.1 (*C*H_2 α fatty chain); 31.4 (s, *C*H_2 fatty chain); 29.3 (s, *C*H_2 fatty chain); 29.1 (s, *C*H_2 fatty chain); 28.8 (s, *C*H_2 fatty chain); 28.7 (s, *C*H_2 fatty chain); 28.3 (s, *C*H_2 fatty chain); 22.2 (s, *C*H_2 fatty chain); 14.0 (s, *C*H_3 fatty chain); mass (ESI QqTOF) *m*/*z* calcd for $C_{32}H_{62}O_{12}SNa$ $[M + Na]^+$: 693.3860, found 693.3857.

3-(Hexadecylsulfinyl)-2-methoxypropan-1-ol (8). To a stirred solution of **4b** [49] (198 mg, 0.571 mmol, 1.0 eq.) in dry CH_2Cl_2 (5 mL) is added very slowly mCPBA (77%) (128 mg, 0.571 mmol, 1.0 eq.) in dry CH_2Cl_2 (5 mL) at 0 °C. The mixture is stirred at 0 °C and the reaction is followed by TLC (CH_2Cl_2/MeOH: 95/5). The mixture is quenched with a saturated aqueous solution of $NaHSO_3$ (10 mL). The aqueous layer is extracted three times with CH_2Cl_2 (3 × 20 mL) and the combined organic layers are washed with a saturated aqueous solution of $NaHCO_3$ (30 mL) and brine (30 mL). The organic layer is dried on $MgSO_4$, filtered and concentrated to give **8** (193 mg; 93% yield) as a white solid. R_f (CH_2Cl_2/MeOH: 95/5): 0.39; ^{1}H NMR ($CDCl_3$, 500.133): 3.80–3.70 (m, 2 H, C*H* sn-2 + H_a CH_2 sn-3); 3.40–3.35 (m, 1 H, H_b CH_2 sn-3); 3.40–3.35 (2 s, 3 H, OCH_3 two diastereoisomers); 2.98–2.61 (m, 4 H, CH_2 sn-1 + CH_2 α fatty chain); 1.68 (qt, 2 H, J_{HH} = 7.0 Hz, CH_2 β fatty chain); 1.38–1.17 (m, 26 H, CH_2 fatty chain); 0.80 (t, 3 H, J_{HH} = 7.0 Hz, CH_3 fatty chain); ^{13}C NMR ($CDCl_3$, 75.475): 76.1–75.8 (*C*H sn-2, two diastereoisomers); 62.3–62.0 (*C*H_2 sn-3, two diastereoisomers); 57.9–57.1 (O*C*H_3, two diastereoisomers); 55.7–52.6 (*C*H_2 sn-1, two diastereoisomers); 53.2 (CH_2 α fatty chain); 31.9 (*C*H_2 fatty chain); 29.7 (*C*H_2 fatty chain); 29.5 (*C*H_2 fatty chain); 29.3 (*C*H_2 fatty chain); 29.2 (*C*H_2 fatty chain); 28.8 (*C*H_2 fatty chain); 22.7 (*C*H_2 fatty chain); 14.1 (*C*H_3 fatty chain); mass (ESI QqTOF): *m*/*z* calcd for $C_{20}H_{42}O_3SNa$ $[M + Na]^+$: 385.2752, found 385.2759.

1-*S*-Hexadecylsulfinyl-2-*O*-methyl-rac-thioglycer-3-yl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1–4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (9). To a stirred solution of **6b** (150 mg, 0.16 mmol, 1.0 eq.) in dry CH_2Cl_2 (5 mL) is added very slowly mCPBA (77%) (34 mg, 0.16 mmol, 1.0 eq.) in dry CH_2Cl_2 (5 mL) at 0 °C. The mixture is stirred at 0 °C and the reaction is followed by TLC (petroleum ether/ethyl acetate: 3/7). The mixture is quenched with a saturated aqueous solution of $NaHSO_3$ (10 mL). The aqueous layer is extracted three times with CH_2Cl_2 (3 × 20 mL) and the combined organic layers are washed with a saturated aqueous solution of $NaHCO_3$ (30 mL) and brine (30 mL). The organic layer is dried upon $MgSO_4$, filtered and concentrated to give **9** (148 mg; 97% yield; estimated purity: >95%). R_f (petroleum spirit/ethyl acetate: 7/3): 0.10; ^{1}H NMR ($CDCl_3$, 399.972): 5.34 (d, 1 H, J_{HH} = 3.2 Hz, $H_{4'}$); 5.19 (td, 1 H, J_{HH} = 9.8 Hz, J_{HH} = 2.8 Hz, H_3); 5.10 (dd, 1 H, J_{HH} = 10.4 Hz, J_{HH} = 8.0 Hz, $H_{2'}$); 4.96–4.90 (m, 2 H, H_2 + $H_{3'}$); 4.53–4.46 (m, 3 H, H_1 + H_{6a} + $H_{1'}$); 4.12–4.06 (m, 3 H, H_{6b} + $H_{6'a}$ + $H_{6'b}$); 3.98–3.61 (m, 6 H, H_4 + H_5 + $H_{5'}$ + CH_2 sn-3 + CH sn-2); 3.46–3.38 (4 s, 3 H, OCH_3 diastereoisomers); 2.98–2.61 (m, 4 H, CH_2 sn-1 + CH_2 α fatty chain); 2.14 (s, 3 H, CH_3 acetyl); 2.11 (s, 3 H, CH_3 acetyl); 2.05 (s, 3 H, CH_3 acetyl); 2.03 (s, 3 H, CH_3 acetyl); 2.02 (s, 6 H, 2 CH_3 acetyl); 1.95 (s, 3 H, CH_3 acetyl); 1.75–1.71 (m, 2 H, CH_2 fatty chain); 1.32–1.23 (m, 26 H, CH_2 fatty chain); 0.87 (t, 3 H, J_{HH} = 6.8 Hz, CH_3 fatty chain); ^{13}C NMR ($CDCl_3$, 75.474): 170.3 (s, C═O); 170.1 (s, C═O); 170.0 (s, C═O); 169.7 (s, C═O); 169.0 (s, C═O); 101.1 (s, $C_{1'}$); 101.0–100.6 (C_1 diastereoisomers); 76.1 (s, C_4); 74.0 (m, CH sn-2); 72.8 (s, C_3); 72.6 (s, C_5); 71.6(s, C_2); 71.0 ($C_{3'}$); 70.7 ($C_{5'}$); 70.3 (m, CH_2 sn-3); 69.1 (s, $C_{2'}$); 66.6 (s, $C_{4'}$); 61.8 (s, C_6); 60.8 (s, $C_{6'}$); 58.5 (m, OCH_3, diastereoisomers); 55.9 (m, CH_2 sn-1, diastereoisomers); 52.8 (m, CH_2 α fatty chain); 31.9 (s, CH_2 fatty chain); 29.7 (s, CH_2 fatty chain); 29.5 (s, CH_2 fatty chain); 29.4 (s, CH_2 fatty chain); 29.2 (s, CH_2 fatty chain); 28.8 (s, CH_2 fatty chain); 22.7 (s, CH_2 fatty chain); 20.9 (s, OCH_3); 20.8 (s, OCH_3); 20.6 (s, OCH_3); 20.5 (s, OCH_3); 14.1 (s, CH_3 fatty chain); mass (ESI QqTOF): *m*/*z* calcd for $C_{46}H_{77}O_{20}S$ $[M + H]^+$: 981.4729, found 981.4731.

1-*S*-Hexadecylsulfinyl-2-*O*-methyl-rac-thioglycer-3-yl-β-D-galactopyranosyl-(1–4)-β-D-glucopyranoside (10 – Ohmline-4-SO). K_2CO_3 (2.8 mg, 0.02 mmol, 0.5 eq.) is added to a solution of **9** (40 mg, 0.04 mmol, 1.0 eq.) in MeOH (10 mL). The mixture is stirred at room temperature overnight. Amberlyst IR-120 (H^+) is added and the mixture is stirred for 30 minutes at room temperature. The reaction mixture is warmed to reflux, quickly filtered and concentrated to give **10** in a quantitative yield (28 mg); ^{1}H NMR (DMSO-d_6, 500.133): 5.20–5.13 (m, 1 H, O*H*); 5.07 (brs, 1 H, O*H*); 4.76 (brs, 1 H, O*H*); 4.66–4.62 (m, 2 H, 2 O*H*); 4.57–4.49 (m, 2 H, 2 O*H*); 4.25–4.18 (m, 2 H, H_1 + $H_{1'}$); 3.94–3.25 (m, 17 H, H_3 + H_4 + H_5 + H_{6a} + H_{6b} + $H_{2'}$ + $H_{3'}$ + $H_{4'}$ + $H_{5'}$ + $H_{6'a}$ + $H_{6'b}$ + C*H* sn-2 + CH_2 sn-3 + OCH_3); 3.09–3.02 (m, 1 H, H_2); 3.09–2.80 (m, 2 H, CH_2 sn-1); 2.77–2.49 (m, 2 H, CH_2 α fatty chain); 1.65–1.60 (m, 2 H, CH_2 β fatty chain); 1.39–1.32 (m, 2 H, CH_2 fatty chain); 1.32–1.20 (m, 26 H, CH_2 fatty chain); 0.86 (t, 3 H, J_{HH} = 6.8 Hz, CH_3 fatty chain); ^{13}C NMR (DMSO-d_6, 125.771): 103.8 (s, $C_{1'}$); 103.0–102.8 (C_1 diastereoisomers); 80.7–80.6 (C_4 diastereoisomers); 75.5 (s, $C_{5'}$); 74.9 (brs, C_3 + C_5); 74.7–73.7 (m, *C*H sn-2 diastereoisomers); 73.2 (s, C_2); 73.1 (s, $C_{3'}$); 70.5 ($C_{2'}$); 69.6–69.2 (*C*H_2 sn-3, diastereoisomers); 68.1 ($C_{4'}$); 60.5–60.4 (2s, C_6 + $C_{6'}$); 57.3–56.6 (m, O*C*H_3 diastereoisomers); 54.5–51.8 (m, *C*H_2 sn-1 diastereoisomers); 52.5–51.3 (m, *C*H_2 α fatty chain diastereoisomers); 31.3 (s, *C*H_2 fatty chain); 29.0 (s, *C*H_2 fatty chain); 28.8 (s, *C*H_2 fatty chain); 28.7 (s, *C*H_2 fatty chain); 28.1 (s, *C*H_2 fatty chain); 22.1 (s, *C*H_2 fatty chain); 13.9 (s, *C*H_3 fatty chain); mass (ESI QqTOF): *m*/*z* calcd for $C_{32}H_{63}O_{13}S$ $[M + H]^+$: 687.3989, found 687.3982.

3-(Hexadecylsulfonyl)-2-methoxypropan-1-ol (11).[49] To a stirred solution of **4b**[49] (200 mg, 0.577 mmol, 1.0 eq.) in dry CH_2Cl_2 (10 mL) is added mCPBA (500 mg, 2.885 mmol, 5.0 eq.) dissolved in a few mL of dry CH_2Cl_2 at 0 °C. The mixture is stirred at room temperature for 4 hours. The mixture is quenched with a saturated aqueous solution of $NaHSO_3$ (10 mL). The aqueous layer is extracted three times with CH_2Cl_2 (3 × 20 mL) and the combined organic layers are washed with a saturated aqueous solution of $NaHCO_3$ (20 mL) and brine (20 mL). The organic layer is dried with $MgSO_4$, filtered and concentrated to give **11** (209 mg; 98% yield). R_f (CH_2Cl_2/MeOH: 95/5): 0.66; ^{1}H NMR ($CDCl_3$, 399.992): 3.85–3.82 (m, 2 H, C*H* sn-2 + H_a CH_2 sn-3); 3.63–3.59 (m, 1 H, H_b CH_2 sn-3); 3.43 (s, 3 H, OCH_3); 3.37 (dd, 1 H, J_{HH} = 14.8 Hz, J_{HH} = 8.0 Hz, H_a CH_2 sn-1); 3.10–3.00 (m, 3 H, H_b CH_2 sn-1 + CH_2 α fatty chain); 1.82 (qt, 2 H, J_{HH} = 6.6 Hz, CH_2 β fatty chain); 1.41 (qt, 2 H, J_{HH} = 6.8 Hz, CH_2 fatty chain); 1.36–1.23 (m, 24 H, CH_2 fatty chain); 0.87 (t, 3 H, J_{HH} = 6.6 Hz, CH_3 fatty chain); ^{13}C NMR ($CDCl_3$, 75.475): 76.4 (*C*H sn-2); 62.0 (*C*H_2 sn-3); 57.6 (O*C*H_3); 55.0 (*C*H_2 sn-1); 54.6 (CH_2 α fatty chain); 32.0 (*C*H_2 fatty chain); 29.7 (*C*H_2 fatty chain); 29.6 (*C*H_2 fatty chain); 29.4 (*C*H_2 fatty chain); 29.3 (*C*H_2 fatty chain); 29.1 (*C*H_2 fatty chain); 28.5 (*C*H_2 fatty chain); 22.7 (*C*H_2 fatty chain); 21.9 (*C*H_2 fatty chain); 14.2 (*C*H_3 fatty chain); mass (ESI QqTOF): *m*/*z* calcd for $C_{20}H_{42}O_4SNa$ $[M + Na]^+$: 401.2702, found 401.2701.

1-*S*-Hexadecylsulfonyl-2-*O*-methyl-rac-thioglycer-3-yl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1–4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (12). A solution of lactose trichloroacetimidate **5**[60] (202 mg, 0.26 mmol, 1.0 eq.) and **11** (100 mg, 0.26 mmol, 1.02 eq.) in dry CH_2Cl_2 (5 mL) is stirred with molecular sieves 4 Å for one hour under an inert atmosphere. At 0 °C, $BF_3{\cdot}Et_2O$ (13 μL, 0.10 mmol, 0.4 eq.) is added dropwise and the mixture is stirred for 17 hours at room temperature under an inert atmosphere. The mixture is quenched by the addition of water (3 mL). The organic layer is washed twice with aqueous saturated $NaHCO_3$ solution (2 × 3 mL) and an aqueous saturated NaCl solution (3 mL). The organic layer is dried with $MgSO_4$, filtered and concentrated. The crude compound is purified by chromatography on silica gel (eluent: petroleum spirit/ethyl acetate: 1/1) to give **12** (140 mg; 54% yield) as a colorless oil. R_f (petroleum spirit/ethyl acetate: 1/1): 0.35; ^{1}H NMR ($CDCl_3$, 399.972): 5.34 (d, 1 H, J_{HH} = 3.2 Hz, $H_{4'}$); 5.19 (t, 1 H, J_{HH} = 9.8 Hz, H_3); 5.12 (dd, 1 H, J_{HH} = 9.8 Hz, J_{HH} = 8.0 Hz, $H_{2'}$); 4.96–4.87 (m, 2 H, H_2 + $H_{3'}$); 4.52–4.47 (m, 3 H, H_1 + H_{6a} + $H_{1'}$); 4.12–4.06 (m, 3 H, H_{6b} + $H_{6'a}$ + $H_{6'b}$); 3.93–3.87 (m, 3 H, $H_{5'}$ + CH sn-2 + H_a CH_2 sn-3); 3.78 (t, 1 H, J_{HH} = 9.8 Hz, H_4); 3.70–3.59 (m, 2 H, H_5 + H_b CH_2 sn-3); 3.41–3.40 (2 s, 3 H, OCH_3 two diastereoisomers); 3.28–3.12 (m, 1 H, H_a CH_2 sn-1); 3.10–2.90 (m, 3 H, H_b CH_2 sn-1 + CH_2 α fatty chain); 2.16 (s, 3 H, CH_3 acetyl); 2.14 (s, 3 H, CH_3 acetyl); 2.12 (s, 3 H, CH_3 acetyl); 2.05 (s, 3 H, CH_3 acetyl); 2.03 (s, 6 H, 2 CH_3 acetyl); 1.95 (s, 3 H, CH_3 acetyl); 1.83–1.80 (m, 2 H, CH_2 fatty chain); 1.42–1.24 (m, 26 H, CH_2 fatty chain); 0.87 (t, 3 H, J_{HH} = 6.6 Hz, CH_3 fatty chain); ^{13}C NMR ($CDCl_3$, 75.474): 170.3 (s, C═O); 170.1 (s, C═O); 170.0 (s, C═O); 169.7 (s, C═O); 169.6 (s, C═O); 169.1 (s, C═O); 101.1 (s, $C_{1'}$); 100.8–100.4 (C_1 two diastereoisomers); 76.1 (s, C_4); 75.2–74.9 (CH sn-2, two diastereoisomers); 72.8 (s, C_3); 72.6 (s, C_5); 71.5(s, C_2); 71.0 ($C_{3'}$); 70.7 ($C_{5'}$); 69.1 (s, $C_{2'}$); 68.5 (CH_2 sn-3); 66.6 (s, $C_{4'}$); 61.8 (s, C_6); 60.8 (s, $C_{6'}$); 58.0–57.7 (OCH_3, two diastereoisomers); 55.0 (CH_2 sn-1, two diastereoisomers); 54.8 (CH_2 α fatty chain); 31.9 (s, CH_2 fatty chain); 29.7 (s, CH_2 fatty chain); 29.1 (s, CH_2 fatty chain); 28.5 (s, CH_2 fatty chain); 22.7 (s, CH_2 fatty chain); 21.8 (s, CH_2 fatty chain); 20.8 (s, OCH_3); 20.6 (s, OCH_3); 20.5 (s, OCH_3); 14.1 (s, CH_3 fatty chain); mass (MALDI-TOF; matrix dithranol: 100 mg mL^{-1}): *m*/*z* calcd for $C_{46}H_{76}O_{21}SNa$ $[M + Na]^+$: 1019.4497, found 1019.4607. Mass (ESI QqTOF): *m*/*z* calcd for $C_{46}H_{76}O_{21}SNa$ $[M + Na]^+$: 1019.4497, found 1019.4493.

1-*S*-Hexadecylsulfonyl-2-*O*-methyl-rac-thioglycer-3-yl-β-D-galactopyranosyl-(1–4)-β-D-glucopyranoside (13 – Ohmline-4-SO_2). NaOMe (0.5 M solution in MeOH, 48 μL, 1.3 mg, 0.03 mmol, 0.3 eq.) is added to a solution of **12** (80 mg, 0.08 mmol, 1.0 eq.) in MeOH (10 mL). The mixture is stirred at room temperature for 5 hours. Amberlyst IR-120 (H^+) is added and the mixture is stirred for 30 minutes at room temperature. The reaction mixture is warmed to reflux, quickly filtered and concentrated to give the compound **13** (53 mg) in a quantitative yield. ^{1}H NMR (DMSO-d_6, 399.972): 5.28–5.12 (m, 2 H, 2 O*H*); 4.85 (brs, 1 H, O*H*); 4.71–65 (m, 2 H, 2 O*H*); 4.60–4.52 (m, 2 H, 2 O*H*); 4.25–4.18 (m, 2 H, H_1 + $H_{1'}$); 3.89–3.74 (m, 3 H, H_{6a} + C*H* sn-2 + H_a CH_2 sn-3); 3.61–3.30 (m, 16 H, H_3 + H_4 + H_5 + H_{6b} + $H_{2'}$ + $H_{3'}$ + $H_{4'}$ + $H_{5'}$ + $H_{6'a}$ + $H_{6'b}$ + H_b CH_2 sn-3 + OCH_3 + CH_2 sn-1); 3.08–3.02 (m, 3 H, H_2 + CH_2 α fatty chain); 1.68 (qt, 2 H, J_{HH} = 7.6 Hz, CH_2 β fatty chain); 1.36–1.23 (m, 26 H, CH_2

fatty chain); 0.85 (t, 3 H, J_{HH} = 6.8 Hz, CH_3 fatty chain); ^{13}C NMR (DMSO-d_6, 125.803): 103.9 (s, $C_{1'}$); 103.0–102.8 (C_1 two diastereoisomers); 80.7 (C_4) 75.5 (*C*H sn-2); 75.0 (brs, $C_{5'}$ + C_3 + C_5); 73.2 (s, C_2); 73.1 (s, $C_{3'}$); 70.6 ($C_{2'}$); 68.9–68.4 (*C*H_2 sn-3, two diastereoisomers); 68.1 ($C_{4'}$); 60.5–60.4 (2s, C_6 + $C_{6'}$); 57.1 (O*C*H_3); 54.1 (*C*H_2 sn-1); 53.7 (*C*H_2 α fatty chain); 31.3 (s, *C*H_2 fatty chain); 29.0 (s, *C*H_2 fatty chain); 28.7 (s, *C*H_2 fatty chain); 28.5 (s, *C*H_2 fatty chain); 27.7 (s, *C*H_2 fatty chain); 22.1 (s, *C*H_2 fatty chain); 21.1 (s, *C*H_2 fatty chain); 14.0 (s, *C*H_3 fatty chain); mass (ESI QqTOF): *m*/*z* calcd for $C_{32}H_{62}O_{14}SNa$ $[M + Na]^+$: 725.3758, found 725.3755.

2-Methoxy-but-3-en-1-ol (14a). To a stirred solution of butadiene oxide (575 μL, 7.13 mmol, 1.0 eq.) in methanol (7 mL) is added Al(OTf)$_3$ (3.4 mg, 0.007 mmol, 0.001 eq.) in methanol (3 mL) dropwise. The mixture is stirred at 100 °C for 1 hour (oil bath temperature). The solvent is removed carefully under vacuum (T = 25 °C, P = 160 mbar) to obtain quantitatively the compound **14a** as a colorless liquid (728 mg). R_f (petroleum spirit/ethyl acetate: 1/1): 0.65; ^{1}H NMR ($CDCl_3$, 399.972): 5.65–5.57 (m, 1 H, H_3); 5.28–5.22 (m, 2 H, CH_2 H_4); 3.67–3.63 (m, 1 H, H_2); 4.51–3.45 (m, 2 H, CH_2 H_1); 3.28 (s, 3 H, O*C*H_3); ^{13}C NMR ($CDCl_3$, 75.5): 134.9 (*C*H alkene); 119.4 (*C*H_2 alkene); 83.5 (*C*H); 65.3 (*C*H_2); 56.6 (O*C*H_3); mass (MALDI-TOF; matrix DCTB 10 mg mL^{-1}): *m*/*z* calcd for $C_5H_{10}O_2$ $[M]^+$: 102.0681, found 102.0659.

2-Ethoxy-but-3-en-1-ol (14b). To a stirred solution of butadiene oxide (575 μL, 7.13 mmol, 1.0 eq.) in ethanol (7 mL) is added Al(OTf)$_3$ (3.4 mg, 0.007 mmol, 0.001 eq.) in ethanol (3 mL) dropwise. The mixture is stirred at 100 °C for 1 hour (heater block insert). The solvent is removed carefully under vacuum (T = 25 °C, P = 6 mbar) to obtain the compound **14b** (1.44 g; 87% yield) as a colorless liquid. R_f (petroleum spirit/ethyl acetate: 1/1): 0.67; ^{1}H NMR ($CDCl_3$, 399.920): 5.73–5.65 (m, 1H, H_3); 5.34–5.25 (m, 2H, CH_2 H_4); 3.85–3.81 (m, 1 H, H_2); 3.69–3.49 (m, 3 H, CH_2 H_1 + CH_2OEt); 3.43–3.38 (m, 1 H, CH_2 $H_{1'}$); 1.22–1.19 (t, 3H, J_{HH} = 7.2 Hz CH_3OEt); ^{13}C NMR ($CDCl_3$, 75.5): 135.3 (*C*H alkene); 117.8 (*C*H_2 alkene); 81.4 (*C*H); 64.9 (*C*H_2OEt); 63.8 (*C*H_2); 14.8 (*C*H_3OEt).

4-(Hexadecylthio)-2-methoxybutan-1-ol (15a). Compound **14a** (200 mg, 1.96 mmol, 1.0 eq.) and 1-hexadecanethiol (1.81 mL, 5.87 mmol, 3.0 eq.) are mixed under argon. DMPA (20 mg, 10% wt) is added and the solution is placed under UV for 24 hours at room temperature. The product is purified by chromatography on silica gel (eluent: petroleum spirit/ethyl acetate: 8/2) to give pure **15a** with 32% yield (218 mg). R_f (petroleum spirit/ethyl acetate: 8/2): 0.50; ^{1}H NMR ($CDCl_3$, 399.992): 3.73 (ABX, part A, dd, 1 H, J_{HH} = 11.6 Hz, J_{HH} = 3.6 Hz, H_a CH_2 H_A); 3.51 (ABX, part B, dd, 1 H, J_{HH} = 11.6 Hz, J_{HH} = 5.2 Hz, H_b CH_2 H_A); 3.40 (s, 4 H, H_B + O*C*H_3); 2.57 (t, 2 H, J_{HH} = 7.0 Hz, CH_2 α fatty chain); 2.51 (t, 2 H, J_{HH} = 7.2 Hz, CH_2 H_D); 1.88 (h, 1 H, J_{HH} = 6.8 Hz, H_a CH_2 H_C); 1.77 (h, 1 H, J_{HH} = 6.8 Hz, H_b CH_2 H_C); 1.59 (qt, 2 H, J_{HH} = 7.4 Hz, CH_2 β fatty chain); 1.35–1.119 (m, 26 H, CH_2 fatty chain); 0.88 (t, 3 H, J_{HH} = 6.6 Hz, CH_3 fatty chain); ^{13}C NMR ($CDCl_3$, 75.474): 80.2 (C_B); 63.4 (C_A); 57.3 (O*C*H_3); 32.2 (*C*H_2 α fatty chain); 31.9 (*C*H_2 fatty chain); 30.6 (C_C); 29.7; 29.5; 29.3; 29.2; 28.9; 27.9 (C_D + *C*H_2 fatty chain); 22.7 (*C*H_2 fatty chain); 14.1 (*C*H_3 fatty chain); mass (ESI QqTOF) *m*/*z* calcd for $C_{21}H_{44}O_2SNa$ $[M + Na]^+$: 383.2960, found 383.2959.

4-(Hexadecylthio)-2-ethoxybutan-1-ol (15b). Compound **14b** (400 mg, 3.44 mmol, 1.0 eq.) and 1-hexadecanethiol (3.2 mL, 10.3 mmol, 3.0 eq.) are mixed under nitrogen. DMPA (20 mg, 10% wt) is added and the solution is placed under UV for 24 hours at room temperature. The product is purified by chromatography on silica gel (eluent: pentane/ethyl acetate: 8/2) to give **15b** (460 mg; 36% yield) as a colorless liquid. R_f (pentane/ethyl acetate: 8/2): 0.41; ^{1}H NMR ($CDCl_3$, 399.922): 3.67–3.62 (m, 1H, CH_a sn3); 3.57–3.52 (m, 2H, CH_2 –OEt); 3.48–3.43 (m, 2 H, CH_b sn3 + C*H* sn2); 2.55 (t, 2 H, J_{HH} = 7.2 Hz, CH_2 α S); 2.48 (t, 2 H, J_{HH} = 7.2 Hz, CH_2 α S fatty chain); 2.13 (s, 1H, OH); 1.83–1.78 (m, 1H, CH_a sn1); 1.73–1.69 (m, 1H, CH_b sn1); 1.55–1.49 (m, 2H, *CH*$_2$ βS fatty chain); 1.34–1.31 (m, 2H; CH_2 γS fatty chain), 1.21 (s, 23H, CH_2 fatty chain); 1.17 (t, 3H, CH_3 –OEt); 0.84 (t, 3H, CH_3 fatty chain); ^{13}C NMR ($CDCl_3$, 75.474): 78.5 (CH sn2); 65.3 (CH_2 –OEt); 63.97 (CH_2 sn3); 32.3; 32.0; 31.2; 29.8; 29.6; 29.5; 29.4; 29.0; 28.1 (CH_2 αS + CH_2 fatty chain); 22.8 (CH_2 sn1) 15.7 (CH_3 –OEt); 14.2 (CH_3 fatty chain); mass (Maldi TOF, Matrix HCCA): *m*/*z* calcd for $C_{22}H_{46}O_2SNa$ $[M + Na]^+$: 397.311, found 397.620.

1-*O*-[4-(Hexadecylthio)-2-methoxybutyl]-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1–4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (16a). A solution of lactose trichloroacetimidate **5** [60] (425 mg, 0.54 mmol, 1.0 eq.) and **15a** (200 mg, 0.55 mmol, 1.02 eq.) in dry CH_2Cl_2 (10 mL) is stirred with molecular sieves 4 Å for one hour under an inert atmosphere. At 0 °C, $BF_3{\cdot}Et_2O$ (27 μL, 0.216 mmol, 0.4 eq.) is added dropwise and the mixture is stirred for 15 hours at room temperature under an inert atmosphere. The mixture is quenched by the addition of water (3 mL). The organic layer is washed twice with aqueous saturated $NaHCO_3$ solution (2 × 3 mL) and an aqueous saturated NaCl solution (3 mL). The organic layer was dried on $MgSO_4$, filtered and concentrated to give **16a** which was purified by chromatography on silica gel (eluent: petroleum spirit/ethyl acetate: 8/2 to 6/4) to give **16a** (180 mg) in 35% yield. R_f (petroleum spirit/ethyl acetate: 6/4): 0.65; ^{1}H NMR ($CDCl_3$, 399.992): 5.34 (d, 1 H, J_{HH} = 2.8 Hz, $H_{4'}$); 5.19 (t, 1 H, J_{HH} = 9.4 Hz, H_3); 5.11 (dd, 1 H, J_{HH} = 10.2 Hz, J_{HH} = 7.8 Hz, $H_{2'}$); 4.95–4.87 (m, 2 H, H_2 + $H_{3'}$); 4.52–4.45 (m, 3 H, H_1 + H_{6a} + $H_{1'}$); 4.11–4.03 (m, 3 H, H_{6b} + $H_{6'a}$ + $H_{6'b}$); 3.87–3.80 (m, 2 H, $H_{5'}$ + H_a CH_2 H_A); 3.80 (t, 1 H, J_{HH} = 9.4 Hz, H_4); 3.59–3.56 (m, 1 H, H_5); 3.53–3.39 (m, 2 H, CH H_C + H_b CH_2 H_A); 3.36–3.35 (2 s, 3 H, OCH_3 diastereoisomers); 2.55–2.52 (m, 2 H, CH_2 H_D); 2.48 (t, 2 H, J_{HH} = 7.4 Hz, CH_2 α fatty chain); 2.13 (s, 3 H, CH_3 acetyl); 2.10 (s, 3 H, CH_3 acetyl); 2.04 (s, 3 H, CH_3 acetyl); 2.02 (s, 9 H, 3 CH_3 acetyl); 1.94 (s, 3 H, CH_3 acetyl); 1.72–1.70 (m, 2 H, CH_2 H_C); 1.56 (qt, 2 H, J_{HH} = 7.4 Hz, CH_2 β fatty chain); 1.34–1.19 (m, 26 H, CH_2 fatty chain); 0.87 (t, 3 H, J_{HH} = 6.8 Hz, CH_3 fatty chain); ^{13}C NMR ($CDCl_3$, 75.474): 170.4 (s, C═O); 170.2 (s, C═O); 170.1 (s, C═O); 169.9 (s, C═O); 169.7 (s, C═O); 169.2 (s, C═O); 101.2 (s, $C_{1'}$); 100.8 (s, C_1); 78.6–78.4 (C_B); 76.4 (s, C_4); 72.8 (s, C_3); 72.7 (s, C_5); 71.7(s, C_2); 71.1 ($C_{3'}$); 70.8 ($C_{5'}$); 72.2–70.3 (C_A); 69.2 (s, $C_{2'}$); 66.7 (s, $C_{4'}$); 62.1 (s, C_6);

60.9 (s, $C_{6'}$); 58.3–57.7 (m, OCH_3, diastereoisomers); 32.3 (m, CH_2 α fatty chain); 32.0 (s, CH_2 fatty chain); 31.6 (s, C_C); 29.8; 29.5; 29.4; 29.0; 27.9(C_D + CH_2 fatty chain); 22.8 (s, CH_2 fatty chain); 21.0 (s, OCH_3); 20.9 (s, OCH_3); 20.7 (s, OCH_3); 20.6 (s, OCH_3); 14.2 (s, CH_3 fatty chain); mass (MALDI TOF; matrix dithranol: 100 mg mL^{-1}): *m*/*z* calcd for $C_{47}H_{78}O_{19}S$ $[M + Na]^+$: 1001.4756, found 1001.4678.

1-*O*-[-4-(Hexadecylthio)-2-ethoxybutyl]-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1–4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (16b). A solution of lactose trichloroacetimidate **5** [60] (1.44 g, 1.84 mmol, 1.5 eq.) and **15b** (460 mg, 1.23 mmol, 1.0 eq.) in dry CH_2Cl_2 (15 mL) is stirred with molecular sieves 4 Å for one hour under an inert atmosphere. At 0 °C, $BF_3{\cdot}Et_2O$ (160 µL, 0.98 mmol, 0.8 eq.) is added dropwise and the mixture is stirred for 15 hours at room temperature under an inert atmosphere. The mixture is quenched by addition of water (5 mL). The organic layer is washed twice with aqueous saturated $NaHCO_3$ solution (2 × 10 mL) and an aqueous saturated NaCl solution (10 mL). The organic layer is dried on $MgSO_4$, filtered and concentrated to give **16b** which was purified by chromatography on silica gel (eluent: pentane/ethyl acetate: 7/3) to give **16b** (500 mg; 41% yield). R_f (pentane/ethyl acetate: 7/3): 0.4; ^{1}H NMR ($CDCl_3$, 500.133): 5.2 (d, 1 H, $^3J_{HH}$ = 3.0, $H_{4'}$); 5.07 (t, 1 H, J_{HH} = 9.5 Hz, H_3); 4.97–4.94 (dd, 1 H, J_{HH} = 10.5 Hz, J_{HH} = 8.0 Hz, $H_{2'}$); 4.85–4.82 (m, 1 H, $H_{3'}$); 4.78–4.73 (q, 1H, J_{HH} = 9.50 Hz, J_{HH} = 18 Hz, H_2); 4.43–4.34 (m, 3 H, H_1 + H_{6a} + $H_{1'}$); 4.01–3.94 (m, 3 H, $H_{6a'}$ + $H_{6b'}$ + H_{6b}); 3.80 (t, 1 H, J_{HH} = 7.0 Hz, $H_{5'}$); 3.68 (t, 2H, J_{HH} = 9.0 Hz, H_4 + H_{A1}); 3.50–3.30 (m, 5H, H_5 + H_B + H_{A2} + CH_2 –OEt diastereoisomers); 2.45–2.33 (m, 4 H, H_D + CH_2 fatty chain); 2.03–1.81 (m, 25 H, C*H*$_3$ acetyl); 1.61–1.54 (m, 2 H, C*H*$_2$ H_C); 1.42 (qt, 2 H, J_{HH} = 7.4 Hz, C*H*$_2$ β fatty chain); 1.23 (m, 2H, C*H*$_2$ γ fatty chain); 1.11 (s, 26 H, C*H*$_2$ fatty chain); 1.0 (t, 3H, J_{HH} = 7.0 Hz, CH_3 –OEt); 0.74 (t, 3 H, J_{HH} = 7.0 Hz, C*H*$_3$ fatty chain); ^{13}C NMR ($CDCl_3$, 125.771): 169.94(s, *C*═O); 169.92 (s, *C*═O); 169.8 (s, *C*═O); 169.6 (s, *C*═O); 169.4 (s, *C*═O); 169.2 (s, *C*═O); 169.0 (s, *C*═O); 168.7 (s, *C*═O); 100.7 (s, $C_{1'}$); 100.33 + 100.28 (s, C_1α + β); 76.4–76.1 (C_B); 75.9 (s, C_4); 72.6 (s, C_3); 72.3 (s, C_5); 72.1 (C_{A1}); 71.3 (s, C_2); 70.7 ($C_{3'}$); 70.4 (C_{A2}) 70.3 ($C_{5'}$); 68.8 (s, $C_{2'}$); 66.4 (s, $C_{4'}$); 65.6–65.0 (OCH_2 diastereoisomers); 61.7 (s, $C_{6'}$); 61.0 (s, C_6); 31.7–31.5 (m, C*H*$_2$ α fatty chain + C_c); 30.0 (s, C*H*$_2$ fatty chain); 29.3, 29.2, 29.0, 28.6, 27.5, 26.5 (C_D + C*H*$_2$ fatty chain); 22.3 (s, C*H*$_2$ fatty chain); 20.6–20.1 (m, O*C*H$_3$); 15.3 (s, OEt); 13.8 (CH_3 fatty chain). Mass (Maldi TOF, Matrix HCCA): *m*/*z* calcd for $C_{48}H_{80}O_{19}SK$ $[M + K]^+$: 1031.465, found 1031.567.

1-*O*-[-4-(Hexadecylthio)-2-methoxybutyl]-β-D-galactopyranosyl-(1–4)-β-D-glucopyranoside (17a – Ohmline-5S). NaOMe (0.5 M solution in MeOH, 33 µL, 0.90 mg, 0.017 mmol, 0.1 eq.) is added to a solution of **16a** (163 mg, 0.17 mmol, 1.0 eq.) in methanol (10 mL). The mixture is stirred at room temperature for one hour. Amberlyst IR-120 (H^+) is added and the mixture is stirred for 30 minutes at room temperature. The reaction mixture is warmed to reflux, quickly filtered and concentrated to give **17a** (99 mg; 87% yield). ^{1}H NMR (DMSO-d_6, 300.131): 5.12–5.09 (m, 2 H, 2 O*H*); 4.77 (brs, 1 H, O*H*); 4.67–64 (m, 2 H, 2 O*H*); 4.54–4.50 (m, 2 H, 2 O*H*); 4.22–4.19 (m, 2 H, H_1 + $H_{1'}$); 3.78–3.75 (m, 2 H, H_{6a} + H_a C*H*$_2$ H_A); 3.61–3.29 (m, 15 H, H_3 + H_4 + H_5 + H_{6b} + $H_{2'}$ + $H_{3'}$ + $H_{4'}$ + $H_{5'}$ + $H_{6'a}$ + $H_{6'b}$ + H_b C*H*$_2$ H_A + C*H* H_B + OC*H*$_3$); 3.02–3.00 (m, 1 H, H_2); 2.54–2.44 (m, 4 H, C*H*$_2$ H_D + C*H*$_2$ α fatty chain); 1.72–1.62 (m, 2 H, C*H*$_2$ H_C); 1.52 (qt, 2 H, J_{HH} = 6.6 Hz, C*H*$_2$ β fatty chain); 1.36–1.23 (m, 26 H, C*H*$_2$ fatty chain); 0.87 (t, 3 H, J_{HH} = 6.8 Hz, C*H*$_3$ fatty chain); ^{13}C NMR (DMSO-d_6, 75.474): 103.9 (s, $C_{1'}$); 102.9–102.7 (C_1 diastereoisomers); 80.8 (C_4); 78.0 (C_B); 75.5 (s, $C_{5'}$); 75.0 (s, C_3); 74.9 (s, C_5); 73.2 (s, C_2); 73.1 (s, $C_{3'}$); 70.5 ($C_{2'}$); 70.2–69.8 (C_A, diastereoisomers); 68.1 ($C_{4'}$); 60.6–60.4 (2s, C_6 + $C_{6'}$); 57.0–56.8 (O*C*H$_3$, diastereoisomers); 31.3 (*C*H$_2$ α fatty chain); 32.1 (C_C); 29.0; 28.7; 28.6; 28.2; 27.1 (C_D + *C*H$_2$ fatty chain); 22.1 (s, *C*H$_2$ fatty chain); 13.9 (s, *C*H$_3$ fatty chain). Mass (ESI, 100 mg mL^{-1}): *m*/*z* calcd for $C_{33}H_{64}O_{12}SNa$ $[M + Na]^+$: 707.40, found 707.40 $[M + Na]^+$.

1-*O*-[-4-(Hexadecylthio)-2-ethoxybutyl]-β-D-galactopyranosyl-(1–4)-β-D-glucopyranoside (17b – Ohmline-2-OEt-5S). Potassium carbonate (0.021 mg, 0.15 mmol, 0.5 eq.) is added to a solution of **16b** (300 mg, 0.31 mmol, 1.0 eq.) in methanol (20 mL). The mixture is stirred at room temperature for 6 hours. Amberlyst IR-120 (H^+) is added and the mixture is stirred for 30 minutes at room temperature. The reaction mixture is warmed to reflux, quickly filtered and concentrated to give the compound **17b** (211 mg; 99% yield; estimated purity). ^{1}H NMR (DMSO-d_6, 500.251): 5.2–8 (m, 2 H, 2 O*H*); 4.8–4.3 (m, 4 H, O*H*); 4.67–64 (m, 2 H, 2 O*H*); 4.22–4.19 (m, 2 H, H_1 + $H_{1'}$); 3.76–3.71 (m, 2 H, H_{6a} + C*H*$_2$ H_A); 3.61–3.41 (m, 15H, H_3 + H_4 + H_5 + H_{6b} + $H_{2'}$ + $H_{3'}$ + $H_{4'}$ + $H_{5'}$ + $H_{6'a}$ + $H_{6'b}$ + H_b C*H*$_2$ H_A + C*H* H_B + C*H*$_2$ –OEt); 3.0–2.98 (m, 1 H, H_2); 2.51–2.44 (m, 4 H, C*H*$_2$ H_D + C*H*$_2$ α fatty chain); 1.60 (m, 1H, CH_{C1}); 1.49 (m, 1H, CH_{C2}); 1.52 (m, 1 H, C*H*$_2$ β fatty chain); 1.33–1.22 (m, 25 H, C*H*$_2$ fatty chain); 1.1–1.06 (m, 2H, C*H*$_2$ –OEt); 0.87 (t, 3 H, J_{HH} = 7.0 Hz, C*H*$_3$ fatty chain); ^{13}C NMR (DMSO-d_6, 125.804): 103.8 (s, $C_{1'}$); 102.8 + 102.7 (C_1 diastereoisomers); 80.8 (C_4); 76.2 (C_B); 75.5 (s, $C_{5'}$); 75.0 (s, C_3); 74.8 (s, C_5); 73.2 (s, C_2); 73.1 (s, $C_{3'}$); 70.7 (C_{A1} diastereoisomers); 70.5 ($C_{2'}$); 70.3 (C_{A2}, diastereoisomers); 68.1 ($C_{4'}$); 64.4 + 64.3 (2s, CH_2 –OEt diastereoisomers); 60.5–60.3 (2s, C_6 + $C_{6'}$); 31.7 (*C*H$_2$ α fatty chain); 32.1 (C_C); 40.0; 28.9; 28.8; 28.7; 28.6; 28.1; 27.1 (C_D + *C*H$_2$ fatty chain); 22.1 (s, *C*H$_2$ fatty chain); 15.5 (s, CH_3 –OEt diastereoisomers); 13.9 (s, *C*H$_3$ fatty chain). Mass (ESI QqTOF): *m*/*z* calcd for $C_{34}H_{66}O_{12}SNa$ $[M + Na]^+$: 721.4173, found 721.4171.

Cell culture

HEK293T was obtained from the American Type Culture Collection (ATCC) and cultured in Dulbecco's modified Eagle's medium, supplemented with 10% (v : v) fetal bovine serum (Lonza, France). The cells were grown under a humidified atmosphere at 37 °C with 95% air and 5% CO_2. HEK293T cells were transduced as previously described to achieve stable expression of homotetramer SK3 channels.[16,29] Human cancer cell lines MDA-MB-435s, HCT116 and PC3 were purchased at the American Type Culture Collection and grown respectively in Alpha Dulbecco's modified Eagle's medium (USA, Manassas, Virginia), McCoy's 5A medium and RPMI medium

containing 10% foetal bovine serum (Hyclone, Lonza, Levallois-Perret, France) at 37 °C, 5% CO_2 and at moisture saturation as already described.[17,18,58] We have tested compounds on these cell lines because we already demonstrated that the SK3 channel is expressed in these cells and promotes their calcium entries (including SOCE, see below) and their migration.[17–19]

Electrophysiological recordings

Experiments were performed in the whole-cell configuration of the patch-clamp technique. The patch pipettes were filled with solution containing 145 mM KCl, 1 mM $MgCl_2$, 1 mM Mg-ATP, 0.7 mM $CaCl_2$, 1 mM EGTA and 10 mM HEPES (PCa 6). The pH was adjusted to 7.2 with KOH. The bath solution contained 140 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 0.33 mM NaH_2PO_4, and 11.5 mM D-glucose. The pH was adjusted to 7.4 with NaOH. Patch-clamp experiments and data analyses were conducted as previously described.[26] The effects of compounds on HEK293T cells expressing SK3 were measured using a ramp protocol from +100 mV to −100 mV with a holding potential of 0 mV (500 ms duration; 4 s intervals) to inactive endogenous potassium currents. The amplitude of the apamin-sensitive current was obtained by subtraction of the amplitude of the current before and after the application of 100 nmol L^{-1} apamin (a specific SKCa blocker).

In vitro cell viability and cell migration assay

Cell viability was determined using the tetrazolium salt reduction method (MTT) and cell migration assay was performed in 24-well plate cell culture inserts with a pore size of 8 μm as previously described.[61] HCT116 and PC3 cell migration was performed as already described.[18,54] Briefly, after 24 h, stationary cells were removed from the top side, whereas migrated cells in the bottom side of the inserts were fixed with DAPI (Sigma-Aldrich) and then counted. Three independent experiments were performed and these were done in triplicate.

Ca^{2+} entry measurements

Time-lapse [Ca^{2+}] cytosolic measurements were performed using a FlexStation III fluorometer (Molecular Devices). HCT116 and PC3 cells were seeded on 96-well flat clear-bottom black microplates (#3603, Corning) at a density of 3.10^4 cells per well. HCT116 and PC3 cells were grown on fibronectin-coated microplates. One day after seeding, HCT116 and PC3 cells were incubated with 4 mmol L^{-1} Fura-2/AM in culture medium for 35 minutes at 37 °C protected from light. Afterward, the cells were gently washed three times with HEPES buffered saline (140 mmol L^{-1} NaCl, 1.13 mmol L^{-1} $MgCl_2$, 4.7 mmol L^{-1} KCl, 2 mmol L^{-1} $CaCl_2$, 10 mmol L^{-1} D-glucose, and 10 mmol L^{-1} HEPES, pH 7.4) and left for 5 minutes at room temperature protected from light. For store operated Ca^{2+} entry (SOCE) experiments, cells were put in HEPES buffered saline Ca^{2+}-free solution and treated with thapsigargin (4 μM) (T7458, Life-Technologies): to deplete the intracellular store before injection of 2 mM $CaCl_2$ after 10 minutes. The maximum of fluorescence (peak of calcium influx (F340/F380)) after injection of 2 mM Ca^{2+} is measured and normalized to control conditions. Data acquisition was performed using SoftMax pro software. Fura-2-loaded cells were alternately illuminated with 340 and 380 nm. Fluorescence emission was collected at 510 nm. Figures showing Ca^{2+} traces display averages from several wells per condition and are representative of several independent recordings.

Vasorelaxation experiments

Animals. Male Wistar rats (350–450 g) were purchased from CER Janvier (Le Genest, St Isle, France). All experiments involving animals were approved by the institutional ethical committee (Comité d'éthique en expérimentation animal Val de Loire, Tours, France, Permit number: 2016090711251954) and carried out in accordance with European guidelines on animal experimentation and the French "Ministère de l'Enseignement supérieur, de la Recherche et de l'Innovation"

Preparation of isolated mesenteric arteries. Rats were anaesthetised with a bolus injection of sodium pentobarbital (60 mg kg^{-1}, i.p.). After intravenous injection of heparin (500 IU kg^{-1}) the mesenteric bed irrigating the ileum was removed and immediately placed in a Krebs–Henseleit solution containing 119 mM NaCl, 4.7 mM KCl, 1.18 mM KH_2PO_4, 1.17 mM $MgSO_4$, 1.36 mM $CaCl_2$, 25 mM $NaHCO_3$ and 5.5 mM glucose. After dissection of surrounding tissue, the second-order mesenteric resistance arteries were cut into 1.5–2 mm ring segments and were individually mounted in a Mulvany–Halpern myograph (Multi Myograph system-610, DMT, Aarhus, Denmark). The 9 ml organ chamber was filled with Krebs' solution continuously bubbled with 95% O_2/5% CO_2 and maintained at 37 °C.

Experiment protocols. Experiments were started after an equilibration period of at least 30 minutes. The mesenteric arteries were stretched to a tension of 3 mN mm^{-1} which corresponds to the physiological conditions. The measurements of isometric force (mN mm^{-1}) were performed according to the methods described by Mulvany and Halpern.[62] Isometric wall tensions of mesenteric arteries were measured with force transducers connected to a Powerlab system and recorded using Chart 5 software. To evaluate the vasorelaxant effect of the compounds, the mesenteric artery rings were precontracted with U46619 (3 μM), an agonist of thromboxane A2 receptors. The role of the endothelium in the relaxation effect induced by **7b** (1 μM) has been tested in mesenteric artery rings with or without the endothelium. The endothelium was removed by gently rubbing the mesenteric artery luminal surface with a human hair. The integrity or functional removal of the endothelium was examined from the endothelium-dependent relaxation response to acetylcholine (10 μM).

Statistics

Statistical analyses have been performed using SigmaStat Software (version 3.0.1a, Systat Software, Inc). Unless otherwise indicated, data were expressed as mean ± standard error of the

mean (N, number of experiments and n, number of cells from independent experiments). For comparison between more than two means we used Kruskal–Wallis one way analysis of variance followed by Dunn's or Dunnet's *post hoc* tests as appropriate. Comparisons between two means were made using the Mann–Whitney test and using Student *t*-text for vasorelaxation experiments. Differences were considered significant when $p < 0.05$.

Author contributions

HC, CV, and PAJ conceived the study and the new analogues of Ohmline and CMS, AB and HC performed the organic synthesis and the characterization of the new amphiphiles. KM, CP, VM and CV conceived the biological studies. DF performed *in vitro* cell viability and cell migration assays. AC performed the electrophysiological recordings. MG performed Ca^{2+} entry measurements. BLZ performed the mesenteric artery tone measurements. PAJ and CV wrote the paper with input from all authors.

Conflicts of interest

There are no conflicts to declare.

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