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First synthesis of polyoxin M

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Abstract—Chiral enolate derived from (4*R*)-4-*tert*-butyldiphenylsilyloxymethyl-4-butanolide **10** with lithium hexamethyldisilyazide (LiHMDS) was treated with trisyl azide, followed by addition of TMSCl to give (2*S*,4*R*)-2-azido-4-[(*tert*-butyldiphenylsilyloxy)methyl]-4-butanolide **8** (53%), from which the first total synthesis of polyoxin M (**1**) was achieved in overall 3.2% yield (13 steps) from D-glutamic acid. Moreover, the synthesis of the reported synthetic intermediate (2*S*,4*R*)-4-hydroxyornithine congener **6** for biphenomycins A and B was also achieved in overall 4.1% yield (12 steps) from D-glutamic acid.

1. Introduction

Polyoxin M (**1**) is a class of peptidyl nucleoside antibiotics isolated from the culture broths of *Streptomyces cacao*i var *asoensis*.[1] All members of the polyoxin family possess 1-(5′-amino-5′-deoxy-β-D-allofuranuronosyl)pyrimidines such as thymine polyoxin C and uracil polyoxin C (**3**) as a basic component. The biological activity of the polyoxins is very characteristic because of their specific action against phytopathogenic fungi and the human fungal pathogen (e.g., *Candida albicans*), and lack of activity against other microorganisms, plants, fish, and mammals.[1] The site of action of the polyoxins was reported to be responsible for cell wall chitin biosynthesis.[2] In the preceding paper, we reported a short-path synthesis of methyl (methyl-2,3-*O*-isopropylidene-α-L-talofuranoside)uronate from methyl 2,3-*O*-isopropylidene-dialdo-D-ribofuranoside and its application to the total syntheses of thymine polyoxin C and uracil polyoxin C (**3**).[3] We also reported a convenient synthesis of the *N*-protected L-carbamoyl-polyoxamic acid derivative and its application to the total syntheses of polyoxins J,[4a] L,[4a] B,[4b] and D.[4b] Retrosynthetically, the synthesis of **1** can be achieved by amide formation between the left-half α-amino acid congener (**2**) and the right-half **3**. On the other hand, biphenomycins A (**4**) and B (**5**) were isolated from the cultured broth of *Streptomyces griseorubiginosus* No. 43708. These antibiotics are active in vitro and in vivo against bacteria and are especially potent against Gram-positive bacteria.[5] For the synthesis of **4** and **5**, (2*S*,4*R*)-4-hydroxyornithine congener (**6**), protected in three different ways, is thought to be an important intermediate.[6] We now describe the synthesis of polyoxin M (**1**) and (2*S*,4*R*)-4-hydroxyornithine congener (**6**) based on the electrophilic azide transfer to chiral enolate (Scheme 1).

2. Results and discussion

2.1. Total synthesis of polyoxin M (1)

For the synthesis of **2** or **6**, (2*S*,4*R*)-2-azido-4-protected hydroxymethyl-4-butanolide congener **7** or **8** is thought to be an important intermediate. These azide compounds, **7** or **8**, could be obtained by the diastereoselective azide transfer to chiral enolate derived from the (4*R*)-protected hydroxymethyl-4-butanolide **9** or **10**. By applying the reported method,[7] the synthesis of (4*R*)-**9** or (4*R*)-**10** was achieved by tritylation or silylation of (4*R*)-γ-hydroxymethyl-γ-butyrolactone derived from D-glutamic acid. Concerning the diastereoselective introduction of a substituent at the 2-position in (4*S*)-**9** or (4*S*)-**10**, three examples were reported as shown in Scheme 2. The first example is the efficient enantioselective construction of quarternary carbon centers by the sequential dialkylation of (4*S*)-**9**[8] and the second one is the diastereoselective introduction (2α:2β=7:1) of a hydroxyl group at the 2-position in (4*S*)-**10**.[9] The third one is the diastereoselective introduction (2β:2α=93:7) of a 1,2-bis(*N*-Boc)hydrazino group at the 2-position in (4*R*)-**10**.[10] On the other hand, treatment of chiral enolate derived from *N*-acyloxazolidone **A** with 2,4,6-triisopropylbenzenesulfonyl azide (trisyl azide), followed by addition of AcOH was reported to give (2*S*)-azido carboximides **B** with high diastereoselectivity[11] as shown in Scheme 2.

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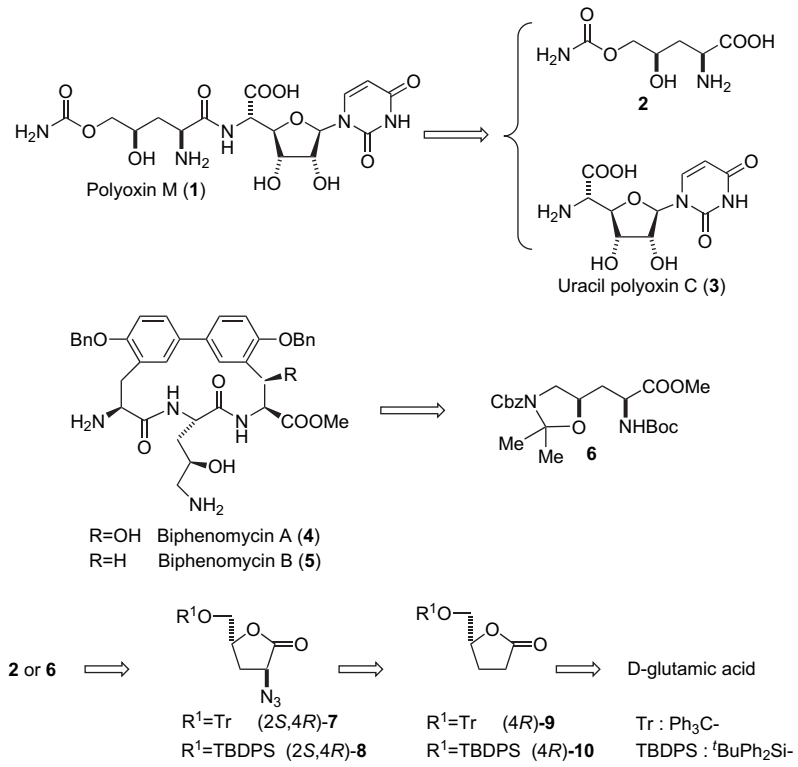
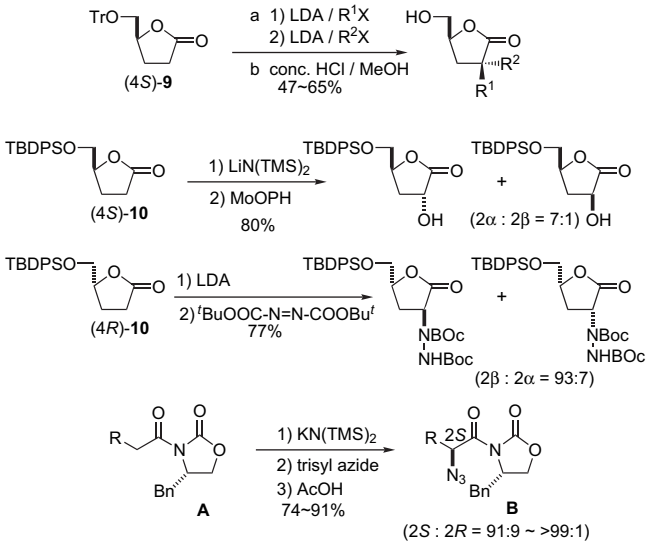


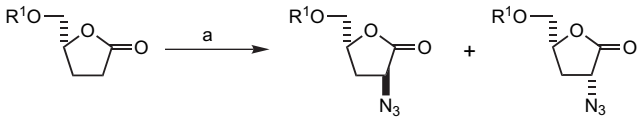
Scheme 1.



Scheme 2.

On consideration of these reports, our attention was focused only on the electrophilic azide transfer to the (4*R*)-protected hydroxymethyl-4-butanolide **9** or **10**. Chiral enolate derived from (4*R*)-**9** with lithium hexamethyldisilyazide (LiHMDS) was treated with trisyl azide, followed by addition of AcOH to give (2*S*)-**7** (37%) and (2*R*)-**11** (12%) (Table 1, entry 1). Change of the counter metal cation to sodium or potassium caused decrease of the yield of **7** (Table 1, entries 2 and 3). Treatment of chiral enolate derived from (4*R*)-**10** with trisyl azide, followed by addition of AcOH provided (2*S*)-**8** (33%) and (2*R*)-**12** (13%) (Table 1, entry 4), while change of AcOH to trimethylsilyl chloride (TMSCl) brought about a remarkable increase of the yield of (2*S*)-**8** (53%) along with (2*R*)-**12** (28%) (Table 1, entry 5). In the case of the electrophilic azide transfer to an enolate, the quench reagent was found to be an essential ingredient for successful azide transfer.[11] Surprisingly, AcOH proved to be superior to the silylating agents, TMSCl or TMSOTf, or strong acid TFA, while TMSCl was found to be a more effective quench agent in the present case. The structure of (2*S*)-**8** was determined by NMR analysis including NOE experiment as shown in Figure 1.

Table 1



a: 1) base / THF, 2) 2,4,6-triisopropylbenzenesulfonyl azide, 3) acid.

Entry	R^1	Base	Acid	Product (yield)	
1	Tr	LiHMDS	AcOH	**7** (37%)	**11** (12%)
2	Tr	NaHMDS	AcOH	**7** (25%)	**11** (trace)
3	Tr	KHMDS	AcOH	**7** (11%)	**11** (trace)
4	TBDPS	LiHMDS	AcOH	**8** (33%)	**12** (13%)
5	TBDPS	LiHMDS	TMSCl	**8** (53%)	**12** (28%)

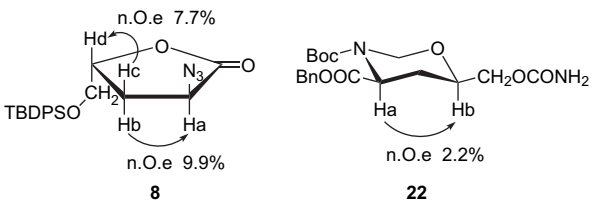
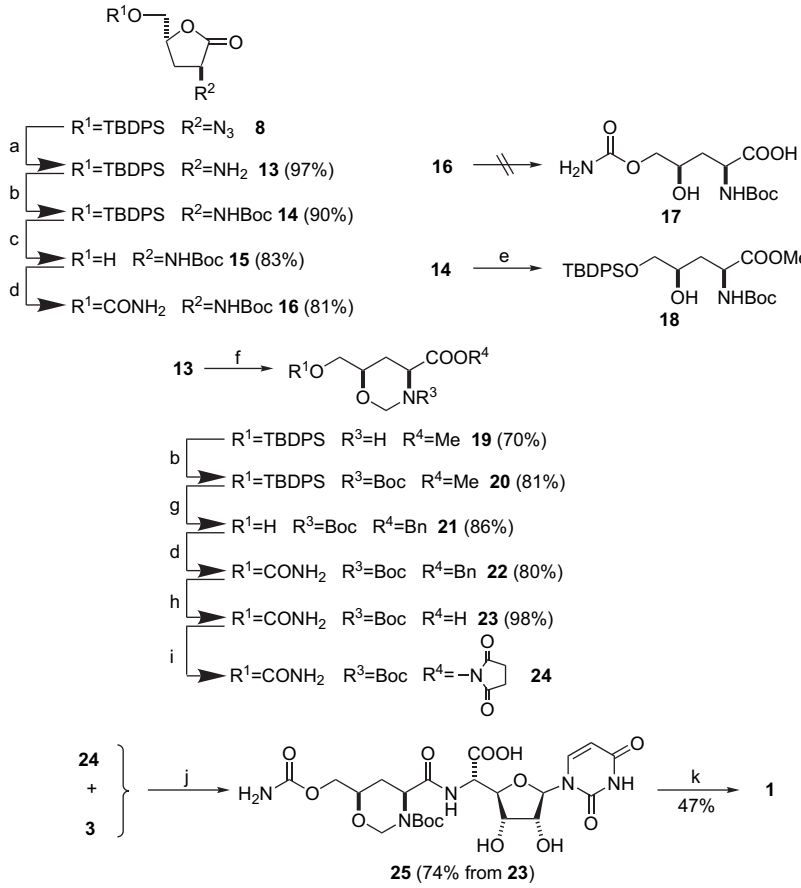


Figure 1.

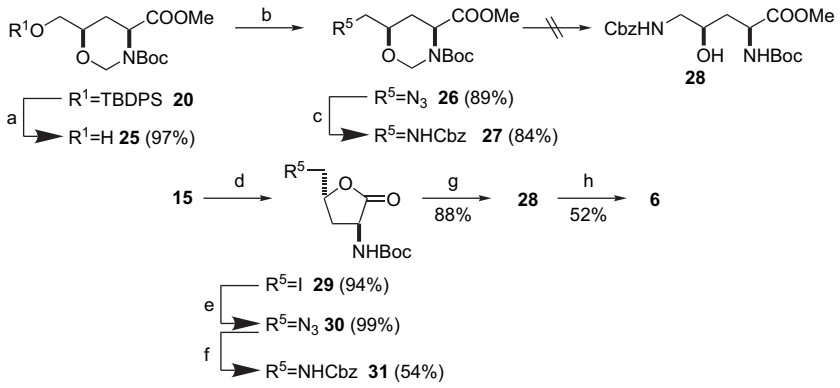
Then conversion of (2*S*)-**8** to the left-half congener **24** corresponding to **2** was carried out. Reduction of (2*S*)-**8** with Ph_3P and H_2O gave the amine **13** (97%), which was treated with $(Boc)_2O$ to afford the *N*-Boc compound **14** (90%). Deprotection of the silyl group in **14** provided an alcohol **15** (83%), which was converted to carbamoyl compound **16** in 81% yield. Alkaline hydrolysis of **16** did not give the desired γ-hydroxy acid **17**, while cleavage of the lactone ring of **14**, followed by esterification provided γ-hydroxy ester **18**. Protection of the alcohol group in **18** as a silyl group did not occur or treatment of **18** with *N*-methyl-*N*-(*tert*-butyldimethylsilyl) trifluoroacetamide gave the γ-lactone **14**. For the purpose of the double protection of the hydroxyl group and NHBoc group as a six-membered ring form, treatment of **18** with 3,3-dimethoxypropane and PPTS afforded only the starting **18**, while treatment of **18** with 3,3-dimethoxypropane and TsOH, or CSA provided γ-lactone **14**. By applying the reported procedure,[12] alkaline hydrolysis of (2*S*)-amino-γ-lactone **13**, followed by acetal formation with formaldehyde gave the six-membered ring compound **19** in 70% yield. Protection of the secondary amino group in **19** as a Boc group gave **20** (80%), which was subjected to consecutive trans-esterification and desilylation to afford an alcohol **21** in 86% yield. Conversion of **21** to the carbamoyl compound **22** (80%), followed by catalytic hydrogenation yielded the desired carboxylic acid **23** in 98% yield. The structure of **22** was reconfirmed by NMR analysis including NOE experiment as shown in Figure 1. Treatment of carboxylic acid **23** with *N*-hydroxysuccinimide in the presence of *N*,*N*-dicyclohexylcarbodiimide (DCC) in DMSO[13] provided an active ester **24**, which was coupled with uracil polyoxin C (**3**) in the presence of $(i\text{-}Pr)_2NEt$ to give the dipeptide **25** in 75% yield from **23**. Removal of the *N*-Boc and *N*,*O*-acetal protecting groups upon acid hydrolysis provided polyoxin M (**1**) ($[\alpha]_D^{25}$ +46.9 (*c* 0.29, H_2O), mp 215–220 °C (dec)) in 47% yield. The spectral data (^1H and ^{13}C NMR) of the synthetic **1** were identical with those of the natural polyoxin M (**1**) given by Dr. T. Yano. The specific rotation of synthetic **1** was in good agreement with that ($[\alpha]_D$ +49.9 (H_2O)) of the reported natural product (**1**)[1] (Scheme 3).

2.2. Synthesis of intermediate (6) for biphenomycins A and B

Deprotection of the silyl group in **20** provided an alcohol **25** (97%), which was subjected to consecutive trifluoromethanesulfonylation and azidation to give an azide **26** in overall 89% yield. A catalytic hydrogenation of **26**, followed by treatment of benzyl chloroformate (CbzCl) afforded NHCbz compound **27** (84%), deprotection of the acetal group of which did not occur. On the other hand, treatment of an alcohol **15** with iodine in the presence of Ph_3P and imidazole gave an iodide **29** (94%), which was treated with NaN_3 to provide azide **30** in 99% yield. Reduction of **30** with Ph_3P and H_2O gave the amine, which was treated with CbzCl to



Scheme 3. Reagents and conditions: (a) (1) Ph_3P, (2) H_2O; (b) $(Boc)_2O$/dioxane; (c) HF·Py/THF/pyridine; (d) (1) 4-nitrophenyl chloroformate/pyridine/Et_3N/THF, (2) NH_3/MeOH; (e) (1) NaOH aq/THF, (2) H^+, (3) CH_2N_2/Et_2O; (f) (1) NaOH aq/THF, (2) HCHO aq, (3) H^+, (4) CH_2N_2/Et_2O; (g) (1) BnOH/Ti(O-*i*-Pr)$_4$/benzene, (2) $Bu_4N^+F^-$/THF; (h) H_2/Pd–C/MeOH; (i) *N*-hydroxysuccinimide/DCC/AcOEt; (j) *i*-Pr_2NEt/DMSO; (k) $CF_3COOH/MeOH/H_2O$.



Scheme 4. Reagents and conditions: (a) HF·Py/THF/pyridine; (b) (1) Tf_2O/pyridine/CH_2Cl_2, (2) NaN_3/DMF; (c) (1) H_2/Pd–C/MeOH, (2) benzyl chloroformate/7% aq $NaHCO_3$/dioxane; (d) I_2/Ph_3P/imidazole/benzene; (e) NaN_3/DMF; (f) (1) Ph_3P/THF, (2) H_2O, (3) benzyl chloroformate/7% aq $NaHCO_3$/dioxane; (g) (1) NaOH aq, (2) H^+, (3) CH_2N_2/Et_2O; (h) 2,2-dimethoxypropane/PPTS.

afford the *N*-Cbz compound **31** (54%). Alkaline hydrolysis of **31**, followed by esterification with CH_2N_2 provided the desired methyl ester **28** (88%), which was treated with 2,2-dimethoxypropane and PPTS to afford the intermediate **6** ($[\alpha]_D^{24}$ +8.73 (*c* 1.50, $CHCl_3$)) for biphenomycins A and B in 52% yield. The spectral data (^1H and ^{13}C NMR) of the synthetic **6** were identical with those of the reported (2*S*,4*R*)-4-hydroxyornithine congener **6**.[6] The specific rotation of synthetic **6** was in good agreement with that ($[\alpha]_D^{20}$ +9.1 (*c* 1.09, $CHCl_3$)) of the reported **6**[6] (Scheme 4).

3. Conclusion

Chiral enolate derived from (4*R*)-4-*tert*-butyldiphenylsilyloxymethyl-4-butanolide **10** with lithium hexamethyldisilyazide (LiHMDS) was treated with trisyl azide, followed by addition of TMSCl to give (2*S*,4*R*)-2-azido-4-[(*tert*-butyldiphenylsilyloxy)methyl]-4-butanolide **8** (53%), from which the first total synthesis of polyoxin M (**1**) was achieved in overall 3.2% yield (13 steps) from D-glutamic acid. Moreover, the synthesis of the reported intermediate (2*S*,4*R*)-4-hydroxyornithine congener **6** for biphenomycins A and B was also achieved in overall 4.1% yield (12 steps) from D-glutamic acid.

4. Experimental

4.1. General

All melting points were measured on a Yanaco MP-3S micro melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on JEOL EX 400 spectrometer in $CDCl_3$. High-resolution mass spectra (HRMS) and the fast atom bombardment mass spectra (FABMS) were obtained with JEOL JMS-DX 303 spectrometer. IR spectra were recorded with a JASCO FTIR-300 spectrometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. All evaporations were performed under reduced pressure. For column chromatography, silica gel (Kieselgel 60) was employed.

4.1.1. (4*R*)-4-[(Trityloxy)methyl]-4-butenolide 9 and (4*R*)-4-[(*tert*-butyldiphenylsilyloxy)methyl]-4-butanolide 10. (i) To a solution of D-glutamic acid (10.07 g, 0.068 mol) in concd HCl (20 ml) and H_2O (40 ml) was added slowly a solution of $NaNO_2$ (7.0 g, 0.102 mol) in H_2O (20 ml) at −5 °C and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was evaporated in vacuo at below 50 °C to give a residue, which was shaken with AcOEt. The precipitate was filtered off and washed with AcOEt. The filtrate and washing were combined, and dried over $MgSO_4$. Evaporation of the solvent afforded (4*R*)-γ-carboxy-γ-butyrolactone (8.28 g, 93%) as a colorless syrup. $[\alpha]_D^{22}$ −5.53 (*c* 1.14, MeOH); NMR (acetone-d_6): δ 2.29–2.35 (1H, m), 2.51–2.55 (2H, m), 2.57–2.69 (1H, m), 5.00 (1H, dd, *J*=4.4, 8.4 Hz), 10.34 (1H, br s). FABMS: 131 $(M+1)^+$. (ii) To a solution of (4*R*)-γ-carboxy-γ-butyrolactone (5.48 g, 0.042 mol) in THF (100 ml) was added slowly 2 M $BH_3\cdot Me_2S$ in THF solution (25.3 ml, 0.0506 mol) at −20 °C and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with aqueous NH_4Cl and AcOEt. The organic layer was washed with brine, and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (60 g, CHC_3/MeOH=100:1) to give (4*R*)-γ-hydroxymethyl-γ-butyrolactone (2.58 g, 53%) as a colorless oil. $[\alpha]_D^{24}$ −38.36 (*c* 1.35, EtOH); NMR (acetone-d_6): δ 2.05–2.15 (1H, m), 2.23–2.32 (1H, m), 2.46–2.51 (2H, m), 3.62 (1H, dd, *J*=4.4, 12.0 Hz), 3.76 (1H, dd, *J*=3.2, 12.0 Hz), 4.14 (1H, br s), 4.45–4.59 (1H, m). FABMS: 117 $(M+1)^+$. (iii) To a solution of (4*R*)-γ-hydroxymethyl-γ-butyrolactone (1.03 g, 8.9 mmol) in pyridine (5 ml) was added trityl chloride (TrCl, 3.72 g, 13.3 mmol) and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine, and dried over $MgSO_4$. Evaporation of the organic solvent provided a residue, which was chromatographed on silica gel (40 g, *n*-hexane/AcOEt=10:1) to give **9** (2.97 g, 93%) as colorless needles. (4*R*)-**9**: mp 150–151 °C (*n*-hexane) $[\alpha]_D^{28}$ −25.3 (*c* 1.02, $CHCl_3$); IR (KBr): 1774 cm^{-1}. NMR: δ 1.99–2.07 (1H, m), 2.21–2.28 (1H, m), 2.46–2.54 (1H, m), 2.68 (1H, ddd, *J*=6.8, 10.0, 18.0 Hz), 3.15 (1H, dd, *J*=4.4, 10.6 Hz), 3.42 (1H, dd, *J*=3.6, 10.6 Hz), 4.61–4.66 (1H, m), 7.22–7.26 (3H, m), 7.28–7.33 (6H, m), 7.41–6.44 (6H, m). Anal. Calcd for $C_{24}H_{22}O_3$: C, 80.42; H, 6.19%. Found: C, 80.69; H, 6.26%. (iv) To a solution of (4*R*)-γ-hydroxymethyl-γ-butyrolactone (2.03 g, 17.5 mmol) in DMF (20 ml) were added *tert*-butyldiphenylsilyl chloride (TBDPSCl, 5.68 g,

20.7 mmol) and imidazole (2.34 g, 34.4 mmol), and the whole mixture was stirred for 1 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine, and dried over $MgSO_4$. Evaporation of the organic solvent provided a residue, which was chromatographed on silica gel (60 g, *n*-hexane/AcOEt=50:1) to give **10** (5.81 g, 94%) as colorless prism. (4*R*)-**10**: mp 77–78 °C (*n*-hexane) $[\alpha]_D^{25}$ −28.55 (*c* 1.28, $CHCl_3$); IR (KBr): 1772 cm^{-1}. NMR: δ 1.06 (9H, s), 2.20–2.30 (2H, m), 2.46–2.55 (1H, m), 2.63–2.72 (1H, m), 3.69 (1H, dd, *J*=3.2, 11.2 Hz), 3.88 (1H, dd, *J*=3.2, 11.2 Hz), 4.57–4.61 (1H, m), 7.37–7.46 (6H, m), 7.65–7.68 (4H, m). Anal. Calcd for $C_{21}H_{26}O_3Si$: C, 71.15; H, 7.39%. Found: C, 71.44; H, 7.56%.

4.1.2. (2*S*,4*R*)-2-Azido-4-[(trityloxy)methyl]-4-butanolide 7 and (2*R*,4*R*)-2-azido-4-[(trityloxy)methyl]-4-butanolide 11. (i) (Entry 1, Table 1) To a well-stirred solution of (4*R*)-(trityloxy)methyl-4-butenolide **9** (0.354 g, 0.99 mmol) in THF (4 ml) at −78 °C was added 1 M solution of lithium bis(trimethylsilyl) amide (LiHMDS) in THF (1.1 ml, 1.1 mmol) and the whole mixture was stirred for 30 min. To the above reaction mixture was added a solution of 2,4,6-triisopropylbenzenesulfonyl azide (0.383 g, 1.24 mmol) in THF (4 ml) and the whole mixture was stirred for 30 min at the same temperature. To the above reaction mixture was added AcOH (0.4 ml) and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with 7% aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (30 g) to give **7** (0.146 g, 37%) as colorless needles from *n*-hexane/AcOEt=30:1 elution and **11** (0.049 g, 12%) as colorless needles from *n*-hexane/AcOEt=20:1 elution. (2*S*,4*R*)-**7**: mp 135–137 °C (*n*-hexane); $[\alpha]_D^{25}$ −85.3 (*c* 0.83, $CHCl_3$); IR (KBr): 2114, 1779 cm^{-1}. NMR: δ 2.09–2.17 (1H, m), 2.32 (1H, ddd, *J*=2.8, 8.8, 13.2 Hz), 3.06 (1H, dd, *J*=2.8, 10.8 Hz), 3.60 (1H, dd, *J*=2.8, 10.8 Hz), 4.58 (1H, t, *J*=8.8 Hz), 4.63–4.67 (1H, m), 7.24–7.28 (3H, m), 7.30–7.34 (6H, m), 7.37–7.40 (6H, m). HRMS (FAB) Calcd for $C_{24}H_{22}O_3N_3$ (M^++H; *m*/*z*) 400.1662. Found 400.1613. (2*R*,4*R*)-**11**: mp 147–149 °C (*n*-hexane); $[\alpha]_D^{25}$ +65.3 (*c* 0.68, $CHCl_3$); IR (KBr): 2110, 1779 cm^{-1}. NMR: δ 2.04 (1H, dt, *J*=10.4, 12.8 Hz), 2.50 (1H, ddd, *J*=6.0, 8.8, 12.8 Hz), 3.26 (1H, dd, *J*=5.0, 10.6 Hz), 3.36 (1H, dd, *J*=3.8, 10.6 Hz), 4.32 (1H, dd, *J*=8.8, 10.4 Hz), 4.50–4.57 (1H, m), 7.23–7.33 (9H, m), 7.42–7.45 (6H, m). HRMS (FAB) Calcd for $C_{24}H_{22}O_3N_3$ (M^++H; *m*/*z*) 400.1662. Found 400.1602. (ii) (Entry 2, Table 1) To a well-stirred solution of (4*R*)-**9** (0.358 g, 1.0 mmol) in THF (4 ml) at −78 °C was added 0.6 M solution of sodium bis(trimethylsilyl) amide (NaHMDS) in toluene (1.9 ml, 1.1 mmol) and the whole mixture was stirred for 30 min. To the above reaction mixture was added AcOH (0.3 ml) and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was worked up in the same way as (i) to give **7** (0.10 g, 25%). (iii) (Entry 3, Table 1) To a well-stirred solution of (4*R*)-**9** (0.358 g, 1.0 mmol) in THF (4 ml) at −78 °C was added 0.5 M solution of potassium bis(trimethylsilyl) amide (KHMDS) in toluene (2.2 ml, 1.1 mmol) and the whole mixture was stirred for 30 min. To the above reaction mixture was added AcOH (0.3 ml) and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was worked up in the same way as (i) to give **7** (0.045 g, 11%).

4.1.3. (2*S*,4*R*)-2-Azido-4-[(*tert*-butyldiphenylsilyloxy)-methyl]-4-butanolide 8 and (2*R*,4*R*)-2-azido-4-[(*tert*-butyldiphenylsilyloxy)methyl]-4-butanolide 12. (i) (Entry 4, Table 1) To a well-stirred solution of (4*R*)-(*tert*-butyldiphenylsilyloxy)methyl-4-butenolide **10** (1.0 g, 2.8 mmol) in THF (10 ml) at −78 °C was added 1 M solution of lithium bis(trimethylsilyl) amide (LiHMDS) in THF (3.4 ml, 3.4 mmol) and the whole mixture was stirred for 30 min. To the above reaction mixture was added a solution of 2,4,6-triisopropylbenzenesulfonyl azide (1.05 g, 0.4 mmol) in THF (10 ml) and the whole mixture was stirred for 30 min at the same temperature. To the above reaction mixture was added AcOH (0.75 ml) and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with 7% aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (50 g) to give **8** (0.368 g, 33%) as colorless needles from *n*-hexane/AcOEt=30:1 elution and **12** (0.145 g, 13%) as colorless oil from *n*-hexane/AcOEt=10:1 elution. (2*S*,4*R*)-**8**: mp 72–74 °C (*n*-hexane); $[\alpha]_D^{25}$ −108.9 (*c* 1.0, $CHCl_3$); IR (KBr): 2107, 1778 cm^{-1}. NMR: δ 1.06 (9H, s), 2.20 (1H, dt, *J*=8.8, 13.2 Hz), 2.52–2.58 (1H, m) 3.64 (1H, dd, *J*=2.4, 11.4 Hz), 3.92 (1H, dd, *J*=2.8, 11.4 Hz), 4.54 (1H, t, *J*=8.8 Hz), 4.60–4.64 (1H, m), 7.39–7.48 (6H, m), 7.61–7.65 (4H, m). Anal. Calcd for $C_{21}H_{25}N_3O_3Si$: C, 63.77; H, 6.37; N, 10.62%. Found: C, 63.91; H, 6.41; N, 10.42%. (2*R*,4*R*)-**12**: $[\alpha]_D^{24}$ +52.9 (*c* 1.13, $CHCl_3$); IR (KBr): 2110, 1784 cm^{-1}. NMR: δ 1.06 (9H, s), 2.18 (1H, ddd, *J*=9.8, 10.4, 13.0 Hz), 2.52 (1H, ddd, *J*=6.2, 9.0, 13.0 Hz), 3.72 (1H, dd, *J*=4.0, 11.6 Hz), 3.89 (1H, dd, *J*=3.6, 11.6 Hz), 4.33 (1H, dd, *J*=9.0, 10.4 Hz), 4.50 (1H, ddd, *J*=3.6, 6.2, 9.8 Hz), 7.38–7.45 (6H, m), 7.64–7.67 (4H, m). HRMS (FAB) Calcd for $C_{21}H_{26}N_3O_3Si$ (M^++H; *m*/*z*) 396.1744. Found 396.1741. (ii) (Entry 5, Table 1) To a well-stirred solution of (4*R*)-**10** (2.0 g, 5.6 mmol) in THF (20 ml) at −78 °C was added 1 M solution of lithium bis(trimethylsilyl) amide (LiHMDS) in THF (6.8 ml, 6.8 mmol) and the whole mixture was stirred for 30 min. To the above reaction mixture was added a solution of 2,4,6-triisopropylbenzenesulfonyl azide (2.1 g, 6.8 mmol) in THF (20 ml) and the whole mixture was stirred for 30 min at the same temperature. To the above reaction mixture was added trimethylsilyl chloride (TMSCl, 3.3 ml) and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was worked up in the same way as (iv) to give **8** (1.182 g, 53%) and **12** (0.625 g, 28%).

4.1.4. (2*S*,4*R*)-2-Amino-4-[(*tert*-butyldiphenylsilyloxy)-methyl]-4-butanolide 13. A mixture of **7** (2.06 g, 5.2 mmol) and triphenylphosphine (Ph_3P, 1.65 g, 6.3 mmol) in THF (30 ml) was stirred for 30 min at room temperature. To the above reaction mixture was added H_2O (0.5 ml) and the whole mixture was heated with stirring for 4 h at 60 °C. The reaction mixture was evaporated to give a residue, which was chromatographed on silica gel (60 g, *n*-hexane/AcOEt=1:1) to afford (2*S*,4*R*)-**13** (1.87 g, 97%) as a colorless oil. (2*S*,4*R*)-**13**: $[\alpha]_D^{25}$ −25.94 (*c* 0.69,

$CHCl_3$); IR (KBr): 3425, 1781 cm^{-1}. NMR: δ 1.05 (9H, s), 2.07–2.17 (1H, m), 2.63 (1H, ddd, J=2.0, 9.4, 13.2 Hz), 3.65 (1H, dd, J=2.8, 11.4 Hz), 3.88 (1H, dd, J=2.8, 11.4 Hz), 3.99 (1H, t, J=9.4 Hz), 4.55–4.59 (1H, m), 7.38–7.48 (6H, m), 7.62–7.70 (4H, m). HRMS (FAB) Calcd for $C_{21}H_{28}NO_3Si$ (M^++H; m/z) 370.1838. Found 370.1852.

4.1.5. (2*S*,4*R*)-2-*tert*-Butoxycarbonylamino-4-[(*tert*-butyldiphenylsilyloxy)methyl]-4-butanolide 14. A mixture of **13** (1.33 g, 3.6 mmol), di-*tert*-butyl dicarbonate [$(Boc)_2O$, 0.9 g, 4.3 mmol] and Et_3N (0.73 g, 7.2 mmol) in dioxane (20 ml) was stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (50 g, *n*-hexane/AcOEt=5:1) to give **14** (1.53 g, 90%) as colorless oil. (2*S*,4*R*)-**14**: $[\alpha]_D^{25}$ −22.69 (*c* 1.3, $CHCl_3$); IR (KBr): 3418, 1788, 1715 cm^{-1}. NMR: δ 1.06 (9H, s), 1.46 (9H, s), 2.35–2.43 (1H, m), 2.70–2.76 (1H, m), 3.65 (1H, dd, J=2.6, 11.6 Hz), 3.90 (1H, dd, J=2.6, 11.6 Hz), 4.53–4.59 (1H, m), 5.10 (1H, br s), 7.38–7.47 (6H, m), 7.63–7.67 (4H, m). Anal. Calcd for $C_{26}H_{35}NO_5Si\cdot H_2O$: C, 64.03; H, 7.65; N, 2.87%. Found: C, 64.07; H, 7.35; N, 2.57%.

4.1.6. (2*S*,4*R*)-2-*tert*-Butoxycarbonylamino-4-hydroxymethyl-4-butanolide 15. A mixture of **14** (2.88 g, 6.1 mmol), HF·pyridine complex (1.22 g, 12.3 mmol) in a mixed solvent [THF (20 ml)/pyridine (20 ml)] was stirred for two days at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (50 g, $CHCl_3$/MeOH=100:1) to give **15** (1.18 g, 83%) as colorless dust. (2*S*,4*R*)-**15**: mp 202–203 °C ($CHCl_3$/MeOH); $[\alpha]_D^{25}$ −45.33 (*c* 0.3, $CHCl_3$); IR (KBr): 3348, 2963, 1735, 1696 cm^{-1}. NMR (DMSO-d_6): δ 1.37 (9H, s), 2.19–2.31 (2H, m), 3.46 (1H, ddd, J=2.4, 5.6, 12.0 Hz), 3.58 (1H, ddd, J=2.8, 5.6, 12.0 Hz), 4.34 (1H, q, J=9.4 Hz), 4.52–4.56 (1H, m), 5.15 (1H, t, J=5.6 Hz), 7.31 (1H, d, J=9.4 Hz). HRMS (FAB) Calcd for $C_{10}H_{18}NO_5$ (M^++H; m/z) 232.1185. Found 232.1204.

4.1.7. (2*S*,4*R*)-2-*tert*-Butoxycarbonylamino-4-carbamoyloxymethyl-4-butanolide 16. To a solution of **15** (0.092 g, 0.39 mmol) in THF (10 ml) was added pyridine (0.19 g, 2 mmol), Et_3N (0.075 g, 0.74 mmol), and 4-nitrophenyl chloroformate (0.23 g, 1.1 mmol) at −20 °C and the reaction mixture was stirred for 30 min at the same temperature. To the above reaction mixture was added saturated NH_3/MeOH (5 ml) and the whole mixture was stirred for 1 h at 0 °C. The reaction mixture was evaporated and the resulting residue was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (10 g, *n*-hexane/AcOEt=1:2) to give **16** (0.088 g, 81%) as colorless oil. (2*S*,4*R*)-**16**: NMR (DMSO-d_6): δ 1.38 (9H, s), 2.19–2.28 (2H, m), 4.04–4.09 (2H, m), 4.30 (1H, q, J=8.8 Hz), 4.70–4.75 (1H, m), 6.53 (1H, br s), 6.77 (1H, br s), 7.44 (1H, d, J=8.8 Hz). FABMS: 297 $(M+Na)^+$.

4.1.8. Methyl (2*S*,4*R*)-2-*tert*-butoxycarbonylamino-4-hydroxy-5-*tert*-butyldiphenylsilyloxy pentanoate 18. To a solution of **14** (0.19 g, 0.4 mmol) in THF (2 ml) was added 2 M NaOH solution (3 ml) at 0 °C and the reaction mixture was stirred for 1 h at the same temperature. The reaction mixture was acidified with 10% HCl solution and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was treated with CH_2N_2/Et_2O solution to provide a crude oily product. It was chromatographed on silica gel (10 g, *n*-hexane/AcOEt=5:1) to give **18** (0.13 g, 64%) as colorless oil. (2*S*,4*R*)-**18**: NMR: δ 1.06 (9H, s), 1.43 (9H, s), 1.85–1.93 (2H, m), 3.53 (1H, dd, J=6.8, 10.2 Hz), 3.63 (1H, dd, J=5.6, 10.2 Hz), 3.76 (3H, s), 3.84–3.90 (1H, m), 4.37–4.39 (1H, m), 5.47 (1H, br s), 7.37–7.46 (6H, m), 7.63–7.67 (4H, m). FABMS: 502 (M^++1).

4.1.9. (4*S*,6*R*)-6-*tert*-Butyldiphenylsilyloxymethyl-4-methoxycarbonyl-tetrahydro-2*H*-1,3-oxazine 19. To a solution of **13** (0.605 g, 1.6 mmol) in THF (2 ml) was added 2 M NaOH solution (3 ml) at 0 °C and the reaction mixture was stirred for 1 h at the same temperature. To the above reaction mixture was added 37% aqueous HCHO (1 ml) and the reaction mixture was stirred for 12 h at the same temperature. The reaction mixture was acidified with 10% HCl solution and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was treated with CH_2N_2/Et_2O solution to provide a crude oily product. It was chromatographed on silica gel (10 g, *n*-hexane/AcOEt=5:1) to give **19** (0.473 g, 70%) as colorless oil. (4*S*,6*R*)-**19**: $[\alpha]_D^{23}$ −4.36 (*c* 0.55, $CHCl_3$); IR (KBr): 3439, 1744 cm^{-1}. NMR: δ 1.06 (9H, s), 1.33–1.42 (1H, m), 1.93 (1H, dt, J=2.4, 12.8 Hz), 3.55–3.59 (1H, m), 3.63–3.78 (3H, m), 3.75 (3H, s), 4.23 (1H, d, J=10.8 Hz), 4.67 (1H, d, J=10.8 Hz), 7.35–7.44 (6H, m), 7.65–7.69 (4H, m). HRMS (FAB) Calcd for $C_{23}H_{32}NO_4Si$ (M^++H; m/z) 414.2132. Found 414.2163.

4.1.10. (4*S*,6*R*)-3-*tert*-Butoxycarbonylamino-6-*tert*-butyldiphenylsilyloxymethyl-4-methoxycarbonyl-tetrahydro-2*H*-1,3-oxazine 20. A mixture of **19** (0.472 g, 1.14 mmol), di-*tert*-butyl dicarbonate [$(Boc)_2O$, 0.49 g, 2.2 mmol], and Et_3N (0.34 g, 3.3 mmol) in dioxane (5 ml) was stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (10 g, *n*-hexane/AcOEt=20:1) to give **20** (0.472 g, 81%) as colorless oil. (4*S*,6*R*)-**20**: $[\alpha]_D^{26}$ −23.29 (*c* 1.1, $CHCl_3$); IR (KBr): 1750, 1706 cm^{-1}. NMR (pyridine-d_5, 90 °C): δ 1.13 (9H, s), 1.45 (9H, s), 2.16–2.30 (2H, m), 3.67 (3H, s), 3.81 (1H, dd, J=5.0, 10.6 Hz), 3.88 (1H, dd, J=5.0, 10.6 Hz), 3.94 (1H, dq, J=5.0, 9.6 Hz), 4.51 (1H, dd, J=6.0, 10.0 Hz), 5.03 (1H, d, J=9.4 Hz), 5.23 (1H, d, J=9.4 Hz), 7.38–7.42 (6H, m), 7.79–7.83 (4H, m). Anal. Calcd for $C_{28}H_{39}NO_6Si$: C, 65.47; H, 7.65; N, 2.73%. Found: C, 65.55; H, 7.55; N, 2.72%.

4.1.11. (4*S*,6*R*)-4-Benzyloxycarbonyl-3-*tert*-butoxycarbonylamino-6-hydroxymethyl-tetrahydro-2*H*-1,3-oxazine 21. A mixture of **20** (1.2 g, 2.24 mmol), benzyl alcohol

(5.05 g, 46.7 mmol), and Ti(O-*i*-Pr)$_4$ (0.332 g, 1.17 mmol) in benzene (40 ml) was stirred for 12 h at reflux. The reaction mixture was diluted with 7% aqueous $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (50 g, *n*-hexane/EtOAc=20:1) to give an oily product. To a solution of the above oily product in THF (10 ml) was added 1 M $Bu_4N^+F^-$/THF solution (5 ml) and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (50 g, *n*-hexane/AcOEt=2:1) to give **21** (0.706 g, 86%) as colorless oil. (4*S*,6*R*)-**21**: $[\alpha]_D^{25}$ −29.88 (*c* 1.23, $CHCl_3$); IR (KBr): 3425, 1748, 1703 cm^{-1}. NMR (pyridine-d_5, 90 °C): δ 1.43 (9H, s), 2.22–2.37 (2H, m), 3.75 (1H, dd, *J*=4.4, 11.6 Hz), 3.81 (1H, dd, *J*=5.2, 11.6 Hz), 3.89–3.96 (1H, m), 4.58 (1H, dd, *J*=6.0, 9.6 Hz), 5.04 (1H, d, *J*=9.6 Hz), 5.10 (1H, br s), 5.24–5.31 (3H, m), 7.24–7.32 (3H, m), 7.42 (2H, d, *J*=7.2 Hz). Anal. Calcd for $C_{18}H_{25}NO_6$: C, 61.52; H, 7.17; N, 3.99%. Found: C, 61.26; H, 7.41; N, 4.06%.

4.1.12. (4*S*,6*R*)-4-Benzyloxycarbonyl-3-*tert*-butoxycarbonylamino-6-carbamoyloxymethyl-tetrahydro-2*H*-1,3-oxazine 22. To a solution of **21** (0.646 g, 1.84 mmol) in THF (15 ml) was added pyridine (0.945 g, 12 mmol), Et_3N (0.372 g, 3.7 mmol), and 4-nitrophenyl chloroformate (1.112 g, 5.52 mmol) at −20 °C and the reaction mixture was stirred for 30 min at the same temperature. To the above reaction mixture was added saturated NH_3/MeOH (5 ml) and the whole mixture was stirred for 1 h at 0 °C. The reaction mixture was evaporated and the resulting residue was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (10 g, *n*-hexane/AcOEt=2:1) to give **22** (0.58 g, 80%) as colorless oil. (4*S*,6*R*)-**22**: $[\alpha]_D^{23}$ −33.12 (*c* 0.77, $CHCl_3$); IR (KBr): 3444, 1734, 1716, 1701 cm^{-1}. NMR (pyridine-d_5, 90 °C): δ 1.42 (9H, s), 2.18–2.22 (2H, m), 4.00–4.08 (1H, m), 4.22 (1H, dd, *J*=4.8, 11.6 Hz), 4.32 (1H, dd, *J*=6.0, 11.6 Hz), 4.56 (1H, t, *J*=7.2 Hz), 5.01 (1H, d, *J*=9.6 Hz), 5.22 (1H, d, *J*=9.6 Hz), 5.26 (1H, d, *J*=12.4 Hz), 5.30 (1H, d, *J*=12.4 Hz), 6.67 (2H, br s), 7.24–7.33 (3H, m), 7.41–7.43 (2H, m). Anal. Calcd for $C_{19}H_{26}N_2O_7 \cdot 1/4H_2O$: C, 57.20; H, 6.70; N, 7.02%. Found: C, 56.91; H, 6.88; N, 6.98%.

4.1.13. (4*S*,6*R*)-3-*tert*-Butoxycarbonylamino-6-carbamoyloxymethyl-tetrahydro-2*H*-1,3-oxazin-3-carboxylic acid 23. A mixture of **22** (0.54 g, 1.37 mmol) and 10% Pd–C (0.1 g) in MeOH (10 ml) was subjected to a catalytic hydrogenation for 1 h at ordinary temperature. The reaction mixture was filtered with the aid of Celite and the filtrate was evaporated to give **23** (0.409 g, 98%) as amorphous solid. (4*S*,6*R*)-**23**: $[\alpha]_D^{20}$ −40.98 (*c* 0.82, $CHCl_3$); IR (KBr): 3443, 3367, 1733, 1715, 1703 cm^{-1}. NMR (pyridine-d_5, 90 °C): δ 1.48 (9H, s), 2.31–2.38 (2H, m), 4.08–4.14 (1H, m), 4.29 (1H, dd, *J*=4.4, 11.6 Hz), 4.44 (1H, dd, *J*=6.4, 11.6 Hz), 4.70 (1H, d, *J*=7.6 Hz), 5.20 (1H, d, *J*=9.6 Hz), 5.31 (1H, d, *J*=9.6 Hz), 6.66 (2H, br s), 7.77 (1H, br s). Anal. Calcd for $C_{12}H_{20}N_2O_7$: C, 47.36; H, 6.62; N, 9.21%. Found: C, 47.21; H, 6.84; N, 9.11%.

4.1.14. Coupling reaction of (4*S*,6*R*)-23 and uracil polyoxcin C (3). A mixture of **23** (0.077 g, 0.25 mmol), *N*-hydroxysuccinimide (0.032 g, 0.28 mmol), and *N*,*N*-dicyclohexylcarbodiimide (DCC, 0.058 g, 0.28 mmol) in AcOEt (5 ml) was stirred for 1 h at room temperature. The reaction mixture was evaporated to give a crude residue **24**. To a solution of the above residue in DMSO (3 ml) was added a mixture of uracil polyoxin C (**3**, 0.088 g, 0.28 mmol) and *i*-Pr_2NEt (0.13 ml, 0.506 mmol) in DMSO (1 ml) and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was directly subjected to column chromatography (silica gel, 10 g, $CHCl_3$/MeOH=1:1) to afford **25** (0.107 g, 74%) as amorphous solid. **25**: mp 207–210 °C (dec); $[\alpha]_D^{25}$ −4.3 (*c* 0.6, MeOH); IR (KBr): 3401, 1685, 1670, 1637, 1625 cm^{-1}. ^{1}H NMR (pyridine-d_5, 90 °C): δ 1.45 (9H, s), 2.34–2.37 (2H, m), 4.08–4.12 (1H, m), 4.27 (1H, dd, *J*=4.8, 11.2 Hz), 4.40 (1H, dd, *J*=6.4, 11.2 Hz), 4.70–4.78 (2H, m), 4.91–4.94 (1H, m), 5.04–5.07 (1H, m), 5.17 (1H, d, *J*=9.8 Hz), 5.22 (1H, d, *J*=9.8 Hz), 5.29–5.32 (1H, m), 5.79 (1H, d, *J*=7.8 Hz), 6.39 (1H, d, *J*=3.6 Hz), 6.66 (2H, br s), 7.94 (1H, d, *J*=7.8 Hz). ^{13}C NMR (pyridine-d_5, 90 °C): δ 27.9, 28.4, 55.6, 56.6, 66.5, 71.3, 71.8, 71.8, 74.8, 81.3, 86.0, 90.9, 103.2, 141.5, 150.3, 152.3, 155.3, 158.0, 164.2, 172.2. HRMS (FAB) Calcd for $C_{22}H_{32}N_5O_{13}$ (M^++H; *m*/*z*) 574.1997. Found 574.1932.

4.1.15. Polyoxin M (1). To a solution of **25** (0.107 g, 0.187 mmol) in a mixed solvent [MeOH (2 ml)/H_2O (2 ml)] was added CF_3COOH (2 ml) at 0 °C and the reaction mixture was stirred for 12 h at room temperature. The reaction mixture was evaporated to give a crude residue, which was directly subjected to column chromatography (ODS, 10 g, H_2O) to afford **1** (0.040 g, 47%) as amorphous solid. **1**: mp 215–220 °C (dec); $[\alpha]_D^{25}$ +46.9 (*c* 0.29, H_2O); IR (KBr): 3423, 1677, 1655, 1648, 1637, 1631 cm^{-1}. ^{1}H NMR (D_2O): δ 1.76–1.86 (1H, m), 1.94–1.99 (1H, m), 3.79 (1H, dd, *J*=6.0, 11.2 Hz), 3.89 (1H, dd, *J*=3.6, 11.2 Hz), 3.92–3.97 (1H, m), 4.04–4.12 (2H, m), 4.21–4.28 (1H, m), 4.33 (1H, t, *J*=6.0 Hz), 4.62–4.66 (1H, m), 5.60 (1H, d, *J*=4.0 Hz), 5.70 (1H, d, *J*=8.0 Hz), 7.38 (1H, d, *J*=8.0 Hz). ^{13}C NMR (D_2O): δ 33.0, 51.3, 53.7, 66.4, 67.6, 69.4, 71.8, 81.9, 90.5, 102.1, 142.0, 151.0, 158.6, 165.4, 168.9, 170.7. HRMS (FAB) Calcd for $C_{16}H_{24}N_5O_{11}$ (M^++H; *m*/*z*) 462.1472. Found 462.1495.

4.1.16. (4*S*,6*R*)-3-*tert*-Butoxycarbonylamino-6-hydroxymethyl-4-methoxycarbonyl-tetrahydro-2*H*-1,3-oxazine 25. A mixture of **20** (0.35 g, 0.68 mmol), HF·pyridine complex (0.135 g, 1.36 mmol) in a mixed solvent [THF (5 ml)/pyridine (5 ml)] was stirred for two days at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (10 g, *n*-hexane/AcOEt=3:2) to give **25** (0.182 g, 97%) as colorless oil. (4*S*,6*R*)-**25**: NMR (pyridine-d_5): δ 1.44 (9H, s), 2.18–2.31 (2H, m), 3.69 (3H, s), 3.75 (1H, dd, *J*=4.8, 11.2 Hz), 3.82 (1H, dd, *J*=5.4, 11.2 Hz), 3.89–3.95 (1H, m), 4.51 (1H, dd, *J*=6.4, 9.6 Hz),

4.80 (1H, br s), 5.03 (1H, d, J=9.6 Hz), 5.27 (1H, d, J=9.6 Hz). FABMS: 276 (M^++1).

4.1.17. (4*S*,6*R*)-6-Azidomethyl-3-*tert*-butoxycarbonylamino-4-methoxycarbonyl-tetrahydro-2*H*-1,3-oxazine 26. To a solution of **25** (0.615 g, 2.23 mmol) in CH_2Cl_2 (10 ml) was added pyridine (0.883 g, 11.2 mmol) and $(CF_3SO_2)_2O$ (0.892 g, 3.35 mmol) at 0 °C and reaction mixture was stirred for 15 min at the same temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with 10% aqueous HCl, brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product. To a solution of the above crude product in DMF (10 ml) was added NaN_3 (0.219 g, 3.37 mmol) and the reaction mixture was stirred for 4 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (10 g, *n*-hexane/AcOEt=10:1) to give **26** (0.596 g, 89%) as colorless needles. (4*S*,6*R*)-**26**: NMR (pyridine-d_5): δ 1.44 (9H, s), 2.04–2.18 (2H, m), 3.28 (1H, dd, J=4.4, 13.2 Hz), 3.33 (1H, dd, J=6.0, 13.2 Hz), 3.70 (3H, s), 3.87–3.93 (1H, m), 4.47 (1H, dd, J=6.0, 9.6 Hz), 4.98 (1H, d, J=9.6 Hz), 5.24 (1H, d, J=9.6 Hz). FABMS: 301 (M^++1).

4.1.18. (4*S*,6*R*)-6-Benzyloxycarbonylaminomethyl-3-*tert*-butoxycarbonylamino-4-methoxycarbonyl-tetrahydro-2*H*-1,3-oxazine 27. A mixture of **26** (0.433 g, 1.44 mmol) and 10% Pd–C (0.1 g) in MeOH (10 ml) was subjected to a catalytic hydrogenation for 1 h at ordinary temperature. The reaction mixture was filtered with the aid of Celite and the filtrate was evaporated to give a crude amine. To a solution of the crude amine in dioxane (20 ml) was added 7% aqueous $NaHCO_3$ (4 ml) and 30% benzyl chloroformate/toluene solution (1.62 g, 2.85 mmol) and the reaction mixture was stirred for 1 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (10 g, *n*-hexane/AcOEt=2:1) to give **27** (0.493 g, 84%) as colorless oil. (4*S*,6*R*)-**27**: NMR (pyridine-d_5): δ 1.44 (9H, s), 2.09–2.20 (2H, m), 3.44–3.46 (1H, m), 3.67 (3H, s), 3.62–3.69 (1H, m), 3.89–3.96 (1H, m), 4.45 (1H, dd, J=6.0, 9.6 Hz), 4.92 (1H, d, J=9.6 Hz), 5.22–5.27 (3H, m), 7.21–7.34 (3H, m), 7.40–7.43 (2H, m). FABMS: 431 (M^++Na).

4.1.19. (2*S*,4*R*)-2-*tert*-Butoxycarbonylamino-4-iodomethyl-4-butanolide 29. To a solution of **15** (0.30 g, 1.3 mmol) in benzene (50 ml) were added Ph_3P (0.51 g, 1.94 mmol), imidazole (0.177 g, 2.6 mmol), and I_2 (0.495 g, 1.95 mmol) and the reaction mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with saturated $NaHSO_3$ solution and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (20 g, *n*-hexane/AcOEt=3:1) to give **29** (0.416 g, 94%) as colorless dust. (2*S*,4*R*)-**29**: mp 141–142 °C; $[\alpha]_D^{22}$ −44.8 (c 1.04, $CHCl_3$); IR (KBr): 3340, 1789, 1679 cm^{-1}. NMR (acetone-d_6): δ 1.42 (9H, s), 2.46 (2H, dd, J=5.0, 9.8 Hz), 3.55 (1H, dd, J=5.4, 10.6 Hz), 3.60 (1H, dd, J=5.8, 10.6 Hz), 4.47–4.54 (1H, m), 4.73–4.79 (1H, m), 6.64 (1H, br s). HRMS (FAB) Calcd for $C_{10}H_{17}INO_4$ (M^++H; m/z) 342.0203. Found 342.0312.

4.1.20. (2*S*,4*R*)-2-*tert*-Butoxycarbonylamino-4-azidomethyl-4-butanolide 30. A mixture of **29** (0.415 g, 1.2 mmol) and NaN_3 (0.119 g, 1.83 mmol) in DMF (10 ml) was stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (20 g, *n*-hexane/AcOEt=3:1) to give **30** (0.307 g, 99%) as colorless dust. (2*S*,4*R*)-**30**: mp 103–104 °C (*n*-hexane/AcOEt); $[\alpha]_D^{22}$ −80.0 (c 0.93, $CHCl_3$); IR (KBr): 3373, 2122, 1776, 1682 cm^{-1}. NMR: δ 1.45 (9H, s), 2.38–2.55 (2H, m), 3.53 (1H, dd, J=4.0, 13.2 Hz), 3.68 (1H, dd, J=3.6, 13.2 Hz), 4.40–4.50 (1H, m), 4.73–4.80 (1H, m), 5.32 (1H, d, J=6.4 Hz). Anal. Calcd for $C_{10}H_{16}N_4O_4$: C, 46.87; H, 6.29; N, 21.86%. Found: C, 46.59; H, 6.27; N, 21.90%.

4.1.21. (2*S*,4*R*)-4-Benzyloxycarbonylaminomethyl 2-*tert*-butoxycarbonylamino-4-butanolide 31. A mixture of **30** (0.103 g, 0.4 mmol) and Ph_3P (0.125 g, 0.48 mmol) in THF (5 ml) was stirred for 5 h at room temperature. To the above reaction mixture was added H_2O (0.5 ml) and the whole mixture was heated with stirring for 5 h at 60 °C. The reaction mixture was evaporated to give a crude amine. To a solution of the crude amine in dioxane (10 ml) was added 7% aqueous $NaHCO_3$ (2 ml) and 30% benzyl chloroformate/toluene solution (0.343 g, 0.6 mmol) and the reaction mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (10 g, *n*-hexane/AcOEt=4:1) to give **31** (0.079 g, 54%) as colorless dust. (2*S*,4*R*)-**31**: mp 73–74 °C (*n*-hexane/AcOEt); $[\alpha]_D^{25}$ −11.60 (c 0.75, $CHCl_3$); IR (KBr): 3353, 1771, 1701 cm^{-1}. NMR: δ 1.49 (9H, s), 2.27–2.34 (2H, m), 2.42–2.48 (1H, m), 3.31–3.38 (1H, m), 3.45–3.50 (1H, m), 4.21–4.30 (1H, m), 4.71 (1H, br s), 5.10 (2H, s), 5.27 (1H, d, J=6.8 Hz), 5.42–5.45 (1H, m), 7.29–7.37 (5H, m). HRMS (FAB) Calcd for $C_{18}H_{25}N_2O_6$ (M^++H; m/z) 365.1662. Found 365.1741.

4.1.22. Methyl (2*S*,4*R*)-5-benzyloxycarbonylamino-2-*tert*-butoxycarbonylamino-4-hydroxypentanoate 28. To a solution of **31** (0.078 g, 0.21 mmol) in THF (5 ml) was added 2 M NaOH solution (1 ml) at 0 °C and the reaction mixture was stirred for 30 min at the same temperature. The reaction mixture was acidified with 10% HCl solution and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was treated with CH_2N_2/Et_2O solution to provide a crude oily product. It was chromatographed on silica gel (10 g, $CHCl_3$/MeOH=100:1) to give **28** (0.073 g, 88%) as colorless oil. (2*S*,4*R*)-**28**: $[\alpha]_D^{25}$ −8.5 (c 0.6, $CHCl_3$); IR (KBr): 3370, 1771, 1701 cm^{-1}. NMR: δ 1.42 (9H, s), 1.80–1.87 (1H, m), 1.90–1.98 (1H, m), 3.08–3.15 (1H, m), 3.30–3.34 (1H, m), 3.58–3.62 (1H, m), 3.71 (3H, s), 3.83–3.89 (1H, m), 4.38 (1H, br s), 5.08 (2H, s), 5.51 (1H, t, J=5.4 Hz),

5.57–5.59 (1H, m), 7.29–7.33 (5H, m). HRMS (FAB) Calcd for $C_{19}H_{29}N_2O_7$ (M^++H; *m*/*z*) 397.1950. Found 397.1992.

4.1.23. Acetonide formation of 28 (synthesis of 6). A mixture of **28** (0.073 g, 0.18 mmol), dimethoxypropane (5 ml), and pyridinium *p*-toluenesulfonate (PPTS, 0.002 g) in DMF (1 ml) was stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the organic solvent provided a crude oily product, which was chromatographed on silica gel (5 g, $CHCl_3$/MeOH=200:1) to give **6** (0.042 g, 52%) as colorless oil. (2*S*,4*R*)-**6**: $[\alpha]_D^{24}$ +8.73 (*c* 1.50, $CHCl_3$); IR (KBr): 3426, 1747, 1711 cm^{-1}. ^1H NMR: δ 1.44 (9H, s), 1.50 (3H, s), 1.58 (3H, s), 2.01–2.05 (1H, m), 2.12–2.16 (1H, m), 3.11–3.18 (1H, m), 3.74 (3H, s), 3.77–3.81 (1H, m), 4.13–4.20 (1H, m), 4.37 (1H, br s), 5.10–5.15 (2H, m), 5.30–5.32 (1H, m), 7.29–7.36 (5H, m). ^{13}C NMR: δ 24.2, 26.1, 28.3, 35.8, 50.5, 51.3, 52.4, 66.5, 70.8, 80.1, 94.1, 127.9, 128.0, 128.5, 136.6, 152.2, 155.2, 172.4. HRMS (FAB) Calcd for $C_{22}H_{33}N_2O_7$ (M^++H; *m*/*z*) 437.2288. Found 437.2330.

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