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# Configurational Assignment of Polyene Macrolide Antibiotics Using the [$^{13}$C]Acetonide Analysis

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Structure determination of natural products is one of the classic activities of organic chemists, and structural proof has been one of the historical justifications for total synthesis. Komppa's 1903 synthesis of camphor confirmed the structure proposed earlier by Bredt, and thus established total synthesis as a final arbitrator of natural product structure.[1] The advent of a very accurate and reliable method for structure determination—X-ray crystallography—gradually undermined the role of total synthesis in confirming structure. Some classes of natural products, however, have proved to be largely unsuitable for crystallographic analysis, and in these cases partial and total syntheses often play an important role in their structural assignments. In 1988 we initiated a program to investigate the synthesis of polyacetate natural products. The largest family of interest, the polyene macrolide antibiotics, includes over 200 members.[2] At the outset of our investigations, the complete stereochemical structure was known for only three of these complex natural products: amphotericin B[3] and mycoticin A and B.[4] In an offshoot of our synthetic work, we identified a very reliable method for distinguishing between *syn*- and *anti*-1,3-diols by inspection of the $^{13}$C chemical shifts of the corresponding acetonides.[5] Described in this Account is the development of the [$^{13}$C]acetonide method, the configurational assignments of polyene macrolides roflamycoin[6] and filipin III (Figure 1),[7] and the verification of these assignments by chemical synthesis.

As more powerful spectroscopic methods are developed, the role of synthesis in confirming structure becomes less important.[8] The 2-D [$^{13}$C]acetonide method described at the end of this account is one such method, and its potential is illustrated in the structure elucidation of dermostatin A.[9]

## The [$^{13}$C]Acetonide Method

The [$^{13}$C]acetonide method relies upon the divergent conformations of *syn*- and *anti*-1,3-diol acetonides. As illustrated in Figure 2, *syn*-1,3-diol acetonides prefer chair conformations where one of the acetal methyl groups is axial and the other is equatorial. The axial methyl group has a $^{13}$C chemical shift of ca. 20 ppm, while the equatorial methyl group has a chemical shift of ca. 30 ppm. *anti*-1,3-Diol acetonides adopt twist-boat conformations, where the two acetal methyl groups are in nearly identical environments and thus both have $^{13}$C chemical shifts of ca. 25 ppm. The chemical shifts of *syn*- and *anti*-1,3-diol acetonides are distinctively different, and the relative configuration of 1,3-diol acetonides can be assigned by inspection of their $^{13}$C NMR spectra.[5]

The idea for a [$^{13}$C]acetonide analysis appears obvious in retrospect. The conformational difference between syn and anti acetonides was known, and was the basis for a coupling-constant analysis used to assign the relative configuration of 1,3-diol acetonides.[10] The conformation of various substituted 1,3-dioxanes had been established by the work of Eliel, Pihlaja, and Ridell.[11] Though we were unaware of his work at the time of our initial report, Buchanan had even commented upon the $^{13}$C chemical shifts of acetonide methyl groups.[12] Our idea for this analysis derived from symmetry considerations,[13] where the approximate $C_2$-symmetry of an *anti*-1,3-diol acetonide results in almost identical chemical shifts for the two nearly homotopic methyl groups. In contrast, the approximate meso symmetry of a *syn*-1,3-diol acetonide results in the two methyl groups being diastereotopic, and each having a different chemical shift. This symmetry-based analysis is independent of the conformational preferences of dioxanes and is equally valid for 1,2-diol acetonides.[14] Our principal contributions in this area have been to establish the generality of the $^{13}$C chemical-shift correlation and to develop it as a tool for structure determination.

How general is the $^{13}$C chemical-shift correlation for *syn*- and *anti*-1,3-diol acetonides? The chemical shift differences correspond to a change in conformation between syn and anti acetonide rings. Most 1,3-dioxanes exist in chair conformations, and the *syn*-1,3-diol acetonides are typical examples. The twist-boat conformation of an *anti*-1,3-diol acetonide is the exception, and results from severe 1,3-diaxial interactions that destabilize the two chair conformations (Figure 2).[15] When the *anti*-

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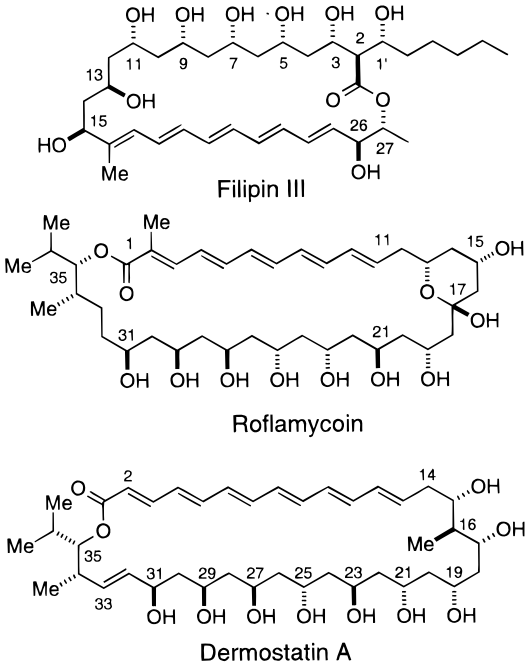



**FIGURE 1.** Structures of polyene macrolides filipin III, roflamycoin, and dermostatin A.

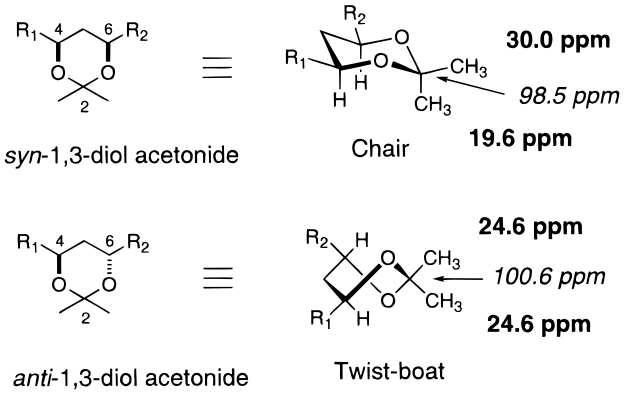



**FIGURE 2.** $^{13}$C NMR chemical shift correlation for *syn*- and *anti*-1,3-diol acetonides (*cis*- and *trans*-4,6-dialkyl-2,2-dimethyl-1,3-dioxanes).

4,6-substituents on an acetonide are as large as methyl, for example, the twist-boat conformation is favored by over 2 kcal/mol.[16] At the other extreme, an axial hydrogen substituent is well tolerated in a chair conformation. Somewhere between hydrogen and methyl the conformational preference crosses-over from a chair to a twist-boat. To identify the crossover region, we carried out a literature search on 1,3-diol acetonides and evaluated the correlation between the C2-methyl chemical shifts and the relative configuration.[17] With $sp^3$ carbon atoms at the C4 and C6 positions of the 1,3-dioxane, the correlation worked well in every case, including many examples with C5-substituents. The *anti*-dioxanes with an $sp^2$ carbon at the C4 position show chemical shifts in the 23−26 ppm range, still quite close to the average values listed in Figure 2. The more polar aldehyde substituent falls outside of this range, indicating a significant chair population. In one case, to be discussed later, macrocyclic constraints forced a 4-alkene substituent to adopt the axial position

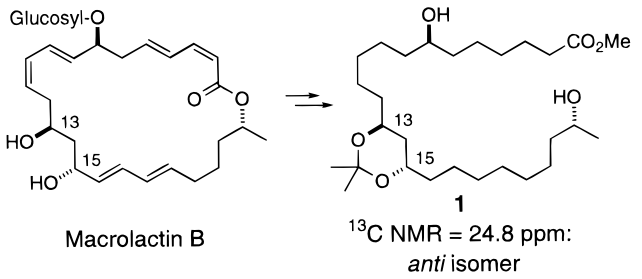



**FIGURE 3.** Relative configuration of the macrolactin B C13−C15 diol by [$^{13}$C]acetonide analysis. Isotopically enriched [$^{13}$C]acetone was used to enhance sensitivity.

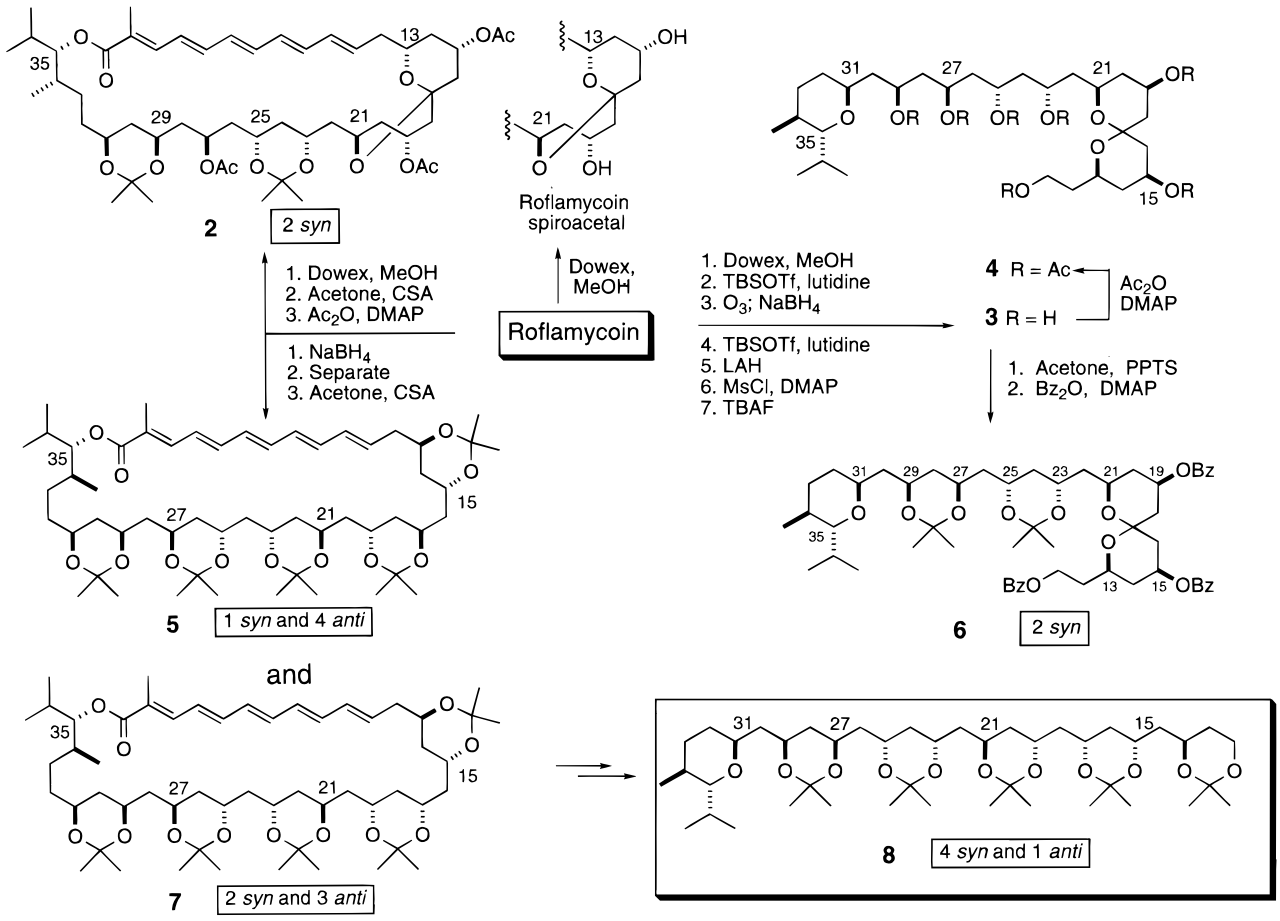
in a chair conformation. The chemical shift correlation only seriously breaks down with *sp* substituents. A C6-alkyne substituent leads to energetically balanced chair and twist-boat conformations, and the more electronegative nitrile strongly favors the axial-chair arrangement. Thus the simple correlation from Figure 2 holds for essentially all 4,6-substituents except nitrile and alkyne.

Evans extended the $^{13}$C chemical correlation to polypropionate chains.[18] He also pointed out that the chemical shift at the C2 acetal carbon correlated well with syn and anti configuration: the average *syn*-1,3-diol acetonide has a C2 acetal at 98.5 ppm, while the anti-1,3-diol acetonide has an acetal chemical shift of 100.6 ppm. When numerous methyl groups are present, it is much easier to locate the acetal carbon than the acetonide methyl groups in the $^{13}$C NMR spectrum. Unfortunately the syn and anti acetal chemical shifts do overlap. Examining the acetonides from our literature search, we found that if 100.0 ppm were taken as an arbitrary dividing line between syn and anti acetals, then 10 of 145 or 7% of the syn acetals would be misassigned as anti, and 3 of 76 or 4% of the anti isomers would be misassigned as syn.[17] The methyl chemical shifts lead to no such ambiguity. Acetal chemical shifts are very easy to identify, but syn and anti assignments should be confirmed by identification of the methyl chemical shifts.

## Macrolactin

The first configurational assignment of a natural product using the [$^{13}$C]acetonide method was that of macrolactin B.[19] The macrolactins, particularly macrolactin A, are of interest because of their antiviral activity: they protect human T-lymphoblast cells against HIV viral replication.[20] Very limited quantities of macrolactin B were available, so the derivatization was carried out with 98% 1,3-$^{13}$C-labeled acetone to increase the sensitivity of the method. Macrolactin B was hydrogenated and hydrolyzed in HCl-methanol prior to acetonide formation. The $^{13}$C chemical shift of 24.8 ppm for the acetonide methyl groups of compound **1** indicated an anti configuration of the C13−C15 diol (Figure 3). Further degradation and Moshers' ester analysis[21] led to the configurational assignment of macrolactin B as shown in Figure 3. The configuration of macrolactin F was in accord with that of macrolactin B, suggesting that stereochemistry was invariant within the family. The recent synthesis of macrolactin A by Smith

**Scheme 1**



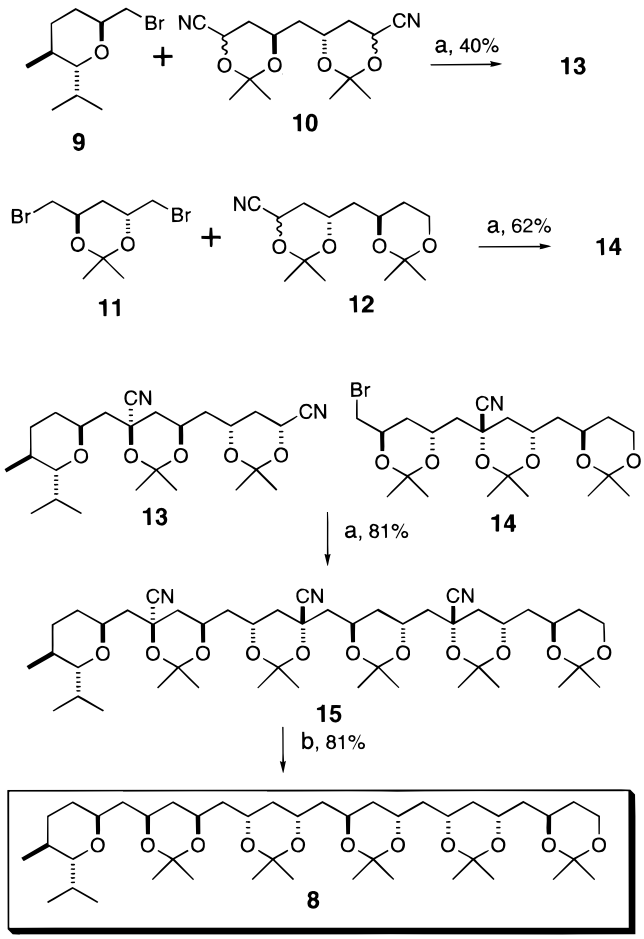

confirms its stereochemical homology with macrolactin B.[22] The [13C]acetonide method has been instrumental in the configurational assignment of a number of natural products,[23] and has also been widely used to confirm the configuration of synthetic intermediates containing 1,3-diols.[24] In the following sections we will focus on the structure assignment of complex *polyol* chains using the [13C]acetonide method.

## Roflamycoin

Roflamycoin is a polyene macrolide antibiotic isolated from a strain of *Streptomyces roseoflavus*.[25] Like amphotericin B, it forms sterol-dependent ion channels in cell membranes.[26] The flat structure of roflamycoin was reported in 1981,[27] and we determined its stereochemical configuration in 1994 as part of a collaborative effort with Rolf Schlegel.[6] The configuration of roflamycoin was assigned by preparing a number of polyacetonide derivatives and analyzing their relative configurations as illustrated in Scheme 1. Roflamycoin is a cyclic hemiacetal that rapidly closes to its spiroacetal on treatment with acid. Standard $^1$H NMR analysis of the roflamycoin spiroacetal gave the relative configurations of C13−C15 and C19−C21 as anti, and C15−C19 as syn. The major acetonide derivative formed from the spiroacetal, compound **2**, had acetonide rings at C23−C25 and C29−C31, and both were found to be syn by [13C]acetonide analysis. Degradation of roflamycoin gave tetrahydropyran **3**. Formation of diacetonide derivative **6** from **3** and subsequent [13C]acetonide analysis revealed that C23−C25 and C27−C29 were syn. The relative configuration of the C31−C35 segment was assigned by standard $^1$H NMR analysis of tetrahydropyran **4**. The only stereochemical relationships remaining to be assigned were C21−C23 and C25−C27.

One of the shortcomings of the original [13C]acetonide method is that it only indicates the total *number* of syn and anti rings in a given polyacetonide, but it does not help one *to assign* each ring as either syn or anti. Consider the pentaacetonide derivative **5**. It was prepared by reduction of the C17 ketone in roflamycoin, separation of the resulting alcohol isomers, and treatment of the polyol with acetone and acid. All of the alcohols are protected as acetonides in **5**, so there is no ambiguity about the *positions of the acetonide rings*. The $^{13}$C chemical shifts indicate that pentaacetonide **5** contains four anti rings and one syn ring. There are five different ways to arrange one syn and four anti acetonides, but the [13C]acetonide method does not indicate which one of the acetonide rings is syn. Further information is needed to solve this problem. In the case of **5**, arriving at a unique solution is simple: the C29−C31 acetonide in compound **2** was identified as syn, and thus must be syn in compound **5**. The remaining acetonides are all anti, including the C21−C23 and C25−C27 rings. These last two relative

**Scheme 2**[a]




[a] (a) $LiNEt_2$, THR, −78 °C; then RBr, 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU), −23 to 15 °C. (b) Li, $NH_3$, THF, −78 °C.

stereochemical assignments, combined with the absolute configuration of the C35 alcohol determined by Mosher's analysis,[21] complete the stereochemical assignment of roflamycoin. A general solution for assigning each acetonide ring in a polyacetonide as either syn or anti will be presented in the structure determination of dermostatin A.

The configuration of roflamycoin was verified by synthesis of a degradation fragment.[6b] Compound **8,** containing all of the stereogenic centers in roflamycoin, was prepared by oxidative degradation of pentaacetonide **7** (Scheme 1). Synthesis of the degradation fragment was designed around our cyanohydrin acetonide coupling methodology.[28] Each of the chiral building blocks, compounds **9**−**12** in Scheme 2, was prepared using standard enantioselective reactions including Ru−BINAP hydrogenation of ketones[29] and Brown allylborane additions.[30] Monoalkylation of dinitrile **10** with bromide **9** gave the product **13** in 40% yield (Scheme 2).Alkyl bromide **14** was prepared by alkylation of nitrile **12** with an excess of the $C_2$-symmetric dibromide **11**. An excess of the dibromide was used to ensure a preponderance of the monoalkylated product. Alkylation of the nitrile anion **13** with a modest excess of the bromide **14** gave the completed carbon skeleton in very good yield. In the final step, the three cyano groups were removed by a syn-selective Li/$NH_3$ reduction to give polyacetonide **8** as a single stereoisomer. The spectra of synthetic **8** matched that of the degradation product, confirming the configurational assignment of roflamycoin. The synthesis of **8** was highly convergent but was marred by the low yield of the first alkylation reaction. By using a different disconnection to avoid this problematic alkylation, we have recently completed a total synthesis of roflamycoin.[31]

## Filipin III

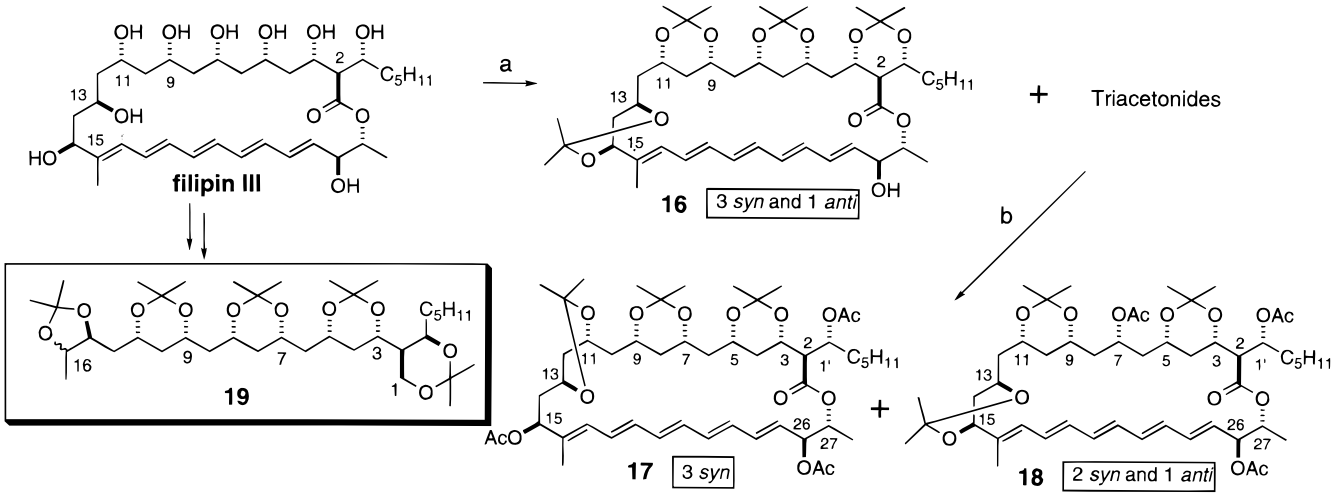
Filipin is a polyene macrolide isolated from *Streptomyces filipinensis*.[32] The principal component is filipin III, whose flat structure was first correctly identified by Ceder and Ryhage.[33] Filipin does not form ion channels, but it does aggregate with cholesterol and disrupt membranes.[34] In fact, it is often used as a probe for membrane-bound cholesterol.[35]

Filipin III decomposes upon treatment with strong acid, but it was possible to form polyacetonide derivatives by treatment with acetone, 2,2-dimethoxypropane (DMP), and pyridinium *p*-toluenesulfonate (PPTS). The three polyacetonides formed, compounds **16**-**18** in Scheme 3, were separated by flash chromatography after acetylation. In a single polyacetonide derivative, every other pair of alcohols is tied into a ring and [$^{13}$C]acetonide analysis can only provide the relative configuration for half of the alcohol pairs. At least two frame-shifted polyacetonide derivatives are required to solve the relative configuration of a polyol chain. Protection of filipin III produces just such a pair of frame-shifted derivatives, compounds **16** and **17**. The 2-D [$^{13}$C]acetonide method developed later would have allowed filipin's polyol configuration to be solved with two derivatives alone, but the standard [$^{13}$C]acetonide method required the analysis of all three polyacetonides.

The [$^{13}$C]acetonide analysis of polyacetonides **16**−**18** provides almost all of the stereochemical information necessary for the structural assignment of filipin III. The [$^{13}$C]acetonide analysis of compounds **16**−**18** showed that tetraacetonide **16** had three syn and one anti acetonide rings, triacetonide **17** had three syn acetonides, and triacetonide **18** contained two syn and one anti acetonides. Compound **17** has only syn acetonides and directly leads to the stereochemical assignment of C3−C5, C7−C9, and C11−C13 as syn. Both **16** and **18** have one anti ring, and the only acetonide ring in **16** that is not present in **18**, C3−C5, has already been assigned as syn by analysis of compound **17**. Thus the *same* anti acetonide ring is present in both **16** and **18** and must be located at either C9−C11 or C13−C15. The two remaining acetonide rings in **16**, C1′-C3 and C5−C7, must be syn. The number of possibilities have been narrowed down to two: there is one anti relationship in the polyol chain and it is either at C13−C15 or C9−C11.

The anti acetonide ring in compounds **16** and **18** was unique in that the $^{13}$C chemical shifts of the acetal methyl groups were found at ca. 25 and 31 ppm. In our previous studies no other acetonide ring had displayed such $^{13}$C

**Scheme 3**[a]




[a] (a) [1,3-$^{13}C_2$]Acetone, 2,2-DMP, PPTS, THF. (b) $Ac_2O$, DMAP, THF.

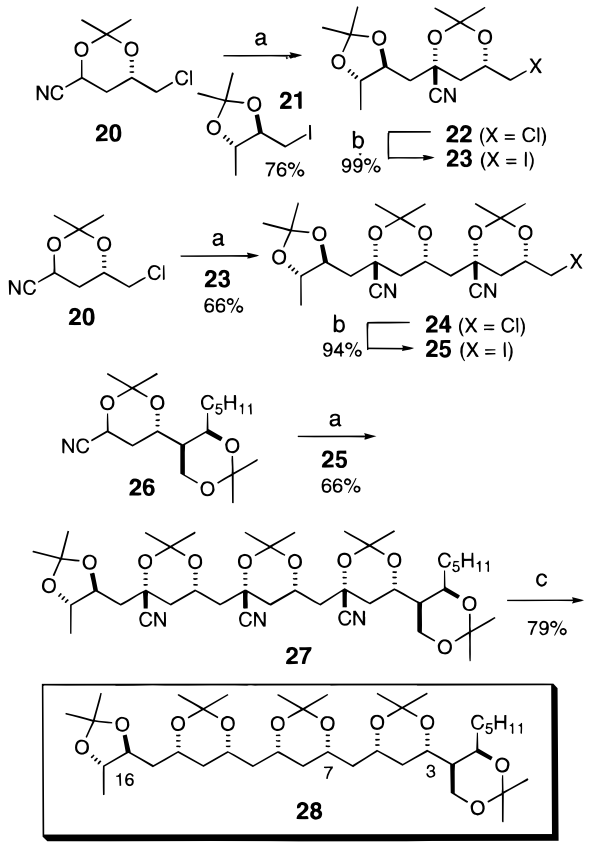
chemical shifts, and a prediction for the position of the anti acetonide ring in **16** resulted from our interpretation of these data. The 25 and 31 ppm methyl shifts were certainly not consistent with a syn acetonide, the methyl shifts of which are almost invariant. The most reasonable interpretation was that the methyl shifts arose from an anti acetonide ring in a chair conformation. Support was found in the work of Pihlaja, who showed that 2,2,4,4,6-pentamethyl-1,3-dioxane adopts a chair conformation due to disubstitution at the 4-position.[11d] This ring was analogous to an anti acetonide in a chair conformation: it had an axial methyl substituent at the 4-position and an equatorial methyl substituent at the 6-position. The chemical shifts of its acetal methyl groups were 24.9 and 32.0 ppm, in good agreement with the spectra of **16** and **18**. Apparently, the conformational constraint imposed by the macrocycle forces the anti acetonide ring in compounds **16** and **18** to adopt a chair conformation rather than the twist-boat conformation normally preferred. This is the only example of which we are aware, in which a macrocyclic conformation overcomes the local conformational preference. The unusual chair conformation of the anti acetonide also provided a clue to its location. The C9−C11 acetonide in **16** is flanked by two methylene groups, while the C13−C15 acetonide is flanked by a methylene and an alkene. One of these substitutents must occupy an axial position, and computational studies have shown that an axial methylene is much less favorable than an axial alkene.[16] Thus the conformational preference of a macrocycle would be more likely to shift the balance and favor a chair conformation with an alkene-substituted acetonide than with a methylene substituted acetonide. This reasoning suggested but did not prove that the anti acetonide in compounds **16** and **18** was located at the C13−C15 position. Further experimental work supported this view, and in conjunction with other degradation studies lead us to propose the filipin III configuration illustrated in Scheme 3.

The configuration of the polyol portion of filipin III was confirmed by synthesis of the C1′-C16 degradation fragment **19**.[7b] Ozonolysis of the filipin III polyene followed by reduction and acetonide formation gave degradation compound **19** as a mixture of epimers at the C16 position (Scheme 3). Synthesis of an authentic sample of **19** was based on our iterative all-syn polyol strategy.[36] The 1,3-diol synthon **20** was deprotonated and alkylated to build a chain to one side, or activated by displacement of the chloride with an iodide to build a chain in the other direction. Each of the chiral building blocks—**20**, **21**, and **26**—were prepared as single enantiomers by asymmetric synthesis (Scheme 4). Alkylation of *trans*-dioxolane **21** with chloromethyl cyanohydrin **20** gave the first cyanohydrin adduct **22**, which was converted to the iodide **23** by treatment with potassium iodide. Alkylation of another equivalent of cyanohydrin **20** with iodide **23** gave the bis-adduct **24** in good yield. Activation of the chloride by iodide displacement gave **25**, and alkylation of cyanohydrin **26** with iodide **25** gave the pentaacetonide **27**. The three cyano groups of compound **27** were then removed by $Li/NH_3$ reduction to give a single stereoisomer of pentaacetonide **28** in 79% yield. The $^1$H NMR spectrum of **28** was identical with that of the major trans isomer of the degradation product, confirming the stereochemical assignment for the polyol portion of filipin III. Our cyanohydrin acetonide coupling strategy facilitated the rapid and convergent syntheses of protected polyols **8** and **28**.

## The Two-Dimensional [$^{13}$C]Acetonide Method

The principal limitation of the [$^{13}$C]acetonide method when applied to polyacetonides is that one cannot tell which $^{13}$C acetal methyl peak belongs to which acetonide ring. In the case of roflamycoin, numerous different acetonide and spiroacetal derivatives were prepared to identify the complete configuration. If each acetonide ring could have been assigned as either syn or anti, the complete relative configuration of roflamycoin would have been solved with just two polyacetonide derivatives, **7** and **8**. Similarly, all the information required to assign the

**Scheme 4**[a]




[a] (a) $LiNEt_2$; DMPU/THF, −78 °C. (b) KI, 18-crown-6, xylenes, 140 °C. (c) Li, $NH_3$, THF, −78 °C.

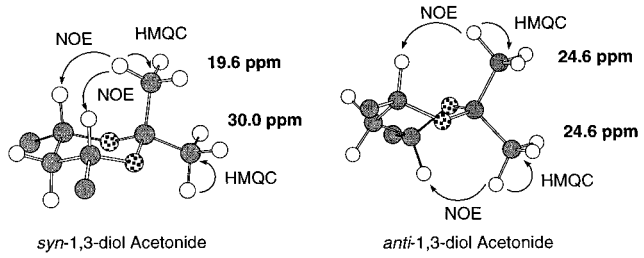



**FIGURE 4.** Overview of configurational assignments of polyol chains using [$^{13}$C]acetonide analysis, HMQC correlations, and NOE data.

filipin III C1′−C15 polyol configuration can be found in the frame-shifted acetonides **16** and **17** (Scheme 3). The key to solving polyacetonide structures is to link the easily identified syn and anti $^{13}$C-methyl shifts to specific protons along the polyol chain. Two simple 2-D NMR experiments accomplish exactly that. An HMQC experiment ties the $^{13}$C chemical shifts of the methyl groups to specific methyl peaks in the proton spectra, and a ROESY or NOESY experiment connects the methyl peaks in the proton spectra to carbinol protons in the same ring. Figure 4 illustrates the combined techniques. Others have used NOE experiments to assign the configuration of a single acetonide,[37] but the added complexity of a polyacetonide is not well handled by this strategy.

The application of these 2-D NMR techniques to the [$^{13}$C]acetonide method was obviously desirable. We did not develop it originally because the $^{1}$H methyl region was very poorly resolved in $CDCl_3$, the solvent in which all of the acetonide correlations were developed. As more systems were explored, it became clear that the [$^{13}$C]-acetonide correlations hold up well in other solvents, and that the proton methyl region is normally well resolved in benzene-$d_6$. Simultaneous improvements in instrumentation increased the practical resolution of 2-D NMR methods. The 2-D [$^{13}$C]acetonide method worked well with simple model compounds and showed several advantages over the conventional [$^{13}$C]acetonide analysis, including greater sensitivity due to inverse detection and easier identification of the methyl acetal peaks. The first serious application was a reexamination of a filipin III derivative. The 25 ppm peak in triacetonide **18** correlated with a 1.54 ppm proton singlet, and that in turn showed a ROESY cross-peak with the C13-carbinol proton. The position of the anti acetonide in the filipin III was positively identified by these nondestructive NMR experiments, and our speculation about the C15 alkene adopting an axial position in the anti acetonide of **18** was confirmed. In each case that we have examined, the position of *each* syn and anti acetonide ring in a polyacetonide can be assigned using an HMQC experiment combined with a ROESY or NOESY experiment.

## Dermostatin

Dermostatin A is a polyene macrolide antibiotic isolated from *Streptomyces viridogriseus* that shows antifungal activity comparable to amphotericin B.[38] Its flat structure was reported in 1970,[39] but its configuration remained unknown. Its 12 stereogenic centers, along with the unbroken nine-center polyol chain made it a particularly challenging target for stereochemical elucidation. The derivatization of dermostatin A is outlined in Scheme 5. Standard acetonide formation gave three separable polyacetonide fractions that were individually acetylated. Two of the fractions were single components, and the third was a mixture of two polyacetonides that were resolved by HPLC. Dermostatin A has nine free alcohols, and four of the five possible tetraacetonide derivatives were isolated in this two-step protection sequence. Although each of the polyacetonides could be analyzed, the two frame-shifted acetonides **33** and **34** contain all of the stereochemical information necessary to determine the relative configuration of C15−C31 in dermostatin.

The stereochemical assignment of dermostatin cannot be completed using the original [$^{13}$C]acetonide method. The $^{13}$C NMR analysis of tetraacetonides **31**, **32**, and **33** showed that each had three syn acetonides and one anti acetonide. Tetraacetonide **34** was shown to have two syn and two anti acetonide rings by the same method. None of the dermostatin derivatives had only syn or anti rings, so unlike roflamycoin and filipin III, none of the acetonides could be assigned as syn or anti simply by inspection. There are four different ways to arrange the three syn and one anti ring in **33**, and six different ways to arrange the two syn and two anti rings in **34**.[40] None of the acetonide rings in **33** and **34** overlap, so multiplying

**Scheme 5**

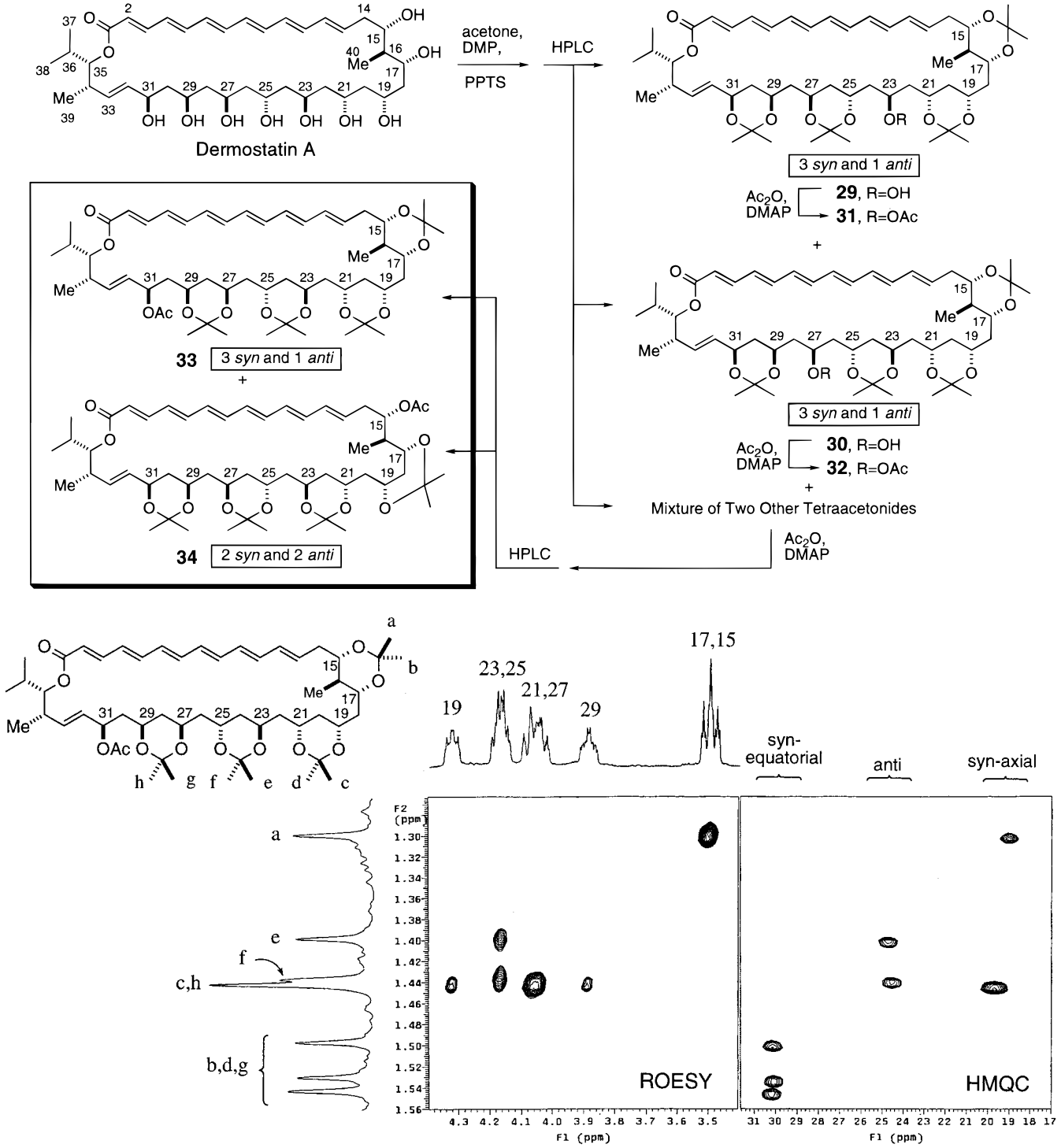



**FIGURE 5.** ROESY (left) and HMQC (right) of the acetonide methyl region for compound 33.

the possibilities gives 24 different stereoisomers that are consistent with the $^{13}C$ NMR data for **33** and **34**. The [$^{13}C$]-acetonide analyses of **31** and **32** introduce additional constraints that reduce the number of possible diastereomers from 24 to six, but that is it. The C15−C17 ring in **33** can be assigned as syn on the basis of $^{1}H$ coupling constants, but that only reduces the number of diastereomers to five. Many other polyacetonides potentially derived from dermostatin A have been considered, but none of them lead to a unique stereochemical solution by the standard [$^{13}C$]acetonide analysis.

The configurational assignment of dermostatin A was trivial using the 2-D [$^{13}C$]acetonide method. The HMQC and ROESY data for tetraacetonide **33** are plotted in Figure 5, with the methyl region of the common $^{1}H$ axis shown to the left. The acetal methyl groups in the HMQC spectrum were classified as syn axial (20 ppm), anti (25 ppm), or syn equatorial (30 ppm) by inspection. The

assignments of the carbinol peaks were made by DQF−COSY analysis. Two of the syn-axial methyl groups, c and h, overlapped in both the $^{13}$C and $^{1}$H NMR spectra, at 19.5 and 1.44 ppm, respectively, but the remaining six methyl signals were easily identified in the HMQC spectrum. The two anti methyl groups at 25 ppm correlated with peaks e and f in the methyl proton region, and these two signals showed ROE cross-peaks with the C25 and C23 carbinol signals at 4.18 ppm. Thus the anti ring in **33** was located at C23−C25, and the three remaining acetonide rings in **33** were syn. A similar analysis of tetraacetonide **34** placed the syn rings at C17−C19 and C29−C31 and the anti rings at C21−C23 and C25−C27. The relative configuration of the C15−C31 section of dermostatin A was completely assigned by analysis of only two derivatives generated from a single protection sequence. Degradation led to the stereochemical assignment at C34 and C35, and the absolute configuration of the polyol was determined by advanced Mosher's analysis of **29**.[21] Using the 2-D $^{13}$C analysis, the relative configuration of the dermostatin polyol was assigned using less than 35 mg of material[41] and was only possible by using the 2-D [$^{13}$C]acetonide method.

The [$^{13}$C]acetonide method arose out of an endeavor to develop new methods for the synthesis of polyols. It has become a useful tool for the stereochemical assignment of polyol chains, and the new 2-D [$^{13}$C]acetonide method promises to be even more powerful. Many of the configurational assignments made using the [$^{13}$C]acetonide analysis have been confirmed by partial or total synthesis. Synthetic chemistry, developed hand-in-hand with new strategies for NMR analysis, forms a powerful synergy for the structure elucidation of natural products.

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