portion was dried over magnesium sulfate and concentrated to give an 82% yield of the acetonide **5d**. The diastereomers were separated via preparative SiO_2 TLC (2 developments, methylene chloride) to give the higher R_f material **5d** (major isomer) as pale yellow crystals: mp 89–90 °C; ^{1}H NMR (400 MHz, $CDCl_3$) major isomer (higher R_f) δ 7.28–7.25 (m, 4 H), 7.19 (m, 1 H), 7.02 (d, J = 8.79 Hz, 2 H), 6.77 (d, J = 8.79 Hz, 2 H), 5.24 (d, J = 5.26 Hz, 1 H), 4.83 (t, J = 5.42 Hz, 1 H), 3.75 (s, 3 H), 2.66 (m, 1 H), 2.02 (m, 1 H), 1.73 (m, 1 H), 1.56 (m, 1 H), 1.35 (s, 3 H), 1.33 (s, 3 H); ^{1}H NMR (400 MHz, $CDCl_3$) minor isomer (lower R_f) δ 7.25–7.21 (m, 3 H), 7.17–7.14 (m, 2 H), 7.12–7.09 (m, 2 H), 6.80 (d, J = 8.81 Hz, 2 H), 5.23 (m, 1 H), 4.83 (m, 1 H), 3.76 (s, 3 H), 2.66 (m, 1 H), 2.01 (m, 1 H), 1.71 (m, 1 H), 1.53 (m, 1 H), 1.37 (s, 3 H), 1.33 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) major isomer 157.55, 145.50, 137.94, 128.28, 128.10, 127.80, 125.73, 113.47, 109.31, 85.63, 80.61, 58.25, 55.14, 33.13, 30.61, 26.23, 24.01; ^{13}C NMR (100 MHz, $CDCl_3$) minor isomer 157.44, 146.27, 137.42, 129.15, 128.11, 127.30, 125.94, 113.20, 109.30, 85.52, 80.66, 58.28, 55.04, 33.49, 30.64, 26.27, 24.02; IR (KBr pellet) 2940, 1600, 1505, 1245, 1027 cm^{-1}; MS (EI 70 eV) m/z 324 (M$^+$, 21), 210 (100); HRMS (EI 70 eV) m/z (M$^+$) calcd for $C_{21}H_{24}O_3$ 324.1725, obsd 324.1722.

X-ray Structure Determination of 2c (Major Isomer). Single crystals of the major isomer of the bromo-substituted diol **2c** were grown by the uninduced crystallization of the initial oil upon standing for an extended period of time: monoclinic space group $P2_1/a$; a = 9.125 (5) Å, b = 12.793 (1) Å, c = 12.849 (2) Å, β = 103.866 (5)°, V = 1456.3 (8) Å^3, Z = 4, d(calcd) = 1.520 g cm^{-3}. For 1252 unique, observed ($>3\sigma(I)$) reflections and 181 parameters, the discrepancy indices are R = 0.033 and R_w = 0.045. The intensity data was obtained at 20 °C with a Rigaku AFC5R four circle autodiffractometer system using graphite monochromated Cu Kα radiation and a 12-kW rotating anode generator. The cell constants and an orientation matrix for data collection were obtained from a least-squares refinement using the setting angles of 25 centered reflections in the range $25 < 2\theta < 30$. Scans were made at a speed of 32 deg min^{-1} in omega. The weak reflections ($I < 10.0\ \sigma$) were rescanned (maximum of two rescans). The intensities of three standard reflections were measured after every 150 reflections and remained constant throughout the data collection; no decay correction was applied. The crystallographic calculations were performed using the TEXSAN program.[23] An emperical absorption correction, using the DIFABS program, was applied which resulted in transmission factors ranging from 0.91 to 1.00. The data were corrected for Lorentz and polarization effects. The structure was solved by direct methods. The non-hydrogen atoms were refined anisotropically. The hydrogen atoms were included in calculated positions for the final full-matrix least-squares refinement cycles but were not refined.

Acknowledgment. We wish to thank the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research.

Supplementary Material Available: Tables of bond lengths, bond angles, and crystallographic, positional, and thermal parameters for **2c** (8 pages); tables of observed and calculated structure factors for **2c** (8 pages). Ordering information is given on any current masthead page.

Calicheamicins, a Novel Family of Antitumor Antibiotics. 4. Structure Elucidation of Calicheamicins $\beta_1{}^{\mathrm{Br}}$, $\gamma_1{}^{\mathrm{Br}}$, $\alpha_2{}^{\mathrm{I}}$, $\alpha_3{}^{\mathrm{I}}$, $\beta_1{}^{\mathrm{I}}$, $\gamma_1{}^{\mathrm{I}}$, and $\delta_1{}^{\mathrm{I}}$

May D. Lee,* Theresa S. Dunne, Conway C. Chang, Marshall M. Siegel,
George O. Morton, George A. Ellestad, William J. McGahren, and Donald B. Borders

Contribution from the American Cyanamid Company, Medical Research Division, Lederle Laboratories, Pearl River, New York 10965. *Received June 26, 1991*

Abstract: The details of the structural assignment of the potent antitumor antibiotic, calicheamicin $\gamma_1{}^{\mathrm{I}}$ (**6**, $C_{55}H_{74}IN_3O_{21}S_4$), is reported. Methanolysis studies on **6** and *N*-acetylcalicheamicin $\gamma_1{}^{\mathrm{I}}$ (**8**, $C_{57}H_{76}IN_3O_{22}S_4$) permitted the structural assignment of the glycosidic chain. Details of the spectral analysis supporting the assignments of the 3-*O*-methyl-α-L-rhamnopyranoside (D-ring) and the methyl 2,4-dideoxy-3-*O*-methyl-4-(*N*-acetyl-*N*-ethylamino)-α-L-xylopyranoside (E-ring) is reported. The structure of calicheamicinone (**32**, $C_{18}H_{17}NO_5S_3$), containing a bicyclo[7.3.1]tridec-9-ene-2,6-diyne system and a methyl trisulfide, was elucidated by a series of chemical degradation studies, which included an unexpected free radical cycloaromatization reaction. The presence of 4,6-dideoxy-4-(hydroxyamino)-β-D-glucopyranoside (A-ring) and its N–O glycosidic linkage to the thio sugar (B-ring) was ascertained by X-ray crystallography of **24** ($C_{36}H_{40}INO_{13}S_2$), a degradation product of **6**. The chemical structures of calicheamicins $\beta_1{}^{\mathrm{Br}}$ (**1**), $\gamma_1{}^{\mathrm{Br}}$ (**2**), $\alpha_2{}^{\mathrm{I}}$ (**3**), $\alpha_3{}^{\mathrm{I}}$ (**4**), $\beta_1{}^{\mathrm{I}}$ (**5**), and $\delta_1{}^{\mathrm{I}}$ (**7**) were assigned by correlating their ^{1}H and ^{13}C NMR data with that of calicheamicin $\gamma_1{}^{\mathrm{I}}$. By tracking the biological activities of the degradation products, the enediyne system of calicheamicinone was shown to be essential for the DNA-damaging abilities of the calicheamicins. A mechanism whereby the enediyne could be triggered to cyclize via a 1,4-diyl, the putative DNA cleaving species, is proposed.

Microbial fermentation is a well-known source of compounds of diverse chemical structures and biological activities. Key to the discovery of new biologically active compounds from this rich source is a sensitive and specific assay. The biochemical induction assay (BIA) has been shown to be exquisitely sensitive to certain DNA-damaging antitumor agents although not all DNA-damaging agents respond to this test.[1] At the beginning of our search for novel antitumor agents, several major structural classes of fermentation-derived antitumor agents were known to be BIA positive. These were represented by the pluramycins,[2] mitomycins,[3]

---

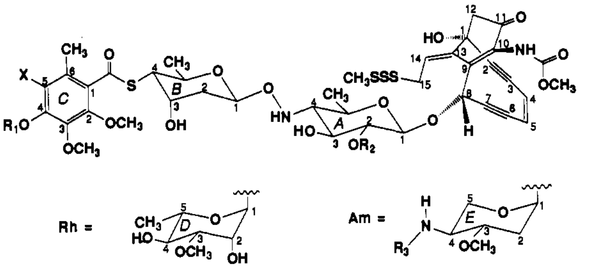
(1) (a) Elespuru, R. K.; Yarmolinsky, M. B. *Environ. Mutagen.* **1979,** *1,* 55–78. (b) Elespuru, R. K.; White, R. J. *Cancer Res.* **1983,** *43,* 2819–2830.

(2) (a) Byrne, K. M.; Gonda, S. K.; Hilton, B. D. *J. Antibiot.* **1985,** *38* (8), 1040–1049. (b) Sequin, U.; Furukawa, M. *Tetrahedron* **1978,** *34,* 3623–3629. (c) Sequin, U. *Tetrahedron* **1978,** *34,* 761–767. (d) Kondo, S.; Miyamoto, M.; Naganawa, H.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1977,** *30* (12), 1143–1145. (e) Furukawa, M.; Hayakawa, I.; Ohta, G.; Iitaka, Y. *Tetrahedron* **1975,** *31,* 2989–2995.

(3) Remers, W. A. *The Chemistry of Antitumor Antibiotics*; John Wiley & Sons: New York, 1979.

0002-7863/92/1514-985$03.00/0

Table I. Chemical Structures of Calicheamicins β_1^{Br}, γ_1^{Br}, α_2^{I}, α_3^{I}, β_1^{I}, γ_1^{I}, and δ_1^{I}



calicheamicin	X	R_1	R_2	R_3
β_1^{Br} (**1**)	Br	Rh	Am	$(CH_3)_2CH$
γ_1^{Br} (**2**)	Br	Rh	Am	CH_3CH_2
α_2^{I} (**3**)	I	H	Am	CH_3CH_2
α_3^{I} (**4**)	I	Rh	H	
β_1^{I} (**5**)	I	Rh	Am	$(CH_3)_2CH$
γ_1^{I} (**6**)	I	Rh	Am	CH_3CH_2
δ_1^{I} (**7**)	I	Rh	Am	CH_3

bleomycins,[3] streptonigrins,[4] some anthracyclines,[3] and neocarzinostatin and related macromolecular antitumor antibiotics.[5] The calicheamicins, produced by *Micromonospara echinospora* ssp. *calichensis*,[6] were discovered through a concerted effort in identifying BIA positive microbial fermentation products outside the six structural classes above. The esperamicins, a related family of antitumor antibiotics produced by the fermentation of *Actinomadura verrucosospora* were discovered at Bristol-Myers Laboratories at approximately the same time.[7] Antibiotics FR-900405 and FR-900406 discovered at Fujisawa and the veractamycins discovered at Parke-Davis were closely related or identical to the esperamicins.[8]

The calicheamicins complex contained a series of 15–20 compounds with closely related chemical structures; the components were named according to their relative TLC mobility. The first members of the complex to be isolated were calicheamicins β_1^{Br} (**1**) and γ_1^{Br} (**2**).[9] The presence of Br and S in **1** was suggested by high-resolution EIMS analysis and confirmed by electron spectroscopy for chemical analysis (ESCA). In order to obtain sufficient quantities of **1** and **2** for structure elucidation and complete biological evaluation, strain and fermentation improvement experiments were undertaken.[9] During this study, calicheamicins α_2^I (**3**), α_3^I (**4**), β_1^I (**5**), γ_1^I (**6**), and δ_1^I (**7**), containing I instead of Br, were discovered by conducting the fermentations in the presence of sodium iodide. Calicheamicin γ_1^I (**6**) was the major component of the iodinated calicheamicin complex. The degradation and high-field NMR studies which led to the structural assignments of the calicheamicins were carried out only with calicheamicin γ_1^I. Structures for the other calicheamicins (Table I) were assigned by correlating their spectral data with those of calicheamicin γ_1^I.

Approach toward Solving the Structure of Calicheamicin γ_1^I. The analytical and spectroscopic data of calicheamicin γ_1^I (**6**), including FABMS, ESCA, elemental analysis, and ^1H and ^{13}C NMR, revealed the following structural information: (1) a molecular weight of 1367 and a molecular formula of $C_{54-56}H_{73-77}IN_3O_{20-22}S_4$; (2) the presence of four glycosidic units in the molecule; (3) each molecule also contained 11 methyl groups, seven of which were not bonded to sp^3 carbons; and (4) of the remaining carbons, 17 had no attached protons, two of which resonated in the ketone carbonyl region (191.9 and 192.4 ppm), nine in the olefinic carbon region, and six in the 72–101 ppm region. Thus, the structure of calicheamicin γ_1^I was composed of four glycosides and an aglycon. The identities of the glycosidic units were not apparent due to considerable overlaps in the NMR signals. On the basis of the above observations, two approaches toward derivatization and degradation studies were under taken initially. (1) Since no carbon signals were present in the amide carbonyl region, the nitrogens were likely basic, and selective *N*-acetylation introduced an acetyl group on one of the nitrogens and afforded *N*-acetylcalicheamicin γ_1^I (**8**), which facilitated the molecular formula determination by high-resolution FABMS. (2) Carefully controlled methanolysis permitted the isolation and identification of the individual glycosidic units as their methyl glycosides and the isolation and characterization of the calicheamicin pseudoaglycon (**21**), which was a partially degraded fragment containing the aglycon. In order to solve the structure of the aglycon, further degradation studies were carried out on the calicheamicin pseudoaglycon. It was first converted by an unexpected cycloaromatization process via a benzene-1,4-diyl diradical to compound **23**, which was further degraded via retro-aldol cleavage to afford the crystalline compound **24**. The enediyne structure of the calicheamicin aglycon was finally assigned on the basis of the structures of **23** and a degradation product (**28**) containing no glycosidic units.

N-Acetylation Studies and the Molecular Formula of *N*-Acetylcalicheamicin γ_1^I (8). Selective *N*-acetylation studies were first carried out on an analytical scale using a calicheamicin γ_1^I sample containing ~10% calicheamicin β_1^I. TLC-bioautography of the reaction mixture revealed that the presumptive *N*-acetylcalicheamicin γ_1^I was much less active in the BIA and that the calicheamicin β_1^I (**5**) in the mixture was not acylated. Careful comparison of the ^1H and ^{13}C NMR data of the two calicheamicins showed that the difference between the γ_1^I and β_1^I components was an ethyl group versus an isopropyl group. The difference in the reactivity of the two calicheamicins under the very mild (Ac_2O/MeOH) *N*-acetylation conditions suggested that *a $NHCH_2CH_3$ unit was present in calicheamicin γ_1^I while a $NHCH(CH_3)_2$ unit was present in calicheamicin β_1^I* instead.[10]

^1H and ^{13}C NMR data revealed that *N*-acetylcalicheamicin γ_1^I was a mono-*N*-acetyl derivative of calicheamicin γ_1^I. It gave an intense M + H ion in both the sulfolane and dithiothreitol/dithioerythritol matrices, and its molecular formula was determined to be $C_{57}H_{76}IN_3O_{22}S_4$ (HRFABMS M + H *m/z* 1410.2954 Δ 0.26 mmu). The molecular formula for calicheamicin γ_1^I, calculated to be $C_{55}H_{74}IN_3O_{21}S_4$, was confirmed by a high-resolution FABMS measurement using the sulfolane matrix (M + H *m/z* 1368.2878 Δ 5.7 mmu). The ^1H–^1H COSY data of *N*-acetylcalicheamicin γ_1^I confirmed the presence of four glycosides and further identified them as a 2,6-dideoxypyranoside, two 6-deoxypyranosides, and a lesser defined 2-deoxy sugar.

Methanolysis of Calicheamicin γ_1^I (6) and *N*-Acetylcalicheamicin γ_1^I (8) Using HCl/MeOH. Three major anthrone and $CuSO_4/H_3PO_4$ positive compounds were found in the methyl glycoside fraction of the methanolysate of **6**. Two of these (**9** and

---

(4) Balitz, D. M.; Bush, J. A.; Bradner, W. T.; Doyle, T. W.; O'Herron, F. A.; Nettleton, D. E. *J. Antibiot.* **1982**, *35* (3), 259–265 and references therein.

(5) Hensens, O. D.; Goldberg, I. H. *J. Antibiot.* **1989**, *42* (5), 761–768 and references therein.

(6) (a) Lee, M. D.; Dunne, T. S.; Siegel, M. M.; Chang, C. C.; Morton, G. O.; Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3464–3466. (b) Lee, M. D.; Dunne, T. S.; Chang, C. C.; Ellestad, G. A.; Siegel, M. M.; Morton, G. O.; McGahren, W. J.; Borders, D. B. *Ibid.* **1987**, *109*, 3466–3468.

(7) (a) Golik, J.; Clardy, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Ohkuma, H.; Saitoh, K.; Doyle, T. W. *J. Am. Chem. Soc.* **1987**, *109*, 3461–3462. (b) Golik, J.; Clardy, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Ohkuma, H.; Saitoh, K.; Doyle, T. W. *Ibid.* **1987**, *109*, 3462–3464.

(8) (a) Iwami, M.; Kiyoto, S.; Nishikawa, M.; Terano, H.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiot.* **1985**, *38*, 835–839. (b) Kiyoto, S.; Nishikawa, M.; Terano, H.; Kohsaka, M.; Aoki, H.; Imanaka, H.; Kawai, Y.; Uchida, I.; Hashimoto, M. *J. Antibiot.* **1985**, *38*, 840–848. (c) Bunge, R. J. H.; Hurley, T. R.; Smitka, T. A.; Willmer, N. E.; Brarkiewicz, A. J.; Steinman, C. E.; French, J. C. *J. Antibiot.* **1984**, *37*, 1566–1571. (d) Wilton, J. H.; Hokanson, G. C.; French, J. C. *J. Chem. Soc., Chem. Commun.* **1985**, 919–920. (e) Wilton, J. H.; Rithner, C. D.; Hokanson, G. C.; French, J. C. *J. Antibiot.* **1986**, *39*, 1349–1350. (f) Mamber, S. W.; Okasinski, W. G.; Pinter, C. D.; Tunac, J. B. *J. Antibiot.* **1987**, *40*, 1044–1049.

(9) (a) Lee, M. D.; Manning, J. K.; Williams, D. R.; Kuck, N. A.; Testa, R. T.; Borders, D. B. *J. Antibiot.* **1989**, *42*, 1070–1087. (b) Maiese, W. M.; Lechevalier, M. P.; Lechevalier, H. A.; Korshalla, J.; Kuck, N.; Fantini, A.; Wildey, M. J.; Thomas, J.; Greenstein, M. *J. Antibiot.* **1989**, *42*, 558–563.

(10) *N*-Acetylcalicheamicin β_1^I could be prepared by adding a trace amount of triethylamine to the reaction mixture.

Table II. ^{1}H NMR (300 MHz, CDCl$_3$) and ^{13}C NMR Data of **15** (α Anomer)

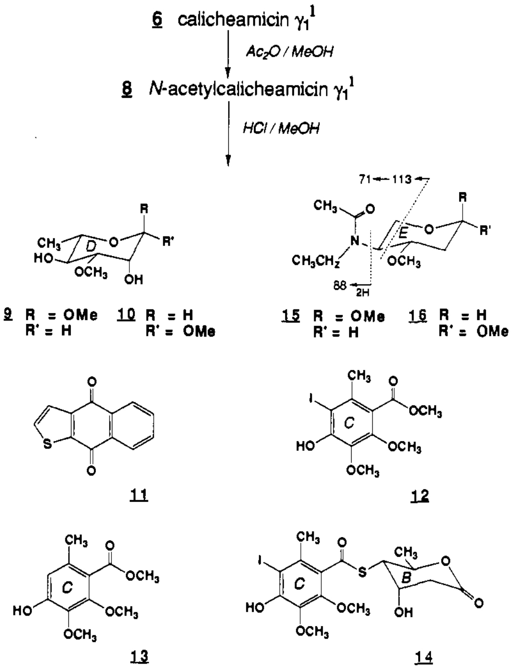
atom no.	^{13}C	^{1}H (mult, *J* (Hz), intgrtn)	^{1}H–^{1}H COSY
1E	98.9	4.80 (bs, 1 H)	1.54, 2.35
2E	35.3	1.54 (m, 1 H, ax)	2.35, 3.69, 4.80
		2.35 (dt, 13.4, 4.2, 1 H, eq)	1.54, 3.69, 4.80
3E	71.8, 72.7	3.69 (m, 1 H)	1.54, 2.35, 3.59
4E	54.9, 56.4	3.59 (m)	3.69, 3.32
5E	54.7, 55.5	3.32 (m)	3.59, 3.87
		3.87, 4.05 (2 m, 1 H)	
1E-OCH$_3$	59.9	3.30 (s, 3 H)	
3E-OCH$_3$	59.2, 59.7	3.34, 3.36 (2 s, 3 H)	
4E-NCH_2CH$_3$	36.8	3.14, 3.42 (2 m, 6.9, 2 H)	1.13, 1.20
4E-NCH$_2$$\mathit{CH}_3$	14.7, 15.3	1.13, 1.20 (2 t, 7.0, 3 H)	3.14, 3.42
4E-N*C*OCH$_3$	171.4, 171.0		
4E-NCOCH_3	22.3	2.12, 2.15 (2 s, 3 H)	

10) were isolated from the methanolysate of a crude calicheamicin complex. The third compound, presumably a methyl glycoside of the 4-amino sugar (ring-E), was quite unstable. The ^{1}H NMR, ^{13}C NMR, and high-resolution EIMS data clearly showed that **9** and **10** were the α- and β-methyl glycosides of a 6-deoxyhexopyranose containing an *O*-methyl ether group. The mannopyranose configuration was assigned on the basis of the vicinal proton coupling constants of the ring protons, which showed that H-2 was equatorial while H-3, H-4, and H-5 were axial (Table 1, supplementary material). The ^{13}C NMR data of **9** were practically identical to those reported previously, and the possibility of a 2-*O*-methyl or a 4-*O*-methyl substitution was ruled out since the δ_C values of these OCH$_3$ groups were 2–3 ppm higher.[11] The carbon chemical shifts of the anomeric carbon and of the C-1-methoxy carbon of **9** (α-) and **10** (β-) were consistent with those reported for the α- and β-methyl glycosides of D-mannopyranose and 6-deoxy-D-mannopyranose.[12] Since the optical rotation observed for the α anomer (**9**, $[\alpha]^{26}{}_D = -44°$) was more negative than that for the β anomer (**10**, $[\alpha]^{26}{}_D = +127°$), **9** and **10** were assigned the L configuration.[13]

The presumptive methyl glycoside of the amino sugar was absent from the methanolysate of *N*-acetylcalicheamicin $\gamma_1{}^I$; instead, a new and less polar degradation product was observed. Other than this new compound, the TLC profiles of the methanolysates of *N*-acetylcalicheamicin $\gamma_1{}^I$ (**8**) and calicheamicin $\gamma_1{}^I$ (**6**) were identical. This and observations made during *N*-acetylation studies suggested that one of the glycosides in calicheamicin $\gamma_1{}^1$ was an ethylamino sugar which was converted to an *N*-acetyl-*N*-ethylamino sugar in *N*-acetylcalicheamicin $\gamma_1{}^1$.

The dichloromethane-soluble portion of the total methanolysate from a preparative-scale methanolysis of analytically pure *N*-acetylcalicheamicin $\gamma_1{}^I$ was subjected to repeated chromatography with the objective of isolating the methyl glycosides of the putative *N*-acetyl-*N*-ethylamino sugar and as many other methanolysis products as possible. Compounds **12**, **11**, **13**, **14**, **15** (**16**), **9**, and **10**, in order of increasing polarity, were isolated (Scheme I). The chemical structure of compound **14**, isolated as colorless prisms, was determined by X-ray crystallography and established the substitution pattern of the hexasubstituted benzene ring.[14] The chemical structures of compounds **12** and **13** were determined by comparing their NMR and EIMS data to those of compound **14**. *The isolation and identification of naphtho[2,3-b]thiophene-4,10-dione (**11**) was considered to be an anomaly at this stage*, since there was no such structural element in calicheamicin $\gamma_1{}^1$ on the basis of its NMR data.

Scheme I



The chemical structures of **15** and **16** were by no means evident at first. They could not be separated by either normal or reverse-phase chromatography. Different methanolysis conditions yielded mixtures with **15**/**16** ratios varying from >9/1 to 7/3 based on ^{13}C NMR analysis. The NMR data were further complicated by the diamagnetic anisotropy of the amide nitrogen.[15] Doubling of the signals was observed in both ^{1}H and ^{13}C NMR for both the protons and the carbons of C-3–C-5 of the pentopyranose ring (Table II).[16]

The ^{1}H NMR data confirmed that **15** was the methyl glycoside of an *N*-acetyl-*N*-ethylamino sugar containing a methyl ether substitution. ^{1}H–^{1}H COSY studies revealed **15** to be a 2-deoxy sugar, and the coupling constants of its anomeric proton indicated it to be an α-methyl glycoside. The chemical shifts (98.9 and 101.4 ppm) of the anomeric carbons of **15** and **16** suggested that they were pyranosides,[12] narrowing the structure of **15** to a 2-deoxypentopyranoside with a methoxy and an *N*-acetyl-*N*-ethylamino group on two of the remaining three carbons of the pyranose ring. Close examination of the ^{1}H–^{1}H COSY data of *N*-acetylcalicheamicin $\gamma_1{}^1$ (**8**) showed that the *N*-acetyl-*N*-ethylamino sugar in **8** was present in primarily one anisotropic form and that H-4 (δ_H 2.96) was coupled to a pair of methylene protons in addition to H-3. Three strong fragment ions in the HREIMS of **15**, $C_6H_{11}NO$, C_4H_9N, and $C_4H_{10}NO$, confirmed that C-5 was unsubstituted and that the *N*-acetyl-*N*-ethylamino group was on C-4. The coupling patterns of the two protons on C-2 were very different

---

(11) (a) Toman, R.; Karacsonyi, S.; Palovcik, R. *Carbohydr. Res.* **1977**, *56*, 191–194. (b) Liptak, A.; Nanasi, P.; Neszmelyi, A.; Wagner, H. *Tetrahedron Lett.* **1980**, *36*, 1261–1268.

(12) Bock, K.; Pedersen, C. *Advances in Carbohydrate Chemistry and Biochemistry*; Academic Press, Inc.: New York, 1983; Vol. 41, pp 27–66.

(13) (a) Schallenberger, R. S. *Advanced Sugar Chemistry, Principles of Sugar Stereochemistry*; AVI Publishing Company, Inc.: Westport, CT, 1982; pp 160–170. (b) An optical rotation for **9** [$[\alpha]^{22}{}_D = -61°$ (*c* 1.3, CHCl$_3$)] was reported in ref 11a.

(14) The crystallographic data of compound **14** can be found in the supplementary material of ref 6a. **14** was isolated as a minor degradation product from one methanolysis experiment only. Its formation at all is presumably due to the hydroxyamino glycosidic linkage between A-ring and B-ring.

(15) Stewart, W. E.; Siddall, T. H., III. *Chem. Rev.* **1970**, *70* (5), 517–551.

(16) Raising the probe temperature to 55 °C did not coalesce the signals.

Table III. ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR Data of Calicheamicin Pseudoaglycon (**21**)[a]

atom no.	^{13}C (mult)	^1H (mult, *J* (Hz), intgrtn)	^1H–^1H COSY	atom no.	^{13}C (mult)	^1H (mult, *J* (Hz), intgrtn)	^1H–^1H COSY
1	72.5 (s)			1A	103.5 (d)	4.60 (d, 7.7, 1 H)	3.64
2[b]	100.4 (s)			2A	74.5 (d)	3.64 (bt, 8.2)	4.60, 3.98
3	87.5 (s)			3A	70.1 (d)	3.98 (bt, 10.0)	3.64, 2.44
4	124.1 (d)	5.89 (s, 1 H)	5.93	4A	67.2 (d)	2.44 (t, 9.8)	3.98, 3.79
5	124.2 (d)	5.89 (s, 1 H)		5A	69.6 (d)	3.79 (m)	2.44, 1.39
6	83.9 (s)			6A	17.8 (q)	1.39 (d, 6.1, 3 H)	3.79
7[b]	98.7 (s)			1B	99.7 (d)	5.06 (bd, 10, 1 H)	1.78, 2.04
8	71.3 (d)	5.99 (s, 1 H)	5.89	2B	36.8 (t)	1.78 (bdd, 13, 10, 1 H)	5.06, 4.32, 2.04
9	130.6 (s)					2.04 (md, 13, 1 H)	5.06, 4.32, 1.78
10	136.3 (s)			3B	68.2 (d)	4.32 (m, 1 H)	1.78, 2.04, 3.72
11	191.8 (s)			4B	51.6 (d)	3.72 (dd, 11, 2.3)	4.32, 4.07
12	53.3 (t)	2.84 (d, 16.8, 1 H)	3.22	5B	69.1 (d)	4.07 (m)	3.72, 1.43
		3.22 (d, 16.8, 1 H)	2.84	6B	19.1 (q)	1.43 (d, 6.2, 3 H)	4.07
13	140.7 (s)[d]			1C	126.8 (s)		
14	127.5 (d)	6.45 (dd, 9.8, 5.3, 1 H)	3.87, 4.12	2C	148.8 (s)		
15	39.1 (t)	3.87 (m)	6.45, 4.12	3C	136.4 (s)		
		4.12 (m)	6.45, 3.87	4C	150.9 (s)		
10-NH*C*OOCH$_3$	154.3 (s)			5C	84.4 (s)		
10-NHCOO*CH$_3$*	53.6 (q)	3.77 (s, 3 H)		6C	133.2 (s)		
15-SSSCH$_3$	22.8 (q)	2.52 (s, 3 H)		1C-CO	192.0 (s)		
				2C-OCH$_3$	61.5 (q)	3.91 (s, 3 H)	
				3C-OCH$_3$	61.0 (q)	3.88 (s, 3 H)	
				6C-CH$_3$	24.7 (q)	2.34 (s, 3 H)	

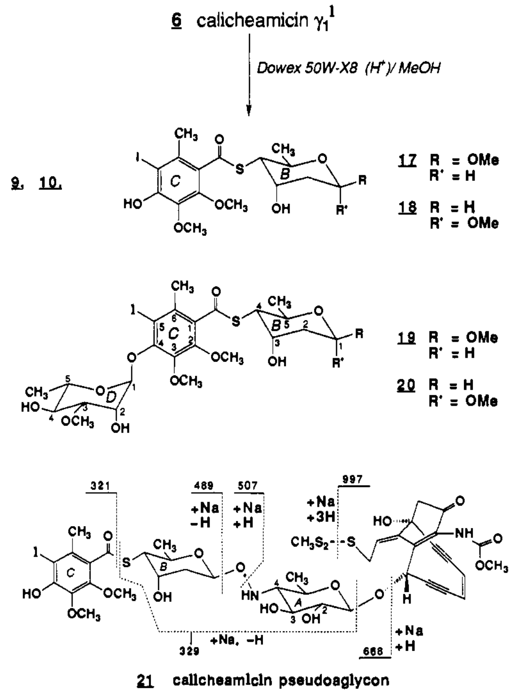
[a] ^1H–^{13}C correlations of atoms 1–15 were determined by single-frequency off-resonance decoupling (SFORD); correlations for the other atoms were made by analogy to compound **23**. [b,c] The assignments for these carbons could be reversed. [d] Very low intensity signal.

from each other, suggesting that H-3 was axial since H-1 was equatorial. The bulky *N*-acetyl-*N*-ethylamino group on C-4 was necessarily equatorial, and the coupling pattern of H-3 was consistent with 1,2-diaxial interactions. Insufficient pure **15** was isolated from the methanolysate to determine its specific rotation. However, the specific rotation of a 7/3 mixture (based on relative signal intensities of the anomeric carbons in the ^{13}C NMR spectrum) of **15** (α-) and **16** (β-) was determined ($[\alpha]^{20}_D$ −40°, *c* 0.627, $CHCl_3$) and found to be identical to that calculated for a 7/3 mixture of pure **15** and **16** prepared by synthesis from L-serine.[17] Thus, the chemical structure of **15** derived from the methanolysis of *N*-acetylcalicheamicin $\gamma_1{}^I$ was confirmed as methyl 2,4-dideoxy-3-*O*-methyl-4-(*N*-acetyl-*N*-ethylamino)-α-L-xylopyranoside. It is an α-glycoside in the intact calicheamicin $\gamma_1{}^I$ since the corresponding anomeric proton in both calicheamicin $\gamma_1{}^I$ and *N*-acetylcalicheamicin $\gamma_1{}^I$ showed only the small coupling constants (2–3 Hz) attributable to equatorial–equatorial and equatorial–axial interactions with the two protons on C-2E.

Methanolysis of Calicheamicin $\gamma_1{}^I$ (6) Using Dowex 50W-X8/MeOH. In order to obtain higher molecular weight and BIA positive degradation products, a number of methanolysis conditions were investigated. The methanolysis of **6** catalyzed by a strong cation exchange resin (Dowex 50) in the hydrogen form permitted the isolation of **17**, **18**, **19**, **20**, and the BIA positive calicheamicin pseudoaglycon **21** (Scheme II). Reasonably good yields (~50%) of **21** could be achieved by loading a concentrated solution of calicheamicin $\gamma_1{}^I$ in methanol at a low flow rate on a Dowex 50W-X8 column, using no more resin than necessary, followed by eluting the column with large quantities of methanol. The early eluate contained mostly **9** and **10** with small amounts of **19** and **20**, and the late eluate contained **17**, **18**, and **21**, suggesting that the 3-*O*-methylrhamnoside linkage was the most labile followed by the thio sugar linkage and the ethylamino sugar linkage under the experimental conditions. Calicheamicin pseudoaglycon (**21**) was extremely acid and base labile; the reaction process described above allowed it to be removed from the acidic resin environment immediately after it was formed and made it possible to prepare large quantities of this important intermediate for the many studies to be described below. All attempts at recovering the ethylamino sugar from the Dowex 50 failed.

The ^1H and ^{13}C NMR data (Table II, supplementary material) of **17** and **18** revealed that they were a pair of α- and β-methyl glycosides containing the aromatic ring of **14**. The proton resonances of the glycosidic portion of **17** bore close resemblance to that of the 2,6-dideoxypyranoside unit defined by the ^1H–^1H COSY data of *N*-acetylcalicheamicin $\gamma_1{}^I$. With the molecular formula of **17** ($C_{17}H_{23}IO_7S$) and the structure of **14** on hand, it was not difficult to assign the structures of **17** and **18**. The chemical structures of **19** and **20**, as a result, were apparent on the basis of their NMR data. The glycosidic linkage of the 3-*O*-methyl-L-rhamnopyranoside (D-ring) in **19** was assigned to be α, since the general trend of the chemical shifts of the D-ring protons were similar to that of **9** rather than **10**.

Scheme II

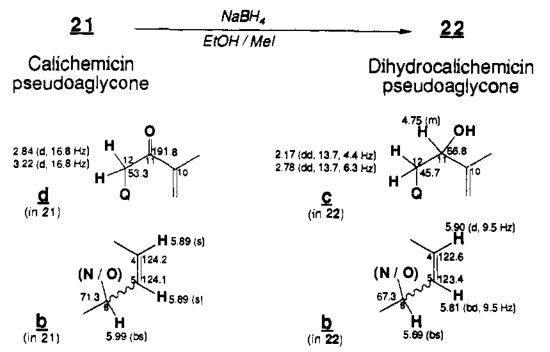


Partial Structure of the Calicheamicin Pseudoaglycon (21). The spectral data (Table III) of calicheamicin pseudoaglycon (**21**, $C_{40}H_{47}IN_2O_{15}S_4$) revealed that it was calicheamicin $\gamma_1{}^I$ without the 3-*O*-methyl-L-rhamnose (D-ring) and the 4-ethylamino sugar (E-ring). Numerous signal overlaps in the ^1H NMR spectrum of calicheamicin $\gamma_1{}^I$ disappeared with the removal of the two

---

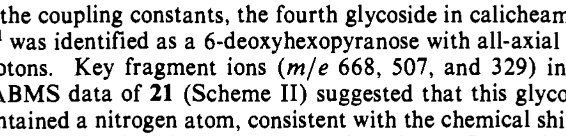
(17) Kahne, D.; Yang, D.; Lee, M. D. *Tetrahedron Lett.* **1990**, *31* (1), 21–22.

Scheme III



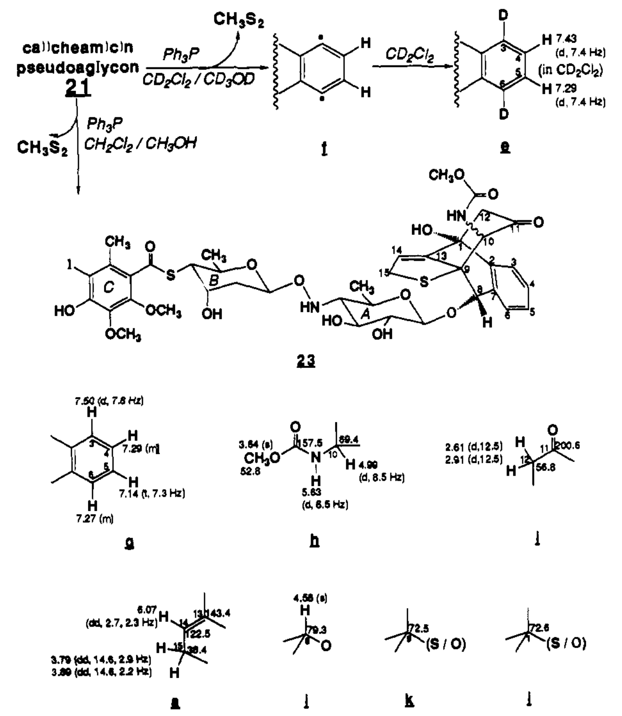
glycosides. On the basis of ^{1}H–^{1}H COSY and careful examination of the coupling constants, the fourth glycoside in calicheamicin $\gamma_1{}^1$ was identified as a 6-deoxyhexopyranose with all-axial ring protons. Key fragment ions (*m*/*e* 668, 507, and 329) in the FABMS data of **21** (Scheme II) suggested that this glycoside contained a nitrogen atom, consistent with the chemical shift of H-4A (δ_H 2.44) which was too high-field for an O-substitution on C-4A. The mass spectral data also located this glycosidic unit between the B-ring and the aglycon.

Once all of the carbon and proton signals of the two glycosides and the hexasubstituted benzene ring were assigned, the remaining signals observed in the ^{1}H and ^{13}C NMR spectra of **21** were attributed to the aglycon. Similar signal assignments were made for the polysaccharide chains (rings A, B, C, D, and E) of calicheamicin $\gamma_1{}^1$ (**6**) and *N*-acetylcalicheamicin $\gamma_1{}^{\mathrm{I}}$ (**8**) (Table 3, supplementary material). Careful comparison showed that the ^{1}H and ^{13}C NMR signals of the aglycon portion of **21**, **6**, and **8** were practically identical. Thus, *the aglycon of calicheamicin*, calculated to *have a molecular formula of* $C_{18}H_{16}NO_4S_3$, contained an OCH_3 group (δ_H 3.77, δ_C 53.6), a CH_3 group (δ_H 2.52, δ_C 22.8) on a fully substituted olefinic carbon (or possibly as a $SSCH_3$ or $SSSCH_3$ group),[18] an isolated methylene group (δ_H 2.84, 3.22, δ_C 53.3), an α,β-unsaturated ketone (δ_C 191.8, IR 1680 cm^{-1}), an isolated $=CH-CH_2$ unit (**a**), and a vinyl ABX spin system (**b**) where one of the protons of a pair of adjacent olefinic protons was long range coupled to a methine proton on a carbon bearing a heteroatom. The two olefinic protons in **b** collapsed to a 2-proton singlet in **21** and occasionally in **4**, suggesting that *the chemical environments on either side of the double bond in* **b** *were equivalent*. The above structural units accounted for 14 of the protons and nine of the carbons of the aglycon portion of **21** ($C_{18}H_{16}NO_4S_3$). The remaining two protons were presumably exchangeable, while the remaining nine carbons (δ_C 72.5, 83.9, 87.5, 98.7, 100.4, 130.6, 136.3, 140.7, and 154.3) did not have attached protons.



Sodium Borohydride Reduction of the Calicheamicin Pseudoaglycon (21). In order to provide an extra proton for 2D NMR studies to connect the structural units defined so far, it was desirable to reduce the α,β-unsaturated ketone to an alcohol. Sodium borohydride was the obvious reducing agent; however, it could also reduce the thioester linkage between ring-B and ring-C, producing a free thiol which could complicate the reaction. *In order to trap the thiolate if formed, the reduction was carried out using methyl iodide as a cosolvent* (Scheme III). The BIA positive dihydrocalicheamicin pseudoaglycon (**22**) was the major reaction product. When methyl iodide was omitted from the reaction, very little **22** was detected; instead, the reaction mixture was composed of intractable decomposition products. By serendipity, *methyl iodide functioned as a buffer for the sodium borohydride reduction.* In the absence of methyl iodide, the reaction mixture was too basic for **21** or **22** to remain intact, since both compounds were extremely base labile.

Scheme IV



Careful NMR studies on dihydrocalicheamicin pseudoaglycon (**22**), including ^{1}H–^{1}H COSY and ^{1}H–^{13}C correlation spectroscopy, showed the presence of partial structure **c** in **22**. Since the methylene protons and the proton vicinal to the new OH were coupled only to one another, the α-carbon of the α,β-unsaturated ketone must be substituted, the CH_2 group must be the isolated methylene unit, and partial structure **d** must be present in **21**. The chemical shifts of the methine proton (H-8), as well as the corresponding methine carbon in **b**, were also significantly affected by the reduction, suggesting that partial structures **d** and **b** were in close proximity. It was not at all clear, however, how structural units **a**, **b**, **d**, and the remaining carbons could be connected to give the structure of *calicheamicinone, defined as* $C_{18}H_{17}NO_5S_3$, containing 11 double bond equivalents.

Reaction of Triphenylphosphine with the Calicheamicin Pseudoaglycon (21). In an attempt to elucidate the nature of the three sulfur atoms in calicheamicinone, calicheamicin $\gamma_1{}^1$ was treated with triphenylphosphine. A derivative (calicheamicin ϵ) containing the entire polysaccharide chain and four unexpected contiguous aromatic protons was obtained along with triphenylphosphine sulfide and methyl mercaptan.[19] As expected, the NMR spectrum of calicheamicin ϵ was congested with signal overlaps. In order to study the reaction product between triphenylphosphine and the aglycon more carefully, the reaction was carried out with calicheamicin pseudoaglycon (**21**). Immediately after the addition of triphenylphosphine, **21** disappeared from the reaction mixture and a number of undefined intermediates could be detected by TLC. At the end of ca. 3 h, most of these intermediates converged to a major reaction product, **23** (Scheme IV). A few drops of methanol were added to the reaction mixture originally to ensure complete solution of **21**; it was subsequently found that without the methanol the reaction was more sluggish and the yield of **23** lower. The reaction was also cleaner and more reproducible, and yields of **23** were higher if it was carried out under argon.

---

(18) Bremser, W.; Franke, B.; Wagner, H. *Chemical Shift Ranges in Carbon-13 NMR Spectroscopy*; Verlag Chemie GmbH: Weinheim, Germany, 1982.

(19) Ellestad, G. A.; Hamann, P. R.; Zein, N.; Morton, G. O.; Siegel, M. M.; Pastel, M.; Borders, D. B.; McGahren, W. J. *Tetrahedron Lett.* **1989**, *30* (23), 3033–3036.

Table IV. ^{1}H NMR[a] (400 MHz, $CDCl_3$[b]) and ^{13}C NMR Data[c] of the Cyclized Calicheamicin Pseudoaglycon (**23**)

atom no.	^{13}C (mult)	^{1}H (mult, *J* (Hz), intgrtn)	^{1}H–^{1}H COSY	long range ^{1}H–^{13}C	
				^{1}H to ^{13}C	^{13}C to ^{1}H
1	72.8 (s)				2.61, 2.91
2	141.1 (s)				2.91, 4.58, 7.29
3	124.3 (d)	7.50 (d, 7.8, 1 H)	7.29	124.3, 130.4, 132.2	7.29, 7.50
4	130.4 (d)	7.29 (m, 1 H)	7.50, 7.14	124.3, 141.1	7.50
5	128.4 (d)	7.14 (t, 7.3, 1 H)	7.28, 7.50		
6	131.1 (d)	7.27 (m, 1 H)	7.14	132.2	
7	132.2 (s)				4.58, 7.27, 7.50
8	79.3 (d)	4.58 (s, 1 H)		72.5, 79.3, 132.2, 141.1, 143.4	4.58, 4.99
9	72.5 (s)				4.58, 4.99, 6.07
10	69.4 (d)	4.99 (d, 8.5, 1 H)	5.63	69.4, 72.5, 79.3, 157.5, 200.6	4.99
11	200.6 (s)				4.99, 2.91, 2.61
12	56.8 (t)	2.61 (d, 12.5, 1 H)	2.91	72.8, 143.4, 200.6	2.91
		2.91 (d, 12.5, 1 H)	2.61	56.8, 72.8, 141.1, 200.6	
13	143.4 (s)				2.61, 3.74, 3.89, 4.58, 6.07
14	122.5 (d)	6.07 (dd, 2.7, 2.3, 1 H)	3.74, 3.89	38.4, 72.5, 122.5, 143.4	3.74, 3.89, 6.07
15	38.4 (t)	3.74 (dd, 14.6, 2.9)	3.89, 6.07	122.5, 143.4	6.07
		3.89 (dd, 14.6, 2.2)	3.74, 6.07	122.5, 143.4	
10-NH*C*OOCH$_3$	157.5 (s)				3.64, 4.99
10-NHCOO*CH$_3$*	52.8 (q)	3.64 (s, 3 H)		52.8, 157.5	3.64
10-NH		5.63 (d, 8.5, 1 H)	4.99		
1A	103.9 (d)	4.78 (d, 8.1, 1 H)	3.15		
2A	75.2 (d)	3.15 (t, 8.5, 1 H)	3.85, 4.78		
3A	69.8 (d)	3.85 (t, 9.8)	2.23, 3.15		
4A	67.3 (d)	2.23 (t, 9.7, 1 H)	3.85, 3.66		1.28
5A	69.2 (d)	3.66 (dq, 9.7, 6.3)	2.23, 1.28		1.28
6A	18.0 (d)	1.28 (d, 6.1, 3 H)	3.66	18.0, 67.3, 69.2	1.28
1B	99.9 (d)	5.03 (dd, 9.6, 2.1, 1 H)	1.70		4.20
2B	37.2 (t)	1.70 (bt, 10.4, 1 H)	1.95, 5.03		
		1.95 (bd, 11.3, 1 H)	1.70, 4.20	51.4, 68.0	
3B	68.0 (d)	4.20 (m)	1.95, 3.61	69.5, 99.9	1.95, 4.20
4B	51.4 (d)	3.61 (dd, 9.1, 2.6)	4.00, 4.20	192.8	1.33, 1.95
5B	69.5 (d)	4.00 (dq, 10.9, 6.2)	3.61, 1.33		4.20, 1.33
6B	18.9 (q)	1.33 (d, 6.2, 3 H)	4.00	51.4, 69.5	1.33
1C	126.6 (s)				2.27
2C	149.1 (s)				3.79
3C	136.9 (s)				3.78
4C	151.4 (s)				
5C	85.2 (s)				2.27
6C	133.1 (s)				2.27
1C-CO	192.8 (s)				3.61
2C-OCH$_3$	61.5 (q)	3.79 (s, 3 H)		61.5, 149.1	3.79
3C-OCH$_3$	60.9 (q)	3.78 (s, 3 H)		60.9, 136.9	3.78
6C-CH$_3$	24.7 (q)	2.27 (s, 3 H)		24.7, 85.2, 126.6, 133.1	2.27

[a] The proton chemical shifts of this entire set are ~0.1 ppm higher field than normal, probably due to error in calibration. [b] ~80 mg/mL concentration; one drop of CD_3OD was added to clarify the solution. [c] ^{1}H–^{1}H correlation was by phase-sensitive COSY (COSYPHDQ), ^{1}H–^{13}C correlation by XHCORR, and long range ^{1}H–^{13}C correlation by COLOC.

Compound **23** was inactive in the BIA.

The NMR data (Table IV) of **23** confirmed that rings A, B, and C remained intact while the chemical structure of the aglycon portion of **23** was considerably different from that of **21**. The four contiguous aromatic protons were present as expected, and the OCH_3 group and structural unit **a** appeared to have survived the transformation. The isolated methylene unit in **d** also remained. However, the carbon signal due to the adjacent α,β-unsaturated carbonyl shifted from 191.8 to 200.6 ppm (i, Scheme IV). The CH_3 group at δ_H 2.52 (δ_C 22.8) was lost, while the molecular formula of **23** ($C_{39}H_{47}IN_2O_{15}S_2$) differed from that of the pseudoaglycon (**21**, $C_{40}H_{47}IN_2O_{15}S_4$) by the elements of CS_2. Prominent ions due to the loss of CS_2 from M + Na were also observed during the FABMS analyses of **21** and *N*-acetylcalicheamicin $\gamma_1{}^1$ (**8**). *In fact, during the initial FABMS studies of the calicheamicins, a dithiothreitol/dithioerythritol (magic bullet)*[20] *matrix was used and the highest mass ion observed was M + H − CS_2 instead of M + H.*

During the conversion to **23** via the reaction with triphenylphosphine, calicheamicin pseudoaglycon (**21**) must have lost CH_3S_2 and acquired three new hydrogens along the way. The presumptive source of these hydrogens was the solvent (CH_2Cl_2/CH_3OH) used for the reaction. However, when the reaction was carried out in CH_2Cl_2/CD_3OD (1/1), no deuterium incorporation in **23** was observed. The reaction was then carried out in CD_2Cl_2/CD_3OD (1/1) and, surprisingly, two deuterium atoms were incorporated into the new aromatic ring with the four contiguous aromatic protons (**e**), suggesting a free radical mechanism for the formation of the new aromatic ring in **23**. ^{1}H NMR data showed that approximately 12% of the reaction product was not deuterated, providing an internal reference for the NMR analysis. In order to assign the deuterium substitution pattern, the ^{1}H NMR spectra were determined in CD_2Cl_2 and CD_2Cl_2/acetone-d_6. Sufficient differences in the chemical shifts of the aromatic protons were observed in the two solvents, permitting the unambiguous assignment of the deuterium labels at the para positions. *The deuterium labeling pattern and the source of the deuterium atoms suggested the existence of a benzene-1,4-diyl* (**f**) *diradical as an intermediate in the conversion of calicheamicin pseudoaglycon to* **23**. The chemical structure of the precursor of **f**, however, was not at all apparent at this point.

Careful NMR studies of **23**, including long range ^{1}H–^{13}C correlation experiments (COLOC),[21] established the presence of

---

[20] Witten, J. L.; Schaffer, M. H.; O'Shea, M.; Cook, J. C.; Hemling, M. E.; Rinehart, K. L., Jr. *Biochem. Biophys. Res. Commun.* **1984**, *124* (2), 351–358.

[21] (a) Kessler, H.; Griesinger, C.; Zarbock, J.; Loosli, H. R. *J. Magn. Reson.* **1984**, *57*, 331. (b) Kessler, H.; Griesinger, C.; Lautz, J. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 444. (c) Kessler, H.; Griesinger, C.; Lautz, J. *Angew. Chem.* **1984**, *96*, 434.

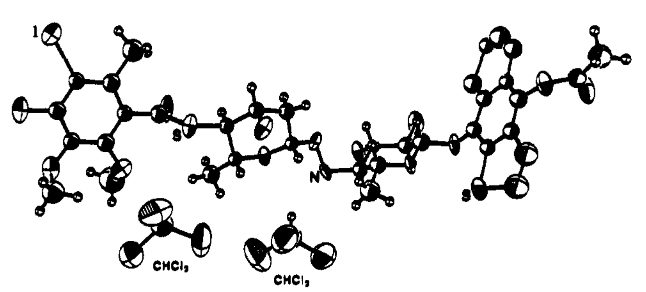
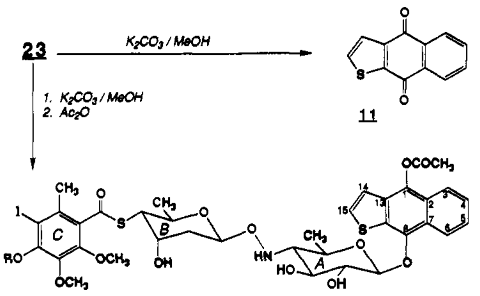


Figure 1. ORTEP drawing with 50% probability ellipsoids for compound **24** with the correct absolute configuration. The hydrogen atoms on the hydroxyl groups are omitted. Each unit cell contains two cocrystallized $CHCl_3$ molecules as shown.

Scheme V



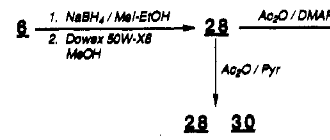
24 R = H
25 R = CH_3CO

structural units **g**, **h**, **i**, **a**, **j**, **k**, and **l** (Scheme IV). To account for the molecular formula of **23**, an exchangeable proton must also be present in structural unit **k** or **l**. The observed interactions through multiple bonds between the protons of the OCH_3 group at δ_H 3.64 and a carbon at δ_C 157.5 as well as between the same carbon and a methine proton doublet at δ_H 4.99, which was coupled (8.5 Hz) to an exchangeable doublet at δ_H 5.63, suggested the presence of partial structure **h**.[22] Long range interactions between the proton at δ_H 4.99 and the carbonyl carbon of **i** and the quaternary carbons of **j** and **k** were also observed. On the basis of the COLOC data, the signal at δ_C 143.4 was assigned unambiguously to the disubstituted olefinic carbon of **a**; this carbon also showed long range interactions with one of the methylene protons of **i** and with the proton in **j**. The proton in **j** (δ_H 4.58), in addition, is long range coupled to the carbon of **k** and two aromatic carbons at δ_C 132.2 and δ_C 141.1. Since the long range coupling paths could occur through two, three, or four bonds, it was not clear how these structural units could be combined to give the structure of **23**.

Retro-Aldol Cleavage of the Cyclized Calicheamicin Pseudoaglycon (23). Treatment of compound **23** with methanolic K_2CO_3 (30 min) afforded a number of new compounds based on TLC analysis of the reaction mixture. Upon workup and chromatographic purification, however, only compound **11** (Scheme I) was isolated, providing further evidence that *11 was a degradation product of calicheamicin $\gamma_1{}^I$*. In order to trap and characterize the reaction intermediate before it was converted to **11**, the reaction of **23** with methanolic K_2CO_3 was quenched after 5 min with excess acetic anhydride, and compounds **24** and **25** were isolated (Scheme V). ^1H NMR data revealed that **24** and **25** were the mono- and diacetates of a compound which retained rings A, B, and C of **23** but lost the OCH_3 group at δ_H 3.64 (**h**) and the methylene protons at δ_H 2.61 and 2.91 (**i**) and gained two more aromatic protons (δ_H 7.90, m and δ_H 8.58, m).

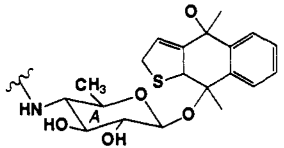
Compound **24** ($C_{36}H_{40}INO_{13}S_2$) crystallized in the triclinic space group, *P*1, with one molecule per unit cell. Its structure as determined by X-ray crystallography is shown in Figure 1.[23]

Scheme VI

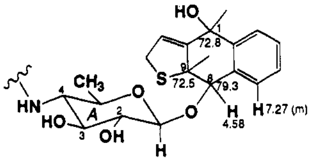


The structure was solved by the direct method, with the nonhydrogen atoms other than carbon refined anisotropically. As *P*1 was an acentric space group, refinements were carried out on both enantiomers. The structure in Figure 1 corresponds to the enantiomer which resulted in lower residual values ($R = 0.0651$, $R_w = 0.0700$ versus $R = 0.0663$, $R_w = 0.0708$). The naphtho[2,3-*b*]thiophene portion of **24** was found to be symmetrically disordered by a pseudomirror normal to the plane of the naphtho[2,3-*b*]thiophene ring and containing the C-1–C-8 vector.

The structure of **24** confirmed the chemical structure of the unusual hydroxyamino sugar (A-ring) and established (1) that both the hydroxyamino sugar (A-ring) and the thio sugar (B-ring) were in the D configuration, (2) the unusual N–O glycosidic linkage between the two sugars, (3) the basic carbon skeleton of the aglycon of **23**, and (4) the location of the glycosidic linkage of the hydroxyamino sugar to the aglycon of **23**. This information in conjunction with partial structures **a** and **g** suggested the existence of partial structure **m** in the aglycon portion of **23**. An NOE difference experiment carried out on **23** showed a strong NOE between δ_H 4.78 (H-1A) and the two protons at δ_H 4.58 (**j**) and δ_H 7.27 (**g**), establishing the proximity of ring-A to structural unit **j** and allowing us to extend the partial structure of **23** to **n**, which included structural units **a**, **l**, **k**, **j**, and **g** and was consistent with the observed long range ^1H–^{13}C interactions.

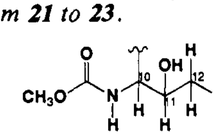


m



n

During the mild base treatment of **23** (Scheme V), a retro-aldol reaction and subsequent aromatization to the naphthothiophene ring system in **24** must have occurred, which resulted in the elimination of structural units **h** and **i** from C-1 and C-9 of **n** and prompted us to assign the chemical structure of **23** as shown. The connectivities between C-9 and C-10, C-10 and C-11, and C-12 and C-1 were consistent with the long range ^1H–^{13}C interactions observed for **23**. The connectivity between C-10 and C-11 was further confirmed by ^1H–^1H COSY analysis of the C-11 alcohol (**26**) derived from **23** where the presence of partial structure **o** was evident based on ^1H–^1H COSY. *The proton on C-10 of 23 is enolizable and must be the third hydrogen aquired during the transformation from 21 to 23.*



o

Isolation of a Modified Dihydrocalicheamicin Aglycon (28) and the Structure of Calicheamicinone (32). When dihydrocalicheamicin $\gamma_1{}^I$ (**27**) was subjected to methanolysis in the presence of Dowex 50, compound **28**, which had lost the entire polysaccharide chain, was isolated. Preliminary NMR studies showed that **28** had lost the same CH_3 group (δ_H 2.52, δ_C 22.8) which was lost during the transformation of **21** to **23**. The remainder of the dihydro aglycon, however, remained intact in **28**. A strong ion at 311 ($C_{17}H_{13}NO_3S$) was observed in the FABMS spectrum of **28**, which was H_3O_2 less than the expected molecular ion if the only difference between **28** and *dihydrocalicheamicinone* ($C_{18}H_{19}NO_5S_3$) was the loss of CH_3S_2. Weak ions observed at *m/z* 329 (EIMS) and at *m/z* 328 (negative FABMS) suggested that *m/e* 311 was $M^+ - H_2O$ and the *molecular formula of 28*

---

[22] Mallams, A. K.; Puar, M. S.; Rossman, R. R. *J. Am. Chem. Soc.* **1981**, *103*, 3938–3940.

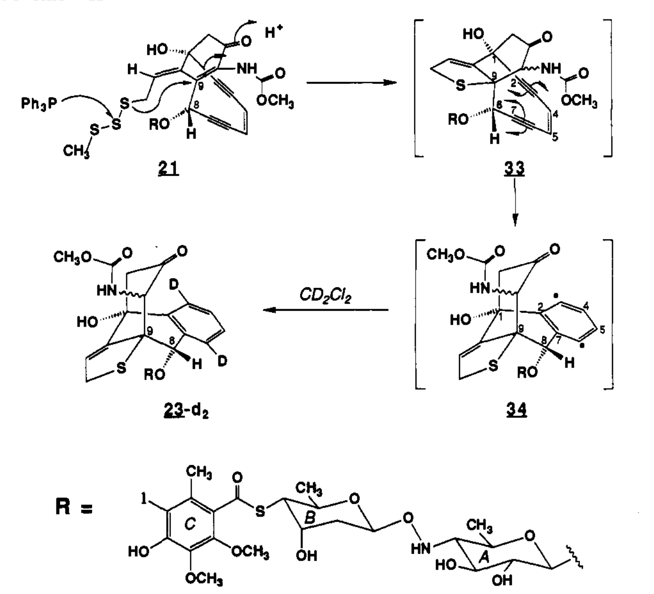
[23] The X-ray analysis was carried out by the Molecular Structure Corporation, 3304 Longmire Dr., College Station, TX 77840-409. The X-ray data could be found in the supplementary material of ref 6b.

Table V. ^{1}H NMR (300 MHz) and ^{13}C NMR Data of **28** and the Aglycon Portion of **22**

	28 (DMSO-d_6)[a]			**22**-aglycon ($CDCl_3$/CD_3OD)		
atom no.	^{13}C (mult)	^{1}H (mult, J (Hz), intgrtn)	^{1}H–^{1}H COSY	^{13}C (mult)	^{1}H (mult, J (Hz), intgrtn)	^{1}H–^{1}H COSY
1	71.0 (s)			71.8 (s)		
2[b]	102.0 (s)			103.5 (s)		
3[c]	87.6 (s)			87.3 (s)		
4	124.5 (d)	5.79 (d, 9.6, 1 H)		123.4 (d)	5.90 (d, 9.5, 1 H)	5.81
5	123.8 (d)	5.75 (d, 9.6, 1 H)		122.6 (d)	5.81 (d, 9.5, 1 H)	5.90, 5.69
6[c]	86.1 (s)			83.9 (s)		
7[b]	99.7 (s)			97.7 (s)		
8	30.7 (d)	4.70 (s, 1 H)		67.3 (d)	5.69 (s, 1 H)	5.81
9	128.2 (s)			124.1 (s)		
10	140.8 (s)			137.3 (s)		
11	67.0 (d)	4.54 (t, 7.2, 1 H)	1.93, 2.51	66.8 (d)	4.75 (m, 1 H)	2.17, 2.73
12	44.2 (t)	1.93 (dd, 12.2, 9.7, 1 H)	2.51, 4.54	45.7 (t)	2.17 (dd, 13.7, 4.4, 1 H)	2.71, 4.75
		2.51 (DD, 12.2, 5.0, 1 h)	1.93, 4.54		2.73 (DD, 13.7, 6.3, 1 h)	2.17, 4.75
13	140.8 (s)			139.4 (s)		
14	117.0 (d)	6.10 (t, 4.0, 1 H)	3.29, 3.59	125.1 (d)	6.36 (dd, 9.0, 6.9, 1 H)	3.89, 4.05
15	24.5 (t)	3.29 (dd, 18.2, 4.3, 1 H)	3.59, 6.10	39.4 (t)	3.89 (dd, 1 H)	4.05, 6.36
		3.59 (ddd, 18.7, 3.8, 1.0, 1 H)	3.29, 6.10		4.05 (dd, 1 H)	3.89, 6.36
10-NH*C*OOCH$_3$	157.4 (s)			155.1 (s)		
10-NHCOO*C*H$_3$	52.9 (q)	3.67 (s, 3 H)		53.0 (q)	3.77 (s, 3 H)	
15-SSSCH$_3$				22.8 (q)	2.56 (s, 3 H)	

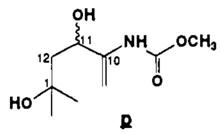
[a] ^{1}H–^{13}C correlation by HETCOR. [b,c] Assignments for these carbons could be reversed.

Scheme VII

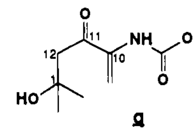


was $C_{17}H_{15}NO_4S$, which was one OH less than expected, if the loss of the polysaccharide chain was due to simple methanolysis leaving a hydroxy group at the original glycosidic linkage.

Compound **28** was converted (Scheme VI) to its monoacetate (**29**), diacetate (**30**), and triacetate (**31**), demonstrating the presence of three exchangeable protons in **28**. Careful analysis of the ^{1}H NMR data revealed that the acetate in **29** was the acetate of the secondary alcohol in partial structure **c**, and the second acetate in **30** was the acetate of a tertiary alcohol adjacent to the methylene in **c**, since one of the methylene protons in **30** and **31** shifted down field by ~0.7 ppm. The third acetate in **31** was an N-acetate on the carbamate nitrogen. These observations indicated that the portion of the structure containing the equivalent of C-1, C-12, C-11, and C-10 of **23** was present in **28**, as shown in partial structure **p**, and structural unit **d** of calicheamicinone could be extended to **q**.

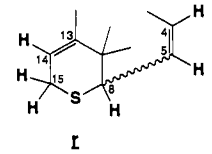


p

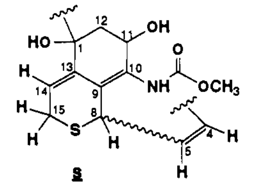


q

In-depth NMR studies (Table V), including ^{1}H–^{1}H COSY, ^{1}H–^{13}C correlation spectroscopy, and side-by-side comparison with the aglycon portion of dihydrocalicheamicin pseudoaglycon (**22**), showed that in **28** significant changes in the carbon chemical shifts occurred for the methine carbon (C-8) in partial structure **b** and for the methylene carbon (C-15) in partial structure **a**. The structure of **23** dictated that the methine carbon in **b** was the point of glycosidic attachment, and the chemical shift of this carbon in both **21** and **22** was consistent with its attachment to two carbon atoms and one oxygen atom. During the formation of **28**, a displacement reaction must have occurred at this carbon and resulted in the loss of the entire polysaccharide chain and the large upfield shift of this carbon to δ_C 30.7. A displacement reaction at this carbon instead of the normal methanolysis reaction would also account for the molecular formula of **28**. To account for its carbon chemical shift within the established structural constraint, this methine carbon must now be bonded either to three carbon atoms or to two carbon atoms and a sulfur atom. Considering the mild reaction conditions used, a carbon nucleophile was deemed unlikely, while a sulfur nucleophile was a real possibility, especially in conjunction with the loss of S_2CH_3. Prompted by the chemical structure of **23**, partial structure **r**, which bridged structural units **a** and **b** with a sulfur atom, was assigned for **28**.



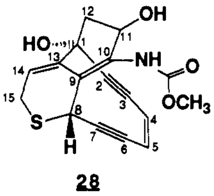
r



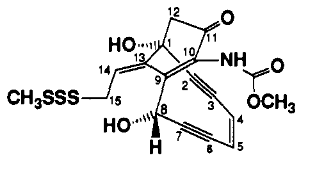
s

The large geminal coupling constant (18.2 Hz) of the C-15 methylene protons in **28** suggested that the thiacyclohexene ring is rigid and prompted us to combine **r** and **p**, again modeling after the structure of **23**, into partial structure **s**. Partial structure **s** accounted for all but four carbon atoms in the molecular formula of **28**. The carbon chemical shifts of these remaining four carbon singlets, 86.1, 87.6, 99.7, and 102.0 ppm, suggested that they could be acetylenic. This was confirmed by the presence of a weak absorption at 2190 cm^{-1} in the IR spectrum of **28**, which further suggested that the acetylenes were conjugated. Since H-4, H-5, and H-8 constituted the vinyl ABX spin system where H-8 was the X proton long range coupled to H-5 and the chemical structure on either side of the C-4–C-5 double bond was equivalent as discussed before, the chemical structure of **28** with the symmetrical enediyne system was assigned.

The structure of **28** required that the S–C-9 bond in **23** was not present in the calicheamicin aglycon and was formed during the transformation from calicheamicin pseudoaglycon (**21**). As a result, the chemical structure of the calicheamicin aglycon,



28



32 calicheamicinone

calicheamicinone, was proposed as **32**, which encompassed the structure of **28** and partial structure **q**. *This assignment rationally accounted for the loss of a S_2CH_3 unit during the formation of* ***23*** *(Scheme IV) and* ***28*** *(Scheme VI), as well as the sulfur-containing ions (CH_3S_2, $CH_3S_2CH_3$, and $CH_3S_3CH_3$) observed in the low-mass region during the initial HREIMS analysis of calicheamicin β_1^{Br}.* A total synthesis of calicheamicinone (**32**) has been reported recently.[24]

As described earlier, the transformation (Scheme IV) of calicheamicin pseudoaglycon (**21**) to **23** occurred via a benzene-1,4-diyl intermediate. In order to account for this experimental observation, the following sequence of events was proposed (Scheme VII): (1) triphenylphosphine attack at the allylic trisulfide, (2) Michael reaction of the resulting thiolate or thiol with the α,β-unsaturated ketone, (3) tautomerization of the resulting enol to the corresponding ketone **33**, (4) cyclization of **33** to generate the 1,4-diyl **34**, and (5) deuterium atom abstraction from solvent CD_2Cl_2 to give the isolated reaction product **23**-d_2. During the Dowex 50W-X8 treatment of dihydrocalicheamicin γ_1^I, in the absence of a driving force for the β-addition at C-9, the thiol displaced the glycoside at C-8 instead, resulting in the formation of **28**.

When Scheme VII was proposed initially,[6b] *it was the only explanation that completely accounted for the experimental data concerning the chemistry and the structure of the calicheamicin aglycon.* The β-addition step was in accordance with the observation that the conversion of **21** to **23** was much slower in the absence of methanol. The cyclization of the enediyne system via a 1,4-diyl was supported by Bergman's work on 1,4-dehydrobenzene.[25] Examination of models revealed that the enediyne system in **33** was considerably more flexible than that in **21** and that it was not possible to build a cyclized model with both bridgehead double bonds present, providing an explanation of why the calicheamicins existed. A number of publications since our initial communications have demonstrated the validity of our supposition and provided real insight and understanding to the cycloaromatization process.[26] According to this model, neither calicheamicin ϵ, which was prepared from calicheamicin γ_1^I as well as isolated from the fermentation broth,[19] nor esperamicin X[7a] were natural products; rather, they were degradation products of the corresponding natural products. The calicheamicins β_1^{Br}, γ_1^{Br}, α_2^I, α_3^I, β_1^I, γ_1^I, and δ_1^I, N-acetylcalicheamicin γ_1^I, the calicheamicin pseudoaglycon (**21**), the dihydrocalicheamicin pseudoaglycon (**22**), and dihydrocalicheamicin γ_1^I (**27**) were potent DNA-damaging agents as demonstrated by their activity in the BIA. Compound **23** and calicheamicin ϵ, however, were completely inactive in the BIA. These observations led us to propose that the cycloaromatization process shown in Scheme VII was responsible for the potent DNA-damaging effects of the calicheamicins and that the 1,4-diyl **34** was the actual active species in the DNA cleavage process.[27] The existence of intermediate **33** and an estimation of its lifetime at physiological temperature was demonstrated by solution NMR studies.[28]

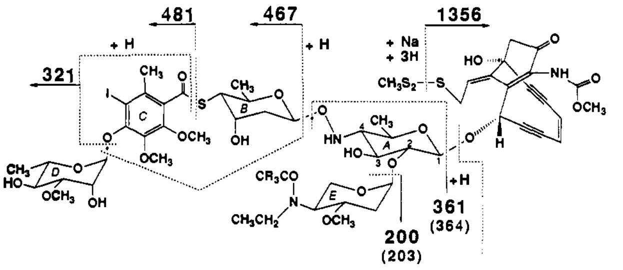


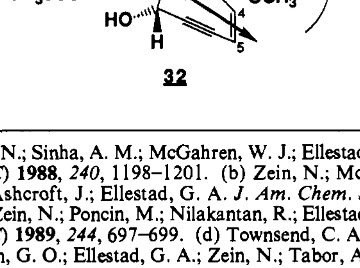
Figure 2. Diagnostic FABMS fragmentation patterns of N-acetylcalicheamicin γ_1^I (**8**) and N-(acetyl-d_3)calicheamicin γ_1^I (**35**). The fragment ions in brackets were observed only in the spectrum of **35**.

Table VI. Chemical Shifts (^{1}H NMR, 300 MHz, $CDCl_3$) of the A-, B-, and D-Ring Protons of **8**, **21**, Tetraacetyl-**21**, Diacetyl-**17**, Triacetyl-**19**, and **36**[a]

atom no.	8	21	tetraacetyl-21	diacetyl-17	triacetyl-19	36
1A	4.60	4.60	4.81			*5.68*
2A	3.62	3.64	*4.95*			3.63
3A	4.03	3.98	*5.47*			*5.50*
4A	2.33	2.44	2.72			2.68
5A	3.69	3.79	3.77			3.81
1B	5.06	5.06	4.87	4.64	4.64	4.88
2Bax	1.78	1.78	1.86	1.89	1.88	1.86
2Beq	2.04	2.04	2.04	2.14	2.05	2.04
3B	4.31	4.32	*5.37*	*5.40*	*5.40*	*5.40*
4B	3.75	3.72	3.77	3.83	3.85	3.82
5B	4.07	4.07	3.94	3.96	3.95	3.98
1D	5.72				5.62	5.63
2D	4.48				*5.74*	*5.75*
3D	3.83				4.03	4.03
4D	3.63				*5.10*	*5.11*
5D	4.19				4.34	4.34

[a] Protons geminal to the acetylated hydroxyls are italicized. Assignments for **8**, **21**, tetraacetyl-**21**, and **36** were determined by ^{1}H–^{1}H COSY.

A characteristic negative Cotton effect (311 nm, $\Delta\epsilon$ −370; 272 nm, $\Delta\epsilon$ +370), centered around the dienone chromophore, was observed for calicheamicin γ_1^I. Assuming that the polarization of the transition moment of the enediyne system was orthogonal to the C-4–C-5 double bond as shown below and the observed circular dichroism was due to exciton coupling,[29] the configuration of calicheamicinone (**32**) as drawn showed the left-handed relationship between the two chromophores and accounted for the



32

---

[24] Haseltine, J. N.; Cabal, M. P.; Mantlo, N. B.; Iwasawa, N.; Yamashita, D. S.; Coleman, R. S.; Danishefsky, S. J.; Schulte, G. K. *J. Am. Chem. Soc.* **1991**, *113*, 3850–3866.

[25] (a) Lockhart, T. P.; Comita, P. B.; Bergman, R. G. *J. Am. Chem. Soc.* **1981**, *103*, 4082–4090. (b) Lockhart, T. P.; Bergman, R. G. *Ibid.* 4091–4096. (c) Wong, H. N. C.; Sondheimer, F. *Tetrahedron Lett.* **1980**, *21*, 217–220.

[26] (a) Magnus, P.; Fortt, S.; Pitterna, T.; Snyder, J. P. *J. Am. Chem. Soc.* **1990**, *112*, 4986–4987. (b) Snyder, J. P. *J. Am. Chem. Soc.* **1990**, *112*, 5367–5369. (c) Nicolaou, K. C.; Ogawa, Y.; Zucarello, G.; Kataoka, H. *J. Am. Chem. Soc.* **1988**, *110*, 7247–7248. (d) Nicolaou, K. C.; Zuccarello, G.; Ogawa, Y.; Schweiger, E. J.; Kumazawa, T. *J. Am. Chem. Soc.* **1988**, *110*, 4866–4868. (e) Magnus, P.; Carter, P. A. *J. Am. Chem. Soc.* **1988**, *110*, 1626–1628. (f) Snyder, J. P. *J. Am. Chem. Soc.* **1989**, *111*, 7630–7632. (g) Mantlo, N. B.; Danishefsky, S. J. *J. Org. Chem.* **1989**, *54*, 2781–2783. (h) Haseltine, J. N.; Danishefsky, S. J.; Schulte, G. *J. Am. Chem. Soc.* **1989**, *111*, 7638–7640.

[27] (a) Zein, N.; Sinha, A. M.; McGahren, W. J.; Ellestad, G. A. *Science (Washington, DC)* **1988**, *240*, 1198–1201. (b) Zein, N.; McGahren, W. J.; Morton, G. O.; Ashcroft, J.; Ellestad, G. A. *J. Am. Chem. Soc.* **1989**, *111*, 6888–6890. (c) Zein, N.; Poncin, M.; Nilakantan, R.; Ellestad, G. A. *Science (Washington, DC)* **1989**, *244*, 697–699. (d) Townsend, C. A.; DeVoss, J. J.; Ding, W.; Morton, G. O.; Ellestad, G. A.; Zein, N.; Tabor, A. B.; Schreiber, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 9669–9670.

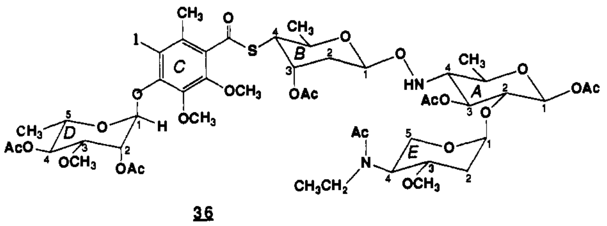
[28] De Voss, J. J.; Hangeland, J. J.; Townsend, C. A. *J. Am. Chem. Soc.* **1990**, *112*, 4554–4556.

[29] (a) Liu, H.-W.; Nakanishi, K. *J. Am. Chem. Soc.* **1982**, *104*, 1178–1185. (b) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy—Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983.

observed negative Cotton effect. *Esperamicin A showed an identical Cotton effect, indicating that its aglycon had the same absolute configuration as 32.*[30]

FABMS Studies and the Assignment of the Glycosidic Linkage of the Ethylamino Sugar. With the chemical structures of the calicheamicin pseudoaglycon (**21**) and compound **19** in hand, the remaining task was to assign the final glycosidic attachment of the ethylamino sugar (E-ring). High-resolution FABMS studies on **8** (Figure 2) gave diagnostic ions at *m/e* 481.0349 ($C_{17}H_{22}IO_8$), 467.0038 ($C_{16}H_{20}IO_6S$), 361.1979 ($C_{16}H_{29}N_2O_7$), and 987.2660 ($C_{39}H_{60}IN_2O_{17}S$), which allowed us to sequence the major structural units very early during the structure elucidation and provided the first evidence for the attachment of the ethylamino sugar to the hydroxyamino sugar. This observation was confirmed by fragment ions at *m/e* 990, 364, and 203, instead of 987, 361, and 200, for *N*-(acetyl-d_3)calicheamicin $\gamma_1{}^I$ (**35**).

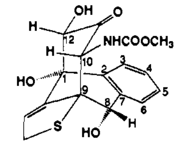
In order to determine whether the glycosidic linkage was at C-2 or C-3 of the hydroxyamino sugar, *N*-acetylcalicheamicin $\gamma_1{}^I$ (**8**) was subjected to peracetylation ($Ac_2O/DMAP/CH_2Cl_2$). The geminal protons of the secondary hydroxyls on the glycosidic rings should show a characteristic downfield shift of ~1 ppm upon acetylation of the corresponding hydroxyls. In addition to heptaacetylcalicheamicin $\gamma_1{}^I$, compound **36** was isolated and selected for ^1H–^1H COSY studies. Unfortunately, it was not possible to assign the resonances of the A-ring protons unambiguously due to extensive signal overlap with the ring protons of the other glycosidic units. However, side-by-side comparison (Table VI) of the glycoside ring-proton resonances of *N*-acetylcalicheamicin $\gamma_1{}^I$ (**8**), calicheamicin pseudoaglycon (**21**), 1,2A,3A,3B-tetraacetylcalicheamicin pseudoaglycon (**37**), diacetyl-**17**, triacetyl-**19**, and the hexaacetyl glycoside **36** allowed us to assign, unambiguously, the glycosidic linkage of the ethylamino sugar (E-ring) at C-2 of the hydroxyamino sugar (A-ring). A total synthesis of **36** has been reported.[31]



36

Structures of Calicheamicins $\beta_1{}^{Br}$, $\gamma_1{}^{Br}$, $\alpha_2{}^I$, $\alpha_3{}^I$, and $\delta_1{}^I$. The structural difference between calicheamicins $\beta_1{}^I$ (**1**) and $\gamma_1{}^I$ (**6**) was noted during the *N*-acetylation studies. The similarities in their chromatographic behavior provided the first evidence that calicheamicins $\beta_1{}^{Br}$ and $\beta_1{}^I$ and calicheamicins $\gamma_1{}^{Br}$ and $\gamma_1{}^I$ were two pairs of closely related structures.[9a] That the difference between the components within each pair was a bromine versus an iodine substitution was predicted by the observation that components $\beta_1{}^I$ and $\gamma_1{}^I$ were produced only in fermentations supplemented with iodide and was confirmed by (1) their practically identical ^1H NMR spectra, (2) the shift of a carbon at δ_C 115 in the brominated analogues to δ_C 93.5 in the iodinated analogues (Table 4, supplementary material), and (3) FABMS determination of their molecular weights.[32] Once the chemical structures of **9** and **15** were assigned, it was evident, on the basis of their ^1H NMR spectra, that calicheamicin $\alpha_2{}^I$ was calicheamicin $\gamma_1{}^I$ without the 3-methoxyrhamnose unit and calicheamicin $\alpha_3{}^I$ was calicheamicin $\gamma_1{}^I$ without the ethylamino sugar unit. These differences were confirmed by their ^{13}C NMR data and their molecular weights. The ^{13}C NMR data of calicheamicins α_2 and α_3 were particularly useful in sorting out the ^{13}C NMR data of the glycosides in calicheamicin $\gamma_1{}^I$. The chemical structure of calicheamicin $\delta_1{}^I$ was first suggested by its mobility in HPLC relative to calicheamicins $\beta_1{}^I$ and $\gamma_1{}^I$[9a] and was confirmed by its ^1H and ^{13}C NMR data and molecular weight determination.

A number of the carbon resonances in the aglycon portion of the calicheamicins were of very low intensity, notably the two bridgehead sp^2 carbons, C-9 and C-13, and one of the acetylene carbons at ~100 ppm. The resonance of C-13 in **4** was observed only in the NOE enhanced proton coupled ^{13}C NMR spectrum. The olefinic protons, H-4 and H-5 in calicheamicin $\alpha_3{}^I$ (**4**), appeared as a two-proton singlet (δ_H 5.88) and showed no long range coupling with H-8 (δ_H 6.00) when the ^1H NMR spectrum was determined in deuteriochloroform. When the same solution contained 10 μL of methanol-d_4, however, the two protons became nonequivalent (δ_H 5.91, d, 9.4 Hz, H-4; δ_H 5.84, dd, 9.4, ~1 Hz, H-5) and H-5 showed long range coupling to H-8 (δ_H 5.99, ~1 Hz). These observations suggested that *the enediyne system could assume more than one conformation depending on its environment and that the extent of the coupling between C-5 and C-8 was dependent on the exact conformation of calicheamicinone.* The protons H-4 and H-5 were not equivalent in dihydrocalicheamicin pseudoaglycon (**22**, Table V) but were equivalent in calicheamicin pseudoaglycon (**21**, Table III). No long range coupling between H-5 and H-8 of 1 Hz or greater was observed for either **21** or **22**. Interactions between H-5 and H-8 were observed, however, in the ^1H–^1H COSY analysis of both **21** and **22**. It was suggested that the stereochemistry at C-8 of calicheamicinone was as shown, which was the opposite of our original publication on the structure of calicheamicin $\gamma_1{}^I$ (**6**).[6b] This suggestion was based on the observation that the propargylic hydrogen (H-8), in a model of the same stereochemistry as we proposed originally, did not exhibit long range coupling with H-5.[33] It appears now that this argument may not be valid on account of the observations described above. However, we favor the revised stereochemistry based on the X-ray structure of **38**,[7a] a degradation product of esperamicin X, which placed the glycosidic linkage at C-8 on the same side of the enediyne plane as the methyl trisulfide.[34] Nature is not likely to produce two series of compounds as closely related to each other as the calicheamicins and the esperamicins in two different configurations at one particular carbon. X-ray crystallography of the aglycon of **23**, which could be prepared from either **23** or calicheamicin ε, would ultimately resolve this problem.



38

The carbamate methyl protons in **1**, **2**, **3**, **5**, **6**, **7**, and **8** were broad and did not integrate to a full 3 H; the same signal was sharp in the spectra of **4**, **21**, **22**, and **23**, suggesting possible interaction between the carbamate group and the ethylamino sugar residue in solution. The H-8 in **4** and **21** appeared at δ_H 5.99 while the same proton in **1**, **2**, **3**, **5**, **6**, **7**, and **8** appeared at δ_H 6.21–6.25, suggesting that the spatial environment of H-8 in solution was affected by the presence of the ethylamino sugar.

Summary. The chemical structure of calicheamicin $\gamma_1{}^I$ was determined by a combination of degradation studies and spectroscopic methods. In this report, we have presented the approach and strategies toward solving the chemical structure of a complex molecule that is not related to any known compounds in the literature. With a few minor complications, the structure of the glycosidic chain was determined by traditional and relatively predictable experiments. The unprecedented enediyne structure of calicheamicinone (**32**) was assigned on the basis of compelling

---

[30] We thank Dr. T. W. Doyle of Bristol-Squibb for a sample of esperamicin A for comparison studies.

[31] Nicolaou, K. C.; Groneberg, R. D.; Miyazaki, T.; Stylianides, N. A.; Schulze, T. J.; Stahl, W. *J. Am. Chem. Soc.* **1990**, *112*, 8193–8195 and references therein.

[32] The ^1H NMR spectra and FABMS data of calicheamicins $\beta_1{}^{Br}$, $\gamma_1{}^{Br}$, $\alpha_2{}^I$, $\alpha_3{}^I$, and $\delta_1{}^I$ are found in ref 9a.

[33] Kende, A. S.; Smith, C. A. *Tetrahedron Lett.* **1988**, *29* (34), 4217–4220.

[34] The proposed structure of **38** in ref 7a is drawn in the opposite enantiomeric configuration; however, as discussed before, the aglycons of esperamicin A and calicheamicin $\gamma_1{}^I$ have the same absolute configuration.

chemical and spectroscopic evidence and, during the structural elucidation process, the existence of a 1,4-dehydrobenzene diradical as a reaction intermediate at ambient temperature was demonstrated. By tracking the biological activities of the degradation products using the BIA, we were able to demonstrate that the enediyne system was essential for the DNA-damaging abilities of the calicheamicins and proposed a mechanism whereby the enediyne could be triggered to cyclize via a 1,4-diyl.[35] The ^1H and ^{13}C NMR spectra of calicheamicin $\gamma_1{}^I$ as well as those of the key degradation products were assigned. The chemical structures of the minor components of the calicheamicin complex, calicheamicins $\beta_1{}^{Br}$ (**1**), $\gamma_1{}^{Br}$ (**2**), $\alpha_2{}^I$ (**3**), $\alpha_3{}^I$ (**4**), $\beta_1{}^I$ (**5**), and $\delta_1{}^I$ (**7**), were also assigned by correlating their ^1H and ^{13}C NMR data with that of calicheamicin $\gamma_1{}^I$.

Experimental Section

General. UV absorption spectra were recorded with a Hewlett-Packard 8450A UV/VIS spectrophotometer. IR spectra were determined on KBr disks using a Nicolet FT-IR spectrometer. Mass spectrometric measurements were carried out in the EI or FAB (glycerol, sulfolane or dithiothreitol/dithioerythritol matrix) ionization mode, using a VG Analytical Instruments Model ZAB-SE mass spectrometer or at the University of Illinois. The ^1H and ^{13}C NMR spectra were recorded using Nicolet NT-300, Brucker AM-500, and AM-400 spectrometers. The samples were prepared in $CDCl_3$. A 10-μL drop of CD_3OD was added to some samples where the $CDCl_3$ solutions were not clear.

Unless indicated otherwise, TLC analyses were carried out using Whatman High-Performance TLC (HPTLC) glass plates, Type HP-KF or Type LHP-KF silica gel, and detected by $UV_{254\,nm}$ quenching, $CuOAc/H_3PO_4$,[36] or anthrone spray. TLC-bioautography studies were carried out using E. Merck silica gel 60 F_{254} precoated aluminum sheets (0.2-mm layer thickness). Preparative column chromatographies were carried out using silica gel 60 (Kieselgel 60, 40–63 μm, E. Merck), Silica Woelm (32–63 μm, Woelm Pharma GmbH & Co.), or Bio-Sil A (20–40 μm, Bio-Rad Laboratories) in closed glass columns fitted with 1/4-28 thread chemically inert Teflon tube end fittings. Rainin Rabbit HPLC pumps equipped with Rainin Electronic Pressure Monitors were used for solvent delivery. Preparative TLCs were carried out using Analtech silica gel GF (1000 μm, 2000 μm, or tapered) precoated glass plates.

***N*-Acetylcalicheamicin $\gamma_1{}^I$ (8).** Acetic anhydride (3 mL) was added dropwise to a methanolic solution (100 mL) of partially purified calicheamicin $\gamma_1{}^I$ (421 mg, 32% pure) cooled in an ice–water bath. The reaction mixture was stirred at 0 °C for 1.5 h, warmed slowly to room temperature, stirred for another 2.5 h, and concentrated in vacuo. The residue was redissolved in ethyl acetate and precipitated by addition of diethyl ether and hexanes. The precipitated crude **8** was purified by chromatography on a Bio-Sil A column (1.5 × 95 cm) eluting with EtOAc/MeOH (96/4). The desired fractions were pooled, concentrated in vacuo, and precipitated from EtOAc by addition of hexanes to give analytically pure *N*-acetylcalicheamicin $\gamma_1{}^I$ (**8**): 107 mg (white amorphous solid), R_f 0.49 (3% 2-propanol in EtOAc saturated with water); $C_{57}H_{76}IN_3O_{22}S_4$ (HRFABMS, M + H m/z 1410.2954 Δ 0.26 mmu); NMR data as shown in Table 3 of the supplementary material.

Methanolysis of Crude Calicheamicin Complex. A sample of the crude complex (13.0 g, 1.2% **6**, 0.1% **5**) suspended in 4% HCl in MeOH (100 mL) was sealed in a high-pressure reaction tube and heated at 90 °C for 4 h. The reaction mixture was cooled, diluted with 300 mL of MeOH, and concentrated to a sticky gum. The black gum was redissolved in 400 mL of MeOH and reconcentrated in order to remove as much of the HCl as possible. The residue was dissolved in 400 mL of MeOH and neutralized by addition of $BaCO_3$. The excess salt was filtered off, and the solution was concentrated to dryness. The residue was partitioned between 200 mL each of CH_2Cl_2 and water. The aqueous solution, after being washed once with CH_2Cl_2, was freeze-dried to give a dark green gum which was triturated with CH_2Cl_2, and the CH_2Cl_2 solution was concentrated to give a crude mixture of **9** and **10**.

Isolation of 9 and 10. The entire mixture of **9** and **10** above was combined with another preparation of the same and chromatographed on two 20 × 20, 2000-μm layer preparative TLC plates developed with EtOAc saturated with 0.1 M phosphate buffer (pH 7.0). Thin strips of the developed plates were removed by Scotch-tape, and the thin layers of silica gel on the tapes were visualized by $CuOAc/H_3PO_4$ and anthrone spray. The bands on the preparative TLC plates corresponding to the anthrone positive bands on the Scotch-tapes were excised, and the glycosides were recovered from the silica gel by washing with CH_2Cl_2/MeOH (7/3) to give partially purified **9** and **10**. **9** and **10** were each further purified by preparative TLC [CH_2Cl_2/MeOH (95/5); 10% 2-propanol in EtOAc saturated with 0.1 M phosphate buffer (pH 7.0)] to give analytically pure products.

9: 120 mg (colorless oil); R_f 0.63 (CH_2Cl_2/MeOH, 90/10), R_f 0.45 [(EtOAc saturated with 0.1 M phosphate buffer (pH 7.0)]; $[\alpha]^{26}{}_D$ −44 ± 5° (c 0.185, EtOH); $C_8H_{16}O_5$ (CIMS, M + NH_4 m/z 210, HREIMS, M − OCH_3 m/z 161.0817 Δ 0.4 mmu); NMR data as shown in Table I of the supplementary material. **10**: 60 mg (low-melting crystalline solid); R_f 0.55 [(CH_2Cl_2/MeOH (90/10)], R_f 0.24 [(EtOAc saturated with 0.1 M phosphate buffer (pH 7.0)]; $[\alpha]^{26}{}_D$ +127 ± 3° (c 0.39, EtOH); CIMS, M + NH_4 m/z 210; NMR data as shown in Table I of the supplementary material.

Methanolysis of *N*-Acetylcalicheamicin $\gamma_1{}^I$ (8). A sample of **8** (448 mg, 94% pure) dissolved in 4% HCl in MeOH (40 mL) was sealed in a high-pressure reaction tube and heated at 50 °C overnight. The reaction mixture was cooled, diluted with MeOH (200 mL), and concentrated to dryness. The residue was redissolved in MeOH (200 mL) and reconcentrated twice to remove dissolved HCl. The final residue was triturated thoroughly with CH_2Cl_2, and the CH_2Cl_2 solution was concentrated to a dark brown gum and chromatographed on a Bio-Sil A column (2.5 × 22 cm). The column was eluted at 5 mL/min with a step gradient of 200 mL each of 0, 0.2, 0.5, 1, 2, 4, and 10% MeOH in CH_2Cl_2, collecting 10-mL fractions. The fractions were analyzed by TLC and combined into 10 fractions; those eluting after **9** and **10** were discarded. Each of the 10 fractions were concentrated in vacuo, and only those containing visible amounts of sample were followed up.

Isolation of 11. The fraction containing **11** from the methanolysis was chromatographed on a Bio-Sil A column [1.5 × 20 cm, hexanes/CH_2Cl_2 (1/1)] to give pure **11**: 5.3 mg (fine yellow crystals); mp 190 °C dec; R_f 0.87 (CH_2Cl_2), R_f 0.39 [hexanes/acetone (1/1)]; HREIMS $C_{12}H_6O_2S$ (M), $C_{11}H_6OS$ (M − CO), $C_{10}H_6S$ (M − 2CO), C_9H_6 (M − 2CO − CS); UV_{max} (MeOH) 327 nm (ϵ 6400), 280 (16 000), 271 (13 000), 251 (29 000), 247 (28 000); ^1H NMR ($CDCl_3$) δ 7.72 (d, $J_{14,15}$ = 4.8, 1 H), 7.75 (d, $J_{14,15}$ = 4.8, 1 H), 7.77 (m, 2 H) 8.25 (m, 2 H).

Isolation of 12. The fraction containing **12** from the methanolysis was chromatographed on a Bio-Sil A column [1.5 × 20 cm, hexanes/acetone (9/1)], and the desired fractions were worked up and recrystallized from CH_2Cl_2/hexanes to give pure **12**: 25 mg (colorless crystals); mp 131 °C; R_f 0.55 (CH_2Cl_2), R_f 0.42 [hexanes/acetone (8/2)]; $C_{11}H_{13}IO_5$ (HREIMS m/z 351.9792 Δ 1.4 mmu); IR (KBr) 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.36 (s, 3 H), 3.88 (s, 3 H), 3.91 (s, 3 H), 3.92 (s, 3 H), 6.36 (s, 1 H, exchangeable); ^{13}C NMR ($CDCl_3$) δ 25.1, 52.2, 60.5, 61.0, 85.0, 120.7, 133.7, 136.9, 149.8, 152.2, 168.2.

Isolation of 13. The fraction containing **13** from the methanolysis was chromatographed twice on a Bio-Sil A column [1.5 × 20 cm, CH_2Cl_2/MeOH (99.5/0.5)] to give pure **13**: ~15 mg (colorless oil); R_f 0.32 (CH_2Cl_2); FABMS m/z 249 (M + Na), 227 (M + H), 195 (M − OCH_3); IR (neat) 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.23 (s, 3 H), 3.88 (s, 3 H), 3.90 (s, 6 H), 5.84 (bs, 1 H), 6.56 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 19.4, 52.1, 60.9, 61.3, 112.3, 120.5, 132.4, 137.3, 150.2, 150.4, 168.1.

Isolation of 14. The fraction containing **14**, chromatographing just before **15** from the methanolysis, was chromatographed on a Bio-Sil A column [0.9 × 40 cm, EtOAc/hexanes (6/4)] to give crystalline **14**: 4 mg (colorless crystals); R_f 0.4 [CH_2Cl_2/MeOH (96/4)], R_f 0.55 [EtOAc/hexanes (6/4)]; EIMS m/z 482 (M^+), 321, 195; IR (KBr) 1720, 1675 cm^{-1}; ^1H NMR ($CDCl_3/CD_3OD$) δ 1.56 (d, J = 6.4, 3 H, H-6B), 2.35 (s, 3 H, H-6C), 2.82 (dd, J = 18.1, 3.4, 1 H, H-2B), 2.90 (dd, J = 18.1, 3.8, 1 H, H-2B), 3.888 (s, 3 H), 3.892 (s, 3 H) 3.94 (dd, J = 11.0, 2.5, 1 H, H-4B), 4.33 (bq, J = ~3, 1 H, H-3B), 4.83 (dq, J = 10.8, 6.5, 1 H, H-5B), 5.31 (s, 1 H); ^{13}C NMR δ 19.8, 24.7, 38.6, 49.2, 61.1, 61.5, 66.7, 74.3, 84.5, 126.1, 133.3, 136.4, 148.9, 151.0, 168.2 (signal for thio ester CO missing); X-ray crystallographic data can be found in the supplementary material of reference 6a.

Isolation of 15. The fraction containing **15** from the methanolysis was chromatographed on a Bio-Sil A column [0.9 × 40 cm, EtOAc/MeOH (99/1)], and the desired fractions were further purified on a reversed-phase column [0.9 × 23 cm, Separalyte C18, 40 μm (Analytichem), MeOH/H_2O (55/45)] to give >90% pure **15**: ~2 mg (colorless oil); R_f 0.39 [CH_2Cl_2/MeOH (96/4)], R_f 0.30 (EtOAc), R_f 0.44 [Whatman LHPKC_{18} precoated glass plate, MeOH/0.1 M NaCl (55/45)]; IR (neat) 1642 cm^{-1}; HREIMS $C_{11}H_{21}NO_4$ (M^+ 231.1458 Δ 1.2 mmu), $C_6H_{11}NO$ (113.0815 Δ 2.6 mmu), $C_4H_{10}NO$ (88.0758 Δ 0.4 mmu), and

---

(35) For further evidence, see: (a) Zein, N.; Sinha, A. M.; McGahren, W. J.; Ellestad, G. A. *Science (Washington, DC)* **1988**, *240*, 1198–1201. (b) Zein, N.; McGahren, W. J.; Morton, G. O.; Ashcroft, J.; Ellestad, G. A. *J. Am. Chem. Soc.* **1989**, *111*, 6888–6890. (c) Zein, N.; Poncin, M.; Nilakantan, R.; Ellestad, G. A. *Science (Washington, DC)* **1989**, *244*, 697–699. (d) Townsend, C. A.; DeVoss, J. J.; Ding, W.; Morton, G. O.; Ellestad, G. A.; Zein, N.; Tabor, A. B.; Schreiber, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 9669–9670.

(36) Touchstone, J. C.; Chen, J. C.; Beaver, K. M. *Lipids* **1979**, *15* (1), 61–62.

C_4H_9N (71.0740 Δ 0.5 mmu); ^{1}H and ^{13}C NMR data as shown in Table II.

The *N*-acetyl-*N*-ethylamino sugar **15** was also prepared from the methanolysis of *N*-acetylcalicheamicin γ_1^1 (**8**) via the Dowex 50-X8 procedure. From the methanolysate of 512 mg of **8** (95% pure), 25 mg of a 7/3 mixture of **15**/**16** ($[\alpha]^{20}_D$ −40° (*c* 0.627, $CHCl_3$)) was isolated.

Dowex 50W-X8 Catalyzed Methanolysis of Calicheamicin γ_1^1. A methanolic solution (5 mL) of partially purified calicheamicin γ_1^I (**6**, 408 mg, 65% pure) was passed slowly (via the use of a peristaltic pump) through a column (1.5 × 30 cm), which was packed with CH_2Cl_2 and MeOH prewashed Dowex 50W-X8 (50–100 mesh, H^+ form) and preequilibrated with methanol. The column effluent was monitored by TLC and was recycled back onto the column until no **6** (R_f 0.29, 3% 2-propanol in EtOAc saturated with water) was detected. The column was eluted with 4 L of MeOH over 18 h. The first 50 mL of the eluate was pooled with the dark colored effluent and concentrated to a brown solid, which was triturated with *tert*-butyl methyl ether. The solution was concentrated to give a mixture containing **9**, **10**, **19**, and **20**. The remaining eluate (~4 L) from the Dowex 50W-X8 column was collected in one vessel and concentrated in vacuo to a light yellow oil, which was triturated with *tert*-butyl methyl ether. The insoluble solids were separated from the solution by centrifugation to give crude **21**, and concentration of the solution gave a mixture of **9**, **10**, **17**, **18**, **19**, and **20**.

Isolation of 17 and 18. The mixture of **9**, **10**, **17**, **18**, **19**, and **20** above was chromatographed on a Bio-Sil A column (1.5 × 42 cm) eluting with EtOAc saturated with 0.1 M phosphate buffer (pH 7.0) to give a mixture of **17** and **18**. Pure **17** and **18** were obtained by repeated column (1.5 × 28 cm) chromatography on Bio-Sil A, eluting with CH_2Cl_2/CH_3OH (99/1).

17: 16 mg (white solid); R_f 0.86 [(EtOAc saturated with 0.1 M phosphate buffer (pH 7.0)], R_f 0.32 [CH_2Cl_2/MeOH (98.5/1.5)]; $C_{17}H_{23}IO_7S$ (HREIMS 498.0182 Δ 2.7 mmu); ^{1}H and ^{13}C NMR data as shown in Table II of the supplementary material. **18**: 11 mg (white solid); R_f 0.86 [(EtOAc saturated with 0.1 M phosphate buffer (pH 7.0)], R_f 0.54 [CH_2Cl_2/MeOH (98.5/1.5)]; ^{1}H and ^{13}C NMR data as shown in Table II of the supplementary material.

Isolation of 19 and 20. The mixture of **9**, **10**, **19**, and **20** obtained above was combined with similar mixtures obtained from three similar reactions and chromatographed on a Silica Woelm column (1.5 × 25 cm), eluting with EtOAc saturated with 0.1 M phosphate buffer (pH 7.0). Fractions containing the $UV_{254\,nm}$ quenching band eluting just before **9** were pooled and further purified by preparative TLC [two 1000-μm layer plates, CH_2Cl_2/MeOH (96/4)] to give **19** and **20**.

19: 8 mg (white solid); R_f 0.58 [EtOAc saturated with 0.1 M phosphate buffer (pH 7.0)], R_f 0.32 [CH_2Cl_2/MeOH (96/4)]; $C_{24}H_{35}IO_{11}S$ (HRFABMS, M + Na 681.0863 Δ 2.0); ^{1}H and ^{13}C NMR data as shown in Table II of the supplementary material. **20**: 12 mg (white solid); R_f 0.58 [EtOAc saturated with 0.1 M phosphate buffer (pH 7.0)], R_f 0.46 [CH_2Cl_2/MeOH (96/4)]; ^{1}H NMR ($CDCl_3$) δ 1.30 (d, *J* = 6.2, 3 H, H-6D), 1.41 (d, *J* = 6.3, 3 H, H-6B), 2.08 (td, *j* = 14.4, 3.3, 1 H, H-2Bax), 2.14 (bd, *J* = 14.4, 1 H, H-2Beq), 2.37 (s, 3 H, 6C-CH_3), 3.40 (s, 3 H, 1B-OCH_3), 3.57 (s, 3 H, 3D-OCH_3), 3.64 (t, *J* = 9.5, 1 H, H-4D), 3.81 (m, 1 H), 3.83 (s, 3 H), 3.84 (m, 1 H), 3.90 (s, 3 H), 4.11 (m, H-5B), 4.20 (dq, *J* = 9.5, 6.2, 1 H, H-5D), 4.48 (bs, 1 H, H-2D), 4.90 (bs, 1 H, H-1B), 5.73 (bs, 1 H, H-1D).

Isolation of Calicheamicin Pseudoaglycon (21). The crude **21** obtained from the methanolysis was redissolved in ethyl acetate and precipitated by addition of hexane to yield 121 mg of 82% pure **21**. This was further purified by chromatography on Bio-Sil A [0.9 × 25 cm column, CH_2Cl_2/MeOH (95/5)]. The desired fractions were pooled, concentrated in vacuo, and precipitated from EtOAc by addition of hexanes to give analytically pure **21**: 73 mg (white solids); R_f 0.41 [CH_2Cl_2/MeOH (95/5)], R_f 0.75 [EtOAc saturated with 0.1 M phosphate buffer (pH 7)]; $C_{40}H_{47}IN_2O_{15}S_4$ (HRFABMS, M + Na *m*/*z* 1073.0810 Δ 0.8 mmu); NMR data as shown in Table III.

Dihydrocalicheamicin Pseudoaglycon (22). To a solution of calicheamicin pseudoaglycon (**21**, 112 mg) in EtOH (25 mL), cooled in a ice–water bath, was first added MeI (10 mL) followed by a solution (12 mL) of 0.025 M ethanolic $NaBH_4$ in 2-mL portions. After remaining at 0 °C for 20 min, the reaction mixture was decomposed by addition of acetic acid (1 M solution in EtOH, 1.2 mL) and concentrated to a golden yellow residue. The oily residue was dissolved in EtOAc and concentrated in vacuo to remove the last trace of EtOH, and the resulting solids were redissolved in EtOAc and the insolubles filtered off. The solution was concentrated to a small volume and precipitated by addition of hexanes to give 128 mg of crude **22**, which was purified by column chromatography on Bio-Sil A [1.5 × 42 cm, CH_2Cl_2/MeOH, (97/3)] to give analytically pure **22**: 42 mg (white solid); R_f 0.58 [EtOAc saturated with 0.1 M phosphate buffer (pH 7)]; FABMS M + Na 1075, M + K 1091; ^{1}NMR ($CDCl_3/CD_3OD$) δ 1.43 (d, *J* = 6, 3 H, H-6A), 1.44 (d, *J* = 6, 3 H, H-6B), 1.81 (mt, *J* = 12, 1 H, H-2Bax), 2.05 (bd, *J* = 13, 1 H, H-2Beq), 2.17 (dd, *J* = 14.7, 4.4, 1 H, H-12), 2.34 (s, 3 H, 6C-CH_3), 2.43 (t, *J* = 9.7, 1 H, H-4A), 2.56 (s, 3 H, $SSSCH_3$), 2.73 (dd, *J* = 13.7, 6.3, 1 H, H-12), 3.67 (t, *J* = 8.4, 1 H, H-2A), 3.74 (dd, *J* = 10, 2.5, 1 H, H-4B), 3.77 (s, 3 H, 10-$NCOOCH_3$), 3.79 (m, H-5A), 3.88 (s, 3 H, 3C-OCH_3), 3.89 (m, H-15), 3.90 (s, 3 H, 2C-OCH_3), 3.96 (m, H-3A), 4.05 (m, H-15), 4.09 (m, 1 H, H-5B), 4.32 (m, 1 H, H-3B), 4.60 (d, *J* = 7.8, 1 H, H-1A), 4.75 (m, 1 H, 11-H), 5.08 (dd, *J* = 9.8, 1.0, H-1B), 5.69 (bs, 1 H, H-8), 5.81 (bd, *J* = 9.5, 1 H, H-5), 5.90 (d, *J* = 9.5, 1 H, H-4), 6.36 (dd, *J* = 9.0, 6.9, 1 H, H-14); ^{13}C NMR δ 17.8 (q, C-6A), 19.0 (q, C-6B), 22.8 (q, $SSSCH_3$), 24.7 (q, 6C-CH_3), 36.9 (t, C-2B), 39.4 (t, C-15), 45.7 (t, C-12), 51.6 (d, C-4B), 53.0 (q, 10-$NCOOCH_3$), 61.0 (q, 3C-OCH_3), 61.3 (q, 2C-OCH_3), 66.8 (d, C-11), 67.2 (d, C-4A), 67.3 (d, C-8), 68.1 (d, C-3B), 69.2 (d, C-5B), 69.9 (d, C-5A, C-3A), 71.8 (s, C-1), 73.8 (d, C-2A), 83.9 (s, C-6), 84.6 (s, C-5C), 87.3 (s, C-3), 97.7 (s, C-7), 99.9 (d, C-1B), 101.8 (d, C-1A), 103.5 (s, C-2), 122.6 (d, C-5), 123.4 (d, C-4), 124.1 (s, C-9), 125.1 (d, C-14), 126.7 (s, C-1C), 133.2 (s, C-6C), 136.5 (s, C-3C), 137.3 (s, C-10), 139.4 (s, C-13), 148.9 (s, C-2C), 151.0 (s, C-4C), 155.1 (s, 10-NCO).

Conversion of 21 to 23 by Triphenylphosphine. A solution of **21** (281 mg, 80% pure) in CH_2Cl_2/MeOH (2/1, 60 mL) was purged with argon. Triphenylphosphine (140 mg) was added to the solution and the reaction mixture was stirred under argon for 3 h. Hexanes (100 mL) were added to the reaction mixture, and it was concentrated to give an off-white solid. The solid was triturated with hexane (50 mL), and the hexane insolubles were triturated with CH_2Cl_2. The CH_2Cl_2 solution was concentrated in vacuo and precipitated by addition of hexanes to give crude **23**. The crude **23** was purified by column (1.5 × 22 cm) chromatography on Silica Woelm [32–63 μm, CH_2Cl_2/MeOH (95/5)], and the desired fractions were concentrated and precipitated from hexanes to give 74 mg of 90% pure **23**. This was further purified by preparative TLC [two 20 × 20 cm, 2000-μm plates, EtOAc saturated with 0.1 M phosphate buffer (pH 7)], and the major $UV_{254\,nm}$ quenching band (R_f 0.4) was worked up to give 46 mg of analytically pure **23**: R_f 0.23 [CH_2Cl_2/MeOH (94/6)], R_f 0.62 [EtOAc saturated with 0.1 M phosphate buffer (pH 7)]; $C_{39}H_{47}IN_2O_{15}S_2$ (HRFABMS, M + Na *m*/*z* 997.1370 Δ 1.0 mmu); NMR data as shown in Table IV.

Retro-Aldol Cleavage of 23 and the Isolation of 24 and 25. A sample of **23** (46 mg, 90% pure) was dissolved in methanol (4 mL), and a saturated methanolic solution of K_2CO_3 (~0.2 M, 4 mL) was added. The reaction mixture turned bright yellow immediately; after remaining at room temperature for 5 min, it was cooled in a ice water bath and treated with 400 μL of acetic anhydride. The reaction mixture was allowed to remain at 4 °C for 2 h, neutralized with methanolic K_2CO_3, and concentrated to dryness in vacuo. The CH_2Cl_2-soluble portion of the residue was purified by preparative TLC [two 20 × 20 cm, 2000-μm plates, CH_2Cl_2/MeOH (94/6)]; and the two major $UV_{254\,nm}$ quenching, $UV_{366\,nm}$ blue fluorescent bands, chromatographing close to each other, were worked up together, and the mixture was rechromatographed on four preparative TLC plates [20 × 20 cm, 1000-μm layer, CH_2Cl_2/MeOH, (94/6)] to give pure **24** and **25**.

24: 7.5 mg (crystalline solid); R_f 0.42 [CH_2Cl_2/MeOH, (95/5)]; $C_{36}H_{40}INO_{13}S_2$ (HRFABMS M + K 924.0650 Δ 2.7 mmu); ^{1}H NMR δ 1.29 (d, *J* = 6.0, 3 H), 1.44 (d, *J* = 6.2, 3 H), 1.73 (m, 1 H), 1.98 (md, *J* = 13.0, 1 H), 2.34 (s, 3 H), 2.55 (t, *J* = 9.5, 1 H), 2.59 (s, 3 H), 3.62 (dd, *J* = 9.5, 6.0, 1 H), 3.72 (dd, *J* = 10.9, 2.5, 1 H), 3.86 (s, 3 H), 3.91 (s, 3 H), 3.96 (m, 1 H), 4.01 (m, 1 H), 4.10 (m, 1 H), 4.28 (m, 1 H), 5.04 (dd, *J* = 10.1, 1.8, 1 H), 5.12 (d, *J* = 7.4, 1 H), 7.26 (d, *J* = 5.7, 1 H), 7.48 (d, *J* = 5.7, 1 H), 7.52 (m, 1 H), 7.52 (m, 1 H), 7.91 (m, 1 H), 8.58 (m, 1 H); recrystallized from a mixture of methanol and chloroform to give crystals suitable for X-ray crystallography. **25**: 5 mg (off white solid); R_f 0.57 [CH_2Cl_2/MeOH (95/5)]; $C_{38}H_{42}INO_{14}S_2$ (HRFABMS M + Na 950.0971 Δ 1.8 mmu); ^{1}H NMR δ 1.29 (d, *J* = 6.1, 3 H), 1.44 (d, *J* = 6.2, 3 H), 1.73 (bt, *J* = 11.0, 1 H), 1.98 (bd, *J* = 13.0, 1 H), 2.35 (s, 3 H), 2.40 (s, 3 H), 2.55 (t, *J* = 9.5, 1 H), 2.58 (s, 3 H), 3.62 (dd, *J* = 9.5, 6.1, 1 H), 3.74 (dd, *J* = 10.8, 2.5, 1 H), 3.82 (s, 3 H), 3.87 (s, 3 H), 3.93 (m, 1 H), 4.00 (m, 1 H), 4.08 (m, 1 H), 4.28 (m, 1 H), 5.04 (dd, *J* = 10.0, 1.6, 1 H), 5.12 (d, *J* = 7.4, 1 H), 7.26 (d, *J* = 5.7, 1 H), 7.47 (d, *J* = 5.7, 1 H), 7.52 (m, 1 H), 7.52 (m, 1 H), 7.90 (m, 1 H), 8.58 (m, 1 H); ^{13}C NMR δ 17.6 (q), 19.0 (q), 20.8 (q), 20.9 (q), 24.8 (q), 36.8 (t), 51.5 (d), 60.9 (q), 61.6 (q), 67.7 (d), 68.2 (d), 69.1 (d), 69.2 (d), 70.1 (d), 74.9 (d), 93.2 (s), 99.8 (d), 104.2 (d), 119.6 (d), 120.8 (d), 122.6 (d), 124.7 (s), 125.3 (d), 125.5 (s), 125.8 (d), 129.4 (d), 130.8 (s), 132.0 (s), 132.4 (s), 133.2 (s), 137.1 (s), 143.1 (s), 145.1 (s), 146.7 (s), 149.9 (s), 167.7 (s), 169.4 (s), 191.5 (s).

Preparation of 26. The aromatized calicheamicin pseudoaglycon **23** (30 mg) was reduced with sodium borohydride following the procedure described above for the preparation of dihydrocalicheamicin pseudoaglycon **22**. The reaction mixture was purified by preparative TLC [CH_2Cl_2/MeOH (94/6)] to give **26**: 9 mg (white solids); R_f 0.60

[CH_2Cl_2/MeOH (94/6)]; ^{1}H NMR ($CDCl_3$), with assignments made by ^{1}H–^{1}H COSY, δ 2.23 (m, 2 H, H-12), 3.75 (s, 3 H, 10-$NCOOCH_3$), 3.87 (2 H, H-15), 3.98 (H-11), 4.66 (m, 1 H, H-10), 4.76 (bs, 1 H, H-8), 5.44 (d, J = 10.0, 1 H, 10-NH), 5.88 (m, 1 H, H-14), 7.33 (bt, J = 7.5, 1 H, H-4/5), 7.40 (bt, J = 7.5, 1 H, H-4/5). 7.63 (bd, J = 7.5, 1 H, H-3/6), 7.66 (bd, J = 7.5, 1 H, H-3/6), the signals for the glycosidic portion are identical to those of **23**.

Preparation of Modified Aglycon 28. Calicheamicin $\gamma_1{}^1$ (268 mg) was reduced with sodium borohydride following the procedure described above for the preparation of dihydrocalicheamicin pseudoaglycon **22**. The reaction mixture, following concentration, was passed through a Sep-Pak Silica cartridge, and dihydrocalicheamicin $\gamma_1{}^1$ (**27**) was eluted with CH_2Cl_2/CH_3OH (96/4). Without further purification, **27** was dissolved in a small amount of MeOH and loaded onto a Dowex 50W-X8 column (1 × 18 cm) packed in MeOH. The column eluate was recycled back onto the column for 18 h, and the column was then eluted with 500 mL of MeOH. Phosphate buffer (0.1 M, pH 6) was added to the combined methanolic eluate, and the mixture was concentrated in vacuo until the MeOH was removed and water insolubles were precipitated. The aqueous solution was combined with the aqueous wash of the precipitate and passed through a Sep-Pak C_{18} cartridge. The cartridge was first eluted with excess water to remove phosphate salt and was then eluted sequentially with 20%, 60%, and 95% MeOH in water. The 95% MeOH eluate was worked up and further purified by preparative TLC [Whatman HPTLC, CH_2Cl_2/MeOH (96/4), EtOAc saturated with 0.1 M phosphate buffer (pH 6.0)] and HPLC [Waters μ-porasil, CH_2Cl_2/MeOH (96/4)] to give analytically pure **28**: 5 mg; EIMS m/z 329 (M^+ weak); negative FABMS m/z 328 (M − H, weak); HREIMS m/e 311.0643 ($C_{17}H_{13}NO_3S$ Δ 1.7); UV_{max} (MeOH) 239 nm (ϵ, 3160), 267 (3020); IR (KBr), 3300, 3050, 2190, 1710, 1520, 1240, 1060 cm^{-1}; ^{1}H and ^{13}C NMR data as shown in Table V.

Preparation of 29 and 30. A solution of the modified aglycon **28** (4 mg) in pyridine (0.2 mL) was allowed to react with acetic anhydride (20 μL) at room temperature overnight. The reaction mixture was quenched by addition of MeOH and concentrated in vacuo, and the last trace of solvent and reagent was removed under high vacuum. Purification by preparative TLC [Whatman HPTLC, CH_2Cl_2/MeOH (98/2)] and HPLC [Waters μ-Porasil, CH_2Cl_2/MeOH (98.5/1.5)] gave analytically pure **29** and **30**.

29: 11-*O*-acetyl-**28**; HREIMS m/e 311.0626 ($C_{17}H_{13}NO_3S$ M^+ − HOAc Δ 1.0 mmu); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.08 (s, 3 H, 11-$OCOCH_3$), 2.15 (dd, J = 12.5, 7.2, 1 H, H-12), 2.67 (dd, J = 12.5, 7.2, 1 H, H-12), 3.35 (dd, J = 18.8, 4.2, 1 H, H-15), 3.68 (dd, J = 18.5, 4.2, 1 H, H-15), 3.71 (s, 3 H, 10-$NHCOOCH_3$), 4.70 (s, 1 H, H-8), 5.61 (t, J = 7.2, 1 H, H-11), 5.75 (d, J = 9.5, 1 H, H-4), 5.80 (dd, J = 9.5, 1.2, 1 H, H-5), 6.20 (t, J = 4.2, 1 H, H-14). **30**: 1,11-di-*O*-acetyl-**28**; HREIMS m/z 353.0713 ($C_{19}H_{15}NO_4S$ M^+ − HOAc Δ 0.9 mmu); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.08 (s, 3 H, 11-$OCOCH_3$), 2.15 (s, 3 H, 1-$OCOCH_3$), 2.15 (dd, J = 12.4, 7.0, 1 H, H-12), 3.25 (dd, J = 12.4, 7.0, 1 H, H-12), 3.36 (dd, J = 18.8, 4.2, 1 H, H-15), 3.68 (dd, J = 18.8, 4.2, 1 H, H-15), 3.71 (s, 3 H, 10-$NHCOOCH_3$), 4.70 (s, 1 H, H-8), 5.60 (t, J = 7.0, 1 H, H-11), 5.78 (d, J = 9.5, 1 H, H-4), 5.80 (d, J = 9.5, 1 H, H-5), 6.20 (t, J = 4.2, 1 H, H-14).

Preparation of 31. A solution of the modified aglycon **28** (3 mg) in CH_2Cl_2 (0.5 mL) was treated with acetic anhydride (20 μL) and 4-(dimethylamino)pyridine (0.2 mg) overnight. The reaction mixture, after quenching with MeOH, was concentrated in vacuo to give a residue which was purified via a Sep-Pak Silica cartridge, eluting with CH_2Cl_2 and 1–10% MeOH in CH_2Cl_2. Compound **31**, eluted with 1–2% MeOH in CH_2Cl_2, was further purified by preparative TLC [Whatman HPTLC, CH_2Cl_2/MeOH (96/4)] and HPLC [Waters μ-Porasil, CH_2Cl_2/MeOH (98.5/1.5)] to give analytically pure **31**: 10-*N*-1,11-di-*O*-acetyl-**28**; HREIMS m/e 395.0837 ($C_{21}H_{17}NO_5S$ M^+ − HOAc Δ 0.9 mmu); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.08 (s, 3 H, 11-$OCOCH_3$), 2.12 (dd, J = 12.4, 7.0, 1 H, H-12), 2.17 (s, 3 H, 1-$OCOCH_3$), 2.52 (s, 3 H, 10-$NCOCH_3$), 3.30 (dd, J = 12.4, 7.0, 1 H, H-12), 3.40 (dd, J = 18.8, 4.2, 1 H, H-15), 3.69 (dd, J = 18.8, 4.2, 1 H, H-15), 3.72 (s, 3 H, 10-$NHCOOCH_3$), 4.70 (s, 1 H, H-8), 5.90 (m, 3 H, H-4, H-5, H-11), 6.32 (t, J = 4.2, 1 H, H-14).

Preparation of 36. A CH_2Cl_2 (80 mL) solution of *N*-acetylcalicheamicin $\gamma_1{}^1$ (**8**, 280 mg) stirred at room temperature was treated with a 10-fold excess of 4-(dimethylamino)pyridine (244 mg) followed by a 10-fold excess of acetic anhydride (189 μL). The reaction mixture, after stirring at room temperature overnight, was quenched with 200 μL of MeOH, evaporated to ca. 5 mL, diluted with 30 mL of hexane, and evaporated to a brown residue, which was purified by preparative TLC [EtOAc/hexanes (50/50)] to give a mixture containing **36** and the penta-, hexa-, and heptaacetylcalicheamicin $\gamma_1{}^1$. Repetitive normal-phase [CH_2Cl_2/MeOH (97/3)] and reversed-phase [Whatman KC_{18}, 20 × 20, 1000 μm, CH_3CN/H_2O (80/20)] preparative TLC gave **36**: ^{1}H NMR ($CDCl_3$), with assignments determined by ^{1}H–^{1}H COSY, δ 1.11/1.18 (t, J = 6, 3 H, 4E-NCH_2*CH_3*), 1.20 (d, J = 6, 3 H, H-6D), 1.41 (d, J = 6, 6 H, H-6A, H-6B), 1.51 (m, 1 H, H-2E), 1.86 (m, 1 H, H-2B), 2.04 (H-2B), 2.07 (s, 3 H, $OCOCH_3$), 2.10/2.11 (s, 3 H, $OCOCH_3$), 2.13 (s, 6 H, $OCOCH_3$), 2.15 (s, 3 H, $OCOCH_3$), 2.17 (s, 3 H, $OCOCH_3$), 2.18 (H-4E), 2.22 (H-2E), 2.34 (6C-CH_3), 2.68 (bt, J = 10, 1 H, H-4A), 3.27/3.28 (s, 3 H, 3E-OCH_3), 3.30/3.47 (4E-NCH_2), 3.44 (s, 3 H, 3D-OCH3), 3.56/3.87 (H-5E), 3.62 (H-3E), 3.63 (H-2A), 3.81 (H-5A), 3.82 (H-5B), 3.82/4.04 (H-5E), 3.83 (s, 3 H, 3C-OCH3), 3.85 (s, 3 H, 2C-OCH3), 3.98 (H-5B), 4.03 (dd, J = 10, 4.0, 1 H, H-3D), 4.34 (dq, J = 6.3, 4.1, 1 H, H-5D), 4.88 (bd, J = 10, 1 H, H-1B), 5.01 (m, 1 H, H-1E), 5.11 (t, J = 10, 1 H, H-4D), 5.40 (m, 1 H, H-3B), 5.49/5.51 (t, J = 10, 1 H, H-3A), 5.63 (m, 1 H, H-1D), 5.66/5.68 (d, J = 9, 1 H, H-1A), 5.75 (m, 1 H, H-2D).

Peracetylation of Crude Calicheamicin Pseudoaglycon. A CH_2Cl_2 (60 mL) solution of the crude calicheamicin pseudoaglycon (211 mg) obtained from the Dowex 50W-X8 catalyzed methanolysis was stirred at room temperature and was treated with a 10-fold excess of 4-(dimethylamino)pyridine (244 mg) followed by a 10-fold excess of acetic anhydride (189 μL). The reaction mixture, after being stirred at room temperature for 5 h, was quenched with 200 μL of MeOH, evaporated to ca. 5 mL, diluted with 30 mL of hexane, and evaporated to a brown residue, which was purified by preparative TLC [EtOAc/hexanes (50/50)] to give a mixture containing the tetra-, penta-, and hexaacetyl-**21** compounds and a mixture containing diacetyl-**17** and triacetyl-**19**.

Purification of 1,2A,3A,3B-Tetraacetylcalicheamicin Pseudoaglycon (37). The mixture above containing the tetra-, penta-, and hexaacetyl-**21s** was chromatographed on four reversed-phase preparative TLC plates [Whatman KC_{18}, 20 × 20, 1000 μm, CH_3CN/H_2O (80/20)]. The most polar band was worked up and further purified by normal-phase preparative TLC [CH_2Cl_2/MeOH (97/3)] to give **37**: 21 mg (white solids); R_f 0.30 [CH_2Cl_2/MeOH (99/1)], 0.32 [EtOAc/hexanes (50/50)], 0.49 [Whatman KC_{18}, CH_3CN/H_2O (90/10)]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (d, J = 6.2, 3 H, H-6B), 1.43 (d, J = 6.2, 3 H, H-6A), 1.86 (m, 1 H, H-2), 2.04 (H-2), 2.07 (s, 3 H, $OCOCH_3$), 2.08 (s, 3 H, $OCOCH_3$), 2.16 (s, 3 H, $OCOCH_3$), 2.18 (s, 3 H, $OCOCH_3$), 2.32 (s, 3 H, 6C-CH_3), 2.48 (s, 3 H, 15-$SSSCH_3$), 2.72 (t, J = 9.7, 1 H, H-4A), 2.81 (d, J = 16.9, 1 H, H-12), 3.48 (dd, J = 15.6, 4.1, 1 H, H-15), 3.77 (s, 3 H, 10-$NHCOCH_3$), 3.77 (H-5A), 3.77 (H-4B), 3.85 (s, 3 H, 3C-OCH_3), 3.87 (H-15), 3.90 (s, 3 H, 2C-OCH_3), 3.94 (d, J = 16.9, 1 H, H-12), 3.94 (H-5B), 4.81 (d, J = 8.1, 1 H, H-1A), 4.87 (bd, J = 9.7, 1 H, H-1B), 4.95 (t, J = 8.5, 1 H, H-2A), 5.37 (m, 1 H, H-3B), 5.47 (t, J = 9.5, 1 H, H-3A), 5.86 (dd, J = 9.5, 1.1, 1 H, H-5), 5.93 (d, J = 9.6, 1 H, H-4), 6.01 (d, J = 1.1, 1 H, H-8), 6.42 (m, 1 H, H-14).

Purification of Diacetyl-17 and Triacetyl-19. The mixture containing diacetyl-**17** and triacetyl-**19** was separated by preparative TLC [CH_2Cl_2/MeOH (98/1)] to give diacetyl-**17**: 4 mg (white solids); R_f 0.63 [CH_2Cl_2/MeOH (99/1)], 0.65 [EtOAc/hexanes (50/50)], 0.49 [Whatman KC_{18}, CH_3CN/H_2O (90/10)]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (d, J = 6.1, 3 H, H-6B), 1.89 (mt, J = 11, 1 H, H-2B), 2.08 (s, 3 H, 3B-$OCOCH_3$), 2.14 (md, J = 11, 1 H, H-2B), 2.34 (s, 3 H, 6C-CH_3), 2.40 (s, 3 H, 4C-$OCOCH_3$), 3.51 (s, 3 H, 1B-OCH_3), 3.82 (s, 3 H, 3C-OCH_3), 3.83 (m, 1 H, H-4B), 3.87 (s, 3 H, 2C-OCH_3), 3.96 (dq, J = 10, 6, 1 H, H-5B), 4.64 (bd, J = 9, 1 H, H-1B), 5.40 (m, 1 H, H-3B). Triacetyl-**19**: 7 mg (white solids); R_f 0.31 [CH_2Cl_2/MeOH (99/1)], 0.72 [EtOAc/hexanes (50/50)], 0.40 [Whatman KC_{18}, CH_3CN/H_2O (90/10)]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (d, J = 6.2, 3 H, H-6D), 1.43 (d, J = 6.0, 3 H, H-6B), 1.88 (mt, J = 9.5, 1 H, H-2B), 2.05 (m, 1 H, H-2B), 2.07 (s, 3 H, 3B-$OCOCH_3$), 2.13 (s, 3 H, 2/4D-$OCOCH_3$), 2.16 (s, 3 H, 2/4D-$OCOCH_3$), 2.34 (s, 3 H, 6C-CH_3), 3.44 (s, 3 H, 3D-OCH_3), 3.51 (s, 3 H, 1B-OCH_3), 3.83 (s, 3 H, 3C-OCH_3), 3.85 (m, 1 H, H-4B), 3.86 (s, 3 H, 2C-OCH_3), 3.95 (m, 1 H, H-5B), 4.03 (dd, J = 9.6, 3.6, 1 H, H-3D), 4.34 (dq, J = 9.1, 6.3, 1 H, H-5D), 4.64 (bd, J = 9.1, 1 H, H-1B), 5.10 (t, J = 9.2, 1 H, H-4D), 5.40 (m, 1 H, H-3B), 5.62 (bs, 1 H, H-1D), 5.74 (m, 1 H, H-2D).

Acknowledgment. We thank Professors C. Townsend, K. L. Rinehart, Jr., and K. Nakanishi, and Dr. N. Colthup for helpful discussions, Mrs. J. K. Manning and Mrs. L. Barbieri for technical assistance, and Professor C. Townsend and Dr. P. T. Northcote for helpful suggestions on the manuscript.

Supplementary Material Available: Tables of ^{1}H and ^{13}C NMR data for **9** and **10**, ^{1}H and ^{13}C NMR data for **17**, **18**, and **19**, ^{1}H and ^{13}C NMR assignments for calicheamicin $\gamma_1{}^I$ (**6**) and *N*-acetylcalicheamicin $\gamma_1{}^I$ (**8**), and ^{13}C NMR correlations of calicheamicins $\beta_1{}^{Br}$ (**1**), $\beta_1{}^I$ (**5**), $\gamma_1{}^{Br}$ (**2**), $\gamma_1{}^1$ (**6**), $\alpha_2{}^I$ (**3**), $\alpha_3{}^I$ (**4**), and $\delta_1{}^1$ (**7**) (10 pages). Ordering information is given on any current masthead page.