

HETEROCYCLES, Vol. 80, No. 1, 2010, pp. 579 - 591. © The Japan Institute of Heterocyclic Chemistry
Received, 28th July, 2009, Accepted, 25th August, 2009, Published online, 26th August, 2009
DOI: 10.3987/COM-09-S(S)82

SYNTHESIS OF MACROSPHELIDES CONTAINING A HETEROCYCLIC SIDE CHAIN AS A NOVEL APOPTOSIS INDUCER[†]

**Yuji Matsuya,* Ayana Hori, Tomomi Kawamura, Heba F. Emam, Kanwal
Ahmed, Da-Yong Yu, Takashi Kondo, Naoki Toyooka, and Hideo Nemoto**

Graduate School of Medicine and Pharmaceutical Sciences, University of
Toyama, 2630 Sugitani, Toyama 930-0194, Japan (matsuya@pha.u-toyama.ac.jp)

Abstract – Synthesis of macrosphelides containing a pyridyl or benzimidazolyl side chain is described. The synthesis was accomplished through sequential assembling of suitable chiral blocks and ring-closing metathesis (RCM) with a high efficiency. These novel derivatives were evaluated on the apoptosis-inducing activity against human lymphoma cells.

INTRODUCTION

Macrosphelides are natural macrolides isolated from *Microsphaeropsis* sp. FO-5050 and *Periconia byssoides*, and characterized by a 16-membered tri-lactone framework (Figure 1).¹ These macrolide compounds have been reported to exhibit potent cell-cell adhesion inhibitory activity on human leukemia HL-60 cells and human-umbilicalvein endothelial cells (HUVECs).² Besides these reports, we have recently revealed that macrosphelides and analogous compounds exert apoptosis-inducing activity against human lymphoma U937 cells.³ In addition, these effects were significantly enhanced in combination with mild hyperthermia.⁴ Encouraged by these new findings, we have pushed a further project on design, synthesis, and biological evaluation of macrosphelides and relating compounds.^{5,6} In the course of our ongoing research, we found that several artificial macrosphelides (**1** and **2**), incorporated with some heteroaromatic side chain at the 15-position, exhibited improved apoptosis-inducing activity compared with natural macrosphelides.⁶ These molecular designs were inspired by extensive studies on epothilones, 16-membered natural macrolides possessing extraordinarily potent anti-tumor activity and apoptosis-inducing activity against broad tumor cell lines.⁷ These results prompted us to further investigate the bioactivities of heteroaromatic variants, as a part of the structure-activity relationship studies of macrosphelides. In this paper, we describe the synthetic study of three new derivatives (**3**, **4**, and **5**) based on a ring-closing metathesis (RCM) strategy, and their apoptosis-inducing activity.

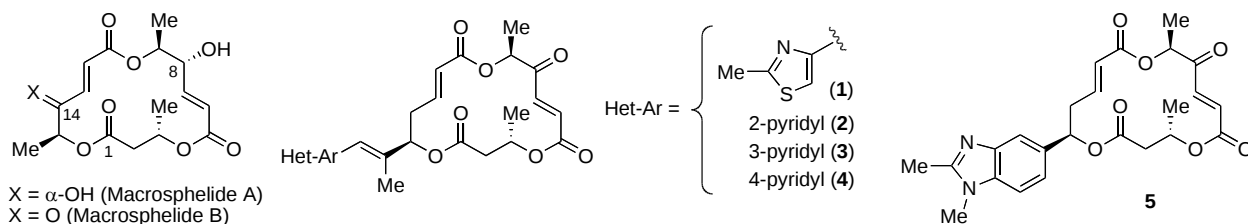
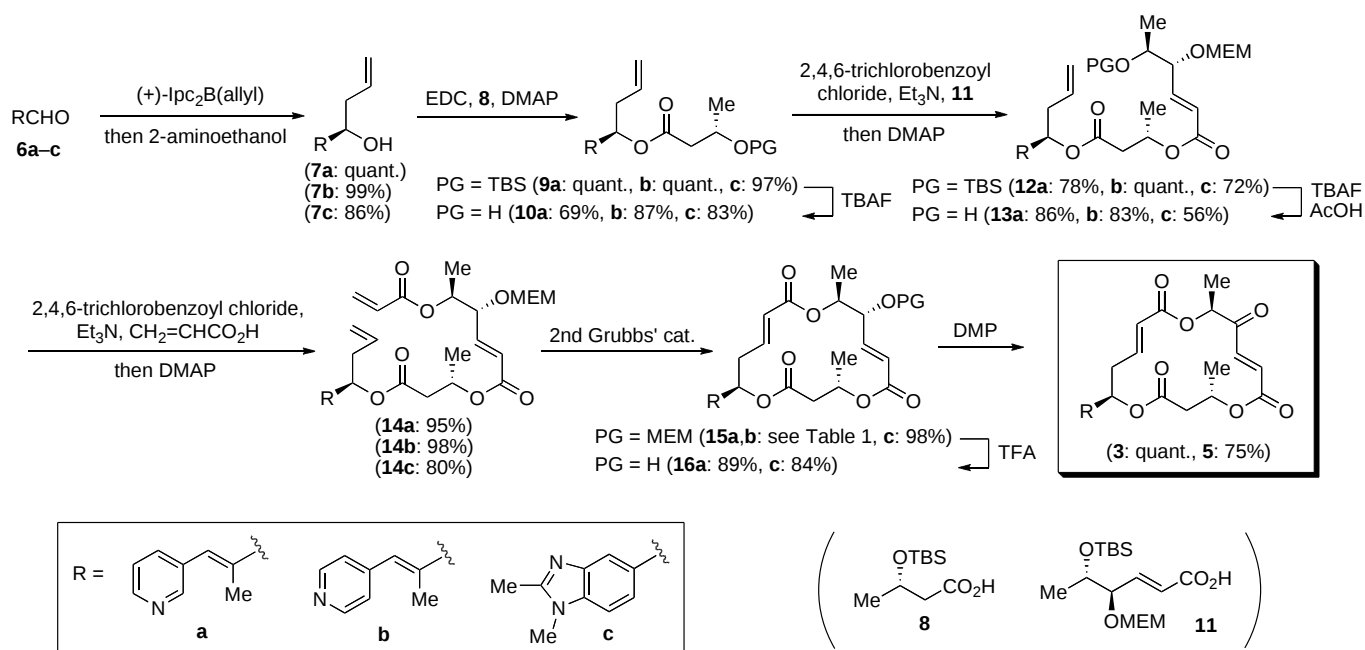


Figure 1. Natural Macrophelides and Artificial Analogues

RESULTS AND DISCUSSION

Thiazole side chain, found in natural epothilones, has been reported to play an important role for interacting with the target heterodimeric protein (tubulin), mainly due to action as a hydrogen-bonding acceptor.⁷ Therefore, the pyridine ring, possessing more basic nitrogen, can be a good surrogate of the thiazole ring, and the 2-pyridyl analogue was revealed to exhibit more potent anti-tumor activity.⁸ On the other hand, the position of nitrogen was decisively important; namely, corresponding 3- and 4-pyridyl analogues significantly decreased in the activity.⁸ We also observed that macrophelide with the thiazole side chain (1) and the 2-pyridyl side chain (2) showed comparable level of apoptosis-inducing activity at 1 μ M concentrations,⁶ although the mechanism of action remained unclear. For seeking a similarity of biological profiles of macrophelides and epothilones, comparison of the bioactivities among isomeric pyridyl macrophelides seemed to be meaningful. In addition, effects of the benzimidazolyl substituent on the bioactivity was also investigated, in the light of recent findings that introduction of such substituents brought about positive results on anti-tumor activity of epothilones.⁹



Scheme 1. Synthesis of Macrophelide Analogues 3 and 5

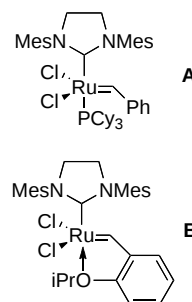
Three new derivatives **3–5** were planned to be synthesized via a common synthetic pathway depicted in Scheme 1. The aldehydes **6a–c**, prepared by modified procedures of reported methods,^{6,10} were subjected to asymmetric allylation utilizing (+)-*B*-allyldiisopinocampheylborane¹¹ to give chiral homoallyl alcohols **7a–c** with a high enantiomeric purity,¹² which was estimated by a standard Mosher's ester analysis (> 98% ee). Dehydrative condensation of these alcohols with chiral carboxylic acid **8**,⁶ followed by desilylation afforded alcohols **10a–c**, which were subsequently conducted to the second condensation with chiral carboxylic acid **11**⁶ according to the Yamaguchi's method¹³ to produce diesters **12a–c** with high efficiency. Removal of the TBS group of **12a–c** upon treatment with TBAF required addition of AcOH to avoid retro-Michael elimination at the C2–C3 position. Although acryloylation of the resulting alcohols **13a–c** were first performed using acryloyl chloride as a reagent, this transformation resulted in considerably low yields (10–20%) probably due to quaternalization of the heteroaromatic moiety and subsequent side reactions. Thus we employed the Yamaguchi's method again to achieve the preparation of RCM substrates **14a–c**.

Table 1. RCM Reaction of the Substrates **14a** and **14b**

Entry	Substrate	Catalyst ¹⁾	Conditions	Yield (%) ²⁾
1	14a	A	CH ₂ Cl ₂ , rt, 24 h	21
2	14a	A	CH ₂ Cl ₂ , reflux, 24 h	46
3	14a	B	toluene, 80°C, 2 h	36
4	14a	A	TsOH (1 eq.), CH ₂ Cl ₂ , reflux, 24 h	66
5	14b	A	CH ₂ Cl ₂ , rt, 24 h	0
6	14b	A	CH ₂ Cl ₂ , reflux, 24 h	0 ³⁾
7	14b	B	CH ₂ Cl ₂ , reflux, 24 h	0 ³⁾
8	14b	A	TsOH (1 eq.), CH ₂ Cl ₂ , reflux, 24 h	0 ³⁾

1) 30 mol% of the catalyst was used for all reactions.

2) Isolation yields. 3) Afforded a complex mixture.



In our continuous studies regarding macrocyclizations,^{6,14} the standard RCM conditions (10 mol% of 2nd Grubbs' catalyst **A**, CH₂Cl₂, rt, overnight) have worked well for macrocyclization. However, the RCM reactions of **14a** and **14b** were very sluggish (Table 1, entries 1 and 5), while the substrate **14c**, having benzimidazole moiety, smoothly cyclized in excellent yield (98%) under these conditions. These were unexpected results for us, because the corresponding 2-pyridyl compound **2** could be efficiently synthesized under the same conditions.⁶ These difficulties may be attributed to relatively high Lewis basicity of the pyridine nitrogen at the less hindered positions; namely ligation effects to the ruthenium complex. Consequently, we examined the RCM cyclization of **14a** and **14b**, searching for optimal reaction conditions (Table 1). Gentle heating (catalyst **A**, CH₂Cl₂, reflux) increased the yield of **15a** (entry 2), while Hoveyda-Grubbs catalyst **B** gave a poor result (entry 3). Pretreatment of the substrate with 1 equiv. of TsOH, however, significantly improved the cyclization yield up to 66% (entry 4),¹⁵ thus indicating that masking of the nitrogen lone pair by protonation was crucial for the RCM of the substrate

14a. On the other hand, the substrate **14b** did not produce the desired product under the refluxing conditions (entries 6–8), although the reasons remained unclear. The MEM group of **15a** and **15c** was removed upon treatment with TFA, and finally alcohols **16a** and **16c** were subjected to Dess-Martin oxidation. Thus, the syntheses of the target compounds **3** and **5** were accomplished, except for the 4-pyridyl compound **4**.

Apoptosis inducing activity of new compounds synthesized in this study was investigated, and the results of comparison between 2-pyridyl (**2**) and 3-pyridyl (**3**) derivatives are presented in Table 2. To our regret, the benzimidazolyl derivative (**5**) did not exhibit the activity (data not shown). The assay was performed using a human lymphoma cell line (U937), and assessment of early apoptosis and secondary necrosis was carried out by flow cytometry of annexin V/FITC and propidium iodide (PI) staining cells.⁶ As shown in Table 2, a great disparity was observed between the 2-pyridyl and the 3-pyridyl compounds on the apoptosis-inducing effects. For example, the 2-pyridyl compound **2** induced apoptotic cell death (*ca.* 9% with 18% secondary necrosis) at 20 μ M concentration, while the 3-pyridyl compound **3** only gave the values of the control level at the same concentration. Although detailed mechanism of action remains unclear at present, these results suggest that the nitrogen atom on the pyridyl substituent installed at a suitable position may play an important role for interactions with a living substance controlling an apoptotic program. Further studies for elucidation of the action mechanisms of macrophelide analogues will be performed in our laboratory.

Table 2. Effects of the Compounds **2** and **3** on the Apoptotic Cell Death¹⁾

	control	compound 2			compound 3		
		5 μ M	20 μ M	40 μ M	5 μ M	20 μ M	40 μ M
early apoptosis	1.03	4.65	9.16	20.7	0.75	1.21	7.83
secondary necrosis	0.62	5.51	17.5	56.9	1.11	1.30	4.87

1) The data represent fraction of cells (%). Human lymphoma U937 cells were treated with 5–40 μ M concentrations of each compound for 12 h. Percentage of early apoptotic and secondary necrotic cells were measured by flow cytometry using annexin V-FITC and PI staining. Results are presented as means of trials.

EXPERIMENTAL

All nonaqueous reactions were carried out under an Ar atmosphere. Reagents were purchased from commercial sources and used as received. Anhydrous solvents were prepared by distillation over CaH₂, or purchased from commercial sources. ¹H and ¹³C NMR spectra were obtained on a Varian Gemini 300 instrument, using chloroform peak as an internal reference. Mass spectra were measured on a JEOL D-200 or a JEOL AX 505 mass spectrometer, and the ionization method was electron impact (EI, 70 eV). IR spectra were recorded on a JASCO FT/IR-460Plus spectrometer. Column chromatography was carried

out by employing Cica Silica Gel 60N (spherical, neutral, 40-50 μm or 63-210 μm). Aldehydes **6a** and **6b** were prepared by Wittig reaction of commercially available 3- or 4-formylpyridines in accordance with the reported procedure.⁶ Aldehyde **6c** was synthesized according to the reported method.¹⁰ Details of biological evaluation experiments have already been reported.⁶

Asymmetric Allylation of Compounds **6a–c**

(+)-Ipc₂B(allyl) (1.0 M pentane solution, 6.0 mL, 6 mmol) was added to a solution of the aldehyde **6** (4 mmol) in Et₂O (16 mL) at -100°C , and the mixture was stirred for 1 h at -100°C . After addition of MeOH (1.6 mL), the mixture was warmed to room temperature, and then 2-aminoethanol (2.4 mL, 40 mmol) was added. After stirring for 24 h, the mixture was diluted with sat. aq. NH₄Cl and extracted with CH₂Cl₂, dried over MgSO₄, and evaporated. The residue was purified by silica gel column chromatography to afford the compounds **7a–c** as colorless oil (**7a, b**) and colorless solid (**7c**). Optical purity was determined by a standard Mosher's ester analysis ($> 98\%$ ee). **7a**: Yield quant.; ¹H-NMR (CDCl₃): δ 8.50 (1H, s), 8.43 (1H, d, $J = 4.9$ Hz), 7.57 (1H, d, $J = 7.7$ Hz), 7.25 (1H, dd, $J = 7.7, 4.9$ Hz), 6.49 (1H, s), 5.91–5.77 (1H, m), 5.19 (1H, d, $J = 17$ Hz), 5.16 (1H, d, $J = 10$ Hz), 4.24 (1H, d, $J = 7.4$ Hz), 2.43 (2H, q, $J = 7.4$ Hz), 1.87 (3H, s); ¹³C-NMR (CDCl₃): δ 149.89, 147.15, 142.27, 135.85, 134.15, 133.22, 122.97, 121.69, 118.24, 75.98, 40.18, 14.01; IR (neat): 3276 cm⁻¹; MS (EI): m/z 189 (M⁺); HRMS Calcd for C₁₂H₁₅NO: 189.1154 (M⁺), found: 189.1158; $[\alpha]_{\text{D}}^{24} +16.9$ ($c = 1.165$, CHCl₃). **7b**: Yield 99%; ¹H-NMR (CDCl₃): δ 8.41 (2H, d, $J = 4.5$ Hz), 7.08 (2H, d, $J = 4.5$ Hz), 6.41 (1H, s), 5.79 (1H, ddt, $J = 17, 9.9, 7.1$ Hz), 5.10 (1H, d, $J = 17$ Hz), 5.05 (1H, d, $J = 9.9$ Hz), 4.59–4.25 (1H, br), 4.19 (1H, t, $J = 6.3$ Hz), 2.57 (2H, dd, $J = 7.1, 6.3$ Hz), 1.85 (3H, s); ¹³C-NMR (CDCl₃): δ 148.81, 145.50, 144.95, 134.24, 123.52, 122.34, 117.42, 75.79, 39.97, 14.19; IR (neat): 3031 cm⁻¹; MS (EI): m/z 189 (M⁺); HRMS Calcd for C₁₂H₁₅NO: 189.1154 (M⁺), found: 189.1143; $[\alpha]_{\text{D}}^{25} +23.9$ ($c = 1.050$, CHCl₃). **7c**: Yield 86%; mp 124–125°C; ¹H-NMR (CDCl₃): δ 7.62 (1H, s), 7.28 (1H, d, $J = 8.2$ Hz), 7.24 (1H, d, $J = 8.2$ Hz), 5.85–5.76 (1H, m), 5.14 (1H, dd, $J = 16, 1.4$ Hz), 5.09 (1H, dd, $J = 10, 1.9$ Hz), 4.85 (1H, t, $J = 6.6$ Hz), 3.71 (3H, s), 2.59–2.54 (4H, m), 2.59 (1H, brs), 2.00 (1H, br); ¹³C-NMR (CDCl₃): δ 151.90, 141.90, 138.24, 134.86, 120.10, 117.34, 116.14, 108.42, 73.64, 44.08, 29.82, 13.66; IR (KBr): 3162, 1522 cm⁻¹; MS (EI): m/z 216 (M⁺); HRMS Calcd for C₁₃H₁₄N₂O₂: 216.1038 (M⁺), found: 216.1025; $[\alpha]_{\text{D}}^{25} +45.0$ ($c = 1.20$, CHCl₃).

Dehydrative Condensation of Alcohol **7a–c** and Carboxylic Acid **8**

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide Hydrochloride (EDC, 2 mmol) was added to a stirred solution of the alcohol **7** (1 mmol), the carboxylic acid **8** (2 mmol), and 4-dimethylaminopyridine (DMAP, 0.1 mmol) in CH₂Cl₂ (5 mL) at 0°C under Ar atmosphere. After continuous stirring at room temperature

for several hours (monitored by TLC), the solvent was evaporated to leave a residue, which was triturated with ether and filtered through Celite. Evaporation of the solvent followed by chromatography on silica gel afforded the corresponding ester **9a–c** as colorless oil. **9a**: Yield quant.; $^1\text{H-NMR}$ (CDCl_3): δ 8.49 (1H, s), 8.44–8.43 (1H, m), 7.54 (2H, d, $J = 8.0$ Hz), 7.27–7.22 (1H, m), 6.45 (1H, s), 5.73 (1H, ddq, $J = 17, 15, 10$ Hz), 5.34 (1H, q, $J = 6.9$ Hz), 5.12 (1H, dd, $J = 17, 1.6$ Hz), 5.07 (1H, dd, $J = 10, 1.6$ Hz), 4.31–4.24 (1H, m), 2.54 (2H, dd, $J = 15, 6.9$ Hz), 2.48–2.36 (2H, m), 1.86 (3H, s), 1.19 (3H, d, $J = 6.0$ Hz), 0.87 (9H, s), 0.06 (3H, s), 0.04 (3H, s); $^{13}\text{C-NMR}$ (CDCl_3): δ 169.92, 149.58, 147.11, 137.67, 135.44, 132.72, 132.33, 123.65, 122.55, 117.45, 65.37, 44.63, 37.23, 25.53, 23.59, 17.72, 13.77, $-4.72, -5.11$; IR (neat): 1737 cm^{-1} ; MS (EI): m/z 389 (M^+); HRMS Calcd for $\text{C}_{22}\text{H}_{35}\text{NO}_3\text{Si}$: 389.2386 (M^+), found: 389.2357; $[\alpha]_{\text{D}}^{25} +20.8$ ($c = 1.115, \text{CHCl}_3$). **9b**: Yield quant.; $^1\text{H-NMR}$ (CDCl_3): δ 8.55 (2H, d, $J = 6.0$ Hz), 7.16 (2H, d, $J = 6.0$ Hz), 6.44 (1H, s), 5.80–5.65 (1H, m), 5.72 (1H, t, $J = 6.6$ Hz), 5.12 (1H, d, $J = 17$ Hz), 5.09 (1H, d, $J = 10$ Hz), 4.25–4.33 (1H, m), 2.48–2.36 (4H, m), 1.90 (3H, s), 1.20 (3H, d, $J = 6.0$ Hz), 0.87 (9H, s), 0.07 (3H, s), 0.05 (3H, s); $^{13}\text{C-NMR}$ (CDCl_3): δ 170.08, 148.76, 144.81, 139.94, 132.67, 124.70, 123.47, 117.72, 77.42, 65.50, 44.71, 37.33, 25.65, 23.71, 17.86, 14.19, $-4.59, -5.00$; IR (neat): 1735 cm^{-1} ; MS (EI): m/z 389 (M^+); HRMS Calcd for $\text{C}_{22}\text{H}_{35}\text{NO}_3\text{Si}$: 389.2386 (M^+), found: 389.2394; $[\alpha]_{\text{D}}^{24} +16.1$ ($c = 0.94, \text{CHCl}_3$). **9c**: Yield 97%; $^1\text{H-NMR}$ (CDCl_3): δ 7.64 (1H, s), 7.27 (1H, d, $J = 7.4$ Hz), 7.22 (1H, d, $J = 7.4$ Hz), 5.90 (1H, t, $J = 6.9$ Hz), 5.72–5.61 (1H, m), 5.05 (1H, dd, $J = 16, 1.4$ Hz), 5.00 (1H, dd, $J = 10, 1.9$ Hz), 4.24–4.20 (1H, m), 3.71 (3H, s), 2.60 (3H, s), 2.74–2.31 (4H, m), 1.14 (3H, d, $J = 6.0$ Hz), 0.86 (9H, s), 0.04 (3H, s), 0.01 (3H, s); $^{13}\text{C-NMR}$ (CDCl_3): δ 170.45, 152.15, 142.07, 135.20, 133.81, 133.30, 120.96, 117.66, 116.75, 108.45, 75.53, 65.63, 44.98, 41.11, 29.82, 25.76, 23.83, 13.71, $-4.58, -4.91$; IR (neat): 1732 cm^{-1} ; MS (EI): m/z 416 (M^+); HRMS Calcd for $\text{C}_{23}\text{H}_{36}\text{N}_2\text{O}_3\text{Si}$: 416.2464 (M^+), found: 416.2495; $[\alpha]_{\text{D}}^{26} +38.5$ ($c = 1.70, \text{CHCl}_3$).

Desilylation of TBS Ether **9a–c**

A 1 M solution of tetra-*n*-butylammonium fluoride (TBAF) in THF (2 mL, 2 mmol) was added to a stirred solution of the TBS ether **9** (1 mmol) in THF (2 mL) at room temperature under Ar atmosphere, and the mixture was stirred for several hours at room temperature (monitored by TLC). The solvent was evaporated off to leave a residue, which was dissolved in AcOEt and the resulting organic layer was washed with water and brine, and dried over MgSO_4 . Evaporation of the solvent left a residue, which was chromatographed on silica gel to give the corresponding alcohol **10a–c** as colorless oil. **10a**: Yield 69%; $^1\text{H-NMR}$ (CDCl_3): δ 8.50 (1H, s), 8.43 (1H, d, $J = 4.8$ Hz), 7.60 (1H, d, $J = 7.7$ Hz), 7.30–7.25 (1H, m), 6.45 (1H, s), 5.80–5.65 (1H, m), 5.30 (1H, t, $J = 6.7$ Hz), 5.15 (1H, d, $J = 18$ Hz), 5.09 (1H, d, $J = 10$ Hz), 4.16–4.09 (1H, m), 2.60–2.45 (4H, m), 1.88 (3H, s), 1.25 (3H, d, $J = 6.3$ Hz); $^{13}\text{C-NMR}$ (CDCl_3): δ 170.97, 149.31, 146.90, 135.67, 132.63, 132.38, 123.48, 122.68, 117.58, 77.62, 77.19, 63.81, 43.31, 37.17,

22.60, 13.79; IR (neat): 3309, 2974, 1733 cm^{-1} ; MS (EI): m/z 275 (M^+); HRMS Calcd for $\text{C}_{16}\text{H}_{21}\text{NO}_3$: 275.1521 (M^+), found: 275.1513; $[\alpha]_{\text{D}}^{26} +34.6$ ($c = 1.06$, CHCl_3). **10b**: Yield 87%; ^1H -NMR (CDCl_3): δ 8.38 (2H, d, $J = 5.9$ Hz), 7.01 (2H, d, $J = 5.9$ Hz), 6.32 (1H, s), 5.70–5.55 (1H, m), 5.24 (1H, t, $J = 6.9$ Hz), 5.00 (1H, d, $J = 17$ Hz), 4.96 (1H, d, $J = 10$ Hz), 4.30–4.19 (1H, br), 4.19–4.09 (1H, m), 2.47–2.27 (4H, m), 1.77 (3H, s), 1.13 (3H, d, $J = 6.3$ Hz); ^{13}C -NMR (CDCl_3): δ 170.92, 148.85, 144.42, 139.58, 132.53, 124.52, 123.32, 117.68, 63.80, 43.38, 37.15, 22.69, 14.08; IR (neat): 3010 cm^{-1} ; MS (EI): m/z 275 (M^+); HRMS Calcd for $\text{C}_{16}\text{H}_{21}\text{NO}_3$: 275.1521 (M^+), found: 275.1499; $[\alpha]_{\text{D}}^{26} +28.1$ ($c = 1.00$, CHCl_3). **10c**: Yield 83%; ^1H -NMR (CDCl_3): δ 7.65 (1H, s), 7.26 (1H, d, $J = 9.3$ Hz), 7.25 (1H, d, $J = 9.3$ Hz), 5.95 (1H, t, $J = 6.0$ Hz), 5.75–5.63 (1H, m), 5.09 (1H, dd, $J = 16$, 1.4 Hz), 5.04 (1H, dd, $J = 10$, 1.9 Hz), 4.12–4.09 (1H, m), 3.71 (3H, s), 3.00 (1H, br), 2.60 (3H, s), 2.75–2.46 (4H, m), 1.19 (3H, d, $J = 6.3$ Hz); ^{13}C -NMR (CDCl_3): δ 171.76, 152.32, 142.20, 135.33, 133.53, 133.21, 120.80, 117.85, 116.67, 108.58, 75.93, 64.04, 43.15, 41.02, 29.87, 22.49, 13.75; IR (neat): 3389, 1731, 1519 cm^{-1} ; MS (EI): m/z 302 (M^+); HRMS Calcd for $\text{C}_{17}\text{H}_{22}\text{N}_2\text{O}_3$: 302.1630 (M^+), found: 302.1631; $[\alpha]_{\text{D}}^{25} +65.2$ ($c = 1.00$, CHCl_3).

Esterification of Alcohol **10a–c** with Carboxylic Acid **11**

To a solution of the carboxylic acid **11** (1.2 mmol) and Et_3N (1.5 mmol) in toluene (10 mL) was added 2,4,6-trichlorobenzoyl chloride (1.2 mmol) at room temperature under Ar atmosphere, and the resulting mixture was stirred at room temperature for 0.5 h. The alcohol **10** (1 mmol) and DMAP (1 mmol) were added, and the reaction mixture was stirred for several hours. After the reaction completed (by TLC), the mixture was diluted with benzene, and washed with sat. aq. NaHCO_3 and brine, and dried over MgSO_4 . The solvent was evaporated off to leave a residue, which was chromatographed on silica gel to afford the corresponding ester **12a–c** as colorless oil. **12a**: Yield 78%; ^1H -NMR (CDCl_3): δ 8.69–8.51 (1H, br), 8.50–8.45 (1H, br), 7.60 (1H, d, $J = 7.7$ Hz), 7.35–7.25 (1H, m), 6.83 (1H, dd, $J = 16$, 6.3 Hz), 6.45 (1H, s), 5.95 (1H, d, $J = 16$ Hz), 5.79–5.65 (1H, m), 5.49–5.30 (2H, m), 5.13 (1H, d, $J = 18$ Hz), 5.09 (1H, d, $J = 10$ Hz), 4.72–4.66 (2H, m), 4.03 (1H, t, $J = 5.5$ Hz), 3.89–3.73 (3H, m), 3.66–3.51 (2H, m), 3.38 (3H, s), 2.76 (1H, dd, $J = 16$, 6.9 Hz), 2.55–2.50 (2H, m), 1.86 (3H, s), 1.33 (3H, d, $J = 6.3$ Hz), 1.14 (3H, d, $J = 6.3$ Hz), 0.86 (9H, s), 0.04 (3H, s), 0.02 (3H, s); ^{13}C -NMR (CDCl_3): δ 168.98, 164.84, 149.61, 147.24, 145.84, 137.84, 136.06, 132.92, 132.80, 124.00, 123.14, 117.97, 93.84, 79.85, 79.77, 78.13, 71.60, 70.51, 67.31, 67.17, 58.99, 41.07, 37.46, 25.84, 25.79, 19.94, 18.07, 14.03, –4.51, –4.71; IR (neat): 1722 cm^{-1} ; MS (EI): m/z 605 (M^+); HRMS Calcd for $\text{C}_{32}\text{H}_{51}\text{NO}_8\text{Si}$: 605.3384 (M^+), found: 605.3399; $[\alpha]_{\text{D}}^{26} -19.6$ ($c = 0.97$, CHCl_3). **12b**: Yield quant.; ^1H -NMR (CDCl_3): δ 8.60–8.52 (2H, br), 7.12 (2H, d, $J = 4.9$ Hz), 6.80 (1H, dd, $J = 16$, 6.3 Hz), 6.39 (1H, s), 5.93 (1H, d, $J = 16$ Hz), 5.69 (1H, ddd, $J = 17$, 10, 7.1 Hz), 5.38–5.28 (2H, m), 5.11 (1H, d, $J = 17$ Hz), 5.07 (1H, d, $J = 10$ Hz), 4.73–4.64 (2H, m), 4.02–3.99 (1H, m), 3.80–3.58 (2H, m), 3.52–3.49 (3H, m), 3.35 (3H, s), 2.73 (1H, dd, $J = 15$, 7.1 Hz), 2.54 (1H, dd, $J =$

16, 7.1 Hz), 2.50–2.44 (2H, m), 1.85 (3H, s), 1.31 (3H, d, $J = 6.3$ Hz), 1.12 (3H, d, $J = 6.3$ Hz), 0.84 (9H, s), 0.02 (3H, s), 0.00 (3H, s); ^{13}C -NMR (CDCl_3): δ 168.93, 164.82, 149.31, 145.88, 144.58, 139.60, 132.67, 125.09, 123.57, 123.11, 118.06, 93.81, 79.73, 77.92, 71.60, 70.50, 67.28, 67.16, 58.99, 41.05, 37.41, 25.79, 19.95, 18.06, 14.29, -4.51 , -4.72 ; IR (neat): 1732, 1717 cm^{-1} ; MS (EI): m/z 605 (M^+); HRMS Calcd for $\text{C}_{32}\text{H}_{51}\text{NO}_8\text{Si}$: 605.3384 (M^+), found: 605.3413; $[\alpha]_{\text{D}}^{25} -16.3$ ($c = 1.295$, CHCl_3). **12c**: Yield 72%; ^1H -NMR (CDCl_3): δ 7.63 (1H, s), 7.29 (1H, d, $J = 9.3$ Hz), 7.24 (1H, d, $J = 9.3$ Hz), 6.85 (1H, dd, $J = 13$, 6.3 Hz), 5.94 (1H, t, $J = 6.7$ Hz), 5.90 (1H, dd, $J = 16$, 1.4 Hz), 5.74–5.60 (1H, m), 5.33–5.30 (1H, m), 5.06 (1H, dd, $J = 15$, 1.4 Hz), 5.03 (1H, dd, $J = 10$, 1.9 Hz), 4.76–4.62 (2H, m), 4.03 (1H, t, $J = 5.2$ Hz), 3.71 (3H, s), 3.38 (3H, s), 3.89–3.60 (3H, m), 3.59–3.51 (2H, m), 2.60 (3H, s), 2.78–2.46 (4H, m), 1.28 (3H, d, $J = 6.3$ Hz), 1.15 (3H, d, $J = 6.3$ Hz), 0.86 (9H, s), 0.04 (3H, s), 0.02 (3H, s); ^{13}C -NMR (CDCl_3): δ 169.16, 164.86, 152.32, 145.67, 133.61, 133.22, 123.23, 120.98, 117.89, 116.88, 108.62, 93.88, 79.82, 75.98, 71.65, 70.54, 69.87, 67.42, 67.16, 58.99, 53.43, 41.07, 41.03, 29.95, 29.74, 25.82, 19.92, 18.09, 13.82, -4.50 , -4.69 ; IR (neat): 1713, 1643 cm^{-1} ; MS (EI): m/z 632 (M^+); HRMS Calcd for $\text{C}_{33}\text{H}_{52}\text{N}_2\text{O}_8\text{Si}$: 632.3538 (M^+), found: 632.3493; $[\alpha]_{\text{D}}^{25} +8.9$ ($c = 1.43$, CHCl_3).

Desilylation of TBS Ether **12a–c**

A 1 M solution of TBAF in THF (4 mL, 4 mmol) was added to a stirred solution of the TBS ether **12** (0.2 mmol) and acetic acid (0.3 mL, 5.2 mmol) in THF (1.2 mL) at room temperature under Ar atmosphere, and the mixture was stirred for 1 to 3 days at room temperature. The ethereal solution of the residue resulting from the evaporation of the solvent was washed with water, sat. aq. NaHCO_3 , and brine, and dried over MgSO_4 . Evaporation of the solvent left a residue, which was chromatographed on silica gel to give the corresponding alcohol **13a–c** as colorless oil. **13a**: Yield 86%; ^1H -NMR (CDCl_3): δ 8.50–8.49 (1H, br), 8.47–8.40 (1H, br), 7.55 (1H, d, $J = 7.6$ Hz), 7.25 (1H, dd, $J = 7.6$, 4.9 Hz), 6.82 (1H, dd, $J = 16$, 6.3 Hz), 6.43 (1H, s), 5.99 (1H, d, $J = 16$ Hz), 5.71 (1H, ddd, $J = 17$, 8.4, 7.1 Hz), 5.43–5.33 (2H, m), 5.12 (1H, d, $J = 8.4$ Hz), 5.08 (1H, d, $J = 8.4$ Hz), 4.74–4.65 (2H, m), 4.17–4.14 (1H, m), 3.92–3.56 (3H, m), 3.55–3.52 (2H, m), 3.37 (3H, s), 3.01–2.91 (1H, br), 2.73 (1H, dd, $J = 17$, 7.7 Hz), 2.57 (1H, dd, $J = 15$, 5.5 Hz), 2.46 (2H, dd, $J = 11$, 7.1 Hz), 1.84 (3H, s), 1.33 (3H, d, $J = 6.3$ Hz), 1.11 (3H, d, $J = 6.6$ Hz); ^{13}C -NMR (CDCl_3): δ 168.96, 164.74, 149.74, 147.30, 144.15, 137.84, 135.93, 132.82, 123.95, 123.58, 122.93, 117.95, 94.28, 80.80, 78.12, 71.58, 68.93, 68.85, 67.57, 67.44, 58.92, 41.13, 37.43, 20.00, 17.80, 14.08; IR (neat): 3422, 1719 cm^{-1} ; MS (EI): m/z 491 (M^+); HRMS Calcd for $\text{C}_{26}\text{H}_{37}\text{NO}_8$: 491.2519 (M^+), found: 491.2483; $[\alpha]_{\text{D}}^{25} -18.7$ ($c = 0.76$, CHCl_3). **13b**: Yield 83%; ^1H -NMR (CDCl_3): δ 8.52 (2H, d, $J = 4.9$ Hz), 7.12 (2H, d, $J = 4.9$ Hz), 6.81 (1H, dd, $J = 16$, 6.0 Hz), 6.39 (1H, s), 5.97 (1H, d, $J = 16$ Hz), 5.74–5.63 (1H, m), 5.38–5.30 (2H, m), 5.11 (1H, d, $J = 9.3$ Hz), 5.09 (1H, d, $J = 9.3$ Hz), 4.70–4.68 (2H, m), 4.63–4.14 (1H, m), 3.91–3.88 (1H, m), 3.66–3.60 (2H, m), 3.54–3.51 (2H, m), 3.36 (3H, s), 2.73 (1H,

dd, $J = 16$, 7.4 Hz), 2.56 (1H, dd, $J = 15$, 7.4 Hz), 2.49–2.44 (2H, m), 1.85 (3H, s), 1.32 (3H, d, $J = 6.3$ Hz), 1.09 (3H, d, $J = 6.6$ Hz); ^{13}C -NMR (CDCl_3): δ 168.95, 164.73, 149.29, 144.61, 144.03, 139.65, 132.67, 125.07, 123.57, 123.48, 118.06, 94.31, 80.75, 77.96, 71.58, 68.88, 67.50, 58.94, 41.08, 37.41, 19.97, 17.69, 14.29; IR (neat): 3441, 1719 cm^{-1} ; MS (EI): m/z 491 (M^+); HRMS Calcd for $\text{C}_{26}\text{H}_{37}\text{NO}_8$: 491.2519 (M^+), found: 491.2522; $[\alpha]_{\text{D}}^{24} -32.8$ ($c = 1.235$, CHCl_3). **13c**: Yield 56%; ^1H -NMR (CDCl_3): δ 7.70 (1H, s), 7.32 (1H, d, $J = 8.2$ Hz), 7.28 (1H, d, $J = 8.2$ Hz), 6.83 (1H, dd, $J = 16$, 6.4 Hz), 5.93 (1H, dd, $J = 16$, 1.4 Hz), 5.91 (1H, t, $J = 6.9$ Hz), 5.66–5.64 (1H, m), 5.38–5.35 (1H, m), 5.05 (1H, dd, $J = 17$, 1.6 Hz), 5.02 (1H, dd, $J = 10$, 1.1 Hz), 4.72–4.59 (2H, m), 4.20 (1H, br), 4.14 (1H, t, $J = 4.4$ Hz), 3.78 (3H, s), 3.38 (3H, s), 3.89–3.52 (5H, m), 2.75 (3H, s), 2.69–2.54 (4H, m), 1.28 (3H, d, $J = 6.3$ Hz), 1.14 (3H, d, $J = 6.3$ Hz); ^{13}C -NMR (CDCl_3): δ 168.96, 164.65, 144.61, 135.38, 133.66, 132.69, 123.18, 122.21, 118.00, 114.94, 109.52, 93.92, 80.30, 75.50, 71.43, 68.62, 67.18, 67.15, 58.72, 53.35, 52.63, 40.84, 40.74, 30.19, 19.76, 18.28, 12.57; IR (neat): 3424, 1731, 1652 cm^{-1} ; MS (EI): m/z 518 (M^+); HRMS Calcd for $\text{C}_{27}\text{H}_{38}\text{N}_2\text{O}_8$: 518.2618 (M^+), found: 518.2628; $[\alpha]_{\text{D}}^{24} +7.8$ ($c = 1.64$, CHCl_3).

Acryloylation of Alcohol **13a–c**

In accordance with the synthesis of the compounds **12**, acrylic acid (0.8 mmol) and the alcohol **13a–c** (0.2 mmol) gave the ester **14a–c** as colorless oil. **14a**: Yield 95%; ^1H -NMR (CDCl_3): δ 8.51 (1H, s), 8.48 (1H, d, $J = 4.6$ Hz), 7.60 (1H, d, $J = 8.0$ Hz), 7.29 (1H, dd, $J = 8.0$, 4.6 Hz), 6.81 (1H, dd, $J = 16$, 6.0 Hz), 6.45 (1H, s), 6.40 (1H, d, $J = 17$ Hz), 6.16 (1H, dd, $J = 17$, 10 Hz), 6.03 (1H, d, $J = 16$ Hz), 5.83 (1H, d, $J = 10$ Hz), 5.73 (1H, ddt, $J = 17$, 10, 8.2 Hz), 5.42–5.34 (1H, m), 5.37 (1H, t, $J = 6.7$ Hz), 5.13 (1H, d, $J = 8.4$ Hz), 5.09 (1H, d, $J = 8.4$ Hz), 4.79–4.72 (1H, m), 4.72–4.66 (2H, m), 4.40–4.36 (1H, m), 3.81–3.59 (2H, m), 3.54–3.51 (2H, m), 3.37 (3H, s), 2.76 (1H, dd, $J = 15$, 7.5 Hz), 2.57 (1H, dd, $J = 15$, 5.9 Hz), 2.54–2.48 (2H, m), 1.86 (3H, s), 1.35 (3H, d, $J = 6.3$ Hz), 1.22 (3H, d, $J = 6.6$ Hz); ^{13}C -NMR (CDCl_3): δ 169.01, 165.18, 164.66, 149.06, 146.73, 143.71, 143.43, 138.26, 136.61, 132.77, 130.99, 128.27, 124.02, 123.79, 118.10, 93.75, 78.15, 71.58, 71.40, 67.66, 67.26, 67.21, 59.04, 41.10, 37.49, 29.76, 20.00, 14.98, 14.11; IR (neat): 2934, 1723 cm^{-1} ; MS (EI): m/z 545 (M^+); HRMS Calcd for $\text{C}_{29}\text{H}_{39}\text{NO}_9$: 545.2625 (M^+), found: 545.6213; $[\alpha]_{\text{D}}^{25} -23.3$ ($c = 0.735$, CHCl_3). **14b**: Yield 98%; ^1H -NMR (CDCl_3): δ 8.61–8.50 (2H, br), 7.16 (2H, d, $J = 4.1$ Hz), 6.77 (1H, dd, $J = 15$, 6.0 Hz), 6.41 (1H, s), 6.40 (1H, d, $J = 17$ Hz), 6.10–6.00 (2H, m), 5.83 (1H, d, $J = 10$ Hz), 5.80–5.65 (1H, m), 5.40–5.29 (2H, m), 5.13–5.05 (3H, m), 4.72–4.64 (2H, m), 4.39–4.25 (3H, m), 3.81–3.56 (2H, m), 3.54–3.51 (2H, m), 3.37 (3H, s), 2.79–2.40 (2H, m), 1.87 (3H, s), 1.34 (3H, d, $J = 6.0$ Hz), 1.18 (3H, d, $J = 6.3$ Hz); ^{13}C -NMR (CDCl_3): δ 168.95, 164.60, 157.23, 154.76, 149.06, 145.54, 143.27, 139.96, 132.67, 131.04, 128.27, 127.95, 125.05, 124.00, 118.15, 100.56, 93.73, 77.97, 71.58, 67.63, 67.31, 59.82, 59.04, 41.07, 37.46, 33.75, 20.00, 15.07, 14.37; IR (neat): 1734, 1717 cm^{-1} ; MS (EI): m/z 545 (M^+); HRMS Calcd for $\text{C}_{29}\text{H}_{39}\text{NO}_9$: 545.2625 (M^+), found:

545.6208; $[\alpha]_{\text{D}}^{25} -23.3$ ($c = 0.735$, CHCl_3). **14c**: Yield 80%; $^1\text{H-NMR}$ (CDCl_3): δ 7.67 (1H, s), 7.27 (1H, d, $J = 6.9$ Hz), 7.24 (1H, d, $J = 6.9$ Hz), 6.76 (1H, dd, $J = 16, 6.2$ Hz), 6.39 (1H, d, $J = 17$ Hz), 6.01 (1H, t, $J = 10$ Hz), 6.14–5.90 (2H, m), 5.83 (1H, dd, $J = 10, 1.4$ Hz), 5.71–5.61 (1H, m), 5.34–5.30 (1H, m), 5.05 (1H, t, $J = 6.3$ Hz), 5.02 (1H, t, $J = 8.2$ Hz), 4.71–4.58 (2H, m), 4.38 (1H, t, $J = 5.6$ Hz), 3.74 (3H, s), 3.36 (3H, s), 3.79–3.50 (5H, m), 2.66 (3H, s), 2.78–2.49 (4H, m), 1.29 (3H, d, $J = 6.3$ Hz), 1.19 (3H, d, $J = 6.6$ Hz); $^{13}\text{C-NMR}$ (CDCl_3): δ 169.08, 165.21, 164.63, 153.79, 150.63, 143.32, 138.81, 132.12, 131.02, 128.27, 124.07, 118.76, 113.60, 110.41, 100.57, 93.67, 73.67, 71.60, 71.48, 67.58, 67.20, 65.87, 59.03, 41.02, 40.87, 30.94, 29.77, 20.02, 15.39; IR (neat): 1723 cm^{-1} ; MS (EI): m/z 572 (M^+); HRMS Calcd for $\text{C}_{30}\text{H}_{40}\text{N}_2\text{O}_9$: 572.2734 (M^+), found: 572.2684; $[\alpha]_{\text{D}}^{24} +10.5$ ($c = 0.65$, CHCl_3).

RCM of Substrates **14a–c**

Grubbs' ruthenium catalyst (second generation) (10 μmol) was added to a solution of substrate **14** (0.1 mmol) in CH_2Cl_2 (100 mL) under Ar atmosphere. For the substrate **14a**, pretreatment with TsOH hydrate (0.1 mmol) was performed in refluxing CH_2Cl_2 for 0.5 h. After reflux for 24 h on an oil bath, the solvent was evaporated to afford a residue, which was chromatographed on silica gel to give the compound **15a** and **15c** as colorless oil. **15a**: Yield 66%; $^1\text{H-NMR}$ (CDCl_3): δ 8.70–8.59 (2H, br), 8.14–8.09 (1H, br), 7.82–7.75 (1H, br), 6.78 (1H, dd, $J = 16, 7.4$ Hz), 6.73 (1H, dd, $J = 16, 7.4$ Hz), 6.59 (1H, s), 5.95 (1H, d, $J = 16$ Hz), 5.87 (1H, d, $J = 16$ Hz), 5.52–5.49 (1H, m), 5.34–5.24 (1H, m), 4.98–4.90 (1H, m), 4.77–4.71 (2H, m), 4.15–4.10 (1H, m), 3.81–3.65 (2H, m), 3.57–3.54 (2H, m), 3.39 (3H, s), 2.79–2.54 (4H, m), 1.95 (3H, s), 1.41 (3H, d, $J = 6.0$ Hz), 1.34 (3H, d, $J = 6.3$ Hz); $^{13}\text{C-NMR}$ (CDCl_3): δ 169.42, 168.88, 164.08, 151.31, 144.92, 143.64, 136.60, 131.04, 129.84, 128.10, 125.36, 124.31, 118.45, 94.02, 78.72, 71.55, 67.78, 67.39, 67.31, 59.03, 41.36, 37.12, 29.74, 19.79, 15.10, 14.19; IR (neat): 1722 cm^{-1} ; MS (EI): m/z 517 (M^+); HRMS Calcd for $\text{C}_{27}\text{H}_{35}\text{NO}_9$: 517.2311 (M^+), found: 517.2345. **15c**: Yield 98%; $^1\text{H-NMR}$ (CDCl_3): δ 7.70 (1H, s), 7.30 (1H, d, $J = 7.1$ Hz), 7.27 (1H, d, $J = 7.1$ Hz), 6.90–6.75 (2H, m), 6.05 (1H, d, $J = 17$ Hz), 6.01–5.95 (1H, m), 5.83 (1H, d, $J = 17$ Hz), 5.38–5.30 (1H, m), 5.15 (1H, q, $J = 6.9$ Hz), 4.77–4.65 (2H, m), 4.45–4.20 (1H, m), 3.77 (3H, s), 3.70–3.55 (4H, m), 3.39 (3H, s), 2.80–2.49 (4H, m), 1.35 (3H, d, $J = 6.3$ Hz), 1.28 (3H, d, $J = 6.3$ Hz); $^{13}\text{C-NMR}$ (CDCl_3): δ 169.92, 169.39, 165.31, 164.42, 144.66, 143.26, 142.48, 125.12, 124.29, 123.52, 120.72, 109.05, 93.91, 93.16, 74.17, 71.48, 71.22, 67.70, 67.16, 59.79, 58.91, 40.77, 34.64, 30.10, 29.22, 20.08, 16.80, 13.50; IR (neat): $1731, 1652\text{ cm}^{-1}$; MS (EI): m/z 544 (M^+); HRMS Calcd for $\text{C}_{28}\text{H}_{36}\text{N}_2\text{O}_9$: 544.2452 (M^+), found: 544.2421; $[\alpha]_{\text{D}}^{24} -13.4$ ($c = 1.94$, CHCl_3).

Removal of the MEM Group of Compounds **15a** and **15c**

Trifluoroacetic acid (TFA, 1 mL) was added to a solution of the MEM ether **15** (0.1 mmol) in CH_2Cl_2 (1

mL) at 0°C under Ar atmosphere. After continuous stirring for 24 h at room temperature, the reaction mixture was diluted with CH₂Cl₂ and washed by sat. aq. NaHCO₃, and then dried over MgSO₄. The solvent was evaporated to afford a residue, which was chromatographed on silica gel to give the alcohol **16a** and **16c** as colorless oil. **16a**: Yield 89%; ¹H-NMR (CDCl₃): δ 8.79–8.42 (2H, br), 8.15 (1H, br), 7.79–7.65 (1H, br), 6.87 (1H, dd, *J* = 16, 9.3 Hz), 6.84 (1H, dd, *J* = 16, 6.3 Hz), 6.66 (1H, s), 5.92 (2H, d, *J* = 16 Hz), 5.88–5.57 (1H, m), 5.39–5.32 (1H, m), 4.91–4.83 (1H, m), 4.50–4.21 (1H, br), 4.15–4.07 (1H, m), 2.83–2.64 (4H, m), 1.96 (3H, s), 1.40 (3H, d, *J* = 6.3 Hz), 1.30 (3H, d, *J* = 6.3 Hz); ¹³C-NMR (CDCl₃): δ 171.27, 166.46, 149.02, 138.38, 129.17, 124.53, 123.22, 122.76, 109.48, 102.04, 78.38, 75.16, 73.58, 69.26, 61.36, 47.71, 41.82, 37.98, 20.07, 18.08, 14.51; IR (neat): 3446, 1726 cm⁻¹; MS (EI): *m/z* 429 (M⁺); HRMS Calcd for C₂₃H₂₇NO₇: 429.1787 (M⁺), found: 429.1804; [α]_D²⁴ +34.5 (*c* = 0.31, MeOH). **16c**: Yield 84%; ¹H-NMR (CDCl₃): δ 7.77 (1H, s), 7.44 (1H, d, *J* = 7.1 Hz), 7.23 (1H, d, *J* = 7.1 Hz), 6.88 (1H, dd, *J* = 16, 5.2 Hz), 6.04 (1H, t, *J* = 5.5 Hz), 5.89 (1H, dd, *J* = 16, 1.4 Hz), 5.50–5.34 (1H, m), 4.91 (1H, t, *J* = 6.1 Hz), 4.43 (1H, dd, *J* = 8.2, 7.7 Hz), 4.23 (1H, t, *J* = 5.2 Hz), 3.79 (3H, s), 3.79–3.64 (1H, m), 2.72–2.26 (4H, m), 2.63 (3H, s), 1.39 (3H, d, *J* = 6.3 Hz), 1.22 (3H, d, *J* = 6.3 Hz), 0.97 (1H, br); ¹³C-NMR (CDCl₃): δ 169.16, 168.72, 164.95, 164.21, 164.09, 145.39, 143.37, 123.73, 122.63, 122.03, 121.54, 110.36, 75.90, 74.15, 73.64, 70.11, 67.15, 59.38, 53.36, 31.37, 29.32, 28.88, 19.61, 17.67; IR (neat): 3351, 1717, 1652 cm⁻¹; MS (EI): *m/z* 456 (M⁺); HRMS Calcd for C₂₄H₂₈N₂O₇: 456.1897 (M⁺), found: 456.1873; [α]_D²⁵ +25.6 (*c* = 0.88, CHCl₃).

DMP Oxidation of Alcohol **16a** and **16c** (Synthesis of Macrospinelide Analogues **3** and **5**)

To a solution of the alcohol **16** (0.1 mmol) in CH₂Cl₂ (2 mL) was added Dess-Martin periodinane (DMP, 0.2 mmol), and the mixture was stirred for several hours at room temperature (monitored by TLC). The reaction was quenched with sat. aq. NaHCO₃, and the mixture was extracted with CH₂Cl₂. The organic layer was dried over MgSO₄, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel to afford the compounds **3** (colorless oil) and **5** (colorless solid). **3**: Yield quant.; ¹H-NMR (CDCl₃): δ 8.01 (1H, d, *J* = 8.0 Hz), 7.79 (1H, d, *J* = 9.3 Hz), 7.45 (1H, dd, *J* = 9.3, 8.0 Hz), 7.26 (1H, d, *J* = 16 Hz), 7.06 (1H, dd, *J* = 15, 8.2 Hz), 6.92 (1H, s), 6.66 (1H, s), 6.57 (1H, d, *J* = 16 Hz), 6.13 (1H, d, *J* = 15 Hz), 5.63 (1H, t, *J* = 4.6 Hz), 5.42–5.32 (1H, m), 5.05 (1H, q, *J* = 7.1 Hz), 2.82–2.73 (4H, m), 2.22 (3H, s), 1.48 (3H, d, *J* = 7.1 Hz), 1.45 (3H, d, *J* = 6.9 Hz); ¹³C-NMR (CDCl₃): δ 176.95, 171.93, 170.10, 168.08, 151.76, 139.88, 129.85, 127.55, 125.98, 124.04, 123.51, 117.65, 109.29, 102.04, 98.70, 78.03, 70.16, 66.96, 55.49, 41.28, 37.97, 16.84, 14.51; IR (neat): 1718, 1704 cm⁻¹; MS (EI): *m/z* 427 (M⁺); HRMS Calcd for C₂₃H₂₅NO₇: 427.1631 (M⁺), found: 427.1647; [α]_D²⁴ +15.0 (*c* = 0.315, MeOH). **5**: Yield 75%; mp 128–130°C; ¹H-NMR (CDCl₃): δ 7.78 (1H, s), 7.35–7.26 (3H, m), 7.09–7.05 (1H, m), 6.69 (1H, d, *J* = 16 Hz), 6.15–6.10 (1H, m), 6.11 (1H, d, *J* = 16 Hz), 5.38–5.31 (1H,

m), 5.16 (1H, q, $J = 7.1$ Hz), 3.75 (3H, s), 2.80–2.70 (4H, m), 2.18 (3H, s), 1.52 (3H, d, $J = 7.1$ Hz), 1.33 (3H, d, $J = 6.3$ Hz); ^{13}C -NMR (CDCl_3): δ 197.51, 168.79, 164.60, 162.95, 145.55, 140.51, 133.81, 132.30, 131.49, 130.34, 127.32, 122.64, 121.01, 108.81, 75.03, 74.07, 68.47, 40.45, 29.72, 29.32, 28.87, 19.24, 16.54; IR (KBr): 1725, 1614 cm^{-1} ; MS (EI): m/z 454 (M^+); HRMS Calcd for $\text{C}_{24}\text{H}_{26}\text{N}_2\text{O}_7$: 454.1740 (M^+), found: 454.1717; $[\alpha]_{\text{D}}^{25} +20.9$ ($c = 0.82$, MeOH).

ACKNOWLEDGEMENTS

This work was supported by THE FUGAKU TRUST FOR MEDICINAL RESEARCH, and by Grant-in-Aid for Scientific Research (C) (No. 19590098) from Japan Society for the Promotion of Science (JSPS).

REFERENCES AND NOTES

- † Dedicated to Prof. Emeritus, Akira Suzuki, Hokkaido University, on the occasion of his 80th birthday.
1. M. Hayashi, Y.-P. Kim, H. Hiraoka, M. Natori, S. Takamatsu, T. Kawakubo, R. Masuma, K. Komiyama, and S. Ômura, *J. Antibiot.*, 1995, **48**, 1435; T. Sunazuka, T. Hirose, Y. Harigaya, S. Takamatsu, M. Hayashi, K. Komiyama, S. Ômura, P. A. Sprengeler, and A. B. Smith III, *J. Am. Chem. Soc.*, 1997, **119**, 10247; T. Yamada, M. Iritani, M. Doi, K. Minoura, T. Ito, and A. Numata, *J. Chem. Soc., Perkin Trans. 1*, 2001, 3046. For a review; Y. Matsuya and H. Nemoto, *Heterocycles*, 2005, **65**, 1741, and references cited therein.
 2. S. Takamatsu, Y.-P. Kim, M. Hayashi, H. Hiraoka, M. Natori, K. Komiyama, and S. Ômura, *J. Antibiot.*, 1996, **49**, 95.
 3. K. Ahmed, Q.-L. Zhao, Y. Matsuya, D.-Y. Yu, L. B. Feril Jr., H. Nemoto, and T. Kondo, *Chem. Biol. Interact.*, 2007, **170**, 86.
 4. K. Ahmed, Q.-L. Zhao, Y. Matsuya, D.-Y. Yu, T. L. Salunga, H. Nemoto, and T. Kondo, *Int. J. Hyperthermia*, 2007, **23**, 353.
 5. Y. Matsuya, T. Matsushita, K. Sakamoto, and H. Nemoto, *Heterocycles*, 2009, **77**, 483; K. Ahmed, Y. Matsuya, H. Nemoto, S. F. H. Zaidi, T. Sugiyama, Y. Yoshihisa, T. Shimizu, and T. Kondo, *Chem. Biol. Interact.*, 2009, **177**, 218.
 6. Y. Matsuya, T. Kawaguchi, K. Ishihara, K. Ahmed, Q.-L. Zhao, T. Kondo, and H. Nemoto, *Org. Lett.*, 2006, **8**, 4609; Y. Matsuya, Y. Kobayashi, T. Kawaguchi, A. Hori, Y. Watanabe, K. Ishihara, K. Ahmed, Z.-L. Wei, D.-Y. Yu, Q.-L. Zhao, T. Kondo, and H. Nemoto, *Chem. Eur. J.*, 2009, **15**, 5799.
 7. For recent review, K. C. Nicolaou, F. Roschangar, and D. Vourloumis, *Angew. Chem. Int. Ed.*, 1998, **37**, 2014; K.-H. Altmann, M. Wartmann, and T. O'Reilly, *Biochim. Biophys. Acta*, 2000, **1470**, M79.

8. K. C. Nicolaou, R. Scarpelli, B. Bollbuck, B. Werschkun, M. M. A. Pereira, M. Wartmann, K.-H. Altmann, D. Zaharevitz, R. Gussio, and P. Giannakakou, [*Chem. Biol.*, 2000, **7**, 593](#).
9. K.-H. Altmann, G. Bold, G. Caravatti, A. Flörsheimer, V. Guagnano, and M. Wartmann, [*Bioorg. Med. Chem. Lett.*, 2000, **10**, 2765](#); F. Cachoux, T. Isarno, M. Wartmann, and K.-H. Altmann, *Angew. Chem. Int. Ed.*, 2005, **44**, 7469; F. Cachoux, T. Isarno, M. Wartmann, and K.-H. Altmann, [*ChemBioChem*, 2006, **7**, 54](#).
10. K.-H. Altmann, G. Bold, G. Caravatti, and A. Flörsheimer, PCT Int. Appl. WO2000037473.
11. For a review, P. V. Ramachandran, *Aldrichimica Acta*, 2002, **35**, 23.
12. Absolute configurations were deduced from the empirical rule (see ref. 11).
13. J. Inanaga, K. Hirata, H. Saeki, T. Katsuki, and M. Yamaguchi, [*Bull. Chem. Soc. Jpn.*, 1979, **52**, 1989](#).
14. Y. Matsuya, T. Kawaguchi, and H. Nemoto, [*Org. Lett.*, 2003, **5**, 2939](#); T. Kawaguchi, N. Funamori, Y. Matsuya, and H. Nemoto, [*J. Org. Chem.*, 2004, **69**, 505](#).
15. V. Gracias, A. F. Gasielki, and S. W. Djuric, [*Org. Lett.*, 2005, **7**, 3183](#); Y. Chen, H. V. R. Dias, and C. J. Lovely, [*Tetrahedron Lett.*, 2003, **44**, 1379](#).