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The diastereoselective asymmetric total synthesis of NG-391, a neuronal cell-protecting molecule

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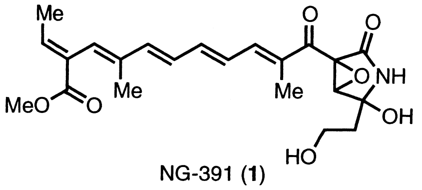
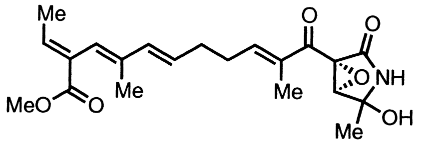
Abstract—The stereocontrolled total synthesis of (+)-NG-391, a neuronal cell-protecting molecule, is described along with the determination of its absolute stereochemistry. The following reactions in this synthesis are particularly noteworthy: (1) The stereoselective construction of the conjugated (*E*,*E*,*E*,*E*,*E*)-pentaene from an (*E*,*E*,*E*)-alcohol using an IBX oxidation followed by stereoselective Horner–Emmons reaction. (2) The (*E*)-selective Knoevenagel condensation of a β-ketonitrile with a chiral 2-alkoxyaldehyde prepared from (*S*)-malic acid. (3) A diastereoselective epoxidation.

NG-391 (**1**), which was isolated from a *Fusarium* sp. TF-0452 by a group at Taisho corporation, shows neurotrophic activity and an effect on neurite outgrowth.[1] Recently Kakeya and Osada et al., reisolated NG-391 from a *Fusarium* sp. RK 97-94 together with lucilactaene, a cell cycle inhibitor in p53-transfected cancer cells.[2] NG-391 may have potential for development as a new drug for various neurodegenerative diseases such as dementia. Structurally, it contains the 3-alkenoyl-3,4-epoxy-2-pyrrolidinone moiety and a labile, substituted pentaene, and its absolute stereochemistry is not known.

In 1998 we succeeded in the first total synthesis of epolactaene,[3] a neurotrophic reagent isolated by Kakeya and Osada et al.[4] Not only do NG-391 and epolactaene have similar biological properties, but they also have a very close structural similarity, both containing an epoxy-lactam and an unsaturated, hydrophobic side-chain. In order to elucidate the mechanism of action of these drugs, the examination of structure–reactivity relationships of NG-391, epolactaene and their derivatives is highly desirable, and to this end a flexible total synthesis of NG-391 is required. From the synthetic point of view, the synthesis of NG-391 is more challenging, and expected to be much more difficult than that of epolactaene, owing to the substituted, conjugated pentaene, labile to acid, base, light, and oxidative conditions. In this paper we will describe the diastereoselective first synthesis of the naturally-occuring enantiomer of NG-391, thus determining its absolute stereochemistry.



NG-391 (**1**)



epolactaene (**2**)

As initially we thought that construction of the unstable pentaene should be left to the last stages of the total synthesis, the following retrosynthetic scheme was designed (Scheme 1). Mild oxidation of selenium derivative **2** would afford NG-391, and the epoxy lactam moiety of **2** could be constructed from β-ketonitrile **3** using a Knoevenagel reaction[5] and epoxidation as key steps. β-Ketonitrile **3** was to be synthesized from the triene derivative **4** used in the synthesis of epolacatene.[3a]

The triene derivative **4** was synthesized stereoselectively from tetrahydropyran-2-ol by our reported method.[3a] Oxidation of alcohol **4** with SO_3·pyridine[6] afforded aldehyde **5** quantitatively (Scheme 2). α-Phenylselenation of aldehyde **5** was achieved using *N*,*N*-diethylbenzeneselenamide,[7] and the subsequent Horner–Emmons reaction of **6** proceeded stereoselectively, affording γ-phenylseleno-*E*-α,β-unsaturated ester **7** in 48% yield over 2 steps, along with the deselenated side product. The methyl ester **7**

Keywords: NG-391; natural product; IBX oxidation; Knoevenagel reaction.

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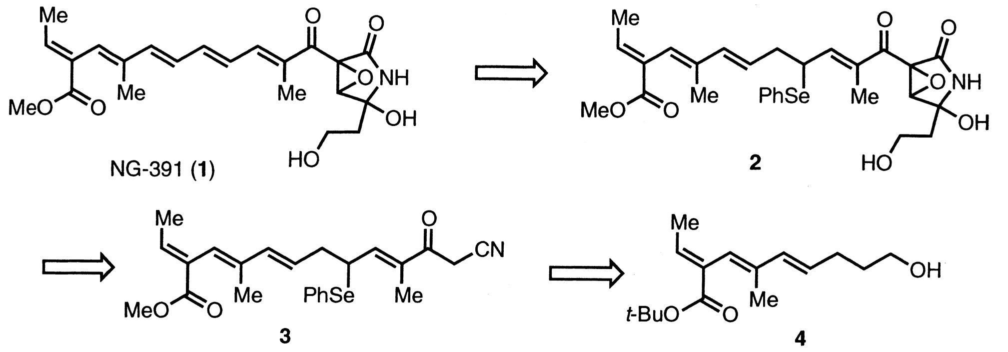


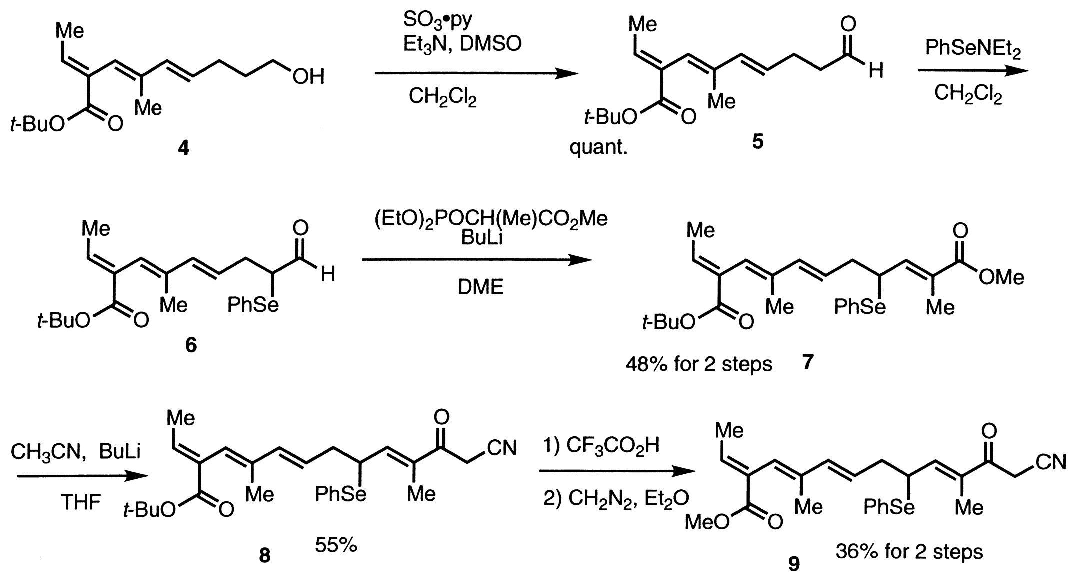
Scheme 1. The retrosynthesis of NG-391.

reacted with $LiCH_2CN$ to afford β-ketonitrile **8** in 55% yield. The transformation of *t*-butyl ester **8** to methyl ester **9** by treatment with CF_3CO_2H, and the subsequent reaction with CH_2N_2 is not efficient, affording methyl ester **9** in low yield (36% for **2** steps). The low yield of the last 5 steps from **5** due to the phenylseleno substituent prompted us to modify the synthetic route.

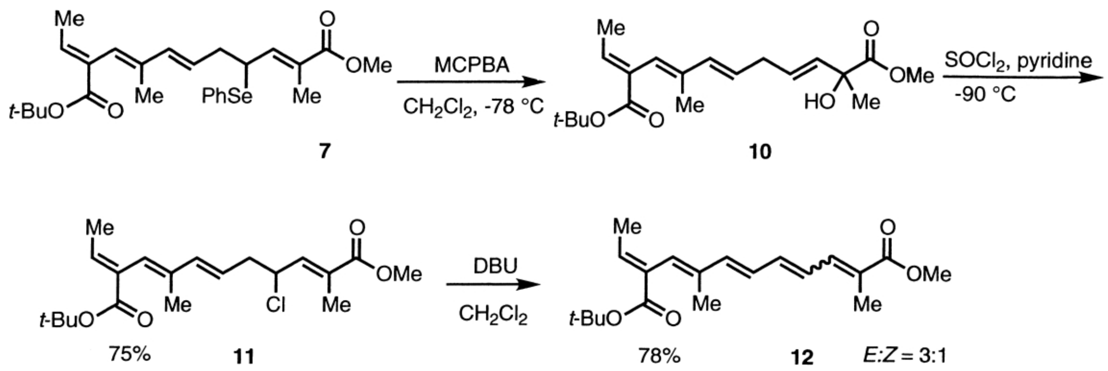
During the course of these synthetic studies, the synthesis of the pentaene moiety was examined in order to check its stability (Scheme 3). When γ-seleno-α,β-unsaturated ester **7** was treated with MCPBA at low temperature, α-hydroxy-*E*-β,γ-unsaturated ester **10** was formed via [2,3]sigmatropic rearrangement[8] instead of the expected pentaene **12**. Though the one step conversion of γ-seleno-α,β-unsaturated ester **7** to pentaene **12** had not been achieved, a 2 step transformation of α-hydroxy-β,γ-unsaturated ester **10** to pentaene **12** was developed: when the α-hydroxy-β,γ-unsaturated ester **10** was treated with $SOCl_2$ in pyridine, a [3,3]sigmatropic rearrangement proceeded, affording γ-chloro-*E*-α,β-unsaturated ester **11** in 75% yield. Elimination of HCl occurred on treatment of **11** with DBU in CH_2Cl_2 at rt, affording pentaene **12** in 78% yield as an inseparable *E*/*Z* mixture (*E*/*Z*=3:1). Although the *E*/*Z* selectivity could not be improved by altering the base or temperature, the stability of the pentaene moiety could be examined. It was found to be tolerant of exposure to weak acid and base as long as there are two electron-withdrawing carbonyl groups attached at each end of the pentaene, but that it isomerizes gradually under illumination.

With these results in hands, we decided that it should be possible to prepare NG-391 by constructing the epoxy-lactam moiety during the last stages of the total synthesis after the formation of the pentaene portion, provided that the reactions employed in making the epoxy-lactam moiety were performed under mild conditions.

We next focused in detail on the stereoselective synthesis of pentaene **12** (Scheme 4). After some experimentation, we found that (*E*,*E*,*E*)-alcohol **4** was effectively oxidized with IBX (*o*-iodoxybenzoic acid, 5 equiv.) in the presence of TsOH at 60°C for 3 h under Nicolaou's conditions,[9] to afford (*E*,*E*,*E*,*E*)-tetraene **13** selectively in 60% yield. Subsequent Horner–Emmons reaction with methyl diethylphosphonopropionate under Heathcock's conditions[10] afforded (*E*,*E*,*E*,*E*,*E*)-pentaene **12** stereoselectively. As the pentaene **12** had been synthesized in only 2 steps from alcohol **4** with very high stereoselectivity, the remaining steps towards total synthesis were examined further. The reaction of methyl ester **12** with $LiCH_2CN$ (2 equiv.) proceeded at −90°C after 20 min to afford β-ketonitrile **14** in good yield (98%) without affecting the *t*-butyl group. After brief treatment of *t*-butyl ester **14** with CF_3CO_2H in

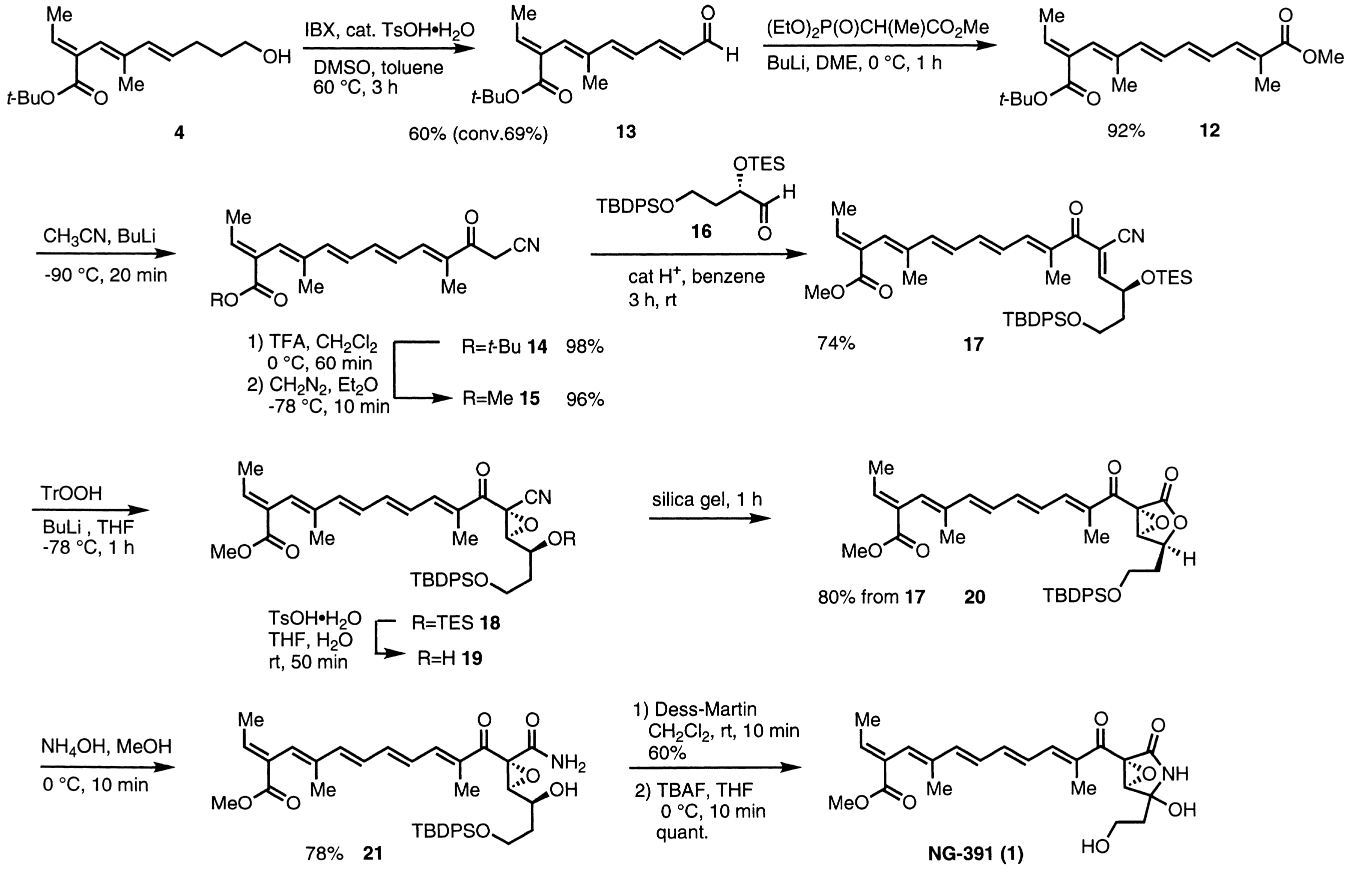


Scheme 2. The first attempt for the synthesis of pentaene moiety.

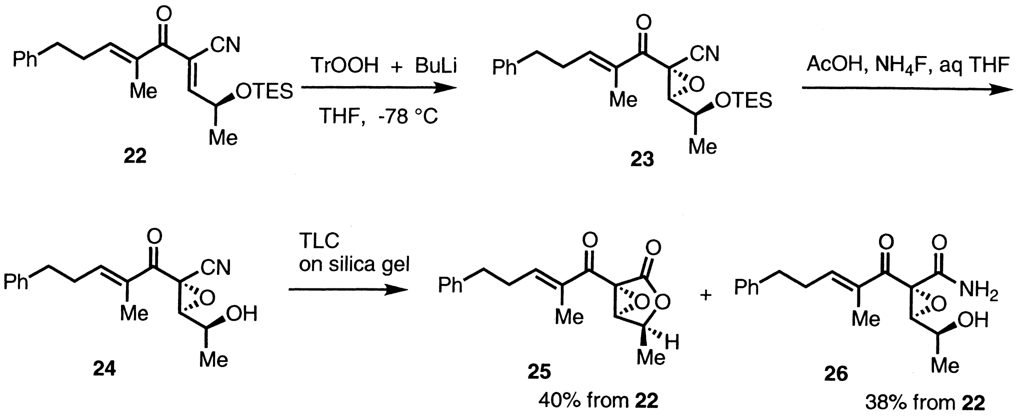


Scheme 3. The second attempt for the synthesis of pentaene (**12**).

CH_2Cl_2 at 0°C for 1 h, the resulting carboxylic acid was treated with diazomethane at −78°C,[11] affording methyl ester **15** in 96% yield. Knoevenagel condensation of β-ketonitrile **15** with (*S*)-4-(*t*-butyldiphenylsiloxy)-2-triethylsiloxybutanal (**16**),[12] prepared from (*S*)-malic acid, proceeded efficiently in the presence of a catalytic amount of ethylenediammonium diacetate in benzene at rt for 3 h, affording *E*-Knoevenagel adduct **17** stereoselectively. The TES protecting group of **17** is essential for high yield in the Knoevenagel reaction, as reaction with the corresponding aldehyde having the more bulky TBS protecting-group proceeded slowly, affording the condensation product in low yield. Epoxidation with tritylperoxide in the presence of BuLi proceeded stereoselectively at low temperature from the side opposite the TES group, to give epoxynitrile **18** without affecting the conjugated pentaene. As epoxynitrile **18** and the following several compounds are labile, the next reactions were conducted without purification. Treatment of epoxynitrile **18** with TsOH at rt for 50 min removed the TES protecting group, affording hydroxynitrile **19**. The hydrolysis of the nitrile to an amide occurred under very mild reaction conditions during TLC (thin layer chromatography) on silica gel via intramolecular hydroxyl group assistance, and without damaging the labile pentaene to afford lactone **20** in 80% yield over 3 steps from the Knoevenagel condensation product **17**. Treatment of lactone **20** with ammonia in MeOH at 0°C for 10 min gave hydroxyamide **21** in 78% yield. Oxidation with the Dess–Martin periodinane[3b,c,13] in CH_2Cl_2 for 10 min gave the epoxy lactam in 60% yield, and the final of the *t*-butyldiphenylsilyl group was effectively achieved with tetra-*n*-butylammonium fluoride in THF for 10 min at 0°C, affording NG-391 (**1**)



Scheme 4. Total synthesis of NG-391 (**1**).



Scheme 5. Determination of the stereochemistry of the model epoxide.

quantitatively as an appropriately 5:1 diastereomeric mixture at the acetal position of γ-lactam moiety.

We determined the stereochemistry of **18** as (12*R*,13*S*), based on the following model study which was conducted in the total synthesis of epolactaene (Scheme 5).[14]

The epoxidation was performed by the model alkene **22**, giving one diastereomer **23** stereoselectively. The epoxide **23** was transferred to the previously synthesized lactone **25**, the srereochemistry of which was already determined by the difference NOE experiment.[3a]

Synthetic NG-391 exhibited identical properties to those reported for the natural substance[1] (^{1}H, ^{13}C NMR and IR). Comparison of the optical rotation (synthetic NG-391: $[\alpha]_D^{25}=+41.7$ (c=0.37, MeOH), natural NG-391:[1] $[\alpha]=+39.3$ (c=0.5, MeOH)), determines the absolute stereochemistry to be as shown in **1**.

In summary, the first total synthesis of NG-391 has been accomplished in an enantio- and highly diastereoselective manner, enabling determination of the absolute stereochemistry. The synthesis has several noteworthy features. In particular the conjugated, substituted pentaene moiety was stereoselectively synthesized from our previously reported triene **4** in a short sequence using the IBX oxidation and Horner–Emmons reactions.

1. Experimental

1.1. General procedures

All reactions were carried out under argon and monitored by thin-layer chromatography using Merck 60 F_{254} precoated silica gel plates (0.25 mm thickness). Specific optical rotations were measured using a JASCO P-1020 polarimeter. FTIR spectra were recorded on an Horiba FT-720 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker AM400 instrument. High-resolution mass spectral analyses (HRMS) were carried out using JEOL JMS-SX 102A. Preparative thin-layer chromatography was performed using Wakogel B-5F purchased from Wako Pure Chemical Industries, Tokyo, Japan. Flash column chromatography was performed using silica gel Merck Art 7734.

1.1.1. (3*E*,5*E*,7*E*)-2-[(*E*)-Ethylidene]-4-methyl-9-oxo-nona-3,5,7-trienoic acid *tert*-butyl ester (13). To a toluene and DMSO mixed solution (1:1, 0.27 mL) of alcohol **4**[3a] (20.7 mg, 0.078 mmol) was added IBX (109 mg, 0.389 mmol) and $TsOH\cdot H_2O$ (3.0 mg, 0.016 mmol) at rt and the reaction mixture was stirred for 10 min at rt then for 3 h at 60°C. The reaction was quenched by the addition of saturated $NaHCO_3$ solution, and the organic materials were extracted with ethyl acetate four times, then the combined organic extracts were washed with $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo after filtration. Purification by flash column chromatography (ethyl acetate/hexane=1/20) gave 12.1 mg (60%) of aldehyde **13**.

^{1}H NMR ($CDCl_3$) δ=1.46 (9H, s), 1.67 (3H, d, J=7.9 Hz), 1.69 (3H, s), 6.17 (1H, dd, J=13.7, 7.9 Hz), 6.29 (1H, s), 6.45 (1H, dd, J=15.2, 11.0 Hz), 6.78 (1H, d, J=15.2 Hz), 6.85 (1H, q, J=7.0 Hz), 7.17 (1H, dd, J=15.2, 11.0 Hz), 9.55 (1H, d, J=7.9 Hz); ^{13}C NMR ($CDCl_3$) δ 14.3, 15.7, 28.0, 80.8, 125.9, 131.1, 131.3, 131.8, 137.1, 139.4, 146.6, 152.3, 165.8, 193.5; IR (neat) 2978, 1707, 1678, 1280, 1255, 1132, 1012, 850, 572 cm^{-1}; HRMS (EI): calcd for $C_{16}H_{22}O_3$: 262.1569, found: 262.1581.

1.1.2. (2*E*,4*E*,6*E*,8*E*)-10-[(*E*)-Ethylidene]-2,8-dimethyl-undeca-2,4,6,8-tetraenedioic acid 11-*tert*-butyl ester 1-methyl ester (12). To a DME (0.5 mL) solution of methyl 2-(diethylphosphono)propanate (115 mg, 0.51 mmol) was added a hexane solution of BuLi (1.45 M, 0.30 mL, 0.43 mmol) at 0°C. The reaction mixture was stirred for 10 min at this temperature, then a DME (0.6 mL) solution of aldehyde **13** (45.0 mg, 0.17 mmol) was added at rt. After a further 1 h of stirring, a buffer solution was added and the organic materials were extracted with ethyl acetate, then the combined organic phases were dried over anhydrous Na_2SO_4, and concentrated in vacuo after filtration. Purification by flash column chromatography (ethyl acetate/hexane=1/5) gave 52.6 mg (92%) of ester **12**.

^{1}H NMR ($CDCl_3$) δ 1.49 (9H, s), 1.61 (3H, s), 1.63 (3H, d, J=7.0 Hz), 3.74 (3H, s), 6.12 (1H, s), 6.36 (1H, dd, J=15.1, 10.0 Hz), 6.81 (1H, q, J=7.0 Hz), 6.51 (1H, dd, J=14.6, 10.8 Hz), 6.52 (1H, d, J=15.1 Hz), 6.58 (1H, dd, J=14.6, 10.0 Hz), 7.21 (1H, d, J=10.8 Hz); ^{13}C NMR ($CDCl_3$) δ 12.7, 14.4, 15.7, 28.1, 51.7, 80.5, 126.4, 127.4, 127.6, 128.1, 132.2, 137.5, 138.5, 138.6, 139.8, 140.3, 166.3, 168.8; IR (neat) 2977, 1708, 1590, 1367, 1153, 991, 750 cm^{-1}; HRMS (FAB): calcd for $C_{20}H_{28}O_4$: 332.1988, found: 332.1978.

1.1.3. (3*E*,5*E*,7*E*,9*E*)-12-Cyano-2-[(*E*)-ethylidene]-4,10-dimethyl-11-oxododeca-3,5,7,9-tetraenoic acid *tert*-butyl ester (14). To a THF (1.2 mL) solution of CH_3CN (0.019 mL, 0.361 mmol) was added BuLi (1.45 M, 0.11 mL, 0.153 mmol) at −78°C and the reaction mixture was stirred for 1 h at this temperature. To the reaction mixture was added a THF (2 mL) solution of ester **12** (30.0 mg, 0.090 mmol) at −90°C. The reaction was stirred for 10 min, then was quenched by the addition of a buffer solution. The organic materials were extracted with ethyl acetate, and the combined organic phases were dried over anhydrous Na_2SO_4, and concentrated in vacuo after filtration. Purification by flash column chromatography (ethyl acetate/hexane=1/5) gave 30.1 mg (98%) of ketonitrile **14**.

^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s), 1.67 (3H, s), 1.68 (3H, d, *J*=6.9 Hz), 1.93 (3H, s), 3.77 (2H, s), 6.19 (1H, s), 6.41 (1H, dd, *J*=15.0, 10.7 Hz), 6.59 (1H, dd, *J*=15.1, 11.1 Hz), 6.61 (1H, d, *J*=14.6 Hz), 6.72 (1H, dd, *J*=14.6, 10.7 Hz), 6.83 (1H, q, *J*=6.9 Hz), 7.1 (1H, d, *J*=11.1 Hz); ^{13}C NMR ($CDCl_3$) δ 11.7, 14.3, 15.7, 28.1, 28.2, 80.7, 114.4, 127.1, 127.7, 128.9, 132.0, 133.2, 137.4, 138.9, 141.5, 142.6, 142.9, 166.1, 187.7; IR (neat) 2979, 2256, 1704, 1666, 1585, 1367, 1282, 993, 850, 732 cm^{-1}; HRMS (FAB): calcd for [$C_{17}H_{19}NO_3$−*tert*Bu]: 285.1365, found: 285.1348.

1.1.4. (3*E*,5*E*,7*E*,9*E*)-12-Cyano-2-[(*E*)-ethylidene]-4,10-dimethyl-11-oxododeca-3,5,7,9-tetraenoic acid. To a CH_2Cl_2 (0.6 mL) solution of ketonitrile **14** (28.0 mg, 0.082 mmol) was added CF_3COOH (0.6 mL) at 0°C and the reaction mixture was stirred for 1 h at 0°C. The solvent and CF_3COOH were removed in vacuo, affording the carboxylic acid, which was used directly in the next reaction.

^{1}H NMR ($CDCl_3$) δ 1.72 (3H, s), 1.77 (3H, d, *J*=7.1 Hz), 1.94 (3H, s), 3.79 (2H, s), 6.23 (1H, s), 6.45 (1H, dd, *J*=15.1, 10.6 Hz), 6.62 (1H, dd, *J*=15.1, 10.9 Hz), 6.63 (1H, d, *J*=14.4 Hz), 6.73 (1H, dd, *J*=14.4, 10.6 Hz), 7.02 (1H, d, *J*=10.9 Hz), 7.11 (1H, q, *J*=7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 11.8, 14.3, 16.2, 77.2, 127.4, 127.6, 128.4, 133.4, 138.6, 142.0, 142.7, 142.9, 170.0, 187.6; IR (neat) 2923, 2260, 1666, 1616, 1585, 1234, 995, 755 cm^{-1}; HRMS (FAB): calcd for $C_{17}H_{19}O_3$: 285.1365, found: 285.1363.

1.1.5. (3*E*,5*E*,7*E*,9*E*)-12-Cyano-2-[(*E*)-ethylidene]-4,10-dimethyl-11-oxododeca-3,5,7,9-tetraenoic acid methyl ester (15). To an ether (4 mL) solution of the crude carboxylic acid was added an ether solution of CH_2N_2 at −78°C. The solvent was removed in vacuo. Purification by flash column chromatography (ethyl acetate/hexane=1/5) gave 23.5 mg (96% for 2 steps) of ketonitrile **15**.

^{1}H NMR ($CDCl_3$) δ 1.68 (3H, s), 1.72 (3H, d, *J*=7.1 Hz), 1.93 (3H, s), 3.70 (3H, s), 3.78 (2H, s), 6.20 (1H, s), 6.42 (1H, dd, *J*=15.2, 10.7 Hz), 6.59 (1H, dd, *J*=15.2, 11.0 Hz), 6.61 (1H, d, *J*=14.6 Hz), 6.72 (1H, dd, *J*=14.6, 10.7 Hz), 6.95 (1H, q, *J*=7.1 Hz), 7.02 (1H, d, *J*=11.0 Hz); ^{13}C NMR ($CDCl_3$) δ 11.7, 14.2, 15.9, 28.2, 51.9, 114.3, 127.4, 128.1, 128.3, 130.4, 133.4, 137.9, 141.1, 142.2, 142.8, 142.9, 167.4, 187.6; IR (neat) 2951, 2256, 1712, 1666, 1585, 1434, 1253, 993, 732 cm^{-1}; HRMS (FAB): calcd for $C_{18}H_{21}NO_3$: 299.1521, found: 299.1467.

1.1.6. (14*S*)-(3*E*,5*E*,7*E*,9*E*,12*E*)-16-(*tert*-Butyldiphenylsiloxy)-12-cyano-2-[(*E*)-ethylidene]-4,10-dimethyl-11-oxo-14-triethylsiloxyhexadeca-3,5,7,9,12-pentaenoic acid methyl ester (17). To a benzene (0.4 mL) solution of ketonitrile **15** (45.0 mg, 0.150 mmol) was added (*S*)-aldehyde **16** (214 mg, 0.469 mmol) and ethylenediammonium diacetate (8.0 mg, 0.045 mmol), and the reaction mixture was stirred for 3 h at rt. After the solvent was removed in vacuo, rapid flash column chromatography on florisil gel (ethyl acetate/hexane=1/10) gave the crude olefin **17** (81.8 mg). Purification on silica gel caused substantial decomposition of the product.

^{1}H NMR ($CDCl_3$) δ 0.61–0.67 (6H, m), 0.93–0.97 (9H, m), 1.04 (9H, s), 1.70 (3H, s), 1.74 (3H, d, *J*=7.3 Hz), 3.36 (3H, s), 3.75–3.85 (2H, m), 4.98 (1H, q, *J*=6.7 Hz), 6.21 (1H, s), 6.38–6.48 (1H, m), 6.58–6.62 (2H, m), 6.98 (1H, q, *J*=7.3 Hz), 7.03–7.10 (2H, m 7.35–7.40 (6H, m), 7.63–7.68 (4H, m); ^{13}C NMR ($CDCl_3$) δ 4.7, 4.9, 6.7, 12.5, 14.2, 15.9, 19.1, 26.8, 40.1, 59.9, 68.4, 113.7, 127.4, 127.7, 127.8, 128.2, 129.7, 130.4, 133.4, 133.5, 135.5, 135.6, 138.0, 140.4, 141.8, 142.2, 142.6, 161.2, 167.4, 188.2; IR (neat) 2954, 1718, 1585, 1245, 1112, 1085, 731, 701, 615 cm^{-1}; HRMS (FAB): calcd for $[C_{44}H_{60}O_5NSi_2+H]^+$: 738.4010, found: 738.4058; $[\alpha]_D^{24}$=+7.0 (*c*=0.55, MeOH).

1.1.7. (12*R*,13*S*,14*S*)(3*E*,5*E*,7*E*,9*E*)-16-(*tert*-Butyldiphenylsiloxy)-12-cyano-2-[(*E*)-ethylidene]-4,10-dimethyl-11-oxo-14-triethylsiloxy-12,13-epoxyhexadeca-3,5,7,9-pentaenoic acid methyl ester (18). To a THF solution (2 mL) of tritylperoxide (122 mg, 0.325 mmol) was added a hexane solution of BuLi (0.187 mL, 1.45 M, 0.27 mmol) at −78°C, and the reaction mixture was stirred at this temperature for 1 h. To this reaction mixture was added a THF (3 mL) solution of crude olefin **17**, and the mixture was stirred for 1 h at −78°C. The reaction was quenched with a buffer solution and the organic materials were extracted with ethyl acetate, and combined organic phases were dried over anhydrous Na_2SO_4, and concentrated in vacuo after filtration. The crude epoxide was used directly in the next reaction.

1.1.8. (3*E*,5*E*,7*E*,9*E*)-11-{(1*R*,4*S*,5*S*)-4-[(2-*tert*-Butyldiphenylsiloxy)ethyl]-2-oxo-3,6-dioxabicyclo[3.1.0]hex-1-yl}-2-[(*E*)-ethylidene]-4,10-dimethyl-11-oxoundeca-3,5,7,9-tetraenoic acid methyl ester (20). To the crude epoxide **18** was added THF (1.2 mL), H_2O(70 μL), and TsOH·H_2O (20 mg) at 0°C and the reaction mixture was stirred for 1 h at rt. After addition of a saturated $NaHCO_3$ solution, the organic materials were extracted with ethyl acetate, and the organic phases were dried over anhydrous Na_2SO_4, and concentrated in vacuo after filtration. Purification by rapid flash column chromatography (ethyl acetate/hexane=1/5) gave crude hydroxynitrile **19**. The crude hydroxynitrile **19** was charged on to a thin-layer chromatography plate and developed by ethyl acetate/hexane mixed solvent (1/1). 51.0 mg of lactone **20** was obtained in 80% over 3 steps.

^{1}H NMR ($CDCl_3$) δ 1.05 (9H, s), 1.71 (3H, s), 1.73 (3H, d, J=7.3 Hz), 1.87 (3H, s), 3.74 (3H, s), 3.81–3.92 (2H, m), 4.23 (1H, s), 4.80–4.87 (1H, m), 6.22 (1H, s), 6.45 (1H, dd, J=15.2, 10.5 Hz), 6.70 (1H, q, J=7.3 Hz), 6.98 (1H, q, J=7.3 Hz), 7.35–7.48 (6H, m), 7.61–7.68 (4H, m); ^{13}C NMR ($CDCl_3$) δ 11.4, 14.2, 15.9, 19.2, 26.9, 35.2, 51.2, 51.9, 59.1, 60.4, 63.4, 127.8, 127.9, 128.1, 128.3, 129.9, 130.4, 133.1, 133.5, 135.5, 138.0, 140.5, 142.4, 143.5, 145.1, 167.4, 168.5, 187.8; IR (neat) 2929, 1787, 1680, 1635, 1112, 991, 735 cm^{-1}; HRMS (FAB): calcd for $C_{38}H_{44}O_7Si$: 640.2856, found: 640.2855; $[\alpha]_D^{25}$=−20.3 (c=0.953, MeOH).

1.1.9. (14*R*,15*S*,16*S*)(3*E*,5*E*,7*E*,9*E*)-16-(*tert*-Butyldiphenylsiloxy)-12-carbamoyl-4,10-dimethyl-2-[(*E*)-ethylidene]-12,13-epoxy-14-hydroxy-11-oxohexadeca-3,5,7,9-tetraenoic acid methyl ester (21). To a MeOH (2 mL) solution of lactone **20** (51.1 mg, 0.080 mmol) was added NH_4OH solution (30%, 1.0 mL) at 0°C, and the reaction mixture was stirred for 20 min at this temperature. The reaction was quenched with a buffer solution and the organic materials were extracted with $CHCl_3$ four times, then the combined organic phases were dried over anhydrous Na_2SO_4, and concentrated in vacuo after filtration. Purification by preparative thin layer chromatography (ethyl acetate/hexane=2/1) gave 40.0 mg (78%) of hydroxyamide **21**.

^{1}H NMR ($CDCl_3$) δ 1.04 (9H, s), 1.69 (3H, s), 1.72 (3H, d, J=7.4 Hz), 1.92 (3H, s), 3.23 (1H, d, J=8.0 Hz), 3.73 (3H, s), 3.82–3.89 (1H, m), 3.89–3.97 (1H, m), 5.74 (1H, bs), 6.18 (1H, s), 6.43 (1H, dd, J=15.1, 10.6 Hz), 6.53 (1H, bs), 6.57 (1H, d, J=15.7 Hz), 6.65 (1H, dd, J=14.6, 11.2 Hz), 6.75 (1H, dd, J=14.6, 10.6 Hz), 6.96 (1H, q, J=7.4 Hz), 7.27–7.47 (7H, m), 7.58–7.70 (4H, m); ^{13}C NMR ($CDCl_3$) δ 11.4, 14.2, 15.9, 19.1, 26.8, 36.0, 51.9, 61.9, 64.2, 65.9, 68.8, 71.6, 127.8, 127.9, 128.0, 128.4, 129.9, 132.6, 132.9, 135.5, 138.0, 140.4, 142.0, 143.3, 146.0, 168.9, 167.5, 192.9; IR (neat) 3454, 2929, 1714, 1693, 1652, 1581, 1428, 1249, 1111, 991, 734, 703, 505 cm^{-1}; HRMS (FAB): calcd for $C_{38}H_{47}NO_7Si$: 657.3122, found: 657.3124; $[\alpha]_D^{23}$=−49.7 (c=0.65, $CHCl_3$).

1.1.10. (3*E*,5*E*,7*E*,9*E*)-11-{(1*R*,5*S*)-4-[2-(*tert*-Butyldiphenylsiloxy)ethyl]-4-hydroxy-2-oxo-3-aza-6-oxabicyclo-[3.1.0]hex-1-yl}-2-[(*E*)-ethylidene]-4,10-dimethyl-11-oxoundeca-3,5,7,9-tetraenoic acid methyl ester. To a CH_2Cl_2 (0.2 mL) solution of hydroxyamide **21** (13.0 mg, 0.0197 mmol) was added Dess–Martin periodinane (16.8 mg, 0.0395 mmol) at 0°C, and the reaction mixture was stirred for 2 h at rt. The reaction was quenched with a saturated $NaHCO_3$ solution. The organic materials were extracted with ethyl acetate, and the combined organic extracts were washed with a saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, and concentrated in vacuo after filtration. Purification by preparative thin layer chromatography (ethyl acetate/hexane=3/2) gave 7.7 mg (60%) of lactam.

^{1}H NMR ($CDCl_3$) δ 1.07 (9H, s), 1.69 (3H, s), 1.97 (3H, s), 2.02 (3H, d, J=1.2 Hz), 2.01–2.12 (2H, m), 3.84–3.91 (1H, m), 3.72 (3H, s), 3.96 (1H, d, J=7.2 Hz), 4.01–4.09 (1H, m), 4.91 (1H, bs), 6.19 (1H, s), 6.45 (1H, dd, J=15.2, 10.4 Hz), 6.58 (1H, d, J=15.2 Hz), 6.66 (1H, dd, J=14.6, 10.8 Hz), 6.73 (1H, dd, J=14.6, 10.4 Hz), 6.95 (1H, q, J=7.2 Hz), 7.13–7.47 (7H, m), 7.59–7.67 (4H, m); ^{13}C NMR ($CDCl_3$) δ 11.4, 14.2, 15.9, 19.0, 35.9, 52.2, 60.4, 61.7, 63.7, 77.3, 85.5, 127.8, 128.0, 128.1, 128.5, 130.3, 130.4, 132.1, 133.9, 135.5, 138.1, 140.4, 141.9, 143.0, 144.4, 167.4, 169.2, 189.3; IR (neat) 3401, 2929, 1722, 1581, 1428, 1265, 1112, 991, 734, 703 cm^{-1}; HRMS (FAB): calcd for $C_{38}H_{43}NO_7$ Si: 655.2965, found: 655.2957; $[\alpha]_D^{24}$=+33.5 (c=0.57, MeOH).

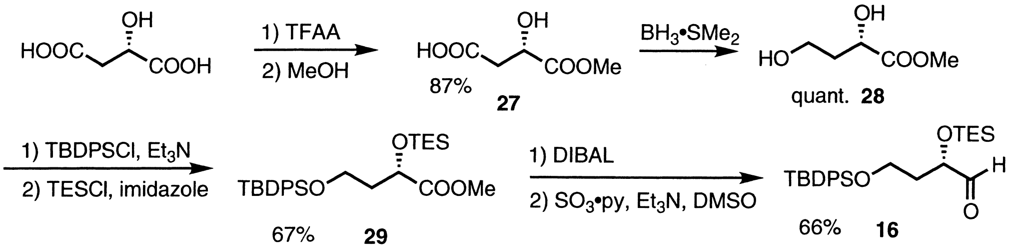
1.1.11. NG-391 (1).[1] To a THF (0.1 mL) solution of lactam (6.5 mg, 0.001 mmol) was added a THF solution of tetra-*n*-butylammonium fluoride (1.0 M, 0.02 mL, 0.02 mmol) at 0°C, and the reaction mixture was stirred for 10 min at 0°C. The reaction was quenched with a saturated NH_4Cl solution, and the organic materials were extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo after filtration. Purification by preparative thin layer chromatography (ethyl acetate/hexane=3/1) gave 4.5 mg of NG-391 (**1**) quantitatively.

^{1}H NMR ($CDCl_3$) δ 1.68 (3H, s), 1.73 (3H, d, J=7.0 Hz), 1.86 (3H, s), 2.02 (1H, s), 2.04–2.13 (2H, m), 3.72 (3H, s), 3.87–3.97 (1H, m), 4.00 (1H, s), 4.02–4.12 (2H, m), 6.20 (1H, s), 6.38–6.53 (2H, m), 6.57–6.69 (2H, m), 6.75 (1H, dd, J=14.6, 10.7 Hz), 6.92–7.02 (2H, m), 7.45 (1H, d, J=11.4 Hz); ^{13}C NMR ($CDCl_3$) δ 11.3, 14.2, 15.9, 35.8, 51.9, 58.3, 61.7, 63.6, 85.3, 127.9, 128.4, 130.3, 133.8, 138.0, 140.5, 142.2, 143.5, 145.3, 167.4, 169.8, 190.0; IR (neat) 3404, 2929, 1722, 1581, 1428, 1265, 991 cm^{-1}; HRMS (FAB): calcd for $[C_{22}H_{27}NO_7+H]^+$: 418.1866, found: 418.1839; $[\alpha]_D^{25}$=+41.7 (c=0.37, MeOH).

1.2. Synthesis of chiral aldehyde 16

Aldehyde **16** was prepared from (*S*)-malic acid according to the following scheme via the known ester **28** by a modification of the procedure of Gong and Lynn.[15] (*S*)-Malic acid was treated with trifluoroacetic anhydride, then with MeOH, affording hydroxy carboxylic acid **27** in 87% yield. Reduction of the carboxylic acid was effectively carried out with $BH_3{\cdot}SMe_2$, affording dihydroxy ester **28**. As the hydroxy-ester easily cyclized to a lactone, **28** was treated with *tert*-butyldimethylsilylchloride and triethylsilylchloride successively to give ester **29** in moderate yield. Reduction of ester **29** and oxidation of the resulting alcohol afford chiral aldehyde **16** in 66% yield over 2 steps (Scheme 6).

1.2.1. (*S*)-2-Hydroxysuccinic acid 1-methyl ester (27). To (*S*)-malic acid (3.54 g, 26.3 mol) was added trifluoroacetic anhydride (14.8 mL), and the reaction mixture was stirred for 40 min. After removal of the remaining trifluoroacetic anhydride in vacuo, MeOH (15 mL) was added to the remaining solid, which was stirred for 1.5 h at rt. Volatile materials were removed in vac*uo* and the residue was crystallized from ether/hexane to give 3.38 g (87%) of esther **27**.



Scheme 6. Synthesis of chiral aldehyde **16**.

^{1}H NMR ($CDCl_3$) δ 2.82 (1H, dd, J=16.7, 6.1 Hz), 2.89 (1H, dd, J=16.7, 4.2 Hz), 3.80 (3H, s), 4.32 (1H, dd, J=6.1, 4.2 Hz); ^{13}C NMR ($CDCl_3$) δ 35.9, 52.4, 59.2, 68.9, 175.3; mp 70.0–71.0°C; $[\alpha]_D^{23}$=−15.6 (c=1.33, MeOH).

1.2.2. (S)-2,4-Dihydroxybutyric acid methyl ester (28). To a THF (15 mL) solution of ester **27** (1.42 g, 9.59 mmol) was added $BH_3{\cdot}SMe_2$ (3.7 mL) at 0°C. After stirring the reaction for 2 h at rt, it was quenched with MeOH. The solvent was removed in vacuo, and the remaining oil was evaporated from MeOH several times to remove the methylborate, giving 1.28 g of diol **28** quantitatively, which was used in next reaction without further purification owing to its easy lactonization.

^{1}H NMR ($CDCl_3$) δ 1.85–1.96 (2H, m), 2.02–2.12 (2H, m), 2.41–2.79 (2H, br), 3.80 (3H, s), 3.76–3.98 (2H, m); ^{13}C NMR ($CDCl_3$) δ 19.1, 26.8, 36.3, 52.3, 60.5, 68.6, 127.6, 129.7, 133.2, 135.5, 175.3; IR (neat) 3501, 2931, 1735, 1427, 1220, 1112, 823, 701, 613, 505 cm^{-1}.

1.2.3. (*S*)-4-(*tert*-Butyldiphenylsiloxy)-2-triethylsiloxy-butyric acid methyl ester (29). To a CH_2Cl_2 (6 mL) solution of ester **28** (1.57 g, 11.7 mmol), triethylamine (1.96 mL, 14.1 mmol) and DMAP (143 mg, 1.17 mmol) was added *tert*-butyldiphenylsilylchloride (3.55 g, 12.9 mmol) at 0°C, and the reaction mixture was stirred for 13 h at rt. After a buffer solution had been added, the organic materials were extracted with ethyl acetate, and the combined organic extracts were washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo after filtration. The crude hydroxyester was used directly in the next step. To a DMF (12 mL) solution of the crude hydroxyester (4.37 g, 11.7 mmol) was added imidazole (1.43 g, 21.1 mmol) and triethylsilylchloride (2.65 g, 17.6 mmol) at 0°C, and the reaction mixture was stirred for 2 h at rt. After a buffer solution had been added, the organic materials were extracted with ethyl acetate, and the combined organic extracts were washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo after filtration. Purification by flash column chromatography (ethyl acetate/hexane=1/10) gave 3.81 g (67%) of ester **29**.

^{1}H NMR ($CDCl_3$) δ 0.60 (6H, q, J=7.8 Hz), 0.94 (9H, s), 1.04 (9H, t, J=7.8 Hz), 1.82–1.90 (1H, m), 1.93–2.22 (1H, m), 3.67 (3H, s), 3.67–3.82 (2H, m), 4.45 (1H, d, J=4.6 Hz); ^{13}C NMR ($CDCl_3$) δ 4.6, 6.7, 19.2, 26.8, 38.1, 51.7, 59.7, 68.8, 127.6, 129.6, 133.8, 135.5, 178.3; IR (neat) 2954, 2933, 2877, 1758, 1428, 1139, 1112, 823, 701, 505 cm^{-1}; $[\alpha]_D^{25}$=−43.4 (c=1.10, $CHCl_3$); HRMS (FAB): calcd for $[C_{27}H_{43}O_4Si_2+H]^+$: 487.2700, found: 487.2690.

1.2.4. (*S*)-4-(*tert*-Butyldiphenylsiloxy)-2-triethylsiloxy-butan-1-ol. To a CH_2Cl_2 (14 mL) solution of ester **29** (3.81 g, 7.83 mmol) was added a hexane solution of DIBAL (0.95 M, 28.8 mL, 27.4 mmol) at −50°C, and the reaction mixture was stirred for 1 h at −10°C. To the reaction mixture were added MeOH (1.44 mL, 32.9 mmol) and $Na_2SO_4{\cdot}10H_2O$(13.2 g, 43.9 mmol), and stirring was continued for 40 min. After filtration of the inorganic materials, the solvent was removed in vacuo to give 2.36 g (66%) of the alcohol, which was used in the next reaction without purification.

^{1}H NMR ($CDCl_3$) δ 0.59 (6H, q, J=7.8 Hz), 0.93 (9H, t, J=7.8 Hz), 1.04 (9H, s), 1.70–1.82 (2H, m), 2.18 (1H, t, J=6.6 Hz), 3.43–3.51 (1H, m), 3.55–3.62 (1H, m), 3.71 (2H, t, J=5.9 Hz), 3.95–4.02 (1H, m), 7.33–7.43 (6H, m), 7.62–7.68 (4H, m); IR (neat) 3436, 2933, 2877, 1471, 1427, 1112, 1006, 738, 721 cm^{-1}; HRMS (FAB): calcd for $[C_{26}H_{43}O_3Si_2+H]^+$: 459.2751, found: 459.2805; $[\alpha]_D^{25}$=+12.2 (c=0.95, $CHCl_3$).

1.2.5. (*S*)-4-(*tert*-Butyldiphenylsiloxy)-2-triethylsiloxy-butanal (16). To a CH_2Cl_2 (0.68 mL) solution of alcohol (315.0 mg, 0.687 mmol) was added triethylamine (0.48 mL), DMSO (0.68 mL) and $SO_3{\cdot}$pyridine (328 mg, 2.06 mmol) at 0°C, and the reaction mixture was stirred for 1.5 h. After a buffer solution had been added, the organic materials were extracted with ethyl acetate three times, and the combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, concentrated in vacuo after filtration to give 313 mg of aldehyde **16** in quantitative yield.

^{1}H NMR ($CDCl_3$) δ 0.64 (6H, q, J=7.9 Hz), 0.97 (9H, t, J=7.9 Hz), 1.04 (9H, s), 1.83–1.99 (2H, m), 3.67–3.73 (1H, m), 3.83–3.90 (1H, m), 4.24 (1H, t, J=5.3 Hz), 7.36–7.44 (6H, m), 7.65–7.70 (4H, m), 9.72 (1H, s); ^{13}C NMR ($CDCl_3$) δ 4.8, 5.0, 6.7, 19.0, 26.8, 36.4, 58.8, 74.7, 127.7, 129.6, 133.5, 135.6, 204.2; IR (neat) 2956, 2859, 1738, 1427, 1113, 1009, 702, 505.

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