Crystal Structure and Total Synthesis of Globomycin: Establishment of Relative and Absolute Configurations

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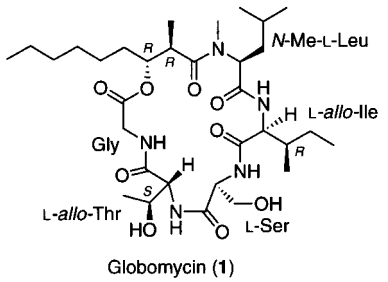
Received July 17, 2000

Globomycin (**1**) was isolated as a cyclic depsipeptide antibiotic against Gram-negative bacteria,[1,2] and as the first natural product[1c,3] which contains both L-*allo*-Ile and L-*allo*-Thr, in 1978. **1** has been proven to be a specific inhibitor of signal peptidase II (prolipoprotein signal peptidase),[4] which processes the acylated precursor form of lipoproteins into apolipoprotein and signal peptide in *Escherichia coli*.[5] A breakthrough in lipoprotein research was the finding that signal peptidase II activity is specifically inhibited by **1**.[5,6] To our knowledge, **1** is the only specific inhibitor of signal peptidase II known for the present. **1** causes the accumulation of the acylated forms of lipoprotein in the cytoplasmic membrane and, consequently, death of the cell.[7a] Therefore, signal peptidase II represents an attractive target for developing a new class of antibiotics that works with a different mechanism from currently available drugs. Globomycin (**1**) has been used routinely to demonstrate the acylation of newly identified lipoprotein,[8] and since then it has been widely used for controlling the maturation of the lipopeptides.[7] Although **1** has been an invaluable tool in studies of lipoprotein biosynthesis[9] to date, its structure remained obscure for almost two decades. An initial structure elucidation of **1** was only able to determine the absolute stereochemistry of the L-*allo*-Thr-L-Ser-L-*allo*-Ile moiety. However, the relative and the absolute stereochemistry of the 3-hydroxy-2-methylnonanoyl-*N*-Me-Leu moiety still remained ambiguous.[1c,2b,10,11] Herein we wish to report the complete stereochemical structure of **1** from the X-ray diffraction analysis and asymmetric total synthesis of **1**.



Globomycin (**1**)

Figure 1.

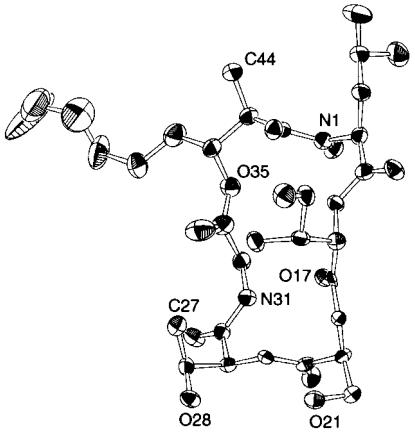


Figure 2. ORTEP drawing of globomycin (**1**).

The structure of **1**, which was unequivocally determined by X-ray crystallography, revealed the following two points: the relative stereochemistry of **1** is as shown in Figure 2, and all the amides are in the trans conformation.[12] The crystal structure of **1** also suggested that the carbonyl oxygen of the L-*allo*-Ile forms an intramolecular hydrogen bond with the NH of glycine (the O17−N31 distance is 2.8 Å).

To develop the synthetic route to various globomycin analogues in search of a more effective inhibitor of signal peptidase II, and to investigate an SAR study of its interesting inhibition activity, we explored a total synthesis of **1**. Our synthetic route to **1** consists of (i) an asymmetric synthesis of (2*R*,3*R*)-3-hydroxy-2-methylnonanoic acid (**2**), (ii) convergent coupling of three components (**2**, **7**, and **11**), and (iii) macrolactamization (Scheme 1).

Synthesis of (+)-**2** was achieved by an *anti*-selective asymmetric boron-mediated aldol reaction which was reported by Masamune.[13] *O*-Benzyl-L-serine allyl ester (**3**) was condensed with *N*-Boc-L-*allo*-isoleucine (**4**) in the presence of the 1*H*-benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP)[14] reagent to provide dipeptide **5** in 97% yield. The Boc group was subsequently removed by using 4 N HCl in ethyl acetate, and the resulting residue was coupled with *N*-Boc-*N*-

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(10) Omoto et al. presumed that the relative stereochemistry of 3-hydroxy-2-methylnonanoic acid is anti by ^{1}H NMR. See ref 2b.

(11) Partial racemization of *N*-Me-Leu easily occurred during acid hydrolysis. See refs 1c and 2b.

(12) X-ray data for **1**: $C_{32}H_{57}N_5O_9$, colorless prisms, hexagonal, $P6_1$, $a = b = 26.711(3)$ Å, $c = 9.884(4)$ Å, $\alpha = \beta = 90.0000°$, $V = 6107(2)$ Å^3, $Z = 6$, $d_{calc} = 1.070$ g/cm^3, $R = 0.053$, $Rw = 0.070$, GOF = 1.16.

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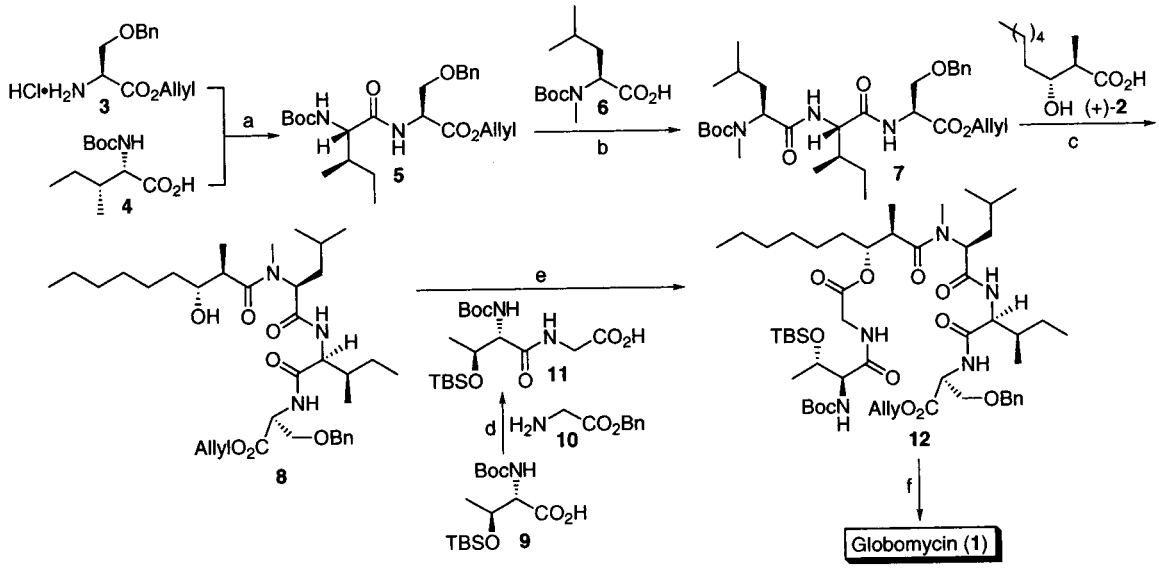
10.1021/ja002547j CCC: $19.00
Published on Web 09/27/2000

Scheme 1[a]



[a] Conditions: (a) PyBOP, Et_3N, 0 °C, CH_2Cl_2 (97%); (b) (i) 4 N HCl−AcOEt, room temperature; (ii) PyBOP, **6**, Et_3N, CH_2Cl_2, 0 °C (2 steps, 95%); (c) (i) 4 N HCl−AcOEt, room temperature; (ii) (+)-**2**, DEPC, Et_3N, THF, 0 °C (2 steps, 95%); (d) (i) **10**, PyBop, CH_2Cl_2, 0 °C to room temperature (93%); (ii) Pd/C−H_2, room temperautre (100%); (e) **11**, DMAP, DCC, cat. CSA, CH_2Cl_2, 0 °C to room temperature (96%); (f) (i) *n*-Bu_4NF, AcOH, THF, 0 °C to room temperature (99%); (ii) cat. $Pd(PPh_3)_4$, morpholine, THF, room temperature (97%); (iii) TFA, CH_2Cl_2, room temperature; (iv) HATU, *i*-Pr_2NEt, THF, 0 °C to room temperature (2 steps, 45%); (v) $Pd(OH)_2$, room temperature (96%).

Me-L-leucine (**6**) under PyBOP conditions to afford tripeptide **7** in 95% yield. Deprotection of the Boc group in **7**, followed by diethyl phosphorocyanidate (DEPC)-induced amide bond formation with the (2*R*,3*R*)-3-hydroxy-2-methylnonanoic acid (**2**), completed the formation of *N*-acyl tripeptide **8** in 95% yield. The dipeptide fragment **11** was prepared by a condensation between *N*-Boc-*O*-TBS-L-*allo*-threonine (**9**) and glycine benzyl ester (**10**) with the PyBOP method, followed by hydrogenolysis over 10% Pd/C to give dipeptide **11** in 93% yield. The esterification of the tripeptide **8** with the dipeptide unit **11** using DCC and DMAP[15] in the presence of a catalytic amount of 10-camphorsulfonic acid (CSA) afforded the protected depsipeptide **12** in 96% yield. Removal of the TBS group using TBAF (99%) was followed by deprotection of the allyl group with $Pd(PPh_3)_4$ (97%) and *N*-terminal Boc with TFA to afford the amine as its salt which was then set for macrolactamization. This intermediate underwent a cyclization in the presence of HATU[16] and *N*,*N*′-diisopropylethylamine yielding *O*-benzyl globomycin (45% in two steps). Finally, removal of the benzyl group by hydrogenolysis afforded globomycin (**1**) in 96% yield [mp 114−116 °C, $[\alpha]^{24}_D$ +23.8° (*c* 0.50, MeOH)]. All physical properties [mixed mp, 500 MHz ^1H (16 mM) and 125 MHz ^{13}C (18 mM) NMR, $[\alpha]_D$, and IR] of synthetic **1** are identical with those of the authentic sample.[17] Thus, the correlation of synthetic and natural **1** establishes the absolute configuration as shown in Figure 1.

The ^1H NMR spectrum suggests that **1** exists as a mixture of two rotational isomers in solution. The ratio is dependent on the solvent (major/minor[18] = 6:1 in $CDCl_3$, 3:1 in CD_3OD at 23 °C). The examination of NOESY experiments suggests that these

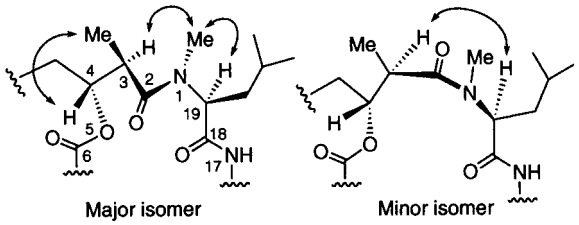


Figure 3. Conformation of the *N*-methyl amide moiety with selected NOESY correlations in CD_3OD.[19]

isomers are derived from the rotation of the acyl *N*-methyl amide moiety. The correlation among the *N*-methyl group, C3−H, and C19−H in the major isomer indicated the trans conformation for the amide moiety which is a similar result to that of the X-ray analysis. However, the correlation between C3−H and C19−H in the minor isomer suggests that it adopted the cis-form in the amide moiety (Figure 3). Synthetic **1** against *Escherichia coli* ATCC 11303 showed the same degree of antimicrobial activity (MIC = 0.2 μg/mL) as that initially observed for natural globomycin.[1b] However, the antimicrobial activity of the *O*-benzyl globomycin was extremely diminished. This result suggests that the serine hydroxyl group of globomycin is quite essential for the antimicrobial activity.

In summary, the relative and absolute configurations of globomycin (**1**) were determined by X-ray analysis and an asymmetric total synthesis which was achieved by convergent coupling of three components followed by macrolactamization. Further synthetic investigations for SAR study are currently underway.

Acknowledgment. We thank Dr. S. Miyakoshi (Sankyo Co., Ltd.) for the antimicrobacterial assay of globomycin.

Supporting Information Available: X-ray structural information and stereoview of **1**, experimental procedures, and characterization data for all new compounds, and ^1H and ^{13}C spectra of natural and synthetic **1** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) Natural globomycin: mp 115−116 °C (from CH_3CN), $[\alpha]^{23}_D$ +24.1° (*c* 0.50, MeOH). For isolation and purification of natural **1**, see ref 1b.

(18) The isomer ratio was determined by ^1H NMR analysis of *N*-Me protons.

(19) The same NOESY correlations for major isomer were measured in $CDCl_3$.