# Studies on Chemical Modification of Monensin IX.   Synthesis of 26-Substituted Monensins and Their Na$^+$ Ion Transport Activity

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The C-26 modified monensin derivatives, 26-*O*-benzoylmonensin (3), 26-*O*-benzylmonensin (4) and 26-phenylaminomonensin (5) were prepared from monensin (1). Na$^+$ ion transport activity through biological membrane and antibacterial activity of 3—5 were evaluated and compared with the activities reported for a 26-phenylurethane derivative (2). Among these compounds, 5 showed the largest Na$^+$ ion transport and antibacterial activities. In these compounds, the formation of head-to-tail hydrogen bonds was suggested to be an important factor for Na$^+$ ion transport and antibacterial activities.

**Key words**   C-26 modified monensin; sodium ion transport activity; antibacterial activity; 26-phenylaminomonensin

Monensin (**1**), a polyether isolated from *Streptomyces cinnamonensis*, is a potent antibiotic[1] and anticoccidial[2] agent. These features are believed to be caused by its Na$^+$ ion transport activity. Various chemical modifications of monensin have been carried out by substitution or oxidation of 7-OH group[3] and condensation of the carboxyl group with amino acid or alcohols.[4] However, there are few examples of modification at the C-26 position, except for the preparation of 26-*O*-urethane derivatives reported by Westley *et al.*[5] They reported that 26-*O*-phenylcarbamoyl monensin (**2**) showed strong antibacterial and anticoccidial activities. The aromatic ring is reported to contribute to trapping and/or stabilizing the guest ions in the host molecules.[6] In addition, the phenyl group usually increases the lipophilicity of the molecule, and the higher lipophilicity of ionophores is believed to induce more potent biological activities. So, the activity of **2** was recognized as quite reasonable. But no one has confirmed whether or not urethane is the most suitable linkage between the C-26 position and phenyl group to increase the antibacterial activities. Thus we attempted to introduce a phenyl moiety at C-26 of monensin as ether, ester and amine. In this paper, we report the preparation of novel C-26 modified monensin derivatives having a phenyl group, 26-*O*-benzoylmonensin (**3**), 26-*O*-benzylmonensin (**4**) and 26-phenylaminomonensin (**5**), and the comparison of their Na$^+$ ion transport and antibacterial activities with those of **2**.

## Results

**Chemistry**   The route to **3** is shown in Chart 1. The direct esterification of 26-*O* position by anhydrides was unsuccessful, probably due to the presence of hydrogen bonds between carboxyl group and 1,2-diol at C-25 and C-26. Thus the carboxyl group of **1** was protected as benzyl ester by treatment with benzyl bromide in the presence of K$_2$CO$_3$ to

give **6**.[4b,c] The acylation of 26-OH was performed by treatment of **6** with benzoic anhydride in pyridine. In the $^1$H-NMR spectrum of the product, the signals of 26-H$_2$ appeared downfield relative to those of **6**. Cleavage of the benzyl ester of the compound by catalytic hydrogenation gave **3**. The FABMS of **3** supported the structure.

The route to **4** is shown in Chart 2. The 7-OH of **7**[7] was protected as benzoate followed by cleavage of the silyl ether at the 26-*O* position to give **8**. Compound **8** was then treated with benzyl bromide in the presence of NaH and *n*-Bu$_4$NI to give 26-*O*-benzyl derivative (**9**) in 62.3% yield. In the $^1$H-NMR spectrum of **9**, the signals due to benzyl position appeared at $\delta$ 4.52 and 4.65. Then the methyl and benzoyl esters were hydrolyzed in aqueous alkaline followed by the exchange of 25-OMe to OH in 0.01 mol/l FeCl$_3$ in *iso*-PrOH–H$_2$O to yield **4**. In the $^1$H-NMR spectrum of **4**, the methyl signals due to 25-OMe and methyl ester and aromatic proton signals due to benzoate disappeared.

The synthetic course to **5** is summarized in Chart 3. Protected 25-formylmonensin (**10**)[7] was reacted with aniline to give the Schiff base, which was reduced by NaBH$_3$CN to amine (**11**). The formation of amine was confirmed by $^1$H-NMR spectrum and FAB-MS. Deprotection of **11** was carried out in the same manner as **4** to give desired **5**.

**Na$^+$ Ion Transport Activity**   The Na$^+$ ion transport activity of **1** and the C-26 modified compounds (**2**—**5**) through human erythrocyte membrane was determined by measuring intracellular Na$^+$ ion concentration using the $^{23}$Na-NMR method as described before.[8] The Na$^+$ ion transport activity of **2**—**5** at $10^{-6}$ M is shown in Fig. 2. Hemolysis induced by the tested samples was not observed during the assay.

Compounds **3** and **5** initially caused a rapid increase of the intracellular Na$^+$ ion concentration ([Na$_{in}^+$]) and then the rate gradually decreased to reach a plateau. This alteration of [Na$_{in}^+$] was similar to that induced by **1**. The increase of
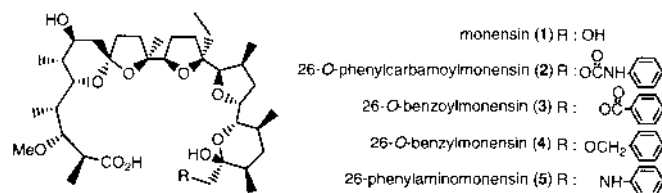


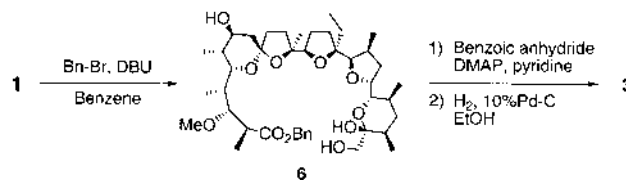Fig. 1.   Chemical Structures of Monensin (**1**) and C-26 Modified Monensins (**2**—**5**)
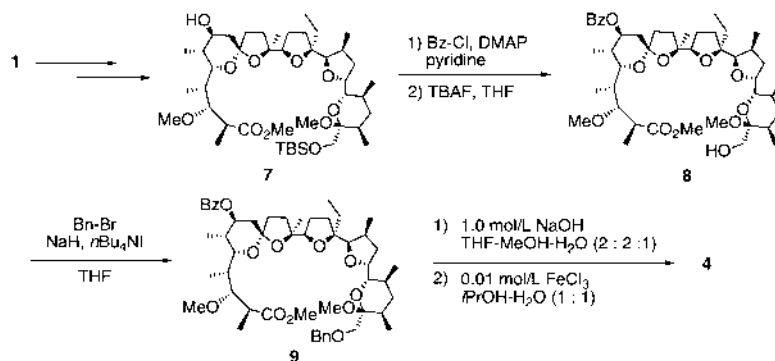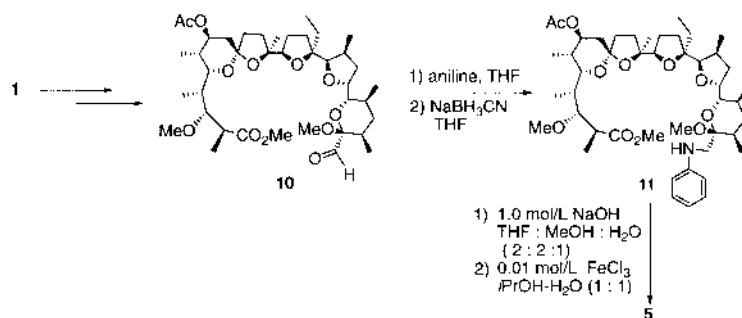


Chart 1

Chart 2



Chart 3


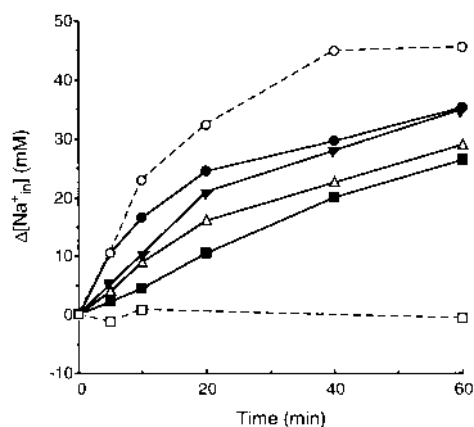
Fig. 2. Time Course of $\Delta[Na^+_{in}]$ Induced by $10^{-6}$ M Monensin (**1**) and C-26 Derivatives

**1** (○ dotted line), **2** (△), **3** (▼), **4** (■), **5** (●), DMSO (□ dotted line).

Table 1. Antibacterial Activity of **2**—**5**

| | MIC[a] ($\mu$g/ml) | | | | |
|---|---|---|---|---|---|
| | Control | **2** | **3** | **4** | **5** |
| *Staphylococcus aureus* | 0.39[b] | 0.78 | 0.78 | 0.78 | 0.39 |
| *Streptococcus pneumoniae* | 0.10[c] | 0.78 | 0.78 | 1.56 | 0.78 |
| *Enterococcus faecalis* | 3.13[c] | 3.13 | 3.13 | 6.25 | 1.56 |
| *Mycobacterium intracellulare* | 1.56[d] | 1.56 | 1.56 | 3.13 | 1.56 |
| *Clostridium perfringens* | 0.78[b] | 0.39 | 0.39 | 0.39 | 0.20 |
| *Mycoplasma gallisepticum* | 0.05[e] | 6.25 | 6.25 | 12.5 | 6.25 |

*a*) Minimum inhibitory concentration. *b*) Vancomycin. *c*) Rifampicin. *d*) Cycloserine. *e*) Tylosin.

$[Na^+_{in}]$ of compound **2** gave an approximately linear plot. The increase of $[Na^+_{in}]$ within the first 5 min ($\Delta[Na^+_{in}]^5$) is believed to represent the initial rate of increase of $[Na^+_{in}]$, which is an important indicator of the $Na^+$ ion permeability of the compounds. Compound **5** induced almost the same $\Delta[Na^+_{in}]^5$ as **1**, 10.5 mM for **5** and 10.4 mM for **1**. The $\Delta[Na^+_{in}]^5$ value of the other compounds was 4.0 mM (**2**), 5.3 mM (**3**) and 2.3 mM (**4**).

**Antibacterial Activity** The values of minimum inhibitory concentration against various bacteria were measured by agar dilution method (Table 1). Vancomycin was used as a control for *Staphylococcus aureus* and *Clostridium perfringens*, rifampicin for *Streptcoccus pneumoniae* and *Enterococcus faecalis*, cycloserine for *Mycobacterium intracellulare*, and tylosin for *Mycoplasma gallisepticum*. Compound **5** showed higher antibacterial activity than **2** against *S. aureus*, and *C. perfringens*. The activity of **5** against *S. aureus*, *M. intracellulare*, *E. faecalis* and *C. perfringens* was comparable to or stronger than that of the corresponding control compounds. Compound **3** showed the same antibacterial activity as **2**, and that of compound **4** was the weakest among these monensin derivatives.

**Discussion**

Among the derivatives (**2**—**5**), **5** showed the highest $Na^+$ ion transport activity, and the value of $\Delta[Na^+_{in}]^5$ of **5** was 2.5 times larger than that of the phenylurethane derivative (**2**). The value of $\Delta[Na^+_{in}]^5$ decreased in the order **5**>**3**>**2**>**4**. Compound **5** was potent against the tested bacteria and the antibacterial activity also decreased in the order **5**>**3**=**2**>**4**. This result implied that higher $Na^+$ ion permeability of the compounds tended to induce higher antibacterial activity, as we reported on 7-*O*-alkylmonensins.[8] Westley *et al.* reported
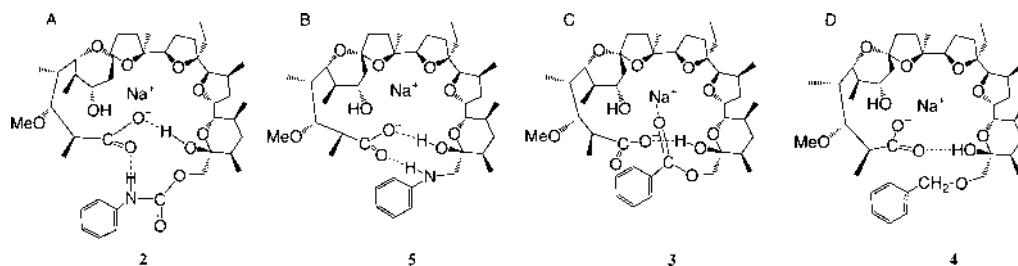
Fig. 3.   Proposed Head-to-Tail Hydrogen Bonds and Pseudocyclic Conformations of **2**—**5** Complexing with Na$^+$
A: 26-*O*-phenylcarbamoylmonensin (**2**), B: 26-phenylaminomonensin (**5**), C: 26-*O*-benzoylmonensin (**3**), D: 26-*O*-benzylmonensin (**4**).

that **2** exhibited higher antibacterial activity than **1**[5b)] in spite of having lower Na$^+$ ion permeability than **1**. This is probably due to the presence of a phenyl group in monensin. Some monensin derivatives with a phenyl group showed potent antibacterial activities in spite of their relatively low Na$^+$ ion permeability,[9)] suggesting that the phenyl group in these derivatives might enhance their antibacterial activities.

We also examined the lipophilicity of these derivatives on TLC (SiO$_2$, chloroform : MeOH=10 : 1). Compounds **2**, **3** and **5** showed almost the same *Rf* values as monensin (**1**) (*Rf*: **1**=0.57, **2**, **3**, **5**=0.55), while that of **4** was much lower (*Rf*=0.43). The pseudocyclic conformation of monensin sodium is believed to be stabilized by two intracellular hydrogen bonds between two oxygens of the carboxylate at one end of the molecule and two OH groups at C-25 and C-26 at the other end. Compounds **2** and **5** could also form two hydrogen bonds, one between N–H at C-26 and O at the carboxylate and the second between OH at C-25 and the other O at the carboxylate (Figs. 3A, B). In the case of **3**, the ester part should be unable to form a hydrogen bond with the carboxylate, but the carbonyl oxygen at the ester could coordinate to complexing Na$^+$ ion and the ester should contribute to stability of the pseudocyclic complex together with the hydrogen bond between carboxylate and 25-OH (Fig. 3C). Thus, compounds **2**, **3** and **5** could exhibit the lipophilicity comparable to **1**. In contrast, **4** could form only one head-to-tail hydrogen bond between 25-OH and the carboxylate, and the 26-*O*-benzyl group could not contribute to the stability of the Na$^+$ complex nor to the pseudocyclic conformation (Fig. 3D). Probably for this reason, **4** showed lower lipophilicity, which resulted in lower Na$^+$ ion transport activity and lower antibacterial activity than the other derivatives.

In summary, we prepared the C-26 modified monensin derivatives, **3**—**5** and compared their Na$^+$ ion transport and antibacterial activities with those of reported 26-phenylurethane derivative (**2**). Among these compounds, **5** showed the largest Na$^+$ ion transport activity and the highest antibacterial activity, and the formation of head-to-tail hydrogen bonds was suggested to be an important factor for Na$^+$ ion transport and antibacterial activities. As **5** can be the lead compound for preparation of more potent C-26 modified monensins, additional investigation is now in progress.

### Experimental

**General**   The FABMS and high resolution (HR) FABMS were measured with a JEOL JMS DX-505 or a SX-102 mass spectrometer, and the IR spectra with a Shimadzu FTIR-8100 spectrometer. The $^1$H-NMR spectra were measured with a JEOL EX-270, Lambda-400 or $\alpha$-500 spectrometer using tetramethylsilane as an internal standard. The following abbreviations are used: s, singlet; d, doublet; t, triplet; dd, doublet-of-doublets; td, triplet-of-doublets; qd, quartet-of-doublets; ddd, doublet-of-doublets-of-doublets; quin, quintet; m, multiplet; br, broad. Optical rotations were measured on a JASCO DIP-140 or DIP-1000 digital polarimeter. TLC was carried out on precoated plates (Kieselgel 60 F$_{254}$, 0.25 mm thick, Merck no. 5715), and spots were detected by illumination with an ultraviolet lamp or by spraying 1% Ce(SO$_4$)$_2$–10% H$_2$SO$_4$, followed by heating. Column chromatography was performed on Silica gel BW-200 (Fuji Davison Chemicals Co., Ltd.).

**Monensin Benzyl Ester (6)**[4b,c)]   Compound **6** was prepared under different condition from the reported method. The solution of monensin (**1**, 700 mg), benzyl bromide (249 $\mu$l, 2 eq) and 1.8-diazabicyclo [5.4.0] undec-7-ene (DBU) (234 $\mu$l, 1.5 eq) in benzene (15 ml) was refluxed for 2.5 h, poured into brine, and extracted with EtOAc. The organic layer was dried over MgSO$_4$, and evaporated *in vacuo*. The residue was chromatographed on silica gel (EtOAc : hexane=1 : 2) to give **6** (673 mg, 82%). Colorless syrup. $[\alpha]_D^{25}$: +41° (*c*=0.35, CHCl$_3$). IR (KBr, cm$^{-1}$): 1740 (C=O); $^1$H-NMR (CDCl$_3$, $\delta$): 2.69 (1H, m, 2-H), 3.24 (3H, s, 3-OCH$_3$), 3.26, 4.22 (each 1H, both d, *J*=11.0 Hz, 26-H$_2$), 3.57 (1H, t-like, *J*=4.9 Hz, 3-H), 3.63 (1H, dd, *J*=6.9, 9.9 Hz, 21-H), 3.75 (1H, m, 21-H), 3.87 (1H, br s, 7-H), 3.93 (1H, d-like, *J*=4.8 Hz, 17-H), 3.99 (1H, d-like, *J*=9.2 Hz, 5-H), 4.11 (1H, m, 20-H), 5.14, 5.19 (each 1H, both d, *J*=12.5 Hz, OCH$_2$Ar), 7.31—7.40 (5H, m, Ar). FAB-MS (*m/z*): 783 (M+Na)$^+$.

**26-*O*-Benzoylmonensin Benzyl Ester**   Benzoic anhydride (7.4 mg) and 4-dimethylaminopyridine (DMAP) (1.0 mg) were added to a solution of monensin benzyl ester (**6**, 10.0 mg) in pyridine (0.5 ml) at room temperature. The mixture was stirred at this temperature for 18 h, diluted with EtOAc, then washed with 5% HCl, 10% NaHCO$_3$, and brine. The organic layer was dried over Na$_2$SO$_4$, filtered and evaporated *in vacuo*. The residue was chromatographed on silica gel (hexane : EtOAc=3 : 1) to give 26-*O*-benzoylmonensin benzyl ester (12.9 mg, 99%). Colorless syrup. $[\alpha]_D^{25}$: +60° (*c*=0.27, CHCl$_3$); IR (CHCl$_3$, cm$^{-1}$): 1724 (C=O); $^1$H-NMR (CDCl$_3$, $\delta$): 2.67 (1H, m, 2-H), 3.26 (3H, s, 3-OMe), 3.54—3.58 (1H, overlapped, 3-H), 3.56 (1H, dd, *J*=5.6, 10.2 Hz, 13-H), 3.74 (1H, d-like, *J*=2.4 Hz, 7-H), 3.90 (1H, d, *J*=3.9 Hz, 17-H), 4.03 (1H, dd, *J*=2.2, 9.0 Hz, 5-H), 4.24 (1H, dd, *J*=3.2, 8.8 Hz, 21-H), 4.25—4.28 (1H, overlapped, 20-H), 4.26, 4.35 (each 1H, both d, *J*=11.2 Hz, 26-H$_2$), 5.15, 5.20 (each 1H, both d, *J*=12.3 Hz, CH$_2$-Ar), 7.28—7.58, 8.04—8.07 (10H, m, Ar). FABMS (*m/z*): 887 (M+Na)$^+$.

**26-*O*-Benzoylmonensin (3)**   A mixture of 26-*O*-benzoylmonensin benzyl ester (61.0 mg) and 10% Pd–C (10.0 mg) in EtOH (2.5 ml) was stirred under H$_2$ atmosphere at room temperature and 1 atm for 2.0 h. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was chromatographed on silica gel (chloroform : EtOAc=1 : 1) to yield **3** (46.6 mg, 84.6%). Colorless syrup. $[\alpha]_D^{25}$: +84° (*c*=0.45, CHCl$_3$); IR (CHCl$_3$, cm$^{-1}$): 1720 (C=O); $^1$H-NMR (CDCl$_3$, $\delta$): 2.65 (1H, qd, *J*=6.8, 9.8 Hz, 2-H), 3.23 (1H, dd, *J*=2.1, 9.9 Hz, 3-H), 3.36 (3H, s, 3-OMe), 3.49 (1H, t-like, *J*=7.7 Hz, 13-H), 3.76 (1H, br, 7-H), 4.03 (1H, d, *J*=4.1 Hz, 17-H), 4.10 (1H, dd, *J*=2.1, 10.6 Hz, 5-H), 4.15 (1H, dd, *J*=1.7, 11.5 Hz, 21-H), 4.31 (1H, m, 20-H), 4.40, 4.53 (each 1H, both d, *J*=12.0 Hz, 26-H$_2$), 7.43 (2H, t-like, *J*=7.6 Hz, 3′-H), 7.55 (1H, t-like, *J*=7.4 Hz, 4′-H), 8.05 (2H, d, *J*=7.3 Hz, 2′-H). FAB-MS (*m/z*): 797 (M+Na)$^+$. HR-FAB-MS: Calcd for C$_{43}$H$_{66}$O$_{12}$Na: 797.4452 (M+Na)$^+$. Found: 797.4454.

**7-*O*-Benzoyl-25-*O*-methyl-26-*O*-*tert*-butyldimethysilylmonensin Methyl Ester**   To a solution of 25-*O*-methyl-26-*O*-*tert*-butyldimethylsilylmonensin methyl ester (**7**, 246.5 mg) in pyridine (6.5 ml) was added benzoyl chloride (2.4 ml) and DMAP (6.5 mg). The mixture was stirred at room temperature for 3.0 h, diluted with EtOAc, washed with 5% HCl, 10% NaHCO$_3$ and brine. The organic layer was dried over Na$_2$SO$_4$, filtered and evaporated to dryness. The residue was chromatographed on silica gel (chloroform) to

give 7-*O*-benzoyl derivative (260.9 mg, 93.9%). Colorless syrup. $[\alpha]_D^{25}$: +58° (*c*=0.46, CHCl$_3$); IR (CHCl$_3$, cm$^{-1}$): 1720, 1740 (C=O); $^1$H-NMR (CDCl$_3$, $\delta$): 0.04 (6H, s, Si(CH$_3$)$_2$), 0.89 (9H, s, C(CH$_3$)$_3$), 2.60 (1H, qd, *J*=7.3, 6.7 Hz, 2-H), 3.21 (3H, s, 25-OMe), 3.32 (3H, s, 3-OMe), 3.41 (1H, dd, *J*=3.7, 9.8 Hz, 3-H), 3.46 (1H, t-like, *J*=4.9 Hz, 13-H), 3.47 (3H, s, 1-CO$_2$Me), 3.50 (1H, dd, *J*=6.7, 8.5 Hz, 21-H), 3.59, 3.62 (each 1H, both d, *J*=10.4 Hz, 26-H$_2$), 4.00 (1H, d, *J*=2.3 Hz, 17-H), 4.13 (1H, dd, *J*=1.8, 9.1 Hz, 5-H), 4.22 (1H, ddd, *J*=3.1, 6.8, 9.3 Hz, 20-H), 5.01 (1H, d-like, *J*=2.4 Hz, 7-H), 7.25—8.18 (5H, m, Ar). FAB-MS (*m/z*): 940 (M+Na+1)$^+$.

**7-*O*-Benzoyl-25-*O*-methylmonensin Methyl Ester (8)** A mixture of 7-*O*-benzoyl-25-*O*-methyl-26-*O*-tert-butyldimethylsilylmonensin methyl ester[7] (273.2 mg) and *n*-Bu$_4$NF 3H$_2$O (141.0 mg) in tetrahydrofuran (THF) (5.0 ml) was stirred under Ar atmosphere at room temperature for 2.5 h. The reaction mixture was poured into brine, and extracted with EtOAc. The organic layer was dried over Na$_2$SO$_4$, filtered and evaporated under reduced pressure. The residue was chromatographed on silica gel (chroloform : EtOAc=10 : 1) to give **8** (226.8 mg, 94.7%). Colorless syrup. $[\alpha]_D^{25}$: +24° (*c*=0.28, CHCl$_3$); IR (CHCl$_3$, cm$^{-1}$): 1700, 1730 (C=O); $^1$H-NMR (CDCl$_3$, $\delta$): 2.60 (1H, qd, *J*=6.6, 5.6 Hz, 2-H), 3.28 (3H, s, 25-OMe), 3.32 (3H, s, 3-OMe), 3.46 (1H, t-like, *J*=5.3 Hz, 13-H), 3.48 (3H, s, 1-CO$_2$Me), 3.51 (1H, m, 21-H), 3.55, 3.71 (each 1H, both d, *J*=11.2 Hz, 26-H$_2$), 3.93 (1H, d, *J*=4.0 Hz, 17-H), 4.13 (1H, dd, *J*=1.7, 10.3 Hz, 5-H), 4.26 (1H, td, *J*=8.9, 3.6 Hz, 20-H), 5.01 (1H, br, 7-H), 7.27—8.18 (5H, m, Ar). FAB-MS (*m/z*): 826 (M+Na+1)$^+$.

**7-*O*-Benzoyl-25-*O*-methyl-26-*O*-benzylmonensin Methyl Ester (9)** To a solution of **8** (249.0 mg) in THF (5.5 ml), NaH (18.6 mg), benzylbromide (0.26 ml) and *n*-Bu$_4$NI (114.5 mg) were added. The mixture was stirred under N$_2$ atmosphere at room temperature for 8.0 h, quenched by the addition of NH$_4$Cl solution and diluted with EtOAc. The organic layer was washed with brine, dried over Na$_2$SO$_4$, filtered, and evaporated to dryness. The residue was chromatographed on silica gel (hexane : EtOAc=8 : 1) to give 26-*O*-benzyl derivative (172.3 mg, 62.3%). Colorless syrup. $[\alpha]_D^{25}$: +67° (*c*=0.41, CHCl$_3$); IR (CHCl$_3$, cm$^{-1}$) : 1717, 1736 (C=O); $^1$H-NMR (CDCl$_3$, $\delta$): 2.59 (1H, qd, *J*=7.1, 5.4 Hz, 2-H), 3.23 (3H, s, 25-OMe), 3.32 (3H, s, 3-OMe), 3.43 (1H, dd, *J*=3.4, 9.7 Hz, 21-H), 3.44—3.47 (1H, overlapped, 3-H), 3.47 (3H, s, 1-CO$_2$Me), 3.47, 3.60 (each 1H, both d, *J*=10.2 Hz, 26-H$_2$), 3.51 (1H, dd, *J*=6.8, 8.1 Hz, 13-H), 4.01 (1H, d, *J*=4.1 Hz, 17-H), 4.12 (1H, dd, *J*=2.0, 9.0 Hz, 5-H), 4.24 (1H, m, 20-H), 4.52, 4.65 (1H, d, *J*=12.2 Hz, CH$_2$Ar), 5.01 (1H, d-like, *J*=2.7 Hz, 7-H), 7.27—8.18 (10H, m, Ar). FAB-MS (*m/z*): 915 (M+Na)$^+$.

**25-*O*-Methyl-26-*O*-benzylmonensin** To a solution of 7-*O*-benzoyl-25-*O*-methyl-26-*O*-benzylmonensin methyl ester (**9**, 57.7 mg) in THF–MeOH (1 : 1, 4.0 ml) was added 5 mol/l NaOH (1.0 ml). The mixture was stirred at room temperature for 5.0 h, and extracted with EtOAc. The organic layer was washed with brine, dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The residue was chromatographed on silica gel (chroloform : MeOH=40 : 1) to give 25-*O*-methyl-26-*O*-benzylmonensin (48.4 mg, 96.2%). Colorless syrup. $[\alpha]_D^{25}$: +51° (*c*=0.41, CHCl$_3$); IR (CHCl$_3$, cm$^{-1}$): 1732 (C=O); $^1$H-NMR (CDCl$_3$, $\delta$): 2.65 (1H, quin-like, *J*=6.8 Hz, 2-H), 3.23 (3H, s, 25-OMe), 3.37 (3H, s, 3-OMe), 3.44 (1H, dd, *J*=3.4, 9.7 Hz, 21-H), 3.47, 3.59 (each 1H, both d, *J*=10.7 Hz, 26-H$_2$), 3.53 (1H, t-like, *J*=4.8 Hz, 3-H), 3.65 (1H, dd, *J*=6.6, 8.6 Hz, 13-H), 3.77 (1H, d-like, *J*=2.9 Hz, 7-H). 4.02 (1H, dd, *J*=2.2, 9.5 Hz, 5-H), 4.03 (1H, d, *J*=3.9 Hz, 17-H), 4.26 (1H, m, 20-H), 4.52, 4.65 (each 1H, both d, *J*=12.2 Hz, CH$_2$Ar), 7.24—7.34 (5H, m, Ar). FAB-MS (*m/z*): 797 (M+Na)$^+$.

**26-*O*-Benzylmonensin (4)** 25-*O*-Methyl-26-*O*-benzylmonensin (34.2 mg) was added to 0.01 mol/l FeCl$_3$ in *iso*-PrOH–H$_2$O (5.0 ml, 1 : 1), and the solution was stirred for 4.0 h at room temperature. The resulting solution was partitioned between EtOAc and H$_2$O, and the organic layer was washed with brine, dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The residue was chromatographed on silica gel (chloroform : MeOH= 50 : 1) to give **4** (22.4 mg, 67.0%). Colorless syrup. $[\alpha]_D^{25}$: +43° (*c*=0.33, CHCl$_3$); IR (CHCl$_3$, cm$^{-1}$): 1730 (C=O); $^1$H-NMR (CDCl$_3$, $\delta$): 2.61 (1H, qd, *J*=6.8, 9.8 Hz, 2-H), 3.24 (1H, dd, *J*=1.7, 9.8 Hz, 3-H), 3.38 (3H, s, 3-OMe), 3.41 (1H, dd, *J*=5.8, 9.5 Hz, 13-H), 3.48, 3.56 (each 1H, both d, *J*=12.0 Hz, 26-H$_2$), 3.86 (1H, br, 7-H), 4.08 (1H, dd, *J*=2.9, 10.0 Hz, 21-H), 4.10 (1H, dd, *J*=2.2, 9.8 Hz, 5-H), 4.13 (1H, d, *J*=3.4 Hz, 17-H), 4.37 (1H, m, 20-H), 4.97, 5.03 (each 1H, both d, *J*=13.2 Hz, CH$_2$Ar), 7.23—7.34, 7.42—7.44 (5H, m, Ar). FAB-MS (*m/z*): 783 (M+Na)$^+$. HR-FAB-MS: Calcd for C$_{43}$H$_{68}$O$_{11}$Na: 783.4659 (M+Na)$^+$. Found: 783.4681.

**7-*O*-Acetyl-25-*O*-methyl-26-phenylaminomonensin Methyl Ester (11)** A solution of **10**[7] (123.3 mg) and aniline (0.76 ml) in THF (5.0 ml) was stirred at room temperature under Ar atmosphere. After 3.0 h, NaBH$_3$CN (31.5 mg) was added, and the mixture was stirred an additional 2.5 h. Then reaction mixture was diluted with EtOAc, and the organic layer was washed

with brine, dried over Na$_2$SO$_4$, filtered and evaporated to dryness. The residue was chromatographed on silica gel (hexane : EtOAc=10 : 1) to give **11** (112.1 mg, 82.4%). Colorless syrup. $[\alpha]_D^{25}$: +84° (*c*=0.32, CHCl$_3$); IR (CHCl$_3$, cm$^{-1}$): 1730 (C=O); $^1$H-NMR (CDCl$_3$, $\delta$): 2.04 (3H, s, CH$_3$CO), 2.63 (1H, p-like, *J*=6.8 Hz, 2-H), 3.21, 3.36 (each 1H, both d, *J*=12.0 Hz, 26-H$_2$), 3.30 (3H, s, 25-OMe), 3.34 (3H, s, 3-OMe), 3.34—3.36 (1H, overlapped, 3-H), 3.51 (1H, dd, *J*=3.7, 9.5 Hz, 21-H), 3.58 (1H, dd, *J*=6.6, 8.3 Hz, 13-H), 3.74 (3H, s, 1-CO$_2$Me), 3.88 (1H, dd, *J*=1.7, 10.0 Hz, 5-H), 4.02 (1H, d, *J*=3.9 Hz, 17-H), 4.28 (1H, m, 20-H), 4.71 (1H, d-like, *J*=2.4 Hz, 7-H), 6.63 (2H, d, *J*=7.8 Hz, 2'-H), 6.67 (1H, t-like, *J*=7.2 Hz, 4'-H), 7.16 (2H, t-like, *J*=7.8 Hz, 3'-H). FAB-MS (*m/z*): 838 (M+Na)$^+$.

**25-*O*-Methyl-26-phenylaminomonensin** To a solution of **11** (67.5 mg) in MeOH–THF (1 : 1, 3.2 ml) was added 5 mol/l NaOH (0.8 ml). The mixture was stirred at room temperature for 5.0 h, neutralized with 10% aqueous citric acid, and extracted with EtOAc. The organic layer was washed with brine, dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The residue was chromatographed on silica gel (chloroform : MeOH=50 : 1) to give 25-*O*-methy-26-phenylaminomonensin (60.6 mg, 93.5%). Colorless syrup. $[\alpha]_D^{25}$: +68° (*c*=0.32, CHCl$_3$); IR (CHCl$_3$, cm$^{-1}$): 1732 (C=O); $^1$H-NMR (CDCl$_3$, $\delta$): 2.65 (1H, m, 2-H), 3.22, 3.30 (each 1H, both d, *J*=12.0 Hz, 26-H$_2$), 3.30 (3H, s, 25-OMe), 3.37 (3H, s, 3-OMe), 3.50 (1H, m, 21-H), 3.54 (1H, t-like, *J*=4.9 Hz, 3-H), 3.66 (1H, dd, *J*=6.6, 8.5 Hz, 13-H), 3.74 (1H, d-like, *J*=2.7 Hz, 7-H), 4.00—4.03 (1H, overlapped, 5-H), 4.03 (1H, d, *J*=3.7 Hz, 17-H), 4.30 (1H, m, 20-H), 6.63 (2H, d, *J*=7.8 Hz, 2'-H), 6.68 (1H, t, *J*=7.3 Hz, 4'-H), 7.16 (2H, t-like, *J*=7.3 Hz, 3'-H). FAB-MS (*m/z*): 783 (M+Na+1)$^+$.

**26-Phenylaminomonensin (5)** 25-*O*-Methy-26-phenylaminomonensin (65.2 mg) was added to 0.01 mol/l FeCl$_3$ in *iso*-PrOH–H$_2$O (1 : 1, 5.0 ml), and the solution was stirred for 1.0 h at room temperature. The resulting solution was partitioned between EtOAc and H$_2$O, and the organic layer was washed with brine, dried over Na$_2$SO$_4$, and concentrated under reduced pressure. The residue was chromatographed on silica gel (chloroform : MeOH= 30 : 1) to give **5** (41.3 mg, 64.0%). Colorless syrup. $[\alpha]_D^{25}$: +62° (*c*=0.32, CHCl$_3$); IR (CHCl$_3$, cm$^{-1}$): 1728 (C=O); $^1$H-NMR (CDCl$_3$, $\delta$): 2.61 (1H, qd, *J*=6.6, 10.4 Hz, 2-H), 3.06, 3.82 (each 1H, both d, *J*=14.6 Hz, 26-H$_2$), 3.30 (1H, dd, *J*=2.0, 10.2 Hz, 3-H), 3.35 (1H, dd, *J*=5.0, 10.6 Hz, 13-H), 3.39 (3H, s, 3-OMe), 3.51 (1H, d, *J*=3.9 Hz, 17-H), 3.88 (1H, br, 7-H), 3.88—3.90 (1H, overlapped, 21-H), 4.23 (1H, d-like, *J*=11.2 Hz, 5-H), 4.34 (1H, m, 20-H), 6.59 (1H, t-like, *J*=7.2 Hz, 4'-H), 6.83 (2H, d, *J*=7.6 Hz, 2'-H), 7.05 (2H, t-like, *J*=7.8 Hz, 3'-H). FAB-MS (*m/z*): 769 (M+Na+1)$^+$. HR-FAB-MS: Calcd for C$_{42}$H$_{67}$O$_{10}$NNa: 768.4663 (M+Na)$^+$. Found: 768.4618.

**26-*O*-Phenylcarbamoylmonensin (2)** Compound **2** was prepared by the reported method.[5] Colorless syrup. $[\alpha]_D^{25}$: +83° (*c*=0.26, CHCl$_3$); IR (CHCl$_3$, cm$^{-1}$): 1717, 1738 (C=O); $^1$H-NMR (CDCl$_3$, $\delta$): 2.68 (1H, qd, *J*=6.8, 9.5 Hz, 2-H), 3.27 (1H, dd, *J*=2.3, 9.6 Hz, 3-H), 3.37 (3H, s, 3-OMe), 3.40 (1H, dd, *J*=5.9, 9.3 Hz, 13-H), 3.80 (1H, d-like, *J*=1.7 Hz, 7-H), 3.91 (1H, d, *J*=4.1 Hz, 17-H), 3.99 (1H, dd, *J*=1.5, 11.5 Hz, 5-H), 4.14—4.17 (1H, overlapped, 21-H), 4.16, 4.42 (each 1H, both d, *J*=11.2 Hz, 26-H$_2$), 4.28 (1H, m, 20-H), 6.99 (1H, t, *J*=7.3 Hz, 4'-H), 7.24 (2H, t-like, *J*=7.4 Hz, 3'-H), 7.47 (2H, br *J*=8.1 Hz, 2'-H). FAB-MS (*m/z*): 813 (M+Na+1)$^+$. HR-FAB-MS: Calcd for C$_{43}$H$_{67}$O$_{12}$NNa: 812.4561 (M+Na)$^+$. Found: 812.4607.

**Na$^+$ Ion Permeability** The Na$^+$ ion permeability of compounds **1**—**5** in a biological membrane was measured by use of $^{23}$Na-NMR spectroscopy and human erythrocyte as described.[8]

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