Esperamicins, a novel class of potent antitumor antibiotics. 3. Structures of esperamicins A1, A2, and A1b

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Methanolysis of **1** yielded the 2-deoxy-L-fucose derivative **2** identical in all respects with that obtained on methanolysis of esperamicins A_1 and A_{1b}[3b] as well as a new product, **3**. Compound **3** was obtained as white crystals, mp 223 °C. The IR spectrum of **3** showed bands for ketone, urethane, and hydroxyl functions.[10] The UV spectrum of **3** was uninformative with only very weak absorbances above 230 nm. A molecular formula of $C_{17}H_{17}NO_6S$ (MW 363) was determined by high-resolution mass spectroscopy. From the ^1H and ^{13}C NMR spectra, the presence of a number of substructural fragments could be deduced, i.e., a disubstituted aromatic ring, an allylic group $=CHCH_2S$, three isolated methine groups bearing heteroatoms, a ketone carbonyl, and two heteroatom substituted quaternary carbons.[11] Assignment of an unambiguous structure based on the data was not possible; consequently, crystals of **3** grown from methanol-chloroform were subjected to X-ray analysis.[12]

Figure 2 is a computer-generated perspective drawing of the final X-ray model. The X-ray experiment defined only the relative, not the absolute, stereochemistry. The tetracyclic core of the molecule can be dissected into smaller rings to discuss the conformation. There is a six-membered ring, atoms C1-C5 and C13, which is in a chair conformation. With respect to this ring, the sulfur substituent at C1 is equatorial, as are O5, O4, and N1. Substituents C6 and C12 are axial and form part of a cyclohexene ring—atoms C5, C6, C11, C12, C1, and C13. This ring is in the expected half-chair conformation; i.e., atoms C5, C6, C11, and C12 are planar with C13 above and C1 below this plane. The dihydrothiophene ring is planar with all torsional angles less than 2°. There is some bond lengthening around C1 which is indicative of strain.

It remained to establish the points of attachment of the 2-deoxy-L-fucose fragment to the core. The mass spectra of compounds **1** and **3** permit us to assign the point of attachment as being at C4. In the mass spectrum of **3**, major ions were observed at m/z 146 and 218. The exact mass of the m/z 146 ion establishes this fragment as $C_5H_8NO_4$. This corresponds to cleavage through bonds C1-C2 and C4-C5 with hydrogen transfer to this fragment. The fragment ion at m/z 218 is consistent with the aromatic side of this fragment without hydrogen transfer.[13] Similarly in the EI mass spectrum of **1**, major fragmentation ions were observed at m/z 540 and 216. The m/z 540 ion is consistent with the analogous C1-C2, C4-C5 cleavage in which the 2-deoxy-L-fucose chromophore is glycosidically attached to the C4 hydroxyl. The m/z 216 ion is consistent with the aromatic side of the fragment with hydrogen transfer. Attachment of the 2-deoxy-L-fucose at either C5 or C12 is inconsistent with the mass spectral fragmentation pattern of **1**. Further support for this assignment from ^1H and ^{13}C NMR comparisons of **1** and **3** was available, e.g., the shift of the C4 carbon from δ 85.1 to 82.7 on going from **1** to **3** with a corresponding shift of the proton signal from δ 4.68 to 4.49. Little or no chemical shift differences for the C5 and C12 resonances were observed. The asssignment of the α-glycosidic linkage in **1** was made on the basis of the C1'-H coupling constants to the C2' protons.

With the structure of esperamicin X (**1**) in hand, assignment of the NMR spectra data to specific structural features was accomplished. Comparison of the spectra of esperamicin X with those of esperamicin A_1 revealed numerous similarities between them, notably, the common presence of the 2-deoxy-L-fucose-aromatic chromophore, the allylic methylene attached to a heteroatom, the presence of the $NHCO_2Me$ function, and the presence of the quaternary carbon at C5 (δ 77.3). A number of differences were also noted, especially the presence of the aromatic disubstituted ring and the quaternary carbon at 74.7 ppm in **1** and not in esperamicin A. Reconciliation of these structural similarities and differences between **1** and esperamicin A_1 is the subject of the following communication[14] in this issue.

Registry No. 1, 107175-47-3; **2**, 99407-56-4; **3**, 107175-48-4.

Supplementary Material Available: Tables of fractional coordinates, thermal parameters, interatomic distances, interatomic angles, and torsional angles for **3** (4 pages). Ordering information is given on any current masthead page.

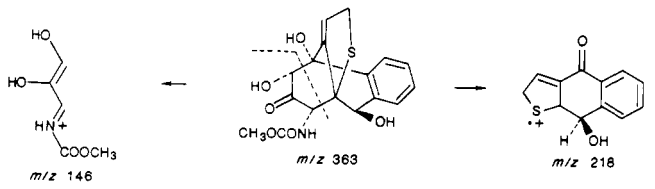
(9) The resonances attributable to the core are listed below. For those assigned to the 2-deoxy-L-fucose chromophore, see ref 3b: ^1H NMR of **1** (CD_3CN at 360 MHz) δ 7.71 (1 H, d, J = 7.5 Hz, C7-H), 7.27-7.41 (3 H, m, C8-H, C9-H, C10-H), 6.27 (1 H, t, J = 2.3 Hz, C14-H), 6.01 (1 H, br s, N*H*-CO_2Me), 5.21 (1 H, d, J = 9.9 Hz, C2-H), 4.68 (1 H, d, J = 2.3 Hz, C4-H), 4.48 (1 H , obsc, C12-H), 4.60 (1 H, s, C5-O*H*), 3.90 (2 H, m, obsc, C15-H_2), 3.65 (3 H, s, $NHCO_2CH_3$), and 3.59 (1 H, d, J = 2.9 Hz, C12-O*H*). ^{13}C NMR of **1** (CD_3CN at 90 MHz) δ 74.4 (C1), 68.4 (C2), 201.2 (C3), 85.1 (C4), 76.4 (C5), 137.5 (C6), 128.2 (C7), 129.0 (C8), 129.1 (C9), 132.0 (C10), 135.5 (C11), 71.9 (C12), 142.2 (C13), 125.6 (C14), 38.9 (C15), 158.1 (CO_2Me), 53.1 (CO_2Me).

(10) IR bands at 3360, 3070, 2960, 2920, 2850, 2255, 1722, 1525, 1454, 1330, 1255, 1181, 1140, 1075, 1033, 910, 775, and 736 cm^{-1}.

(11) ^1H NMR of **3** (CD_3CN at 360 MHz) δ 7.49 (1 H, d, J = 8.1 Hz, C7-H), 7.23-7.35 (3 H, m, C8-H, C9-H, C10-11), 6.21 (1 H, t, J = 2.6 Hz, C14-H), 5.99 (1 H, br s, N*H*CO_2Me), 5.22 (1 H, d, J = 9.9 Hz, C2-H), 4.50 (1 H, obsc, C5-O*H*), 4.49 (1 H, obsc, C4-H), 4.48 H, obsc, C12-H), 3.88 (2 H, m, C15-H2), 3.62 (3 H, s, CO_2CH_3), 3.52 (1 H, d, J = 3.0 Hz, C12-O*H*), 3.36 (1 H, d, J = 5.1 Hz, C4-O*H*). ^{13}C NMR of **3** (CD_3CN at 90 MHz) δ 74.6 (C1), 68.0 (C2), 202.6 (C3), 82.7 (C4), 77.3 (C5), 136.9 (C6), 127.9 (C7), 129.1 (C8), 129.1 (C9), 131.9 (C10), 135.4 (C11), 71.9 (C12), 142.0 (C13), 125.5 (C14), 39.0 (C15), 158.0 (NHCO_2Me), 53.2 (CO_2Me).

(12) Details of the X-ray determination are found in the supplementary material.

(13) Major ions in the mass spectrum of **3** have been confirmed by exact mass measurements.



(14) Golik, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Ohkuma, H.; Saitoh, K.; T. W. Doyle, J. Am. Chem. Soc., following paper in this issue.

Esperamicins, a Novel Class of Potent Antitumor Antibiotics. 3. Structures of Esperamicins A_1, A_2, and A_{1b}

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In the preceding communication in this issue, the structure elucidation of esperamicin X was described.[2] We now report the structure elucidations of esperamicins A_1, A_2, and A_{1b} (compounds **1a-c**, respectively, Figure 1) through chemical degradation and the analysis of the spectra of the degradation products. Esperamicin A_1 (**1a**) contains four sugars and an aromatic chromophore which are attached at two points to a bicyclic core. Of the four sugars in **1a**, three have not previously been reported. The central core contains a number of unique functionalities within a bicyclo[3.7.1] system; an allylic trisulfide attached to the bridging atom, a 1,5-diyn-3-ene system, and an α,β-unsaturated ketone in

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(2) 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.*, preceding paper in this issue.

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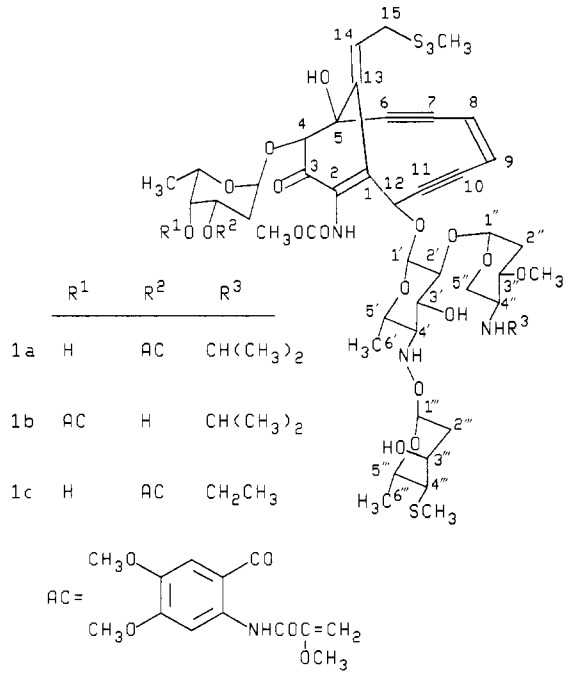


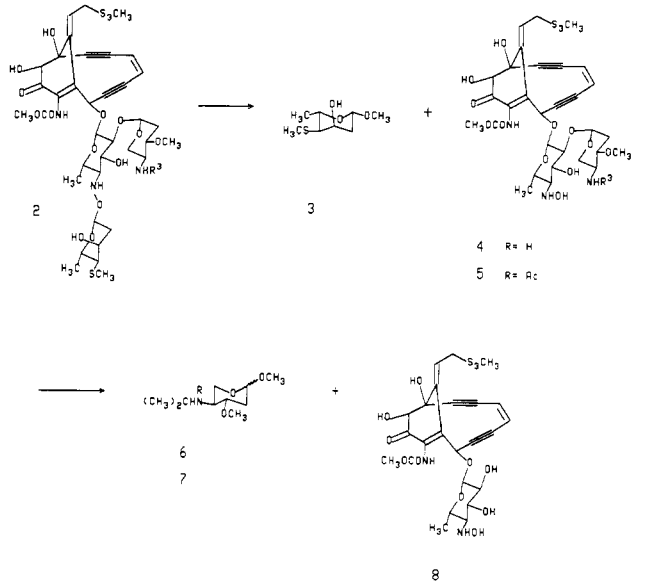
Figure 1. Structures of esperamicins A_1, A_{1b}, A_2—absolute configuration of bicyclo[3.7.1] core and trisaccharide portion arbitrarily chosen.

which the double bond is at the bridgehead. It is the unique interaction of these three functionalities which most probably accounts for the extreme potency and activites observed.

The complexity of the spectra of esperamicins **1a–c** precluded a direct assignment of structure. Consequently a series of degradations of **1a** were embarked upon. Earlier we reported that methanolysis of **1a** and **1b** gave isomeric 2-deoxy-L-fucose derivatives.[3] In addition to these products, both **1a** and **1b** produced a single common product, esperamicin C (**2**). Methanolysis of **2** yielded the methyl glycosides of thiomethyl sugar **3**[4] and esperamicin D (**4**). Further methanolysis of **4** led to a very complex mixture of products from which amino sugar **6** was isolated in low yield. The acetamide **5** was formed and methanolyzed to *N*-acetyl amino sugar **7** obtained as a mixture of anomers. In addition to **7**, we also isolated esperamicin E (**8**) (Scheme I). To date, attempts to prepare the aglycone of esperamicin E or to isolate the novel hydroxyamino sugar have failed presumably due to their instabiulity under the reaction conditions.[5]

Compound **3** was obtained as a mixture of α- and β-anomers readily separable on silica gel. On the basis of the NMR spectra, the compounds were identified as the methyl 2,4,6-trideoxy-4-(methylthio)-*ribo*-hexopyranosides. Comparison of the NMR spectra of both anomers of **3** with the spectra of esperamicins A_1 (**1a**) and C (**2**) permitted us to assign the β-configuration to the sugar in the intact glycosides.[6] In view of the low yield and relative instability of **6** methanolysis was carried out on **5**. Compound **7** was isolated as a mixture of anomers which were identified as the α- and β-methyl glycosides of 4-(*N*-isopropyl-*N*-acetyl)-2,4-dideoxy-3-*O*-methyl-*threo*-pentopyranose by high-resolution mass spectroscopy (HRMS) and NMR spectroscopy.[7] A comparison of the coupling constants for the α- and β-glycosides of **7** with those for C1″–H in **1a**, **2**, and **4** allowed us to assign

Scheme I. Degradation of Esperamicin C (**2**)



the α-configuration to the amino sugar in the intact glycoside. Especially intesting with respect to the position of substitution of the *N*-isopropyl amino sugar on the hydroxyamino sugar were the differences in the chemical shifts of the glycosidic proton (C1″–H) in the intact glycosides **4** and **5** and those observed in **6** and **7** (C1″–H appears at δ 5.50 in **4** and **5** and at δ 4.75 in compounds **6** and **7**). We ascribe δ 0.75 shift to the effect of the diynene upon the C1″–H and consequently have assigned C2′ as the position of attachment of the amino sugar to the hydroxyamino sugar. This assignment was further supported by analysis of the ^{13}C NMR spectra of esperamicin D (**4**) and esperamicin E (**8**).[6] A 5.5 ppm shift in the resonance for the C2′ carbon is observed in going from **4** to **8** (from δ 81.8 in **4** to 76.3 in **8**). Assignment of the point of attachment of the thiomethyl sugar (TMS) to the hydroxylamino sugar (HAS) was arrived at as follows. Peracetylation of esperamicin D (**4**) gave a compound in which significant ^1H NMR shifts for the C3′H and C1″H were observed (C3′H shifted from δ 3.88 to δ 5.26 and C1′H shifted from δ 5.56 to δ 5.01). Similarly peracetylation of esperamicin C (**2**) resulted in analogous shifts for the C3′H and C1″H. This ruled out the C3′OH as the point of attachment of the TMS leaving the hydroxylamino function at C4′ as the probable point of attachment. The lack of significant ^1H and ^{13}C NMR shifts for the C4′ position on going from **2** to **4** ruled out attachment to the nitrogen of the hydroxylamine. On the basis of this evidence, we conclude that the point of attachment of the TMS to the HAS must be the oxygen of the hydroxylamino function at C4′.

The structure of the hydroxyamino sugar in the esperamicins was established by analysis of the HRMS and NMR spectra of compounds **1a**, **2**, **4**, and especially 8. The molecular formula of esperamicin E (**8**) was established as $C_{24}H_{24}N_2O_{10}S_3$ by using high- and low-resolution (TSP)MS.[9] Fragmentation ions were observed at *m/z* 422 and 178 and *m/z* 440 and 162 corresponding to cleavages of the C12–O and C1′–O bonds, respectively. Similar cleavages were observed for esperamicins A (**1a**), C (**2**), and D (**4**), resulting in fragmentation ions at *m/z* 509 and 493 for **1a** and **2** and *m/z* 349 and 333 for **4**. The former ions were measured at high resolution and helped establish the molecular formula for the carbohydrate fragment in E as $C_6H_{12}NO_5$ (*m/z* 178). The

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

(4) Wilton, J. H.; Rithner, C. D. Hokanson, G. C.; French, J. C. *J. Antibiot.* **1986**, *39*, 1349–1350.

(5) Given the allylic–propargylic nature of the C12–O bond, we would expect preferential cleavage of this bond rather than cleavage of the C1′–O bond.

(6) The molecular formulae for both α- and β-anomers were established as $C_8H_{16}O_3S$ by using high-resolution FABMS. The NMR spectra of compounds **1a**, **2**, **3β**, **3α**, **4**, **5**, **7**, and **8** are listed in supplementary material.

(7) The elemental formulae for both **6** and **7** were established by using high-resolution FABMS.

(8) Major ions correspond to the following cleavages.

$$C_{59}H_{81}N_4O_{22}S_4 \rightarrow C_{22}H_{41}N_2O_9S + C_{22}H_{41}N_2O_8S + C_{18}H_{33}NO_6S$$

(9) In addition to the matrix effects in FABMS reported earlier,[3] we have now discovered that molecules in the esperamicin class readily lose a CH_2S_2 fragment via cleavage of the trisulfide and ring closure via Michael addition to the bridgehead enone under the conditions of FABMS experiments. Use of the thermospray technique gave M + H ions for **1**, **2**, **4**, and **8** at *m/z* 1325, 932, 772, and 601, respectively.

^{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^I

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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

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