ORGANIC LETTERS

2003
Vol. 5, No. 1
93−96

Complete Stereochemistry of Tetrafibricin

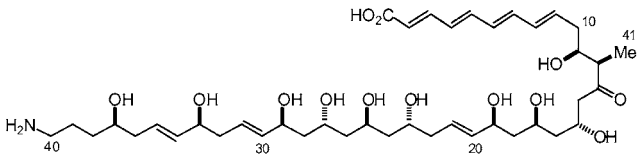
Yoshihisa Kobayashi, Werngard Czechtizky, and Yoshito Kishi*

Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138

kishi@chemistry.harvard.edu

Received November 15, 2002

ABSTRACT

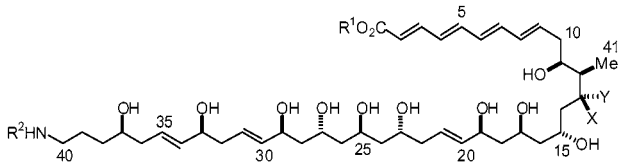


Tetrafibricin (1)

With use of the NMR databases in achiral and chiral solvents, the complete stereochemistry of tetrafibricin (1) has been elucidated without degradation of the carbon framework.

In 1993, Kamiyama and co-workers at Nippon Roche reported the isolation of tetrafibricin (**1**) from *Streptomyces neyagawaensis*.[1] Tetrafibricin (**1**) is the nonpeptidic fibrinogen receptor inhibitor, which shows potent antiaggregation activities on human platelets.[2] Although the gross structure was elucidated through extensive two-dimensional NMR analysis,[3] the stereochemistry still remains to be addressed (Figure 1). We have developed the concept and logic for a universal NMR database approach to assign the relative and absolute configuration of an unknown compound without degradation and/or derivatization.[4] The feasibility, reliability, and applicability of this approach have been demonstrated through the stereochemical assignment of the desertomycin/oasomycin class of natural products,[5] as well as the mycolactones.[6] In this letter, using the NMR database in achiral and chiral solvents, we report the complete stereostructure of tetrafibricin (**1**) without degradation of the carbon framework.

According to the analytical procedure we have previously developed, tetrafibricin (**1**) consists of five independent stereoclusters, i.e., the stereoclusters containing the C11−C12, C15−C19, C23−C29, C33, and C37 stereogenic centers, respectively. For assignment of the complete stereochemistry, it is necessary to establish both the absolute and relative configuration for the first three stereoclusters and the absolute



1: **tetrafibricin** $R^1 = R^2 = H$, X = Y = O (ketone)
2: R^1 = Me, R^2 = Ac, X = H, Y = OH
3: R^1 = Me, R^2 = Ac, X = OH, Y = H

Figure 1. Structure of tetrafibricin (**1**) and 13-dihydro-tetrafibricin **2** and **3**.

(1) Kamiyama, T.; Umino, T.; Fujisaki, N.; Fujimori, K.; Satoh, T.; Yamashita, Y.; Ohshima, S.; Watanabe, J.; Yokose, K. *J. Antibiot.* **1993**, *46*, 1039−1046.

(2) (a) Satoh, T.; Yamashita, Y.; Kamiyama, T.; Watanabe, J.; Steiner, B.; Hadvary, P.; Arisawa, M. *Thromb. Res.* **1993**, *72*, 389−400. (b) Satoh, T.; Yamashita, Y.; Kamiyama, T.; Arisawa, M. *Thromb. Res.* **1993**, *72*, 401−412. (c) Satoh, T.; Kouns, W. C.; Yamashita, Y.; Kamiyama, T.; Steiner, B. *Biochem. J.* **1994**, *301*, 785−791. (d) Satoh, T.; Kouns, W. C.; Yamashita, Y.; Kamiyama, T.; Steiner, B. *Biochem. Biophys. Res. Commun.* **1994**, *204*, 325−332.

(3) Kamiyama, T.; Itezono, Y.; Umino, T.; Satoh, T.; Nakayama, N.; Yokose, K. *J. Antibiot.* **1993**, *46*, 1047−1054.

(4) NMR database for relative stereochemistry: (a) Kobayashi, Y.; Lee, J.; Tezuka, K.; Kishi, Y. *Org. Lett.* **1999**, *1*, 2177−2180. (b) Lee, J.; Kobayashi, Y.; Tezuka, K.; Kishi, Y. *Org. Lett.* **1999**, *1*, 2181−2184. For absolute stereochemistry: (c) Kobayashi, Y.; Hayashi, N.; Tan, C.-H.; Kishi, Y. *Org. Lett.* **2001**, *3*, 2245−2248. (d) Hayashi, N.; Kobayashi, Y.; Kishi, Y. *Org. Lett.* **2001**, *3*, 2249−2252. (e) Kobayashi, Y.; Hayashi, N.; Kishi, Y. *Org. Lett.* **2001**, *3*, 2253−2255.

10.1021/ol0272895 CCC: $25.00
Published on Web 12/13/2002

configuration for the last two. If there were a method available for assigning the relative configuration at the remote stereogenic centers, the problem in hand could be simplified significantly.

In this context, we wondered whether the stereochemical information of one stereocluster is transferred to the other through a ketone via a hydrogen-bonding network.[7] If a meaningful degree of communication were detected between the two stereoclusters, the C11−C19 portion could be considered as a single stereocluster. With this expectation, we first studied the NMR behavior of the *syn*- and *anti*-1,5-dihydroxy-3-ketones (Figure 2). Experimentally, however,

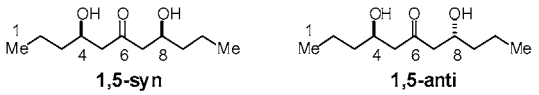


Figure 2. Structures of 1,5-dihydroxy-3-ketones.

it was found that the degree of the communication between the C4 and C8 carbons is negligibly small.[8] Thus, we searched for an alternative method and recognized that, if the C13 ketone is reduced to the corresponding alcohol(s), the two independent stereoclusters in question should be treated as one stereocluster; consequently, the relative configuration between the original two stereoclusters could be assigned. Interestingly, Kamiyama and co-workers already reported the detailed NMR data in DMSO-d_6 for two C13 alcohols **2** and **3** derived from **1**.[3] For this reason,[9] we focused on **2** and **3**.

Because of its self-contained nature, the center carbon indicated by the dot in the five-carbon framework, shown in the box in Figure 3, exhibits a unique chemical shift, which is dependent on the relative stereochemistry of substituents present within this framework but independent from the stereochemistry of substituents present outside this framework. The chemical shift in NMR databse **A** for the central carbon, indicated by the dot (Figure 3), of *syn/syn*-, *syn/anti*- and *anti/syn*-, and *anti/anti*-1,3,5-triols was found to be around 68, 66, and 64 ppm in DMSO-d_6, respectively, but was found to be insensitive to the functionalities present outside of this carbon framework.[10] Thus, a chemical shift analysis of the central carbon in a 1,3,5-triol moiety allows

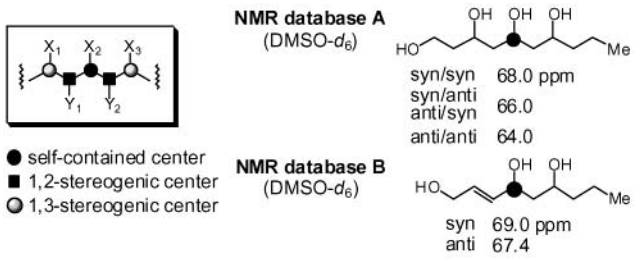


Figure 3. NMR databases **A** and **B** for elucidation of the relative configuration of 1,3,5-triols and 1,3-diols.

us to predict its relative configuration. The following predictions can be made from the NMR data reported for **2**.[3] The C15 chemical shift (δ = 67.8 ppm in DMSO-d_6) is typical for *syn*/*syn*-1,3,5-triols, suggesting the C13/C15/C17 relative configuration to be syn/syn. Similarly, the C25 (63.9 ppm) and C27 (63.8 ppm) chemical shifts are typical for *anti*/*anti*-1,3,5-triols, predicting the relative configuration of both C23/C25/C27 and C25/C27/C29-triols to be anti/anti, respectively, and consequently the relative configuration of the C23−C29 tetraol to be all anti.

On the contrary, as the reported C17 chemical shift (67.2 ppm) lies between the value expected for typical *syn*/*syn*- and *syn*/*anti*-(or *anti*/*syn*-)1,3,5-triols, these data alone do not yield a conclusive assignment. In this connection, the NMR database we have previously reported provides valuable information. The chemical shift marked with a dot in NMR database **B** is diagnostic for distinguishing *syn*- from *anti*-diols (Figure 3).[10] The C19 chemical shift reported for **2** is 69.4 ppm, very close to the value found for the *syn*-diol (C17/C19) of NMR database **B**.[11] Thus, the relative configuration at C15/C17/C19 must be either syn/syn or anti/syn but not syn/anti. Combined with the C13/C15/C17 relative configuration (syn/syn, vide ante), the possibility of anti/syn can be eliminated, thereby establishing the C15/C17/C19 relative configuration to be syn/syn. Consistent with this conclusion, the C15 chemical shift reported for **3** (C13-epimer of **2**) is 65.9 ppm, a typical value for a *syn*/*anti*-(or *anti*/*syn*-)1,3,5-triol, predicting the C13/C15/C17 relative configuration of **3** to be anti/syn.

As reported previously, the methyl group marked by a dot in the NMR database **C** (Figure 4) is a degenerate, self-contained carbon, and its chemical shift is diagnostic for assigning the relative configuration of 2-methyl-1,3-diols.[12] The C41 chemical shift (δ = 7.5 ppm in DMSO-d_6) reported for **2** suggests its C11/C12/C13 relative configuration to be syn/syn as indicated in Figure 1. This assignment is further supported by the C41 chemical shift (9.8 ppm) reported for **3** (C13-epimer of **2**), which indicates the C11/C12/C13 relative configuration to be syn/anti.

(5) (a) Kobayashi, Y.; Tan, C.-H.; Kishi, Y. *Angew. Chem., Int. Ed.* **2000**, *39*, 4279−4281. (b) Tan, C.-H.; Kobayashi, Y.; Kishi, Y. *Angew. Chem., Int. Ed.* **2000**, *39*, 4282−4284. (c) Kobayashi, Y.; Tan, C.-H.; Kishi, Y. *J. Am. Chem. Soc.* **2001**, *123*, 2076−2078.

(6) (a) Benowitz, A. B.; Fidanze, S.; Small, P. L. C.; Kishi, Y. *J. Am. Chem. Soc.* **2001**, *123*, 5128−5129. (b) Fidanze, S.; Song, F.; Szlosek-Pinaud, M.; Small, P. L. C.; Kishi, Y. *J. Am. Chem. Soc.* **2001**, *123*, 10117−10118.

(7) β-Hydroxycarbonyl compounds are known to form hydrogen-bonds between the hydroxyl groups and the lone pairs of the carbonyl oxygen in a solid state by X-ray and in a solution state by IR and ^{1}H NMR. Sharma, V.; Simard, M.; Wuest, J. D. *J. Org. Chem.* **1994**, *59*, 7785−7792.

(8) For details, see Supporting Information.

(9) There are two additional reasons for this decision: (1) DMSO and MeOH have been extensively tested for the NMR database approach; however, the NMR data of **1** were reported only in D_2O. (2) The chemical stability of **2** and **3** appears to be better than that of **1** (ref 3).

(10) Kobayashi, Y.; Tan, C.-H.; Kishi, Y. *Helv. Chim. Acta* **2000**, *83*, 2562−2571.

(11) By employing this database, the relative configuration at C27/C29 is also assigned as anti (C29 of **2**, 67.7 ppm).

(12) Other examples of 2-methyl-1,3-diol for anti/syn (or syn/anti) and anti/anti are found at 9.8−10.6 ppm and 11.0−12.2 ppm in DMSO-d_6, respectively. For details, see ref 5c.

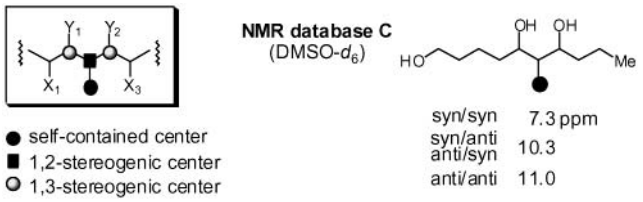


Figure 4. Structures of NMR database **C** for elucidation of the relative configuration of 2-methyl-1,3-diols.

Overall, the analysis of the ^{13}C chemical shifts reported for the 13-dihydrotetrafibricins (**2** and **3**) allowed us to assign the relative configuration at C11−C19 and C23−C29 as indicated (Figure 1). To establish the complete stereostructure of tetrafibricin (**1**), it was necessary to establish the absolute configuration of at least one stereogenic center present in each of the four stereoclusters C11−C19, C23−C29, C33, and C37. The NMR database approach in chiral solvents recently developed in this laboratory is ideally suited for this purpose.

Two types of chiral solvents, mono- and bidentate solvents, represented by DMBA (*N*,α-dimethylbenzylamine)[4c] and BMBA-*p*-Me (vide infra),[13] have been developed; the bidentate solvents are powerful for assigning the absolute configuration of a stereochemically isolated, saturated secondary alcohol (separated by a two-or-more methylene bridge from other alcohols), whereas the monodentate solvents are for assigning the absolute configuration of more than two alcohol-containing stereoclusters.

Our first experiment was to determine the ^{13}C chemical shift differences ($\Delta\delta_{R-S}$) for **3** in perdeuterated (*R*)- and (*S*)-DMBA.[14,15] Figure 5 shows the significant ^{13}C $\Delta\delta_{R-S}$

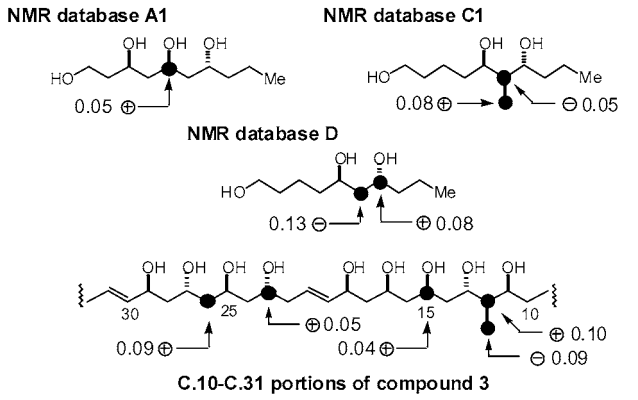


Figure 5. Observed signs and values (in parts per million) of $\Delta\delta_{R-S}$ in chiral NMR solvent, DMBA, of compound **3** and NMR database **A1**, **C1**, and **D** for elucidation of the absolute configuration.

detected.[16] A comparison of these $\Delta\delta_{R-S}$ values with the $\Delta\delta_{R-S}$ values observed for NMR databases **A1**, **C1**, and **D**[4d] allowed us to conclude the absolute configuration of the C10−C30 portion of **3** as indicated.

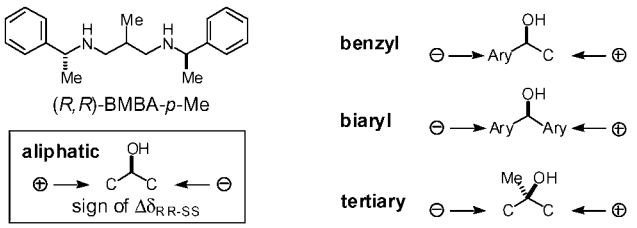


Figure 6. Structure of bidentate chiral NMR solvent, BMBA-*p*-Me, and the general trend of $\Delta\delta_{RR-SS}$ for the adjacent carbon exhibited by isolated alcohols.

Our second experiment was to establish the ^{13}C NMR chemical shift differences ($\Delta\delta_{RR-SS}$) for **3** in perdeuterated (*R*)- and (*S*)-BMBA-*p*-Me (Figure 6).[17,18] As long as both adjacent carbons are sp^3-carbons, a secondary alcohol exhibits the general trend of $\Delta\delta_{RR-SS}$ for the adjacent carbon as summarized in the box in Figure 6.[13] However, we have recently observed the opposite trend of $\Delta\delta_{RR-SS}$ for benzyl, biaryl, and tertiary alcohols.[19] Specifically related to the current case, we synthesized and studied the behavior of $\Delta\delta_{RR-SS}$ of **E** and **F** (Figure 7).[20,21] Upon comparison with

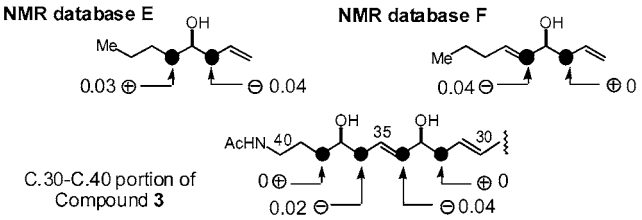


Figure 7. Observed signs and values (in parts per million) of $\Delta\delta_{RR-SS}$ in bidentate chiral NMR solvent, BMBA-*p*-Me, of NMR databases **E** and **F** for the absolute configuration and compound **3**.

these NMR databases, the $\Delta\delta_{RR-SS}$ observed for the C32/C34 and the C36/C38 carbons of **3** (and **2**) suggested the absolute configurations at C33 and C37 as shown in Figure 7.

(13) Kobayashi, Y.; Hayashi, N.; Kishi, Y. *Org. Lett.* **2002**, *4*, 411−414.

(14) We gratefully acknowledge Dr. Kamiyama at Nippon Roche for a generous gift of 20 mg of tetrafibricin (**1**). By following Kamiyama's procedure (ref 3), this sample was converted to a mixture (12 mg) of **2** and **3**.

(15) For details, see Supporting Information of ref 4c.

(16) For detailed conditions of the measurement, see Supporting Information.

(17) For preparation of perdeuterated BMBA-*p*-Me, see Supporting Information.

(18) ^{13}C NMR data of **2** and **3** were obtained in a 5:2:1 w/w mixture of perdeuterated BMBA-*p*-Me (350 mg) and $CDCl_3$ (140 mg)/DMSO-d_6 (70 mg).

(19) Kobayashi, Y.; Hayashi, H.; Kishi, Y. Unpublished results.

(20) Compounds **E** and **F** were prepared by Brown's asymmetric allylation. For details, see Supporting Information.

(21) ^{13}C NMR data of NMR databases **E** and **F** were obtained in BMBA-*p*-Me (350 mg)/$CDCl_3$ (140 mg) (5:2 w/w).

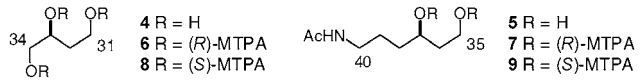


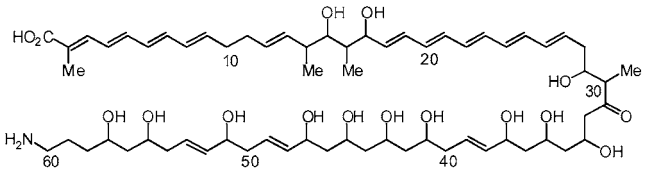
Figure 8. Mosher's esters of degradation products of tetrafibricin (**1**).

For the case of a stereocluster containing more than two groups, more than two independent sets of $\Delta\delta_{R-S}$ can, in principle, be found for assigning the absolute configuration of a given stereocluster. In other words, a cross-checking system is installed for this method. Indeed, the two independent sets of $\Delta\delta_{R-S}$ in DMBA were found, to assign the absolute configuration for both the C11−C19 and C23−C29 stereostructures (Figure 5).

On the other hand, only one set of the $\Delta\delta_{RR-SS}$ is available for deducing the absolute configuration of a given isolated alcohol: the absolute configuration at C33 and C37 was predicted from one set of $\Delta\delta_{RR-SS}$ in BMBA-*p*-Me (Figure 7).

Partly because of this reason and partly because this was the first example of an application of the BMBA-*p*-Me method to an unknown natural product, we decided to confirm the predicted absolute configuration at C33 and C37 by chemical synthesis. In practice, we first synthesized (*R*)- and (*S*)-Mosher esters **6**−**9** of (*S*)-butane-1,2,4-triol (**4**) and (*R*)-diol amide **5** (Figure 8).[22] With these authentic samples in hands, we then subjected a mixture of **2** and **3** to ozonolysis, reduction, and esterification with Mosher acid chloride, to furnish the expected degradation products. Upon comparison of ^{1}H NMR spectra, the two corresponding (*R*)-Mosher esters derived from the natural product were found to be identical to synthetic (*R*)-Mosher esters **6** and **7** but different from synthetic (S)-Mosher esters **8** and **9**, confirming the predicted absolute configuration at C33 and C37.

Last, we should comment on the structure of two natural products, linearmycin A (**10**)[23] and lienomycin (**11**),[24] closely related to tetrafibricin (**1**) (Figure 9). There is no stereochemical information available for **10**. However, on the basis of the chemical shifts reported for **10**, we predict the relative configuration at C15/C16/C17, C35/C37, C41/C43/C45/C47,



linearmycin A (**10**): The reported ^{13}C-NMR chemical shift of C16Me, C37, C43, C45 and C57 suggests the relative configuration to be *syn*/*syn*-C15/C16/C17, *syn*-C35/C37, *anti*/*anti*/*anti*-C41/C43/C45/C47, *syn*-C55/C57, respectively.

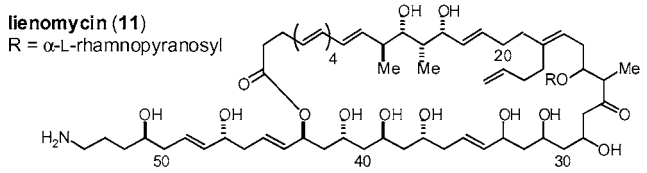


Figure 9. Reported structures of linearmycin A (**10**) and lienomycin (**11**).

and C55/C57 to be syn/syn, syn, anti/anti/anti, and syn, respectively.[25] In the case of lienomycin (**11**), the partial stereostructure was deduced by Nakanishi and co-workers through a combination of degradation, $[\alpha]_D$, ^{1}H NMR, and MS analysis (Figure 9).[26] We believe that the method reported here can be extended to establish the complete stereostructure of both linearmycin A (**10**) and lienomycin (**11**).

In conclusion, we have elucidated the complete stereostructure of tetrafibricin (**1**) via an NMR database approach in achiral and chiral solvents. This conclusion has been derived from the analyses of ^{13}C NMR profiles of 13-dihydrotetrafibricin only. In our view, this example further illustrates the reliability, applicability, and versatility of the universal NMR database approach in achiral and chiral solvents.

Acknowledgment. We gratefully acknowledge Dr. Kamiyama at Nippon Roche for a generous gift of tetrafibricin. Financial support from the National Institutes of Health (NS 12108) is gratefully acknowledged.

Supporting Information Available: Experimental procedures and data for compounds mentioned herein. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0272895

(22) Compound **5** was prepared by acid-catalyzed hydrolysis of the benzylidene acetal reported in ref 26.

(23) (a) Sakuda, S.; Guce-Bigol, U.; Itoh, M.; Nishimura, T.; Yamada, Y. *Tetrahedron Lett*. **1995**, *36*, 2777−2780. (b) Sakuda, S.; Guce-Bigol, U.; Itoh, M.; Nishimura, T.; Yamada, Y. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2315−2319.

(24) Gauze, G. F.; Maksimova, T. S.; Olhovatova, O. L.; Kudrina, J. S.; Iltchenko, G. B.; Kotchetkova, G. V.; Volkava, L. I. *Antibiotiki* **1971**, *5*, 387−390.

(25) NMR data of **10** were reported in CD_3OD. For the detailed analysis, see Supporting Information.

(26) Pawlak, J.; Nakanishi, K.; Iwashita, T.; Borowski, E. *J. Org. Chem.* **1987**, *52*, 2896−2901.