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The Total Synthesis of Piclavines A1–4 and their Biological Evaluation

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Abstract—The total synthesis of piclavines A1, A2, A3, and A4 has been achieved starting from compound **10** with enantiomeric enhancement. Their biological activities (antibacterial, antimicrobacterial, and antiviral activities and inhibition of cell growth) were evaluated. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

The indolizidine ring system is found in many biologically active and structurally interesting alkaloids.[1] Among these alkaloids, piclavines A1–4 (**1**–**4**), the first indolizidine alkaloids in the marine biosphere, extracted from the tunicate *Claveline picta* exhibit interesting antimicrobial activities.[2] So far, among four isomers of **1**–**4**, the synthesis of piclavine A4 (**4**) has been reported only once,[3] the synthesis of the other three isomers has never been reported. In addition, their biological activities have been evaluated as a mixture of piclavines A1–4.[2] Accordingly, we were stimulated to the development of a comprehensive synthetic program for these alkaloids. Our interest in this field has been focused on the potential strategies based on the enantiomeric enhancement[4] caused by the twofold or more application of the Sharpless asymmetric dihydroxylation (AD) reactions.[5] In this letter we describe a new synthesis of **1**–**4** with high enantiomeric purity via iterative AD reaction together with their biological activities (antibacterial, antimicrobacterial, and antiviral activities and inhibition of cell growth).

Synthesis

Recently we developed a general access to 5-substituted indolizidines **5** (all four stereoisomers of indolizidine 209D) with high enantio-enhancement (92–98% ee) via a sequence of 2-fold AD reactions starting from an achiral *N*-pentenylphthalimide (**6**).[6] The two stereogenic centers in **5** were constructed with high enantio-enhancement via a sequence of twofold AD reactions as shown in Scheme 1. We embarked on the synthesis of **1**–**4** using the synthetic intermediates **10** derived from (*R*)-**8**. According to our reported procedure,[6] the pyrrolidine (*R*)-**8** was successively subjected to $(DHQD)_2$-PYR[7] ligand-induced AD reaction followed by epoxidation, to give the epoxide (2*R*-[4*R*])-**10** together with its inseparable diastereomer.[8] The regioselective cleavage of the epoxide rings in **10** with lithium acetylide generated from 1-nonyne with *n*-butyl lithium in combination with BF_3–Et_2O to give the secondary alcohols **11**[8] in good yields. The *N*-protecting group exchange of benzyloxycarbonyl (Cbz) for 2,2,2-trichloroethoxycarbonyl (Troc) in **11** was carried out in a two-step sequence [1. iodotrimethylsilane (TMSI);[9] 2. $TrocCl/K_2CO_3$] to afford the Troc carbamates **12**.[8] After mesylation of [2*S*-(4*R*)]-**12**, *N*-deprotection of the resulting mesylate with 10% Cd–Pb[10] gave the desired (5*S*,9*R*-(−)-**13** $\{[\alpha]_D^{25}$ −67.5 (*c* 1.11, CH_2Cl_2)$\}$[11] as a major product and (5*S*,9*S*)-(−)-**13** $\{[\alpha]_D^{25}$ −3.11 (*c* 0.62, CH_3OH)$\}$ as a minor product in a ratio (3.6:1) in 84% yield. Having this result, similar sequence of the epoxide (2*R*-[4*S*])-**10** prepared by $(DHQ)_2$-PYR ligand-induced AD reaction of (*R*)-**8** afforded the desired (5*R*,9*R*)-(−)-**13** $\{[\alpha]_D^{25}$ +9.03 (*c* 0.32, CH_3OH)$\}$[11] as a major product and (5*R*,9*S*)-(−)-**13** $\{[\alpha]_D^{25}$ –22.7 (*c* 1.54, CH_2Cl_2)$\}$ as a minor product in a ratio (2.3:1) in 25% overall yield.

With two diastereomers (5*S*,9*R*)- and (5*R*,9*R*)-**13** in hand, we examined semi-reduction of their triple bonds. Exposure of hydrogen to (5*S*,9*R*)-**13** in the presence of Lindlar catalyst ($Pd/CaCO_3$/Pb) or Rosenmund catalyst (5% Pd–$BaSO_4$) was carried out in order to obtain *cis* olefin product. However, the hydrogenation using

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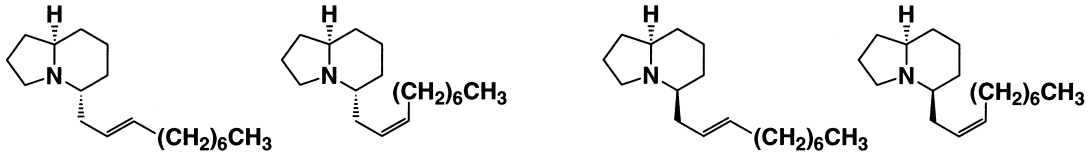
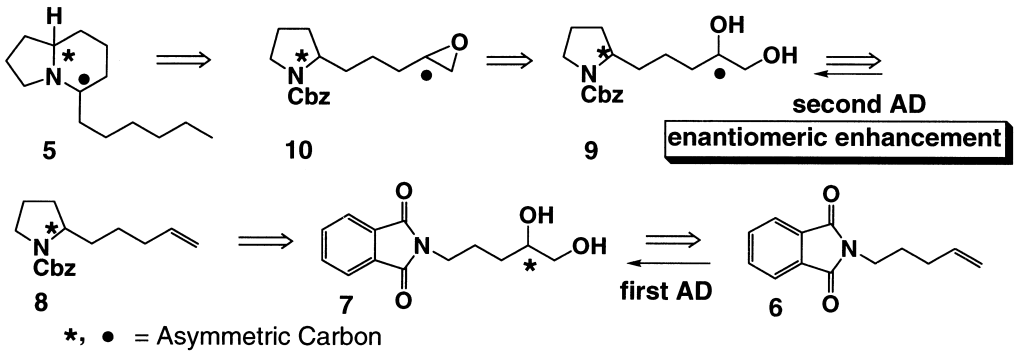
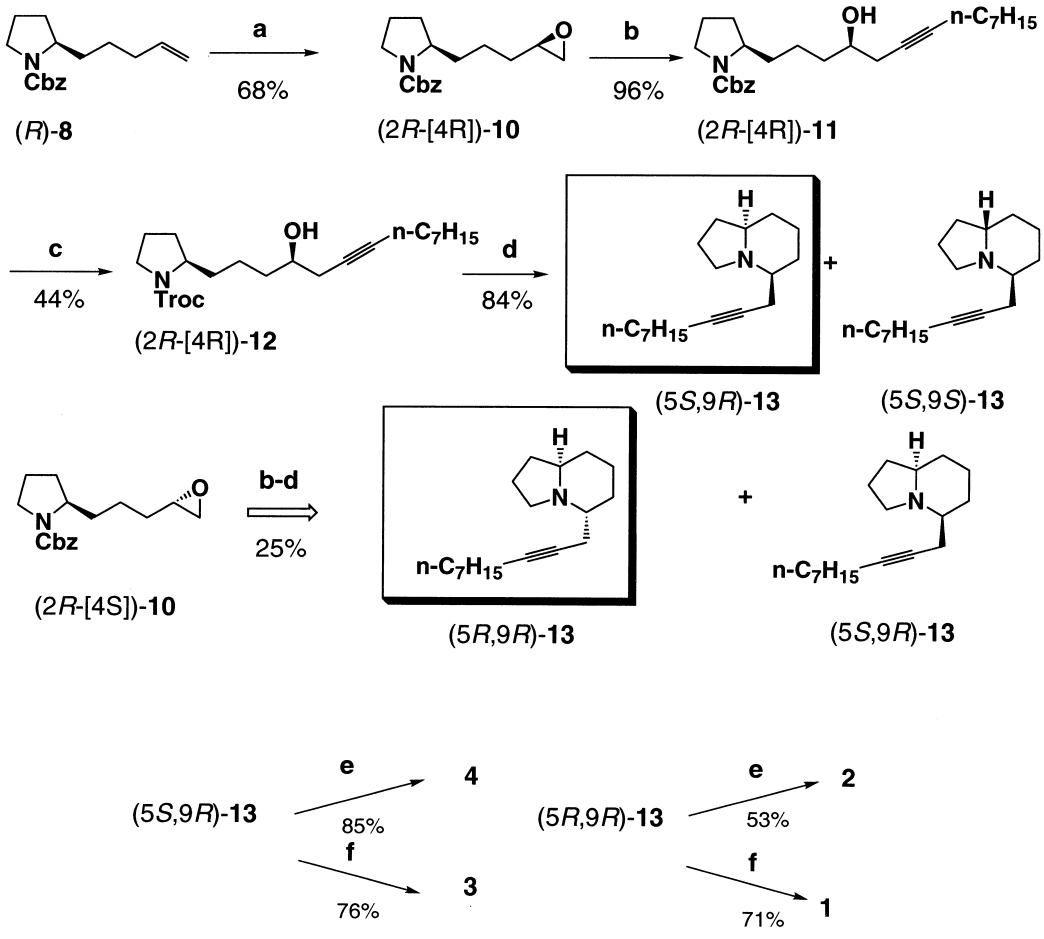


Chart 1.



Scheme 1.



Scheme 2. Reagents and conditions: (a) (1) AD-mix-β [$(DHQD)_2$-PYR ligand], (2) $(CH_3O)_3CCH_3$/PPTS, (3) CH_3COBr, (4) K_2CO_3/MeOH; (b) 1-nonyne/*n*-BuLi/BF_3–Et_2O; (c) (1) TMSI, (2) TrocCl/K_2CO_3; (d) (1) MsCl/pyridine, (2) 10% Cd–Pb; (e) H_2/cat. 5%/Pd–$BaSO_4$ (50 g%); (f) Na/liq. NH_3.

10 g% catalysts did not scarcely proceed, because the tertiary amine in **13** would presumably work as a poison of the catalyst. Accordingly, the use of the large amount (50 g%) of 5% Pd–$BaSO_4$ took place with semi-reduction of (5*S*,9*R*)-**13** to provide piclavine A4 (**4**) $[\alpha]_D^{25}$ −76.5 (*c* 0.63 CH_2Cl_2)} lit.[3] $[\alpha]_D^{20}$ −74.8 (*c* 0.5 CH_2Cl_2)} in 85% yield. Its spectral data were completely consistent with values reported.[3,12] Similarly piclavine A2 (**2**) {$[\alpha]_D^{25}$

+4.03 (ca. 0.21 CH_2Cl_2)}[13] was obtained from (5*R*,9*R*)-**13** in 53% yield. Next, treatment of (5*S*,9*R*)-**13** with sodium in liquid ammonia gave the desired piclavine A3 {$[\alpha]_D^{25}$ −74.3 (*c* 1.30, CH_2Cl_2)[14] containing a *trans*-olefin in 76% yield. In a similar manner (5*R*,9*R*)-**13** was reduced to convert into piclavine A1 {$[\alpha]_D^{25}$−5.6 (*c* 0.84, CH_2Cl_2)}[15] in 71% yield.

Biological evaluation

With all the four piclavines A1–4 (**1**–**4**) in hand, our attention was directed toward their biologically activity. At first, several antibacterial activities for **1**–**4** were tested.[16] Piclavines A1 and A2 showed weak antibacterial activities against S. *peneumoniae*, while piclavines A3 and A4 were almost inactive. Next, antimicrobial testing was examined.[17] Piclavine A2 was found to be feebly active against *C. albicans*. However, other **1**, **3** and **4** showed no pronounced activities against all strains tested. In antiviral test **1**–**4** displayed potent activities, having IC_{50} (1.45–4.84 mg/mL), against Influenza virus A/PR/8. However, all the four had no activities against Herpes simplex virus type 1 as shown Table 1. Finally, inhibition of cell growth was tested. As presented in Table 2, piclavines A1 and A2 inhibited somewhat stronger than A3 and A4 against both MDCK and Vero cells. As a result, it seems the geometry of double bonds in **1**–**4** had little effect on inhibition of cell growth. It was found that the four piclavines A1–4 had delicately their individual biological activities.

In summary, we achieved the first total synthesis of piclavines A1–3 (**1**–**3**) and the second synthesis of piclavine A4 (**4**) starting from an achiral *N*-pentenylphthalimide **6** with high enantio-enhancement via a sequence of twofold AD reactions. Several biological activities (antibacterial, antimicrobacterial, and antiviral activities and inhibition of cell growth) of **1**–**4** were evaluated.

Table 1. Antiviral activity of **1**–**4**. IC_{50} (μg/mL)[a]

	Herpes	Influenza
1	ND[b]	4.84
2	ND	3.72
3	ND	1.45
4	ND	4.20

[a]ND = Not detected owing to toxicity
[b]Culture medium: 2% FCS, 1% methylcellulose addition E′-MEM (Herpes): 1% BSA, 0.8% agar noble, 3 mg/mL, acetyl trypsin addition E′-MEM (Influenza). Virus amount of inoculation: 100 PFU/well (Herpes): 70 PFU/well (Influenza).

Table 2. Inhibition of cell growth of **1**–**4**. CC_{50} (μg/mL)[a]

	Vero	MDCK
1	9.18	8.92
2	7.67	9.28
3	42.3	20.1
4	40.9	17.1

[a]Culture medium: 10% FCS, E′-MEM. Cell amount of inoculation: 3×10^3 cell/well (vero). 2×10^3 cell/well (MDCK).

Acknowledgements

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References and Notes

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8. Only the major diastereomers (**10**–**12**) are shown in Scheme 2.
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11. Ees of (5*S*,9*R*)-**13** and (5*R*,9*R*)-**13** were estimated to be 98% ee and 94% ee, respectively, based on ref 6.
12. Data for **4**: $[\alpha]_D^{27}$ −76.4 (*c* 0.63, CH_2Cl_2); IR (neat) 2926, 2854, 2780, 754 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.87 (3 H, t, *J* = 6.32 Hz), 1.07–1.50 (14H, m), 1.61–2.12 (12H, m), 2.36–2.44 (1H, m), 3.25–3.31 (1H, m), 5.31–5.47 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.42, 20.75, 22.95, 24.92, 27.72, 29.47, 29.59, 29.91, 30.81, 31.28, 31.34, 32.14, 33.02, 51.92, 64.09, 65.24, 126.52, 131.59; HRMS calcd for $C_{18}H_{33}N$ (M^+) 263.2613, found 263.2591.
13. Data for **2**: $[\alpha]_D^{27}$ +4.03 (*c* 0.21, CH_2Cl_2); IR (neat) 2926, 2855, 803 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.88 (3H, t, *J* = 6.59 Hz), 1.12–1.86 (20H, m), 2.00–2.29 (4H, m), 2.45–2.47 (1H, m), 2.69 (1H, q, *J* = 8.79 Hz), 2.86 (1H, td, *J* = 3.30, 8.79 Hz), 2.99–3.04 (1H, m), 5.25–5.46 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.42, 1 9.55, 21.10, 22.96, 27.41, 27.71, 29.09, 29.50, 29.59, 29.97, 30.76, 32.13, 49.16, 55.65, 56.02, 127.477, 131.514; HRMS calcd for $C_{18}H_{33}N$ (M^+) 263.2613, found 263.2624.
14. Data for **3**: $[\alpha]_D^{26}$ −73.6 (*c* 1.30, CH_2Cl_2); IR (neat) 2926, 2854, 2780, 1458, 1437, 1129, 968 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.87 (3H, t, *J* = 6.59 Hz), 1.04–1.49 (13H, m), 1.56–2.02 (12H, m), 2.38–2.44 (1H, m), 3.26 (1H, td, *J* = 2.20, 8.52 Hz), 5.36–5.47 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.42, 20.73, 22.95, 24.89, 29.41, 29.44, 29.84, 30.76, 31.25, 32.13, 32.93, 38.56, 51.90, 64.10, 65.26, 127.11, 132.74; HRMS calcd for $C_{18}H_{33}N$ (M^+) 263.2613, found 263.2634.
15. Data for **1**: $[\alpha]_D^{26}$ −5.60 (*c* 0.84, CH_2Cl_2); IR (neat) 2925, 2853, 1458, 967, 754 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.87 (3 H, t, *J* = 6.59 Hz), 1.08–1.85 (17H, m), 1.94–2.11 (3H, m), 2.23–2.29 (1H, m), 2.38–2.48 (1H, m), 2.65 (1H, q, *J* = 8.79 Hz), 2.83 (1H, td, *J* = 3.11, 8.65 Hz), 2.96–3.02 (1H, m), 5.25–5.46 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.43, 19.44, 21.14, 22.95, 26.73; HMRS calcd for $C_{18}H_{33}N$ (M^+) 263.2613, found 263.2585
16. Bacteria strains such as *S. aureus* FDA209P, *S. aureus* F-1924, *E. coli* NIHJÅA, *S. macrescens* IID 620, *P. aeruginosa* IFO3445, *P. aeruginosa* S-1165, *E. faecalis* D-617, and *S. pneuemonia* IID553 were used.
17. Fungi such as *C. albicans* TIMM 1623, *A. fumigatus* TIMM 0063, *C. neoformans* TIMM 0354 were used.