Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron 63 (2007) 2798–2804
Tetrahedron

Total synthesis of (+)-FR-900493 and establishment of its absolute stereochemistry☆

Shinpei Hirano, Satoshi Ichikawa* and Akira Matsuda*

Faculty of Pharmaceutical Sciences, Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan

Received 9 January 2007; revised 24 January 2007; accepted 25 January 2007
Available online 30 January 2007

Abstract—The first total synthesis of (+)-FR-900493, an antibacterial nucleoside antibiotic possessing a 6′-*N*-alkyl-5′-*O*-aminoribosyl-glycyluridine structure, is described, and its relative and absolute stereochemistries were established. Key elements of the approach include the early-stage introduction of the aminoribose moiety and two sequential reductive alkylations of an amino group at the 6′-position. This synthetic strategy could be applicable to the synthesis of related nucleoside antibiotics, such as the more potent antibacterial nucleoside antibiotics, muraymycins.

1. Introduction

As modern clinical settings and practices change, the consequent spread of infectious diseases caused due to drug-resistant bacterial strains is causing considerable concern to the world public health community.[1] The need for antibacterial drugs with new mechanisms of actions is critical given the global implications. FR-900493 (Fig. 1, **1**), which was isolated from the culture broth of *Bacillus cereus* in 1989, belongs to a class of antibacterial nucleoside antibiotics that are active against both Gram-positive and Gram-negative bacteria.[2] Muraymycins (MRYs), which are structurally related antibiotics,[3] exhibited more promising antibacterial activity, and the mode of action is considered to be inhibitors of MraY (translocase I), which is involved in the biosynthesis of the bacterial cell wall peptidoglycan. Attention, therefore, has been focused on MraY as a new target for the development of antibacterial agents[4,5] since this enzyme is highly conserved across bacterial species and is essential for the growth of the bacteria.[6] Because of their structural and biological similarities, it has been suggested that FR-900493 might possess the same mode of action as the MRYs. Having the lowest molecular weight and simplest chemical structure of these natural inhibitors, FR-900493 theoretically should be a good lead for the development of novel antibacterial agents. It possesses a 6′-*N*-alkyl-5′-*O*-aminoribosyl-glycyluridine structure, and chemical studies on the compound have established its two-dimensional structure but not its relative and absolute stereochemistries. We anticipated that **1** would possess a similar stereochemistry to that of the MRYs at the 5′- and 6′-position, as shown in Figure 1. Herein, we report the first total synthesis of

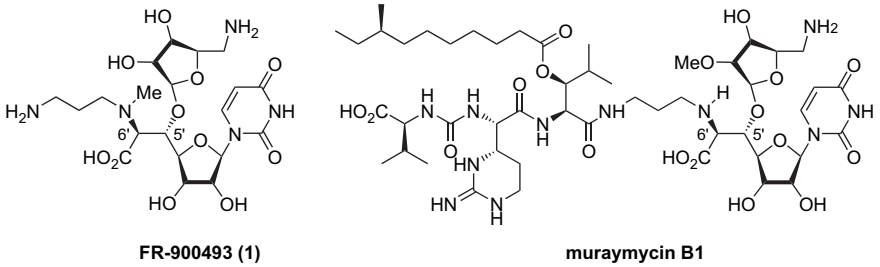


Figure 1. Structures of FR-900493 and muraymycin B1.

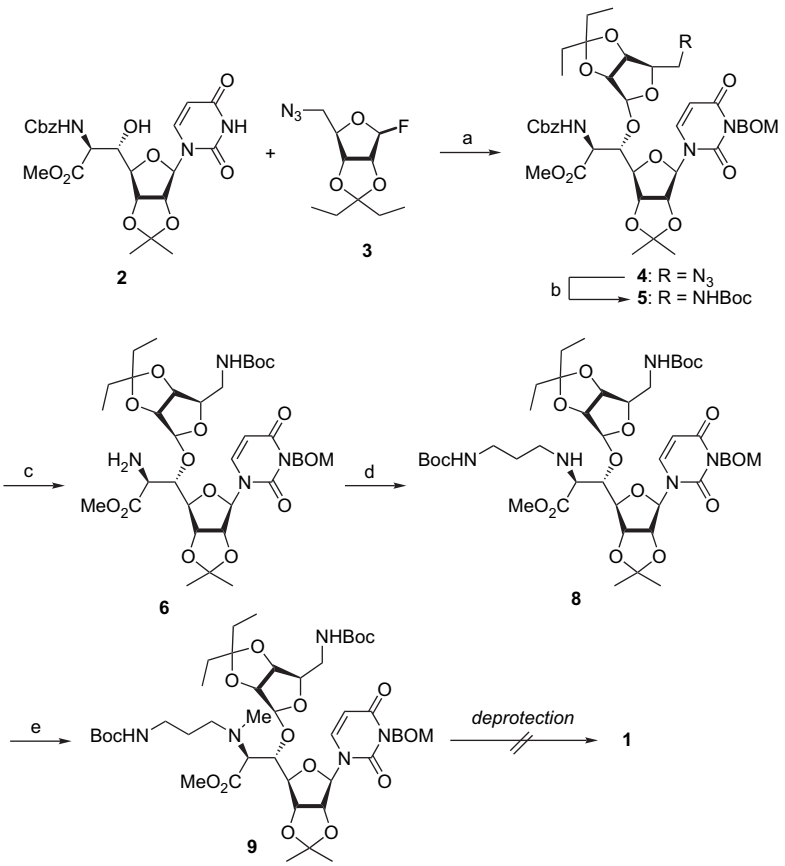
☆ This work was supported financially by a Grant-in-Aid from the Ministry of Education, Science, Sports, and Culture. We thank Fujisawa Pharmaceutical for generous supply of a sample of natural FR-900493. This paper constitutes Part 246 of Nucleosides and Nucleotides: for part 245 in this series, see: Hikishima, S.; Minakawa, N.; Kuramoto, K.; Ogata, S.; Matsuda, A. *Chembiochem* **2006**, *7*, 1970–1975.
* Corresponding authors. Tel.: +81 11 706 3228; fax: +81 11 706 4980 (A.M.); tel.: +81 11 706 3763; fax: +81 11 706 4980 (S.I.); e-mail addresses: ichikawa@pharm.hokudai.ac.jp; matuda@pharm.hokudai.ac.jp

0040–4020/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2007.01.055

FR-900493, and the determination of its relative and absolute stereochemistries.

2. Results and discussions

The initial attempts to synthesize **1** are outlined in Scheme 1. During the course of our first total synthesis of (+)-caprazol, a core structure of the antituberculosis antibiotics caprazamycins, a precursor of the key 5′-*O*-aminoribosyl-glycyluridine **4** was synthesized by β-selective ribosylation of **2** with a fluoride donor **3** as previously reported.[7] Namely, the ribosyl fluoride **3**[8] protected with a 3-pentylidene group was activated with $BF_3 \cdot Et_2O$[9] at −30 °C in the presence of **2**, and the corresponding ribosides were obtained in 80% yield with good β-selectivity (α/β=4/96). The pure β-riboside **4** was easily obtained by simple crystallization from AcOEt. The azide group in **4** was reduced to the corresponding amine with PPh_3 in aqueous THF, which was protected with a Boc group to give **5**. Hydrogenolysis of the Cbz group of **5** catalyzed by $Pd(OH)_2$–C afforded the amine **6** in 90% yield. For the installation of the 3-aminopropyl group at the $N^{6'}$-position of **6**, first we attempted a Mitsunobu reaction using the corresponding $N^{6'}$-*o*-nitrobenzenesulfonyl (Ns) derivative of **6**, the chemistry of which was developed by Fukuyama et al.[10] However, sulfonylation of **6** with NsCl and Et_3N in CH_2Cl_2 resulted in elimination at the 5′- and 6′-position. It was suggested that the increased acidity at the 6′-proton by sulfonylation of the 6′-amino group promoted the β-elimination of the aminoribosyloxy group. In addition, the β-elimination might have been thermodynamically favored because steric hindrance was reduced by releasing the sterically encumbered aminoribose moiety installed at the $O^{5'}$-position. Previous study revealed that this class of compounds including **5** was also unstable toward basic conditions resulting in the abovementioned β-elimination.[11] Therefore, we planned to introduce the 3-aminopropyl group at the $N^{6'}$-position by reductive alkylation conducted under weakly acidic conditions.[12] Treatment of the amine **6** and 1 equiv of *N*-Boc-3-aminopropanal **7** with sodium triacetoxyborohydride in dichloromethane in the presence of acetic acid gave selectively the desired mono-alkylated compound **8** in 92% yield. In this reaction, the use of excess **7** resulted in dialkylation of **6**. Subsequent N-methylation by a second reductive alkylation of **8** with paraformaldehyde gave the fully protected FR-900493 **9** in 80% yield. However, deprotection of **9** under various conditions was unsuccessful due to the difficulties in deprotection of the BOM group at the N^3-position of the uracil moiety and hydrolysis of the methyl ester at the 7′-position. Catalytic hydrogenation of **9** to remove the BOM group was a slow process, and elongation of the reaction time and/or harsher conditions resulted in the formation of 5,6-dihydrouridine derivative due to over-reduction of the uracil moiety. Presumably, the tertiary amine at the 6′-position poisons the catalysis. For the hydrolysis of the methyl ester moiety, treatment of **9** with $Ba(OH)_2$ in aqueous THF resulted in decomposition of the material to give the elimination products. Thus, treatment of **9** under basic conditions should be avoided throughout the synthesis.



Scheme 1. Reagents and conditions: (a) $BF_3 \cdot Et_2O$, MS 4 Å, CH_2Cl_2 (80%); (b) Ph_3P, aq THF–benzene, then Boc_2O (two steps, 95%); (c) H_2, $Pd(OH)_2$–C, MeOH (90%); (d) $BocHNCH_2CH_2CHO$ **7**, $NaBH(OAc)_3$, AcOH, CH_2Cl_2 (92%); (e) $(CH_2O)_n$, $NaBH(OAc)_3$, AcOH, CH_2Cl_2 (80%).

In order to avoid problems associated with deprotection, we next planned to remove the BOM group at the N^3-position before the N-alkylations of the 6′-amino group and deprotect all the protecting groups under acidic conditions as shown in Scheme 2. The methyl ester of **5** was first converted to the *tert*-butyl ester **10** by hydrolysis followed by *tert*-butylation of the resulting carboxylic acid with *O*-*tert*-butyl trichloroacetimidate[13] in the presence of $BF_3 \cdot Et_2O$ in CH_2Cl_2 (two steps, 46%). Hydrogenation of **10** resulted in selective deprotection of the Cbz group and the BOM group was not removed. The liberated amine moiety was then protected with a Troc group to give **11** in 79% yield in two steps. The reduction of **10** under more forcing conditions gave results similar to those mentioned above. Next, selective hydrogenolysis of the BOM group at the N^3-position without affecting the Troc group possessing chlorine atoms was examined (Table 1). Under standard conditions using $Pd(OH)_2$–C catalyst in AcOEt or MeOH (entries 1 and 2), reduction of the chlorine atom(s) of the Troc group was observed in addition to the desired BOM deprotection, and a complex mixture of products resulted. It has been reported that the *O*-benzyl group was selectively deprotected in the presence of a Troc group by hydrogenolysis using trichloroacetic acid (TCA) as an additive.[14] Therefore, we carried out the deblocking of the BOM group in the presence of 2,2,2-trichloroethanol (TCE) and obtained **12** in 45% yield (entry 3). Moreover, the use of 2,2,2-trichloroethyl chloroformate (TrocCl) improved the yield of **12** to 77% yield (entry 4). The use of 10 equiv of TCA as an additive proved to be most effective suppressing the collapse of the Troc group to furnish the desired **12** in 92% yield (entry 5). From these results, it would appear that the additive acts not only as a dummy of the Troc group but also as a promoter of the reaction. Treatment of **12** with Zn powder to deprotect the Troc group smoothly afforded the amine **13**. As in the procedure described in the synthesis of **9**, the amine **13** was alkylated by two sequential reductive alkylations to give **15** (80 and 70%, respectively). Finally, a global deprotection of **15** with aqueous 80% TFA successfully afforded (+)-(**1**), $[\alpha]_D^{25}$ +9.4 (*c* 0.3, H_2O) [lit.[2] $[\alpha]_D^{19}$ +11 (*c* 0.7, H_2O)], the properties of which were identical in all respects to those of the natural material. Especially, chemical shifts and coupling constants of H-5′ and H-6′ of synthetic **1** are in good accordance with those of the natural material. Under global deprotection conditions, the conversion was clean and quantitative, and there was no problem with β-elimination. Thus, the correlations of the synthetic and natural **1** confirm the two-dimensional structural assignments, and establish the relative and absolute stereochemistries as those shown in Figure 1.

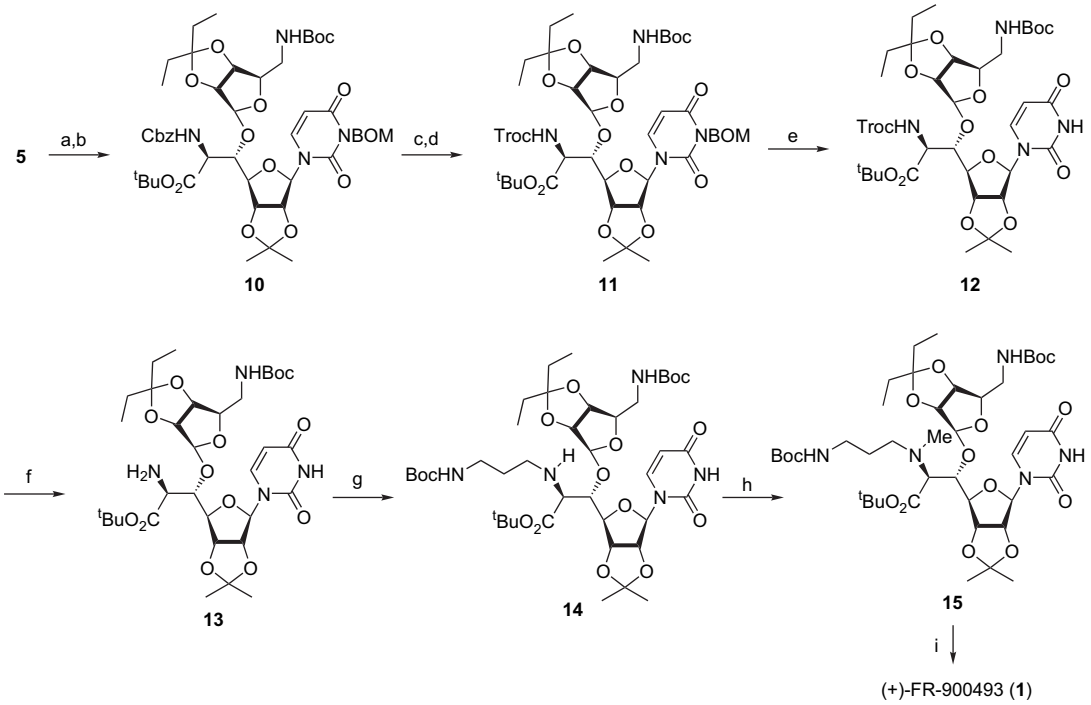
Table 1. Optimization of deprotection conditions of BOM group

Entry	Solvent	Additive (equiv)	Time (min)	Yield of **12** (%)
1	AcOEt	None	120	8
2	MeOH	None	20	30
3	MeOH	TCE (20)	20	45
4	MeOH	TrocCl (10)	20	77
5	MeOH	TCA (10)	20	92

TCE: 2,2,2-trichloroethanol; TrocCl: 2,2,2-trichloroethyl chloroformate; TCA: trichloroacetic acid.

3. Conclusions

The first total synthesis of FR-900493 (+)-(**1**) has been accomplished in 17 steps from uridine and its absolute stereochemistry was determined to be 1′*R*,2′*R*,3′*R*,4′*S*,5′*S*,6′*S*,1″*S*,2″*R*,3″*R*,4″*R*. This synthetic strategy could be applicable to



Scheme 2. Reagents and conditions: (a) $Ba(OH)_2 \cdot 8H_2O$, aq THF; (b) $^tBuOC(NH)CCl_3$, $BF_3 \cdot Et_2O$, CH_2Cl_2 (two steps, 46%); (c) H_2, Pd–C, MeOH; (d) TrocCl, Et_3N, CH_2Cl_2 (two steps, 79%); (e) H_2, $Pd(OH)_2$–C, TCA, MeOH (92%, see Table 1); (f) activated Zn powder, NH_4Cl, MeOH (95%); (g) **8**, $NaBH(OAc)_3$, AcOH, CH_2Cl_2 (80%); (h) $(CH_2O)_n$, $NaBH(OAc)_3$, AcOH, AcOEt (70%); (i) aq 80% TFA (quant).

the synthesis of the MRYs, more potent antibacterial nucleoside antibiotics. Continuous efforts to develop novel antibacterial agents are underway and will be reported in due course.

4. Experimental

4.1. General experimental methods

NMR spectra were obtained on a JEOL EX270, JEOL GX270, JEOL AL400, or Bruker ARX-500, and reported in parts per million (δ) relative to tetramethylsilane (0.00 ppm) as internal standard otherwise noted. Coupling constant (J) was reported in hertz (Hz). Abbreviations of multiplicity were as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Data were presented as follows: chemical shift (multiplicity, integration, coupling constant). Assignment was based on ^{1}H–^{1}H COSY, HMBC, and HMQC NMR spectra. Optical rotations were recorded on JASCO DIP-370 digital polarimeter or JASCO P-1030 polarimeter. FABMS was obtained on a JEOL JMS-HX101 or JEOL JMS-700TZ. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60F_{254} plates. Normal-phase column chromatography was performed on Merck silica gel 5715 or Kanto Chemical silica gel 60N (neutral). Flash column chromatography was performed on Merck silica gel 60.

4.1.1. Amine (6). A mixture of **5** (50 mg, 0.054 mmol) and 10% $Pd(OH)_2$–C (10 mg) in MeOH (2 mL) was vigorously stirred under H_2 atmosphere at room temperature for 30 min. The catalyst was filtered off through Celite pad and the filtrate was concentrated in vacuo. The residue was purified by neutral silica gel column chromatography (2×10 cm, 66% AcOEt–hexane) to afford **6** (38 mg, 90%) as a white foam: $[\alpha]_D^{21}$ +79.4 (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38–7.22 (m, 6H, Ar–*H*, H-6), 5.74 (m, 2H, H-5, N*H*Boc), 5.53 (s, 1H, H-1′), 5.50 (d, 1H, –NCH_aO–, J=9.9 Hz), 5.42 (d, 1H, –NCH_bO–, J=9.9 Hz), 5.10 (d, 1H, H-2′, $J_{2',3'}$=6.0 Hz), 5.01 (s, 1H, H-1″), 4.88 (m, 1H, H-3′), 4.69 (s, 2H, benzyl), 4.55 (m, 2H, H-2″, H-4″), 4.48 (m, 1H, H-3″), 4.31 (d, 1H, H-5′, $J_{5',6'}$=8.3 Hz), 4.22 (m, 1H, H-4′), 3.76 (s, 3H, OMe), 3.70 (m, 1H, H-6′), 3.20 (m, 1H, H-5″a), 3.11 (m, 1H, H-5″b), 1.58 (s, 3H, acetonide), 1.53 (m, 4H, CH_2CH$_3$×2), 1.44 (s, 9H, *t*-Bu), 1.36 (s, 3H, acetonide), 0.76 (m, 6H, CH$_2$CH_3×2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.50, 162.34, 156.01, 150.69, 141.67, 137.63, 128.20, 127.65, 116.14, 114.58, 112.63, 102.08, 96.64, 88.08, 87.01, 86.39, 84.51, 82.05, 81.75, 81.44, 78.99, 72.31, 70.26, 55.57, 52.68, 43.21, 29.38, 28.72, 28.44, 27.14, 25.28, 8.48, 7.28; FABMS-LR *m*/*z* 791 (MH^+); FABMS-HR (NBA) calcd for $C_{38}H_{55}N_4O_{14}$ 791.3715, found 791.3725.

4.1.2. Monoalkylamine (8). A mixture of **6** (20 mg, 0.025 mmol), aldehyde **7** (4.3 mg, 0.025 mmol), and AcOH (7 μL, 0.13 mmol) in CH_2Cl_2 (500 μL) was treated with $NaBH(OAc)_3$ (21 mg, 0.10 mmol) at room temperature for 10 min. The mixture was partitioned between AcOEt and saturated aqueous $NaHCO_3$. The organic phases were washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by neutral silica gel column chromatography (1×5 cm, 50% AcOEt–hexane) to afford **8** (22 mg, 92%) as a white foam: $[\alpha]_D^{21}$ +13.1 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38–7.28 (m, 5H, Ar–*H*), 7.17 (d, 1H, H-6, $J_{6,5}$=8.0 Hz), 5.71 (d, 1H, H-5, $J_{5,6}$=8.0 Hz), 5.63 (m, 1H, N*H*Boc), 5.46 (d, 1H, –NCH_aO–, J=9.9 Hz), 5.44 (s, 1H, H-1′), 5.41 (d, 1H, –NCH_bO–, J=9.9 Hz), 5.15 (d, 1H, H-2′, $J_{2',3'}$=6.1 Hz), 4.92 (s, 1H, H-1″), 4.87 (dd, 1H, H-3′, $J_{3',2'}$=6.1 Hz, $J_{3',4'}$= 4.4 Hz), 4.68 (s, 2H, benzyl), 4.59 (m, 1H, H-4″), 4.53 (d, 1H, H-2″, $J_{2'',3''}$=5.9 Hz), 4.48 (d, 1H, H-3″, $J_{3'',2''}$=5.9 Hz), 4.19 (m, 1H, H-4′), 4.18 (m, 1H, H-5′), 3.74 (s, 3H, OMe), 3.41 (m, 1H, H-6′), 3.19 (m, 3H, H-5″a, H-5″b, H-10′a), 3.03 (m, 1H, H-10′b), 2.87 (m, 1H, H-8′a), 2.35 (m, 1H, H-8′b), 1.57 (s, 3H, acetonide), 1.51 (m, 2H, H-9′a, H-9′b), 1.44 (m, 22H, *t*-Bu×2, CH_2CH$_3$×2), 1.38 (s, 3H, acetonide), 0.76 (m, 3H, CH$_2$CH_3), 0.70 (m, 3H, CH$_2$CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.52, 162.36, 155.94, 150.73, 142.17, 137.58, 128.20, 127.69, 115.92, 114.49, 113.18, 101.97, 97.70, 88.94, 86.64, 86.32, 84.89, 82.59, 81.99, 81.80, 78.90, 72.32, 70.22, 62.21, 52.44, 45.22, 43.33, 37.57, 29.42, 28.66, 28.50, 28.46, 28.41, 28.37, 26.76, 25.14, 8.50, 7.20; FABMS-LR *m*/*z* 948 (MH^+); FABMS-HR (NBA) calcd for $C_{46}H_{70}N_5O_{16}$ 948.4817, found 948.4827.

4.1.3. Dialkylamine (9). A mixture of **8** (24 mg, 0.025 mmol), paraformaldehyde (7.5 mg, 0.25 mmol), and AcOH (14 μL, 0.25 mmol) in CH_2Cl_2 (1 mL) was treated with $NaBH(OAc)_3$ (26 mg, 0.14 mmol) at room temperature for 72 h. The mixture was partitioned between AcOEt and saturated aqueous $NaHCO_3$. The organic phase was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by neutral silica gel column chromatography (1×10 cm, 40% AcOEt–hexane) to afford **9** (19 mg, 80%) as a white foam: $[\alpha]_D^{21}$ +10.0 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38–7.27 (m, 5H, Ar–*H*), 7.18 (d, 1H, H-6, $J_{6,5}$=8.0 Hz), 5.71 (d, 1H, H-5, $J_{5,6}$=8.0 Hz), 5.60 (t, 1H, N*H*Boc, J=7.5 Hz), 5.50 (d, 1H, –NCH$_2$O–, J=9.5 Hz), 5.46 (s, 1H, H-1′), 5.41 (d, 1H, –NCH$_2$O, J=9.5 Hz), 5.32 (br s, 1H, N*H*Boc), 5.17 (d, 1H, H-2′, $J_{2',3'}$=6.1 Hz), 4.88 (s, 1H, H-1″), 4.84 (m, 1H, H-3′), 4.69 (s, 2H, benzyl), 4.58 (d, 1H, H-2″, $J_{2'',3''}$=6.1 Hz), 4.47 (d, 1H, H-3″, $J_{3'',2''}$=6.1 Hz), 4.43 (dd, 1H, H-4′, $J_{4',3'}$= 3.6 Hz, $J_{4',5'}$=9.1 Hz), 4.38 (dd, 1H, H-5′, $J_{5',4'}$=9.1 Hz, $J_{5',6'}$=4.8 Hz), 4.24 (m, 1H, H-4″), 3.71 (s, 3H, OMe), 3.62 (d, 1H, H-6′, $J_{6',5'}$=4.8 Hz), 3.24 (m, 2H, H-5″a, H-10′a), 3.01 (m, 2H, H-10′b, H-5″b), 2.79 (m, 1H, H-8′a), 2.53 (m, 1H, H-8′b), 2.48 (s, 3H, NMe), 1.64 (m, 2H, H-9′a, H-9′b), 1.43 (s, 9H, *t*-Bu), 1.42 (s, 9H, *t*-Bu), 1.40 (s, 3H, acetonide), 1.38 (m, 4H, CH_2CH$_3$×2), 1.37 (s, 3H, acetonide), 0.74 (m, 6H, CH$_2$CH_3×2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.00, 162.50, 156.11, 150.94, 142.11, 137.79, 128.32, 127.78, 116.07, 114.56, 114.21, 102.22, 97.56, 89.00, 86.61, 86.49, 84.83, 82.50, 82.06, 81.90, 78.98, 78.74, 72.40, 70.37, 67.50, 51.45, 43.32, 36.33, 29.43, 28.71, 28.52, 28.44, 26.73, 26.70, 24.94, 8.45, 7.19; FABMS-LR *m*/*z* 962 (MH^+); FABMS-HR (NBA) calcd for $C_{47}H_{72}N_5O_{16}$ 962.4975, found 962.4971.

4.1.4. Cbz-Protected *tert*-butyl ester (10). Barium hydroxide octahydrate (378 mg, 1.2 mmol) was added to a solution of **5** (924 mg, 1.0 mmol) in THF–H_2O (4:1, 10 mL) at room temperature, and the resulting reaction mixture was stirred at

room temperature for 2 h. The reaction mixture was poured onto 0.3 M aqueous HCl and extracted with AcOEt. The organic phase was dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (10 mL), to which $^tBuOC(NH)CCl_3$ (864 mg, 4.0 mmol) and $BF\cdot OEt_2$ (38 μL, 0.3 mmol) were added at 0 °C. The resulting mixture was stirred for 16 h, and partitioned between AcOEt and saturated aqueous $NaHCO_3$. The organic phase was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by neutral silica gel column chromatography (3×15 cm, 33% AcOEt–hexane) to afford **10** (444 mg, two steps, 46%) as a white foam: $[\alpha]_D^{25}$ +29.7 (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.36–7.22 (m, 11H, Ar–*H*, H-6), 5.72 (d, 1H, H-5, $J_{5,6}$=8.0 Hz), 5.53 (br s, 1H, N*H*Boc), 5.53 (s, 1H, H-1′), 5.48 (d, 1H, –NC*H*$_a$O–, *J*=9.8 Hz), 5.41 (d, 1H, –NC*H*$_b$O–, *J*=9.8 Hz), 5.38 (m, 1H, N*H*Cbz), 5.21 (d, 1H, benzyl, *J*=12.3 Hz), 5.04–5.01 (m, 2H, benzyl, H-1″), 4.97 (d, 1H, H-2′, $J_{2',3'}$= 5.3 Hz), 4.84 (m, 1H, H-3′), 4.68 (s, 2H, benzyl), 4.51 (m, 3H, H-6′, H-2″, H-3″), 4.37 (d, 1H, H-5′, $J_{5',6'}$=8.4 Hz), 4.21 (m, 1H, H-4′), 4.18 (m, 1H, H-4″), 3.23 (m, 1H, H-5″a), 3.08 (m, 1H, H-5″b), 1.49 (s, 18H, *t*-Bu×2), 1.46 (m, 4H, C*H*$_2$CH$_3$×2), 1.41 (s, 3H, acetonide), 1.33 (s, 3H, acetonide), 0.75 (m, 6H, CH$_2$C*H*$_3$×2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.06, 162.36, 156.10, 155.96, 150.81, 141.41, 137.75, 136.10, 128.44, 128.26, 128.16, 127.69, 116.48, 114.71, 112.46, 102.22, 96.12, 86.99, 86.16, 84.14, 82.02, 81.14, 79.81, 79.33, 72.34, 70.29, 67.22, 54.86, 52.98, 43.32, 29.34, 28.76, 28.33, 27.01, 25.29, 8.32, 7.21; FABMS-LR *m*/*z* 967 (MH^+); FABMS-HR (NBA) calcd for $C_{49}H_{66}N_4O_{16}$ 967.4552, found 967.4536.

4.1.5. Troc-Protected *tert*-butyl ester (11). A mixture of **10** (150 mg, 0.16 mmol) and 10% Pd–C (15 mg) in MeOH (3 mL) was vigorously stirred under H_2 atmosphere at room temperature for 20 min. The catalyst was filtered off through Celite pad and the filtrate was concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (2 mL), to which TrocCl (29 μL, 0.21 mmol) and triethylamine (29 μL, 0.21 mmol) were added at room temperature. The resulting mixture was stirred for 2 h. The mixture was partitioned between AcOEt and saturated aqueous $NaHCO_3$. The organic phase was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography (4×15 cm, 33% AcOEt–hexane) to afford **11** (127 mg, 79%) as a white foam: $[\alpha]_D^{21}$ +10.3 (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.37–7.30 (m, 5H, Ar–*H*), 7.22 (d, 1H, H-6, $J_{6,5}$=7.5 Hz), 5.74 (d, 1H, H-5, $J_{5,6}$=7.5 Hz), 5.60 (m, 1H, N*H*Boc), 5.50 (s, 1H, H-1′), 5.49 (d, 1H, –NC*H*$_a$O–, *J*=10.1 Hz), 5.44 (br s, 1H, N*H*Troc), 5.42 (d, 1H, –NC*H*$_b$O–, *J*=10.1 Hz), 5.06 (d, 1H, H-2′, $J_{2',3'}$=5.8 Hz), 5.02 (s, 1H, H-1″), 4.97 (d, 1H, H-5′, $J_{5',6'}$= 12.0 Hz), 4.87 (m, 1H, H-3′), 4.68 (s, 2H, benzyl), 4.58 (m, 3H, H-4′, H-2″, H-4″), 4.49 (d, 1H, H-6′, $J_{6',5'}$=9.6 Hz), 4.42 (d, 1H, H-3″, $J_{3'',2''}$=7.0 Hz), 4.21 (m, 2H, C*H*$_2$CCl$_3$), 3.26 (m, 1H, H-5″a), 3.09 (m, 1H, H-5″b), 1.56 (m, 4H, C*H*$_2$CH$_3$×2), 1.50 (s, 9H, *t*-Bu), 1.49 (s, 3H, acetonide), 1.46 (s, 9H, *t*-Bu), 1.37 (s, 3H, acetonide), 0.76 (m, 6H, CH$_2$C*H*$_3$×2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 162.32, 150.73, 141.77, 137.61, 128.21, 127.65, 116.27, 114.63, 112.60, 102.19, 96.76, 87.77, 86.92, 86.26, 84.47, 83.59, 81.98, 81.40, 79.87, 74.63, 72.38, 70.28, 55.19, 43.33, 29.35, 28.81, 28.48, 28.11, 27.11, 25.37, 8.51, 7.32; FABMS-LR *m*/*z* 1029 (MNa^+); FABMS-HR (NBA) calcd for $C_{44}H_{61}N_4O_{16}Na$ 1029.3060, found 1029.3053.

4.1.6. *tert*-Butyl ester (12). A mixture of **11** (7.4 mg, 7.4 μmol), 10% $Pd(OH)_2$–C (5.0 mg), and 2,2,2-trichloroacetic acid (12 mg, 74 μmol) in MeOH (1 mL) was vigorously stirred under H_2 atmosphere at room temperature for 20 min. The catalyst was filtered off through Celite pad and the filtrate was concentrated in vacuo. The residue was purified by preparative TLC (50% AcOEt–hexane) to afford **12** (6.0 mg, 92%) as a colorless glass: $[\alpha]_D^{21}$ +8.77 (*c* 1.22, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 8.99 (br s, 1H, NH-3), 7.27 (d, 1H, H-6, $J_{6,5}$=8.1 Hz), 5.70 (dd, 1H, H-5, $J_{5,6}$= 8.1 Hz, $J_{5,NH}$=0.3 Hz), 5.65 (m, 1H, N*H*Boc), 5.54 (s, 1H, H-1′), 5.49 (m, 1H, N*H*Troc), 5.10 (s, 1H, H-1″), 5.07 (d, 1H, H-2′, $J_{2',3'}$=6.6 Hz), 4.96 (d, 1H, H-5′, $J_{5',6'}$=12.0 Hz), 4.84 (m, 1H, H-3′), 4.57 (m, 3H, H-4′, H-4″, H-6′), 4.47 (d, 1H, H-2″, $J_{2'',3''}$=9.2 Hz), 4.38 (d, 1H, H-3″, $J_{3'',2''}$=9.2 Hz), 4.21 (m, 2H, C*H*$_2$CCl$_3$), 3.27 (m, 1H, H-5″a), 3.10 (m, 1H, H-5″b), 1.55 (m, 4H, C*H*$_2$CH$_3$×2), 1.50 (s, 9H, *t*-Bu), 1.48 (s, 3H, acetonide), 1.45 (s, 9H, *t*-Bu), 1.32 (s, 3H, acetonide), 0.79 (m, 6H, CH$_2$C*H*$_3$×2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 168.96, 162.90, 155.92, 143.14, 116.40, 114.75, 112.20, 102.54, 95.71, 87.40, 86.98, 86.28, 84.24, 83.58, 92.00, 81.36, 79.47, 79.16, 74.63, 55.16, 43.29, 29.43, 28.84, 28.48, 28.09, 28.87, 27.12, 25.34, 8.49, 7.36; FABMS-LR *m*/*z* 909 (MNa^+); FABMS-HR (NBA) calcd for $C_{36}H_{53}Cl_3N_4O_{15}Na$ 909.2471, found 909.2467.

4.1.7. Amine (13). A mixture of **12** (16.1 mg, 0.018 mmol) and NH_4Cl (29.2 mg, 0.54 mmol) in MeOH (1 mL) was treated with activated Zn powder (85% purity, 19.5 mg, 0.18 mmol) at room temperature for 8 h. The insoluble was filtered off through Celite pad and the filtrate was concentrated in vacuo. The residue was purified by neutral silica gel column chromatography (1×7 cm, 66% AcOEt–hexane) to afford **13** (11.7 mg, 91%) as a white foam: $[\alpha]_D^{21}$ +79.4 (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.34 (d, 1H, H-6, $J_{6,5}$=7.3 Hz), 5.90 (br s, 1H, N*H*Boc), 5.73 (d, 1H, H-5, $J_{5,6}$=7.3 Hz), 5.63 (s, 1H, H-1′), 5.07 (s, 1H, H-1″), 5.06 (m, 1H, H-1′), 4.88 (m, 1H, H-3′), 4.63 (m, 1H, H-2″), 4.59 (m, 1H, H-3″), 4.43 (m, 1H, H-5′), 4.30 (m, 1H, 4″), 4.25 (m, 1H, H-4′), 3.67 (m, 1H, H-6′), 3.25 (m, 1H, H-5″a), 3.18 (m, 1H, H-5″b), 1.58 (s, 3H, acetonide), 1.58 (m, 4H, C*H*$_2$CH$_3$×2), 1.52 (s, 9H, *t*-Bu), 1.45 (s, 9H, *t*-Bu), 1.36 (s, 3H, acetonide), 0.81 (m, 6H, CH$_2$C*H*$_3$×2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 185.04, 163.08, 156.06, 149.83, 145.16, 142.97, 116.31, 114.84, 112.22, 102.71, 102.53, 94.93, 87.56, 86.89, 86.40, 84.16, 83.09, 81.97, 81.05, 79.08, 77.19, 55.58, 43.26, 43.19, 29.28, 28.83, 28.51, 28.45, 28.10, 27.21, 25.41, 8.50, 7.42; FABMS-LR *m*/*z* 713 (MH^+); FABMS-HR (NBA) calcd for $C_{33}H_{53}N_4O_{13}$ 713.3609, found 713.3615.

4.1.8. Monoalkylamine (14). A mixture of **13** (28.7 mg, 0.04 mmol), aldehyde **7** (6.9 mg, 0.04 mmol), and AcOH (100 μL) in CH_2Cl_2 (1 mL) was treated with $NaBH(OAc)_3$ (20.4 mg, 0.1 mmol) at room temperature for 1 h. The mixture was partitioned between AcOEt and saturated aqueous $NaHCO_3$. The organic phase was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by neutral silica gel column chromatography (2.5× 10 cm, 60% AcOEt–hexane) to afford **14** (27.6 mg, 80%)

as a white foam: $[\alpha]_D^{25}$ +35.4 (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 8.39 (br s, 1H, NH-3), 7.22 (d, 1H, H-6, $J_{6,5}$=8.0 Hz), 5.81 (m, 1H, N*H*Boc), 5.68 (d, 1H, H-5, $J_{5,6}$=8.0 Hz), 5.48 (s, 1H, H-1′), 5.15 (m, 2H, N*H*Boc, H-2′), 4.97 (s, 1H, H-1″), 4.83 (m, 1H, H-3′), 4.56 (m, 2H, H-4′, H-2″), 4.50 (d, 1H, H-3″, $J_{3'',2''}$=6.0 Hz), 4.23 (m, 1H, H-4″), 4.06 (d, 1H, H-5′, $J_{5',6'}$=8.5 Hz), 3.23 (m, 4H, H-6′, H-5″a, H-10′a, H-10′b), 3.07 (m, 1H, H-5″b), 2.83 (m, 1H, H-8′a), 2.33 (m, 1H, H-8′b), 1.63 (m, 4H, CH_2CH_3×2), 1.56 (s, 3H, acetonide), 1.55 (m, 2H, H-9′a, H-9′b), 1.52 (s, 9H, *t*-Bu), 1.45 (s, 9H, *t*-Bu), 1.43 (s, 9H, *t*-Bu), 1.37 (s, 3H, acetonide), 0.77 (m, 6H, CH_2CH_3×2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.24, 162.82, 156.14, 156.01, 149.89, 143.56, 116.00, 114.74, 112.76, 102.37, 96.48, 88.64, 86.77, 86.67, 84.76, 82.45, 82.07, 81.90, 78.96, 78.80, 77.21, 62.36, 45.32, 43.34, 37.93, 32.94, 29.67, 29.60, 29.47, 29.33, 29.18, 28.82, 28.63, 28.47, 28.36, 28.27, 28.16, 26.89, 25.28, 8.43, 7.30; FABMS-LR *m/z* 870 (MH^+); FABMS-HR (NBA) calcd for $C_{41}H_{67}N_5O_{15}$ 870.4675, found 870.4670.

4.1.9. Dialkylamine (15). A mixture of **14** (5.0 mg, 5.7 μmol), paraformaldehyde (0.5 mg, 16.7 μmol), and AcOH (50 μL) in AcOEt (500 μL) was treated with $NaBH(OAc)_3$ (12.0 mg, 56 μmol) at room temperature for 72 h. The mixture was partitioned between AcOEt and saturated aqueous $NaHCO_3$. The organic phase was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by neutral silica gel column chromatography (1×5 cm, 50% AcOEt–hexane) to afford **15** (3.5 mg, 50%) as a white foam: $[\alpha]_D^{25}$ +30.0 (*c* 1.0, $CHCl_3$); ^1H NMR (CD_3CN, 500 MHz) δ 9.07 (br s, 1H, NH-3), 7.42 (d, 1H, H-6, $J_{6,5}$=8.0 Hz), 5.89 (m, 1H, N*H*Boc), 5.65 (s, 1H, H-1′), 5.58 (d, 1H, H-5, $J_{5,6}$=8.0 Hz), 5.43 (m, 1H, N*H*Boc), 5.04 (m, 2H, H-1″, H-2′), 4.77 (d, 1H, H-3′, $J_{3',2'}$=5.5 Hz), 4.67 (d, 1H, H-2″, $J_{2'',3''}$=5.9 Hz), 4.51 (d, 1H, H-3″, $J_{3'',2''}$= 5.9 Hz), 4.28 (m, 2H, H-4′, H-5′), 4.10 (m, 1H, H-4″), 3.45 (m, 1H, H-6′), 3.19 (m, 1H, H-5″a), 3.04 (m, 3H, H-10′a, H-10′b, H-5″b), 2.76 (m, 1H, H-8′a), 2.53 (m, 1H, H-8′b), 2.40 (s, 3H, NMe), 1.56 (m, 4H, CH_2CH_3×2), 1.53 (m, 2H, H-9′a, H-9′b), 1.52 (s, 3H, acetonide), 1.47 (s, 9H, *t*-Bu), 1.40 (s, 9H, *t*-Bu), 1.39 (s, 9H, *t*-Bu), 1.32 (s, 3H, acetonide), 0.77 (m, 6H, CH_2CH_3×2); ^{13}C NMR (CD_3CN, 125 MHz) δ 170.40, 163.97, 156.89, 151.34, 144.25, 116.67, 115.04, 113.85, 102.89, 94.93, 88.44, 87.43, 87.01, 85.11, 82.91, 82.40, 82.26, 81.43, 79.40, 78.90, 43.95, 38.88, 38.72, 30.31, 29.48, 28.68, 28.62, 28.57, 27.33, 25.50, 8.75, 7.55; FABMS-LR *m/z* 884 (MH^+); FABMS-HR (NBA) calcd for $C_{42}H_{70}N_5O_{15}$ 884.4868, found 884.4879.

4.1.10. (+)-FR-900493 (1). Compound **15** (3.1 mg, 3.5 μmol) was treated with aqueous 80% TFA (1 mL) at room temperature for 1.5 h. The mixture was concentrated in vacuo to give FR-900493 as tri-TFA salt (3.0 mg, quant). $[\alpha]_D^{25}$ +9.4 (*c* 0.3, H_2O); ^1H NMR (D_2O, 500 MHz) δ 7.70 (d, 1H, H-6, $J_{6,5}$=8.2 Hz), 5.89 (d, 1H, H-5, $J_{5,6}$=8.2 Hz), 5.81 (s, 1H, H-1′), 5.28 (s, 1H, H-1″), 4.70 (d, 1H, H-2′, $J_{2',3'}$=3.0 Hz), 4.42 (d, 1H, H-3′, $J_{3',2'}$=3.0 Hz), 4.33 (m, 2H, H-2″, H-3″), 4.17 (m, 3H, H-4′, H-4″, H-5′), 3.47–3.37 (m, 3H, H-5″a, H-10′a, H-10′b), 3.35 (d, 1H, H-6′, $J_{6',5'}$=12.5 Hz), 3.19 (m, 1H, H-5″b), 3.12 (s, 3H, NMe), 3.06 (m, 2H, H-8′a, H-8′b), 2.15 (m, 2H, H-9′a, H-9′b); ^{13}C NMR (D_2O, 125 MHz) δ 167.11, 164.03, 163.68, 152.20, 143.45, 143.34, 120.15, 118.77, 115.87, 109.50, 109.32, 103.14, 93.56, 83.56, 79.72, 78.35, 78.24, 75.86, 73.85, 73.06, 70.18, 55.48, 43.54, 40.96, 37.60, 23.54; FABMS-LR *m/z* 520 (MH^+); FABMS-HR (NBA) calcd for $C_{20}H_{34}N_5O_{11}$ 520.2255, found 520.2251.

For analytical purpose, a part of the TFA salt of **1** was further purified according to the procedure[2] to afford free **1**.

Data for synthetic free **1**: ^1H NMR (D_2O, 500 MHz) δ 7.79 (d, 1H, H-6, $J_{6,5}$=7.8 Hz), 5.83 (d, 1H, H-5, $J_{5,6}$=7.8 Hz), 5.75 (d, 1H, H-1′, $J_{1',2'}$=2.3 Hz), 5.20 (s, 1H, H-1″), 4.33 (dd, 1H, H-2′, $J_{2',3'}$=5.1 Hz, $J_{2',1'}$=2.3 Hz), 4.28 (dd, 1H, H-5′, $J_{5',4'}$ =2.4 Hz, $J_{5',6'}$=8.4 Hz), 4.21 (m, 2H, H-3′, H-2″), 4.16 (dd, 1H, H-3″, $J_{3'',2''}$=6.0 Hz, $J_{3'',4''}$=2.8 Hz), 4.10 (m, 2H, H-4′, H-4″), 3.49 (d, 1H, H-6′, $J_{6',5'}$=8.4 Hz), 3.18 (dd, 1H, H-5″a, $J_{5''a,5''b}$=13.8 Hz, $J_{5''a,4''}$=3.7 Hz), 3.03 (m, 3H, H-5″b, H-10′a, H-10′b), 2.85 (m, 1H, H-8′a), 2.62 (m, 1H, H-8′b), 2.41 (s, 3H, NMe), 1.91 (m, 1H, H-9′a), 1.80 (m, 1H, H-9′b); ^{13}C NMR (D_2O, 125 MHz) δ 175.71, 171.32, 155.20, 141.89, 110.07, 102.44, 91.40, 83.76, 80.78, 78.52, 75.40, 74.20, 71.41, 71.34, 69.98, 52.33, 41.80, 39.00, 38.67, 24.99.

Data for authentic free **1**: ^1H NMR (D_2O, 500 MHz) δ 7.79 (d, 1H, H-6, $J_{6,5}$=7.8 Hz), 5.83 (d, 1H, H-5, $J_{5,6}$=7.8 Hz), 5.75 (d, 1H, H-1′, $J_{1',2'}$=2.3 Hz), 5.20 (s, 1H, H-1″), 4.33 (dd, 1H, H-2′, $J_{2',3'}$=5.1 Hz, $J_{2',1'}$=2.3 Hz), 4.28 (dd, 1H, H-5′, $J_{5',4'}$ =2.4 Hz, $J_{5',6'}$=8.4 Hz), 4.21 (m, 2H, H-3′, H-2″), 4.16 (dd, 1H, H-3″, $J_{3'',2''}$=6.0 Hz, $J_{3'',4''}$=2.8 Hz), 4.10 (m, 2H, H-4′, H-4″), 3.49 (d, 1H, H-6′, $J_{6',5'}$=8.4 Hz), 3.18 (dd, 1H, H-5″a, $J_{5''a,5''b}$=13.8 Hz, $J_{5''a,4''}$=3.7 Hz), 3.03 (m, 3H, H-5″b, H-10′a, H-10′b), 2.85 (m, 1H, H-8′a), 2.62 (m, 1H, H-8′b), 2.41 (s, 3H, NMe), 1.91 (m, 1H, H-9′a), 1.80 (m, 1H, H-9′b).

References and notes

1. Meka, V. G.; Pillai, S. K.; Sakoulas, G.; Wennersten, C.; Venkataraman, L.; DeGirolami, P. C.; Eliopoulos, G. M.; Moellering, R. C., Jr.; Gold, H. S. *J. Infect. Dis. Drug Targets* **2004**, *190*, 311–317.
2. (a) Ochi, K.; Ezaki, M.; Iwani, M.; Komori, T.; Kohsaka, M. EP-333177, 1989; (b) Yoshida, Y.; Yamanaka, H.; Sakane, K. JP H05-78385, 1993.
3. McDonald, L. A.; Barbieri, L. R.; Carter, G. T.; Lenoy, E.; Lotvin, J.; Petersen, P. J.; Siegel, M. M.; Singh, G.; Williamson, R. T. *J. Am. Chem. Soc.* **2002**, *124*, 10260–10261.
4. (a) Bugg, T. D. H.; Lloyd, A. J.; Roper, D. I. *Infect. Disord. Drug Targets* **2006**, *6*, 85–106; (b) Kimura, K.; Bugg, T. D. H. *Nat. Prod. Rep.* **2003**, *20*, 252–273.
5. (a) Dini, C. *Curr. Top. Med. Chem.* **2005**, *5*, 1221–1236; (b) Hyland, S. A.; Anderson, M. S. *Anal. Biochem.* **2003**, *317*, 156–165; (c) Zawadzke, L. E.; Wu, P.; Cook, L.; Fan, L.; Casperson, M.; Kishinani, M.; Calambur, D. S.; Hofstead, J.; Padmanabha, R. *Anal. Biochem.* **2003**, *314*, 243–252; (d) Stachyra, T.; Dini, C.; Ferrari, P.; Bouhss, A.; van Heijenoort, J.; Mengin-Lecreulx, D.; Blanot, D.; Biton, J.; Bellller, D. L. *Antimicrob. Agents Chemother.* **2004**, *48*, 897–907; (e) Yamashita, A.; Norton, E.; Petersen, P. J.; Rasmussen, B. A.; Singh, G.; Yang, Y.; Mansour, T. S.; Ho, D. M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3345–3350; (f) Sarabia, F.;

Martin-Ortiz, L. *Tetrahedron* **2005**, *61*, 11850–11865; (g) Yamashita, A.; Norton, E. B.; Williamson, R. T.; Ho, D. M.; Sinishtaj, S.; Mansour, T. S. *Org. Lett.* **2003**, *5*, 3305–3308.
6. Boyle, D. S.; Donachie, W. D. *J. Bacteriol.* **1998**, *180*, 6429–6432.
7. Hirano, S.; Ichikawa, S.; Matsuda, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 1854–1856.
8. Mukaiyama, T.; Murai, Y.; Shoda, S. *Chem. Lett.* **1981**, 431–432.
9. Nicolaou, K. C.; Chucholowski, A. R.; Dolle, E.; Randall, J. L. *J. Chem. Soc., Chem. Commun.* **1984**, 1155–1158.
10. Fukuyama, T.; Jow, C.-K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373–6374.
11. Kimura, K.; Kagami, S.; Ikeda, Y.; Takahashi, H.; Yoshihama, M.; Kusakabe, H.; Osada, H.; Isono, K. *J. Antibiot.* **1998**, *51*, 640–646.
12. Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849–3862.
13. Armstrong, A.; Brackenridge, I.; Jackson, R. F. W.; Kirk, J. M. *Tetrahedron Lett.* **1988**, *29*, 2483–2486.
14. Boger, D. L.; Kim, S. H.; Mori, Y.; Weng, J.; Rogel, O.; Castle, S. L.; McAtee, J. J. *J. Am. Chem. Soc.* **2001**, *123*, 1862–1871.