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Chemical transformation of Leustroducsins: synthesis of Leustroducsin B

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Abstract—Chemical transformation of Leustroducsin H to Leustroducsin B has been successfully accomplished in 11 steps including enzymatic hydrolysis of phosphate ester. The process described here enables to differentiate all hydroxyl groups, amino and phosphate functionality so that this process would serve as a useful template for the preparation of whole different kinds of synthetic derivatives in structure activity relationship study. Absolute configuration of the side chain carboxylic acid has been determined as (*S*) configuration by Akasaka's method. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

Leustroducsins (LSNs) A, B (**2**) and C were isolated from the culture broth of *Streptomyces platensis* SANK 60191 by Sankyo's groups (Fig. 1).[1,2] These compounds represent novel microbial metabolites belonging to the phoslactomycin family.[3] Leustroducsin B (LSN-B) was originally purified as a colony-stimulating factor (CSF) inducer. In addition, LSN-B has various biologic activities. In vitro, LSN-B induced granulocyte-CSF (G-CSF) and granulocyte-macrophage-CSF (GM-CSF) production by KM-102 cells.[1] In vivo, LSN-B has been shown to augment host resistance in lethal infection with *Escherichia coli*[4] and to induce thrombocytosis[5] when administered to mice. It was speculated that the stimulation of cytokine production via regulatory pathway by LSN-B is distinct from that of interleukin-1β (IL-1β), bacterial lipopolysaccharide (LPS), or phorbol 12-myristate 13-acetate (PMA) and that this signalling pathway of LSN-B might lead to the induction of a variety of cytokines from primary human bone marrow stromal cells (BMSCs).[6]

Since LSN-B reveals promising biological activity, further intensive investigation is necessary to develop LSN-B as a new drug substance for the treatment of various diseases, however, it is severely restricted due to limited supply of the compound. Since no microbial has not yet been found to produce LSN-B selectively, it is currently only available as a minor component of a mixture of LSNs and repeated HPLC purification is essential for the supply of purified LSN-B, even though large scale fermentation is achieved for multi hundred gram scale LSNs production. Indeed, only 9.83 mg of LSN-B was obtained from 60 l of culture broth after significant amount of purification work efforts.[2] Therefore, chemical synthesis seems to be the only feasible way to provide large quantity of the material.

In synthetic chemistry point of view, LSN-B is a quite challenging molecule. The structure features poly-functionalized carbon framework contains seven chiral centers, unsaturated lactone, primary amine, and phosphate monoester. The absolute configuration of LSN-H was determined to be 4*S*,5*S*,8*R*,9*R*,11*R*,16*R*, and 18*S* by Shibata et al.[7] although, the absolute configuration of 6-methyloctanoic acid on C-18 of LSN-B has not yet been determined. Thanks to the previous report regarding to the preparation of LSN-H, which represents the core structure of the LSNs, LSN-H is available in large quantity from the mixture of LSNs by enzymatic chemoselective hydrolysis with

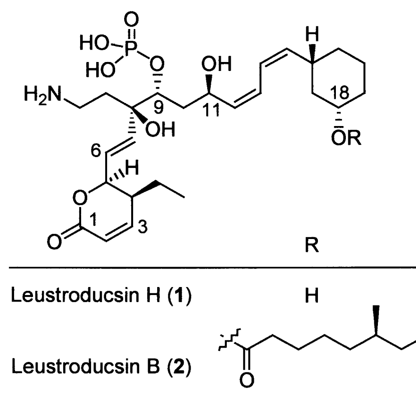

Figure 1. The structures of Leustroducsins.

Keywords: Leustroducsin; chemical transformation; enzymatic hydrolysis.

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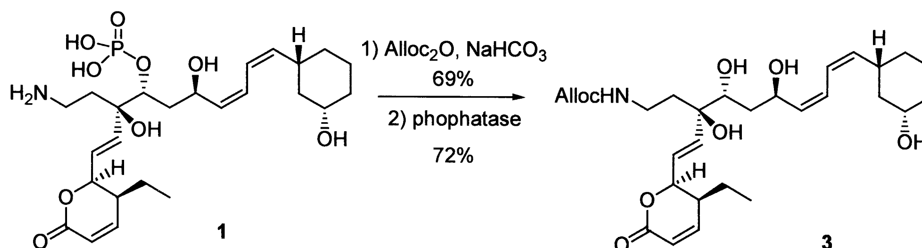


Scheme 1. Dephosphorylation with phosphatase.

porcaine liver esterase (PLE).[8] Thus, the chemical synthesis of LSN-B from LSN-H is an attractive alternative for the chemical supply of LSN-B yet challenging.

In this paper, we report the first example on the chemical transformation of LSN-H to LSN-B. We also report the absolute configuration of 6-methylocanoic acid on C-18 of LSN-B, which is determined to 6′*S* configuration.

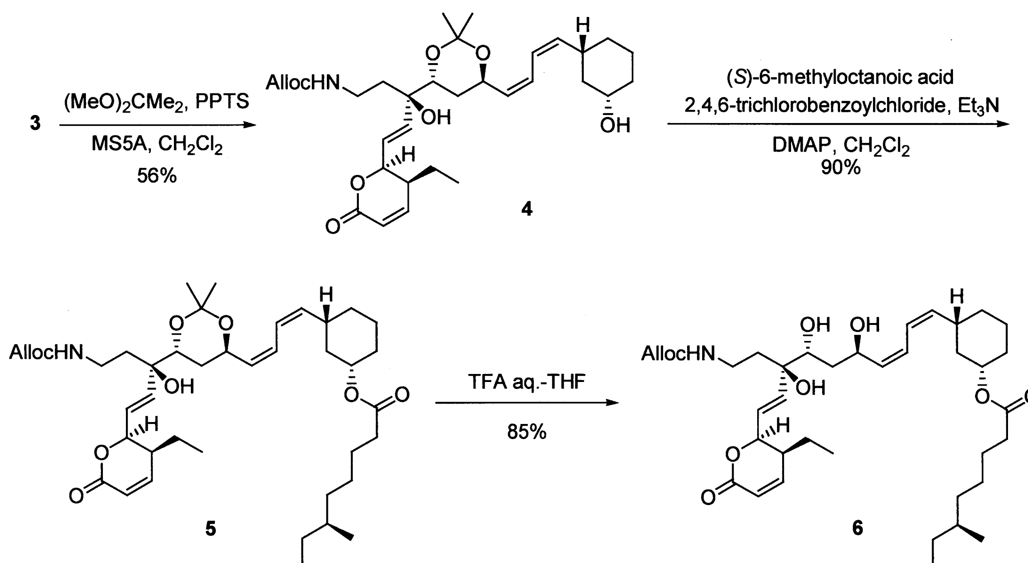
2. Results and discussion

2.1. Chemical transformation of Leustroducsin H to Leustroducsin B

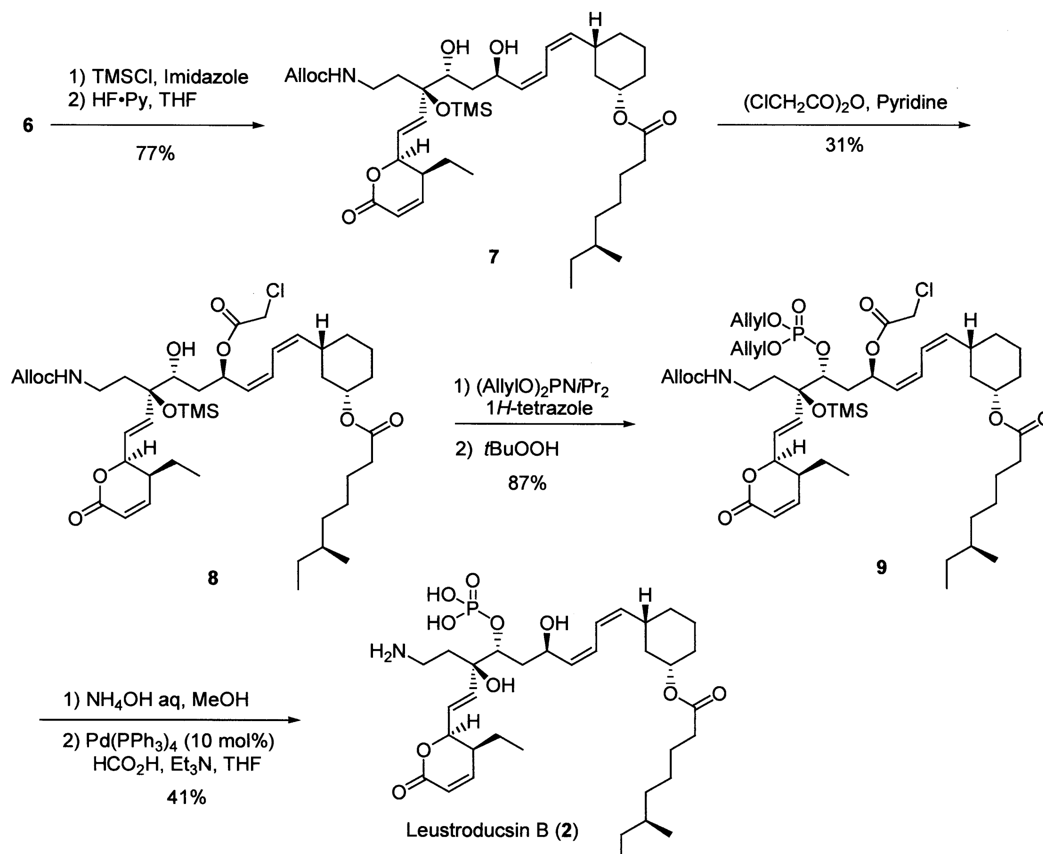
There are several possible strategies to introduce appropriate acyl group onto C-18 hydroxyl group of LSN-H (**1**); 1) direct acylation of **1**, 2) temporally protected phosphate and/or amino group and introduce acyl group at C18 hydroxyl group selectively, and 3) removal of phosphate group and reassemble all functionality including C18 acyl group and phosphate.

The first method is the most straightforward in terms of efficient preparation of LSN-B, however, it should be inevitable non-selective acylation, and is hardly accomplished with conventional chemical transformation. It is undoubted that the amino group and the phosphate monoester of **1** is far more reactive to whatever acylation reagent rather than any hydroxyl group. This made us to initiate the research program from exploration of the second one. After a number of preliminary experiments, it was found that the amino group of **1** is readily protected as various carbamates under conventional reaction condition.[9,10] Meanwhile, the protection of the phosphate monoester of **1** was found to be difficult and troublesome. The phosphate, for instance, is spontaneously cyclized under acylation or condensation conditions to provide five membered cyclic phosphate, presumably due to the unique conformational demand. This clearly indicates the C-8 tertially alcohol should be necessary to protect during transformation. Based on these initial studies, we abandoned the second process, and focused on the stepwise assembly of LSN-B through dephosphorylation (the third option). The major drawback of this route is the long steps (more than 10 steps), which includes many protection-deprotection stages. On the other hand, this route maximize the possibility of LSN-H based derivatization since all functionality could be modified independently. Therefore this route is quite favorable in terms of future drug discovery activity (Scheme 1).

In the early stages of the synthesis, the two major issues are to identify an adequate nitrogen protective group and to develop a mild phosphate ester hydrolysis condition. We initiated the study from the survey of nitrogen protective group. It should be readily removed in a final step under mild condition since the molecule contains both acid and base sensitive functionalities. After preliminary study, we



Scheme 2. Introduction of the side chain.



Scheme 3. Introduction of phosphate ester and final conversion.

found that allyloxycarbonyl (Alloc) group is the most preferable protective group. The acid labile groups such as Boc and *p*-methoxybenzyloxycarbonyl are inexpedient because deprotection of those protective groups under acidic condition caused decomposition of carbon framework or other undesirable reaction. We decided to use Alloc group, which is readily prepared by usual condition to provide Alloc-LSN-H in good yield after reverse phase chromatographic purification. We then pursued to look at subsequent dephosphorylation reaction. Previously, chemical dephosphorylation of LSN-H was reported though the reaction required harsh condition to obtain the dephosphorylated product along with the formylation of the amino group.[6] Thus we investigated the enzymatic reaction utilizing various types of phosphatases.[11] Although, there are many types of enzymes available commercially, most of them did not provide the desired product, presumably due to high structure specificity of the enzymes. After the screening of enzymes, we found that Alkaline phosphatase type-I (Sigma) uniquely possess high catalytic activity. The reaction took place in the presence of the enzyme in pH 9.3 carbonate buffer solution at ambient temperature to provide tetraol **3** in high yield. It should be noted that LSN-H (**1**) is completely intact under the enzymatic hydrolysis condition.

In order for the selective acylation at C-18 position, secondly C-9 and C-11 hydroxyl groups should be protected. We initially designed to protect C-8 and C-9 hydroxyl group as acetonide and C-11 hydroxyl to be protected with chloroacetate so that selective deprotection of the acetal and the introduction of phosphate functionality would be facilitated. The acetalization of 1,2,4-triol usually favors 1,2-derivative (1,3-dioxorane) formation over 2,4-derivative (1,3-dioxane).[12] When **3** were treated with 2,2-dimethoxypropane under various acidic conditions, however, 2,4-derivative **4** was obtained predominantly in most cases. The best condition was utilizing MS5A and catalytic amount of pyridinium *p*-toluenesulfonate (PPTS) to afford the acetonide **4** in 56% along with other isomers, which can be recovered as starting material **3** by acid treatment (see Section 3). The side chain has been successfully assembled by selective acylation at C-18 by Yamaguchi method to provide ester **5** in high yield.[13] Subsequent removal of acetonide protection under acidic condition gave triol **6** in high yield (Scheme 2).

Triol **6** in hand, the next challenge is to introduce phosphorous functionality into C-9 hydroxyl group selectively. Based on the initial study, we know that C-8 tertially alcohol should be protected somehow before installation of phosphorus functionality to prevent intramoleculer interaction of the hydroxyl group with phosphorous group. Trimethylsilyl (TMS) was chosen as the most suitable protection in terms of both selective protection and deprotection in the final step. We found that the tertiary alcohol was selectively protected with TMS group by two-step sequences, protection of all three hydroxyl groups and following selective deprotection of secondary alcohols by HF–pyridine complex at 0°C to provide diol **7**.

Since allylic C-11 hydroxyl group is expected to be more reactive than C-9 hydroxyl group, we surveyed various

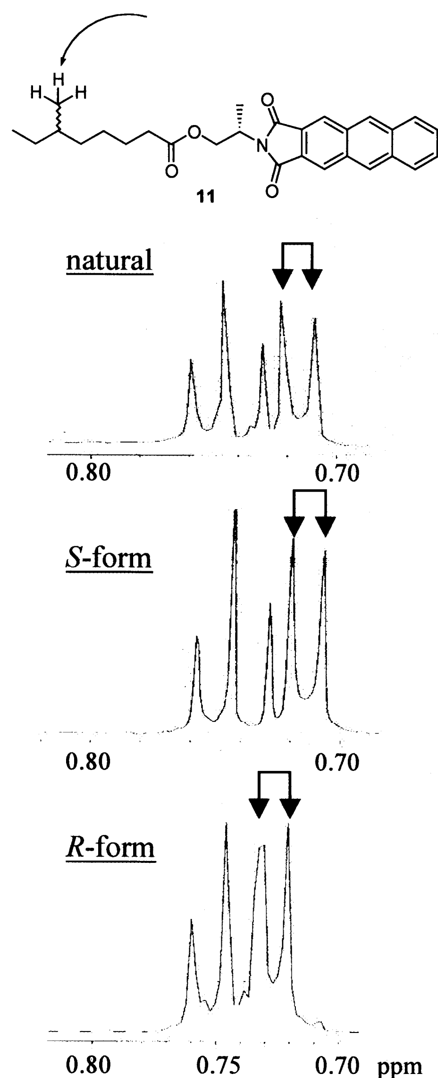


Figure 2. The structure of Akasaka's ester **11** and the doublet of 6-methyl group on **11**.

protective groups for selective protection of C-11 hydroxyl group, however, high selectivity seems to be difficult to achieve. For instance, chloroacetylation of **7** provides desired product **8** in 31% yield along with the regioisomer and diacylated product. Since the undesired products were recyclable by a treatment with ammonium hydroxide solution followed by repeated chloroacetylation, we decided to go through the chemical transformation using this protection. Installation of phosphorus functionality into C-9 position was accomplished by diallylphosphoroamidite method.[14,15] Phosphorylation of alcohol **8** with *O*-diallylphosphorodiisopropylamidite took place spontaneously in the presence of 1*H*-tetrazole, to yield phosphite intermediate, which was oxidized in situ by *t*-butyl hydroperoxide to afford fully assembled phosphate as diallylester form **9** in excellent yield. The final conversion was effectively accomplished by stepwise deprotection of chloroacetyl group with ammonium hydroxide, followed by palladium-catalyzed deprotection of Alloc group and allyl phosphate in the presence of formic acid as reducing agent. It should be noted that TMS group was deprotected during the palladium catalyzed deprotection stage, and LSN-B (**2**) was obtained after reverse phase chromatographic purification.[16] No significant side reaction nor byproduct formation was observed, although the isolated yield was moderate. The all spectral data of synthetic **2** is completely identical to the natural product one (Scheme 3).

2.2. Absolute configuration of the side chain 6-methyl-octanoyl moiety

We preliminary assumed that (6′*S*)-isomer as the natural isomer, since most of terminal isobutyl functionality are existed as (*S*)-form in nature, with best of our knowledge. Meanwhile, to confirm the absolute configuration of the side chain, we also synthesized (6′*R*)-isomer by the synthesis described above, however, ^{1}H NMR spectra of (6′*S*)-LSN-B and (6′*R*)-LSN-B are completely identical. Furthermore, the optical rotation powers of those compounds are quite closed to the natural product. Thus we decided to determine the absolute configuration utilizing Akasaka's method, which features derivatization of the carboxylic acid to the corresponding diastereomeric ester of the chiral anthracene 2,3-dicarboxylic acid derivative, and determination the stereochemistry by HPLC and/or ^{1}H NMR.[17]

The three carboxylic acids, synthetic (*S*) and (*R*)-6-methyloctanoic acid, and carboxylic acid **10** derived from natural LSN-B by alkaline hydrolysis, were subjected esterification with (*S*)-2-(2-hydroxy-1-methylethyl)-1*H*-naphtho[2,3-*f*]-isoindole-1,3(2*H*)-dione. The comparison of ^{1}H NMR spectra of the three esters **11** clearly indicates (*S*)-isomer is the natural form, as shown in Fig. 2.

3. Experimental

3.1. General

All reagents were reagent grade, and were used without further purification unless otherwise noted. All organic solvents were purchased as anhydrous grade, and used without further purification. Phosphatases were purchased from Sigma and Fluka. (*S*)-6-methyloctanoic acid was prepared from (*S*)-6-methyl-1-octanol, which is available from Tokyo Kasei Kogyo, by Jones oxidation. (*R*)-6-methyloctanoic acid was prepared from citroneral by 5 steps as described in the literature.[17]

3.1.1. Tetraol (3). To a mixture of Leustroducsin-H (**1**) (8.30 g, 15.7 mmol) and sodium bicarbonate (4.20 g, 50.0 mmol) in H_2O (60 ml) and 1,4-dioxane (120 ml) was added diallyl dicarbonate (3.30 ml, 20.0 mmol) at ambient temperature, and resulting white suspension was stirred for 2 h. The mixture was concentrated in vacuo, and the residue was purified by flash column chromatography (Cosmosil $75C_{18}$ OPN, 5% CH_3CN to 10% CH_3CN) to afford *N*-alloc-LSN-H (6.70 g) in 69% yield as white amorphous.

^{1}H NMR (500 MHz, CD_3OD) δ 7.11 (dd, J=9.5, 5.5 Hz, 1H), 6.30 (t, J=11.0 Hz, 1H), 6.24 (t, J=11.0 Hz, 1H), 6.07 (dd, J=16.0, 6.5 Hz, 1H), 6.03 (dd, J=10.5, 1.0 Hz, 1H), 5.92 (m, 1H), 5.87 (d, J=16.0 Hz, 1H), 5.45 (dd, J=10.0, 9.0 Hz, 1H), 5.30 (dd, J=9.5, 9.5 Hz, 1H), 5.27 (dd, J=17.0, 1.5 Hz, 1H), 5.16 (d, J=10.0 Hz, 1H), 5.10 (m, 1H), 5.03

(m, 1H), 4.88 (m, 1H), 4.49 (d, J=4.5 Hz, 2H), 4.20 (m, 1H), 3.56 (m, 1H), 3.22 (m, 2H), 2.55 (m, 2H), 2.14 (m, 1H), 1.96–1.62 (m, 5H), 1.60–1.34 (m, 5H), 1.20–1.00 (m, 3H), 0.97 (t, J=7.0 Hz, 3H). Mass (FAB): 614 (M+H). HR-MS calcd for $C_{29}H_{45}NO_{11}P$: 614.2730, found 614.2735.

To a solution of Alloc-LSN-H (1.00 g, 1.60 mmol) in 0.2 M carbonate buffer (pH 9.3) (100 ml) was added alkaline phosphatase type-I (180 mg) at room temperature, and the resulting pale yellow clear mixture was stirred at room temperature for 48 h. During this time, the mixture turned to white suspension. To this suspension was added ethyl acetate (60 ml), and resulting two-phase mixture was stirred additional 30 min. Ethyl acetate phase was separated and the aqueous phase was extracted with ethyl acetate (50 ml×2). Combined organic layers were washed with H_2O, brine, dried over anhydrous sodium sulfate, filtrated and concentrated under reduced pressure. The crude product was purified by silica gel flash column chromatography (CH_2Cl_2–MeOH=15:1, ϕ=3.0×10 cm) to afford tetraol **3** (611 mg) in 72% yield as white amorphous.

Analytical data for tetraol **3**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.97 (dd, J=9.5, 5.5 Hz, 1H), 6.28 (t, J=11.5 Hz, 1H), 6.15 (t, J=11.0 Hz, 1H), 6.05 (d, J=10.0 Hz, 1H), 5.91 (m, 1H), 5.89 (s, 2H), 5.52 (t, J=9.5 Hz, 1H), 5.37 (t, J=10.5 Hz, 1H), 5.36 (m, 1H), 5.29 (dd, J=17.5, 1.5 Hz, 1H), 5.20 (dd, J=11.0, 0.5 Hz, 1H), 5.06 (m, 1H), 4.91 (m, 1H), 4.54 (d, J=5.5 Hz, 2H), 3.81 (m, 1H), 3.62 (m, 1H), 3.29 (m, 1H), 3.22 (m, 1H), 2.51 (m, 1H), 2.45 (m, 1H), 2.00–1.57 (m, 9H), 1.47 (m, 1H), 1.34 (m, 1H), 1.16 (m, 1H), 1.07 (dd, J=12.0, 11.5 Hz, 1H), 1.00 (m, 1H), 0.95 (t, J=7.5 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.13, 156.66, 150.17, 138.88, 135.61, 133.04, 132.86, 125.62, 124.45, 121.48, 120.72, 117.68, 79.91, 73.79, 70.11, 65.66, 65.60, 42.06, 39.23, 37.41, 36.67, 35.18, 35.03, 31.88, 23.84, 21.58, 11.02. Mass (FAB): 534 (M+H). HR-MS calcd for $C_{29}H_{43}NNaO_8$: 556.2886, found 556.2884.

3.1.2. Acetonide (4). To a suspension of powdered molecular sieves 5 A (6.50 g) in dichloromethane (130 ml) were added pyridinium *p*-toluenesulfonate (165 mg, 0.657 mmol) and 2,2-dimethoxypropane (8.00 ml, 65.5 mmol) at room temperature. After stirring for 5 min, a solution of tetraol **3** (3.50 g, 6.57 mmol) in dichloromethane (20 ml) was added, and resulting suspension was stirred for 16 h at room temperature, then all volatile materials were concentrated in vacuo. The residue was suspended in dichloromethane (ca. 20 ml), and the insoluble materials were removed by filtration with a pad of silica gel (ϕ= 5×5 cm). The pad was washed thoroughly with CH_2Cl_2–EtOAc=(1:1). The filtrate was concentrated in vacuo, and the residue was purified by silica gel flash column chromatography (CH_2Cl_2–EtOAc=1:1, ϕ=5.0×5 cm) to afford acetonide **4** (2.09 g) in 56% yield as white amorphous powder along with other isomers (680 mg). The isomers were dissolved in THF (10 ml), and treated with 50% aqueous trifluoroacetic acid (5 ml) at room temperature for 1 h. The mixture was quenched with aqueous saturated sodium bicarbonate (slow addition), and extracted with ethyl acetate (×3). Combined organic phases were washed with H_2O, brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. The residue was purified by silica gel flash column chromatography (CH_2Cl_2–MeOH=15:1, ϕ=5.0×5 cm) to recover tetraol **3** (395 mg).

Analytical data for acetonide **4**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.95 (dd, J=10.0, 5.5 Hz, 1H), 6.33 (t, J= 11.0 Hz, 1H), 6.11 (d, J=11.0 Hz, 1H), 6.06 (d, J= 10.0 Hz, 1H), 5.93 (m, 1H), 5.89 (dd, J=16.0, 5.5 Hz, 1H), 5.82 (d, J=16.0 Hz, 1H), 5.43 (t, J=9.5 Hz, 1H), 5.38 (t, J=10.0 Hz, 1H), 5.30 (dd, J=17.5, 1.5 Hz, 1H), 5.21 (dd, J=10.5, 1.5 Hz, 1H), 5.06 (t, J=4.0 Hz, 1H), 4.69 (m, 1H), 4.55 (d, J=5.0 Hz, 2H), 3.72 (dd, J=9.5, 6.5 Hz, 1H), 3.63 (m, 1H), 3.32 (m, 1H), 3.20 (m, 1H), 2.66 (s, 1H), 2.52 (m, 1H), 2.45 (m, 1H), 2.02–1.90 (m, 3H), 1.80 (m, 2H), 1.70–1.44 (m, 5H), 1.38 (s, 3H), 1.35 (m, 1H), 1.34 (s, 3H), 1.15 (m, 1H), 1.08 (q, J=12.0 Hz, 1H), 0.98 (m, 1H), 0.96 (t, J=7.5 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.85, 149.72, 138.94, 132.97, 130.62, 125.28, 121.62, 120.90, 117.59, 100.84, 71.66, 70.15, 65.51, 63.46, 42.05, 39.40, 35.21, 35.11, 33.01, 31.85, 24.50, 23.83, 11.06. Mass (FAB): 574 (M+H). HR-MS calcd for $C_{32}H_{47}KNO_8$: 612.2939, found 612.2957.

3.1.3. Ester (5). To the solution of (*S*)-6-methyloctanoic acid (0.980 ml, 5.46 mmol), ethyldiisopropylamine (1.10 ml, 6.20 mmol) in THF (10 ml) was added dropwise 2,4,6-trichlorobenzoyl chloride (0.844 ml, 5.40 mmol) at room temperature, and resulting suspension was stirred for 2 h under nitrogen atmosphere to give the mixed anhydride suspension. To a solution of acetonide **4** (2.09 g, 3.64 mmol) and 4-dimethylaminopyridine (DMAP) (757 mg, 6.19 mmol) in dichloromethane (25 ml) was added the mixed anhydride suspension obtained above via cannula in an ice bath under nitrogen atmosphere, and resulting colorless reaction mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo, and the residue was treated with a short silica gel column chromatography (CH_2Cl_2–EtOAc=1:1, ca 300 ml, ϕ=3.0×5 cm). The eluent was concentrated in vacuo, and the residue was purified by silica gel flash column chromatography (hexane–EtOAc=3:2, ϕ=4×15 cm) to afford ester **5** (2.35 g) in 90% yield as colorless viscous oil.

Analytical data for ester **5**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.94 (dd, J=9.5, 5.0 Hz, 1H), 6.32 (t, J=12.0 Hz, 1H), 6.11 (t, J=12.0 Hz, 1H), 6.06 (d, J=10.5 Hz, 1H), 5.94 (m, 1H), 5.90 (dd, J=16.0, 6.0 Hz, 1H), 5.82 (d, J=17.0 Hz, 1H), 5.44 (t, J=9.5 Hz, 1H), 5.35 (t, J=9.5 Hz, 1H), 5.30 (d, J=18.5 Hz, 1H), 5.24 (brs, 1H), 5.20 (d, J=8.5 Hz, 1H), 5.06 (t, J=4.0 Hz, 1H), 4.75 (tt, J=10.5, 5.0 Hz, 1H), 4.68 (dd, J=9.0, 8.5 Hz, 1H), 4.55 (d, J=5.0 Hz, 2H), 3.72 (dd, J=10.0, 6.0 Hz, 1H), 3.33 (m, 1H), 3.18 (m, 1H), 2.63 (s, 1H), 2.58 (m, 1H), 2.44 (m, 1H), 2.26 (t, J=7.5 Hz, 2H), 2.02–1.90 (m, 3H), 1.84 (m, 2H), 1.68–1.55 (m, 6H), 1.48 (m, 2H), 1.37 (s, 3H), 1.34 (s, 3H), 1.40–1.20 (m, 5H), 1.20–1.00 (m, 4H), 0.96 (t, J=8.0 Hz, 3H), 0.85 (t, J= 7.0 Hz, 3H), 0.84 (d, J=6.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.25, 163.79, 149.66, 138.33, 135.33, 132.98, 130.80, 125.56, 125.17, 121.87, 120.90, 117.53, 100.79, 79.90, 76.36, 72.06, 71.60, 65.46, 63.45, 39.42, 38.06, 36.12, 35.01, 34.68, 34.16, 32.94, 31.81, 31.31, 29.36, 26.53, 25.34, 24.89, 24.48, 23.62, 21.56, 19.11, 11.32,

11.04. Mass (FAB): 714 (M+H). HR-MS calcd for $C_{41}H_{63}KNO_9$: 752.4140, found 752.4161.

3.1.4. Triol (6). To a solution of ester **5** (2.25 g, 3.15 mmol) in THF (40 ml) was added 50% aqueous trifluoroacetic acid (16 ml) at room temperature, and the mixture was stirred for 1 h. The reaction mixture was quenched with aqueous saturated sodium bicarbonate, and extracted with ethyl acetate (80 ml, then 30 ml×2). Combined organic phases were washed with H_2O, brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. The residue was purified by silica gel flash column chromatography (CH_2Cl_2–MeOH=20:1, ϕ=3.0×15 cm) to afford triol **6** (1.80 g) in 85% yield as white amorphous powder.

Analytical data for triol **6**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.96 (dd, J=10.0, 6.0 Hz, 1H), 6.26 (t, J=10.5 Hz, 1H), 6.16 (t, J=11.0 Hz, 1H), 6.04 (d, J=10.0 Hz, 1H), 5.90 (m, 1H), 5.89 (s, 2H), 5.51 (t, J=10.0 Hz, 1H), 5.42 (br, 1H), 5.32 (t, J=9.5 Hz, 1H), 5.29 (d, J=17.5 Hz, 1H), 5.19 (d, J=10.0 Hz, 1H), 5.05 (s, 1H), 4.88 (br, 1H), 4.74 (tt, J=10.5, 4.0 Hz, 1H), 4.53 (d, J=5.0 Hz, 1H), 3.81 (d, J=9.5 Hz, 1H), 3.64 (brs, 1H), 3.57 (br, 1H), 3.28 (m, 1H), 3.22 (m, 1H), 3.08 (brs, 1H), 2.57 (m, 1H), 2.45 (m, 1H), 2.26 (t, J=7.5 Hz, 2H), 2.00–1.68 (m, 6H), 1.60 (m, 4H), 1.52–1.20 (m, 8H), 1.14 (m, 3H), 1.02 (m, 1H), 0.94 (t, J=7.5 Hz, 3H), 0.85 (t, J=7.0 Hz, 3H), 0.83 (d, J=6.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.30, 164.13, 156.61, 150.19, 138.01, 135.67, 133.38, 132.86, 125.47, 124.10, 121.80, 120.63, 117.57, 80.04, 73.64, 72.10, 65.58, 65.26, 39.22, 38.03, 37.59, 36.65, 36.11, 35.28, 34.95, 34.67, 34.14, 31.85, 31.27, 29.35, 26.52, 25.31, 23.62, 21.53, 19.10, 11.31, 10.98. Mass (FAB): 674 (M+H). HR-MS calcd for $C_{38}H_{59}NNaO_9$: 696.4087, found 696.4080. $[\alpha]_D^{25}$=123.9 (c=0.740, $CHCl_3$).

3.1.5. TMS ether (7). To a solution of **6** (1.78 g, 2.64 mmol) and imidazole (2.70 g, 39.6 mmol) in dichloromethane (50 ml) was added dropwise chlorotrimethylsilane (3.3 ml, 26.4 mmol) for 10 min, and resulting suspension was stirred for 12 h at room temperature. Methanol (ca. 2 ml) was added to the reaction mixture, and it was stirred for additional 5 min. Upon quenching with H_2O (100 ml), the product was extracted with ethyl acetate (50 ml×2). Combined organic phases were washed with brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. The residual colorless oil was dissolved in toluene (ca. 10 ml), and the mixture was concentrated in vacuo to remove the volatile materials. The residue was dissolved in THF (50 ml), and to this was added hydrogen fluoride pyridine complex (0.50 ml) dropwise in an ice bath. After stirring under ice cooling for 1 h, the reaction mixture was quenched with H_2O (150 ml), and the product was extracted with ethyl acetate (70 ml×3). Combined organic phases were washed with aqueous saturated sodium bicarbonate, H_2O, brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. The residue was purified by silica gel flash column chromatography (hexane–EtOAc= 1:1, ϕ=4.0×15 cm) to afford TMS ether **7** (1.53 g) in 77% yield as colorless viscous oil.

Analytical data for TMS ether **7**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.92 (dd, J=10.0, 6.0 Hz, 1H), 6.23 (t, J=12.0 Hz, 1H), 6.13 (t, J=10.5 Hz, 1H), 6.01 (d, J=9.5 Hz, 1H), 5.91 (d, J=16.5 Hz, 1H), 5.87 (m, 1H), 5.75 (dd, J=16.5, 6.0 Hz, 1H), 5.49 (t, J=8.5 Hz, 1H), 5.28 (t, J=9.5 Hz, 1H), 5.25 (d, J=16.5 Hz, 1H), 5.21 (brs, 1H), 5.15 (d, J=9.5 Hz, 1H), 4.99 (t, J=3.5 Hz, 1H), 4.85 (brs, 1H), 4.71 (m, 1H), 4.51 (d, J=5.0 Hz, 2H), 3.83 (d, J=10.0 Hz, 1H), 3.25 (m, 2H), 2.71 (m, 1H), 2.54 (m, 1H), 2.39 (m, 1H), 2.23 (t, J=6.5 Hz, 2H), 1.90 (m, 4H), 1.78 (d, J=14.0 Hz, 1H), 1.65 (dd, J=15.0, 8.0 Hz, 1H), 1.60–1.18 (m, 12H), 1.10 (m, 3H), 0.98 (brq, J=13.0 Hz, 1H), 0.92 (t, J=8.0 Hz, 3H), 0.81 (t, J=7.0 Hz, 3H), 0.80 (t, J=5.0 Hz, 3H), 0.13 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.19, 163.83, 156.24, 149.80, 137.82, 135.96, 133.57, 132.90, 125.72, 123.96, 121.80, 120.72, 117.32, 80.08, 79.60, 73.26, 72.03, 65.38, 65.29, 39.31, 37.98, 37.45, 36.54, 36.04, 34.86, 34.07, 31.79, 31.22, 29.28, 26.45, 25.26, 23.56, 21.46, 19.04, 11.25, 10.98, 2.45. Mass (FAB): 746 (M+H). HR-MS calcd for $C_{41}H_{67}KNO_9Si$: 784.4222, found 784.4229. IR ($CHCl_3$) 3454 (w), 2960 (m), 2937 (m), 1721 (s), 1514 (m), 1463 (m), 1255 (s), 1173 (m).

3.1.6. Chloroacetate (8). To a solution of **7** (1.53 g, 2.05 mmol) and pyridine (0.186 ml, 2.30 mmol) in dichloromethane (35 ml) was added chloroacetic anhydride (351 mg, 2.05 mmol) at room temperature, and the reaction mixture was stirred for 1 h at this temperature. It was concentrated in vacuo, and the residue was subjected to short silica gel column chromatography (hexane–EtOAc=1:1, ϕ=4.0×10 cm) to separate acylated products and unreacted starting material (361 mg). The products were further purified by silica gel flash column chromatography (hexane–EtOAc=3:2, ϕ=3.0×40 cm) to afford desired product **8** (527 mg) in 31% yield as colorless viscous oil along with other isomers (623 mg). The isomers were converted to starting material **7** by treating with pH 9.5 sodium carbonate buffer to remove all acyl group(s).

Analytical data for chloroacetate **8**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.94 (dd, J=9.5, 6.0 Hz, 1H), 6.34 (t, J=11.5 Hz, 1H), 6.25 (t, J=10.5 Hz, 1H), 6.04 (d, J=9.5 Hz, 1H), 5.96 (td, J=11.0, 3.0 Hz, 1H), 5.90 (d, J=16.0 Hz, 1H), 5.89 (m, 1H), 5.79 (dd, J=15.5, 5.0 Hz, 1H), 5.40 (t, J=11.0 Hz, 1H), 5.34 (t, J=10.0 Hz, 1H), 5.27 (d, J=16.5 Hz, 1H), 5.18 (d, J=11.0 Hz, 1H), 5.06 (brs, 1H), 5.02 (t, J=5.0 Hz, 1H), 4.73 (tt, J=10.5, 4.0 Hz, 1H), 4.53 (d, J=4.0 Hz, 2H), 4.05 (ABq, J=14.5 Hz, 1H), 4.02 (ABq, J=14.5 Hz, 1H), 3.51 (dd, J=9.5, 2.5 Hz, 1H), 3.25 (m, 2H), 2.73 (s, 1H), 2.54 (m, 1H), 2.42 (m, 1H), 2.24 (t, J=7.5 Hz, 2H), 1.96–1.77 (m, 6H), 1.58 (m, 3H), 1.46 (m, 2H), 1.37 (m, 1H), 1.28 (m, 5H), 1.12 (m, 3H), 1.01 (qd, J=12.5, 4.0 Hz, 1H), 0.95 (t, J=7.5 Hz, 3H), 0.83 (t, J=7.0 Hz, 3H), 0.82 (t, J=6.0 Hz, 3H), 0.16 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.27, 167.05, 163.70, 156.20, 149.66, 139.31, 135.28, 132.91, 127.74, 126.57, 126.28, 121.52, 120.86, 117.42, 79.84, 79.46, 72.68, 72.04, 69.92, 65.36, 40.95, 39.33, 38.00, 36.58, 36.10, 35.48, 35.07, 34.66, 34.14, 31.73, 31.26, 29.35, 26.51, 25.32, 23.58, 21.56, 19.10, 11.31, 11.04, 2.46. Mass (FAB): 822 (M+H). HR-MS calcd for $C_{43}H_{68}{}^{35}ClKNO_{10}Si$: 860.3938, found 860.3927. IR ($CHCl_3$) 3454 (w), 2959 (m), 2938 (m), 1722 (s), 1513 (m), 1464 (m), 1255 (s), 1174 (m).

3.1.7. Phosphate ester (9). To a solution of alcohol **8**

(525 mg, 0.638 mmol) and 1*H*-tetrazole (224 mg, 3.20 mmol) in dichloromethane (7 ml) and acetonitrile (7 ml) was added dropwise *O*-diallyl phosphorodiisopropylamidite (0.420 ml, 1.60 mmol) at room temperature. After stirring for 1 h, the mixture was cooled in an ice bath, and to this were added dropwise methanol (ca. 0.5 ml) and 5–6 M *t*-butyl hydroperoxide nonane solution (0.80 ml) subsequently. After stirring for 1 h at this temperature, the reaction mixture was quenched with 1 M aqueous sodium sulfite (ca. 20 ml), and the product was extracted with *t*-butyl methyl ether (TBME) (30 ml×1, then 20 ml×2). Combined organic phases were washed with H_2O, brine, dried over anhydrous sodium sulfate, filtrated, and concentrated in vacuo. The crude product was purified by silica gel flash column chromatography (hexane–EtOAc=3:2, ϕ=3.0×15 cm) to afford desired product **9** (543 mg) in 87% yield as colorless oil.

Analytical data for **9**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.96 (dd, *J*=10.0, 6.0 Hz, 1H), 6.35 (t, *J*=12.0 Hz, 1H), 6.31 (t, *J*=11.5 Hz, 1H), 6.05 (d, *J*=9.5 Hz, 1H), 6.02–5.83 (m, 4H), 5.79 (dd, *J*=15.5, 5.0 Hz, 1H), 5.39 (m, 3H), 5.28 (m, 3H), 5.24 (t, *J*=9.0 Hz, 1H), 5.20 (t, *J*=9.5 Hz, 1H), 5.05 (t, *J*=4.0 Hz, 1H), 4.97 (m, 1H), 4.74 (m, 1H), 4.54 (m, 6H), 4.12 (ABq, *J*=15.0 Hz, 1H), 4.04 (ABq, *J*=15.0 Hz, 1H), 3.25 (m, 2H), 2.55 (m, 1H), 2.41 (m, 1H), 2.26 (t, *J*=8.0 Hz, 2H), 2.10 (m, 2H), 1.94 (m, 3H), 1.82 (m, 1H), 1.64–1.20 (m, 15H), 1.14 (m, 3H), 1.01 (dq, *J*=12.0, 3.0 Hz, 1H), 0.95 (t, *J*=6.5 Hz, 3H), 0.85 (t, *J*=7.0 Hz, 3H), 0.83 (d, *J*=6.5 Hz, 3H), 0.24 (s, 9H). Mass (FAB): 982 (M+H). HR-MS calcd for $C_{49}H_{77}{}^{35}ClKNO_{13}PSi$: 1020.4227, found 1020.4223. IR ($CHCl_3$) 3451 (w), 2959 (m), 1721 (s), 1514 (m), 1463 (m), 1256 (s), 1022 (s), 990 (s).

3.1.8. Leustroducsin B (2). To a solution of **9** (447 mg) in methanol (12 ml) was added 28% aqueous ammonium hydroxide (4 ml) under ice cooling. The resulting suspension was stirred for 2 h, then concentrated in vacuo. The residual pale yellow oil was dissolved in THF (20 ml), and to this solution were added tetrakis(triphenylphosphine)-palladium (53 mg), 98% formic acid (0.690 ml), and triethylamine (0.633 ml) at room temperature. The reaction mixture was stirred at room temperature for 20 min, then at 50°C for 3 h. It was allowed to cool to room temperature, and volatile materials were removed in vacuo. The residue was purified by flash column chromatography (Cosmosil $75C_{18}$ OPN, 50% CH_3CN in H_2O) to afford Leustroducsin B (**2**) (125 mg) in 41% yield.

Analytical data for **2**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.03 (dd, *J*=9.5, 5.0 Hz, 1H), 6.23 (m, 2H), 6.02 (dd, *J*=15.5, 7.0 Hz, 1H), 5.96 (dd, *J*=10.5, 1.5 Hz, 1H), 5.88 (dd, *J*=15.0, 1.0 Hz, 1H), 5.40 (brt, *J*=9.0 Hz, 1H), 5.25 (brt, *J*=9.5 Hz, 1H), 5.05 (m, 1H), 4.90 (brt, *J*=9.0 Hz, 1H), 4.67 (tt, *J*=11.0, 4.0 Hz, 1H), 4.22 (td, *J*=10.0, 2.5 Hz, 1H), 3.05–2.93 (m, 2H), 2.57 (m, 1H), 2.50 (td, *J*=9.0, 5.0 Hz, 1H), 2.22 (t, *J*=7.5 Hz, 2H), 2.15 (td, *J*=7.0, 14.5 Hz, 1H), 1.92–1.74 (m, 4H), 1.66–1.35 (m, 8H), 1.34–1.17 (m, 7H), 1.08 (m, 2H), 1.00 (m, 1H), 0.90 (t, *J*=7.0 Hz, 3H), 0.82 (t, *J*=7.0 Hz, 3H), 0.80 (d, *J*=6.5 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 175.02, 166.35, 152.68, 138.15, 137.22, 135.22, 127.64, 124.25, 123.69, 121.03, 82.32, 78.45, 77.72, 73.87, 64.63, 40.54, 40.54, 39.37, 37.33, 37.08, 36.11, 35.54, 35.45, 34.29, 33.07, 32.41, 30.52, 27.60, 26.44, 24.65, 22.73, 19.58, 11.73, 11.38. Mass (FAB, negative): 668 $(M-H)^-$. HR-MS calcd for $C_{34}H_{55}O_{10}NP$: 668.3567, found 668.3555. $[\alpha]_D{}^{25}$=99.4 (*c*=0.700, MeOH).

3.1.9. 6-Methyloctanoic acid (10) (from natural LSN-B). A mixture of LSN-B (4.0 mg, 6.0 μmol), 4 M NaOH (1.5 ml) and THF (0.5 ml) was stirred at room temperature for 24 h, and quenched with 2 M HCl aqueous solution to adjust pH ~1. It was extracted with TBME (×3), and combined organic phases were washed with brine, dried over anhydrous sodium sulfate, filtered and evaporated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc=1:1, ϕ=0.8×5 cm) to afford 6-methyloctanoic acid (0.5 mg) in 50% yield as colorless oil.

Analytical data for 6-methyloctanoic acid (**10**): ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.36 (t, *J*=8.0 Hz, 2H), 1.62 (m, 3H), 1.32 (m, 4H), 1.12 (m, 2H), 0.86 (t, *J*=6.5 Hz, 3H), 0.85 (d, *J*=6.0 Hz, 3H).

3.1.10. Ester 11 derived from natural 6-methyloctanoic acid (10). To a solution of 6-methylocanoitc acid (from natural LSN-B, 1.0 mg, 6.3 μmol) and triethylamine (10.5 μl, 7.6 μmol) in THF (0.5 ml) was added ClCOO*i*Bu (8.2 μl, 6.3 μmol) at room temperature, and the mixture was stirred for 1 h. It was concentrated in vacuo, and the residue was dissolved in CH_2Cl_2 (1 ml). To this solution was added (*S*)-2-(2-hydroxy-1-methylethyl)-1*H*-naphtho[2,3-*f*]-isoindole-1,3(2*H*)-dione (19 mg, 6.3 μmol) and DMAP (12 mg, 0.10 mmol) at room temperature, and the mixture was stirred for 6 h. It was passed through silica gel column chromatography (CH_2Cl_2, ϕ=0.8×5 cm) to afford ester **11** (2.4 mg) in 85% yield as pale yellow oil.

Analytical data for ester **11** derived from natural 6-methyloctanoic acid (**10**): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.65 (s, 2H), 8.51 (s, 2H), 8.09 (m, 2H), 7.63 (m, 2H), 4.74 (m, 1H), 4.62 (dd, *J*=11.5, 9.5 Hz, 1H), 4.44 (dd, *J*=11.5, 5.0 Hz, 1H), 2.23 (m, 2H), 1.57 (d, *J*=7.0 Hz, 3H), 1.60–0.90 (m, 9H), 0.74 (t, *J*=7.0 Hz, 3H), 0.71 (d, *J*=6.5 Hz, 3H).

3.1.11. Ester 11 derived from (*S*)-6-methyloctanoic acid. The sample was prepared as described above.

Analytical data for the ester **11** derived from (*S*)-6-methyloctanoic acid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.64 (s, 2H), 8.50 (s, 2H), 8.08 (m, 2H), 7.63 (m, 2H), 4.74 (m, 1H), 4.62 (dd, *J*=11.5, 9.5 Hz, 1H), 4.44 (dd, *J*=11.5, 5.0 Hz, 1H), 2.23 (td, *J*=7.5, 2.0 Hz, 2H), 1.57 (d, *J*=7.0 Hz, 3H), 1.55–0.90 (m, 9H), 0.74 (t, *J*=7.0 Hz, 3H), 0.71 (d, *J*=6.5 Hz, 3H).

3.1.12. Ester 11 derived from (*R*)-6-methyloctanoic acid. The sample was prepared as described above.

Analytical data for the ester **11** derived from (*R*)-6-methyloctanoic acid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.63 (s, 2H), 8.49 (s, 2H), 8.08 (m, 2H), 7.62 (m, 2H), 4.75 (m, 1H), 4.62 (dd, *J*=11.5, 9.5 Hz, 1H), 4.45 (dd, *J*=11.5, 5.0 Hz, 1H),

2.23 (t, J=7.0 Hz, 2H), 1.58 (d, J=7.0 Hz, 3H), 1.55–0.90 (m, 9H), 0.75 (t, J=7.0 Hz, 3H), 0.73 (d, J=6.5 Hz, 3H).

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