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CYANOGLYCOSYLATION ACCOMPANIED BY RING-OPENING OF SPIROSTANOLS

Akihiko Tobari,[a] Hiroshi Miyamae,[b] Akira Nagasawa,[c] Junichi Koyanagi,[a] Masami Kawase,[a] and Setsuo Saito[a*]

[a]Faculty of Pharmaceutical Sciences, Josai University, Keyakidai 1-1, Sakado, Saitama 350-0295, [b]Department of Chemistry, Josai University, Keyakidai 1-1, Sakado, Saitama 350-0295, [c]Faculty of Sciences, Saitama University, Shimo-Ohkubo 255, Urawa, Saitama 338-8570, Japan

Abstract -- The reaction of 3-*O*-acetylsarsasapogenin (**7**), which has no hydroxyl group susceptible to glycosylation, with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**5**) in the presence of a mixed catalyst, $Hg(CN)_2$ and $HgBr_2$, caused by cleavage the F-ring to give a 22-α-cyano-3,26-di-*O*-acetyl-5β-furostan derivative (**8**) and five monoglycosides (**9**-**13**) which were also products resulted from the cleavage of the F-ring of **7** accompanied by glycosylation. The trigger of the cleavage of the F-ring was speculated that a cyano anion generated from $Hg(CN)_2$ used as catalyst attacked at C-22 of **7** from α-site to open the F-ring, then the six products were produced. The orientation of the attacking CN^- group to C-22 of **7** was determinated on the basis of the X-Ray structure of **17**.

INTRODUCTION

In a series of our studies for structure-activity relationships, we previously reported that glycosylations of methyl glycyrrhetinate (**1**)[1-7] with various pyranosyl halide in the presence of silver triflate (AgOTf)[8,9] as catalyst gave various 3-*O*-glycosides. Furthermore we reported the synthesis of timosaponin A-III (**2**) by stepwise glycosylations using AgOTf as the catalyst from sarsasapogenin (**3**) *via* a monoglycoside derivative (**4**), and confirmed that a disaccaride, 2-*O*-

(β-D-glucopyranosyl)-β-D-galactopyranose was linked at *O*-3 of the aglycon.[10] In order to obtain **2** and other sarsasapogenin diglycosides having various pyranoses as the terminal sugar components in much larger amount for supply to pharmacological assay tests, we attemped to use a mixed catalyst, $Hg(CN)_2$-$HgBr_2$,[11,12] instead of AgOTf, because AgOTf was commercially expensive. However, when the monoglycoside (**4**) reacted with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**5**)[13] in the presence of the mixed catalyst, many products, which were thought to be changed in the structure of aglycon moiety and were difficult to isolate, were obtained, but not desired diglycoside derivative (**6**). Thus, as it was thought that the aglycon structure was changed in the reaction of **4** with **5** in the presence of the mixed catalyst, 3-*O*-acetylsarsasapogenin (**7**) was employed in the same reaction in order to simplify the results and consequently several monoglycosides were isolated in spite of protecting the OH group at *O*-3 on the aglycon with acetyl group.

In this paper we report the structural analysis of the products in the glycosylation of **7** with **5** in the presence of $Hg(CN)_2$ and $HgBr_2$.

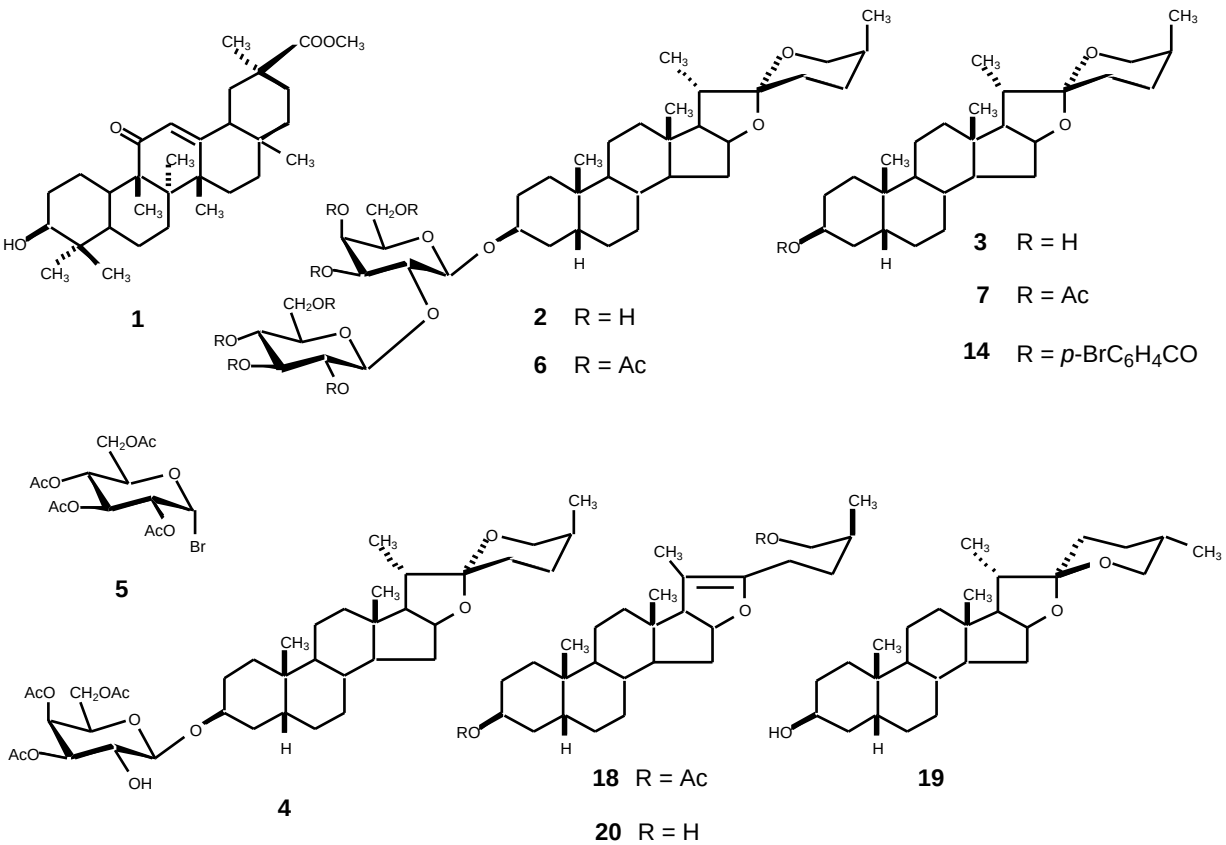


Figure 1

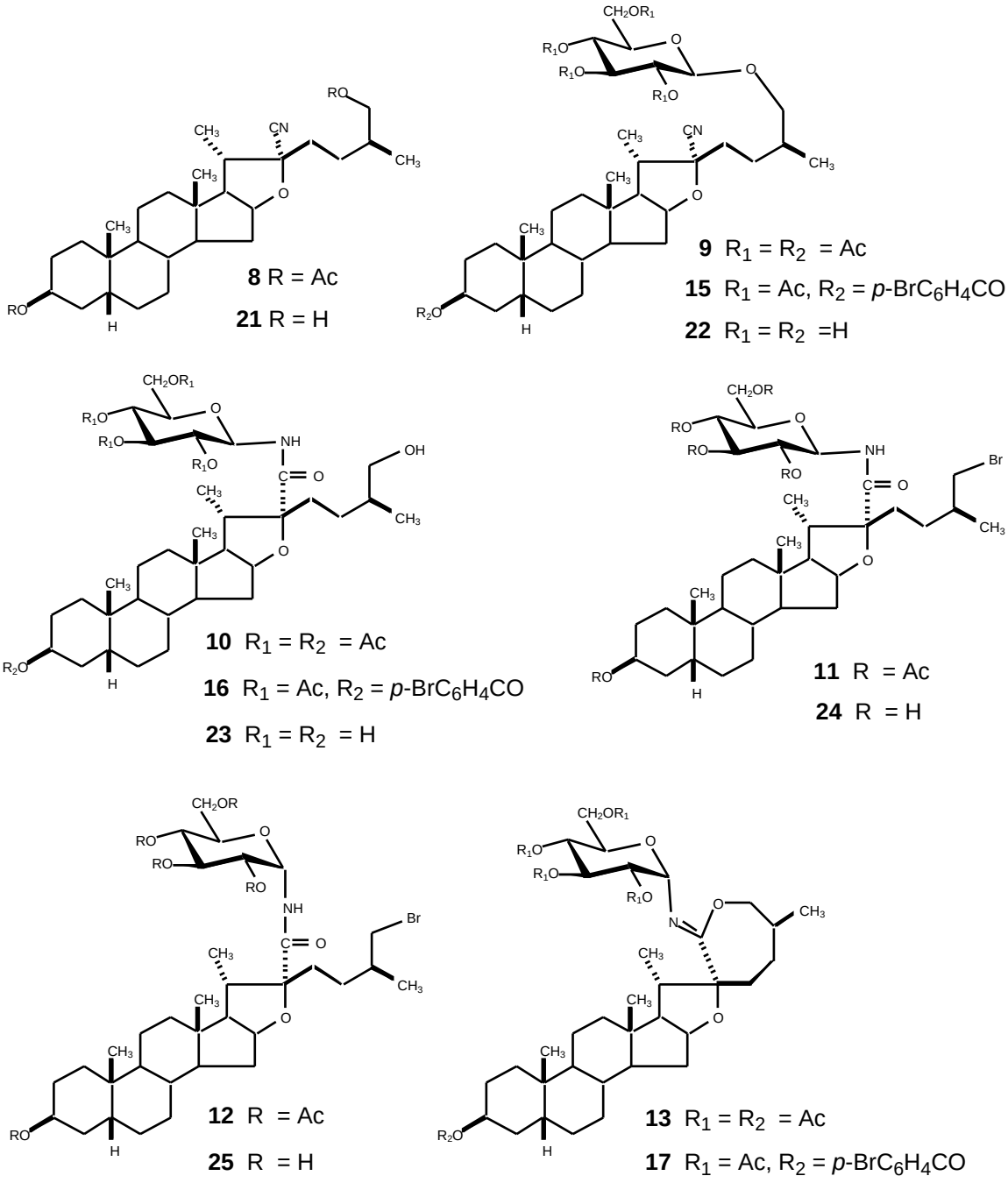


Figure 2

RESULTS AND DISCUSSION

Glycosylation of 3-*O*-acetylsarsasapogenin (**7**) with acetylated glucopyranosyl bromide (**5**) in the presence of a mixed catalyst, $Hg(CN)_2$ and $HgBr_2$, in dry benzene/nitromethane (1 : 1) at 65 °C gave six products (**8**-**13**) in 14.1, 16.6, 14.4, 3.6, 5.6 and 14.7% yields, respectively. In the ^1H NMR spectra, compounds (**9**-**13**) exhibited proton signals on the acetylated glucopyranosyl rings, although **8** showed no proton due to the pyranosyl ring, which suggests that **9**-**13** are monoglucopyranoside derivatives. On the elemental analyses, it became

apparent that **8**-**13** possessed a nitrogen atom in the molecules. As the source of the nitrogen atom was thought to come from nitromethane used as one of solvents, the same reaction was carried out without nitromethane. However, the latter reaction gave the same products in similar yields to the foregoing reaction. Furthermore no reaction occurred without $Hg(CN)_2$ (data not shown). Therefore it was consequently thought that the nitrogen atom came from $Hg(CN)_2$ used as the catalyst.

Compound (**8**), mp 104-106 °C, showed a quasimolecular ion $[M + Na]^+$ peak at *m/z* 550 in the FABMS spectrum. In the comparison of ^1H NMR spectra, compound (**8**) showed two acetyl methyl signals at δ 2.04 and 2.06, whereas the starting material **7** showed one acetyl methyl signal at δ 2.04. The chemical shift of H-16 (δ 4.60) of **8** was different from that of **7** (δ 4.41). In addition the signal paterns and chemical shifts at the 26 position of **8** were different from those of **7**; the signals of H-26a and H-26b of **8** were observed at δ 3.89 and 3.95 as doublet of doublets (*J* = 10.9 and 6.6 Hz, and *J* = 10.9 and 5.9 Hz), respectively, on the other hand, those of **7** at δ 3.30 (d, *J* = 11.0 Hz) and δ 3.95 (dd, *J* = 11.0 and 2.8 Hz), respectively. These results suggest that the structure of the E and/or F ring is different between **8** and **7**. The ^{13}C NMR spectrum of **8** showed twenty eight carbon signals in addition to four signals due to two acetyl groups, while that of **7** showed twenty seven signals in addition to two signals due to an acetyl group. The extra carbon of **8** was predicted to be due to a CN group derived from $Hg(CN)_2$ as mentioned above. The prediction was confirmed from the evidence that a carbon signal was observed at δ 118.5 in the spectrum in **8**, that was assigned to the carbon of the newly introduced CN group. Furthermore, the spiro carbon signal at C-22 which was observed at δ 109.7 in **7** was disappeared, instead a new signal was observed at δ 88.9 in **8**. The heteronuclear multiple bond connection (HMBC) spectral data of **8** showed that an acetyl carbonyl carbon at δ 171.2 correlated to the proton signals at the 26 position (δ 3.89 and 3.95), the carbon signal (δ 118.5) due to the CN group correlated to the signals (δ 1.75 and 1.87) due to methylene protons at the 23 position, and the newly introduced carbon signal at δ 88.9 correlated to CH_3-21 (δ 1.19). These spectral data indicate that compoud (**8**) has a structure in which the six-membered F ring in **7** is opened, and an acetyl group and a CN group are substituted at *O*-26 and C-22, respectively, as shown in Figure 2, although the configuration at C-22 is obscure at this moment.

In the ^{1}H NMR spectrum, compound (**9**) (mp 167-169 °C, a $[M + Na]^{+}$ peak at *m/z* 838) exhibited an anomeric proton signal at δ 4.48 (d, *J* = 7.9 Hz), and signals on the steroidal skeleton of **9** were superimposable on those of **8** except for the proton signal due to the 26 position. In the HMBC spectrum of **9**, the anomeric proton correlated to C-26 (δ 75.1), and protons at the 23 position correlated to the carbon due to the CN group. These spectral data suggest that **9** is 26-*O*-β-D-glucopyranoside of **8**.

Compound (**10**) showed a $[M + Na]^{+}$ peak at *m/z* 856 that was a larger mass number by 18 (corresponding to H_2O) than **9**. The ^{1}H NMR spectrum of compound (**10**) showed an anomeric proton at δ 5.20 as a triplet with a coupling constant of 9.5 Hz, which was not exchangable by addition of D_2O. In the ^{13}C NMR spectrm of **10**, the carbon signal due to CN group presented in **9** was disappeared, instead a new signal was observed at δ 174.0 assignable to amido carbonyl group. In the HMBC spectrum of **10**, the new signal at δ 174.0 correlated to the anomeric proton and H-20 (δ 2.14). From these spectral data, it was indicated that the cyano group introduced at C-22 converted to amido group, resulting in glycosylation to give **10**.

Compounds (**11**) and (**12**) showed peaks $[M + Na]^{+}$ and $[M + Na + 2]^{+}$ at *m/z* 918 and 920 with the peak height ratio of 1 : 1 in the FABMS spectra, which suggests that **11** and **12** are monobromides. In the ^{13}C NMR spectra, signals on the steroidal skeletons of **11** and **12** were superimposable on those of **10** except for signal due to C-26. The carbon signals at C-26 of **11** and **12** were observed at δ 40.7 and 41.3 which were shifted by 26.8 and 26.2 ppm, respectively, to higher fields than that of **10** (δ 67.5), which suggests, together with the MS spectral data, that a bromine atom is substituted at C-26 of **11** and **12**. In the ^{1}H NMR spectra, signals of anomeric proton and amido proton of **11** was exhibited at δ 5.19 and 7.35 as a triplet (*J* = 9.2 Hz) and a doublet (*J* = 9.2 Hz), while those of **12** at δ 5.86 and 7.45 as a doublet of doublets (*J* = 9.2 and 5.5 Hz) and a doublet (*J* = 9.2 Hz), respectively. These spectral data indicated that **11** was a 26-brominated compound of β-glcoside (**10**), and **12** was an α-glucoside isomer of **11**.

Compound (**13**) showed a quasimolecular peak $[M + Na]^{+}$ at *m/z* 838 as well as **9**. In the ^{1}H NMR spectrum of **13**, an anomeric proton was observed at δ 5.74 as a doublet with a coupling constant of 4.6 Hz. Therefore **13** was thought to be α-isomer of **9**. However, the ^{13}C NMR spectrum of **13** showed an anomeric carbon signal at δ 79.6 that was higher by 21.4 ppm than

that of **9**. Futhermore, a carbon signal due to a CN group which was showed in the spectrum of **9** was not observed, instead a new carbon signal was exhibited at δ 163.7 which was assignable to an imino carbon. The imino carbon correlated to the anomeric proton and protons at 23 and 26 positions in the HMBC (Figure 3). However, at this moment the structure of **13** was not completley confirmed, and also the configurations at the 22 positions of **9**-**12** were unsolved. The X-Ray analyses of compounds (**8**-**13**) were not carried out, because all those compounds were obtained as fine crystals or amorphous powders.

Hence, in order to obtain a good crystalline product for the X-Ray analysis, 3-*O*-*p*-bromobenzoylsarsasapogenin (**14**) was used as a starting material. The reaction of **14** with **5** in the presence of the mixed catalyst, $Hg(CN)_2$-$HgBr_2$, in benzene/nitromethane (1 : 1) at the same reaction condition as **7** gave compounds (**15**), (**16**) and (**17**) in 17.1, 13.7 and 5.3% yields, respectively. In the ^1H and ^{13}C spectra, signals on the steroidal skeletons and pyranose rings of **15**, **16** and **17** were superimposable on those of **9**, **10** and **13**, respectively, except for signals due to H's-3 and C's-3. These spectral data suggest, together with FABMS data (see EXPERIMENTAL), that compounds (**15**), (**16**) and (**17**) have the same structures as **9**, **10** and **13**, respectively, except for the substituents at *O*-3.

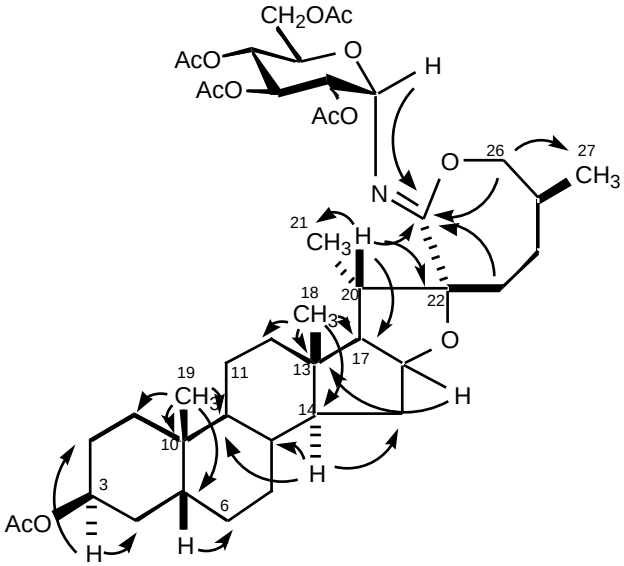


Figure 3. ^1H-^{13}C Long-range correlations observed for compound (**13**).

Although all these *p*-bromobenzoates (**15**-**17**) were obtained as crystals, only **17** was obtained as good crystals for the X-Ray analysis. From the ORTEP structure (Figure 4), it became apparent that **17** was a spiro compound in which a five-membered E ring and a seven-membered etheric F-ring were fused at C-22 and the configuration at the C-22 was *R*. And also it became apparent that a newly introduced CN group changed to the exo imino group in the seven-membered etheric F ring, on the nitrogen atom of which an α-pyranosyl group linked. From the evidences that the ^1H and ^{13}C NMR spectra of **13** were superimposable on those of **17** except for the signals due to the substituents linked to *O*-3, it was deduced that the structures of steroidal moieties of **13** and **17** were the same.

It has been reported that the heating of sarsasapogenin (**3**)[15] in acetic anhydride give 3,26-*O*-diacetylpseudosarsasapogenin (**18**) and that smilagenin (**19**) (an epimer of **3** with respect to C-22) is subject to epimerization at C-22 in hydrochloric acid to give **3**.[16-21] The intermediate of the latter reactions is revealed to be pseudosarsasapogenin (**20**). It can be therefore be presumed that the F-ring on sarsasapogenin is easily opened by some reactions in the glycosylation of **7** with **5** in the presence of the mixed catalyst, $Hg(CN)_2$ and $HgBr_2$.

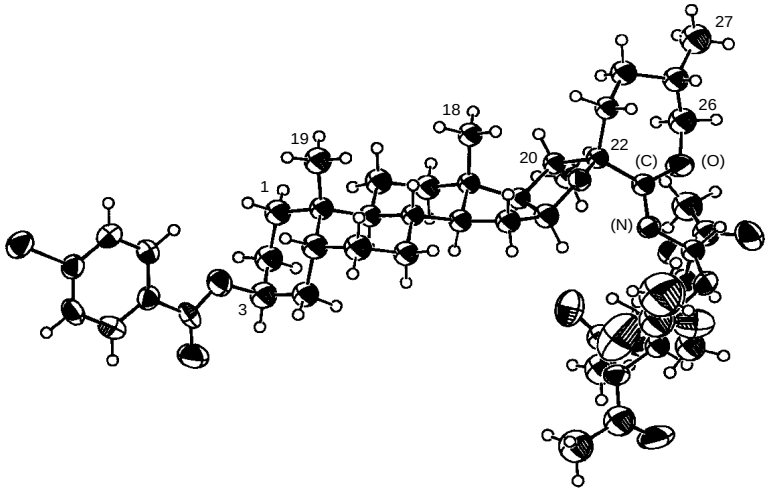


Figure 4. Crystal structure of compound (**17**).

The mechanisms for the formations of products (**9**-**13**) from **7** and the absolute configurations at the 22 positions of compounds (**9**-**12**) were deduced on the basis of the structure of **13** as follows (Figure 5). In the early steps of reaction of **7** with **5** in the presence of catalysts, $Hg(CN)_2$-$HgBr_2$, i) the bond between C-22 and *O*-26 of **7** is cleavaged to produce an intermediate such as an oxonium cation [I]. ii) At the same time bromine anion is released from **5**, and the bromine anion reacts with $Hg(CN)_2$ to generate a cyanide anion. When the attacking of the cyanide anion on the carbon at the 22 position of the intermediate [I] occurred from β-site, the cyanide anion is exerted a bulky hindrance by the CH_3-18 group, on the other hand, such hindrance is not considered by the attack from α-site. Therefore the cyanide anion attacks C-22 of [I] from α-site to produce an intermediate [II]. Compound (**9**) is obtained by the reaction of [II] with **5**, from which **8** might be obtained by the neighboring group participation of acetyl group at C-2' to C-26 as reported by H. Yamada and M. Nishizawa.[22] Compound (**13**) is given after the cyclization of between the side-chain and CN group at C-22 of [II], followed by glycosylation with **5** on the nitrogen atom of the resulting imino group. Compounds (**10**) and α-isomer of **10** (the latter compound is not isolated in this study) might be driven from **13**. Brominations of **10** and its α-isomer at C-26 give products (**11**) and (**12**). Thus, the mechanisms for the formations of products (**8**-**13**) in the reaction of **7** with **5** in the presence of $Hg(CN)_2$-$HgBr_2$ were become apparent, and at the same time it was

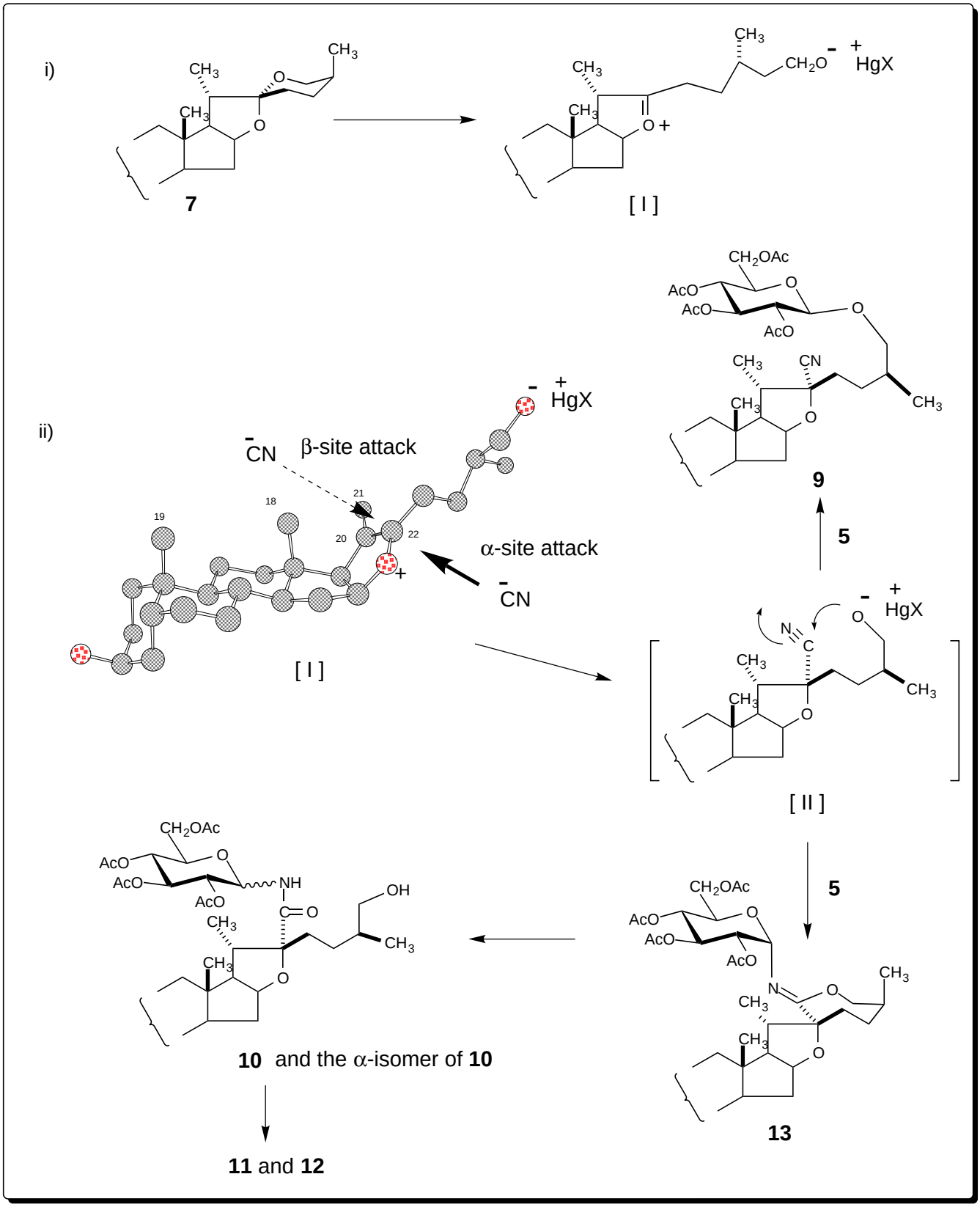
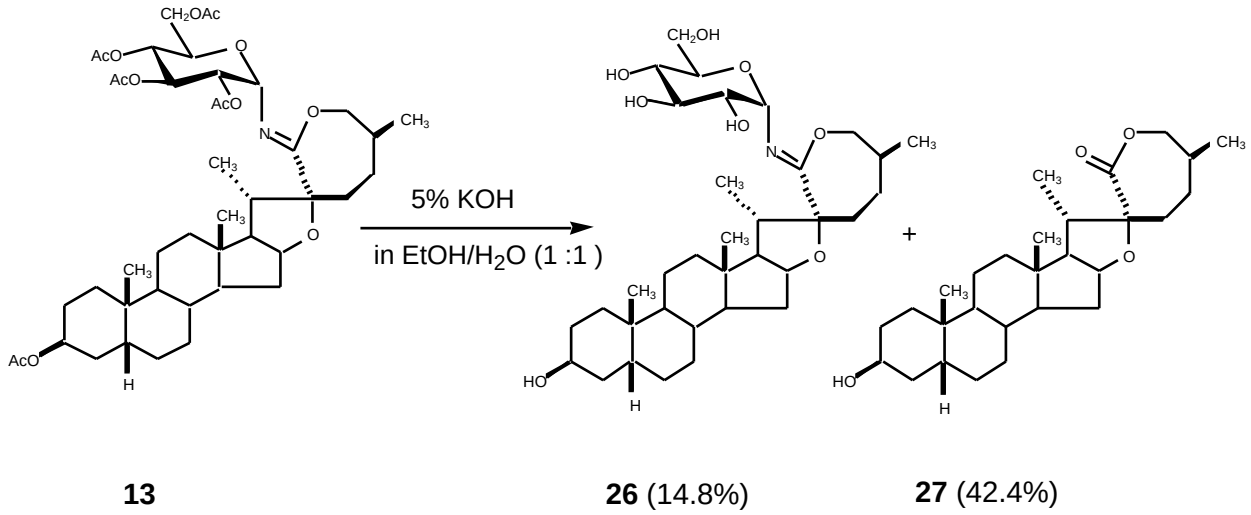


Figure 5. Proposed mechanisms for the formations of compounds (**9**-**13**) in the reaction of **7** with **5** in the presence of mixed catalyst, $Hg(CN)_2$ and $HgBr_2$, in benzene/nitromethane.

deduced that all absolute configurations at C-22 of compounds (**8**-**13**) were *R*. When the reaction of **7** with **5** was carried out in the presence of only $Hg(CN)_2$ as a catalyst, the same products (**8**-**13**) were obtained in almost the same yields as the case of the reaction in the presence of both $Hg(CN)_2$ and $HgBr_2$, though longer reaction times were necessary (data was not shown). This may indicate that $HgBr_2$ acts to release a bromine anion atom from **5** much faster than $Hg(CN)_2$ releases a cyanide anion. Deacetylation of **8**-**12** with 5% KOH in EtOH/H_2O (1 : 1) gave corresponding products (**21**-**25**) in 90.5, 78.5, 70.6, 87.1 and 85.4% yields, respectively. Deacetylation of **13** with 5% KOH in EtOH/H_2O (1 : 1), however, gave two products (**26**) and (**27**) in 14.8 and 42.4% yields, respectively. Compound (**26**) showed a quasimolecular ion $[M + Na]^+$ peak at *m/z* 628 in FABMS spectrum. In the ^1H NMR spectrum of **26**, the signals due to α-glucopyranosyl ring were observed. Compound (**27**) showed a peak $[M + Na]^+$ at *m/z* 467 in the FABMS spectrum. The ^1H NMR spectrum of **27** exhibted no signal due to a pyranose moiety. In the ^{13}C NMR spectrum of **27**, a carbon signal due to lactone carbonyl group formed the seven membered F-ring was observed at δ 172.8. The formation of lactone ring was further confirmed by IR spectrum (1720 cm^{-1}). The further treatment of **26** with 5% KOH in EtOH/H_2O (1 : 1) gave **27** quantitatively. Therefore lactone ring of **27** was confirmed to be formed by hydrolysis during hydrolysis of **26**.



13 **26** (14.8%) **27** (42.4%)

Figure 6. Hydorlyzed products of compound (**13**) in 5% KOH in EtOH/H_2O (1 : 1).

EXPERIMENTAL

General

Sarsasapogenin (**3**) was obtained by acid hydrolysis of timosaponin A-III (**2**) in accordance with the preparation method by Saito *et al*.[14] Other chemicals and solvents were of reagent grade, and were obtained from commercial sources. Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. The TLC utilized Kieselgel 60 F_{254} (E. Merck), and spots were detected by spraying the plates with $Ce(SO_4)_2$/10% H_2SO_4 (1 : 9) reagent, followed by heating at 100 °C for 5 min. Column chromatography was carried out on a Wakogel C-200, and the eluates were monitored by TLC. An SSC-6300 HPLC instrument (Senshu Scientific Co. Ltd.) was employed for analytical HPLC using a DOCOSIL (10 x 250 mm; flow rate, 1.0 mL/min, column temp, 40 °C) column, and was further equipped with an SSC autoinjector 6310 and an SSC fraction collector 6320 for preparative HPLC using a DOCOSIL (10 x 250 mm; flow rate, l.0 mL/min, column temp, 40 °C) preparative column. ^{1}H and ^{13}C NMR at 500 and 125 MHz, respectively, as well as ^{1}H-^{1}H and ^{1}H-^{13}C COSY, DEPT and HMBC spectra, were obtained with a JEOL JNM-A500 FT NMR spectrometer. TMS was used as an internal standard, and chemical shifts are given in ppm. FABMS were recorded on a JEOL JMS-DX 300 mass spectrometer. IR specctrum was recorded on HITACHI Infrared Spectrophotometer 260-10.

3-*O*-Acetyl sarsasapogenin (7)

Sarsasapogenin (**3**) was acetylated in the usual way to give quantitatively 3-*O*-acetyl sarsasapogenin (**7**), which showed a quasimolecular ion peak $[M + Na]^+$ at *m/z* 479; ^{1}H NMR ($CDCl_3$) (only assignable signals were listed) δ 5.06 (1H, br s, H-3), 4.41 (1H, dd, *J* = 14.3, 7.6 Hz, H-16), 3.95 (1H, dd, *J* = 11.0, 2.8 Hz, H-26a), 3.30 (1H, d, *J* = 11.0 Hz, H-26b), 2.04 (3H, s, COC**H**$_3$), 1.08 (3H, d, *J* = 7.0 Hz, CH_3-21), 0.99 (3H, d, *J* = 6.9 Hz, CH_3-27), 0.98 (3H, s, CH_3-19), 0.76 (3H, s, CH_3-18); ^{13}C NMR ($CDCl_3$) δ 170.7 (**C**OCH_3), 109.7 (C-22), 81.0 (C-16), 70.7 (C-3), 65.1 (C-26), 62.1 (C-17), 56.4 (C-14), 42.1 (C-20), 40.7 (C-13), 40.2 (C-12), 40.0 (C-9), 37.3 (C-5), 35.3 (C-8), 35.0 (C-10), 31.7 (C-15), 30.7 (C-1), 30.6 (C-4), 27.1 (C-25), 26.4 (C-6), 26.4 (C-7), 26.0 (C-24), 25.8 (C-23), 25.0 (C-2), 23.9 (C-19), 21.5 (CO**C**H_3), 20.9 (C-11), 16.5 (C-18), 16.0 (C-27), 14.3 (C-21).

Glycosylation of 3-*O*-acetyl sarsasapogenin (7)

To a solution of 3-*O*-acetyl sarsasapogenin (**7**) (4.24 g, 9.25 mmol) in dry benzene/nitromethane (1 : 1, 50 mL), 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**5**) (7.2 g, 17.5 mmol), $Hg(CN)_2$ (4.40 g, 17.5 mmol), and $HgBr_2$ (6.41 g, 17.9 mmol) were added, then the mixture was stirred at 65 °C for 24 h. The mixture was filtered and the resulting filtrate was poured into cold water (150 mL), then extracted with CH_2Cl_2 (100 mL x 3). The combined CH_2Cl_2 extracts were successively washed with $NaHCO_3$-saturated water and water, then dried over $MgSO_4$, and filtered. The filtrate was evaporated to give a residue which was subjected to column chromatography (a gradient of 0-10% AcOEt in benzene), followed by application of preparative HPLC (35 : 65 H_2O/acetone), to give compounds (**8**) (690 mg, 14.1%), mp 104-106 °C after recrystallization from ether, FABMS (*m/z*): $[M + Na]^+$= 550; ^1H NMR ($CDCl_3$) (only assignable signals were listed) δ 5.07 (1H, br s, H-3), 4.60 (1H, m, H-16), 3.95 (1H, dd, *J* = 10.9, 5.9 Hz, H-26a), 3.89 (1H, dd, *J* = 10.9, 6.6 Hz, H-26b), 2.06 and 2.04 (each 3H, s, COC**H**$_3$), 1.20 (3H, d, *J* = 6.9 Hz, CH_3-21), 0.99 (3H, s, CH_3-19), 0.96 (3H, d, *J* = 6.6 Hz, CH_3-27), 0.78 (3H, s, CH_3-18); ^{13}C NMR ($CDCl_3$) δ 171.2 and 170.7 (**C**OCH_3), 118.5 (CN), 88.9 (C-22), 84.3 (C-16), 70.6 (C-3), 68.9 (C-26), 63.1 (C-17), 56.7 (C-14), 42.1 (C-20), 41.1 (C-13), 39.8 (C-9), 39.7 (C-12), 37.2 (C-5), 35.3 (C-8), 35.0 (C-10), 34.6 (C-23), 32.8 (C-25), 31.5 (C-15), 30.7 (C-1), 30.5 (C-4), 28.3 (C-24), 26.3 (C-2), 26.3 (C-6), 24.9 (C-7), 23.8 (C-19), 21.5 and 21.0 (COC**H**$_3$), 20.7 (C-11), 17.1 (C-27), 16.6 (C-18), 16.6 (C-21); *Anal.* Calcd for $C_{32}H_{49}NO_5$: C, 72.83; H, 9.36; N, 2.65. Found: C, 72.58; H, 9.15; N, 2.73), (**9**) (1.25 g, 16.6%), mp 167-169 °C after recrystallization from ether, FABMS (*m/z*): $[M + Na]^+$ = 838; ^1H NMR ($CDCl_3$) (only assignable signals were listed) δ 5.20 (1H, dd, *J* = 9.8, 9.5 Hz, H-3'), 5.08 (1H, dd, *J* = 9.8, 9.8 Hz, H-4'), 5.06 (1H, br s, H-3), 4.98 (1H, dd, *J* = 9.5, 7.9 Hz, H-2'), 4.59 (1H, d, *J* = 12.5, 4.9 Hz, H-16), 4.48 (1H, d, *J* = 7.9 Hz, H-1'), 4.26 (1H, dd, *J* = 12.5, 4.9 Hz, H-6'a), 4.13 (1H, dd, *J* = 12.5, 2.4 Hz, H-6'b), 3.81 (1H, dd, *J* = 9.5, 5.5 Hz, H-26a), 3.68 (1H, ddd, *J* = 9.8, 4.9, 2.4 Hz, H-5'), 3.24 (1H, dd, *J* = 9.5, 7.3 Hz, H-26b), 2.09, 2.05, 2.04, 2.02 and 2.00 (each 3H, s, COC**H**$_3$), 1.19 (3H, d, *J* = 6.9 Hz, CH_3-21), 0.99 (3H, s, CH_3-19), 0.92 (3H, d, *J* = 6.3 Hz, CH_3-27), 0.78 (3H, s, CH_3-18); ^{13}C NMR ($CDCl_3$) δ 170.5, 170.5, 170.1, 169.3 and 169.1 (**C**OCH_3), 118.6 (CN), 101.0 (C-1'), 89.0 (C-22), 84.2 (C-16), 75.1 (C-26), 72.8 (C-3'), 71.7 (C-5'), 71.2 (C-2'), 70.6 (C-3), 68.5 (C-4'), 63.1 (C-17), 62.0 (C-6'), 56.7 (C-14), 42.1 (C-20), 41.1 (C-13), 39.8 (C-9), 39.7 (C-12), 37.2 (C-5), 35.3 (C-25), 35.0 (C-23), 34.9 (C-10), 33.3 (C-8), 31.5 (C-15), 30.7 (C-4), 30.6 (C-1), 28.5 (C-24), 26.3 (C-2), 26.3 (C-6), 24.9 (C-7), 23.8 (C-19),

21.3 (COC**H**$_3$), 20.8 (C-11), 20.6, 20.5, 20.5 and 20.4 (COC**H**$_3$), 17.0 (C-27), 16.6 (C-18), 16.6 (C-21); *Anal.* Calcd for $C_{44}H_{65}NO_{13}$: C, 64.77; H, 8.03; N, 1.72. Found: C, 64.51; H, 7.76; N, 1.73), (**10**) (1.11 g, 14,4%, amorphous powder, FABMS (*m/z*): $[M + Na]^+$ = 856; ^{1}H NMR ($CDCl_3$) (only assignable signals were listed) δ 7.39 (1H, d, *J* = 9.5, NH), 5.30 (1H, dd, *J* = 9.5, 9.5 Hz, H-3'), 5.20 (1H, dd, *J* = 9.5, 9.5 Hz, H-1'), 5.08 (1H, dd, *J* = 9.8, 9.5 Hz, H-4'), 5.06 (1H, br s, H-3), 4.98 (1H, dd, *J* = 9.5, 9.5 Hz, H-2'), 4.50 (1H, dd, *J* = 14.3, 7.2 Hz, H-16), 4.33 (1H, dd, *J* = 12.5, 4.6 Hz, H-6'a), 4.05 (1H, dd, *J* = 12.5, 2.4 Hz, H-6'b), 3.41 (1H, d, *J* = 5.8 Hz, H-26a), 3.41 (1H, d, *J* = 5.8 Hz, H-26b), 3.08 (1H, ddd, *J* = 9.8, 4.6, 2.4 Hz, H-5'), 2.08, 2.05, 2.04, 2.04 and 2.02 (each 3H, s, COC**H**$_3$), 1.10 (3H, d, *J* = 7.3 Hz, CH_3-21), 0.99 (3H, s, CH_3-19), 0.86 (3H, d, *J* = 6.7 Hz, CH_3-27), 0.82 (3H, s, CH_3-18); ^{13}C NMR ($CDCl_3$) δ 174.0 (CONH), 170.7, 170.7, 170.6 ,169.9 and 169.9 (**C**OCH_3), 93.8 (C-22), 83.8 (C-16), 77.8 (C-1'), 73.5 (C-5'), 73.0 (C-3'), 70.6 (C-3), 70.4 (C-2'), 68.4 (C-4'), 67.5 (C-26), 64.3 (C-17), 61.9 (C-6'), 56.3 (C-14), 42.5 (C-20), 41.5 (C-13), 39.9 (C-9), 39.6 (C-12), 37.2 (C-5), 35.8 (C-23), 35.7 (C-25), 35.2 (C-8), 35.0 (C-10), 32.8 (C-15), 30.7 (C-1), 30.6 (C-4), 29.0 (C-24), 26.3 (C-2), 26.3 (C-6), 24.9 (C-7), 23.8 (C-19), 21.5 and 20.8 (CO**C**H_3), 20.7 (CO**C**H_3), 20.7 (C-11) 20.6 (CO**C**H_3 x 2), 17.8 (C-21), 16.4 (C-18), 16.4 (C-27); *Anal.* Calcd for $C_{44}H_{67}NO_{14}$: C, 63.37; H, 8.10; N, 1.68. Found: C, 63.27; H, 8.15; N, 1.57), (**11**) (0.3 g, 3.6%), mp 119-120 °C after recrystallization from ether, FABMS (*m/z*): $[M + Na]^+$ and $[M + Na + 2]^+$ = 918 and 920 (the peak height ratio = 1 : 1); ^{1}H NMR ($CDCl_3$) (only assignable signals were listed) δ 7.35 (1H, d, *J* = 9.2, NH), 5.30 (1H, dd, *J* = 9.2, 9.2 Hz, H-3'), 5.19 (1H, dd, *J* = 9.2, 9.2 Hz, H-1'), 5.07 (1H, dd, *J* = 9.2, 9.2 Hz, H-4'), 5.06 (1H, br s, H-3), 4.97 (1H, dd, *J* = 9.5, 9.2 Hz, H-2'), 4.50 (1H, dd, *J* = 14.3, 5.6 Hz, H-16), 4.33 (1H, dd, *J* = 12.2, 4.6 Hz, H-6'a), 4.05 (1H, dd, *J* = 12.2, 2.0 Hz, H-6'b), 3.81 (1H, m, H-5'), 3.34 (1H, dd, *J* = 9.8, 4.9 Hz, H-26a), 3.27 (1H, dd, *J* = 9.8, 6.1 Hz, H-26b), 2.07, 2.04, 2.03, 2.03 and 2.01 (each 3H, s, COC**H**$_3$), 1.09 (3H, d, *J* = 7.3 Hz, CH_3-21), 0.98 (3H, s, CH_3-19), 0.97 (3H, d, *J* = 6.9 Hz, CH_3-27), 0.82 (3H, s, CH_3-18); ^{13}C NMR ($CDCl_3$) δ 173.9 (CONH), 170.8, 170.6, 170.5, 170.0 and 169.5 (**C**OCH_3), 93.5 (C-22), 83.9 (C-16), 77.8 (C-1'), 73.5 (C-5'), 73.0 (C-3'), 70.6 (C-3), 70.3 (C-2'), 68.4 (C-4'), 64.5 (C-17), 61.9 (C-6'), 56.3 (C-14), 42.3 (C-20), 41.5 (C-13), 40.7 (C-26), 40.0 (C-9), 39.6 (C-12), 37.3 (C-5), 36.0 (C-23), 35.3 (C-25), 35.2 (C-8), 35.0 (C-10), 32.8 (C-15), 30.7 (C-1), 30.6 (C-4), 29.0 (C-24), 26.4 (C-2), 26.4 (C-6), 25.0 (C-7), 23.8 (C-19), 21.5 (CO**C**H_3), 20.7 (C-11 and CO**C**H_3 x 2), 20.6 (CO**C**H_3 x 2), 18.7 (C-27), 17.9 (C-21), 16.4 (C-18); *Anal.* Calcd for $C_{44}H_{66}NO_{13}Br$: C, 58.92; H, 7.42; N, 1.56. Found: C, 58.75; H, 7.34; N, 1.49), (**12**) (0.46 g, 5.6%), mp 180-182 °C after recrystallization

from ether, FABMS (*m/z*): $[M + Na]^+$ and $[M + Na + 2]^+$ = 918 and 920 (the peak height ratio = 1 : 1); ^1H NMR ($CDCl_3$) (only assignable signals were listed) δ 7.45 (1H, d, *J* = 9.2, NH), 5.86 (1H, dd, *J* = 9.2, 5.5 Hz, H-1'), 5.29 (1H, dd, *J* = 9.8, 9.8 Hz, H-3'), 5.19 (1H, dd, *J* = 9.8, 5.5 Hz, H-2'), 5.10 (1H, dd, *J* = 9.8, 9.8 Hz, H-4'), 5.06 (1H, br s, H-3), 4.48 (1H, dd, *J* = 14.3, 7.6 Hz, H-16), 4.34 (1H, dd, *J* = 12.5, 4.0 Hz, H-6'a), 3.99 (1H, dd, *J* = 12.5, 2.5 Hz, H-6'b), 3.85 (1H, m, H-5'), 3.35 (1H, d, *J* = 5.2 Hz, H-26a), 3.35 (1H, d, *J* = 5.2 Hz, H-26b), 2.07, 2.06, 2.05 x 2 and 2.04 (each 3H, s, COC**H**$_3$), 1.08 (3H, d, *J* = 7.3 Hz, CH_3-21), 0.99 (3H, s, CH_3-19), 0.98 (3H, d, *J* = 6.7 Hz, CH_3-27), 0.85 (3H, s, CH_3-18); ^{13}C NMR ($CDCl_3$) δ 173.2 (CONH), 170.7, 170.6, 170.4, 169.4 and 168.7 (**C**OCH$_3$), 94.0 (C-22), 84.0 (C-16), 73.7 (C-1'), 70.5 (C-3), 70.3 (C-3'), 68.6 (C-2'), 68.3 (C-5'), 68.1 (C-4'), 65.1 (C-17), 61.7 (C-6'), 56.5 (C-14), 42.4 (C-20), 41.5 (C-13), 41.3 (C-26), 40.0 (C-9), 39.7 (C-12), 37.2 (C-5), 36.5 (C-23), 35.2 (C-8), 35.0 (C-10 and C-25), 33.1 (C-15), 30.7 (C-1), 30.6 (C-4), 29.5 (C-24), 26.4 (C-6), 26.3 (C-2), 25.0 (C-7), 23.8 (C-19), 21.5 (CO**C**H$_3$), 20.7 (C-11 and CO**C**H$_3$ x 2), 20.6 (CO**C**H$_3$ x 2), 18.6 (C-27), 18.3 (C-21), 16.5 (C-18); *Anal.* Calcd for $C_{44}H_{66}NO_{13}Br$: C, 58.92; H, 7.42; N, 1.56. Found: C, 58.83; H, 7.55; N, 1.55), and (**13**) (1.11 g, 14.7%, mp 104-106 °C after recrystallization from ether, FABMS (*m/z*): $[M + Na]^+$) = 838; ^1H NMR ($CDCl_3$) (only assignable signals were listed) δ 5.74 (1H, d, *J* = 4.6, H-1'), 5.51 (1H, dd, *J* = 9.8, 9.8 Hz, H-3'), 5.13 (1H, dd, *J* = 9.8, 9.5 Hz, H-4'), 5.07 (1H, br s, H-3), 5.00 (1H, dd, *J* = 9.8, 4.6 Hz, H-2'), 4.80 (1H, dd, *J* = 14.8, 7.0 Hz, H-16), 4.45 (1H, ddd, *J* = 9.5, 4.3, 2.1 Hz, H-5'), 4.25 (1H, dd, *J* = 12.2, 4.3 Hz, H-6'a), 4.18 (1H, dd, *J* = 11.3, 2.4 Hz, H-26a), 4.11 (1H, dd, *J* = 11.3, 6.1 Hz, H-26b), 4.11 (1H, dd, *J* = 12.2, 2.1 Hz, H-6'b), 2.10, 2.06, 2.04, 2.01 and 1.96 (each 3H, s, CO**C**H$_3$), 1.20 (3H, d, *J* = 6.7 Hz, CH_3-21), 1.00 (3H, s, CH_3-19), 0.92 (3H, d, *J* = 7.0 Hz, CH_3-27), 0.82 (3H, s, CH_3-18); ^{13}C NMR ($CDCl_3$) δ 170.6, 170.5, 170.0, 169.8 and 169.6 (**C**OCH$_3$), 163.7 (C=N), 93.3 (C-22), 84.0 (C-16), 79.6 (C-1'), 74.8 (C-26), 71.4 (C-2'), 70.6 (C-3), 70.5 (C-3'), 68.8 (C-4'), 68.6 (C-5'), 63.3 (C-17), 62.3 (C-6'), 56.1 (C-14), 45.7 (C-20), 40.6 (C-13), 40.2 (C-12), 39.8 (C-9), 37.1 (C-5), 35.1 (C-8), 34.9 (C-10), 32.6 (C-23), 32.2 (C-24), 31.1 (C-25), 30.6 (C-4), 30.5 (C-1), 29.4 (C-15), 26.3 (C-2 and C-6), 24.8 (C-7), 23.7 (C-19), 21.3 (CO**C**H$_3$), 20.8 (C-11), 20.7 (CO**C**H$_3$ x 2), 20.6 (CO**C**H$_3$ x 2), 16.6 (C-18), 16.4 (C-27), 15.7 (C-21); *Anal.* Calcd for $C_{44}H_{65}NO_{13}$: C, 64.77; H, 8.03; N, 1.72. Found: C, 64.48; H, 8.02; N, 1.68).

3-*O*-*p*-Bromobezoylsarsasapogenin (14)

A solution of **7** (4.0 g, 9.6 mmol) and *p*-bromobenzoyl chloride (6.32 g, 28.8 mmol) in pyridine

(200 mL) was stirred at rt overnight. The reaction mixture was poured into cold water (300 mL), then extracted with CH_2Cl_2 (200 mL x 3). The combined organic extracts were successively washed with 5% HCl solution, $NaHCO_3$-saturated water and water, dried over anhydrous Na_2SO_4, and filtered. The filtrate was evaporated to give a residue that was subjected to column chromatography (a gradient of 0-10% AcOEt in benzene), followed by application of preparative HPLC (H_2O/acetone 15 : 85), to give compound (**14**) (4.95 g, 86.3%). FABMS (*m/z*): $[M + Na]^+$ and $[M + Na + 2]^+$ = 621 and 623 (peak height ratio = 1 : 1); ^1H NMR ($CDCl_3$) (only assignable signals were listed) δ 7.90 (2H, d, *J* = 8.6 Hz, $COC_6\mathbf{H}_4$), 7.57 (2H, d, *J* = 8.6 Hz, $COC_6\mathbf{H}_4$), 5.32 (1H, br s, H-3), 4.42 (1H, ddd, *J* =7.6, 7.6, 6.4 Hz, H-16), 3.96 (1H, dd, *J* =11.2, 2.6 Hz, H-26a), 3.30 (1H, d, *J* = 11.2 Hz, H-26b), 1.08 (3H, d, *J* = 6.9 Hz, CH_3-27), 1.03 (3H, s, CH_3-19), 1.00 (3H, d, *J* = 6.6 Hz, CH_3-21), 0.77 (3H, s, CH_3-18); ^{13}C NMR ($CDCl_3$) δ 165.2 ($\mathbf{C}OC_6H_4$), 131.7, 131.7, 131.0, 130.0, 130.0 and 127.7 ($CO\mathbf{C}_6H_4$), 109.7 (C-22), 80.7 (C-16), 71.8 (C-3), 65.1 (C-26), 62.1 (C-17), 56.4 (C-14), 42.1 (C-20), 40.7 (C-13), 40.2 (C-12), 40.0 (C-9), 37.7 (C-5), 35.3 (C-8), 35.1 (C-10), 31.8 (C-15), 31.1 (C-1), 30.7 (C-4), 27.1 (C-25), 26.5 (C-2 and C-6), 25.9 (C-24), 25.8 (C-23), 25.1 (C-7), 24.1 (C-19), 20.9 (C-11), 16.5 (C-18), 16.1 (C-27), 14.4 (C-21); *Anal.* Calcd for $C_{34}H_{47}O_4Br$: C, 68.10; H, 7.90. Found: C, 67.98; H, 7.99.

Glycosylation of 3-*O*-*p*-bromobezoylsarsasapogenin (14)

The general procedure was employed with **14** (4.0 g, 6.69 mmol), $Hg(CN)_2$ (3.38 g, 13.4 mmol), $HgBr_2$ (4.8 g, 13.4 mmol) dissolved in dry benzene/nitromethane (1 : 1, 50 mL) to give compounds (**15**) (1.1 g, 17.1%), mp 185-183 °C after recrystallization from ether, FABMS (*m/z*): $[M + Na]^+$ and $[M + Na + 2]^+$ = 978 and 980 (peak height ratio = 1 : 1); ^1H NMR ($CDCl_3$) (only assignable signals were listed) δ 7.90 (2H, d, *J* = 8.2 Hz, $COC_6\mathbf{H}_4$), 7.58 (2H, d, *J* = 8.2 Hz, $COC_6\mathbf{H}_4$), 5.32 (1H, br s, H-3), 5.21 (1H, dd, *J* = 9.8, 9.5 Hz, H-3'), 5.10 (1H, dd, *J* = 9.5, 7.9 Hz, H-2'), 5.08 (1H, dd, *J* = 9.8, 9.8 Hz, H-4'), 4.49 (1H, dd, *J* = 13.7, 7.6 Hz, H-16), 4.48 (1H, d, *J* = 7.9 Hz, H-1'), 4.27 (1H, dd, *J* = 12.2, 4.9 Hz, H-6'a), 4.13 (1H, dd, *J* = 12.2, 2.1 Hz, H-6'b), 3.80 (1H, dd, *J* = 9.5, 5.5 Hz, H-26a), 3.70 (1H, ddd, *J* = 9.8, 4.9, 2.1 Hz, H-5'), 3.25 1H, (dd, *J* = 9.5, 7.3 Hz, H-26b), 2.09, 2.05, 2.02 and 2.00 (each 3H, s, $COC\mathbf{H}_3$), 1.20 (3H, d, *J* = 6.7 Hz, CH_3-21), 1.03 (3H, s, CH_3-19), 0.93 (3H, d, *J* = 6.7 Hz, CH_3-27), 0.79 (3H, s, CH_3-18); ^{13}C NMR ($CDCl_3$) δ 170.6, 170.2, 169.3 and 169.3 ($\mathbf{C}OCH_3$), 165.1 ($\mathbf{C}OC_6H_4$), 131.6, 131.6, 131.0, 131.0, 129.9 and 127.7 ($CO\mathbf{C}_6H_4$), 118.5 (CN), 101.0 (C-1'), 88.9 (C- 22), 84.1 (C-16), 75.0 (C-

26), 72.7 (C-3'), 71.7 (C-5'), 71.5 (C-3), 71.1 (C-2'), 68.4 (C-4'), 63.0 (C-17), 61.9 (C-6'), 56.6 (C-14), 42.0 (C-20), 41.0 (C-13), 39.8 (C-9), 39.6 (C-12), 35.3 (C-5 and C-8), 35.0 (C-10), 34.8 (C-23), 33.2 (C-25), 31.4 (C-15), 31.0 (C-1), 30.6 (C-4), 28.4 (C-24), 26.3 (C-2), 26.2 (C-6), 25.0 (C-7), 23.9 (C-19), 20.7 x 2 (C-11 and CO**C**H_3), 20.6 (CO**C**H_3), 20.5 (CO**C**H_3 x 2), 17.0 (C-18), 16.6 (C-21), 16.5 (C-27); *Anal.* Calcd for $C_{49}H_{66}NO_{13}Br$: C, 61.50; H, 6.95; N, 1.46. Found: C, 61.32; H, 6.85; N, 1.50), (**16**) (0.89 g, 13.7%), mp 224-226 °C after recrystallization from ether, FABMS (*m/z*): $[M + Na]^+$ and $[M + Na + 2]^+$ = 996 and 998 (peak height ratio = 1 : 1); ^{1}H NMR ($CDCl_3$) (only assignable signals were listed) δ 7.90 (2H, d, *J* = 8.5 Hz, COC_6**H**$_4$), 7.58 (2H, d, *J* = 8.5 Hz, COC_6**H**$_4$), 7.39 (1H, d, *J* = 9.2 Hz, NH), 5.32 (1H, br s, H-3), 5.30 (1H, dd, *J* = 9.8, 9.5 Hz, H-3'), 5.21 (1H, dd, *J* = 9.5, 7.9 Hz, H-1'), 5.09 (1H, dd, *J* = 9.8, 9.8 Hz, H-4'), 4.99 (1H, dd, *J* = 9.5, 7.9 Hz, H-2'), 4.51 (1H, dd, *J* = 14.3, 7.6 Hz, H-16), 4.34 (1H, dd, *J* = 12.2, 4.9 Hz, H-6'a), 4.06 (1H, dd, *J* = 12.2, 2.1 Hz, H-6'b), 3.82 (1H, ddd, *J* = 9.8, 4.9, 2.1 Hz, H-5'), 3.43 (1H, dd, *J* = 11.9, 6.4 Hz, H-26a), 3.41 (1H, dd, *J* = 11.9, 6.4 Hz, H-26b), 2.09, 2.05, 2.05, and 2.04 (each 3H, s, COC**H**$_3$), 1.11 (3H, d, *J* = 7.0 Hz, CH_3-21), 1.03 (3H, s, CH_3-19), 0.83 (3H, d, *J* = 6.3 Hz, CH_3 -27), 0.83 (3H, s, CH_3-18); ^{13}C NMR ($CDCl_3$) δ 174.0 (CONH), 170.7, 170.7, 170.0 and 169.6 (each **C**OCH_3), 165.2 (**C**OC_6H_4), 131.7, 131.7, 131.1, 131.1, 130.0 and 127.6 (CO**C**$_6H_4$), 93.9 (C-22), 83.8 (C-16), 77.8 (C-1'), 73.5 (C-5'), 73.0 (C-3'), 71.7 (C-3), 70.5 (C-2'), 68.4 (C-4'), 67.5 (C-26), 64.3 (C-17), 61.9 (C-6'), 56.3 (C-14), 42.5 (C-20), 41.5 (C-13), 40.0 (C-9), 39.6 (C-12), 37.7 (C-25), 35.8 (C-23), 35.7 (C-5), 35.2 (C-8), 35.1 (C-10), 32.7 (C-15), 31.1 (C-1), 30.7 (C-4), 26.9 (C-24), 26.4 (C-2 and C-6), 25.1 (C-7), 24.0 (C-19), 20.8 (C-11 and CO**C**H_3), 20.7 and 20.6 (each CO**C**H_3), 17.8 (C-21), 16.5 (C-18), 16.4 (C-27), *Anal.* Calcd for $C_{49}H_{68}NO_{14}Br$: C, 60.36; H, 7.03; N, 1.44. Found: C, 60.18; H, 7.11; N, 1.35) and (**17**) (0.34 g, 5.3%, mp 243-245 °C, FABMS (*m/z*): $[M + Na]^+$ and $[M + Na + 2]^+$ = 978 and 980 (peak height ratio = 1 : 1); ^{1}H NMR ($CDCl_3$) (only assignable signals were listed) δ 7.90 (2H, d, *J* = 8.5 Hz, COC_6**H**$_4$), 7.58 (2H, d, *J* = 8.5 Hz, COC_6**H**$_4$), 5.75 (1H, d, *J* = 4.6 Hz, H-1'), 5.53 (1H, dd, *J* = 9.9, 9.9 Hz, H-3'), 5.34 (1H, br s, H-3), 5.14 (1H, dd, *J* = 9.9, 9.9 Hz, H-4'), 5.02 (1H, dd, *J* = 9.9, 4.6 Hz, H-2'), 4.82 (1H, m, H-16), 4.46 (1H, ddd, *J* = 9.9, 4.3, 2.3 Hz, H-5'), 4.28 (1H, dd, *J* = 12.2, 4.3 Hz, H-6'a), 4.18 (1H, dd, *J* = 12.5, 4.3 Hz, H-26a), 4.06 (1H, dd, *J* = 12.2, 2.3Hz, H-6'b), 3.82 (1H, dd, *J* = 12.5, 5.5 Hz, H-26b), 2.12, 2.07, 2.02 and 1.97 (each 3H, s, COC**H**$_3$), 1.21 (3H, d, *J* = 7.0 Hz, CH_3-21), 1.04 (3H, s, CH_3-19), 0.92 (3H, d, *J* = 6.7 Hz, CH_3-27), 0.83 (3H, s, CH_3-18); ^{13}C NMR ($CDCl_3$) δ 170.8, 170.2, 170.0 and 169.5 (**C**OCH_3), 165.3 (**C**OC_6H_4), 163.9 (C=N), 131.6, 131.6, 131.2, 131.2, 130.0 and 127.8 (CO**C**$_6H_4$), 93.5 (C-22), 84.2 (C-16), 79.8

(C-1'), 71.7 (C-3), 71.6 (C-2'), 70.7 (C-26), 70.7 (C-3'), 68.9 (C-4'), 68.7 (C-5'), 63.5 (C-17), 62.6 (C-6'), 56.3 (C-14), 45.8 (C-20), 40.8 (C-13), 40.4 (C-12), 39.9 (C-9), 35.7 (C-5), 35.3 (C-8), 35.2 (C-10), 32.9 (C-15), 32.8 (C-23), 31.2 (C-25), 31.1 (C-1), 30.7 (C-4), 29.5 (C-24), 26.5 (C-2 and C-6), 25.1 (C-7), 24.1 (C-19), 21.0 (C-11), 20.8, 20.8, 20.7 and 20.6 (each CO**C**H_3), 16.8 (C-18), 16.6 (C-27), 15.9 (C-21); *Anal.* Calcd for $C_{49}H_{66}NO_{13}Br$: C, 61.50; H, 6.95; N, 1.46. Found: C, 60.87; H, 6.82; N, 1.52).

Deacetylation of compound (8)

A solution of compound (**8**) (500 mg, 947 mmol) in 5% KOH in EtOH/H_2O (1 : 1, 2 mL) was allowed to stand overnight at rt. Acetic acid was added at 0 °C to be acidified. The acidified solution was passed through a Diaion HP-20 column chromatography and eluted first with distilled water, then with MeOH. The MeOH eluent was evaporated to give a residue that was subjected to preparative HPLC (H_2O/acetone 1 : 9) to obtain compound (**21**) as a white solid (mp 212-213 °C after recrystallization from MeOH, 380 mg, 90.5%). FABMS (*m/z*): $[M + Na]^+$ = 466; ^1H NMR (C_5D_5N) (only assignable signals were listed) δ 4.71 (1H, dd, *J* = 13.5, 7.6 Hz, H-16), 4.36 (1H, br s, H-3), 3.70 (1H, dd, *J* = 9.4, 6.0 Hz, H-26a), 3.67 (1H, dd, *J* = 9.4, 6.8 Hz, H-26b), 1.24 (3H, d, *J* = 6.7 Hz, CH_3-21), 1.05 (3H, d, *J* = 6.7 Hz, CH_3-27), 1.01 (3H, s, CH_3-19), 0.79 (3H, s, CH_3-18); ^{13}C NMR (C_5D_5N) δ 119.4 (CN), 89.6 (C-22), 84.5 (C-16), 67.3 (C-26), 66.0 (C-3), 63.6 (C-17), 56.7 (C-14), 42.1 (C-20), 41.2 (C-13), 39.9 (C-9), 39.8 (C-12), 36.9 (C-5), 36.4 (C-25), 35.5 (C-8 and C-10), 34.4 (C-15 and C-23), 29.1 (C-1 and C-24), 29.0 (C-4), 28.6 (C-7), 27.1 (C-6), 26.7 (C-2), 24.2 (C-19), 21.0 (C-11), 17.3 (C-21), 16.9 (C-27), 16.6 (C-18); *Anal.* Calcd for $C_{28}H_{45}NO_3$: C, 75.80; H, 10.22; N, 3.16. Found: C, 75.63; H, 10.25; N, 3.00.

Deacetylation of compound (9)

The general procedure was employed with **9** (1.27 g, 1.6 mmol) dissolved in 5% KOH in EtOH/H_2O (1 : 1, 5 mL) to give a residue that was subjected to preparative HPLC (H_2O/MeOH 15 : 85) to afford compound (**22**) as a white solid (mp 116-118 °C after recrystallization from MeOH, 740 mg, 78.5%). FABMS (*m/z*): $[M + Na]^+$ = 628; ^1H NMR (C_5D_5N) (only assignable signals were listed) δ 4.79 (1H, d, *J* = 7.3 Hz, H-1'), 4.71 (1H, dd, *J* = 13.4, 6.4 Hz, H-16), 4.55 (1H, m, H-6'a), 4.38 (1H, br s, H-3), 4.36 (1H, m, H-6'b), 4.18-4.21 (2H, m, H-3' and 4'), 4.00 (1H, m, H-26a), 4.00 (1H, m, H-2'), 3.94 (1H, m, H-5'), 3.44 (1H, dd, *J* = 8.5, 6.7 Hz, H-26b), 1.22 (3H, d, *J* = 6.4 Hz, CH_3-21), 1.00 (3H, s, CH_3-19), 0.97 (3H, d, *J* = 6.4 Hz, CH_3-27), 0.79

(3H, s, CH_3-18); ^{13}C NMR (C_5D_5N) δ 119.4 (CN), 105.0 (C-1'), 89.5 (C-22), 84.5 (C-16), 78.5 (C-3'), 78.5 (C-5'), 75.1 (C-2'), 75.0 (C-26), 71.6 (C-4'), 66.0 (C-3), 63.5 (C-17), 62.8 (C-6'), 56.7 (C-14), 42.1 (C-20), 41.2 (C-13), 39.9 (C-9), 39.8 (C-12), 36.9 (C-5), 35.5 (C-8 and C-10), 35.0 (C-23), 34.4 (C-15), 33.8 (C-25), 31.8 (C-24), 30.6 (C-4), 29.2 (C-1), 28.6 (C-7), 27.0 (C-6), 26.7 (C-2), 24.2 (C-19), 21.0 (C-11), 17.2 (C-21), 17.0 (C-27), 16.7 (C-18); *Anal*. Calcd for $C_{34}H_{55}NO_8 \cdot H_2O$: C, 65.46; H, 9.21; N, 2.25. Found: C, 65.13; H, 9.13; N, 2.31).

Deacetylation of compound (10)

The general procedure was employed with **10** (1.0 g, 1.2 mmol) dissolved in 5% KOH in EtOH/H_2O (1 : 1, 5 mL) to give a residue that was subjected to preparative HPLC (H_2O/MeOH 15 : 85) to afford compound (**23**) as amorphous powder (530 mg, 70.6%). FABMS (*m/z*): $[M + Na]^+$ = 646; ^1H NMR (C_5D_5N) (only assignable signals were listed) δ 8.66 (1H, d, *J* = 9.2 Hz, NH), 5.91 (1H, dd, *J* = 9.2, 8.8 Hz, H-1'), 4.70 (1H, m, H-16), 4.46-4.36 (2H, m, H-6'a and 6'b), 4.38 (1H, br s, H-3), 4.30 (1H, dd, *J* = 9.2, 8.8 Hz, H-4'), 4.22 (1H, dd, *J* = 8.8, 8.8 Hz, H-3'), 4.10 (1H, dd, *J* = 8.8, 8.8 Hz, H-2'), 3.99 (1H, m, H-5'), 3.76 (1H, dd, *J* = 10.3, 5.9 Hz, H-26a), 3.68 (1H, dd, *J* = 10.3, 6.2 Hz, H-26b), 1.43 (3H, d, *J* = 7.0 Hz, CH_3-21), 1.08 (3H, d, *J* = 6.6 Hz, CH_3-27), 1.02 (3H, s, CH_3-19), 0.86 (3H, s, CH_3-18); ^{13}C NMR (C_5D_5N) δ 174.1 (CONH), 94.4 (C-22), 83.6 (C-16), 81.2 (C-1'), 80.1 (C-5'), 79.5 (C-3'), 74.3 (C-2'), 71.5 (C-4'), 67.8 (C-26), 66.1 (C-3), 64.4 (C-17), 62.4 (C-6'), 56.2 (C-14), 43.2 (C-20), 41.5 (C-13), 40.0 (C-9), 40.0 (C-12), 37.2 (C-23), 37.0 (C-5), 36.8 (C-25), 35.6 (C-8), 35.4 (C-10), 34.4 (C-15), 32.8 (C-24), 30.6 (C-4), 28.6 x 2 (C-1 and C-7), 27.1 (C-6), 26.8 (C-2), 24.2 (C-19), 21.1 (C-11), 18.5 (C-21), 17.1 (C-27), 16.6 (C-18); *Anal.* Calcd for $C_{34}H_{57}NO_9 \cdot H_2O$: C, 63.62; H, 9.27; N, 2.18. Found: C, 63.29; H, 9.44; N, 2.09.

Deacetylation of compound (11)

The general procedure was employed with **11** (260 mg, 290 mmol) dissolved in 5% KOH in EtOH/H_2O (1 : 1, 2 mL) to give a residue that was subjected to preparative HPLC (H_2O/MeOH 15 : 85) to afford compound (**24**) as a white solid (mp 176-178 °C after recrystallization from MeOH, 140 mg, 87.1%). FABMS (*m/z*): $[M + Na]^+$ and $[M + Na + 2]^+$ = 708 and 710 (the peak height ratio = 1 : 1); ^1H NMR (C_5D_5N) (only assignable signals were listed) δ 8.91 (1H, d, *J* = 9.5 Hz, NH), 5.92 (1H, dd, *J* = 9.5, 8.9 Hz, H-1'), 4.71 (1H, ddd, *J* = 7.6, 7.0, 7.0 Hz, H-16), 4.46-4.35 (2H, m, H-6'a and 6'b), 4.38 (1H, br s, H-3), 4.31 (1H, dd, *J* = 9.2, 9.2 Hz, H-4'), 4.23 (1H,

dd, *J* = 9.2, 8.9 Hz, H-3'), 4.11 (1H, dd, *J* = 8.9, 8.9 Hz, H-2'), 4.00 (1H, ddd, *J* = 9.2, 7.0, 2.8 Hz, H-5'), 3.48 (1H, dd, *J* = 9.8, 4.3 Hz, H-26a), 3.39 (1H, dd, *J* = 9.8, 6.4 Hz, H-26b), 1.43 (3H, d, *J* = 7.3 Hz, CH_3-21), 1.03 (3H, s, CH_3-19), 1.00 (3H, d, *J* = 6.4 Hz, CH_3-27), 0.89 (3H, s, CH_3-18); ^{13}C NMR (C_5D_5N) δ 173.9 (CONH), 94.0 (C-22), 83.7 (C-16), 81.2 (C-1'), 80.2 (C-5'), 79.6 (C-3'), 74.3 (C-2'), 71.5 (C-4'), 66.0 (C-3), 64.5 (C-17), 62.4 (C-6'), 56.3 (C-14), 43.1 (C-20), 42.3 (C-26), 41.5 (C-13), 40.0 (C-9), 39.9 (C-12), 37.0 (C-5), 37.0 (C-23), 35.7 (C-25), 35.6 (C-10), 35.4 (C-8), 34.4 (C-15), 32.9 (C-24), 30.6 (C-4), 30.0 (C-1), 28.6 (C-7), 27.1 (C-6), 26.8 (C-2), 24.2 (C-19), 21.1 (C-11), 18.7 (C-27), 18.5 (C-21), 16.6 (C-18); *Anal.* Calcd for $C_{34}H_{56}NO_8Br{\cdot}H_2O$: C, 57.95; H, 8.30; N, 1.99. Found: C, 58.01; H, 8.27; N, 2.04.

Deacetylation of compound (12)

The general procedure was employed with **12** (210 mg, 234 mmol) dissolved in 5% KOH in EtOH/H_2O (1 : 1, 2 mL) to give a residue that was subjected to preparative HPLC (H_2O/MeOH 15 : 85) to afford compound (**25**) as a white solid (mp 194-195 °C after recrystallization from MeOH, 170 mg, 85.4%). FABMS (*m/z*): $[M + Na]^+$ and $[M + Na + 2]^+$ = 708 and 710 (the peak height ratio = 1 : 1); ^{1}H NMR (C_5D_5N) (only assignable signals were listed) δ 8.20 (1H, d, *J* = 8.2 Hz, NH), 6.30 (1H, dd, *J* = 8.2, 4.6 Hz, H-1'), 5.01 (1H, ddd, *J* = 7.6, 7.0, 7.0 Hz, H-16), 4.46 (1H, dd, *J* = 11.6, 2.0 Hz, H-6'a), 4.42 (1H, br s, H-3), 4.40 (1H, m, H-3'), 4.39 (1H, m, H-2'), 4.35 (1H, dd, *J* = 11.6, 4.6 Hz, H-6'b), 4.22 (1H, m, H-5'), 4.19 (1H, m, H-4'), 3.48 (1H, dd, *J* = 9.8, 4.6 Hz, H-26a), 3.42 (1H, dd, *J* = 9.8, 6.4 Hz, H-26b), 1.43 (3H, d, *J* = 7.0 Hz, CH_3-21), 1.02 (3H, s, CH_3-19), 0.98 (3H, d, *J* = 6.4 Hz, CH_3-27), 0.89 (3H, s, CH_3-18); ^{13}C NMR (C_5D_5N) δ 174.1 (CONH), 94.2 (C-22), 83.9 (C-16), 77.7 (C-1'), 76.0 (C-3'), 75.2 (C-5'), 71.1 (C-2'), 71.1 (C-4'), 66.1 (C-3), 64.7 (C-17), 62.6 (C-6'), 56.2 (C-13), 43.1 (C-20), 42.1 (C-26), 41.5 (C-13), 40.0 (C-9), 39.9 (C-12), 37.2 (C-23), 37.0 (C-5), 35.7 (C-25), 35.5 (C-10), 35.4 (C-8), 34.4 (C-15), 33.0 (C-24), 30.6 (C-4), 30.2 (C-1), 28.6 (C-7), 27.1 (C-6), 26.8 (C-2), 24.2 (C-19), 21.1 (C-11), 18.6 (C-21), 18.5 (C-27), 16.6 (C-18); *Anal.* Calcd for $C_{34}H_{56}NO_8Br{\cdot}H_2O$: C, 57.95; H, 8.30; N, 1.99. Found: C, 57.67; H, 8.39; N, 2.13.

Deacetylation of compound (13)

The general procedure was employed with **13** (1.0 g, 1.2 mmol) dissolved in 5% KOH in EtOH/H_2O (1 : 1, 5 mL) to give a residue that was subjected to preparative HPLC (H_2O/MeOH 15 : 85) to give compound (**26**) (white solid, mp 156-158 °C after recrystallization from MeOH,

110 mg, 14.8%) and (**27**) (a white solid, mp 193-194 °C after recrystallization from MeOH , 230 mg, 42.2%). FABMS of **26** (*m/z*): $[M + Na]^+$ = 628; ^1H NMR (C_5D_5N) (only assignable signals were listed) δ 6.27 (1H, d, *J* = 4.6 Hz, H-1'), 5.28 (1H, m, H-16), 4.84 (1H, dd, *J* = 8.1, 4.6 Hz, H-2'), 4.45-4.31 (2H, m, H-6'a and 6'b), 4.40 (1H, m, H-3'), 4.38 (1H, br s, H-3), 4.24 (1H, m, H-4'), 4.16 (1H, m, H-5'), 3.72 (1H, dd, *J* = 11.5, 4.8 Hz, H-26a), 3.65 (1H, dd, *J* =11.5, 7.6 Hz, H-26b), 1.18 (3H, d, *J* = 7.0 Hz, CH_3-21), 1.08 (3H, d, *J* = 6.7 Hz, CH_3-27), 1.02 (3H, s, CH_3-19), 0.87 (3H, s, CH_3-18); ^{13}C NMR (C_5D_5N) δ 171.7 (C=N), 94.1 (C-1'), 90.9 (C-22), 84.1 (C-16), 83.2 (C-2'), 76.6 (C-5'), 76.1 (C-3'), 69.4 (C-4'), 67.4 (C-26), 66.1 (C-3), 64.1 (C-17), 62.6 (C-6'), 56.4 (C-14), 49.6 (C-20), 41.2 (C-13), 40.3 (C-12), 40.0 (C-9), 36.9 (C-8), 36.8 (C-25), 35.9 (C-23), 35.5 (C-5), 35.4 (C-10), 34.3 (C-4), 32.8 (C-15), 30.5 (C-6), 28.5 (C-1 and C-24), 27.1 (C-2), 26.7 (C-7), 24.2 (C-19), 21.2 (C-11), 17.2 (C-21), 17.1 (C-27), 16.9 (C-18); *Anal.* Calcd for $C_{34}H_{55}NO_8 \cdot H_2O$: C, 65.46; H, 9.21; N, 2.25 Found: C, 65.22; H, 9.30; N, 2.33). FABMS of **27** (*m/z*): $[M + Na]^+$ = 467; ^1H NMR (C_5D_5N) (only assignable signals were listed) δ 4.85 (1H, dd, *J* = 11.6, 2.1 Hz, H-26a), 4.63 (1H, dd, *J* = 11.8, 7.3 Hz, H-16), 4.39 (1H, br s, H-3), 3.94 (1H, dd, *J* =11.6, 4.9 Hz, H-26b), 1.42 (3H, d, *J* =7.0 Hz, CH_3-21), 1.02 (3H, s, CH_3-19), 0.90 (3H, d, *J* = 7.3 Hz, CH_3-27), 0.86 (3H, s, CH_3-18); ^{13}C NMR (C_5D_5N) δ 172.8 (O-C=O), 93.7 (C-22), 84.0 (C-16), 72.2 (C-26), 66.0 (C-3), 62.6 (C-17), 56.4 (C-14), 46.9 (C-20), 40.8 (C-13), 40.4 (C-12), 40.0 (C-9), 37.0 (C-8), 35.6 (C-5), 35.3 (C-10), 34.4 (C-23), 32.2 (C-15), 31.8 (C-25), 30.6 (C-4), 30.2 (C-6), 29.2 (C-24), 28.6 (C-1), 27.1 (C-2), 26.8 (C-7), 24.2 (C-19), 21.3 (C-11), 16.6 (C-18), 15.4 (C-27), 15.1 (C-21); IR (nujol): 1720 cm^{-1}, *Anal.* Calcd for $C_{28}H_{44}NO_4$: C, 75.63; H, 9.97. Found: C, 75.47; H, 10.01.

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