# Chemistry and Structure of Ganefromycin

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*Received May 18, 1993*•

Ganefromycins are antibiotics produced by *Streptomyces lydicus* sp. *tanzanius* having commercial potential as performance enhancement agents for livestock. Ganefromycins are related to the elfamycin family of antibiotics but contain several unique chemical features which are the source of novel and unexpected chemistry. Reactions under mildly basic conditions resulted in the interconversion of ganefromycins $\alpha$ (1) and $\beta$ (2) by a 1,2-acyl migration. Strong base causes elimination of a trisaccharide whose structure was solved by single-crystal X-ray diffraction analysis of the triacetate of the reduced ring-opened triol. Ammonolysis yields the same rearranged product from either 1 or 2. Evidence is provided for the mechanism of this rearrangement involving elimination of the saccharide to form a transient $\alpha,\beta$-unsaturated carbonyl, Michael addition of ammonia, and intramolecular transacylation. Ozonolysis and acidic methanolysis were employed to obtain simplified compounds for structure determination. Ganefromycin $\beta$ fragments in warm acetic acid solution, releasing the long-chain amino acid. $^{13}C$ NMR data with assignments are provided for the degradation products.

## Introduction

Ganefromycins $\alpha$ (1) and $\beta$ (2) were isolated from a new subspecies of *Streptomyces lydicus*, desinated *tanzanius*, isolated from a pasture near Lake Manyara in Tanzania, Africa. The cultural and physiological characteristics of the organism as well as the biological activity of the ganefromycins have been described.[1] Ganefromycins have a very narrow spectrum of antibacterial activity against human pathogens and show little toxicity, while demonstrating exceptional activity as growth promoting agents in livestock. Aspects of the discovery, isolation, characterization, and biosynthesis of the ganefromycins have been presented previously.[2-4] Ganefromycins belong to the elfamycin[5] family of antibiotics typified by aurodox;[6] however they contain several distinctly different chemical features. These compounds were the first elfamycins to have sugar moieties linked at a position other than O-24.[7] The presence of the trisaccharide at O-21a has a dramatic effect on the chemistry of the antibiotics, especially under basic conditions. Ganefromycins have a truncated backbone structure terminating in a carboxylic acid rather than the substituted pyridone unit found in most of the elfamycin antibiotics. The most closely related antibiotics are the phenelfamycins whose structures were determined through detailed spectroscopic investigations.[8] The phenelfamycin study reported structures and relative stereochemistry for seven components and suggested the identity of phenelfamycins E and F with ganefromycins $\alpha$ and $\beta$, respectively. In the present report the chemistry leading to the structure determination of the ganefromycins is described. It will be shown that the trisaccharide substituent at position 21a confers surprising reactivity upon the compounds under basic conditions and its elimination with ammonia results in a unique unexpected rearrangement reaction.

## Results and Discussion

**Spectral Characterization and Derivatives.** Ganefromycins $\alpha$ (1) and $\beta$ (2) are isomeric having molecular formulas $C_{65}H_{95}NO_{21}$ as determined by HRFABMS. 1 and 2 are readily separable by reverse phase HPLC and are interconvertable under mildly basic conditions (1% $NaHCO_3$) with the equilibrium slightly favoring $\beta$. How the nature of this isomerism was discovered will be defined below, and for the chemical studies, the results for the $\alpha$ isomer (1) will generally be presented. Physicochemical data and spectroscopic characterization for 1 and 2 have been presented previously[2] and these data defined some of the functional groups of the compounds. Of the required 19 units of unsaturation, 3 could be assigned to carbonyl groups and 10 to olefinic bonds on the basis of $^{13}C$ NMR signals (Table I), leaving 6 to be accounted for—apparently as rings. APT results indicated 89 protons bonded to carbon and therefore 6 must be attached to heteroatoms. Of these latter six, four could be further defined by the formation of chemical derivatives. Acetylation of 1 with acetic anhydride in pyridine at room temperature afforded a triacetyl derivative (3), indicating the presence of at least three primary and/or secondary OH groups. A carboxylic acid group in conjugation with a triene system was suggested by the strong UV absorbance at 290 nm.

• Abstract published in *Advance ACS Abstracts*, October 1, 1993.

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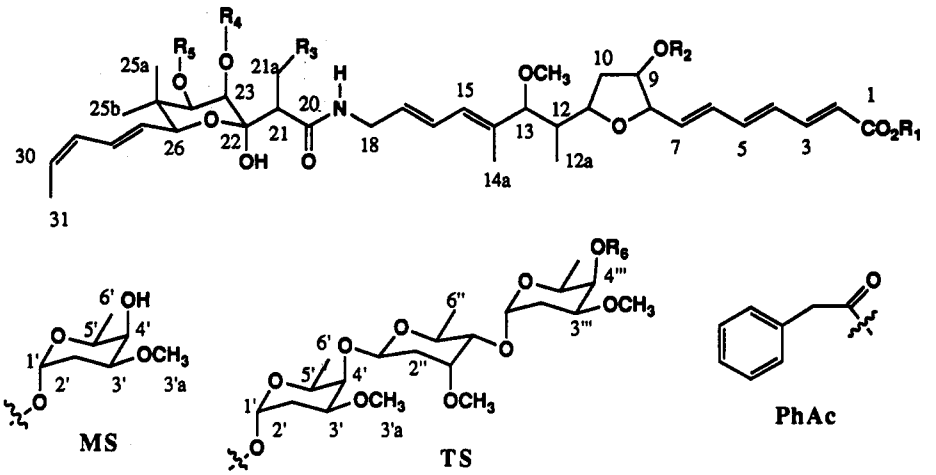


MS TS PhAc

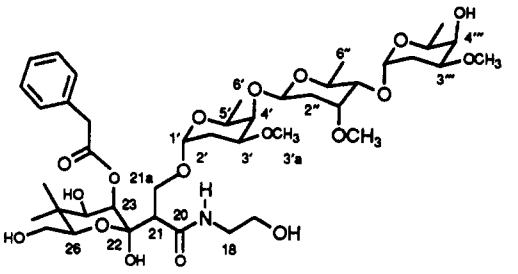
| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ |
|---|---|---|---|---|---|---|
| 1 | H | H | TS | PhAc | H | H |
| 2 | H | H | TS | H | PhAc | H |
| 3 | H | Ac | TS | PhAc | Ac | Ac |
| 4 | $CH_3$ | H | TS | PhAc | H | H |
| 10 | H | H | NHPhAc | H | H | - |
| 11 | $CH_3$ | H | OH | PhAc | H | - |
| 12 | $CH_3$ | Ac | OAc | PhAc | Ac | - |
| 13 | $CH_3$ | H | MS | PhAc | H | - |
| 15 | $CH_3$ | H | TS | H | PhAc | H |

Treatment of 1 with ethereal diazomethane yielded methyl ester 4, thus confirming the acid function. In the UV spectrum of the ester, the long wavelength maximum was shifted to somewhat longer wavelength (298 nm) which was taken as further evidence for conjugation with the triene.

Catalytic hydrogenation of 1 under mild conditions resulted in the formation of tetradecahydro analogue 5 corresponding to the reduction of seven olefinic bonds. Compound 5 had lost the strong UV absorbances at 233 and 290 nm but retained NMR signals in the olefinic region corresponding to a phenyl substituent ($\delta_H$ 7.30, 5H, m; $\delta_C$ 127.1, 128.6 (2×), 129.4 (2×), 133.9). Of these seven olefinic bonds present in the parent compounds, three are in conjugation with the carboxylic acid, forming the unit responsible for the 290-nm absorption, leaving four potentially involved in the chromophore producing the strong maximum at 233 nm. The presence of two separate conjugated diene moieties is consistent with the intensity of this band ($\epsilon$ = 49 800).

In addition to the other obvious functional groups, three aldose sugars were indicated by characteristic NMR signals (Table I) for their anomeric carbons at $\delta_C$ 100.6, 99.0, and 97.1 as well as their corresponding proton resonances at $\delta_H$ 4.98, 4.73, and 4.64, respectively. Further definition of the functional groups and structural fragments and their linkage into the complete structure was accomplished through detailed analysis of a series of degradation products.

**Ozonolysis.** Ozonolysis was initially explored as a possible route to less complex structures. Treatment of 1 with ozone at −78 °C with a reductive workup employing $NaBH_4$ resulted in one major product (6) after chromatographic separation of a complex reaction mixture. Product 6 was only of marginal utility in terms of spectroscopic investigation owing to numerous overlapping oxygenated methine and methylene $^1H$ NMR signals. The compound



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was of value in establishing the proximity of the phenylacetate and trisaccharide substituents, the ketal, geminal dimethyl groups, and the amide group. The phenylacetate ester was represented by an IR band at 1741 $cm^{-1}$, $^{13}C$ NMR signals (Table I) and $^1H$ NMR signals indicative of the phenyl substituent (δ 7.32, 5H, m) and methylene group (δ 3.69, 2H, s). The presence of the trisaccharide group was clearly indicated by $^{13}C$ NMR signals, particularly those of the anomeric carbons (δ 96.93, 98.98, 100.6) as well as $^1H$ NMR doublets arising from the 6-methyl groups at δ 1.18, 1.25, and 1.30, singlets for three methoxy groups at δ 3.39, 3.40, and 3.41, and anomeric proton doublets at δ 4.62, $J$ = 3.0 Hz; 4.72, $J$ = 9.6 Hz; and 4.98, $J$ = 3.5 Hz. A characteristic resonance for a quaternary carbon at $\delta_C$ 97.01 signaled the inclusion of the ketal group found in the parent structure. Evidence for the amide group included an IR band at 1650 $cm^{-1}$, a $^{13}C$ NMR signal at δ 174.4, and a $^1H$ NMR broad triplet for N–H at δ 5.81. The geminal dimethyl group on a quaternary carbon was also represented by high-field singlets ($\delta_H$ 0.86 and 0.97).

Table I. $^{13}C$ NMR Chemical Shifts for Selected Products ($CDCl_3$)

| C | 1 | 2 | 4 | 6 | 7 | 9 | 10* | 11 | 12 | 13 | 15 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 173.3 | 173.6 | 173.2 | 174.4 | | | 175.0 | 173.2 | 172.3 | 173.1 | 173.5 | |
| 32 | 171.4 | 170.6 | 171.2 | 171.4 | | | 172.8 | 171.6 | 170.5 | 171.1 | 170.5 | |
| 1 | 170.4 | 170.0 | 167.3 | | | | 168.1 | 167.4 | 167.3 | 167.3 | 167.2 | 167.4 |
| 3 | 145.9 | 145.6 | 144.3 | | | | 145.3 | 144.4 | 144.3 | 144.4 | 144.3 | 144.3 |
| 5 | 140.3 | 140.2 | 139.7 | | | | 141.0 | 139.8 | 139.6 | 139.8 | 139.8 | 139.8 |
| 14 | 137.2 | 136.7 | 137.0 | | | | 136.6 | 136.3 | 136.9 | 136.9 | 136.2 | 136.6 |
| 7 | 134.4 | 134.4 | 134.1 | | | | 137.1 | 134.2 | 133.2 | 134.2 | 134.3 | 134.2 |
| 34 | 133.9 | 133.9 | 133.8 | 133.8 | | | 136.1 | 133.8 | 134.0 | 133.8 | 133.7 | |
| 6 | 132.0 | 132.0 | 131.8 | | | | 131.3 | 131.8 | 131.7 | 131.7 | 131.5 | 131.9 |
| 4 | 130.1 | 130.1 | 130.0 | | | | 130.2 | 129.9 | 130.2 | 129.9 | 129.7 | 128.5 |
| 36 | 129.6 | 129.1 | 129.4 | 129.4 | | | 129.8 | 129.5 | 129.6 | 129.4 | 128.9 | |
| 38 | 129.6 | 129.1 | 129.4 | 129.4 | | | 129.8 | 129.5 | 129.6 | 129.4 | 128.9 | |
| 29 | 129.0 | 128.9 | 128.9 | | | | 130.1 | 128.8 | 128.8 | 128.8 | 128.9 | |
| 35 | 128.6 | 128.6 | 128.5 | 128.7 | | | 128.9 | 128.5 | 128.4 | 128.3 | 128.3 | |
| 39 | 128.6 | 128.6 | 128.5 | 128.7 | | | 128.9 | 128.5 | 128.4 | 128.3 | 128.3 | |
| 16 | 128.5 | 128.6 | 128.5 | | | | 130.0 | 128.5 | 128.4 | 128.3 | 128.6 | 128.1 |
| 15 | 128.3 | 128.5 | 128.3 | | | | 129.9 | 128.3 | 128.4 | 128.2 | 128.3 | 128.4 |
| 17 | 128.2 | 128.4 | 128.2 | 61.09 | | | 129.4 | 128.0 | 128.2 | 128.1 | 127.7 | 130.0 |
| 27 | 128.2 | 128.2 | 128.0 | 61.19 | | | 127.8 | 127.7 | 128.0 | 128.0 | 127.6 | |
| 28 | 127.5 | 127.8 | 127.3 | | | | 127.2 | 127.4 | 127.4 | 127.2 | 127.3 | |
| 37 | 127.1 | 127.2 | 127.0 | 127.2 | | | 126.8 | 127.0 | 127.0 | 126.8 | 126.9 | |
| 30 | 126.3 | 126.5 | 126.1 | | | | 125.8 | 126.2 | 126.7 | 126.1 | 126.2 | |
| 2 | 120.7 | 120.9 | 120.6 | | | | 121.6 | 120.5 | 120.8 | 120.5 | 120.3 | 120.6 |
| 1‴ | 100.6 | 100.6 | 100.5 | 100.6 | 100.5 | 100.7 | | | | | 100.2 | |
| 1″ | 98.99 | 98.97 | 98.86 | 98.98 | 98.88 | 98.49 | | | | | 98.67 | |
| 22 | 97.30 | 98.40 | 97.11 | 97.01 | | | 99.57 | 97.47 | 97.35 | 97.16 | 98.12 | |
| 1′ | 97.06 | 97.21 | 96.81 | 96.93 | 92.19 | 61.28 | | | | 96.15 | 96.81 | |
| 13 | 89.26 | 89.17 | 89.01 | | | | 89.90 | 89.09 | 89.03 | 89.00 | 88.86 | 89.04 |
| 8 | 83.17 | 83.29 | 83.06 | | | | 84.05 | 83.11 | 81.48 | 83.09 | 82.94 | 83.17 |
| 4″ | 81.70 | 81.64 | 81.51 | 81.69 | 81.57 | 81.90 | | | | | 81.32 | |
| 11 | 77.72 | 77.57 | 77.42 | | | | 77.76 | 77.46 | 77.65 | 77.02 | 77.20 | 77.49 |
| 3″ | 77.02 | 77.52 | 76.80 | 77.25 | 76.90 | 79.22 | | | | | 76.57 | |
| 26 | 76.42 | 76.19 | 76.21 | 76.97 | | | 76.30 | 76.36 | 76.44 | 76.02 | 75.84 | |
| 9 | 74.66 | 75.48 | 74.41 | | | | 74.84 | 74.45 | 76.39 | 74.63 | 75.81 | 74.46 |
| 3‴ | 74.45 | 74.62 | 74.23 | 74.39 | 74.54 | 77.25 | | | | | 75.25 | |
| 3′ | 74.45 | 74.46 | 74.21 | 74.25 | 74.28 | 77.00 | | | | 74.37 | 74.18 | |
| 4′ | 74.28 | 74.25 | 74.14 | 74.25 | 73.62 | 72.99 | | | | 67.29 | 74.18 | |
| 24 | 72.29 | 74.25 | 71.87 | 71.89 | | | 72.74 | 71.84 | 72.88 | 71.77 | 73.95 | |
| 23 | 72.01 | 69.11 | 71.73 | 71.66 | | | 70.64 | 72.10 | 68.74 | 71.77 | 68.78 | |
| 5″ | 69.14 | 68.94 | 69.00 | 69.11 | 69.03 | 70.87 | | | | | 68.54 | |
| 4‴ | 67.61 | 67.53 | 67.36 | 67.55 | 67.42 | 68.94 | | | | | 67.22 | |
| 5′ | 66.49 | 66.57 | 66.22 | 66.43 | 66.21 | 68.26 | | | | 65.57 | 66.22 | |
| 5‴ | 66.08 | 66.08 | 65.88 | 66.03 | 65.92 | 65.37 | | | | | 65.82 | |
| 21a | 63.57 | 64.08 | 63.35 | 63.22 | | | 43.20 | 59.71 | 61.76 | 63.53 | 63.73 | |
| 3″a | 56.53 | 56.49 | 56.34 | 56.52 | 56.40 | 57.35 | | | | | 56.22 | |
| 13a | 56.08 | 56.01 | 55.93 | | | | 55.90 | 55.89 | 56.02 | 55.87 | 55.68 | 55.89 |
| 3′a | 55.63 | 55.62 | 55.48 | 55.90 | 55.61 | 56.79 | | | | 55.26 | 55.30 | |
| 3‴a | 55.45 | 55.43 | 55.30 | 55.45 | 55.37 | 56.13 | | | | | 55.11 | |
| 21 | 49.87 | 49.59 | 49.58 | 49.85 | | | 49.78 | 51.96 | 48.78 | 49.44 | 49.14 | |
| 18 | 41.79 | 41.62 | 41.60 | 42.09 | | | 41.75 | 41.35 | 41.59 | 41.53 | 41.25 | 41.96 |
| 33 | 41.79 | 41.62 | 41.51 | 41.70 | | | 38.52 | 41.48 | 41.53 | 41.53 | 41.25 | |
| 12 | 39.91 | 39.77 | 39.65 | | | | 40.21 | 39.71 | 39.66 | 39.62 | 39.44 | 39.72 |
| 10 | 39.30 | 39.15 | 39.06 | | | | 39.38 | 39.09 | 36.76 | 39.00 | 38.89 | 39.07 |
| 25 | 38.84 | 38.03 | 38.62 | 37.08 | | | 39.83 | 38.58 | 37.68 | 38.59 | 37.69 | |
| 2″ | 33.04 | 32.89 | 32.72 | 32.94 | 32.77 | 33.50 | | | | | 32.59 | |
| 2′ | 31.08 | 31.09 | 30.92 | 31.05 | 31.19 | 31.36 | | | | 29.09 | 30.73 | |
| 2‴ | 29.95 | 29.89 | 29.74 | 29.94 | 29.76 | 28.87 | | | | | 29.56 | |
| 25b | 23.75 | 23.65 | 23.60 | 23.48 | | | 24.23 | 23.64 | 23.50 | 23.61 | 23.31 | |
| 6″ | 18.13 | 18.09 | 17.95 | 18.09 | 18.00 | 18.03 | | | | | 17.78 | |
| 6′ | 17.22 | 17.22 | 17.03 | 17.18 | 17.29 | 16.95 | | | | 16.52 | 16.89 | |
| 6‴ | 17.00 | 17.01 | 16.86 | 17.01 | 16.91 | 16.89 | | | | | 16.70 | |
| 25a | 14.79 | 16.18 | 14.64 | 14.66 | | | 15.57 | 14.64 | 15.94 | 14.66 | 15.85 | |
| 31 | 13.48 | 13.49 | 13.34 | | | | 13.45 | 13.35 | 13.50 | 13.30 | 13.16 | |
| 14a | 10.89 | 10.77 | 10.65 | | | | 10.81 | 10.65 | 10.74 | 10.67 | 10.44 | 10.68 |
| 12a | 10.16 | 10.04 | 9.86 | | | | 10.25 | 9.94 | 10.03 | 9.83 | 9.69 | 9.92 |
| 1-$OCH_3$ | | | 51.42 | | | | | 51.43 | 51.51 | 51.39 | 51.27 | 51.44 |
| other | | | | | | 171.0 | | | 170.3 | | | 166.4 |
| derivatives | | | | | | 170.8 | | | 170.2 | | | 133.0 |
| *acetone-$d_6$ | | | | | | 170.1 | | | 169.9 | | | 131.6 |
| | | | | | | 21.18 | | | 21.01 | | | 131.6 |
| | | | | | | 20.95 | | | 20.76 | | | 128.5 |
| | | | | | | 20.82 | | | 20.50 | | | 128.5 |
| | | | | | | | | | | | | 126.0 |

All of these units could not be precisely connected by 2D techniques to form a single structure because of extensive signal overlap in the range $\delta$ 3–4 in the $^1H$ NMR spectrum.

**Basic Degradation.** Treatment of 1 with $NaOCH_3$ in methanol led to extensive decompostion of the chromophore; however, a trisaccharide (7) was obtained as was

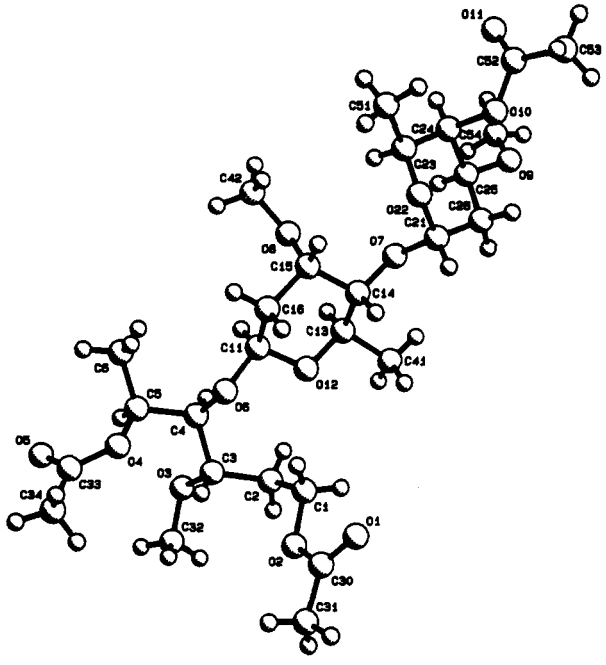
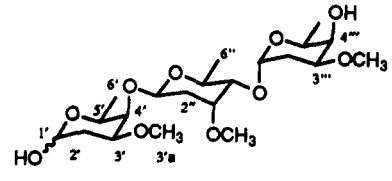


**Figure 1.** Structure of trisaccharide derivative 9.

methyl phenylacetate. Identification of the latter was accomplished by GC/MS analysis and thus confirmed the presence of a phenylacetyl residue in the antibiotic. Reacting the trisaccharide anomeric mixture with sodium borohydride yielded a single ring-opened triol 8, which was converted to the crystalline triacetate 9. The structure of 9 as determined by single crystal X-ray diffraction analysis is shown in Figure 1.[9] Absolute stereochemistry in 9 was established by comparing the optical rotations of methyldiginoside obtained via methanolysis of 9 with literature values.[10]



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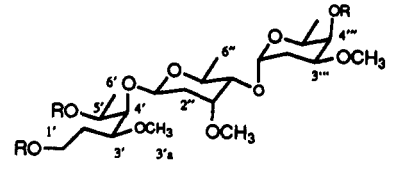

8 R = H
9 R = Ac

A milder basic procedure was sought in an attempt to preserve the chromophoric portion of the antibiotics. To this end, ammonolysis in acetonitrile cosolvent was performed on 1 and 2, resulting in isolation of a single

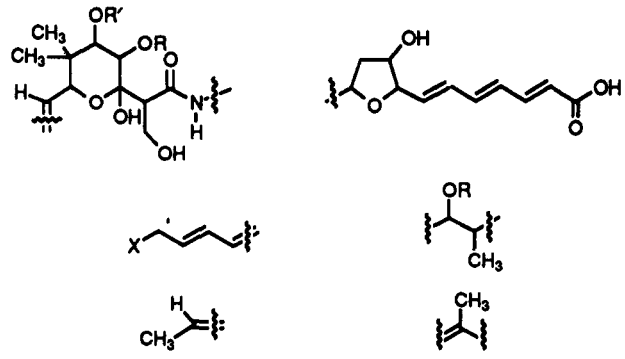

**Figure 2.** Ganefromycin substructures from NMR analysis of 12.

chromophoric product (10) concomitant with liberation of the trisaccharide. Net addition of $NH_3$ was achieved in this process as the chromophore-containing product 10 had the molecular formula $C_{44}H_{60}N_2O_{11}$. There was evidence that the product had rearranged in some manner, since the $^{13}C$ NMR signal for C-21a had shifted from $\delta$ 63.6 to 43.2. In addition, the expected product should have contained an $NH_2$ group derived from ammonia, but a signal for only one new proton bonded to nitrogen was observed in the $^1H$ NMR spectrum of 10. Since the relationship of this product to the parent structure was not clear, it was not further explored until the structures of the ganefromycins were solved. The origin of this compound will be discussed subsequently.

**Acidic Methanolysis.** Methanolysis under acidic conditions (HCl/MeOH) proved to be the most straightforward means for obtaining simplified products, albeit in low yields. By varying acid concentration and monitoring by HPLC, products lacking one, two, or all three sugar units could be obtained. Starting with methyl ester 4, an aglycone 11 was produced, which when converted to triacetate 12 was suitable for detailed spectroscopic analysis. Analysis of the $^1H$ COSY spectrum of 12 in conjunction with long-range correlation experiments provided the substructural fragments shown in Figure 2. These units were combined by analogy to known elfamycins, in particular L-681,217,[11] the first of the class to have the truncated backbone terminating in a carboxylic acid conjugated to the triene system.

Having established the backbone structure of the antibiotics, attention was turned to determining the site of attachment of the trisaccharide. At the time these structures were determined, the only precedent for sugar attachment in this antibiotic family was at O-24, in the case of efrotomycin.[12] Initially[13] the phenelfamycins were also believed to be glycosylated at O-24, as is the only other glycosylated elfamycin UK-69,753.[14] The lability of the trisaccharide under basic conditions, however, could not be reconciled by an analogous structure for ganefromycin. In view of this disparity, several independent methods were employed to establish the position of the

(9) Crystal data: $C_{27}H_{46}O_{13}$, FW = 578.65, space group $P2_12_12_1$ (orthorhombic), $Z = 4$, $a = 8.163(2)$, $b = 48.175(6)$, and $c = 8.064(1)$ Å, $V = 3171(1)$ Å$^3$, $D_c = 1.212$ g cm$^{-3}$. The authors have deposited atomic coordinates for this structure with Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

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(12) Wax, R.; Maiese, W.; Weston, R.; Birnbaum, J. *J. Antibiot.* **1976**, *29*, 670.

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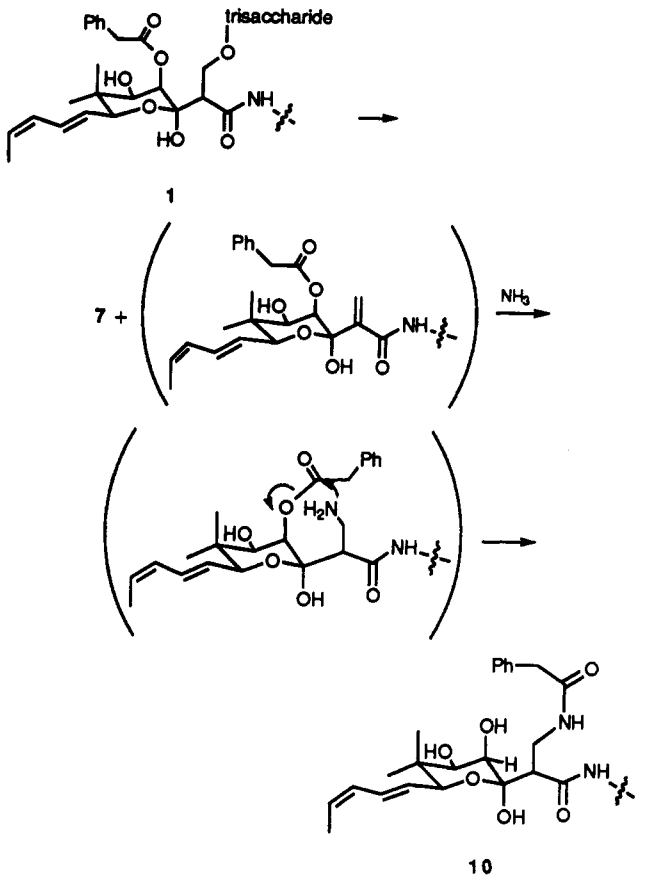
(14) Jefson, M. R.; Bordner, J.; Reese, C. P.; Whipple, E. B. *J. Antibiot.* **1989**, *42*, 1610.

**Scheme I**

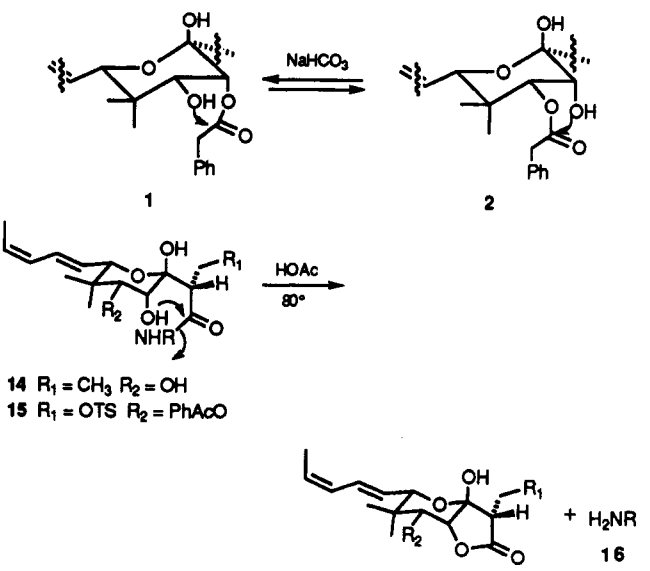



trisaccharide in ganefromycin. First, since the ozonolysis product **6** included the trisaccharide, glycosylation had to be through O-21a, -22, or -24. Linkage at O-21a was established by two lines of evidence. Comparing the $^{13}C$ NMR chemical shift values (Table I) between the aglycon **11** and monosaccharide derivative **13** showed that of the three carbinol carbons, only the signal for C-21a was significantly shifted to higher field in **13** ($\delta$ 63.5 versus 59.7). Spectra of the same two compounds were also compared in a deuterium-induced shift experiment wherein the $^{13}C$ NMR spectra were recorded in the presence of $CH_3OD$ and compared to spectra obtained under the same conditions except using $CH_3OH$. Under these conditions, carbons bearing exchangeable OH groups will show deuterium-induced shifts of their $^{13}C$ NMR signals to slightly higher field.[15] When **11** was studied this way all three signals shifted. In the case of **13**, signals for C-22 and -24 showed the shift, while that of C-21a was unchanged, thereby indicating glycosylation.

**Base-Catalyzed Trisaccharide Elimination.** Trisaccharide liberation under basic conditions must therefore be a simple base-catalyzed elimination resulting in a transient $\alpha,\beta$-unsaturated carbonyl as shown in Scheme I. No intermediates with this structural unit have been isolated in the course of this work, presumably because of their reactivity. The only direct evidence for this proposal comes in the form of the ammonolysis product **10**. In this case the intermediate has evidently been trapped by reaction with $NH_3$ as shown in Scheme I. As indicated, the intermediate primary amine undergoes further reaction via a seven membered transition state to form the phenylacetamide derivative. Spectroscopic evidence for the structure of **10** includes a shift of the $^1H$ NMR signal for H-23 from $\delta$ 5.0 to 3.7, as well as the aforementioned shift for the C-21a signal, and coupling between the 21a methylene protons and the new NH signal as detected via single-frequency irradiation experiments.

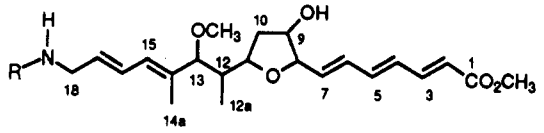
(15) Pfeffer, P. E.; Valentine, K. M.; Parrish, F. W. *J. Am. Chem. Soc.* **1979**, *101*, 1265.

**Scheme II**




**Interconversion of 1 and 2.** The interconversion of **1** and **2** is readily achieved in 1% $NaHCO_3$ resulting in an equilibrium mixture slightly favoring **2** ($K_{eq}$ = [1]/[2] = 0.41). After prolonged equilibration in 1% $NaHCO_3$, decomposition of the **1**/**2** mixture was observed by HPLC analysis. Higher pH conditions such as that obtained with 1% $Na_2CO_3$ resulted in rapid decomposition. Although **1** and **2** are well separated by reverse phase HPLC, their spectroscopic differences are much more subtle as reflected in a comparison of their $^{13}C$ NMR chemical shifts (Table I). The only signals which differ by more than 0.5 ppm between the two isomers are those for C-22 to C-25 and the phenylacetate carbonyl C-32. Characteristic differences are also observed between the $^1H$ NMR spectra of **1** and **2**. Most obvious are the shifts for H-21 ($\delta_H$ 2.30 for **1** versus 3.01 for **2**) and the 25 methyl groups (25a $\delta$ 0.86 to 0.96 and 25b $\delta$ 0.92 to 0.72 from **1** to **2**, respectively). In view of these data, the structure of $\beta$ was assigned as **2**, wherein the phenylacetate ester is transposed to the 24 position. Facile migration of the ester group under basic conditions, as depicted in Scheme II, also explains how the identical ammonolysis product **10** was obtained from both **1** and **2**.

**Fragmentation of 2 in Glacial Acetic Acid.** Further evidence for the structural difference between **1** and **2** was obtained from a comparison of the reactivities of their methyl ester derivatives in glacial acetic acid. Aurodox **14** undergoes an anchimerically assisted fragmentation of the amide bond in warm acetic acid, yielding the free amine portion and a bicyclic lactone as shown in Scheme II.[6] Under identical reaction conditions, the $\beta$ methyl ester **15** fragments, releasing amine **16**, while **4** remains intact, giving no reaction. Lack of reactivity of **4** is further evidence that the 23-OH is esterified with the phenylacetate group and therefore blocked from participating in the intramolecular displacement, while in **15** this OH group is free to react. The amine product was isolated and characterized as its (*p*-bromobenzoylamide) derivative **17**.



16 R = H
17 R = *p*-Br-benzoyl

## Conclusion

The most intriguing chemistry discovered in the course of these experiments resulted from basic treatment of the ganefromycins. Mildly basic conditions, such as treatment with 1% bicarbonate, promoted the migration of the phenacyl group between O-23 and O-24 resulting in an equilibrium mixture slightly biased in favor of ganefromycin $\beta$ (2), which has the equatorial ester at O-24. This interconversion process was an important element leading to the understanding of the nature of the isomerism between 1 and 2. Under highly basic conditions (e.g. sodium methoxide in methanol) only the trisaccharide and methyl phenylacetate were obtained. This elimination of the trisaccharide was evidence for the unique site of glycosylation in these compounds and was the first indication of the reactivity of H-21. The 21 proton is sufficiently acidic to react with concentrated ammonia in acetonitrile solution, giving rise to a transient $\alpha,\beta$-unsaturated carbonyl intermediate, which is then captured by Michael addition of ammonia. The interplay of both processes is highlighted in the formation of **10** by intramolecular transacylation of the intermediate primary amine. In addition to these processes ganefromycin has been shown to undergo a variety of chemical conversions which were more predictable such as acidic methanolysis and ozonolysis. Fragmentation of the backbone amide of ganefromycin $\beta$ in warm acetic acid is a hallmark of the class of compounds wherein the hydroxyl group at O-23 is free to react.

## Experimental Section

$^1$H NMR spectra were recorded at either 300 or 500 MHz and $^{13}$C NMR spectra at 75 or 125 MHz. All chemical shift values are reported in parts per million downfield of internal tetramethysilane. Mass spectra, obtained in the FAB mode, employed a 5:1 mixture of dithiothreitol and dithioerythritol as the matrix (unless specified). Useful meta-adduct molecular ion abundances were obtained of sodium or potassium salts to the matrix prior to low resolution scan acquisition or high resolution mass measurement (R ~ 10 000). High resolution measurements done by peak matching employed an internal standard ion from polyethylene glycol or an appropriate ion from sodium or cesium iodide on the wobble probe. General chromatographic procedures are as follows, unless otherwise specified. HPLC analyses were achieved using Perkin-Elmer 3X3 CR $C_{18}$ cartridge columns. The solvent for all analytical HPLC and reversed phase preparative separations consisted of mixtures of acetonitrile and 0.1 M ammonium acetate, pH 4.5 (buffer). Detection was by UV absorbance at 254 and 280 nm. Preparative separations were obtained either using a Prep-500 system with standard Waters cartridges filled with 55–105 $\mu$m silica gel or $C_{18}$ bonded phase, eluted at a flow rate of 100 mL/min, collecting 300-mL fractions, or with a smaller capacity high pressure pumping system. In the latter case, Rainin Dynamax 21.5 mm × 25 cm $C_{18}$ columns were used for reversed phase separations and Woelm silica was dry packed into glass columns for normal phase chromatography. The flow rate of column eluant was 10 mL/min and 20-mL fractions were collected for all separations using the lower capacity pump. Appropriate fractions from preparative reversed phase separations were pooled and concentrated to remove most of the organic modifier before extraction with ethyl acetate to concentrate the component of interest. Analytical TLC separations were performed with Analtech silica gel GHLF plates.

**Acetylation of Ganefromycin $\alpha$.** Ganefromycin $\alpha$ (1) (400 mg) was dissolved in pyridine (5.0 mL) and treated with acetic anhydride (1.0 mL). The mixture was allowed to stand stoppered at rt for 52 h, when the reaction was quenched by the addition of methanol (10 mL). Solvents were removed under reduced pressure and the residue was chromatographed on a Whatman CCS 2.5 × 50 cm 10-$\mu$m $C_8$ column eluted with 80% methanol/20% water at 10 mL/min. Fractions were collected at 2-min intervals. Fractions 52–73 were combined and extracted with ethyl acetate to obtain the product **3** (200 mg), after freeze drying from *tert*-butyl alcohol. MS (FAB): $[M + K]^+ = m/z$ 1391.

**Preparation of Ganefromycin $\alpha$ Methyl Ester (4).** To 13 g (10.6 mmol) of 1 slurried in ethyl acetate (200 mL) was added ethereal diazomethane (50 mL) (Aldrich process #1). After the solution was allowed to stand for 15 min in a fume hood, an aliquot of the solution was examined by HPLC (50% $CH_3CN$/buffer, isocratic, 2.0 mL/min), which showed that conversion to product was complete. Evaporation of the solvents and vacuum drying left 12.9 g (10.4 mmol) 98% yield, of **4** as a yellow glassy solid.

**Methyl ester 4:** $[\alpha]^{26}_D = 50°$ (c 1.027, MeOH); UV (MeOH) $\lambda_{max}$ nm ($\epsilon$) 232 (57 000), 298 (40 700); IR (KBr) 3420 (br), 2976, 2936, 2911, 2905, 2826 m, 1742 m, 1719 m, 1650, 1619, 1455, 1440, 1411, 1368, 1258, 1100, 1023, 1008, 989, 975 cm$^{-1}$; $^{13}$C NMR see Table I; MS (FAB) $[M + K]^+ = m/z$ 1278, $[M + Na]^+ = m/z$ 1262; HRMS, calcd for $C_{66}H_{97}NO_{21}K = m/z$ 1278.6190, obsd $m/z$ 1278.6246.

**Hydrogenation of Ganefromycin $\alpha$.** Ganefromycin $\alpha$ (1) (1.0 g) was dissolved in methanol (50 mL) and stirred with 10% Pd on carbon (250 mg) under 1 atm $H_2$ for 65 h. The catalyst was removed by filtration through a 0.45-$\mu$m nylon membrane. After removal of the methanol under reduced pressure, one-half of the crude product was subjected to chromatography on silica gel (2.5 × 100 cm) developed with 5% methanol/95% methylene chloride at 3 mL/min. Fractions were collected at 8-min intervals. Fractions 8–24 were combined to yield 360 mg of tetradecahydro derivative **5**: MS(FAB) $[M + K]^+$ = m/z 1278; relevant NMR data are presented in the Results and Discussion section.

**Ozonolysis of E19020$\alpha$, Production of 6.** Ganefromycin $\alpha$ (1) (2.6 g, 2.1 mmol) dissolved in methanol (100 mL) was cooled in a dry ice–acetone bath. Ozone, produced in a Welsbach Model T-408 ozone generator (90 V, 1.0 L/min sample flow at 8 psi), was bubbled through the solution for 25 min. Excess ozone was then removed with a stream of nitrogen. Dimethyl sulfide (1.0 mL) was added and the mixture was stirred at room temperature for 45 min. A solution of sodium borohydride (260 mg) in methanol (10 mL) was added and stirring was continued for 30 min, followed by a second portion of sodium borohydride (250 mg) in methanol (20 mL). Stirring was continued for an additional 15 min. The solution was diluted with water and extracted with ethyl acetate. Evaporation of the ethyl acetate layer yielded 900 mg of crude product. This material was separated on a 300-g silica gel column (2.5 × 105 cm) eluted with 4% methanol in methylene chloride. The flow rate was maintained at 3 mL/min and fractions were collected at 8-min intervals. Fractions were pooled on the basis of TLC analysis (10% methanol in methylene chloride, detection by sulfuric acid charring) to yield 180 mg of the major product **6**.

**Ozonolysis product (6):** $[\alpha]^{26}_D = -76°$ (c 0.487, MeOH); IR (KBr) 3420, 2980, 2940, 2900, 1741, 1650, 1540, 1456, 1365, 1098, 1020, 984 cm$^{-1}$; $^{13}$C NMR see Table I; MS (FAB, thioglycerol) $[M + Na]^+ = m/z$ 896. Anal. Calcd for $C_{41}H_{63}NO_{19}$: C, 56.36; H, 7.22; N, 1.60. Found: C, 55.88; H, 7.14; N, 1.62.

**Preparation of Trisaccharide 7.** Sodium methoxide (1.0 g) was added to a solution of 1 (5.0 g, 4.08 mmol) in methanol and the mixture was stirred for 1 h at rt. Cold, distilled water (100 mL) was added and the pH of the resulting solution was brought to 6–7 by small additions of 3 N HCl. After extraction with two 50-mL portions of ethyl acetate, the aqueous phase was concentrated under reduced pressure and freeze-dried, leaving a yellow powder. This was chromatographed on a 50 × 300 mm silica column eluted with 3% methanol/methylene chloride (7 L) (fractions 1–350) followed by 5% methanol/methylene chloride

(fractions 351–430). Evaporation of solvent from fractions 388–411 gave 800 mg (1.78 mmol, 44% yield) of a white powder containing an anomeric mixture of the trisaccharide 7.

**Trisaccharide 7:** $[\alpha]^{26}_D = -118°$ (*c* 1.048, MeOH); IR (KBr) 3420, 2976, 2938, 2905, 2830, 1449, 1368, 1260, 1098, 1027, 1008, 990, 973 $cm^{-1}$; $^{13}C$ NMR see Table I; MS (FAB) $[M + K]^+ = m/z$ 489; HRMS, calcd for $C_{21}H_{38}O_{10}K = m/z$ 489.2102; obsd $m/z$ 489.2102.

**Trisaccharide Structure Determination.** Trisaccharide 7 (0.85 g) was dissolved in methanol (50 mL), and sodium borohydride (280 mg in 10 mL of methanol–water 1:1) was added. After the solution was stirred for 25 min, the reaction was diluted with water (50 mL) and concentrated in vacuo to remove the methanol. The residual aqueous solution was saturated with sodium chloride and extracted with ethyl acetate (3 × 50 mL). The combined ethyl acetate layers were dried over anhydrous sodium sulfate and then evaporated to yield 0.76 g of crude product. The bulk of this crude product (0.64 g) was dissolved in pyridine (9.0 mL) and acetic anhydride (3.0 mL) was added. The stoppered solution was held at rt for 16 h. Toluene (50 mL) and methanol (10 mL) were added and the solution was concentrated under reduced pressure at 40 °C. The crude residue weighed 0.80 g. The bulk of this product was chromatographed on a silica column (93 g) developed with 1:1 hexane–ethyl acetate at 4.0 mL/min. Fractions were collected at 5-min intervals and were checked by TLC (ethyl acetate) visualized by charring with sulfuric acid. Fractions 15–19 were combined and the solvents were evaporated to yield **9** (420 mg). Fractions 20–27 contained less pure material and these were combined and worked up, yielding 300 mg for further purification. Crystallization of the latter material from 1:1 methylene chloride–hexane yielded needles which were suitable for single crystal X-ray diffraction analysis.

**Triacetate derivative of the open-chain reduced trisaccharide 9:** $[\alpha]_D^{26} = -89°$ (*c* 1.014, MeOH); IR (KBr) 2980, 2938, 2902, 2830, 1741, 1375, 1241, 1166, 1112, 1091, 1028, 1009 $cm^{-1}$; $^{13}C$ NMR see Table I; MS (FAB, MNBA) $[M + Na]^+ = m/z$ 557.

**Identification of Methyl α-L-Diginoside.** Compound **9** (400 mg) was dissolved in methanol (10 mL). Methanolic HCl (10% wt/wt) (10 mL) was added and the mixture was allowed to stand at rt for 85 min. The solution was diluted with methanol (150 mL) and sparged with nitrogen for 60 min. Two 1-g portions of solid $NaHCO_3$ were added and the solvent was removed under reduced pressure, yielding a white solid. The solid was triturated with methylene chloride (2 × 50 mL) and ethyl acetate (2 × 50 mL) to extract the product. Removal of the solvents left a residue of 350 mg. The residue was chromatographed on a column (2.5 × 35 cm) of silica (100 g), developed initially with ethyl acetate/hexane (1/4) and increasing the ethyl acetate content stepwise to 50%. The fractions were analyzed by TLC (ethyl acetate) detected by charring with $H_2SO_4$. One of the major components of this reaction ($R_f$ 0.29) was identified as methyl α-L-diginoside on the basis of $^1H$ and $^{13}C$ NMR and optical rotation comparisons.[10] Data for the isolated compound are as follows: $[\alpha]_D^{26} = -133°$ (*c* 0.23%, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 1.32 (1H, d, $J$ = 6.6 Hz), 1.8–2.0 (2H, m), 3.33 (3H, s), 3.39 (3H, s), 3.61 (1H, ddd, $J$ = 3.1, 5.4, 11.5 Hz), 3.78 (1H, br s), 3.83 (1H, q, $J$ = 3.6 Hz), 4.81 (1H, dd, $J$ = 0.5, 3.5 Hz); $^{13}C$ NMR ($CDCl_3$) δ 16.8, 29.4, 54.8, 55.6, 65.3, 67.7, 74.6, 98.6.

**Preparation of Ammonolysis Product 10.** Ganefromycin α(1) (9.69 g, 7.9 mmol) was dissolved in acetonitrile (250 mL) and concentrated $NH_4OH$ (125 mL) was added. After 14 h, HPLC (42.5% $CH_3CN$/buffer, isocratic, 2.5 mL/min) analysis of the reaction mixture showed complete absence of starting material. The mixture was diluted with water (1000 mL) and the pH was adjusted to approximately 1 with 6 N HCl. The product was extracted from the suspension with three 600-mL portions of ethyl acetate. The ethyl acetate portion was dried over sodium sulfate and then concentrated to give a light brown oil (9 g) which was chromatographed twice using the Prep-500 and a reverse phase cartridge, eluting with 35% acetonitrile/buffer. Combination of appropriate fractions and recovery of product yielded **10** as a yellow powder [1.05 g (0.13 mmol, 17%)]. *When ganefromycin β (2) was reacted under similar conditions, the major product was identical by HPLC retention time and $^1H$ NMR to the product produced as described above.*

**Ammonolysis product 10:** UV $\lambda_{max}$ nm (ε) MeOH 232 (57 700), 289 (41 700); IR (KBr) 3350, 2975, 2925, 1700, 1650, 1620, 1550, 1455, 1382, 1258, 1221, 1100, 1008, 995, 950, $cm^{-1}$; $^{13}C$ NMR see Table I; MS (FAB, thioglycerol), $[M + H]^+ = m/z$ 793; HRMS calcd for $C_{44}H_{61}N_2O_{11} = m/z$ 793.4275, obsd $m/z$ 793.4257.

**Preparation of α Methyl Ester Aglycon 11 and Monosaccharide 13.** Methanolic HCl (3% HCl, 40 mL) was added to a solution of α methyl ester **4** (5.0 g, 4.04 mmol) in methanol (100 mL). The reaction mixture was kept in an ice–water bath and aliquots were periodically examined by HPLC (55% acetonitrile/buffer, isocratic, 2.0 mL/min). After 4 h the aglycon had reached a maximal concentration and the mixture was poured into 200 mL of water. The resulting suspension was extracted twice with 150-mL portions of ethyl acetate. The organic layers were combined, dried over sodium sulfate, and concentrated to give a dark yellow oil (4 g). This oil was chromatographed using the Prep-500 with a silica gel cartridge. A step gradient starting with 60% hexane/ethyl acetate for the first 10.5 L (fractions 1–35 was followed by 50/50 for 13.5 L (fractions 36–80 followed by elution with 60% ethyl acetate/hexane (fractions 81–111). The aglycon methyl ester **11** was obtained by concentration of fractions 61–71 (0.4 mmol, 9.8% yield) and the monosaccharide methyl ester **13** was recovered from fractions 100–106 (0.134 mmol, 3.3% yield).

**α methyl ester monosaccharide 13:** $[\alpha]^{26}_D = +12°$ (*c* 0.933, MeOH); UV (MeOH) $\lambda_{max}$ nm (ε) 233 (27 000), 297 (17 000); IR (KBr) 3400, 2974, 2936, 2828, 1737, 1723, 1650, 1619, 1538, 1455, 1440, 1258, 1122, 1108, 1025, 1006, 987 $cm^{-1}$; 13C NMR see Table I; MS (FAB, mononitrobenzyl alcohol) $[M + K]^+ = m/z$ 990; HRMS, calcd for $C_{52}H_{73}NO_{15}K = m/z$ 990.4617, obs $m/z$ 990.4651.

**α methyl ester aglycon 11:** $[\alpha]^{26}_D = +21°$ (*c* 0.876, MeOH); UV (MeOH) $\lambda_{max}$ nm (ε) 232 (50 000) 298 (34 500); IR (KBr) 3400, 2970, 2930, 2860, 1753, 1719, 1702, 1644, 1619, 1455, 1438, 1258, 1147, 1083, 1006 $cm^{-1}$; $^{13}C$ NMR see Table I; MS (FAB, mononitrobenzyl alcohol) $[M+Na]^+ = m/z$ 830; HRMS, calcd for $C_{45}H_{61}NO_{12}Na = m/z$ 830.4091; obsd $m/z$ 830.4078.

**Preparation of α Methyl Ester Aglycon Triacetate 12.** Ganefromycin α aglycon methyl ester (**11**) (200 mg, 0.248 mmol) was dissolved in pyridine (5 mL) and acetic anhydride (1 mL) was added. The mixture was allowed to stir for 16 h. The mixture was then poured into 1 N HCl (200 mL) and the suspension was extracted with ethyl acetate (200 mL). The ethyl acetate layer was extracted with 1N HCl (200 mL) and the combined acid solution was extracted with ethyl acetate (200 mL). The combined ethyl acetate extract was partitioned against saturated NaCl (25 mL), dried over $Na_2SO_4$, and concentrated to give a light brown oil (175 mg), containing 70% triacetate by HPLC (65% acetonitrile/buffer, isocratic, 2 mL/min). Preparative LC of the oil on a silica gel column (20 × 300 mm) eluted with 75% hexane/ethyl acetate (v/v) gave 110 mg (0.118 mmol, 48% yield) of the α aglycon triacetate **12**.

**α methyl ester aglycon triacetate 12:** $[\alpha]^{26}_D = +16°$ (*c* 1.042, MeOH); UV (MeOH) $\lambda_{max}$, nm (ε) 232 (56 800), 296 (39 900); IR (KBr) 3400, 2938, 2930, 2901, 2897, 1744, 1720, 1650, 1619, 1372, 1253, 1241, 1143, 1039, 1010 $cm^{-1}$; $^{13}C$ NMR see Table I; MS (FAB) $[M + K]^+ = m/z$ 972; HRMS calcd for $C_{51}H_{67}NO_{15}K = m/z$ 972.4148, obsd $m/z$ 972.4129.

**Preparation of β Methyl Ester 15.** Ganefromycin β (**2**) (20 g, 16.3 mmol) was slurred in ethyl acetate (200 mL) and ethereal diazomethane (75 mL) was added. After the solution was allowed to stand for 15 min, HPLC of an aliquot (60% acetonitrile/buffer, isocratic, 2.5 mL/min) showed complete conversion to the methyl ester. Evaporation of the solvents and vacuum drying left a brown powder (20 g), which was used directly as a starting material for chemical modification but was purified for spectroscopic analysis. The crude ester (5 g) was dissolved in ethyl acetate (5 mL) and chromatographed on a 50 × 200 mm silica column eluted with ethyl acetate. Fractions 65–145 were combined on the basis of TLC. Evaporation of the solvent yielded 2.95 g (2.38 mmol, 50% yield) of **15**.

**β methyl ester 15:** $[\alpha]^{26}_D = -18°$ (*c* 1.059, MeOH); UV (MeOH) $\lambda_{max}$ nm (ε) 232 (54 000), 298 (41 000); IR (KBr) 3461, 3370, 2974, 2936, 2903, 2825, 1736, 1719, 1648, 1618, 1546, 1454, 1438, 1366, 1257, 1099, 1023, 1007, 986, 972, $cm^{-1}$; $^{13}C$ NMR, see Table I; MS (FAB), $[M + K] = m/z$ 1278; HRMS calcd for $C_{66}H_{97}NO_{21} = m/z$ 1278.6190, obsd $m/z$ 1278.6246.

**Preparation of (*p*-Bromobenzoyl)amide 17.** Five grams (4.04 mmol) of the crude $\beta$ methyl ester **15** was dissolved in acetic acid (50 mL) and the solution was warmed to 80 °C for 20 min. The solution was then frozen and the acetic acid removed under vacuum. The resulting solid was dissolved in methanol (10 mL) and chromatographed on the Prep-500 with a reverse phase cartridge. The column was first eluted with 10% acetonitrile/buffer (fractions 1–20) then 15% acetonitrile/buffer (fractions 21–50) and the amine was eluted from the column with 20% acetonitrile. Fractions 52–76 were combined and the pH adjusted to 9 with concentrated $NH_4OH$. The solution was extracted with ethyl acetate and the organic phase was concentrated. The resulting oil was dissolved in *tert*-butyl alcohol and freeze dried. Although the product from the above procedure gave only one HPLC peak (20% acetonitrile/buffer, isocratic, 2.5 mL/min), $^1H$ NMR showed solvent impurities. Approximately 50% (260 mg) of the crude amine **16** was dissolved in methylene chloride (20 mL), and DMAP (30 mg) plus *p*-bromobenzoyl chloride (100 mg) were added and the mixture was stirred. After 1 h, the reaction was complete and the mixture was poured into 200 mL of 2 N HCl. The acid suspension was extracted three times with ethyl acetate (100 mL, 25 mL, 25 mL). The combined ethyl acetate portion was shaken with brine, dried over sodium sulfate, and concentrated. The concentrate was dissolved in ethyl acetate (4 mL) and chromatographed on a 20 × 250 mm column of silica gel eluted with a gradient from 20% to 40% ethyl acetate/hexane. Combination of fractions 102 to 115, removal of the solvents, and freeze drying from *tert*-butyl alcohol yielded 197 mg (0.343 mmol, 17% yield from the ester) of **17** as an off-white powder.

**(*p*-Bromobenzoyl)amide 17:** $[\alpha]^{26}_D = +50°$ (*c* 1.023, MeOH); UV (MeOH) $\lambda_{max}$ nm ($\epsilon$) 243 (24 700), 297 (19 200); IR (KBr) 3400, 2974, 2947, 2936, 2822, 1717, 1700 (sh) 1646, 1619, 1592, 1538, 1484, 1438, 1303, 1264, 1141, 1073, 1012, 973, 946, $cm^{-1}$; $^{13}C$ NMR, see Table I; MS (FAB) $[M + K]^+ = m/z$ 612; HRMS, calcd for $C_{29}H_{37}NO_6BrK = m/z$ 612.1363; obsd $m/z$ 612.1363.

**Comparison of $\alpha$ and $\beta$ Methyl Esters toward Amine Liberation in Acetic Acid.** Approximately 20-mg portions of $\alpha$ methyl ester **4** and $\beta$ methyl ester **15** were weighed into separate 1-dram vials and dissolved in glacial acetic acid (1.0 mL). Aliquots (50 $\mu$L) were removed as soon as the materials were dissolved and diluted with 70% acetonitrile/buffer (0.95 mL) for HPLC analysis: 20% acetonitrile/buffer for 3.0 min followed by gradient to 60% acetonitrile from 3.0 to 8.0 min, then isocratic at 60%, 2.0 mL/min flow. The acetic acid solutions were then capped and heated in an 80 °C water bath for 20 min, after which another 50-mL aliquot was removed for HPLC. The chromatograms showed negligible reaction of **4**, whereas **15** had virtually totally reacted, yielding the amine **16**.

**Equilibration of 1 and 2 in $NaHCO_3$ Solution.** Ganefromycins $\alpha$ (**1**) and $\beta$ (**2**) were separately dissolved in dioxane (5 mg/mL) and an equal volume of 2% $NaHCO_3$ in water was added to each solution. The solutions were allowed to stand at rt for several hours and aliquots were removed periodically for HPLC analysis. HPLC was performed with a Rainin Microsorb-MV, 5 $\mu$m, 4.6 mm × 25 cm $C_{18}$ column, eluted with a linear gradient of dioxane (55% to 70% over 25 min) in 1% aqueous acetic acid, holding at the final composition for an additional 5 min, at 1.0 mL/min. In this system, ganefromycin $\alpha$ has a retention time of 24.2 min and $\beta$ 27.3 min. Detection was by UV absorbance at 300 nm. After 43 h, the reaction with **1** had equilibrated to a **1/2** ratio of 0.41 and remained essentially unchanged for an additional 20 h. In the reaction of **2**, at 42 h the **1/2** ratio was 0.37; after 74 h it was 0.41. In both reactions significant decomposition (between 15 and 25%), as indicated by a decrease of the sum of the areas of the HPLC peaks for **1** and **2**, was observed after 40 h.

**Acknowledgment.** We wish to thank D. R. Williams, J. D. Korshalla, and F. Pinho of the Discovery Pilot Plant for their role in providing the starting material for these chemical studies. Drs. M. M. Siegel, T. S. Dunne, J. Ashcroft, and J. C. James and Mr. G. O. Morton are recognized for their expert assistance in providing spectroscopic data. Thanks are due also to Dr. W. M. Maiese for his continued support and encouragement.

**Supplementary Material Available:** $^1H$ NMR spectra of **4, 6, 7, 9, 10, 11, 12, 12, 15,** and **17** (10 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.