^{1}H NMR spectrum of **8** at 360 MHz (CD_3OD) exhibited resonances at δ 6.49 (dd, J_1 = 4.6, J_2 10.5 Hz, 14-H), 6.03 (d, J_1 = 1.8 Hz, 12-H), 5.98 (d, J = 9.6 Hz, 8-H), 5.90 (dd, J_1 = 9.6, J_2 = 1.8 Hz, 9-H), 4.53 (d, J = 7.8 Hz, 1'-H), 4.12 (s, 4-H), 4.12 (dd, J_1 = 10.5, J_2 = 14.7 Hz, 15-Ha), 3.84 (dd, J_1 = 4.7, J_2 = 14.7 Hz, 15-Hb), 3.69 (s, NCO_2CH_3), 3.70 (dd, J_1 = 10.3, J_2 = 9.2 Hz, 3'-H)8 3.66 (dq, J_1 = 6.4, J_2 = 9.6 Hz, 5'-H), 3.35 (dd, J_1 = 9.2, J_2 = 7.8 Hjz, 2'-H), 2.50 (s, SCH_3), 2.26 (dd, J_1 = 9.6, J_2 = 10.3 Hz, 4'-H), 1.36 (d, J = 6.4 Hz, 6'-H).[10] The connectivity and relative stereochemistry of C1'-C6' was unequivocally established as shown. The C4' H resonance at δ 2.26 indicated the position of hydroxyamino substitution, and therefore the sugar is an β-glycoside of 4-(hydroxyamino)-4,6-dideoxyglucose.[11]

The structure of the core of esperamicins A_1 (**1a**), C (**2**), D (**4**), and E (**8**) remained to be established. From an examination of the ^{1}H and ^{13}C NMR spectra of **8**, the following structural features in the core were readily apparent: —CHOR—C≡C—CH=CH—C≡C—, NH—CO_2CH_3, X—C=C—, C=O, CHOH, C=CH—CH_2—S, S—CH_3, —C—OR. The C12H proton adjacent to the diynene chromophore showed long-range coupling to C9–H as well as CCH and CCCH coupling to C11, C1, and C13. The C4–H also exhibited CCCH coupling to C6 and resonated in the ^{13}C at δ 84.4, suggesting possible attachment to the α,β-unsaturated carbonyl group. Especially difficult in the assignment of structure to the esperamicins was the elucidation of the allylic trisulfide portion of the molecule. From high-resolution mass spectroscopy, it was evident that the core of the molecule contained three sulfur atoms. The NMR spectra revealed both an S–methyl group as well as an allylic sulfide. In addition, decomposition of the esperamicins readily resulted in the loss of both methylmercaptan and hydrogen sulfide. A yellow film of elemental sulfur deposited on long-standing solutions of **1a**. On the basis of these observations, we hypothesized the existence of a trisulfide in **1a**, **2b**, **4**, and **8** which was verified by MS–MS and high-resolution FABMS fragmentation of molecular ions.[12]

An important clue to the assemblage of the above structural information to yield a viable structural hypothesis was the isolation and structure elucidation of esperamicin X (see preceding communication in this issue). In esperamicin X, the structural features of the esperamicins were present with the following notable exceptions: the α,β-unsaturated ketone in **8** was replaced by a saturated ketone with concomitant saturation of the C1–C2 double bond; the elements of CH_2S_2 had been eliminated from X; the diynene function in **8** had been replaced by a 1,2-disubstituted benzene ring. It was also apparent from the structure of esperamicin X that it could readily have arisen from cleavage of a trisulfide, Michael addition to the bridgehead double bond, and aromatization of a diynene.

Applying this reasoning, we have assigned the structures of **1a–c**, **2**, **4**, and **8** as shown. In the case of **1b** we had earlier shown it to be isomeric with **1a**, resulting from a shift of the acyl group of the anthranilic acid chromophore from $C3^{iv}$ hydroxyl to $C4^{iv}$ hydroxyl. Esperamicin A_{1b} (**1c**) was shown to differ from **1a** only in the substitution of the amino function of the pentapyranose. The assignment was fully supported by the mass spectral fragmentation pattern of **1c**.[13] In all cases the structural assignment is fully consistent with the spectral data.

Esperamicin X also provides valuable insights into the probable mechanism of action of these compounds. An examination of models shows that the existence of the bridgehead double bond prevents the ends of the diyne from approaching one another closely enough for cyclization to occur. Saturation of the bridgehead double bond permits geometries suitable for ring closure. Ring closure will result in the generation of a biradial[14] capable of H atom abstraction from the sugar phosphate backbone in DNA and resulting in strand scission. Especially important in this regard is the fact that clean double strand DNA breaks due to simultaneous cleavage of each strand are possible under this mechanism. Thus, the esperamicins represent a new class of bioreductively activated DNA-damaging antitumor agents. Their unique structure, high biological activity in murine systems, and possibly unique mechanism of action warrant their further study as potential antitumor agents for the treatment of cancer in man.

Acknowledgment. We gratefully acknowledge the partial support of this work under contract No. 1-CM37556 from the Division of Cancer Treatment, National Institutes of Health. Helpful discussions with Jon Clardy and Koji Nakanishi are gratefully acknowledged.

Registry No. **1a**, 99674-26-7; **1b**, 99674-27-8; **1c**, 88895-06-1; **2**, 107453-55-4; **3** (α-anomer), 107453-56-5; **3** (β-anomer), 107453-57-6; **4**, 107473-04-1; **5**, 107473-05-2; **6** (α-anomer), 107453-58-7; **6** (β-anomer), 107453-59-8; **7** (α-anomer), 107453-60-1; **7** (β-anomer), 107453-61-2; **8**, 107473-06-3.

Supplementary Material Available: Tables of high-resolution FABMS, ^{1}H NMR, and ^{13}C NMR data (10 pages). Ordering information is given on any current masthead page.

(10) Assignments reported in the text and accompanying supplementary material have been confirmed using COSY and 2DJ techniques.

(11) We have synthesized the α- and β-glycosides of 4-(hydroxyamino)-4,6-dideoxyglucose and galactose as model compounds and find that assignment of the C4' resonance at δ 69.5 is consistent with the observed values for these compounds. Toda, S.; Vyas, D., unpublished observations. The ^{13}C NMR of **8** (90 MHz CD_3OD) δ 132.4 (C1), 149.0 (C2), 194.0 (C3), 84.4 (C4), 80.6 (C5), 99.9 (C6), 84.6 (C7), 125.9 (C8), 124.2 (C9), 88.5 (C10), 99.3 (C11), 71.3 (C12), 136.8 (C13), 130.8 (C14), 40.8 (C15), 22.9 (SCH_3), 156.6 (CO_2CH_3), 53.4 (CO_2CH_3), 104.3 (C1'), 76.3 (C2'), 72.0 (C3'), 69.6 (C4'), 72.0 (C5'), 18.8 (C6').

(12) In the high mass region of the mass spectrum of **1a**, **2**, and **4**, ions corresponding to cleavages of CH_2S, CH_2S_2, and CH_2S_3 could be detected.

(13) The mass spectra of **1a** and **2** exhibited a strong ion at m/z 172 corresponding to cleavage of the N-isopropyl sugar. In the mass spectrum of **1c**, the m/z 172 ion was missing, replaced by one at m/z 158. The ^{1}H and ^{13}C NMR spectra of **1c** showed loss of the isopropyl group and its replacement by an *N*-ethyl function.

(14) Aromatization of siimple diynenes to yield biradicals has been demonstrated by Bergman and co-workers. Lockart, T. P.; Comita, P. B.; Bergman, R. C. *J. Am. Chem. Soc.* **1981**, *103*, 4082–4090.

Calichemicins, a Novel Family of Antitumor Antibiotics. 1. Chemistry and Partial Structure of Calichemicin γ_1^1

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

American Cyanamid Co., Medical Research Division Lederle Laboratories, Pearl River, New York 10965

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The calichemicins (also known as the LL-E33288 antibiotics), produced by *Micromonospora echinospora* ssp. calichensis, were discovered during our search for new fermentation-derived antitumor antibiotics.[1] They show extraordinary potency against murine tumors and are approximately 4000-fold more active than adriamycin with optimal dose at 0.5–1.5 μg/kg.[2] The calichemicins represent a novel structure class and are related to three other recently reported families of extremely potent antitumor antibiotics, viz., esperamicins,[3] FR-900406,[4] PD 114,759, and PD

(1) (a) Fantini, A. A.; Korshalla, J. D.; Pinho, F.; Kuck, N. A.; Mroczenski-Wildey, M. J.; Greenstein, M.; Maiese, W. M.; Testa, R. T. *Program and Abstracts*; 26th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, LA, Sept. 1986; American Society for Microbiology: Washington, DC; Abstr 227. (b) Lee, M. D.; Morton, G. O.; Dunne, T. S.; Williams, D. R.; Manning, J. K.; Siegel, M.; Chang, C. C.; Borders, D. B. *Ibid.*; Abstr 228.

(2) Thomas, J. P.; Carvajal, S. G.; Lindsay, H. L.; Citarella, R. V.; Wallace, R. E.; Lee, M. D.; Durr, F. E., ref 1, Abstr. 229.

(3) Konishi, M.; Ohkuma, H.; Saitoh, K.-I.; Kawaguchi, H.; Golik, J.; Dubay, G.; Groenewold, G.; Krishnan, B.; Doyle, T. W. *J. Antibiot.* **1985**, *38*, 1605–1609.

(4) Kiyoto, S.; Nishikawa, M.; Terano, H.; Kohsaka, M.; Aoki, H.; Imanaka, H.; Kawai, Y.; Uchida, I.; Hashimoto, M. *J. Antibiot.* **1985**, *38*, 840–848.

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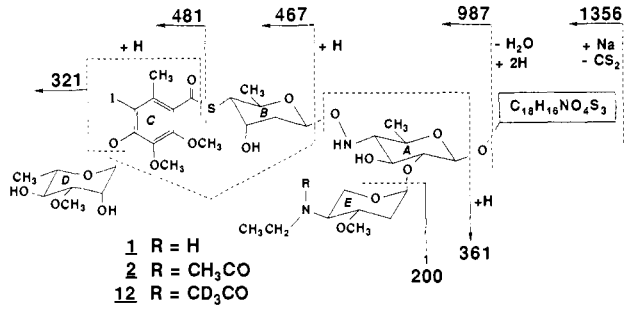
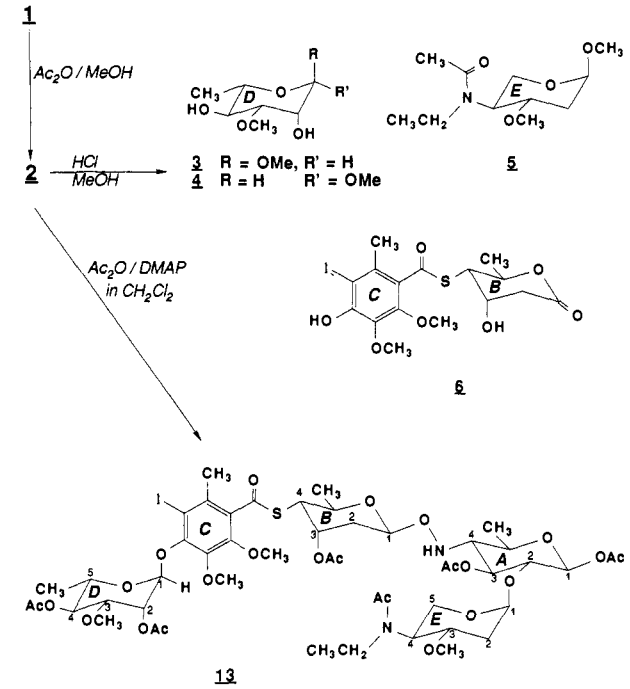


Figure 1. Chemical structures of calichemicin γ_1^1 (**1**), *N*-acetylcalichemicin γ_1^1 (**2**), and *N*-(acetyl-d_3)calichemicin γ_1^1 (**12**). Diagnostic ions observed in the HRFAB mass spectrum of **2** are shown with the corresponding fragmentations.

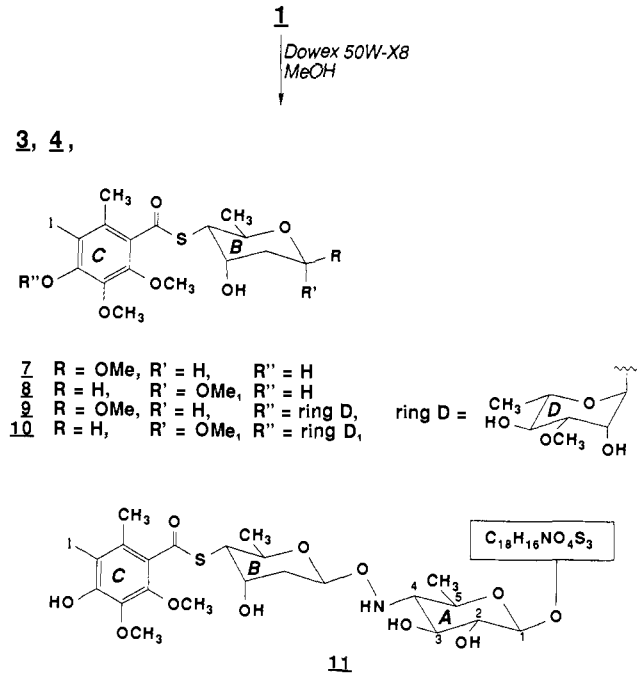
Scheme I



115,208,[5] all produced by *Actinomadura*. In the present report we assign the partial structure of calichemicin γ_1^1 (**1**) (Figure 1), the major component of the calichemicin complex. In the following paper we assign the structure of the aglycon and the complete structure of calichemicin γ_1^1.[6]

Calichemicin γ_1^1 [$[\alpha]^{26}_D$ −124° (*c* 0.98%, EtOH); $C_{55}H_{74}IN_3O_{21}S_4$, HRFABMS weak M + H at *m/z* 1368.2878, Δ 5.7 mmu; anal. C 48.81, H 5.41, N 2.75, I 9.21, S 9.03] was isolated as a white amorphous powder. It was converted to its mono-*N*-acetyl derivative (**2**, $C_{57}H_{76}IN_3O_{22}S_4$, HRFABMS M + H, *m/z* 1410.2954, Δ 2.6 mmu) which upon methanolysis (1% HCl in MeOH, room temperature 3 h, Scheme I) gave methyl 3-*O*-methyl-α-L-rhamnopyranoside (**3**),[7] the corresponding β-methyl glycoside (**4**), **5** ($C_{11}H_{21}NO_4$, HREIMS Δ 1.2 mmu), and **6**. The structure of **5** was established by NMR studies including ^1H–^1H COSY; the acetyl group was introduced during the conversion

Scheme II



of **1** to **2**. The attachment of this ethylamino sugar to the rest of the calichemicin γ_1^1 molecule was assigned on the basis of FABMS and NMR studies described below. The structure of **6** was determined by X-ray crystallography which established the substitution pattern of the hexasubstituted benzene ring.[8] Methanolysis of **1** (Dowex 50W-X8, H^+ form, Scheme II) gave **3**, **4**, **7** ($C_{17}H_{23}IO_7S$, HREIMS Δ 2.7 mmu), **8**, **9** ($C_{17}H_{23}IO_7S$, HRFABMS M + Na Δ 2.0 mmu), **10**, and the pseudoaglycon of calichemicin, **11** ($C_{40}H_{47}IN_2O_{15}S_4$, HRFABMS M + Na Δ 0.8 mmu). The structures of **7**–**10**, were established by comparing their ^1H and ^{13}C NMR data to those of **3**, **4**, and **6**.

The ^1H and ^{13}C NMR data of **11** clearly showed the presence of the glycoside **7** subunit. The existence of a second β-glycosidic unit (ring A) in **11** was also evident (δ_H 4.60, d, J = 7.7 Hz; δ_C 103.5, d, CH-1A). Its structure was assigned on the basis of extensive NMR studies including ^1H–^1H COSY, ^1H–^1H spin–spin decoupling, and ^1H–^{13}C correlation experiments. The chemical shifts of H-4A (δ_H 2.44, t, J = 9.7 Hz) and C-4A (δ_C 67.2, d) suggested an N-substitution at C-4. The unusual N–O glycosidic linkage in **11** and the configurations of rings A and B were revealed by X-ray crystallographic analysis of a degradation product (compound **11**, following paper) derived from **11**.

HRFABMS studies on **2** (Figure 1) gave diagnostic ions (481.0349, $C_{17}H_{22}IO_8$; 467.0038, $C_{16}H_{20}IO_6S$; 361.1979, $C_{16}H_{29}N_2O_7$; 987.2660, $C_{39}H_{60}IN_2O_{17}S$) which allowed us to sequence the structural units and provided the first evidence for the attachment of the ethylamino sugar to ring A.[9] The corresponding fragment ions were observed in the FAB mass spectra of *N*-(acetyl-d_3)calichemicin γ_1^1 (**12**). Strong NOE interaction between the anomeric proton (δ_H 5.73, d, J = 1.6 Hz) of ring D and one of the methoxy protons (δ_H 3.89, s) of ring C was observed for **2**, confirming the close proximity of the 3-*O*-methyl-α-L-rhamnopyranoside to the iodothiobenzoate moiety.

Compound **2**, on treatment with Ac_2O/DMAP/CH_2Cl_2 (Scheme I), afforded compound **13** and the heptaacetate of **1**. Proton–proton COSY analysis of **13** and **2** revealed the characteristic downfield shifts of ~1 ppm for H-1A, H-3A, H-3B, H-2D, and H-4D of **13**, clearly demonstrating that the corresponding carbons in **2** bear hydroxyls.[10] The glycosidic linkage between

(5) (a) Bunge, R. H.; Hurley, T. R.; Smitka, T. A.; Willmer, N. E.; Brankeiwicz, A. J.; Steinman, C. E.; French, J. C. *J. Antibiot.* **1984**, *37*, 1566–1571. (b) Wilton, J. H.; Rithner, C. D.; Hokanson, G. C.; French, J. C. *J. Antibiot.* **1986**, *39*, 1349–1350.

(6) (a) Lee, M. D.; Dunne, T. S.; Chang, C. C.; Ellestad, G. E.; McGahren, W. J.; Morton, G. O.; Siegel, M.; Borders, D. B. *J. Am. Chem. Soc.*, following paper in this issue. (b) The chemical structures of other members of the family, including some containing bromine instead of iodine, will be presented in a separate publication.

(7) (a) Alföldi, J.; Toman, R.; Peciar, C. *Carbohydr. Res.* **1982**, *105*, 258–265. (b) Pozsgay, V.; Nánási, P. *Ibid.* **1980**, *81*, 184–186. (c) Toman, R.; Karácsonyi, S.; Palovcík, R. *Ibid.* **1977**, *56*, 191–194.

(8) The X-ray analysis was carried out by Molecular Structure Corporation, College Station, TX 77840; see supplementary material.

(9) The loss of CS_2 from M + Na is significant, pertaining to the chemical structure of the aglycon. A degradation product derived from **11** with molecular formula CS_2 less than that of **11** is described in the following paper.

rings A and E must therefore be at C-2 of ring A with a free hydroxyl at C-3 in calichemicin γ_1^1. Similar experiments were carried out on the tetraacetate of **11**, confirming that, in **11**, both C-2A and C-3A bear free hydroxyls.

In this report we have shown calichemicin γ_1^1 to consist of four glycosidic units, a hexasubstituted benzene moiety, and an undefined $C_{18}H_{16}NO_4S_3$ unit. The exact configuration of the ethylamino sugar (ring E) is presently unknown; work is in progress to synthesize both of the enantiomers of **5** for optical activity comparison. In the following paper we assign the structure of the calichemicin aglycon ($C_{18}H_{17}NO_5S_3$).[11]

Acknowledgment. We thank J. K. Manning and L. Barbieri for technical assistance and V. Dean for optical rotation measurements.

Supplementary Material Available: Table of ^{13}C NMR chemical shifts of **1-3**, **5**, **7**, **9**, and **11**, ^1H NMR spectra of **1**, **2**, and **11**, summary of crystal data, computer-generated perspective drawing with atom numbering scheme, and table of the atomic positional and thermal parameters of **6** (8 pages). Ordering information is given on any current masthead page.

(10) ^1H NMR chemical shifts of ring A protons determined by ^1H-^1H COSY experiments. **2**: δ 4.62 (1A), 3.62 (2A), 4.04 (3A), 2.31 (4A), 3.74 (5A). **13**: 5.66 (1A), 3.63 (2A), 5.49 (3A), 2.68 (4A), 3.80 (5A).
(11) The calichemicin aglycon is defined as ($C_{18}H_{16}NO_4S_3$)-OH.

Calichemicins, a Novel Family of Antitumor Antibiotics. 2. Chemistry and Structure of Calichemicin γ_1^1

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

American Cyanamid Co., Medical Research Division Lederle Laboratories, Pearl River, New York 10965

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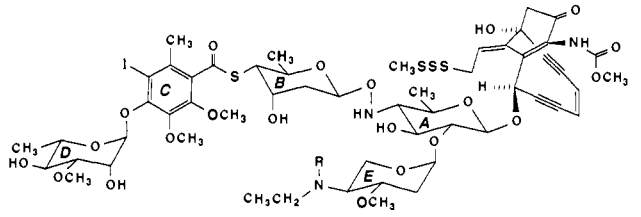
The structures of a number of methanolysis products of calichemicin γ_1^1 ($C_{55}H_{74}IN_3O_{21}S_4$), a potent antitumor antibiotic, are reported in the preceding paper. These degradation studies show calichemicin γ_1^1 to consist of four glycosidic units, a fully substituted iodothiobenzoate moiety, and an undefined $C_{18}H_{17}NO_5S_3$ aglycon.[1] Evidence is presented in the present paper which defines the structure of the aglycon and allows us to assign calichemicin γ_1^1 the structure **1**, containing the unprecedented bi-

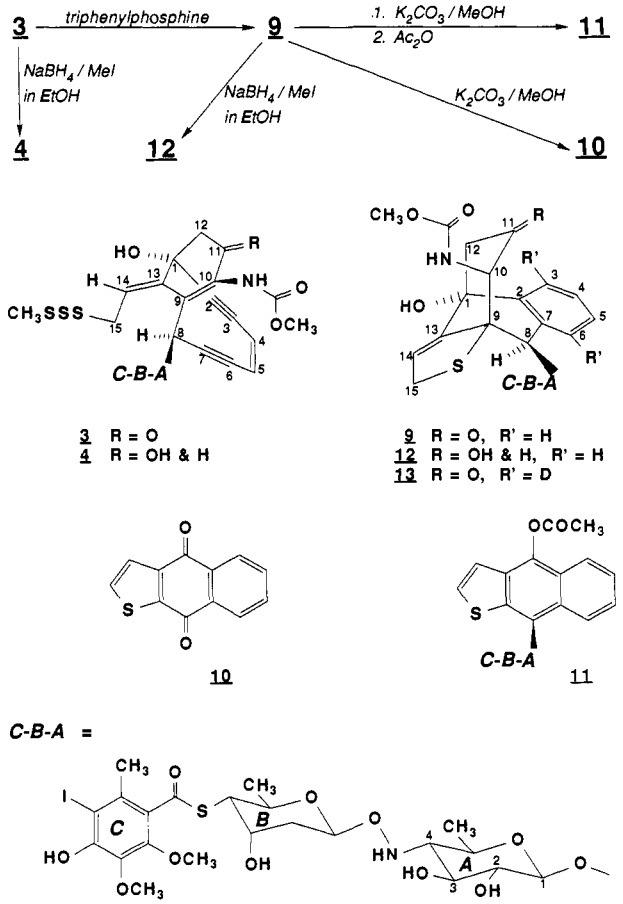


1 calichemicin γ_1^1, R = H
2 *N*-acetylcalichemicin γ_1^1, R = CH_3CO

cyclo[7.3.1]tridec-9-ene-2,6-diyne system, a unique N-O glycosidic linkage, and a methyl trisulfide moiety. Interestingly, the enediyne system can be readily triggered to aromatize via a free radical intermediate[2] by cleavage at the methyl trisulfide moiety. This aromatization process may be responsible for the remarkable DNA damaging effects of the calichemicins and is probably related to the reaction of neocarzinostatin with thiols.[3]

(1) Lee, M. D.; Dunne, T. S.; Siegel, M.; Chang, C. C.; Morton, G. O.; Borders, D. B. *J. Am. Chem. Soc.*, preceding paper in this issue.
(2) (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.

Scheme I



3 R = O
4 R = OH & H

9 R = O, R' = H
12 R = OH & H, R' = H
13 R = O, R' = D

10

11

Extensive NMR studies including ^1H-^1H COSY, ^{13}C DEPT, and ^1H-^{13}C correlation were carried out on **1**, its mono-*N*-acetyl derivative **2**,[1] and the pseudoaglycon **3**.[1] These spectral studies showed that the aglycon of calichemicin γ_1^1 contained two methyl groups (δ_H 2.52, δ_C 22.8, C-15-SSSCH$_3$; δ_H 3.77, δ_C 53.6, C-10-NHCOOCH$_3$),[4] a vinyl ABX system (A δ_H 5.89, δ_C 122.4, CH-4; B δ_H 5.81, δ_C 123.9, CH-5; X δ_H 6.27, δ_C 71.2, CH-8; J_{AB} = 9.5 Hz, J_{BX} = 1.4 Hz),[5] an isolated =CH-CH$_2$- spin system (δ_H 6.45, δ_C 127.5, $^3J_{HCCH}$ = 9.8, 5.3 Hz, CH-14; δ_H 3.87, 4.12, δ_C 39.1, CH$_2$-15),[6] an isolated methylene (δ_H 2.84, 3.22, δ_C 53.5, $^2J_{HCH}$ = 16.8 Hz, CH$_2$-12), and an α,β-unsaturated ketone (δ_C 191.8, C-11; IR 1680 cm^{-1}). These structural units accounted for 14 of the protons and nine of the carbons of the aglycon ($C_{18}H_{17}NO_5S_3$). 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 bear protons.

Treatment of **3** (Scheme I) with $NaBH_4$/EtOH in the presence of methyl iodide gave dihydropseudoaglycon **4** ($C_{40}H_{49}IN_2O_{15}S_4$, FABMS weak M + K at *m/z* 1091 and M + Na at *m/z* 1075; δ_H 2.17 dd, 2.73 dd, δ_C 45.7, $^2J_{HCH}$ = 13.7 Hz, CH$_2$-12; δ_H 4.75 m, δ_C 66.8, CH-11, $^3J_{HCCH}$ = 6.3, 4.4 Hz), establishing the connectivity between the α,β-unsaturated ketone and the isolated

(3) (a) Edo, K.; Mizugaki, M.; Koide, Y.; Seto, H.; Furihata, K.; Otake, N.; Ishida, N. *Tetrahedron Lett.* **1985**, *26*, 331-334. (b) Kappen, L. S.; Goldberg, I. H. *Nucleic Acids Res.* **1985**, *13*, 1637-1648. (c) Povirk, L. F.; Goldberg, I. H. *Biochemistry* **1985**, *25*, 4035-4040. (d) Hensens, O. D.; Dewey, R. S.; Liesch, J. M.; Napier, M. A.; Reamer, R. A.; Smith, J. L.; Albers-Schönberg, G.; Goldberg, I. H. *Biochem. Biophys. Res. Commun.* **1983**, *113*, 538-547.
(4) The NMR data of the aglycon portion of **1-3** are practically identical. Since there is much less signal overlap in the spectra of **3**, the chemical shifts and coupling constants (except for the ABX system) observed in **3** are reported here.
(5) The chemical shifts and coupling constants observed for **2** are reported for the ABX system. The AB portion of the ABX system in **3** collapses into a two-proton singlet; the BX interaction can still be observed in ^1H-^1H COSY experiment.
(6) The chemical shifts of the methylene protons was determined by ^1H-^1H COSY experiment.

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