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Synthesis of enantiomerically pure (*Z*)-(2′*R*)-1-*O*-(2′-methoxyhexadec-4′-enyl)-*sn*-glycerol present in the liver oil of cartilaginous fish

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ABSTRACT

Synthesis of enantiopure (*Z*)-(2′*R*)-1-*O*-(2′-methoxyhexadec-4′-enyl)-*sn*-glycerol **1**, the principal methoxylated glyceryl ether found in Nature, is described by a highly convergent five-step process taking place in 27% overall yield. The synthesis is based on an ether bond formation between the chiral synthon (*R*)-2,3-*O*-isopropylidene-*sn*-glycerol and (*Z*)-(*R*)-1-chlorohexadec-4-en-2-ol employing ground potassium hydroxide and tetra-*n*-butylammonium bromide as a catalyst under solvent free conditions.

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1. Introduction

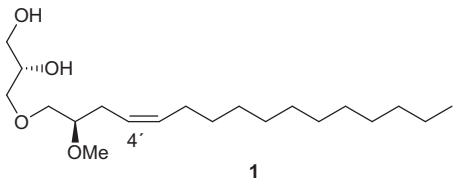
Methoxylated lipids have gained considerable attention from the medical science point of view due to their antibacterial, antifungal, antiviral, and antitumor properties.[1] The naturally occurring methoxylated lipids bearing a methoxyl group at the alkyl chain can be divided into two groups of compounds, that is, the methoxylated fatty acids and the methoxyl substituted alkylglycerols. Hitherto, the only methoxylated alkylglycerols known are those of the 1-*O*-(2′-methoxyalkyl)-*sn*-glycerol type. They are a particular category of 1-*O*-alkyl-*sn*-glycerols, characterized by bearing a methoxyl group at the 2-position of their 1-*O*-alkyl moiety.

The 1-*O*-alkyl-*sn*-glycerols, also known as glyceryl ethers, are widely found in Nature, but in especially high amounts in the liver oil of various cartilaginous fish including shark species.[2,3] In humans, they are widely found in various tissues, usually as minor lipid components.[4] The glyceryl ethers are biologically active compounds considered to have high therapeutic potential.[5,6] These properties are mutually related to their chemical structure, serving as essential precursors of platelet activating factors (PAF) and plasmalogens.[7–9] In the crude liver oil of some cartilaginous fish they are present in their diacylated form and commonly constitute about 25% of the oil.[3,10,11] The methoxylated alkylglycerols have been found to make up 2–4% of the glyceryl ether content of such oils.[12,13] Their occurrence in mammals and especially in humans has also been demonstrated but only in trace quantities.[14]

Structurally, 1-*O*-(2′-methoxyalkyl)-*sn*-glycerols contain a long alkyl chain situated at the *sn*-1 position of the glycerol moiety via an *O*-ether linkage, implying an (*S*)-configuration for the stereogenic center at the glycerol backbone. The second chiral center located at the 2-position of the *O*-alkyl chain possessing the methoxyl group displays an (*R*)-absolute configuration.[15] In general, the alkyl chain components may be saturated, monounsaturated, or polyunsaturated ranging from C_{14} to C_{22}. The monounsaturated analogs are characterized by constituting a Δ4 *Z*-configured double bond.[9,13] A peculiar polyunsaturated derivative, an all *cis*-(2′*R*)-1-*O*-(2′-methoxydocosa-4′,7′,10′,13′,16′,19′-hexaenyl)-*sn*-glycerol, first discovered by Hallgren et al.,[16] is present in appreciable amounts in the liver oil of cartilaginous fish.[13]

The principal 1-*O*-(2′-methoxyalkyl)-*sn*-glycerols found in Nature are the monounsaturated (*Z*)-(2′*R*)-1-*O*-(2′-methoxyhexadec-4′-enyl)-*sn*-glycerol **1**, its *bis*-homo (*Z*)-(2′*R*)-1-*O*-(2′-methoxyoctadec-4′-enyl)-*sn*-glycerol congener, and the saturated (2′*R*)-1-*O*-(2′-methoxyhexadecyl)-*sn*-glycerol **2**. The first one is by far the most abundant methoxylated alkylglycerol derivative found in the liver oil of cartilaginous fish. These derivatives are the same as those first reported in the pioneering work of Hallgren and Stallberg.[9]

Methoxyl substituted glyceryl ethers have been shown to possess antibacterial[17] and antifungal[18] activities, and to inhibit some cancer cell lines[19,20] and metastasis formation in mice.[17,18]



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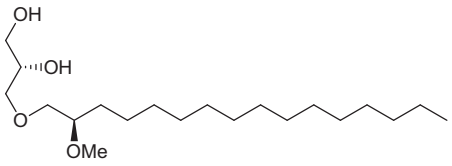

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Furthermore, several studies have demonstrated their immune stimulating properties in mice.[21] A deeper look into these studies shows that in most cases, mixtures of methoxylated alkylglycerols,

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isolated from shark liver oil, or synthetic stereoisomeric mixtures of 1-*O*-(2′-methoxyhexadecyl)glycerol, or (*Z*)-1-*O*-(2′-methoxyhexadec-4-enyl)glycerol were utilized.[17–21] At the same time only few studies have been carried out on pure individual compounds possessing the natural configuration. Presumably, this is due to the low concentrations of methoxylated alkylglycerols present in shark liver oil and the tedious isolation methods needed for their separation into singular compounds. Therefore, the development of new routes to synthesize enantiomerically pure methoxylated alkylglycerols in the laboratory setting is a tempting option to supply such valuable natural compounds.

Stallberg reported on the synthesis of enantiopure (2′*R*)-1-*O*-(methoxyhexadecyl)-*sn*-glycerol **2** in 1990.[15] Herein, we report the first synthesis of enantiomerically pure (*Z*)-(2′*R*)-1-*O*-(2′-methoxyhexadec-4′-enyl)-*sn*-glycerol **1** by a highly convergent five step synthesis, that is, based on ether bond formation between the chiral synthon (*R*)-2,3-*O*-isopropylidene-*sn*-glycerol and (*Z*)-(*R*)-1-chlorohexadec-4-en-2-ol **5**.

2. Results and discussion

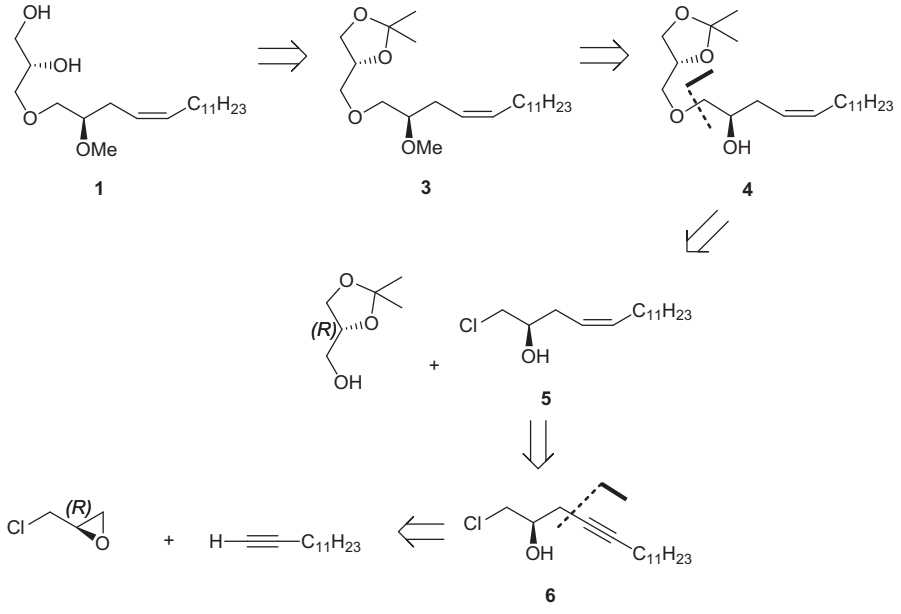
Although the structure of the principal unsubstituted 1-*O*-alkyl-*sn*-glycerols has been known since the early 1920s, it was not until the late 1960s that the first methoxylated alkylglycerols were isolated and their structures elucidated by Hallgren and Stallberg[13] when working with 1-*O*-alkyl-*sn*-glycerols from the unsaponifiable matter of Greenland shark (*Somniousus microcephalus*) liver oil. They found that the mixture of methoxylated alkylglycerols made up to 4% of the glyceryl ether content. Likewise, Hayashi and Takagi[12] have reported the methoxyl substituted alkylglycerol content in three shark species and three ratfish species where they accounted for 0.1% to 0.3% of the total liver lipid content. Similar percentages have been reported in several other aquatic animals, ranging from 0.01% to 0.17% in the non-polar lipids and from 0.06% to 0.47% in the phospholipids.[22] More recently, methoxylated alkylglycerols have been isolated from the brachiopod *Gryphus vitreus*[23] and from the marine sponge *Spirastrella abata*.[24]

Previous studies on the synthesis of unsubstituted 1-*O*-alkyl-*sn*-glycerols have established that the most suitable route for their preparation is via an ether synthesis between a chiral 2,3-*O*-isopropylidene-*sn*-glycerol, (*R*)-solketal, and the corresponding long-chain primary alkyl halides or sulfonates.[25,26]

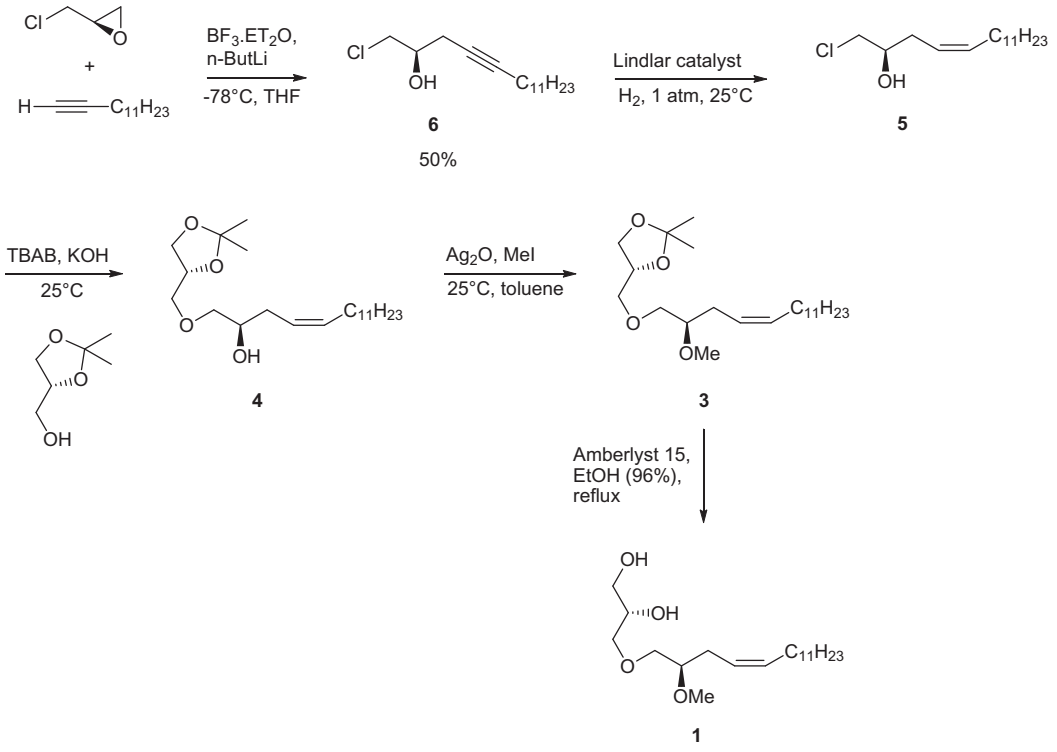
Hallgren and Stallberg[13] reported the first synthesis of the methoxylated alkylglycerol 1-*O*-(2′-methoxyhexadecyl)glycerol **2** as a racemic mixture of diastereomers. The key step of their synthetic route involved a coupling of the potassium salt of racemic solketal with racemic tosylate of 2-methoxyhexadecan-1-ol in 45% yield. Following a similar methodology, Stallberg[27] synthesized the monounsaturated 2′-methoxylated alkylglycerol (*Z*)-1-*O*-(2′-methoxyhexadec-4′-enyl)glycerol **1** also obtained as a mixture of four stereoisomers. More recently, Stallberg[15] performed the first synthesis of the naturally occurring (2′*R*)-1-*O*-(2′-methoxyhexadecyl)-*sn*-glycerol **2** enantiomerically pure. The key step, taking place in 60% yield, involved an ether formation between (*R*)-solketal and (*R*)-2-methoxyhexadecyl *p*-toluenesulfonate in the presence of powdered potassium hydroxide and solvent.

The task of the current synthesis of enantiopure (*Z*)-1-*O*-(2′-methoxyhexadec-4′-enyl)-*sn*-glycerol **1** involves preparing a target molecule possessing two stereogenic centers and a *Z*-configured carbon–carbon double bond. To address that goal and searching for a new efficient synthetic plan the retrosynthetic analysis shown in Scheme 1 was proposed.

The retrosynthetic approach was started with a disconnection of the methyl group from the methoxyl ether moiety of the acetonide protected target molecule **3**. This implies a late-stage incorporation of the methoxyl group by methylation of the hydroxyl group in alcohol adduct **4**. Then, considering previous strategies in the synthesis of regular and substituted glyceryl ethers, the proposed synthetic strategy involves the disconnection of the main ether function at the *O*-alkyl site of **4**. This indicates a coupling between 2,3-*O*-isopropylidene-*sn*-glycerol and the functionalized compound **5**. The *Z*-configured double bond in **5** is easily obtained by partial hydrogenation of the corresponding triple bond in **6** with a Lindlar catalyst. Compound **6** contains three functional groups closely situated and may be constructed by a highly regioselective oxirane ring-opening of (*R*)-epichlorohydrin with the lithium salt of 1-tridecyne. In this manner, the target molecule **1** is disconnected down to three commercially available compounds, including two enantiopure C_3-synthons, offering a synthesis composed of five steps.



Scheme 1.



Scheme 2.

The execution of the proposed synthetic plan is shown in Scheme 2 and was started with the regioselective opening of the epoxide ring in (*R*)-epichlorohydrin with tridec-1-ynyllithium in the presence of boron trifluoride etherate according to a method developed by Yamaguchi and Hirao.[28] This afforded (*R*)-1-chlorohexadec-4-yn-2-ol **6** in 50% yields and 98% ee as was established by ^{1}H NMR analysis of its Mosher ester derivative. In this step BF_3 plays a crucial role by activating the epoxide ring thus directing the nucleophilic attack of the organolithium adduct exclusively to the less substituted carbon in the oxirane ring rather than the methylene group possessing the chloride group. That would result in an inversion of the desired configuration of the initial epichlorohydrin.

Such regioselective control is thought to be induced by boron trifluoride coordination to the oxirane oxygen resulting in the formation of an alkoxyboron trifluoride salt following the nucleophilic attack[29] (Scheme 3). In the absence of such coordination, the liberated alkoxide anion could substitute the chlorine atom, hence forming a new epoxide that in turn may undergo a second attack of the organolithium nucleophile.

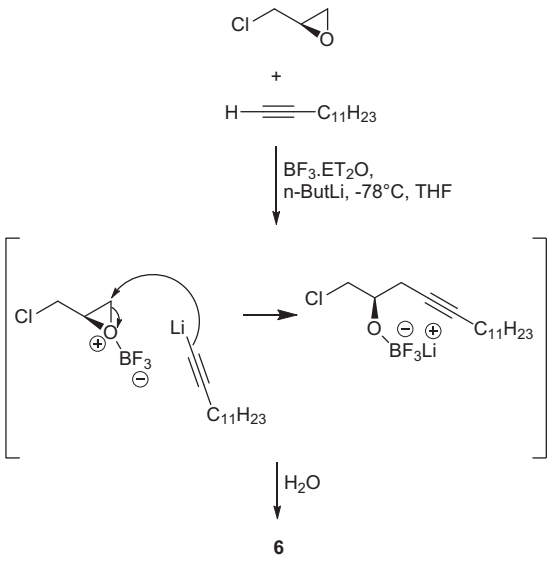
Alternatively, such an epoxide may undergo a base promoted elimination to form hexadec-2-en-4-yn-1-ol **7**. Subsequent hydrogenation of **6** over a Lindlar catalyst afforded the chloroalkenol **5** in 96% yield.

The coupling of (*R*)-solketal with **5** was performed using freshly ground potassium hydroxide in the presence of a catalytic amount of tetra-*n*-butylammonium bromide (TBAB) under solvent free condition at room temperature, affording the highly functionalized key adduct **4** in 62% yield. Scheme 4 shows a plausible mechanism for the reaction where TBAB plays a dual function, as a phase-transfer agent and as a catalyst. TBAB exchanges its bromide anion for the hydroxide anion, which is transferred from the solid phase to the organic phase. Then, the hydroxide ion deprotonates the hydroxyl groups of **5** and the solketal, forming epoxide **8**, and presumably, a tetra-*n*-butylammonium (TBA) isopropylideneglyceroxide salt, respectively. The high reactivity of the ammonium-alkoxide ion-pair is thought to be caused by a low cation–anion interaction energy induced by the large TBA cation rendering the alkoxide anion with higher nucleophilicity.[30] There is evidence from the ^{1}H NMR investigations that **8** was produced slowly as an intermediate and converted to **4** during the 52 h reaction time at room temperature. Under these reaction conditions, however, formation of the elimination side-product, hexadec-2,4-dien-1-ol **9**, was greatly diminished compared to what occurred with other attempted approaches and is described in the discussion below.

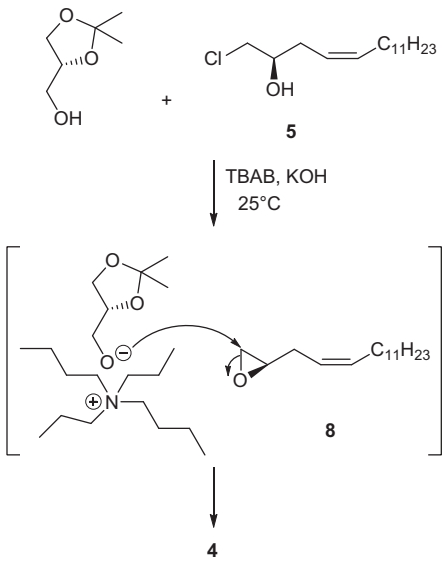
Three other compounds closely related to **5** were screened for the ether synthesis coupling reaction with (*R*)-solketal under various reaction conditions (Scheme 5). For instance, employment of the methoxylated chloroalkyne, 1-chloro-2-methoxyhexadec-4-yne **10**, gave no reaction product when reacted with the solketal. All attempts to couple the protected glycerol with adduct **10** were unsuccessful, although a variety of conditions were screened, including sodium or sodium hydride in THF or DMSO and pulverized potassium hydroxide with TBAB under solvent free conditions.

The closer proximity of the methoxyl group to the chloride was thought to induce some steric effects on nucleophilic attack of the alkoxide. On the other hand, the coupling of (*R*)-solketal with chloroalkynol **6** and its corresponding epoxide derivative, 1,2-epoxyhexadec-4-yne **11**, in the presence of ground potassium hydroxide and TBAB at 30–40 °C furnished the desired coupling adduct, but in low yields, mainly due to the production of high amounts of the elimination side-product hexadec-2-en-4-yn-1-ol **7** as a 1:1 mixture of *E*- and *Z*-isomers. The presence of **7** strongly indicates its formation via **11**, the epoxide form of **6**, although it was not detected during the progress of the reaction involving chloroalkynol **6**, unlike that which was observed during the coupling reaction of chlorohydrin **5**.

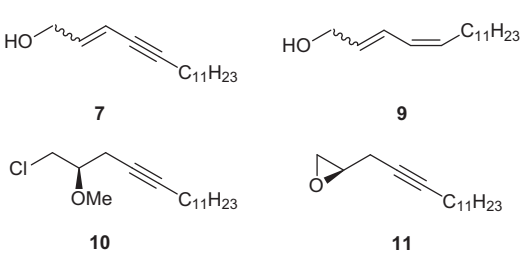
The hydroxyl group in **4** was smoothly methylated by taking advantage of silver oxide and methyl iodide in a toluene suspension containing molecular sieves at room temperature. The mild reaction conditions employed triggered complete methylation without any deterioration of the closely situated *Z*-configured



Scheme 3.



Scheme 4.



Scheme 5.

double bond as was evidenced from the ^{1}H NMR spectrum of **3**. The simple separation of **3** from the reaction mixture consisted of a rapid flush of the reaction suspension through silica gel packed inside a Pasteur pipette eluting with diethyl ether. No further purification was needed and **3** was afforded in excellent yields (93%) and high purity as indicated by ^{1}H and ^{13}C NMR analysis.

Finally, the isopropylidene moiety in **3** was cleaved under mild reaction condition employing Amberlyst® 15, an ion exchanger, in 96% ethanol at gentle reflux.[31] Again, under these conditions, the *Z*-configured double bond in **1** remained stable as was confirmed by ^{1}H NMR spectroscopy. Isolation of the final product **1** was accomplished by simple filtration of the ion exchanger, followed by removal of the solvents, affording **1** in 99% yield and 27% overall yield from (*R*)-epichlorohydrin. The structure of **1** was confirmed by ^{1}H and ^{13}C NMR analysis and two dimensional ^{1}H–^{1}H COSY and HETCOR correlation analysis.

Table 1
^{1}H NMR data of compound **1**

Position	Compound **1**[a] ^{1}H (ppm)	Compound **1**[b] ^{1}H (ppm)
sn-3	3.71 (dd, 1H, *J* = 11.4, 3.9)	3.69 (dd, *J* = 11.5, 3.9)
	3.65 (dd, 1H, *J* = 11.4, 5.2)	3.63 (dd, *J* = 11.5, 5.2)
sn-2	3.86 (m, 1H)	3.85 (m, 1H)
sn-1	3.62 (dd, 1H, *J* = 10.1, 3.6)	3.61 (dd, 1H, *J* = 10.0, 3.8)
	3.55 (dd, 1H, *J* = 10.1, 6.2)	3.53 (dd, 1H, *J* = 9.9, 6.2)
1′	3.57 (dd, 1H, *J* = 10.5, 3.6)	3.56 (dd, 1H, *J* = 10.3, 3.6)
	3.48 (dd, 1H, *J* = 10.5, 6.0)	3.47 (dd, 1H, *J* = 10.5, 6.0)
2′	3.33 (m, 1H)	3.32 (m, 1H)
3′	1.47 (m, 2H)	1.47 (m, 2H)
4′ to 15′	1.26 (m, 24H)	1.25 (m, 24H)
16′	0.88 (t, 3H)	0.87 (t, 3H, *J* = 6.9)
OCH_3	3.40 (s, 3H)	3.39 (s, 3H)

[a] From the literature;[15] measured at 125 MHz in $CDCl_3$; *J* = Hz.
[b] Prepared by hydrogenation of **2**; measured at 400 MHz in $CDCl_3$–D_2O (7:0.1); *J* = Hz.

The fact that no loss of chirality was observed for adduct **6** (98% ee) nor any degradation of the isopropylidene protective moiety was detected throughout the total synthesis, strongly implies a high enantiopurity of the final product **1**. Examination of the ^{1}H NMR spectrum (400 MHz) of the saturated **2** that was prepared from **1** by hydrogenation revealed its excellent agreement with that of **2** found in the literature (125 MHz)[15] as may be seen from Table 1, which shows the details of the comparison.

Table 2
Comparison of optical activity data for **1** and **2**

Compound	**1**[a]	**1**[b]	**2**[c]	**2**[d]
$[\alpha]_D$	−3.0	−3.0	−12.5	−12.0

[a] *c* 0.89, $CHCl_3$, 20 °C; obtained from the hydrogenation of **2**.
[b] *c* 1.3, $CHCl_3$, 20 °C; from the literature.[15]
[c] *c* 0.84, $CHCl_3$, 20 °C; prepared by the presented approach.
[d] *c* 1.3, $CHCl_3$, 25 °C; from the literature.[27]

Although some minor differences can be noticed in Table 1, they are presumably due to the solvent and the concentration differences under which the measurements were performed. Furthermore, comparison of specific rotation data of **1** and **2** with the corresponding data from the literature, firmly establishes the absolute configuration of the stereogenic centers in compound **1** as can be noticed from Table 2.

3. Conclusion

The most abundant methoxylated alkylglycerol present in the liver oil of cartilaginous fish, (*Z*)-(2′*R*)-1-*O*-(2′-methoxyhexadec-4′-enyl)-*sn*-glycerol **1**, has been synthesized in enantiomerically pure form by a highly convergent chemical route. It is anticipated that the facile and efficient synthetic approach described herein will prompt scientists to investigate further on the biological properties of this intriguing compound.

4. Experimental

4.1. General

^{1}H and ^{13}C nuclear magnetic resonance spectra were recorded on a Bruker Avance 400 spectrometer in deuterated chloroform as a solvent, unless otherwise stated, at 400.12 and 100.61 MHz, respectively. Chemical shifts (δ) are quoted in parts per million (ppm) and the coupling constants (J) in hertz (Hz). The following abbreviations are used to describe the multiplicity: s, singlet; d, doublet; t, triplet; dd, doublet of doublets; ddt, doublet of doublets of triplets; m, multiplet. The number of carbon nuclei behind each ^{13}C signal is indicated in parentheses after each chemical shift value, when there is more than one carbon responsible for the peak. All Infrared spectra were conducted on a Nicolet Avatar 360 FT-IR (E.S.P.) Spectrophotometer on a ZnSe plate. The optical activities were measured on an Autopol V from Rudolph Research Analytical, New Jersey USA. Melting points were determined on a Büchi 520 melting point apparatus and are uncorrected. The high-resolution mass spectra (HRMS) were acquired on a Bruker micrOTOF-Q mass spectrometer equipped with an atmospheric pressure chemical ionization chamber (APCI) or an E-spray atmospheric pressure ionization chamber (ESI). All data analysis was done on Bruker software.

All chemicals and solvents were used without further purification unless otherwise stated. (*R*)-2,3-*O*-Isopropylidene-*sn*-glycerol (98%, 99% ee), (*R*)-epichlorohydrin (99%, 98% ee), (*S*)-epichlorohydrin (98%, 97% ee), *n*-butyllithium solution (2.0 M in cyclohexane), quinoline (98%), and all the solvents (HPLC grade) were purchased from Sigma-Aldrich (Steinheim, Germany). Tetra-*n*-butylammonium bromide (⩾99.0%), (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (⩾99.0%, enantiomeric ratio: ⩾99.5:0.5), Amberlyst 15, and Lindlar catalyst were obtained from Fluka (Buchs, Switzerland) and silver(I) oxide from Fischer Scientific UK. Boron trifluoride etherate (~50%), methyl iodide (99%), activated palladium (10% Pd), and potassium hydroxide were purchased from Merck (München or Darmstad, Germany). Molecular sieves 4 Å were obtained from Acros Organics (Geel, Belgium), 1-tridecyne (>99.0%) from GFS Chemicals (Ohio, USA) and preparative TLC plates (250 μm, F-254) from Silicycle (Quebec, Canada).

4.1.1. Synthesis of (*R*)-1-chlorohexadec-4-yn-2-ol 6

To a solution of 1-tridecyne (2.16 g, 11.98 mmol) in freshly distilled tetrahydrofuran (60 ml) under a nitrogen atmosphere, *n*-butyllithium (2.0 M) in cyclohexane (6 ml, 12 mmol) was added at −78 °C and the resulting solution stirred for 20 min. Subsequently, boron trifluoride etherate (1.6 ml, 12.7 mmol) was added to the solution and stirred vigorously for 15 min at −78 °C. Then, (*R*)-epichlorohydrin (1.11 g, 12.0 mmol) was added, after which the solution was stirred for 3 h at −78 °C, and finally left to reach room temperature. The reaction was quenched with water, treated with saturated ammonium chloride solution, and extracted with diethyl ether. After drying over magnesium sulphate and evaporation of the solvents, the concentrate was applied to silica gel column chromatography using diethyl ether/*n*-hexane (10:90 and 20:80) as eluents affording the product **6** as a clear oil (1.63 g, 50% yield). $[\alpha]_D^{20} = -12.2$ (*c* 2.50, ethanol); IR(ZnSe) 3375 (O–H), 2922 (C–H) cm^{-1}; HRMS (APCI): *m/z* calcd for $C_{16}H_{29}OCl + H$: 273.1980; found 273.1981 amu. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.93 (quintet, J = 5.7 Hz, 1H, CH_2CHCH_2), 3.70 (dd, J = 11.1, 4.6 Hz, 1H, CH_2Cl), 3.61 (dd, J = 11.1, 6.1 Hz, 1H, CH_2Cl), 2.48 (ddt, J = 17.6, 7.3, 2.3 Hz, 1H, $CHCH_2C{\equiv}$), 2.54 (ddt, J = 16.4, 5.4, 2.5 Hz, 1H, $\equiv CCH_2$), 2.31–2.17 (br s, 1H, CHO*H*), 2.15 (tt, J = 7.1, 2.4 Hz, 2H, $\equiv CCH_2$), 1.48 (quintet, J = 7.2, 2H, $\equiv CCH_2CH_2$), 1.39–1.32 (m, 2H, $\equiv CCH_2CH_2CH_2$), 1.32–1.18 (m, 14H, CH_2), 0.88 (br t, J = 6.8 Hz, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 83.96 (C≡C), 74.30 (C≡C), 69.98, 48.31, 31.90, 29.61 (2), 29.52, 29.33, 29.11, 28.88, 28.86, 24.70, 22.67, 18.69, 14.10 ppm.

4.1.2. Synthesis of (*Z*)-(*R*)-1-chlorohexadec-4-en-2-ol 5

To a solution of (*R*)-1-chlorohexadec-4-yn-2-ol **6** (1.6 g, 5.87 mmol) and quinoline (59 mg, 0.458 mmol) in dry petroleum ether (100 ml), Lindlar catalyst (169 mg) was added. The resulting suspension was placed in a PARR reactor under hydrogen pressure (1 atm) and stirred for 50 min at room temperature. The reaction mixture was filtered over a pad of Celite, flushed with dichloromethane, and concentrated. Further purification was accomplished by silica gel column chromatography with gradient elution, diethyl ether/petroleum ether (10:90), and pure diethyl ether, affording the product **5** as a clear oil (1.56 g, 96% yield). $[\alpha]_D^{20} = +2.0$ (*c* 1.00, ethanol); IR(ZnSe) 3372 (O–H), 3010 (=C–H *cis*), 2922 (C–H), 1656 (C=C) cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{16}H_{31}OCl + Li$: 281.2218; found 281.2205 amu. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.58 (m, 1H, =C*H*), 5.37 (m, 1H, =C*H*), 3.85 (ddd, J = 13.5, 6.5, 3.7 Hz, 1H, CH_2CHCH_2), 3.64 (dd, J = 11.1, 3.7 Hz, 1H, CH_2Cl), 3.51 (dd, J = 11.1, 6.6 Hz, 1H, CH_2Cl), 2.35 (t, J = 7.2 Hz, 2H, $CHCH_2C{=}$), 2.19–1.90 (br s, 1H, CHO*H*), 2.05 (quartet (br), J = 6.8 Hz, 2H, $=CCH_2$), 1.39–1.32 (m, 2H, $=CCH_2CH_2$), 1.32–1.19 (m, 16H, CH_2), 0.88 (br t, J = 6.9 Hz, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.13 (C=C), 123.36 (C=C), 71.20 (CH_2CHCH_2), 49.60 (CH_2Cl), 32.23 ($CHCH_2CH{=}$), 31.91, 29.65, 29.63, 29.61, 29.55, 29.51, 29.34, 29.30, 27.42 ($=CCH_2$), 22.68, 14.11 (CH_3) ppm.

4.1.3. Synthesis of (*Z*)-(2′*R*)-1-*O*-(2′-hydroxyhexadec-4′-enyl)-2,3-*O*-isopropylidene-*sn*-glycerol 4

To a mixture of (*Z*)-(*R*)-1-chlorohexadec-4-en-2-ol **5** (1.0 g, 3.64 mmol), (*R*)-2,3-*O*-isopropylidene-*sn*-glycerol (480 mg, 3.64 mmol) and tetra-*n*-butylammonium bromide (242 mg, 0.728 mmol), freshly pulverized potassium hydroxide (408 mg, 7.28 mmol) was added and the resulting slurry mixture stirred at room temperature for 52 h. Then, water and diethyl ether were added to the reaction mixture, after which the organic phase was separated, washed three times with water, and once with a brine solution. After evaporation of the solvent, the resulting crude residue was applied to a silica gel column with gradient elution, diethyl ether/petroleum ether (60:40 and 40:60), affording the product **4** as a clear oil (1.34 g, 62% yield). $[\alpha]_D^{20} = -5.7$ (*c* 0.86, ethanol); IR(ZnSe) 3461 (O–H), 2923 (C–H), 1656 (C=C), 1123 and 1054 (C–O–C) cm^{-1}; HRMS (APCI): *m/z* calcd for $C_{22}H_{42}O_4 + NH_4$: 388.3408; found 388.3417 amu. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.56–5.48 (m, 1H, $C{=}CHCH_2CH_2$), 5.42–5.35 (m, 1H, $CHCH_2CH{=}C$), 4.30–4.25 (m, 1H, $CHOC(CH_3)_2$), 4.05 (dd, J = 8.3, 6.5 Hz, 1H, $(CH_3)_2COCH_2$), 3.85–3.78 (m, 1H, C*H*OH), 3.74 (dd, J = 8.3, 6.4 Hz, 1H, $(CH_3)_2COCH_2$), 3.57 (dd, J = 10.2, 5.5 Hz, 1H, $(CH_3)_2COCHCH_2O$), 3.54 (dd, J = 9.6, 3.1 Hz, 1H, OCH_2CHOH), 3.53 (dd, J = 10.0, 2.6 Hz, 1H, $(CH_3)_2COCHCH_2O$), 3.38 (dd, J = 9.8, 7.6 Hz, 1H, OCH_2CHOH), 2.41 (d, J = 3.4 Hz, 1H, O*H*), 2.30–2.18 (m, 2H, $CHOHCH_2C{=}$), 2.02 (quartet (br), J = 6.8 Hz, 2H, $=CCH_2CH_2$), 1.42 (s, 3H, $(CH_3)_2CO$), 1.36 (s, 3H, $(CH_3)_2CO$), 1.33–1.20 (m, 18H, CH_2), 0.88 (br t, 3H, J = 6.9 Hz, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.12 ($C{=}CHCH_2CH_2$), 124.24 ($CHCH_2CH{=}C$), 109.46 ($(CH_3)_2C$), 75.44 (OCH_2CHOH), 74.68 ($CHOC(CH_3)_2$), 72.29 ($(CH_3)_2COCHCH_2O$), 70.21 (C*H*OH), 66.58 ($(CH_3)_2COCH_2$), 31.91, 31.20 ($CHOHCH_2C{=}$), 29.66, 29.63 (2), 29.59, 29.54, 29.33 (2), 27.38 ($=CCH_2CH_2$), 26.72 ($(CH_3)_2CO$), 25.37 ($(CH_3)_2CO$), 22.68 (CH_2CH_3), 14.11 (CH_3) ppm.

4.1.4. Synthesis of (*Z*)-(2′*R*)-1-*O*-(2′-methoxyhexadec-4′-enyl)-2,3-*O*-isopropylidene-*sn*-glycerol 3

To a mixture of (*Z*)-(2′*R*)-1-*O*-(2′-hydroxyhexadec-4′-enyl)-2,3-*O*-isopropylidene-*sn*-glycerol **4** (500 mg, 1.349 mmol), silver oxide (937 mg, 4.047 mmol) and 4 Å molecular sieves (9.37 g) in freshly

distilled toluene (15 ml), methyl iodide (843 μl, 13.49 mmol) was added, and the resulting mixture stirred for 17 h at room temperature under an argon atmosphere. The reaction mixture was filtered over a small cake of silica gel and eluted with diethyl ether. Removal of volatile constituents in vacuo afforded the pure compound **3** as a slightly yellowish oil (480 mg, 93% yield). $[\alpha]_D^{20} = -13.1$ (*c* 0.83, ethanol); IR(ZnSe) 2922 (C–H), 1657 (C=C), 1102 and 1055 (C–O–C) cm^{-1}; HRMS (APCI): *m/z* calcd for $C_{23}H_{44}O_4 + NH_4$: 402.3578; found 402.3576 amu. ^1H NMR (400 MHz, $CDCl_3$): δ 5.51–5.44 (m, 1H, C=C*H*CH_2CH_2), 5.40–5.33 (m, 1H, $CHCH_2CH$=C), 4.26 (quintet, *J* = 5.9 Hz, 1H, C*H*O$C(CH_3)_2$), 4.05 (dd, *J* = 8.3, 6.4 Hz, 1H, $(CH_3)_2COCH_2$), 3.76 (dd, *J* = 8.3, 6.3 Hz, 1H, $(CH_3)_2COCH_2$), 3.57 (dd, *J* = 10.0, 5.3 Hz, 1H, $(CH_3)_2COCHCH_2O$), 3.50 (d, *J* = 5.0 Hz, 2H, OCH_2CHOCH_3), 3.48 (dd, *J* = 10.0, 5.8 Hz, 1H, $(CH_3)_2COCHCH_2O$), 3.41 (s, 3H, OCH_3), 3.37 (quintet, *J* = 5.4 Hz, 1H, C*H*OCH_3), 2.27 (t, *J* = 6.4 Hz, 2H, CH_3OCHCH_2C=), 2.02 (quartet (br), *J* = 6.8 Hz, 2H, =CCH_2CH_2), 1.41 (s, 3H, $(CH_3)_2CO$), 1.36 (s, 3H, $(CH_3)_2CO$), 1.33–1.20 (m, 18H, CH_2), 0.88 (br t, 3H, *J* = 6.9 Hz, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 132.43 (C=CHCH_2CH_2), 124.55 ($CHCH_2CH$=C), 109.30 ($(CH_3)_2C$), 80.08 (C*H*OCH_3), 74.65 (C*H*O$(CH_3)_2$), 73.25 (OCH_2CHOCH_3), 72.37 ($(CH_3)_2COCHCH_2O$), 66.90 ($(CH_3)_2COCH_2$), 57.46 (OCH_3), 31.91, 29.67 ($CHOHCH_2C$=), 29.64 (2), 29.59, 29.56, 29.36, 29.34, 28.85 (CH_3OCHCH_2C=), 27.38 (=CCH_2CH_2), 26.76 ($(CH_3)_2CO$), 25.42 ($(CH_3)_2CO$), 22.68 (CH_2CH_3), 14.11 (CH_3) ppm.

4.1.5. Synthesis of (*Z*)-(2′*R*)-1-*O*-(2′-methoxyhexadec-4′-enyl)-*sn*-glycerol 1

To a solution of (*Z*)-(2′*R*)-1-*O*-(2'methoxyhexadec-4′-enyl)-2,3-*O*-isopropylidene-*sn*-glycerol **3** (359 mg; 0.933 mmol) in 96% ethanol (3 ml), wet Amberlyst 15 (63 mg) was added and refluxed for 5 h and 30 min. After filtration of Amberlyst the solution was concentrated in vacuo, affording the pure compound as slightly yellowish oil (317 mg, 99% yield). $[\alpha]_D^{20} = -12.5$ (*c* 0.84, chloroform); IR(ZnSe) 3402 (O–H), 3010 (=C–H *cis*), 2921 (C–H), 1655 (C=C), 1082 and 1046 (C–O–C) cm^{-1}; HRMS (APCI): *m/z* calcd for $C_{20}H_{40}O_4$ + H: 345.2999; found 345.2999 amu. ^1H NMR (400 MHz, $CDCl_3$–D_2O, 7:0.1) δ 5.52–5.45 (m, 1H, C=C*H*CH_2CH_2), 5.38–5.31 (m, 1H, $CHCH_2CH$=C), 3.88–3.83 (m, 1H, C*H*OH), 3.69 (dd, *J* = 11.5, 4.0 Hz, 1H, CH_2OH), 3.63 (dd, *J* = 11.5, 5.2 Hz, 1H, CH_2OH), 3.61 (dd, *J* = 10.0, 3.7 Hz, 1H, $HOCHCH_2OCH_2$), 3.57 (dd, *J* = 10.4, 3.2 Hz, 1H, OCH_2CHOCH_3), 3.53 (dd, *J* = 10.0, 6.3 Hz, 1H, $HOCHCH_2OCH_2$), 3.46 (dd, *J* = 10.5, 6.2 Hz, 1H, OCH_2CHOCH_3), 3.42 (s, 3H, OCH_3), 3.40–3.35 (m, 1H, C*H*OCH_3), 2.35–2.21 (m, 2H, CH_3OCHCH_2C=), 2.02 (quartet (br), *J* = 6.8 Hz, 2H, =CCH_2CH_2), 1.35–1.20 (m, 18H, CH_2), 0.88 (br t, 3H, *J* = 6.9 Hz, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$–D_2O, 7:0.1) δ 132.74 (C=CHCH_2CH_2), 124.15 ($CHCH_2CH$=C), 80.22 (C*H*OCH_3), 73.39 ($HOCHCH_2OCH_2$), 73.14 (OCH_2CHOCH_3), 70.44 (C*H*OH), 63.85 (CH_2OH), 57.23 (OCH_3), 31.90, 29.66, 29.63 (2), 29.55 (2), 29.34 (2), 28.36 (CH_3OCHCH_2C=), 27.37 (=CCH_2CH_2), 22.68 (CH_2CH_3), 14.10 (CH_3) ppm.

4.1.6. Preparation of (2′*R*)-1-*O*-(2′-methoxyhexadecyl)-*sn*-glycerol 2

A suspension of (*Z*)-(2′*R*)-1-*O*-(2′-methoxyhexadec-4′-enyl)-*sn*-glycerol **1** (35 mg, 0.102 mmol) and 10% Pd in activated charcoal (7 mg) in absolute ethanol (25 ml) was placed in a PARR reactor under a hydrogen pressure (1 atm) and stirred for 2 h and 30 min at room temperature. The reaction mixture was filtered over a pad of Celite and flushed with diethyl ether. Solvent removal in vacuo afforded the pure product **2** as a white wax (34 mg, 96% yield). $[\alpha]_D^{20} = -3.0$ (*c* 0.89, chloroform); IR(KBr) 3420 (O–H), 2918 (C–H), 1121 (C–O–C) cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{20}H_{42}O_4$ + H: 347.3156; found 347.3159 amu. ^1H NMR (400 MHz, $CDCl_3$–D_2O, 7:0.1): δ 3.88–3.83 (m, 1H, C*H*OH), 3.69 (dd, *J* = 11.5, 3.9 Hz, 1H, CH_2OH), 3.63 (dd, *J* = 11.5, 5.2 Hz, 1H, CH_2OH), 3.61 (dd, *J* = 10.0, 3.8 Hz, 1H, $HOCHCH_2OCH_2$), 3.56 (dd, *J* = 10.3, 3.6 Hz, 1H, OCH_2CHOCH_3), 3.53 (dd, *J* = 9.9, 6.2 Hz, 1H, $HOCHCH_2OCH_2$), 3.47 (dd, *J* = 10.5, 6.0 Hz, 1H, OCH_2CHOCH_3), 3.39 (s, 3H, OCH_3), 3.35–3.30 (m, 1H, C*H*OCH_3), 1.54–1.41 (m, 2H, CH_3OCHCH_2C), 1.37–1.18 (m, 24H, CH_2), 0.87 (br t, 3H, *J* = 6.9 Hz, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$–D_2O, 7:0.1): δ 80.37 (C*H*OCH_3), 73.51 (OCH_2CHOCH_3), 73.34 ($HOCHCH_2OCH_2$), 70.45 (C*H*OH), 63.86 (CH_2OH), 57.26 (OCH_3), 31.91, 30.80 (CH_3OCHCH_2C), 29.77, 29.68 (2), 29.66, 29.64 (2), 29,58, 29.56, 29.34, 25,33, 22.68 (CH_2CH_3), 14.10 (CH_3) ppm.

4.1.7. Preparation of (*R*,*R*)-MTPA ester of (*R*)-6

The (*R*)-MTPA ester of **6**, obtained from (*R*)-epichlorohydrin was prepared according to the reported method[32] and purified by preparative TLC with ethyl acetate/petroleum ether (2.5:97.5) as an eluent affording the Mosher ester product as a clear liquid. ^1H NMR (400 MHz, $CDCl_3$): δ 7.60–7.55 (m, 2H, =C–H), 7.43–7.37 (m, 3H, =C–H), 5.34–5.28 (m, 1H, CH_2CHCH_2), 3.76 (dd, *J* = 11.9, 4.7 Hz, 1H, CH_2Cl), 3.69 (dd, *J* = 11.9, 5.9 Hz, 1H, CH_2Cl), 3.59 (m, 3H, OCH_3), 2.72 (ddt, *J* = 17.1, 6.3, 2.4 Hz, 1H, $CHCH_2C$≡), 2.65 (ddt, *J* = 16.7, 5.8, 2.4 Hz, 1H, ≡CCH_2), 2.13 (tt, *J* = 7.1, 2.4 Hz, 2H, ≡CCH_2), 1.45 (quintet, *J* = 7.2, 2H, ≡CCH_2CH_2), 1.38–1.19 (m, 16H, CH_2), 0.88 (br t, *J* = 6.9 Hz, 3H, CH_3) ppm.

4.1.8. Preparation of (*R*,*S*)-MTPA ester of (*S*)-6

A procedure identical to the one described for the preparation of the (*R*,*R*,)-MTPA ester of **6** was followed in detail using (*S*)-**6**. Adduct (*S*)-**6** was obtained from (*S*)-epichlorohydrin by the same methodology previously described for **6**. ^1H NMR (400 MHz, $CDCl_3$): δ 7.61–7.54 (m, 2H, =C–H), 7.43–7.37 (m, 3H, =C–H), 5.31–5.26 (m, 1H, CH_2CHCH_2), 3.88 (dd, *J* = 12.0, 3.8 Hz, 1H, CH_2Cl), 3.77 (dd, *J* = 12.0, 6.4 Hz, 1H, CH_2Cl), 3.60 (m, 3H, OCH_3), 2.58 (m, 2H, $CHCH_2C$≡), 2.07 (tt, *J* = 7.0, 2.4 Hz, 2H, ≡CCH_2), 1.44 (quintet, *J* = 7.0, 2H, ≡CCH_2CH_2), 1.37–1.17 (m, 16H, CH_2), 0.88 (br t, *J* = 6.9 Hz, 3H, CH_3) ppm.

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