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The efficient preparation of α-substituted serine scaffolds as the chiral building blocks for the synthesis of SPT inhibitors

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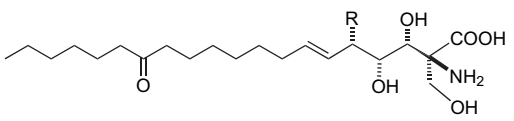
Dedicated to Professor Štefan Toma on the occasion of his 70th birthday

Abstract—An efficient stereoselective synthesis of unusual substituted serine synthons **7**, **10**, **12** is reported. It was shown that highly functionalized furanose **6** has the suitable structure for further synthetic manipulations toward the inhibitors of SPT.

1. Introduction

Serine palmitoyltransferase SPT,[1] a pyridoxal phosphate dependent enzyme, catalyzes the first step in the biosynthesis of sphingolipids that have significant roles as structural cell membrane components and are present essentially in all eucaryotic cells.[2] Inhibitors[1b,3] of SPT include the fungal metabolites myriocin, sphingofungins A–F, mycestericins D–G, viridiofungins, and sulfamysterin (Fig. 1). These naturally occurring compounds with potent and highly selective activity[1c] also have fungicidal and immunosuppressive potential, and thus they are very interesting pharmacological articles. Some of them (e.g., myriocin,[1b,3,4] sphingofungins E and F,[3–5] mycestericins[3]) possess an unusual α-substituted serine framework with contiguous chiral centers as the important structural feature necessary for their biological activity.[6]

From a structural standpoint, the synthesis of the chiral, polar head group (including a tetrasubstituted carbon with nitrogen) of sphingolipid-based compounds such as myriocin and sphingofungins is certainly the most difficult part of the total synthesis. For construction of the quaternary carbon atom part of this hydrophilic polar head, several synthetic approaches have been developed.[3,4,7]



Myriocin (R = H)
Sphingofungin E (R = OH)

Figure 1.

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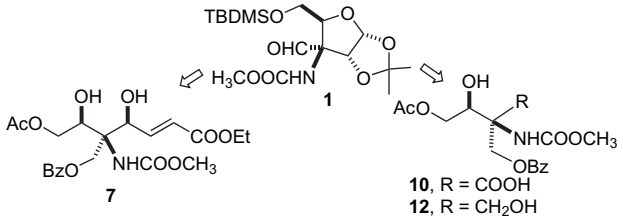
Here we would like to describe the stereoselective synthesis of α-substituted serine synthons as the advanced building blocks for the preparation of SPT inhibitors.

2. Results and discussion

Our analysis depicted in Scheme 1 revealed that protected α-D-xylofuranose scaffold **1** with a tetrasubstituted carbon, provided the functionalized synthetic serine fragments, which after further transformations can be utilized in the total synthesis of SPT inhibitors or other α-substituted amino acid derivatives.

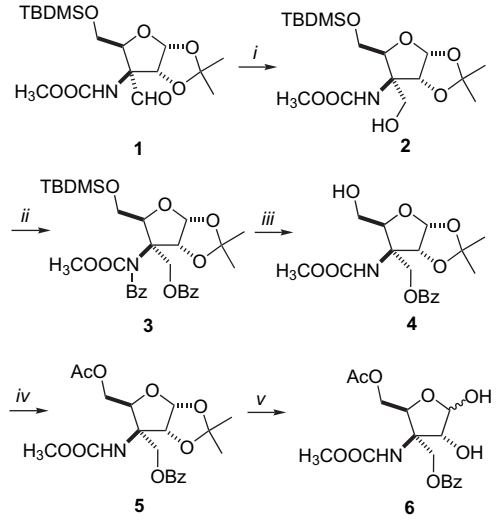


Scheme 1.

We have recently reported the stereoselective synthesis of protected α-D-xylofuranose-3-*C*-carbaldehyde **1**[8] as the precursor of novel furanoid α-substituted α-amino acids possessing the 3*S* configuration on the newly formed quaternary carbon stereocenter.[8] The same stereochemistry was found in inhibitors of SPT having a tetrasubstituted C-2. The same aldehyde **1** was converted into the three advanced precursors: (1) amino-polyol **12**, (2) fully protected α-substituted α-amino acid **10** and (3) (*E*)-α,β-unsaturated ester **7**.

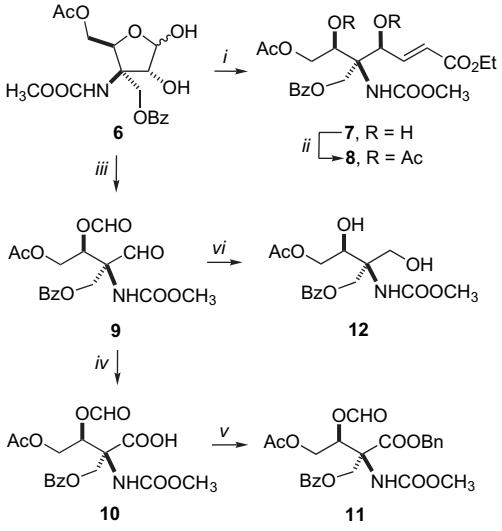
The conversion of **1** into alcohol **2** was achieved using $NaBH_4$ in methanol. The resulting amino alcohol **2** was

treated with benzoyl chloride in pyridine as a solvent to afford the protected derivative **3** in 91% yield (Scheme 2). Desilylation of **3** using tetrabutylammonium fluoride as the global deprotecting agent in THF and in the presence of 4 Å molecular sieve[9] gave the corresponding alcohol **4** in 96% yield. Acetylation of **4** with acetic anhydride in pyridine and in the presence of DMAP afforded the highly functionalized furanose intermediate **5** in 92% yield (Scheme 2).



Scheme 2. Reagents and conditions: (i) $NaBH_4$, MeOH (90%); (ii) BzCl, pyridine (91%); (iii) Bu_4NF, THF, 4 Å sieves (96%); (iv) Ac_2O, pyridine, DMAP, rt (92%); (v) TFA–H_2O, rt (84%).

Acid hydrolysis of **5** removed the acetonide protecting group to provide lactol **6** as the mixture of anomers (α:β=10:7, determined by ^{1}H NMR spectroscopy; the observed coupling constant in the α-anomer $J_{2,1}$=4.5 Hz and in the β-anomer $J_{2,1}$=3.1 Hz), which was then treated with a stabilized ylide ($Ph_3P{=}CHCO_2CH_2CH_3$, CH_2Cl_2) to successfully afford (*E*)-α,β-unsaturated ester **7** exclusively in 85% yield (Scheme 3). Its structure was determined by ^{1}H and ^{13}C NMR spectroscopy (for data see Section 4). The observed coupling constant in **7** ($J_{2,1}$=15.6 Hz) clearly indicated the *trans*-configuration of the double bond. Because of overlap of proton signals in **7** it was not possible to determine all *J* values, therefore the unsaturated ester **7** was characterized in the form of diacetate **8** (Ac_2O, pyridine, DMAP, 89%). The resulting fragment **8** possesses a double bond for the installation of remaining chiral centers by Sharpless dihydroxylation.



Scheme 3. Reagents and conditions: (i) $Ph_3P{=}CHCOOEt$, CH_2Cl_2, benzoic acid, rt (85%); (ii) Ac_2O, pyridine, DMAP, rt (89%); (iii) $NaIO_4$, MeOH–H_2O, rt (78%); (iv) $NaClO_2$, NaH_2PO_4, MeCN–*t*-BuOH–2-methyl-2-butene (68%); (v) BnBr, K_2CO_3, DMF, rt (80%); (vi) $NaBH_4$, MeOH (70%).

Oxidative cleavage[10] of the furanose **6** with sodium metaperiodate in CH_3OH–H_2O provided aldehyde **9**, which was used immediately in the next reaction step without purification (Scheme 3). Its structure was confirmed by ^{1}H NMR spectroscopy (for data see Section 4). The subsequent oxidation of the aldehyde **9** with $NaClO_2$ in CH_3CN–*tert*-butyl alcohol–2-methyl-2-butene afforded the desired protected α-substituted α-amino acid **10** (68%), possessing the correct stereochemistry on the quaternary carbon and proper functionalities for the further transformations (Scheme 3). The resulting acid **10** was converted to its benzyl ester **11** by treatment with benzyl bromide and K_2CO_3 in DMF[11] (80%, Scheme 3) to avoid problems connected with its possible instability. The corresponding ester **11** gave very good resolved NMR spectra. The reduction of the aldehyde **9** with $NaBH_4$ in methanol afforded the partially protected aminopolyol **12** (Scheme 3) and can also be useful for the construction of analogues of myriocin such as FTY 720. This compound does not inhibit SPT but is reported to show a remarkable immunosuppressive activity.[2d,12]

3. Conclusion

In summary, we have found an efficient route to advanced building blocks **7**, **10**, **12**. We have shown that these intermediates possess suitable structure for installation of the remaining functionalities, which are necessary for the construction of SPT inhibitors such as myriocin, sphingofungins, and others. Further studies toward the total synthesis of these inhibitors are in progress in our laboratory.

4. Experimental

4.1. General experimental

All commercially available reagents were used without purification and solvents were dried according to standard procedures. Product purification was carried out using flash chromatography (Merck silica gel 60 (0.040–0.063 mm)). TLC was run on Merck silica gel 60 F_{254} analytical plates; detection was carried out with either UV, iodine, spraying with a solution of phosphomolybdic acid, or with a solution of $KMnO_4$, with subsequent heating. NMR spectra were recorded at room temperature on a Varian Mercury Plus 400 FT NMR spectrometer (400.13 MHz for ^{1}H and 100.6 MHz for ^{13}C) using $CDCl_3$ as the solvent and TMS as internal reference. For ^{1}H δ are given in parts per million relative to TMS (0 ppm) and for ^{13}C relative to $CDCl_3$ (δ=77.0). ^{13}C NMR multiplicities were determined by using a DEPT pulse sequence. Infrared spectra were recorded on a Perkin–Elmer 599 IR spectrometer. Optical rotations

were measured at 20±2 °C on a P3002 Krüss polarimeter and reported as follows: $[\alpha]_D$ (*c* in grams per 100 mL, solvent). The melting points were determined on the Kofler block and are uncorrected.

4.1.1. 5-*O*-(*tert*-Butyldimethylsilyl)-3-deoxy-3-*C*-hydroxy-methyl-1,2-*O*-isopropylidene-3-methoxycarbonylamino-α-D-xylofuranose (2). A solution of known aldehyde **1**[8] (3.49 g, 8.96 mmol) in methanol (70 mL) was treated with $NaBH_4$ (0.37 g, 9.85 mmol) at 0 °C. The reaction mixture was stirred for 10 min at 0 °C and then at room temperature for 20 min, before neutralization with Amberlite IR-120 H^+. The resin was removed by filtration and the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (60 mL) and washed with brine (15 mL). The organic layer was dried (Na_2SO_4), solvent removed under reduced pressure and the resulting residue was subjected to flash column chromatography on silica gel (hexane–ethyl acetate, 7:1) to afford 3.42 g (90%) of alcohol **2**; $[\alpha]_D^{20}$ +20.8 (*c* 0.39, CH_3OH). ^1H NMR (400 MHz, $CDCl_3$): δ 0.11 (3H, s, CH_3), 0.12 (3H, s, CH_3), 0.91 (9H, s, 3×CH_3), 1.33 (3H, br s, CH_3), 1.53 (3H, br s, CH_3), 3.39–3.47 (1H, m, OH), 3.65 (3H, s, CH_3O), 3.97–4.09 (5H, m, H_4, 2×H_5, 2×H_6), 5.07 (1H, d, $J_{2,1}$=3.4 Hz, H_2), 5.92 (1H, d, $J_{2,1}$=3.4 Hz, H_1), 6.98 (1H, br s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.7 (CH_3), −5.6 (CH_3), 18.2 (C), 25.7 (3×CH_3), 26.1 (CH_3), 26.6 (CH_3), 52.1 (CH_3), 60.9 (CH_2), 62.9 (CH_2), 67.6 (C), 79.6 (CH), 83.7 (CH), 104.4 (CH), 112.3 (C), 156.8 (C). Anal. Calcd for $C_{17}H_{33}NO_7Si$: C, 52.15; H, 8.50; N, 3.58. Found: C, 52.05; H, 8.60; N, 3.53.

4.1.2. 5-*O*-(*tert*-Butyldimethylsilyl)-3-*C*-benzoyloxy-methyl-3-deoxy-1,2-*O*-isopropylidene-3-(methoxycarbonyl)benz-amido-α-D-xylofuranose (3). To a solution of alcohol **2** (3.01 g, 7.69 mmol) in pyridine (22.60 mL) was added benzoyl chloride (1.94 mL, 16.91 mmol) at 0 °C. The reaction mixture was stirred for 10 min at 0 °C and then for 40 min at room temperature. After stirring, the reaction mixture was poured into ice water (50 mL) and extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (hexane–ethyl acetate, 13:1) to afford 4.20 g (91%) of compound **3** as a colorless oil; $[\alpha]_D^{20}$ +36.6 (*c* 0.61, $CHCl_3$); ν_{max} (liquid film) 3340, 2973, 1717, 1707, 1690, 1257, 1120, 1060, 833, 713 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 0.07 (3H, s, CH_3), 0.11 (3H, s, CH_3), 0.92 (9H, s, 3×CH_3), 1.33 (3H, br s, CH_3), 1.52 (3H, br s, CH_3), 3.62 (3H, s, CH_3O), 4.02 (1H, dd, $J_{5,4}$= 2.4, 1.5 Hz, H_4), 4.16 (1H, dd, $J_{5,5}$=12.4 Hz, $J_{5,4}$=2.4 Hz, H_5), 4.21 (1H, dd, $J_{5,5}$=12.4 Hz, $J_{5,4}$=1.5 Hz, H_5), 4.54 (1H, d, $J_{6,6}$=11.8 Hz, H_6), 5.17 (1H, d, $J_{2,1}$=3.7 Hz, H_2), 5.26 (1H, d, $J_{6,6}$=11.8 Hz, H_6), 5.92 (1H, d, $J_{2,1}$=3.7 Hz, H_1), 7.41–7.50 (4H, m, Ph), 7.55–7.64 (3H, m, Ph), 8.03–8.06 (2H, m, Ph), 8.10–8.13 (1H, m, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.8 (CH_3), −5.6 (CH_3), 18.1 (C), 25.6 (3×CH_3), 26.1 (CH_3), 26.7 (CH_3), 51.8 (CH_3), 62.3 (CH_2), 62.7 (CH_2), 66.4 (C), 79.9 (CH), 83.1 (CH), 104.1 (CH), 112.4 (C), 128.4 (4×CH), 129.3 (C), 129.6 (2×CH), 129.9 (C), 130.2 (2×CH), 133.1 (CH), 133.7 (CH), 156.1 (C), 166.0 (C), 171.6 (C). Anal. Calcd for $C_{31}H_{41}NO_9Si$: C, 62.08; H, 6.89; N, 2.34. Found: C, 62.15; H, 6.83; N, 2.30.

4.1.3. 3-*C*-Benzoyloxymethyl-3-deoxy-1,2-*O*-isopropylidene-3-methoxycarbonylamino-α-D-xylofuranose (4). To a solution of compound **3** (4.16 g, 6.94 mmol) in dry tetrahydrofuran (69 mL) was added activated 4 Å powdered molecular sieve (1.28 g). The suspension was treated with a 1 M solution of Bu_4NF in THF (6.90 mL, 6.94 mmol) at 0 °C. The reaction mixture was stirred for 10 min at 0 °C and then for 35 min at room temperature. The solid was removed by filtration and solvent evaporated under reduced pressure. The residue was dissolved in ethyl acetate (70 mL) and washed with water (80 mL). The water layer was extracted with further portions of ethyl acetate (2×40 mL). The combined organic layers were dried (Na_2SO_4) and the solvent was evaporated under reduced pressure. The residue was purified by chromatography on silica gel (hexane–ethyl acetate, 2:1) to afford 2.54 g (96%) of compound **4** as a colorless oil; $[\alpha]_D^{20}$ +44.7 (*c* 0.27, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 1.34 (3H, br s, CH_3), 1.54 (3H, br s, CH_3), 2.44–2.51 (1H, m, OH), 3.65 (3H, s, CH_3O), 4.10–4.21 (3H, m, H_4, 2×H_5), 4.60 (1H, d, $J_{6,6}$=11.9 Hz, H_6), 5.15 (1H, d, $J_{2,1}$=3.6 Hz, H_2), 5.26 (1H, d, $J_{6,6}$=11.9 Hz, H_6), 5.95 (1H, d, $J_{2,1}$=3.6 Hz, H_1), 7.16 (1H, br s, NH), 7.44–7.49 (2H, m, Ph), 7.56–7.61 (1H, m, Ph), 8.01–8.05 (2H, m, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.2 (CH_3), 26.8 (CH_3), 52.0 (CH_3), 61.0 (CH_2), 62.8 (CH_2), 66.3 (C), 80.4 (CH), 83.5 (CH), 104.3 (CH), 112.9 (C), 128.6 (2×CH), 129.6 (2×CH), 129.7 (C), 133.2 (CH), 156.0 (C), 166.0 (C). Anal. Calcd for $C_{18}H_{23}NO_8$: C, 56.69; H, 6.08; N, 3.67. Found: C, 56.59; H, 6.15; N, 3.70.

4.1.4. 5-*O*-Acetyl-3-*C*-benzoyloxymethyl-3-deoxy-1,2-*O*-isopropylidene-3-methoxycarbonylamino-α-D-xylofuranose (5). To a solution of derivate **4** (2.54 g, 6.66 mmol) in pyridine (52 mL) were added DMAP (81.3 mg, 0.67 mmol) and acetic anhydride (0.96 mL, 9.99 mmol). Stirring was continued for 30 min at room temperature. Then solvent was removed, the residue was poured into ice water (70 mL) and extracted with CH_2Cl_2 (2×70 mL). The combined organic layers were dried (Na_2SO_4) and solvent was evaporated under reduced pressure. The residue was purified by chromatography on silica gel (hexane–ethyl acetate, 2:1) to afford 2.61 g (92%) of compound **5** as white crystals; mp 152–154 °C; $[\alpha]_D^{20}$ +53.1 (*c* 0.27, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 1.34 (3H, br s, CH_3), 1.55 (3H, br s, CH_3), 2.09 (3H, s, CH_3CO), 3.66 (3H, s, CH_3O), 4.34–4.39 (2H, m, H_4, H_5), 4.59 (1H, d, $J_{6,6}$=12.0 Hz, H_6), 4.63 (1H, dd, $J_{5,5}$=14.7 Hz, $J_{5,4}$=5.7 Hz, H_5), 5.10 (1H, d, $J_{2,1}$= 3.7 Hz, H_2), 5.17 (1H, d, $J_{6,6}$=12.0 Hz, H_6), 5.66 (1H, br s, NH), 5.98 (1H, d, $J_{2,1}$=3.7 Hz, H_1), 7.44–7.49 (2H, m, Ph), 7.57–7.61 (1H, m, Ph), 7.97–8.01 (2H, m, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.7 (CH_3), 26.2 (CH_3), 26.7 (CH_3), 52.3 (CH_3), 62.3 (CH_2), 62.9 (CH_2), 66.1 (C), 79.2 (CH), 83.6 (CH), 104.5 (CH), 112.8 (C), 128.6 (2×CH), 129.5 (C), 129.6 (2×CH), 133.3 (CH), 155.6 (C), 166.0 (C), 170.1 (C). Anal. Calcd for $C_{20}H_{25}NO_9$: C, 56.73; H, 5.95; N, 3.31. Found: C, 56.70; H, 5.98; N, 3.27.

4.1.5. 5-*O*-Acetyl-3-*C*-benzoyloxymethyl-3-deoxy-3-methoxycarbonylamino-D-xylofuranose (6). The compound **5** (0.40 g, 0.95 mmol) was treated with a mixture of TFA–H_2O (8 mL, 8:2). The resulting solution was left under stirring for 1.5 h at room temperature. To the mixture was

added brine (20 mL) and the solution was extracted with ethyl acetate (2×25 mL). The combined organic layers were dried (Na_2SO_4) and solvent was evaporated under reduced pressure. Chromatography of residue on silica gel (hexane–ethyl acetate, 1:1) afforded 0.31 g (84%) of lactol **6** as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.01 (3H, s, $CH_{3\alpha}CO$), 2.10 (3H, s, $CH_{3\beta}CO$), 3.68 (3H, s, $CH_{3\alpha}O$), 3.69 (3H, s, $CH_{3\beta}O$), 4.31–4.36 (2H, m, $H_{4\alpha}$, $H_{4\beta}$), 4.37–4.46 (4H, m, $H_{2\alpha}$, $H_{2\beta}$, $H_{6\alpha}$, $H_{6\beta}$), 4.56–4.61 (4H, m, 2×$H_{5\alpha}$, 2×$H_{5\beta}$), 4.87 (1H, d, $J_{6,6}$=11.8 Hz, $H_{6\beta}$), 5.00 (1H, d, $J_{6,6}$=11.8 Hz, $H_{6\alpha}$), 5.41 (1H, d, $J_{2,1}$=3.1 Hz, $H_{1\beta}$), 5.57 (1H, d, $J_{2,1}$=4.5 Hz, $H_{1\alpha}$), 5.75 (1H, s, NH_α), 5.82 (1H, s, NH_β), 7.43–7.49 (4H, m, $Ph_{\alpha,\beta}$), 7.56–7.62 (2H, m, $Ph_{\alpha,\beta}$), 7.98–8.03 (4H, m, $Ph_{\alpha,\beta}$).

4.1.6. Ethyl (*E*,4*S*,5*R*,6*S*)-7-*O*-acetyl-5-benzoyloxymethyl-4,6-dihydroxy-5-(methoxycarbonylamino)hept-2-enoate (7). To a solution of **6** (32 mg, 0.083 mmol) in dry CH_2Cl_2 (0.80 mL) were added $Ph_3P{=}CHCO_2Et$ (87 mg, 0.25 mmol) and benzoic acid (1 mg, 0.0083 mmol). The reaction mixture was stirred for 22 h at room temperature. Removal of the solvent gave a residue, which was purified by chromatography on silica gel (hexane–ethyl acetate, 1:2) to afford 37.6 mg (85%) of compound **7** as a white solid; mp 115–116 °C; $[\alpha]_D^{20}$ +32.3 (*c* 0.26, $CHCl_3$); ν_{max} (liquid film) 1720, 1713, 1703, 1693, 1233, 1093, 727 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.22 (3H, t, *J*=7.2 Hz, CH_3), 2.07 (3H, s, CH_3CO), 3.66 (3H, s, CH_3O), 4.11 (2H, q, *J*=7.2 Hz, CH_2O), 4.15–4.19 (1H, m, H_4), 4.25 (1H, dd, $J_{5,5}$=11.5 Hz, $J_{5,4}$=7.4 Hz, H_5), 4.50 (1H, dd, $J_{5,5}$=11.5 Hz, $J_{5,4}$=2.0 Hz, H_5), 4.56 (1H, d, $J_{6,6}$=12.0 Hz, H_6), 4.63 (1H, d, $J_{6,6}$=12.0 Hz, H_6), 5.00–5.05 (1H, m, H_3), 5.80 (1H, br s, NH), 6.21 (1H, dd, $J_{2,1}$=15.6 Hz, $J_{3,1}$=1.7 Hz, H_1), 7.20 (1H, dd, $J_{2,1}$=15.6 Hz, $J_{3,2}$=4.6 Hz, H_2), 7.43–7.48 (2H, m, Ph), 7.56–7.61 (1H, m, Ph), 7.97–8.01 (2H, m, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (CH_3), 20.9 (CH_3), 52.8 (CH_3), 60.5 (CH_2), 63.3 (C), 63.8 (CH_2), 66.0 (CH_2), 71.3 (CH), 71.4 (CH), 122.8 (CH), 128.5 (2×CH), 128.9 (C), 129.7 (2×CH), 133.6 (CH), 145.0 (CH), 157.6 (C), 166.1 (C), 166.2 (C), 171.4 (C). Anal. Calcd for $C_{21}H_{27}NO_{10}$: C, 55.62; H, 6.00; N, 3.09. Found: C, 55.70; H, 5.78; N, 3.17.

4.1.7. Ethyl (*E*,4*S*,5*R*,6*S*)-4,6,7-tri-*O*-acetyl-5-benzoyloxymethyl-5-(methoxycarbonylamino)hept-2-enoate (8). To a solution of **7** (20 mg, 0.044 mmol) in pyridine (0.018 mL) were added DMAP (1 mg, 0.008 mmol) and acetic anhydride (10 μL, 0.106 mmol) at room temperature. The reaction mixture was stirred overnight. Removal of solvent gave a residue, which was purified by chromatography on silica gel (hexane–ethyl acetate, 2:1) to afford 21 mg (89%) of compound **8** as a colorless oil; $[\alpha]_D^{20}$ +42.3 (*c* 0.135, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.23 (3H, t, *J*=7.1 Hz, CH_3), 2.01 (3H, s, CH_3CO), 2.06 (3H, s, CH_3CO), 2.12 (3H, s, CH_3CO), 3.66 (3H, s, CH_3O), 4.13 (2H, q, *J*=7.1 Hz, CH_2O), 4.17 (1H, dd, $J_{5,5}$=12.3 Hz, $J_{5,4}$=6.7 Hz, H_5), 4.58 (1H, dd, $J_{5,5}$=12.3 Hz, $J_{5,4}$=3.3 Hz, H_5), 4.83 (2H, m, 2×H_6), 5.39 (1H, br s, NH), 5.64 (1H, dd, $J_{5,4}$=6.7, 3.3 Hz, H_4), 5.88 (1H, dd, $J_{2,1}$=15.7 Hz, $J_{3,2}$=1.5 Hz, H_1), 6.14 (1H, m, H_3), 6.99 (1H, dd, $J_{2,1}$=15.7 Hz, $J_{3,2}$=5.2 Hz, H_2), 7.43–7.49 (2H, m, Ph), 7.56–7.61 (1H, m, Ph), 7.98–8.01 (2H, m, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (CH_3), 20.6 (CH_3), 20.8 (CH_3), 20.8 (CH_3), 52.5 (CH_3), 60.8 (CH_2), 61.5 (CH_2), 62.4 (CH_2), 63.5 (C), 70.6 (CH), 72.4 (CH), 123.3 (CH), 128.6 (2×CH), 129.1 (C), 129.6 (2×CH), 133.5 (CH), 140.2 (CH), 155.4 (C), 165.3 (2×C), 169.0 (C), 169.4 (C), 170.3 (C). Anal. Calcd for $C_{25}H_{31}NO_{12}$: C, 55.86; H, 5.81; N, 2.61. Found: C, 55.94; H, 5.84; N, 3.02.

4.1.8. (2*S*,3*S*)-4-*O*-Acetyl-2-benzoyloxymethyl-3-*O*-formyl-2-(methoxycarbonylamino)butanal (9). To a solution of the diol **6** (0.21 g, 0.55 mmol) in methanol (0.88 mL) was added an aqueous solution of sodium metaperiodate (0.14 g, 0.65 mmol) in water (0.88 mL). The mixture was stirred at room temperature for 1.5 h and then diluted with CH_2Cl_2. The solid part was filtered and solvent was removed under reduced pressure to give 0.164 g (78%) of aldehyde **9** as a colorless oil, which was used immediately in the next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.00 (3H, s, CH_3CO), 3.71 (3H, s, CH_3O), 4.30 (1H, dd, $J_{4,4}$=12.9 Hz, $J_{4,3}$=2.6 Hz, H_4), 4.43 (1H, dd, $J_{4,4}$=12.9 Hz, $J_{4,3}$=1.9 Hz, H_4), 4.89 (1H, d, $J_{5,5}$=11.6 Hz, H_5), 5.05 (1H, d, $J_{5,5}$=11.6 Hz, H_5), 6.02 (1H, m, H_3), 6.16 (1H, br s, NH), 7.41–7.47 (2H, m, Ph), 7.56–7.61 (1H, m, Ph), 7.91–7.94 (2H, m, Ph), 8.20 (1H, s, OCHO), 9.80 (1H, s, CHO). Anal. Calcd for $C_{17}H_{19}NO_9$: C, 53.54; H, 5.02; N, 3.67. Found: C, 53.28; H, 5.23; N, 3.49.

4.1.9. (2*S*,3*S*)-4-*O*-Acetyl-2-benzoyloxymethyl-3-*O*-formyl-2-(methoxycarbonylamino)butanoic acid (10). A solution of $NaClO_2$ (0.359 g, 3.97 mmol) and NaH_2PO_4 (0.447 g, 2.86 mmol) in water (2.30 mL) was added to the solution of aldehyde **9** (0.164 g, 0.43 mmol) in acetonitrile–*t*-BuOH–2-methyl-2-butene (4:4:1, 9.60 mL) at 0 °C over 5 min and then stirred at the same temperature for 3 h. After evaporation of the solvent, the crude material was poured into brine (10 mL) and extracted with ethyl acetate (5×10 mL). The extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was chromatographed on silica gel (CH_2Cl_2–methanol, 95:5) to afford 0.116 g (68%) of carboxylic acid **10** as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.01 (3H, s, CH_3CO), 3.61 (3H, s, CH_3O), 4.21 (1H, dd, $J_{4,4}$=12.4 Hz, $J_{4,3}$=7.8 Hz, H_4), 4.67–4.73 (1H, m, H_4), 4.87 (1H, d, $J_{5,5}$=11.4 Hz, H_5), 4.95 (1H, d, $J_{5,5}$=11.4 Hz, H_5), 5.90 (1H, m, H_3), 6.29 (1H, br s, NH), 7.37–7.43 (2H, m, Ph), 7.51–7.56 (1H, m, Ph), 7.93–7.97 (2H, m, Ph), 8.10 (1H, s, OCHO). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.7 (CH_3), 52.6 (CH_3), 62.6 (C), 62.8 (CH_2), 63.5 (CH_2), 70.9 (CH), 128.4 (2×CH), 129.4 (C), 129.7 (2×CH), 133.3 (CH), 156.2 (C), 160.2 (C), 166.0 (C), 171.4 (C), 172.1 (C). Anal. Calcd for $C_{17}H_{19}NO_{10}$: C, 51.39; H, 4.82; N, 3.53. Found: C, 51.60; H, 4.68; N, 3.27.

4.1.10. Benzyl (2*S*,3*S*)-4-*O*-acetyl-2-benzoyloxymethyl-3-*O*-formyl-2-(methoxycarbonylamino)butanoate (11). K_2CO_3 (51 mg, 0.37 mmol) and benzyl bromide (64 μL, 0.54 mmol) were added to a solution of **10** (0.116 g, 0.29 mmol) in DMF (4.20 mL). The reaction mixture was stirred at room temperature for 1 h, then diluted with water (12 mL) and extracted with Et_2O (2×12 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by chromatography (hexane–ethyl acetate, 3:1) to give 0.11 g (80%) of ester **11** as a colorless oil; $[\alpha]_D^{20}$ +52.0 (*c* 0.35, $CHCl_3$);

ν_{max} (liquid film) 3407, 3010, 1740–1693 (br), 1673, 1237, 713 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 2.01 (3H, s, CH_3CO), 3.66 (3H, s, CH_3O), 4.15 (1H, dd, $J_{4,4}$=12.5 Hz, $J_{4,3}$=7.0 Hz, H_4), 4.62 (1H, dd, $J_{4,4}$=12.5 Hz, $J_{4,3}$=3.0 Hz, H_4), 4.82 (1H, d, $J_{5,5}$=11.3 Hz, H_5), 5.13 (1H, d, $J_{5,5}$=11.3 Hz, H_5), 5.20 (1H, d, $J_{6,6}$=12.0 Hz, H_6), 5.26 (1H, d, $J_{6,6}$=12.0 Hz, H_6), 5.93 (1H, dd, $J_{4,3}$=7.0, 3.0 Hz, H_3), 6.02 (1H, br s, NH), 7.22–7.26 (3H, m, Ph_{Bn}), 7.29–7.33 (2H, m, Ph_{Bn}), 7.37–7.43 (2H, m, Ph_{Bz}), 7.53–7.58 (1H, m, Ph_{Bz}), 7.83–7.87 (2H, m, Ph_{Bz}), 8.00 (1H, s, OCHO). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.6 (CH_3), 52.5 (CH_3), 62.1 (CH_2), 62.5 (CH_2), 63.7 (C), 68.7 (CH_2), 70.3 (CH), 128.4 (2×CH), 128.6 (4×CH), 128.7 (CH), 129.2 (C), 129.6 (2×CH), 133.3 (CH), 134.3 (C), 155.2 (C), 159.1 (C), 165.4 (C), 168.7(C), 170.5 (C). Anal. Calcd for $C_{24}H_{25}NO_{10}$: C, 59.13; H, 5.17; N, 2.87. Found: C, 59.00; H, 5.58; N, 2.47.

4.1.11. (2*S*,3*S*)-4-*O*-Acetyl-2-benzoyloxymethyl-2-(methoxycarbonylamino)-1,3-butanediol (12). To a solution of crude aldehyde **9** (1.12 g, 2.94 mmol) in methanol (20 mL) was added sodium borohydride (167 mg, 4.40 mmol) at 0 °C. The reaction mixture was stirred for 10 min at 0 °C and then at room temperature for 2 h. The solution was neutralized by addition of Amberlite IR-120 H^+. The resin was removed by filtration and filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (hexane–ethyl acetate, 1:1) and afforded 0.73 g (70%) of alcohol **12** as a colorless oil; $[\alpha]_D$ +21.7 (*c* 0.28, $CHCl_3$); ν_{max} (liquid film) 3420, 3017, 1720, 1707, 1697, 1240, 1087, 713 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 2.08 (3H, s, CH_3CO), 3.66 (3H, s, CH_3O), 3.83 (1H, d, $J_{1,1}$=11.9 Hz, H_1), 4.10 (1H, d, $J_{1,1}$=11.9 Hz, H_1), 4.12 (1H, dd, $J_{4,3}$=7.5, 2.7 Hz, H_3), 4.18 (1H, dd, $J_{4,4}$=11.6 Hz, $J_{4,3}$=7.5 Hz, H_4), 4.41 (1H, dd, $J_{4,4}$=11.6 Hz, $J_{4,3}$=2.7 Hz, H_4), 4.55 (1H, d, $J_{5,5}$=11.7 Hz, H_5), 4.63 (1H, d, $J_{5,5}$=11.7 Hz, H_5), 5.58 (1H, br s, NH), 7.43–7.48 (2H, m, Ph), 7.56–7.61 (1H, m, Ph), 8.00–8.04 (2H, m, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.9 (CH_3), 52.7 (CH_3), 61.2 (C), 62.1 (CH_2), 64.0 (CH_2), 65.6 (CH_2), 71.4 (CH), 128.6 (2×CH), 129.1 (C), 129.7 (2×CH), 133.6 (CH), 157.4 (C), 166.6 (C), 171.2 (C). Anal. Calcd for $C_{16}H_{21}NO_8$: C, 54.08; H, 5.96; N, 3.94. Found: C, 54.44; H, 5.68; N, 4.18.

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