

## Total synthesis of spiruchostatin B aided by an automated synthesizer†

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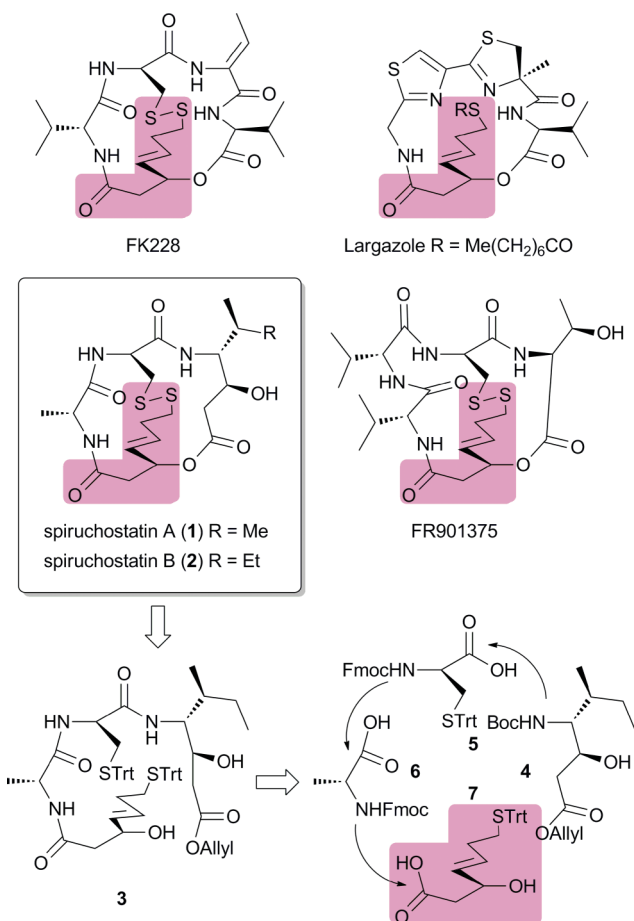
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The total synthesis of a natural product HDAC inhibitor, spiruchostatin B, was successfully achieved. A 5-step synthesis that included an asymmetric aldol reaction was carried out in an automated synthesizer to provide an (*E*)-(*S*)-3-hydroxy-7-thio-4-heptenoic acid segment that is the crucial structure of cysteine-containing, depsipeptidic natural products such as spiruchostatins, FK228, FR901375, and largazole for their inhibitory activity against HDACs.

### Introduction

HDAC (histone deacetylase) inhibitors make up a very important class of compounds because they show promise as prospective targets for cancer chemotherapy.<sup>1</sup> There are 18 HDACs divided into four main sub-types: classes I, II, IV, and the structurally distinct class III.<sup>2</sup> HDAC inhibitor design has concentrated on the class I and class II zinc-dependent enzymes.<sup>3</sup> Although the precise targets of class I and class II HDACs remain uncertain, class I selectivity is now being considered useful for anticancer agents,<sup>4</sup> whereas the inhibition of specific class II HDACs is considered to promote undesired cardiac hypertrophy.<sup>5</sup> Synthetic HDAC inhibitors, such as hydroxamates and short-chain fatty acids, suffer from relatively weak HDAC inhibition (micromolar IC<sub>50</sub>) and lack of class I selectivity.<sup>3</sup> On the other hand, cysteine-containing, depsipeptidic natural products including spiruchostatin A (**1**), B (**2**),<sup>6</sup> FK228,<sup>7</sup> FR901375,<sup>8</sup> and largazole<sup>9</sup> have a high potency and a class I selectivity. All these compounds retain (*E*)-(*S*)-3-hydroxy-7-thio-4-heptenoic acid (emphasized in Scheme 1) that is a crucial structure for inhibitory activity.<sup>10</sup> The development of an efficient synthetic method for the discovery of more potent and class I selective analogues of HDAC inhibitors is very important. Recently, the successful total synthesis of the natural product HDAC inhibitors and their analogues and biological evaluation



**Scheme 1** Structures of cysteine-containing, depsipeptidic natural products and the synthetic strategy for spiruchostatin B (**2**). The (*E*)-(*S*)-3-hydroxy-7-thio-4-heptenoic acid moiety that is a crucial structure for HDAC inhibition is emphasized. Trt = trityl, Boc = *t*-butoxycarbonyl, Fmoc = 9-fluorenylmethoxycarbonyl.

on the basis of spiruchostatin A (**1**),<sup>11–17</sup> B (**2**),<sup>17,18</sup> FK228,<sup>17,19–25</sup> FR901375,<sup>26</sup> and largazole<sup>25,27–39</sup> have been reported.<sup>40</sup> An efficient

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† Electronic supplementary information (ESI) available: Full picture of ChemKonzert and detailed protocol to use ChemKonzert. Copies of NMR spectra of compounds **2–4**, **7**, **10–12**, **13a**, **15**, **16a**, **17–20** are available. See DOI: 10.1039/c0ob01169j

supply of a sufficient amount of (*E*)-(*S*)-3-hydroxy-7-thio-4-heptenoic acid Segment 7 is required for the synthesis of various analogues of HDAC inhibitors.

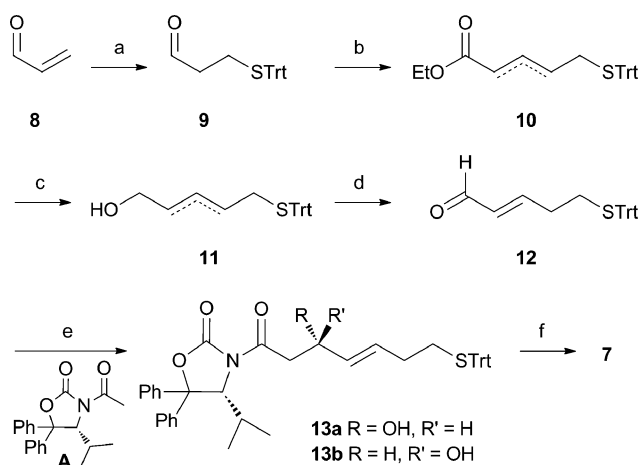
Automation of synthetic procedures improves both the reproducibility and reliability of the syntheses because automated synthesizers minimize the variability of the experimental manipulations.<sup>41</sup> We previously reported a formal total synthesis of taxol using an automated synthesizer that was developed in our laboratory. Use of the ChemKonzert®<sup>42,43</sup> enabled production of a key synthetic intermediate in a 36-step, solution-phase synthesis.<sup>44</sup> We also reported an efficient synthesis of a cyclic ether key intermediate for 9-membered masked enediyne using the ChemKonzert.<sup>45</sup> If synthetic chemists run the reactions in automated synthesizers and digitally store the experimental procedures, anyone could reproduce the results by using the same apparatus and compounds, regardless of the time and place. As a result, synthetic chemists could expend more effort on the advanced and challenging work of construction of new synthetic routes.

Herein, we wish to report the total synthesis of spiruchostatin B (2).  $\beta$ -Hydroxy acid segment 7, which is a common synthetic intermediate for cysteine-containing, depsipeptidic natural products were efficiently supplied with the aid of an automated synthesizer.

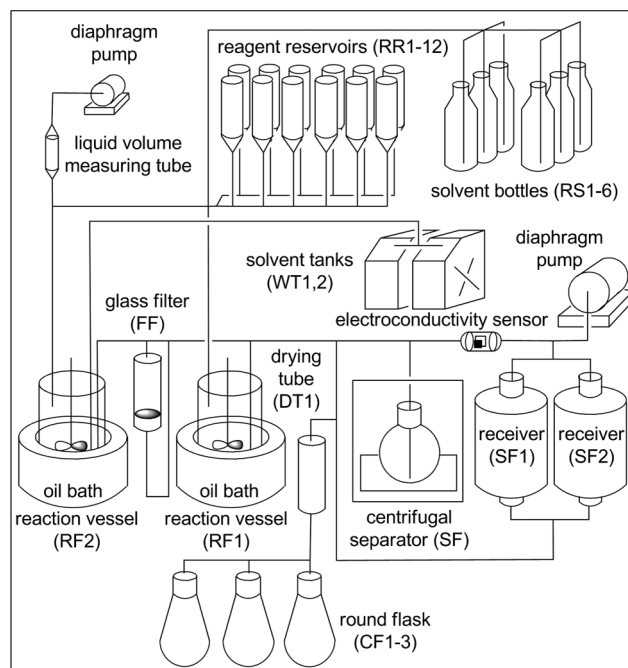
## Results and discussion

The synthetic route for spiruchostatin B (2) is illustrated in Scheme 1. Macrolactonization of *seco*-acid derivative 3 and subsequent disulfide formation is the most crucial step in the construction of the 15-membered bicyclic skeleton of 2. Compound 3 can be prepared by the sequential coupling of *D*-allo-isoleucine-derived (3*S*, 4*R*)-statine allyl ester 4, *N*-Fmoc-*S*-trityl-*D*-cysteine (5), *N*-Fmoc-*D*-alanine (6), and  $\beta$ -hydroxy acid 7.<sup>12,13</sup> For the preparation of  $\beta$ -hydroxy acid 7, the key step is an asymmetric aldol reaction. Since acetate aldols proceed with poor diastereoselectivity with the classical Evans' oxazolidin-2-one auxiliary, we have investigated the potential of Seebach's *N*-acetyl-oxazolidin-2-one A<sup>46-49</sup> (Scheme 2) and observed that the Zr-enolate<sup>50-52</sup> of 13 was effective in this reaction.<sup>12</sup> The developed asymmetric aldol reaction requires careful synthetic manipulation to obtain a high yield and a high stereoselectivity. Therefore, we planned to utilize an automated synthesizer<sup>42,43</sup> to improve both efficiency and reproducibility.<sup>44,45</sup>

$\beta$ -Hydroxy acid 7 was prepared with the use of the automated synthesizer ChemKonzert (Scheme 2).<sup>42</sup> Preparation of 10 from acrolein (8) was carried out as follows. The computer controlling the automated synthesizer was programmed with a specific procedure. Reagents, substrate and solvent were added to the reaction vessel, RF1, the reagent reservoirs, RR1 and RR2, and the solvent bottle, RS1 (Fig. 1). Then, the operation of the automated synthesizer was started. TrtSH was stirred at 25 °C under a nitrogen atmosphere in the reaction vessel, RF1. CH<sub>2</sub>Cl<sub>2</sub>, Et<sub>3</sub>N and acrolein (8) in the solvent bottle, RS1, and reagent reservoirs RR1 and RR2 were added to the reaction vessel, RF1 (Fig. 1).<sup>26</sup> After stirring at 25 °C for 1 h, the resultant mixture was transferred to a round flask, CF1. The entire apparatus was washed with water and acetone from the solvent tanks, WT1 and WT2, then dried under reduced pressure (Fig. 1). The collected solution in CF1 was manually concentrated *in vacuo*. The residue was used for the next reaction without further purification. Reagents, substrate,



**Scheme 2** Synthesis of  $\beta$ -hydroxy acid 7 aided by an automated synthesizer. *Reagents and Conditions:* a) TrtSH, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 25 °C, 1 h; b) monoethyl malonate, DMF, 100 °C, 2 h, 2 steps 88%; c) DIBAL, CH<sub>2</sub>Cl<sub>2</sub>, -20 °C to 25 °C, 2 h, 75%; d) (COCl)<sub>2</sub>, DMSO, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, -78 to 0 °C, 1 h, 56%; e) *n*-BuLi, Cp<sub>2</sub>ZrCl<sub>2</sub>, THF, -78 to 0 °C, 1 h, 84% (13a:13b = 90 : 10); f) NaOH, THF-MeOH-H<sub>2</sub>O, 0 °C, 1 h, 92%. DMAP = 4-(dimethylamino)pyridine, DMF = *N,N*-dimethylformamide, DIBAL = diisobutylaluminium hydride, DMSO = dimethyl sulfoxide, THF = tetrahydrofuran.



**Fig. 1** Schematic diagram of the automated synthesizer, ChemKonzert.

solvent and wash solutions were added to the reaction vessel, RF1, reagent reservoirs, RR1-RR7, solvent bottle, RS1, and wash-solution bottles RS4 and RS5. The operation of the automated synthesizer was then started. A solution of monoethyl malonate and 3-tritylthiopropional (9) in DMF was stirred at 25 °C under a nitrogen atmosphere in the reaction vessel, RF1. A solution of DMAP in DMF in the reagent reservoir, RR3, was added to the reaction vessel, RF1. After stirring at 100 °C for 2 h, the reaction was quenched by the addition of 1 M HCl from the wash-solution bottle, RS4, at 25 °C. After the addition of EtOAc

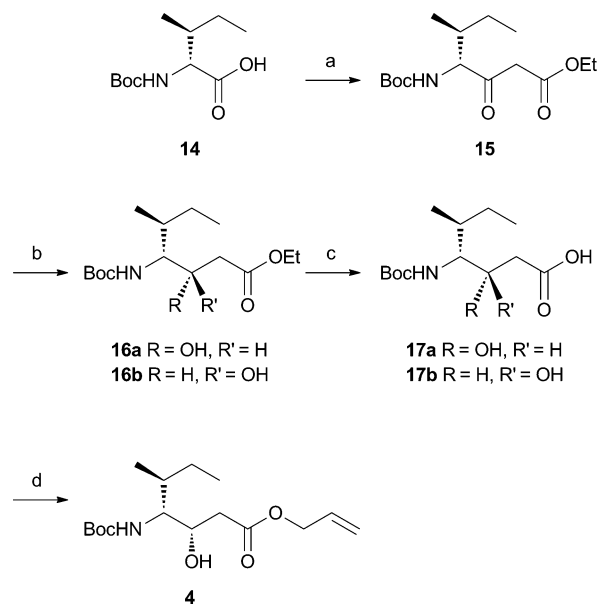
from the solvent bottle, RS1, the mixture was stirred and then transferred to a centrifugal separator, SF. After centrifugation, the two resulting phases were separated by measurement of their electroconductivities with a sensor and were then transferred to two receivers, SF1 and SF2. The aqueous phase in SF1 was taken back to the reaction vessel, RF1. After the addition of EtOAc from the solvent bottle, RS1, the mixture was stirred and then transferred to the centrifugal separator, SF. After performing the extraction procedure twice, the combined organic mixture in the receiver, SF2, was washed with 1 M HCl, 5% aqueous NaHCO<sub>3</sub> and 10% aqueous NaCl from the wash-solution bottles, RS6, RS5, and RS4, in the reaction flask, RF1. The organic layer was separated in the centrifugal separator, SF, and was transferred to the receiver, SF2. The organic layer in SF2 was then passed through an anhydrous Na<sub>2</sub>SO<sub>4</sub> plug, DT1, and was collected in a round flask, CF1. Finally, the entire apparatus was washed with water and acetone, and dried under reduced pressure. The collected solution was purified manually using flash silica gel column chromatography to give a mixture of ethyl (*E*)-5-(tritylthio)pent-2-enoate and ethyl (*E*)-5-(tritylthio)pent-3-enoate **10** in an 88% combined yield.

DIBAL reduction of the ethyl esters and subsequent Swern oxidation were performed by using the automated synthesizer to afford (*E*)-5-(tritylthio)pent-2-enal (**12**)<sup>19,26</sup> as a single product (Scheme 2).

The key step, asymmetric aldol reaction of enal **12**,<sup>12</sup> was carried out with the use of the automated synthesizer (Scheme 2). *n*-BuLi was manually added to a solution of (*R*)-*N*-acetyl-4-isopropyl-5,5-diphenyl-2-oxazolidinone in THF at -78 °C under a nitrogen atmosphere in the reaction vessel, RF1. After stirring for 1 h at the same temperature, a solution of Cp<sub>2</sub>ZrCl<sub>2</sub> in THF, in the reagent reservoir, RR2, was added to the reaction mixture, and then warmed to -30 °C. The resulting mixture was stirred at the same temperature for 1 h and cooled to -50 °C. A solution of the enal **12** in THF, in the reagent reservoir, RR3, was added to the reaction mixture, and then warmed to 0 °C. After stirring for 1 h at the same temperature, the reaction mixture in the reaction vessel, RF1, was poured into 10% aqueous NH<sub>4</sub>Cl in another reaction vessel, RF2. Automated workup and manual silica gel column purification afforded a 90 : 10 mixture of aldols **13a** and **13b** in an 84% combined yield. The diastereomeric mixture was repurified using silica gel column chromatography to give pure **13a**.

Hydrolysis of **13a** was performed manually. After simple purification by silica gel column chromatography, the desired β-hydroxy acid **7**<sup>19</sup> was obtained in 92% yield (Scheme 2).

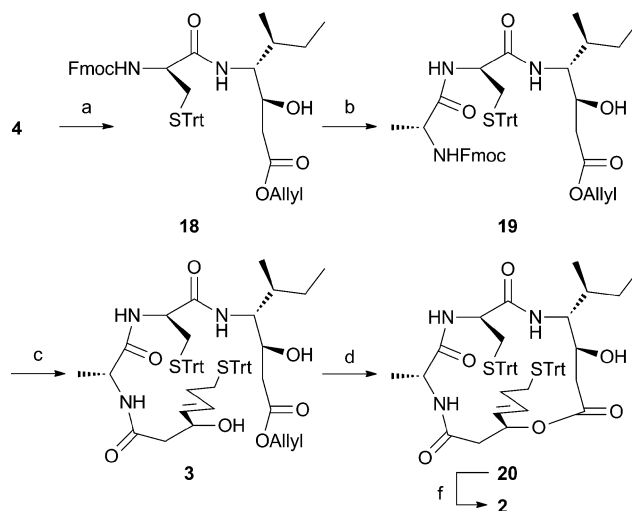
*D*-*allo*-Isoleucine derived statine allyl ester **4** was prepared from *N*-Boc-*D*-*allo*-isoleucine (**14**) (Scheme 3). Condensation with ethyl magnesium malonate,<sup>53</sup> followed by stereoselective reduction with KBH<sub>4</sub> afforded the desired alcohol **16a** as a major isomer. Hydrolysis of the diastereomeric mixture of **16a** and **16b**, and subsequent recrystallization afforded a white solid (17%). After converting the obtained carboxylic acid into its corresponding allyl ester, the ratio of **17a** and **17b** was determined by <sup>1</sup>H NMR analysis (**17a** : **17b** = *ca.* 50 : 50). On the other hand, the carboxylic acid (68%) obtained in the filtrate was converted into its corresponding benzyl ester, and the ratio of **17a** and **17b** was determined by HPLC with a photodiode array (**17a** : **17b** = 95 : 5). The desired product **17a** obtained from the filtrate was treated with allyl bromide to afford the allyl ester **4**.



**Scheme 3** Synthesis of *D*-*allo*-isoleucine derived statine allyl ester **4**. *Reagents and Conditions*: a) carbonyldiimidazole, (EtO<sub>2</sub>CCH<sub>2</sub>CO<sub>2</sub>)<sub>2</sub>Mg, THF, 76%; b) KBH<sub>4</sub>, MeOH, 85% (**16a** : **16b** = 91 : 9); c) LiOH, THF-H<sub>2</sub>O, 68% (**17a** : **17b** = 95 : 5); d) allyl bromide, K<sub>2</sub>CO<sub>3</sub>, DMF, 75%.

#### Total synthesis of spiruchostatin B (2)

Prepared segments were coupled as shown in Scheme 4. In the condensation of cysteine with the statine derivative **4**, the order of reagent addition greatly affected the racemization of cysteine. It is well known that cysteine is highly susceptible to racemization, especially after converting to the corresponding activated ester. In



**Scheme 4** Total synthesis of spiruchostatin B (**2**). *Reagents and Conditions*: a) i) 4 M HCl; ii) **5**, EDCI-HCl, HOBt, DIEA, 2 steps 92%; b) i) Et<sub>2</sub>NH; ii) **6**, EDCI-HCl, HOBt, DIEA, 2 steps 98%; c) i) Et<sub>2</sub>NH; ii) **7**, PyBOP, DIEA, 2 steps 76%; d) i) LiOH, THF-H<sub>2</sub>O; ii) MNBA, DMAP, 2 steps 62%; f) I<sub>2</sub>, MeOH, 82%. EDCI = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, HOBt = 1-hydroxybenzotriazole, DIEA = *N,N*-diisopropylethylamine, PyBOP = benzotriazole-1-yl-oxy-tripyrrolidinophosphonium hexafluorophosphate, MNBA = 2-methyl-6-nitrobenzoic anhydride.

fact, racemization was observed by adding DIEA into the pre-incubated solution of cysteine **5**, hydrochloride salt of deprotected **4**, EDCI·HCl, and HOBt. However, severe racemization (<7%) was avoided by adding DIEA and EDCI·HCl into the premixed solution of cysteine **5**, HOBt, and the hydrochloride salt of deprotected **4**. Compound **3** was prepared from **18** by subsequent condensation with D-alanine (**6**) and β-hydroxy acid **7** in good yield. Unexpectedly, the allyl group of **3** was cleanly removed by using conventional basic hydrolysis conditions to afford *seco*-acid **20**. Macrolactonization of **20** using Shiina's method<sup>54–57</sup> followed by disulfide bond formation, the same procedure used in our total synthesis of spiruchostatin A,<sup>12</sup> was performed to furnish spiruchostatin B (**2**)<sup>18</sup> in high yield. The spectral data of synthetic **2** were in good accordance with those of the natural product.<sup>6</sup>

Reporter gene assay in HEK 293 cell promoted cytomegalovirus (CMV) was performed to evaluate the HDAC inhibitory activity of the synthetic spiruchostatin A (**1**) and B (**2**).<sup>58</sup> ED<sub>50</sub> values of both **1** and **2** were determined to be 200 nM. Almost the same level of HDAC inhibitory activity was observed in the synthetic spiruchostatins.

## Conclusions

In summary, HDAC inhibitor spiruchostatin B (**2**) was successfully synthesized. β-Hydroxy acid **7**, the common synthetic intermediate for cysteine-containing natural product HDAC inhibitors such as spiruchostatins, FK228, FR901375 and largazole was efficiently prepared. A total of 5 steps were successfully performed using an automated synthesizer that was developed by our group -including an asymmetric aldol reaction that requires careful synthetic manipulation to obtain a high yield and a high stereoselectivity. Prepared segments **4–7** were coupled in good yield while suppressing the undesired racemization of a cysteine segment. Shiina's method was employed to form a macrolactone ring and subsequent disulfide formation afforded the desired product. The developed method should be very useful for combinatorial synthesis of cysteine-containing natural product HDAC inhibitor analogues.

## Experimental section

### General

NMR spectra were recorded in the indicated solvents. Chemical shifts are reported in unit of parts per million (ppm) from tetramethylsilane with the solvent resonance as the internal standard (CHCl<sub>3</sub>: δ 7.26 ppm for <sup>1</sup>H, CDCl<sub>3</sub>: δ 77.0 ppm for <sup>13</sup>C). Data are reported as follows: chemical shift, multiplicity (s; singlet, d; doublet, t; triplet, q; quartet, m; multiplet, br; broad), coupling constants (Hz) and integration. All reactions were monitored by thin-layer chromatography using E. Merck silica gel plates (60F-254) pre-coated plates (0.25 mm). TLC visualization was done with UV light and/or 5% ethanolic *p*-anisaldehyde or 10% ethanolic phosphomolybdic acid followed by heating. Flash column chromatography was performed on Silica Gel 60 N, purchased from Kanto Chemical Co. THF was dried by distillation from sodium/benzophenone ketyl. CH<sub>2</sub>Cl<sub>2</sub> was dried by distillation from P<sub>2</sub>O<sub>5</sub>. CH<sub>3</sub>CN and DMF were dried

by distillation from CaH<sub>2</sub>. MeOH was dried by distillation from magnesium contained with a catalytic amount of iodine.

### Ethyl (*E*)-5-(tritylthio)-2-pentenoate and ethyl (*E*)-5-(tritylthio)-3-pent-3-enoate (**10**)

A solution of tritylthiol (10.0 g, 36.2 mmol, 8.3 equiv.) in CH<sub>2</sub>Cl<sub>2</sub> (80 mL) in RF1 was added Et<sub>3</sub>N (7.56 mL, 54.3 mmol, 12.5 equiv., RR1) and acrolein (**8**) (3.44 mL, 4.36 mmol, 1.0 equiv., RR2). After being stirred at room temperature for 1 h, the reaction mixture was transferred to a round flask CF1. After removal of solvent, the crude 3-tritylthiopropenal (**9**) was used in the next reaction without further purification.

Solutions of monoethyl malonate (8.13 g, 61.5 mmol, 1.7 equiv., RR1) in DMF (15 mL, RR1) and 3-tritylthiopropenal (**9**) (12.0 g, 36.2 mmol, 1.0 equiv., RR2) in DMF (60 mL, RR2) were transferred into RF1. The resulting mixture was added DMAP (31.0 mg, 0.254 mmol, 0.007 equiv., RR3) in DMF (8.0 mL, RR3) at room temperature. After being stirred at 100 °C for 2 h, the reaction mixture was quenched by addition of 1 M HCl (60 mL, RS4) and diluted with EtOAc (60 mL, RS1) and transferred to SF. After centrifugation, two phases were separated. The separated aqueous phase in SF1 was taken back to RF1 and extracted with EtOAc (60 mL, RS1). The mixture was transferred to SF and centrifuged, and the two phases were separated. After repeating this extraction process twice, the combined organic phase in SF2 was transferred to RF1. The organic phase was washed with 1 M HCl (60 mL, RS4), 5% aqueous NaHCO<sub>3</sub> solution (60 mL, RS5) and 10% aqueous NaCl solution (60 mL, RS6). The resulting organic phase in SF2 was dried by passing through anhydrous Na<sub>2</sub>SO<sub>4</sub> (DT1) and transferred to a round flask CF1. After removal of solvent, the residue was purified by column chromatography on silica gel (10% EtOAc in hexane) to give a mixture of ethyl (*E*)-5-(tritylthio)-2-pentenoate and ethyl (*E*)-5-(tritylthio)-3-pentenoate **10** (12.8 g, 31.8 mmol, 88%) as a white solid. Ethyl (*E*)-5-(tritylthio)-2-pentenoate: <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>): δ 7.42–7.19 (m, 15H), 6.76 (dt, *J* = 15.5, 6.8 Hz, 1H), 5.69 (d, *J* = 16.0 Hz, 1H), 4.15 (q, *J* = 6.8 Hz, 2H), 2.28 (dd, *J* = 8.2, 6.8 Hz, 2H), 2.17 (dt, *J* = 7.3, 6.8 Hz, 2H), 1.26 (t, *J* = 6.8 Hz, 3H). Ethyl (*E*)-5-(tritylthio)-3-pentenoate: <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>): δ 7.42–7.19 (m, 15H), 5.57 (dt, *J* = 15.4, 6.8 Hz, 1H), 5.44 (dt, *J* = 15.5, 7.3 Hz, 1H), 4.10 (q, *J* = 7.2 Hz, 2H), 2.96 (d, *J* = 6.8 Hz, 2H), 2.80 (d, *J* = 7.2 Hz, 2H), 1.24 (t, *J* = 6.8 Hz, 3H).

### (*E*)-5-(Tritylthio)-2-penten-1-ol and (*E*)-5-(tritylthio)-3-penten-1-ol (**11**)

To a solution of ethyl (*E*)-5-(tritylthio)-2-pentenoate and ethyl (*E*)-5-(tritylthio)-3-pentenoate **10** (4.77 g, 11.8 mmol, 1.0 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (35 mL) in RF1 was added DIBAL (0.99 M in toluene, 36.0 mL, 35.5 mmol, 3.0 equiv.) dropwise at –20 °C and allowed to warm to room temperature. After being stirred for 2 h, the reaction mixture was quenched by addition of Rochelle salt (30 mL, RR7) and stirred for 30 min. The reaction mixture was diluted with 1 M HCl (20 mL, RS6) and EtOAc (100 mL, RS1) and transferred to SF. After centrifugation, two phases were separated. The separated aqueous phase in SF1 was taken back to RF1 and extracted with EtOAc (100 mL, RS1). The mixture was transferred to SF and centrifuged, and the two phases were separated. After repeating

this extraction process twice, the combined organic phase in SF2 was transferred to RF1. The organic phase was washed with 1 M HCl (80 mL, RS6), 5% aqueous NaHCO<sub>3</sub> solution (80 mL, RS5) and 10% aqueous NaCl solution (80 mL, RS4). The resulting organic phase in SF2 was dried by passing through anhydrous Na<sub>2</sub>SO<sub>4</sub> (DT1) and transferred to a round flask CF1. After removal of solvent, the residue was purified by column chromatography on silica gel (20% EtOAc in hexane) to give a mixture of (*E*)-5-(tritylthio)-2-penten-1-ol<sup>19</sup> and (*E*)-5-(tritylthio)-3-penten-1-ol **11** (3.21 g, 8.90 mmol, 75%) as a colorless oil. (*E*)-5-(Tritylthio)-2-penten-1-ol: <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>): δ 7.42–7.18 (m, 15H), 5.55–5.43 (m, 2H), 4.03 (d, *J* = 3.9 Hz, 2H), 2.22 (m, 2H), 2.09 (m, 2H). (*E*)-5-(Tritylthio)-3-penten-1-ol: <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>): δ 7.42–7.18 (m, 15H), 5.55–5.43 (m, 2H), 3.58 (t, *J* = 6.3 Hz, 2H), 2.79 (d, *J* = 4.9 Hz, 2H), 2.22 (m, 2H).

### (*E*)-5-(Tritylthio)-2-pentenal (**12**)

Oxalyl chloride (796 μL, 9.28 mmol, 1.5 equiv.) was placed in RF1 and cooled to –78 °C by attaching EtOH-dry ice bath manually to RF1. A solution of DMSO (1.32 mL, 18.6 mmol, 3.0 equiv., RR1) in CH<sub>2</sub>Cl<sub>2</sub> (27 mL) was added to RF1 at –78 °C. After being stirred at the same temperature for 60 min, a mixture of (*E*)-5-(tritylthio)-2-penten-1-ol and (*E*)-5-(tritylthio)-3-penten-1-ol **11** (2.23 g, 6.19 mmol, 1.0 equiv., RR2) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added dropwise to RF1 at –78 °C and stirred at the same temperature for an additional 30 min. Et<sub>3</sub>N (2.58 mL, 18.6 mmol, 3.0 equiv., RR3) in CH<sub>2</sub>Cl<sub>2</sub> (3.0 mL) was added to RF1 at –78 °C and allowed to warm to 0 °C. After being stirred for 1 h, the reaction mixture was quenched by addition of 1 M HCl (60 mL, RS6) and diluted with EtOAc (80 mL, RS1) and transferred to SF. After centrifugation, two phases were separated. The separated aqueous phase in SF1 was taken back to RF1 and extracted with EtOAc (60 mL, RS1). The mixture was transferred to SF and centrifuged, and the two phases were separated. After repeating this extraction process twice, the combined organic phase in SF2 was transferred to RF1. The organic phase was washed with 5% aqueous NaHCO<sub>3</sub> solution (40 mL, RS5) and 10% aqueous NaCl solution (40 mL, RS4). The resulting organic phase in SF2 was dried by passing through anhydrous Na<sub>2</sub>SO<sub>4</sub> (DT1) and transferred to a round flask CF1. After removal of solvent, the residue was purified by column chromatography on silica gel (8% EtOAc in hexane) to give (*E*)-5-(tritylthio)pent-2-enal (**12**)<sup>19,26</sup> (1.23 g, 3.44 mmol, 56%) as a white solid. <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>): δ 9.43 (d, *J* = 7.7 Hz, 1H), 7.43–7.20 (m, 15H), 6.63 (dt, *J* = 15.4, 6.3 Hz, 1H), 5.98 (dd, *J* = 15.4, 7.7 Hz, 1H), 2.37–2.29 (m, 4H); <sup>13</sup>C NMR (67.8 MHz, CDCl<sub>3</sub>): δ 193.6, 155.6, 144.5, 133.6, 129.5, 127.9, 126.7, 67.0, 31.7, 30.0; IR (solid): 3062, 2931, 2830, 2755, 1964, 1686, 1634, 1592, 1486, 1440, 1396, 1317, 1246, 1208, 1181, 1154, 1112, 1081, 1035, 984, 959, 886, 840, 814, 763, 741, 699, 672, 628, 617, 571, 519, 507, 477, 458 cm<sup>-1</sup>. HRMS (ESI-TOF): calcd for [C<sub>24</sub>H<sub>22</sub>O+NH<sub>4</sub>]<sup>+</sup> 376.1735, found 376.1751.

### (*E*)-(S)-3-Hydroxy-1-[(R)-4-isopropyl-5,5-diphenyl-2-oxazolidinone-3-yl]-7-tritylthio-4-hepten-1-one (**13a**)

(*R*)-*N*-Acetyl-4-isopropyl-5,5-diphenyl-2-oxazolidinone (1.96 g, 3.39 mmol, 2.5 equiv.) placed in RF1 was added THF (12 mL) and added *n*-BuLi (1.59 M in hexane, 2.21 mL, 3.52 mmol,

2.6 equiv.) at –78 °C by attaching EtOH-dry ice bath manually to RF1. After being stirred for 1 h at the same temperature, Cp<sub>2</sub>ZrCl<sub>2</sub> (1.07 g, 3.66 mmol, 2.7 equiv., RR2) in THF (14 mL) was added to RF1 and the reaction mixture was allowed to warm to –30 °C. After being stirred for 1 h, the reaction mixture was cooled to –50 °C and added a solution of (*E*)-5-(tritylthio)-2-pentenal (**12**) (486 mg, 1.35 mmol, 1.0 equiv., RR3) in THF (10 mL) at the same temperature and allowed to warm to 0 °C. After being stirred for 1 h at the same temperature, the reaction mixture was transferred to RF2 to be poured into 10% aqueous NH<sub>4</sub>Cl solution (40 mL) placed in RF2 and diluted with EtOAc (40 mL, RS1) and transferred to SF. After centrifugation, two phases were separated. The separated aqueous phase in SF1 was taken back to RF1 and extracted with EtOAc (60 mL, RS1). The mixture was transferred to SF and centrifuged, and the two phases were separated. After repeating this extraction process twice, the combined organic phase in SF2 was transferred to RF1. The organic phase was washed with 5% aqueous NaHCO<sub>3</sub> solution (100 mL, RS4) and 10% aqueous NaCl solution (100 mL, RS6). The resulting organic phase in SF2 was dried by passing through anhydrous Na<sub>2</sub>SO<sub>4</sub> (DT1) and transferred to a round flask CF1. After removal of solvent, the residue was purified by column chromatography on silica gel (20% EtOAc in hexane) to give a mixture of (*E*)-(S)-3-hydroxy-1-[(R)-4-isopropyl-5,5-diphenyl-2-oxazolidinone-3-yl]-7-tritylthio-4-hepten-1-one (**13a**) and its diastereomer **13b** [771 mg, 1.13 mmol, 84%, **13a**:**13b** = 90 : 10 (the ratio was determined by HPLC with photodiode array)] as a colorless oil. The diastereomeric mixture was purified by column chromatography on silica gel (1% EtOAc in toluene) to give pure **13a** (441 mg, 0.647 mmol, isolated yield was 48%) as a colorless oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.47–7.15 (m, 25H), 5.49 (dt, *J* = 15.0, 6.8 Hz, 1H), 5.37 (m, 2H), 4.47 (m, 1H), 3.15 (dd, *J* = 16.9, 2.9 Hz, 1H), 2.82 (dd, *J* = 16.9, 8.2 Hz, 1H), 2.17 (dd, *J* = 7.7, 7.3 Hz, 2H) 2.00 (m, 3H), 0.87 (d, *J* = 6.8 Hz, 3H), 0.74 (d, *J* = 6.8 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 171.8, 152.9, 144.9, 142.0, 137.9, 131.7, 130.1, 129.5, 128.9, 128.7, 128.4, 128.0, 127.8, 126.5, 125.8, 125.4, 89.6, 68.5, 66.5, 64.5, 42.2, 31.3, 29.9, 21.7, 16.3; IR (neat): 3509, 3060, 3030, 2965, 2927, 2853, 1785, 1699, 1596, 1493, 1466, 1449, 1367, 1319, 1211, 1176, 1094, 1035, 1002, 986, 845, 701, 667, 617, 506 cm<sup>-1</sup>; [α]<sub>D</sub><sup>25</sup> = +85 (*c* 0.81, CHCl<sub>3</sub>). HRMS (ESI-TOF): calcd for [C<sub>45</sub>H<sub>45</sub>NO<sub>3</sub>S+H]<sup>+</sup> 680.3198, found 680.3168.

### (*E*)-(S)-3-Hydroxy-7-tritylthio-4-heptenoic acid (**7**)

To a solution of (*E*)-(S)-3-hydroxy-1-[(R)-4-isopropyl-5,5-diphenyl-2-oxazolidinone-3-yl]-7-tritylthio-4-hepten-1-one (**13a**) (320 mg, 0.470 mmol, 1.0 equiv.) in THF (3.0 mL) and MeOH (3.0 mL) was added 1 M aqueous sodium hydroxide (100 μL, 1.00 mmol, 2.1 equiv.) at 0 °C. After being stirred at the same temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was diluted with EtOAc and quenched with 3 M HCl at 0 °C. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (1.5% MeOH in CHCl<sub>3</sub>) to give (*E*)-(S)-3-hydroxy-7-tritylthio-4-heptenoic acid (**7**)<sup>19</sup> (171 mg, 0.431 mmol, 92%) as a white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.51–7.19 (m, 15H), 5.59 (ddd, *J* = 15.5, 6.8, 6.3 Hz, 1H), 5.42 (dd,

$J = 15.5, 6.3$  Hz, 1H), 4.45 (m, 1H), 2.54 (m, 2H), 2.22 (m, 2H), 2.09 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  176.5, 144.9, 131.6, 130.7, 129.6, 127.9, 126.6, 68.5, 66.6, 41.1, 31.4, 31.3; IR (neat): 3417, 3056, 3030, 2925, 1713, 1595, 1489, 1444, 1280, 1183, 1101, 1083, 1034, 1002, 972, 743, 700, 676, 621, 506,  $\text{cm}^{-1}$ ;  $[\alpha]_{\text{D}}^{25} = -5.0$  ( $c$  1.6,  $\text{CH}_2\text{Cl}_2$ ); HRMS (ESI-TOF): calcd for  $[\text{C}_{26}\text{H}_{26}\text{O}_5\text{S}+\text{Na}]^+$  441.1500, found 441.1495.

#### Ethyl (4*R*,5*S*)-4-(*t*-butoxycarbonylamino)-3-oxo-5-methylheptanoate (15)

To a solution of *N*-Boc-D-*allo*-isoleucine (**14**) (3.56 g, 15.4 mmol, 1.0 equiv.) in THF (45 mL) was added carbonyldiimidazole (4.25 g, 26.2 mmol, 1.7 equiv.). After being stirred at room temperature for 1 h, magnesium ethyl malonate (7.50 g, 26.2 mmol, 1.7 equiv.) was added and the resulting mixture was stirred for 27 h. The reaction mixture was poured into saturated aqueous  $\text{NH}_4\text{Cl}$  and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with saturated aqueous  $\text{NaHCO}_3$ , brine, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (10% EtOAc in hexane) to give ethyl (4*R*,5*S*)-4-(*t*-butoxycarbonylamino)-3-oxo-5-methylheptanoate (**15**)<sup>59</sup> (3.54 g, 11.8 mmol, 76%) as a colorless oil.  $^1\text{H}$  NMR (270 MHz,  $\text{CDCl}_3$ ):  $\delta$  5.04 (d,  $J = 8.9$  Hz, 1H), 4.41 (m, 1H), 4.12 (q,  $J = 7.3$  Hz, 2H), 3.46 (s, 2H), 1.92 (m, 1H), 1.37 (s, 9H), 1.21 (t,  $J = 7.3$  Hz, 3H), 0.95–0.77 (m, 2H), 0.90 (t,  $J = 7.3$  Hz, 3H), 0.71 (d,  $J = 6.9$  Hz, 3H);  $^{13}\text{C}$  NMR (67.5 MHz,  $\text{CDCl}_3$ ):  $\delta$  202.7, 167.0, 156.1, 80.1, 62.9, 61.7, 47.1, 36.2, 28.5, 27.0, 14.3, 14.1, 12.1; IR (neat): 3370, 2972, 2936, 2879, 1749, 1712, 1657, 1504, 1459, 1391, 1367, 1316, 1246, 1167, 1097, 1032, 779  $\text{cm}^{-1}$ ;  $[\alpha]_{\text{D}}^{21} = -30$  ( $c$  1.2,  $\text{CHCl}_3$ ); HRMS (ESI-TOF): calcd for  $[\text{C}_{15}\text{H}_{28}\text{NO}_5+\text{H}]^+$  302.1967, found 302.1962.

#### Ethyl (3*S*,4*R*,5*S*)-4-(*t*-butoxycarbonylamino)-3-hydroxy-5-methylheptanoate (16a)

To a solution of  $\beta$ -keto ester **15** (2.21 g, 7.34 mmol, 1.0 equiv.) in MeOH (40 mL) was added  $\text{KBH}_4$  (1.98 g, 36.7 mmol, 5.0 equiv.) at  $-78$  °C. The reaction mixture was allowed to warm to 0 °C slowly and stirred at the same temperature for 1 h. The reaction mixture was quenched with AcOH at 0 °C and concentrated *in vacuo*. The residue was diluted with EtOAc and water. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with saturated aqueous  $\text{NaHCO}_3$ , brine, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (20% EtOAc in hexane) to give a mixture of ethyl (3*S*,4*R*,5*S*)-4-(*t*-butoxycarbonylamino)-3-hydroxy-5-methylheptanoate **16a**<sup>59,60</sup> and its diastereomer **16b**<sup>59,60</sup> [1.88 g, 6.20 mmol, 85%, **16a**:**16b** = 91 : 9 (the ratio was determined by  $^1\text{H}$  NMR peak area)] as a colorless oil. **16a**:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  4.42 (d,  $J = 10.1$  Hz, 1H), 4.18 (q,  $J = 7.3$  Hz, 2H), 3.90 (m, 1H), 3.64 (m, 1H), 3.25 (d,  $J = 4.4$  Hz, 1H), 2.63 (dt,  $J = 16.5, 2.5$  Hz, 1H), 2.51 (dd,  $J = 16.5, 8.9$  Hz, 1H), 1.93 (m, 1H), 1.43 (s, 9H), 1.38–1.20 (m, 2H), 1.23 (t,  $J = 7.3$  Hz, 3H), 0.92 (t,  $J = 7.3$  Hz, 3H), 0.86 (d,  $J = 6.8$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  173.1, 156.0, 79.1, 68.9, 60.5, 56.6, 38.7, 33.8, 28.2, 26.9, 13.9, 13.0, 11.5; IR (neat): 3451, 3372, 2968, 2935, 2878, 1716, 1701, 1522, 1457, 1391, 1367, 1251, 1171, 1072, 989, 864, 775  $\text{cm}^{-1}$ ;  $[\alpha]_{\text{D}}^{24} = -5.2$

( $c$  0.57, MeOH); HRMS (ESI-TOF): calcd for  $[\text{C}_{15}\text{H}_{31}\text{NO}_5+\text{H}]^+$  304.2124, found 304.2124.

#### (3*S*,4*R*,5*S*)-4-(*t*-Butoxycarbonylamino)-3-hydroxy-5-methylheptanoic acid (17a)

To a mixture of **16a** and **16b** (1.88 g, 6.20 mmol, 1.0 equiv.) in THF (15 mL) was added 1 M aqueous LiOH (15.0 mL, 15.0 mmol, 2.4 equiv.) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was quenched with 1 M HCl at 0 °C and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated *in vacuo*. The residue was recrystallized from EtOAc–hexane to give a mixture of (3*S*,4*R*,5*S*)-4-(*t*-butoxycarbonylamino)-3-hydroxy-5-methylheptanoic acid (**17a**)<sup>61,62</sup> and its diastereomer **17b** [1.05 g, 1.05 mmol, 17%, **17a** : **17b** = 50 : 50, (The mixture of **17a** : **17b** was converted to corresponding allyl esters by treating with  $\text{K}_2\text{CO}_3$ , allyl bromide in DMF. The ratio of **17a** and **17b** was determined by  $^1\text{H}$  NMR analysis) as a white solid. The filtrate was concentrated to afford desired **17a** as a major component [colorless oil, 1.16 g, 4.20 mmol, 68%, **17a**:**17b** = 95 : 5 (The mixture of **17a** and **17b** was converted to corresponding benzyl esters by treating with  $\text{K}_2\text{CO}_3$ , benzyl bromide in DMF. The ratio of **17a** and **17b** was determined by HPLC with photodiode array)]. **17a**:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  4.49 (d,  $J = 9.7$  Hz, 1H), 3.95 (m, 1H), 3.62 (m, 1H), 2.69–2.35 (m, 2H), 1.88 (m, 1H), 1.44 (s, 9H), 1.41–1.22 (m, 2H), 0.93 (t,  $J = 7.3$  Hz, 3H), 0.88 (d,  $J = 6.8$  Hz, 3H).  $[\alpha]_{\text{D}}^{21} = -6.5$  ( $c$  0.75,  $\text{CHCl}_3$ ); HRMS (ESI-TOF): calcd for  $[\text{C}_{13}\text{H}_{26}\text{NO}_5+\text{H}]^+$  276.1811, found 276.1814.

#### Allyl (3*S*,4*R*,5*S*)-4-(*t*-butoxycarbonylamino)-3-hydroxy-5-methylheptanoate (4)

To a mixture of **17a** and **17b** (1.16 g, 4.20 mmol, 1.0 equiv.) in DMF (20 mL) was added  $\text{K}_2\text{CO}_3$  (695 mg, 5.03 mmol, 1.2 equiv.) and allyl bromide (532  $\mu\text{L}$ , 6.29 mmol, 1.5 equiv.). After being stirred at room temperature for 1.5 h, the reaction mixture was poured into 1 M HCl at 0 °C and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with saturated aqueous  $\text{NaHCO}_3$ , brine, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (15% EtOAc in hexane) to give allyl (3*S*,4*R*,5*S*)-4-(*t*-butoxycarbonylamino)-3-hydroxy-5-methylheptanoate (**4**) (1.45 g, 4.60 mmol, quant.) as a colorless oil.  $^1\text{H}$  NMR (270 MHz,  $\text{CDCl}_3$ ):  $\delta$  5.87 (m, 1H), 5.28 (d,  $J = 17.9$  Hz, 1H), 5.20 (d,  $J = 10.2$  Hz, 1H), 4.62 (d,  $J = 5.6$  Hz, 2H), 4.41 (d,  $J = 9.9$  Hz, 1H), 3.91 (m, 1H), 3.65 (m, 1H), 2.69–2.47 (m, 2H), 1.91 (m, 1H), 1.44 (s, 9H), 1.36–1.22 (m, 2H), 0.92 (t,  $J = 7.3$  Hz, 3H), 0.86 (d,  $J = 6.9$  Hz, 3H);  $^{13}\text{C}$  NMR (67.5 MHz,  $\text{CDCl}_3$ ):  $\delta$  173.0, 156.2, 131.9, 118.6, 79.5, 69.2, 65.5, 56.7, 38.7, 34.0, 28.4, 27.1, 13.2, 11.7; IR (neat): 3450, 3374, 2967, 1717, 1699, 1512, 1457, 1392, 1367, 1251, 1172, 1074, 989, 931, 584  $\text{cm}^{-1}$ ;  $[\alpha]_{\text{D}}^{26} = -6.4$  ( $c$  2.9,  $\text{CHCl}_3$ ); HRMS (ESI-TOF): calcd for  $[\text{C}_{16}\text{H}_{30}\text{NO}_5+\text{H}]^+$  316.2124, found 316.2126.

#### Allyl (3*S*,4*R*,5*S*)-4-[(*S*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-3-tritylthiopropionylamino]-3-hydroxy-5-methylheptanoate (18)

D-*allo*-Isoleucine derived statine allyl ester **4** (447 mg, 1.42 mmol, 1.0 equiv.) was treated with HCl (4 M in dioxane, 10 mL) at

0 °C. After being stirred at room temperature for 2 h, the reaction mixture was concentrated *in vacuo*. The crude amine was dissolved in DMF (10 mL) and added Fmoc-D-Cys(Trt)-OH (**5**) (912 mg, 1.56 mmol, 1.1 equiv.), HOBt (287 mg, 2.12 mmol, 1.5 equiv.), DIEA (739 µL, 4.25 mmol, 3.0 equiv.) and EDCI·HCl (408 mg, 2.12 mmol, 1.5 equiv.) at 0 °C. After being stirred at room temperature for 2 h, the reaction mixture was quenched with saturated aqueous NH<sub>4</sub>Cl and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with 1 M HCl, saturated aqueous NaHCO<sub>3</sub>, brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (30% EtOAc in hexane) to give allyl (3*S*,4*R*,5*S*)-4-[(*S*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-3-tritylthiopropionylamino]-3-hydroxy-5-methylheptanoate (**18**) (1.02 g, 1.30 mmol, 2 steps 92%) as a colorless oil and a small amount (<7%) of C2 (cysteine) epimer of **18**. <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>): δ 7.74 (m, 2H), 7.54 (t, *J* = 6.9 Hz, 2H), 7.43–7.17 (m, 19H), 5.83 (m, 2H), 5.28 (d, *J* = 16.8 Hz, 1H), 5.20 (d, *J* = 10.6 Hz, 1H), 4.89 (d, *J* = 6.9 Hz, 1H), 4.55 (d, *J* = 4.6 Hz, 2H), 4.40 (m, 2H), 4.20–3.64 (m, 3H), 4.17 (m, 1H), 2.66–2.43 (m, 4H), 1.89 (m, 1H), 1.22–1.06 (m, 2H), 0.85 (t, *J* = 7.9 Hz, 3H), 0.80 (d, *J* = 6.6 Hz, 3H); <sup>13</sup>C NMR (67.5 MHz, CDCl<sub>3</sub>): δ 173.1, 170.4, 156.1, 144.4, 143.6, 141.4, 131.8, 129.6, 128.2, 127.9, 127.2, 127.0, 125.0, 120.1, 118.7, 68.6, 67.5, 67.1, 65.5, 55.1, 54.3, 47.1, 38.4, 33.7, 33.2, 27.1, 13.3, 11.7; IR (neat): 3316, 2961, 1716, 1665, 1527, 1448, 1248, 1179, 1034, 742, 701 cm<sup>-1</sup>; [α]<sub>D</sub><sup>25</sup> = +1.6 (*c* 1.1, CHCl<sub>3</sub>). HRMS (ESI-TOF): calcd for [C<sub>48</sub>H<sub>50</sub>N<sub>2</sub>O<sub>6</sub>S+H]<sup>+</sup> 783.3468, found 783.3467.

**Allyl (3*S*,4*R*,5*S*)-4-[(*S*)-2-(*R*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionylamino]-3-tritylthiopropionylamino]-3-hydroxy-5-methylheptanoate (**19**)**

*N*-Fmoc cysteine derivative **18** (1.01 g, 1.29 mmol, 1.0 equiv.) in CH<sub>2</sub>Cl<sub>2</sub> (5.0 mL) was added Et<sub>2</sub>NH (5.0 mL). After being stirred at room temperature for 2 h, the reaction mixture was concentrated *in vacuo*. The residue was azeotroped with toluene and hexane twice, then dissolved in DMF (10 mL). To this solution was added *N*-Fmoc-D-Ala-OH (**6**) (441 mg, 1.42 mmol, 1.1 equiv.), HOBt (262 mg, 1.94 mmol, 1.5 equiv.) and EDCI·HCl (373 mg, 1.94 mmol, 1.5 equiv.) at room temperature. After being stirred at the same temperature for 2 h, the reaction mixture was quenched with saturated aqueous NH<sub>4</sub>Cl and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with 1 M HCl, saturated aqueous NaHCO<sub>3</sub>, brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (45% EtOAc in hexane) to give allyl (3*S*,4*R*,5*S*)-4-[(*S*)-2-[(*R*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)propionylamino]-3-tritylthiopropionylamino]-3-hydroxy-5-methylheptanoate (**19**) (1.09 g, 1.27 mmol, 2 steps 98%) as a colorless oil. <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>): δ 7.75 (d, *J* = 7.6 Hz, 2H), 7.55 (m, 2H), 7.47–7.12 (m, 19H), 6.45 (d, *J* = 6.9 Hz, 1H), 6.19 (d, *J* = 7.6 Hz, 1H), 5.82 (m, 1H), 5.30–5.15 (m, 3H), 4.54 (d, *J* = 4.6 Hz, 2H), 4.38 (m, 2H), 4.20–3.98 (m, 5H), 2.90–2.39 (m, 4H), 1.92 (m, 1H), 1.27 (m, 3H), 1.23–1.10 (m, 2H), 1.08–0.78 (m, 6H); <sup>13</sup>C NMR (67.5 MHz, CDCl<sub>3</sub>): δ 172.9, 172.2, 170.1, 156.0, 144.3, 143.8, 143.5, 141.2, 131.9, 129.4, 128.0, 127.7, 127.1, 126.8, 124.9, 120.0, 118.2, 68.4, 67.0, 65.3, 55.6, 52.5, 50.7, 47.0, 38.6, 33.9, 32.9, 27.0, 18.6, 13.3, 11.7; IR (neat): 3306, 2964, 1709, 1647,

1530, 1448, 1220, 758, 742, 701 cm<sup>-1</sup>; [α]<sub>D</sub><sup>26</sup> = +2.6 (*c* 3.1, CHCl<sub>3</sub>); HRMS (ESI-TOF): calcd for [C<sub>51</sub>H<sub>56</sub>N<sub>3</sub>O<sub>7</sub>S+H]<sup>+</sup> 854.3839, found 854.3837.

**Allyl (3*S*,4*R*,5*S*)-4-[(*S*)-2-[(*R*)-2-[(*E*)-(*S*)-3-hydroxy-7-tritylthio-4-heptenoylamino]propionylamino]-3-tritylthiopropionylamino]-3-hydroxy-5-methylheptanoate (**3**)**

To a solution of *N*-Fmoc alanine derivative **19** (191 mg, 0.224 mmol, 1.0 equiv.) in CH<sub>3</sub>CN (5 mL) was added Et<sub>2</sub>NH (1 mL). After being stirred at room temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was azeotroped with toluene and hexane twice, then dissolved in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and CH<sub>3</sub>CN (5 mL). To this solution was added (*E*)-(*S*)-3-hydroxy-7-tritylthio-4-heptenoic acid (**7**) (85.6 mg, 0.216 mmol, 1.0 equiv.), DIEA (113 µL, 0.648 mmol, 3.0 equiv.) and PyBOP (170 mg, 0.324 mmol, 1.5 equiv.) and stirred at room temperature for 2 h. The reaction mixture was quenched with saturated aqueous NH<sub>4</sub>Cl and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (1% MeOH in CHCl<sub>3</sub>) to give allyl (3*S*,4*R*,5*S*)-4-[(*S*)-2-[(*R*)-2-[(*E*)-(*S*)-3-hydroxy-7-tritylthio-4-heptenoylamino]propionylamino]-3-tritylthiopropionylamino]-3-hydroxy-5-methylheptanoate (**3**) (169.0 mg, 0.164 mmol, 2 steps 76%) as a colorless oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.43–7.17 (m, 30H), 6.94 (d, *J* = 7.2 Hz, 1H), 6.09 (m, 2H), 5.85 (m, 1H), 5.45 (m, 1H), 5.34–5.18 (m, 3H), 4.55 (d, *J* = 10.6 Hz, 2H), 4.34–4.25 (m, 2H), 4.03–3.93 (m, 3H), 2.74–2.41 (m, 4H), 2.36–2.05 (m, 7H), 1.89 (m, 1H), 1.33 (d, *J* = 7.2 Hz, 3H), 1.15–1.08 (m, 2H), 0.97–0.80 (m, 6H); <sup>13</sup>C NMR (67.5 MHz, CDCl<sub>3</sub>): δ 173.1, 172.2, 171.8, 170.4, 144.9, 144.3, 132.4, 132.0, 130.4, 129.5, 129.1, 128.2, 128.0, 127.1, 126.7, 118.4, 69.9, 68.6, 67.0, 66.7, 65.4, 55.7, 52.8, 51.0, 44.0, 38.4, 34.1, 33.1, 31.3, 29.8, 27.1, 17.5, 13.7, 11.8; IR (neat): 3273, 1736, 1624, 1528, 1444, 1219, 1168, 744, 699. [α]<sub>D</sub><sup>23</sup> = -4.6 (*c* 1.1, CHCl<sub>3</sub>).

**(2*S*,6*R*,9*S*,12*R*,13*S*)-12-(*S*)-*sec*-Butyl-13-hydroxy-6-methyl-2-[(*E*)-4-tritylthiomethyl-2-butenyl]-9-tritylthiomethyl-1-oxa-5,8,11-triaza-cyclopentadecane-4,7,11,15-tetraone (**20**)**

To a solution of *seco*-acid derivative **3** (49.0 mg, 47.0 µmol, 1.0 equiv.) in THF (2.5 mL) was added water (700 µL) and 1 M aqueous LiOH (150 µL, 150 µmol, 3.2 equiv.) at 0 °C. After being stirred at the same temperature for 1.5 h, the reaction mixture was quenched with 1 M HCl at 0 °C and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of MNBA (32.4 mg, 94.1 µmol, 2.0 equiv.) and DMAP (23.0 mg, 188 µmol, 4.0 equiv.) in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) was added dropwise crude acid in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) over 24 h and stirred at room temperature for further 24 h. The reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (1% MeOH in CHCl<sub>3</sub>) to give (2*S*,6*R*,9*S*,12*R*,13*S*)-12-(*S*)-*sec*-butyl-13-hydroxy-6-methyl-2-[(*E*)-4-tritylthiomethyl-2-butenyl]-9-tritylthiomethyl-1-oxa-5,8,11-triaza-cyclopentadecane-4,7,11,15-tetraone (**20**) (28.5 mg, 293 µmol, 2 steps 62%) as a white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):

$\delta$  7.42–7.19 (m, 30H), 6.83 (d,  $J = 7.9$  Hz, 1H), 6.71 (brs, 1H), 5.86 (brs, 1H), 5.66–5.54 (m, 2H), 5.32 (dd,  $J = 15.5, 6.6$  Hz, 1H), 4.25–4.18 (m, 2H), 3.79 (m, 1H), 3.17 (m, 1H), 2.65–2.37 (m, 6H), 2.17 (t,  $J = 7.3$  Hz, 2H), 2.05–1.83 (m, 3H), 1.34 (d,  $J = 6.9$  Hz, 3H), 1.17–1.11 (m, 2H), 0.92–0.78 (m, 6H); IR (solid): 3294, 3059, 2960, 2926, 1734, 1661, 1542, 1444, 1378, 1252, 1179, 742, 700, 622  $\text{cm}^{-1}$ .  $[\alpha]_{\text{D}}^{27} = -23$  ( $c$  0.095,  $\text{CHCl}_3$ ).

### (–)-Spiruchostatin B (2)

To a solution of iodine (15.1 mg, 59.5  $\mu\text{mol}$ , 10 equiv.) in  $\text{CH}_2\text{Cl}_2$  (9 mL) and MeOH (1 mL) was dropwisely added a solution of trityl thiol **20** (5.8 mg, 6.0  $\mu\text{mol}$ , 1.0 equiv.) in  $\text{CH}_2\text{Cl}_2$  (13.5 mL) and MeOH (1.5 mL) at room temperature. After being stirred at room temperature for 3 h, the reaction mixture was quenched with 10% aqueous  $\text{Na}_2\text{S}_2\text{O}_3$  and saturated aqueous  $\text{NaHCO}_3$ . The aqueous layer was extracted with  $\text{CHCl}_3$ . The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (5% MeOH in  $\text{CHCl}_3$ ) to give spiruchostatin B (**2**) (2.4 mg, 4.9  $\mu\text{mol}$ , 82%) as a white solid. The spectral data of **2** were in good accordance with reported values.<sup>6,18</sup>  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.29 (d,  $J = 10.6$  Hz, 1H), 6.69 (d,  $J = 9.7$  Hz, 1H), 6.46 (m, 1H), 5.82 (brs, 1H), 5.64 (d,  $J = 15.5$  Hz, 1H), 5.50 (brs, 1H), 4.95 (dt,  $J = 8.7, 3.9$  Hz, 1H), 4.66 (m, 1H), 4.21 (dq,  $J = 3.9, 7.2$  Hz, 1H), 3.42 (m, 1H), 3.37 (dd,  $J = 7.0, 13.1$  Hz, 1H), 3.20–3.09 (m, 2H), 2.95–2.88 (m, 1H), 2.85–2.72 (m, 3H), 2.73 (d,  $J = 3.9$  Hz, 2H), 2.57 (d,  $J = 13.1$  Hz, 1H), 2.47 (m, 1H), 2.09 (m, 1H), 1.52 (d,  $J = 7.2$  Hz, 3H), 1.26 (m, 2H), 0.93 (d,  $J = 6.8$  Hz, 3H), 0.92 (t,  $J = 6.8$  Hz, 3H);  $^{13}\text{C}$  NMR (67.5 MHz,  $\text{CDCl}_3$ ):  $\delta$  171.9, 171.0, 170.5, 169.1, 133.6, 128.5, 70.5, 68.2, 61.9, 54.2, 52.2, 41.6, 40.7, 40.7, 39.5, 36.3, 33.5, 27.1, 16.7, 15.3, 11.5; IR (neat): 3340, 2967, 2931, 1713, 1662, 1504, 1216, 1164, 757  $\text{cm}^{-1}$ ;  $[\alpha]_{\text{D}}^{25} = -57$  ( $c$  0.23, MeOH), lit.<sup>6</sup>  $[\alpha]_{\text{D}}^{25} = -59$  ( $c$  0.11, MeOH); HRMS (ESI-TOF): calcd for  $[\text{C}_{21}\text{H}_{34}\text{N}_3\text{O}_6\text{S}_2\text{H}]^+$  488.1889, found 488.1889.

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