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A Minimalist NMR Approach for the Structural Revision of Mucoxin

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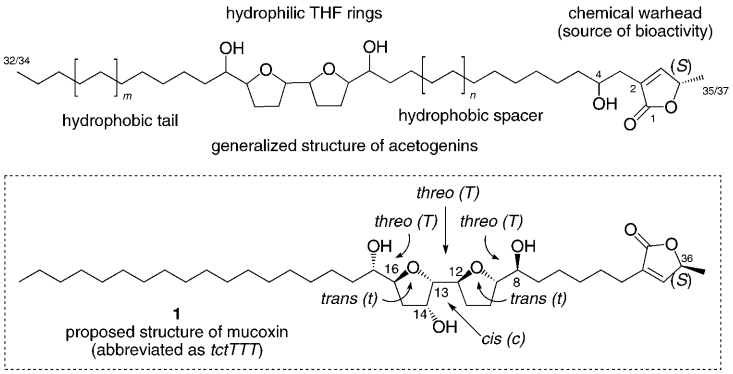
Abstract: In an attempt to revise the structural assignment of mucoxin, and faced with 64 diastereomeric possibilities, we resorted to the synthesis of truncated structures that contained the core stereochemical sites. Twelve stereochemical analogues were synthesized, their ^{1}H and ^{13}C NMR spectra were analyzed and four recurring stereochemical trends were distilled from the data. Applying the observed trends to the diastereomeric population pared the possible choices for the correct structure of mucoxin from 64 to 4. Synthesis of these analogues led to the identification of the correct structure of mucoxin.

Keywords: acetogenins · mucoxin · natural products · NMR spectroscopy · structure elucidation

Introduction

With the exception of X-ray crystallography, which yields unequivocal assignment of structure for organic molecules and new natural products, total synthesis is still the gold standard for proof of structure. With a large number of natural product families that do not succumb to crystallization, the initial structural assignment is often a non-trivial exercise. Expectedly, errors in structure determination are common (most often realized through synthesis of the proposed structure), and the problem is exacerbated since an error in stereochemical determination of one site of the molecules can lead to significant changes in the chemical shift of nearby nuclei, realizing that most natural products are complex molecules containing multiple stereocenters. Error in assignment of one or a combination of stereocenters leads to observed mismatches between the NMR spectra of the authentic natural compound and the synthetic material. Often the magnitude of chemical shift discrepancies for individual centers cannot be used reliably for identification of misidentified stereocenters (particularly with compounds that have contiguous stereocenters), since changes in relative configuration can lead to a number of different conformations, rotamers, and ring geometries. These have a great effect on the chemical environment of the resonating nuclei and thus yield large changes in chemical shift. Therefore, it is not uncommon that a complete set of possible diastereomers must be synthesized in order to discover the real structure of a misidentified natural product. Recently, we faced this exact problem during the synthesis of mucoxin, a reportedly bioactive Annonaceous acetogenin (Scheme 1).[1] With the mismatch observed between the reported NMR spectrum and that of our synthesized material we were faced with the possibility of having to synthesize 64 different diastereomers. To avoid this daunting task we resorted to



Scheme 1. General structure of Annonaceous acetogenins. Most but not all have two disubstituted THF rings flanked by two hydrophobic chains. On one end, a butenolide ring system is a common structural feature for this class of molecules. Hydroxylation of the hydrophobic spacer is seen but is not a common or necessary feature for activity. Dashed box: The proposed structure of mucoxin (arbitrarily drawn with absolute configuration based on the proposed relative configuration) is unique due to the ring hydroxylation.

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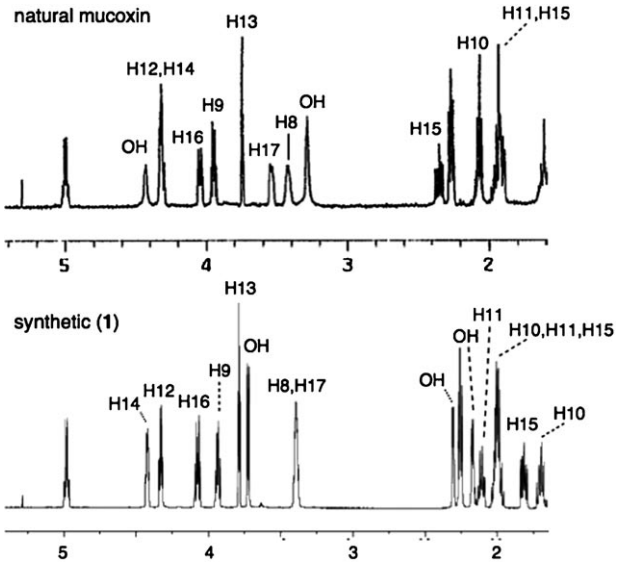
synthesize a few selected diastereomers with the intention of utilizing spectral trends obtained from the study of these diastereomers to focus the field of possibilities to only a handful of choices. Chemical shift correlations have been used previously as a guide for structure determination for acetogenins. Hoye and Suhadolnik et al., in an elegant set of experiments, demonstrated the utility of chemical shift correlations for relative configurational assignment of uvaricin.[2,3] Curran and co-workers performed an exhaustive study of a stereoisomeric library of murisolin, illustrating the use of Mosher ester derivatives for configurational assignment of single THF containing acetogenins.[4,5] Herein, we take a complimentary approach to previous studies to assign the relative configurational relationships in mucoxin. A brief summary of our initial investigation, which led to the latter strategy for the identification of the real structure of mucoxin is provided below.

Annonaceous acetogenins are a series of natural products isolated from the plant family *Annonaceae*, whose members include edible fruits such as cherimoya and custard apple, and are widely distributed in tropical and sub-tropical regions.[1–9] They are found to have potent and diverse biological effects such as cytotoxic, antitumor, antimalarial, pesticidal, and insecticidal activities.[6–13] The acetogenins share a common skeleton characterized by an unbranched C32 or C34 fatty acid ending in an α,β-unsaturated γ-lactone (Scheme 1).[7,12,14] Several oxygenated functional groups, such as tetrahydrofuran (THF), tetrahydropyran (THP), epoxide, hydroxy, and ketone could be present in the structure, as well as double and triple bonds. There is a variety of stereoisomers of the center THF cores, however, all isolated compounds have an *S* configuration of the chiral center in the γ-lactone ring. Generally acetogenins are divided into two broad groups. Classical acetogenins contain up to three 2,5-disubstituted THF units along with a terminal γ-lactone. Nonclassical acetogenins contain either a THP or a hydroxylated THF ring, and in some examples in combination with a 2,5-disubstituted THF ring. Although to date there are more than 400 known acetogenins, their isolation and structure determination can be tedious since only small amounts in complex mixtures are obtained from natural sources.[10,12] Moreover, most acetogenins are waxes or gums, and cannot be crystallized for X-ray crystallographic analysis in order to determine the relative and absolute configuration of their stereogenic centers.

Mucoxin, an example of nonclassical acetogenins, was isolated from the bioactive leaf extracts of *Rollinia mucosa* by McLaughlin and his co-workers in 1996.[15] It has exhibited potent and selective cytotoxic properties against PACA-2 (pancreatic cancer) and MCF-7 (breast cancer) cell lines in a panel of six human solid tumors (in vitro assays), however, limited access to the material (1.8 mg was isolated from natural sources) has hampered further biological evaluations.[15] We have recently disclosed the total synthesis of mucoxin (the proposed structure) and after careful consideration concluded that the stereochemical assignment was wrong.[1] Table 1 summarizes the observed chemical shifts for both

Table 1. ^{1}H NMR comparison of the natural and synthetic mucoxin (NMR spectra recorded in $CDCl_3$).[a]



H–C	**1**[b]	Natural mucoxin	Δδ (**1**-natural)
H8	3.41	3.42	−0.01
H9	3.96	3.95	+0.01
H10	1.69, 2.02	1.91, 2.05	**−0.22**, −0.03
H11	2.02, 2.13	1.91, 2.05	**+0.11**, +0.08
H12	4.35	4.31	+0.04
H13	3.80	3.71	+0.09
H14	4.44	4.32	**+0.12**
H15	1.84, 2.02	1.91, 2.35	−0.07, **−0.33**
H16	4.09	4.04	+0.05
H17	3.41	3.53	**−0.12**
C8	73.6	73.6	0.0
C9	84.1	83.4	+0.7
C12	79.3	78.9	+0.4
C13	83.3	83.5	−0.2
C14	74.7	72.8	**+1.9**
C15	39.0	38.0	**+1.0**
C16	81.6	79.9	**+1.7**
C17	74.3	73.7	+0.6

[a] Deviation greater than 0.1 ppm for proton and 1.0 ppm for carbon are highlighted in bold. [b] The data in all Tables for all synthesized analogues in this study have been referenced to 7.27 ppm ($CDCl_3$) to be comparable with the published data for mucoxin.

the naturally isolated compound and the synthetically prepared material. A close inspection of the data reveals the problem alluded to above with regards to identifying site(s) that are stereochemically misassigned based on the gross changes in chemical shift of each stereocenter; namely, most of the chiral centers exhibit large differences that hamper the formulation of a reasonable hypothesis as to the source of the stereochemical misassignment. Excluding the chiral center in the butenolide, mucoxin has seven closely situated stereocenters, which translates to 64 possible diastereomers. In the present study, we resorted to the synthesis of a small subgroup of diastereomers such that trends in the ^{1}H and ^{13}C NMR spectra could be derived and subsequently used to pare down the number of possible structures. The choice of diastereomers synthesized is critical to provide a sufficient

grouping of various relative stereochemical relationships so that conclusive trends could be obtained. Via the latter strategy, we demonstrate how with 12 diastereomeric structures we were able to narrow the field of 64 possible diastereomers to four candidates, leading to the identification of two stereochemical mistakes and the eventual structural revision of mucoxin.

Results and Discussion

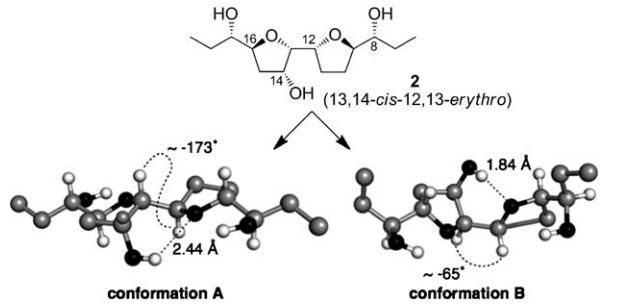
We have re-examined McLaughlin's structural elucidation of mucoxin[15] to delineate any ambiguities in their proposed structure and possible sources of discrepancies between the synthetic and the natural spectra. Based on the reported COSY and HRMS analysis of the natural sample of mucoxin, we find no reason to question the proposed constitutional structure of mucoxin. Therefore, we believe that the differences in the spectra of the synthetic versus natural samples are most likely due to stereochemical mismatches. In the case of natural mucoxin, the relative configuration of the 2,5-substitution for both THF rings (C9–C12 and C13–C16) was assigned as *trans* based on the lack of NOESY correlations. However, lack of NOE can be misleading in the determination of relative configuration, especially with five-membered ring systems.

We next turned to evaluate the assignment for the relative configuration of the C12, C13, and C14 triad. In the NMR spectrum of natural mucoxin, H13 appeared as a pseudo triplet ($J=3$ Hz). Based on this and molecular models, the authors proposed that the rotation of the C12–C13 bond might be restricted possibly due to intramolecular hydrogen bonding between the C14 hydroxyl and the C9–C12 THF ring oxygen.[15] In such a rigid conformation, it was suggested that in order for H13 to maintain a 3 Hz coupling constant with H12 and H14, the relative configuration of the two THF rings must be *threo* and H14 and H13 must be *cis*.

This stereochemical assignment seems tenuous since the only experimental evidence presented is the coupling constant of H13 with H12 and H14. To gain further insight into the conformations of the natural product, we carried out Monte Carlo based conformational studies (MacroModel version 7.0) of the proposed structure and its epimers at C12 and C14. Modeling of the 13,14-*cis*-12,13-*threo* compound led to structures (within 2 kcal) with dihedral angles necessary for the small observed 3 Hz coupling (consistent with the proposed structure). However, similar analysis of the 13,14-*cis*-12,13-*erythro* diastereomer (**2**) provided low energy conformers (within 2 kcal) that could also exhibit the observed 3 Hz coupling. Conformational minimization of 13,14-*cis*-12,13-*erythro* isomer **2** leads to a series of conformers with 50% population in agreement with conformation **B** (Table 2). The ~65° dihedral angle between H12 and H13 would yield a small coupling constant (in order of 3 Hz), consistent with the observed NMR spectrum of the natural product. Notably, the OH–O distance between C14-OH and C9–C12 THF oxygen in **B** is about 1.84 Å whereas that in **A** is 2.44 Å. Thus, the 13,14-*cis*-12,13-*erythro* conformation with strong intramolecular H-bonding results in the expected small coupling constant, and therefore, contrary to the argument used for structural assignment of mucoxin, it is possible for the *cis–erythro* compound to exhibit the observed 3 Hz coupling. As anticipated, the H12/H13 dihedral angle does not change to a great extent in any of the conformers. We further confirmed our modeling results by measuring the NMR spectrum of **1** (proposed structure of mucoxin) in methanol. H13 appears as a triplet with a 3 Hz coupling constant in $CDCl_3$, however, in CD_3OD H13 appeared as a doublet of a doublet ($J=3.3$ and 7.3 Hz). While the coupling constant between H13 and H14 is expected to be more or less independent of the solvent, the J value between H12 and H13 could vary with the nature of the solvent if intramolecular H-bonding is disturbed. Thus, as predicted by modeling the non-H-bonded rotamer (longer OH–O bond length) leads to a >170° dihedral angle and the larger observed coupling constant (7.3 Hz).

In a similar fashion, we have examined the 13,14-*trans*-12,13-*threo* and 13,14-*trans*-12,13-*erythro* systems, and find it difficult to eliminate these possibilities upon further analysis and in comparison to the reported spectroscopic data for mucoxin. At this juncture it became clear that many alternate isomers are possible, and since the synthesis of each isomer would be unfeasible, we resorted to an alternate approach. The goal was to derive guides for NMR chemical shifts that were unique to specific stereochemical relation-

Table 2. Conformational analysis of the 13,14-*cis*-12,13-*erythro* structure.[a]



Conformer	Relative energy [kcal mol^{-1}]	OH–O[b] [Å]	θ_{13-14}[c]	θ_{12-13}[d]
1	0.00	2.44	−34.2	−173
2	0.31	2.44	−34.2	−173
3	0.62	2.43	−34.1	−173
4	0.69	2.37	−32.7	−171
5	0.73	1.84	−40.0	−65.4
6	0.94	1.84	−40.0	−65.7
7	0.95	2.35	−32.6	−173
8	1.13	1.84	−40.0	−65.6
9	1.22	2.44	−34.0	−173
10	1.30	1.85	−39.3	−64.4
11	1.33	1.85	−39.5	−64.5
12	1.43	1.85	−40.0	−64.6
13	1.54	2.44	−33.9	−173
14	1.64	1.85	−39.3	−64.5

[a] Non-stereogenic hydrogen atoms are omitted for clarity. [b] Distance between C14-OH and C9–C12 THF oxygen. [c] H_{13}–H_{14} dihedral angle. [d] H_{12}–H_{13} dihedral angle.

ships. These could be utilized not only to pare down the number of possibilities for mucoxin, but also would add to the library of chemical shift correlations that has been growing through mostly the efforts of Kishi and co-workers.[16–20] Although tedious to accumulate, chemical shift correlations are immensely useful for the structural determination of natural products and also for verification of structural assignment in synthetic projects.

Strategy for selection of representative structures

Figure 1 depicts the list of all possible diastereomeric structures of mucoxin without the inclusion of the C36 stereogenic center (assumed to be *S* by inference from all known structures of acetogenins). Scheme 1 illustrates the short hand codes used to identify each acetogenin. The first three lower case letters refer to the stereochemical relationships that are either *cis* (*c*) or *trans* (*t*). Placing the butenolide on the right hand side, the *cis*/*trans* relationships are read from left to right. Thus, the proposed structure of mucoxin (**1**, Scheme 1) is *tct* (C16–C13 *trans*; C14–C13 *cis*; C12–C9 *trans*). Similarly, *erythro* (*E*) and *threo* (*T*) relationships that would fully describe the stereochemical picture of each diastereomer are given as the last three letters in capitals (C17–C16 *threo*; C13–C12 *threo*; C9–C8 *threo*, thus *TTT* for structure **1**).

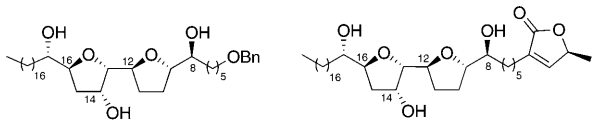
cccEEE	cccTEE	cccETE	cccEET	cccETT	cccTET	cccTTE	*cccTTT*
cctEEE	cctTEE	cctETE	cctEET	**cctETT**	**cctTET**	**cctTTE**	***cctTTT***
ctcEEE	ctcTEE	ctcETE	ctcEET	ctcETT	ctcTET	ctcTTE	ctcTTT
tccEEE	tccTEE	tccETE	tccEET	tccETT	**tccTET**	tccTTE	tccTTT
cttEEE	cttTEE	cttETE	cttEET	cttETT	cttTET	cttTTE	**cttTTT**
ttcEEE	ttcTEE	ttcETE	ttcEET	ttcETT	ttcTET	ttcTTE	*ttcTTT*
tctEEE	tctTEE	tctETE	tctEET	**tctETT**	**tctTET**	**tctTTE**	**tctTTT**
tttEEE	tttTEE	tttETE	tttEET	tttETT	**tttTET**	tttTTE	***tttTTT***

Figure 1. List of 64 possible diastereomers considering only the stereogenic centers between C8 and C17. Isomers in bold were chosen as initial synthetic targets. Isomers highlighted in italics satisfy the restrictions concluded after analysis of the data in Tables 5–8.

As described above, the goal was to select 12 diastereomers for synthesis and complete NMR assignment. Dependence of chemical shift changes in both ^{1}H and ^{13}C NMR spectra as a function of relative stereochemical relationships were sought in order to narrow down the number of diastereomeric possibilities. The selection of the 12 diastereomers was governed by two major factors. First and foremost, it was necessary to have a list of structures that would yield redundant stereochemical relationships at each site except for the location that was changed. This would ensure that if a trend is observed from the chemical shift analysis for each diastereomeric relationship (such as C9–C8 *erythro* vs *threo*), then it must have been immune to stereochemical changes in other parts of the molecule. Second, we relied on the synthetic scheme developed for **1** to synthesize the proposed diastereomers, and therefore took advantage of interjecting into the scheme at opportune locations that would yield the necessary changes with simple chemistry (most often by changing chiral auxiliaries for the Sharpless Asymmetric Dihydroxylation (SAD) reaction or by selective deprotection of hydroxyl groups followed by an oxidation-reduction sequence). Structures in bold in Figure 1 list the chosen diastereomers for synthesis.

Prior to the synthesis of the proposed diastereomers we demonstrated that a truncated structure containing the bis-THF core that includes all stereogenic centers in doubt is sufficient for spectral comparisons, thus eliminating the need to synthesize the full-length molecules. This is illustrated in Table 3, in which the ^{1}H NMR spectrum of the truncated *tctTTT* molecule **A** is compared to the full-length *tctTTT* acetogenin **1** (proposed structure of mucoxin). There was less than 0.02 ppm deviation between protons on C8 to C17 of the truncated and full length molecule. This allowed the use of simpler truncated structures in an effort to determine the structure of mucoxin.

Table 3. ^{1}H NMR comparison of a truncated structure with the full length acetogenin (proposed structure of mucoxin) (NMR spectra recorded in $CDCl_3$).



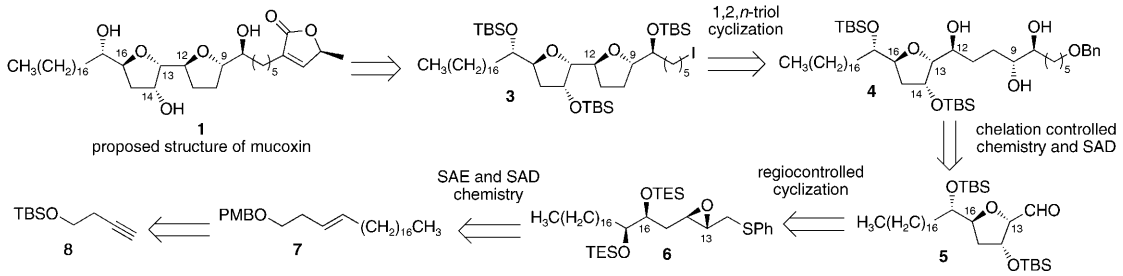
A
tctTTT truncated model

1
tctTTT full length
(proposed structure of mucoxin)

H–C		*tctTTT* Model	Proposed mucoxin	Δδ Value [ppm]
8	H	3.41	3.41	0.00
	C	73.4	73.6	−0.2
9	H	3.95	3.96	−0.01
	C	83.9	84.1	−0.2
10	H	1.71	1.69	+0.02
	H	2.03	2.02	+0.01
11	H	2.03	2.02	+0.01
	H	2.13	2.13	0.00
12	H	4.34	4.35	−0.01
	C	79.0	79.3	−0.3
13	H	3.80 (t, J = 3.4 Hz)	3.80 (t, J = 3 Hz)	0.00
	C	83.1	83.3	−0.2
14	H	4.43	4.44	−0.01
	C	74.4	74.7	−0.3
15	H	1.83	1.84	−0.01
	H	2.03	2.02	+0.01
	C	38.7	39.0	−0.3
16	H	4.09	4.09	0.00
	C	81.3	81.6	−0.3
17	H	3.41	3.41	0.00
	C	74.1	74.3	−0.2

Synthesis of truncated diastereomers of mucoxin

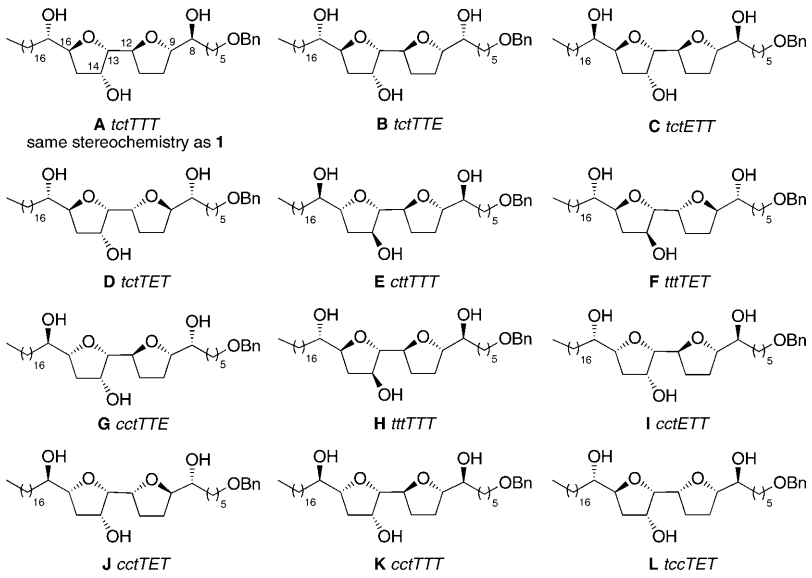
Scheme 2 summarizes the strategies utilized to establish the stereogenic centers in **1**. The stereochemistries of C17–C16 and C9–C8 were established via Sharpless asymmetric dihy-



Scheme 2. Retrosynthetic strategy used to synthesize the proposed structure of mucoxin. Most of the stereogenic centers were established via the use of either Sharpless Asymmetric Dihydroxylation (SAD) or Sharpless Asymmetric Epoxidation (SAE), thus enabling the use of appropriate ligands to effect stereochemical inversion.

droxylation, while the stereochemistry of C14–C13 originates from Sharpless asymmetric epoxidation.[1] The stereochemistry at C12 was arrived at via chelation controlled addition of an organometallic reagent with an incipient aldehyde. The trisubstituted tetrahydrofuran ring in **5** was closed regioselectively with the aid of the thiophenyl directed epoxydiol cyclization,[21] while the disubstituted ring in **3** was furnished through the 1,2,*n*-triol cyclization.[22] Inspection of the synthetic strategy indicates the ease by which stereogenic changes to the molecule can be accomplished. These could be either through changes in the ligands used for asymmetric induction or by inversion of stereochemistry of the three hydroxyl groups present in the target molecule. The synthetic details by which the 12 diastereomers depicted in Scheme 3 were synthesized are relegated to the Supporting Information. It should be noted, however, that the stereochemistry of each diastereomer was rigorously confirmed by a battery of 1D and 2D NMR experiments.

Comparison of spectral data of diastereomers A–L with mucoxin and structural revision

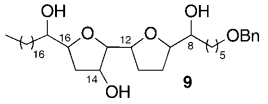


Scheme 3. Structure of synthesized truncated diastereomers of mucoxin.

Upon completion of the syntheses and inspection of the ^{1}H and ^{13}C NMR data, none of the spectra from 12 isomers **A**–**L** (Scheme 3) fully matched the spectra obtained for natural mucoxin. In order to efficiently compare the data for each of the isomers with natural mucoxin, the deviations of ^{1}H and ^{13}C NMR chemical shift between the truncated structures and the reported natural mucoxin are tabulated in Table 4. The data of protons exhibiting differences greater than 0.1 ppm and carbons exhibiting differences greater than 1.0 ppm are shown in bold. A careful analysis of the data enabled the extraction of four reoccurring trends. These are listed below:

1) ***C8–C9 relationship***: Table 5 lists the chemical shifts observed for H8–C8 for analogues **A**–**L**. The chemical shift of H8 in the 8,9-*erythro*-isomers is shifted downfield relative to 8,9-*threo*-isomers. Generally, *erythro* isomers exhibit a chemical shift for H8 that is larger than 3.8 ppm. On the contrary, *threo* isomers have chemical shifts smaller than 3.5 ppm for H8. A similar but opposite trend is observed for the ^{13}C NMR data. The C8 chemical shift of *erythro* isomers falls below 72 ppm, while the *threo* isomers have chemical shifts above 73 ppm. This trend has been previously observed and utilized for predicting the relative configuration of 2,5-disubstituted THF rings that have a hydroxyl group on the carbon adjacent to the ring (Born's rule),[23] and is further verified by the present data for bis-THF systems. The reported H8–C8 chemical shifts for mucoxin are 3.42 and 73.6 ppm, respectively, suggesting a *threo* relationship for C8–C9.
2) ***C16–C17 relationship***: Similar to the observations made above for the 2,5-dis-

Table 4. Chemical shift differences between the truncated analogues and the reported chemical shifts for mucoxin (NMR spectra recorded in $CDCl_3$). $\Delta\delta = \delta$ (ppm) truncated analogue $-\delta$ [ppm] reported mucoxin[a]



9

H–C		A	B	C	D	E	F	G	H	I	J	K	L
8	H	−0.01	**+0.40**	−0.01	−0.02	−0.03	−0.05	**+0.44**	−0.05	−0.01	−0.02	+0.03	−0.02
	C	−0.2	**−2.1**	0.0	+0.2	0.0	+0.3	**−2.3**	+0.3	+0.3	–	+0.1	–
9	H	0.00	+0.05	−0.03	**−0.10**	**−0.11**	**−0.12**	+0.02	**−0.18**	−0.09	−0.12	−0.01	**−0.16**
	C	**+0.5**	**+0.4**	**+0.5**	−0.2	0.0	−0.3	−0.2	−0.1	+0.1	–	+0.2	–
10	H	**−0.20**	**−0.10**	**−0.27**	**−0.2**	**−0.20**	**−0.25**	−0.04	**−0.27**	**−0.28**	**−0.21**	**−0.20**	**−0.17**
	H	−0.05	**−0.15**	−0.04	−0.02	−0.06	−0.06	**−0.18**	−0.09	**−0.11**	−0.04	−0.02	**−0.12**
11	H	**+0.10**	−0.01	+0.02	−0.07	+0.08	−0.09	−0.09	+0.05	+0.05	−0.01	−0.05	+0.02
	H	+0.07	+0.08	+0.07	**+0.16**	−0.06	+0.03	**+0.15**	−0.09	**+0.14**	**+0.12**	**+0.16**	+0.08
12	H	+0.02	+0.09	−0.02	−0.09	**−0.24**	**−0.48**	−0.02	**−0.36**	**−0.19**	−0.08	−0.05	**−0.13**
	C	+0.1	+0.2	+0.2	**−1.1**	+0.3	**+1.3**	+0.3	+0.8	−0.2	–	−0.2	–
13	H	+0.09	+0.06	**+0.16**	0.00	+0.05	+0.02	−0.05	**+0.15**	**−0.14**	**−0.12**	0.00	−0.02
	C	−0.4	−0.2	+0.6	+0.5	**+3.5**	**+4.6**	**+2.3**	**+5.7**	**+2.6**	–	**+2.1**	–
14	H	**+0.11**	+0.09	**+0.12**	**+0.20**	**+0.13**	−0.03	**−0.13**	−0.09	**−0.10**	+0.03	−0.08	**+0.18**
	C	**+1.6**	**+1.6**	**+1.4**	+0.3	**+1.2**	**+1.0**	−0.5	**+1.3**	**−1.2**	–	−0.3	–
15	H	−0.08	**−0.10**	−0.07	−0.08	+0.04	−0.09	−0.04	**−0.12**	**−0.25**	−0.04	+0.01	+0.02
	H	**−0.35**	**−0.33**	**−0.23**	**−0.32**	**−0.25**	+0.06	+0.02	**+0.13**	**−0.14**	+0.01	+0.06	**−0.33**
	C	+0.7	+0.7	**−3.3**	−0.5	−0.1	−0.9	+0.8	+0.1	**−3.7**	–	+0.8	–
16	H	+0.05	+0.03	**+0.19**	+0.08	+0.08	−0.04	+0.02	+0.02	+0.06	−0.06	+0.06	+0.09
	C	**+1.4**	**+1.5**	**+1.3**	**+1.7**	**+1.5**	**+1.2**	+0.3	**+1.6**	**+1.3**	–	+0.3	–
17	H	**−0.12**	**−0.12**	**+0.39**	**−0.13**	**−0.14**	−0.01	−0.04	−0.03	**+0.38**	−0.01	0.00	**−0.13**
	C	+0.4	+0.5	**−2.1**	+0.4	+0.6	+0.7	+0.4	+0.8	**−2.0**	–	+0.4	–

[a] Deviations greater or equal to 0.10 ppm for proton and 1.0 ppm for carbon are highlighted in bold.

Table 5. Proton and carbon chemical shifts of H8–C8 for analogues **A**–**L** (NMR spectra recorded in $CDCl_3$).

H–C	C8–C9 *erythro*		C8–C9 *threo*									
	B	**G**	**A**	**C**	**D**	**E**	**F**	**H**	**I**	**J**	**K**	**L**[a]
H8	3.82	3.86	3.41	3.41	3.40	3.39	3.37	3.37	3.41	3.40	3.45	3.40
C8	71.5	71.3	73.4	73.6	73.8	73.6	73.9	73.9	73.9	74.0	73.7	–

[a] Overlapping chemical shifts did not allow for an absolute assignment of C8 in analogue **L**.

Table 6. Proton and carbon chemical shifts of H17–C17 for analogues **A**–**L** (NMR spectra recorded in $CDCl_3$).

H–C	C16–C17 *erythro*		C16–C17 *threo*									
	C	**I**	**A**	**B**	**D**	**E**	**F**	**G**	**H**	**J**	**K**	**L**[a]
H17	3.92	3.91	3.41	3.41	3.40	3.39	3.52	3.49	3.50	3.52	3.53	3.40
C17	71.6	71.7	74.1	74.2	74.1	74.3	74.4	74.1	74.5	73.8	74.1	–

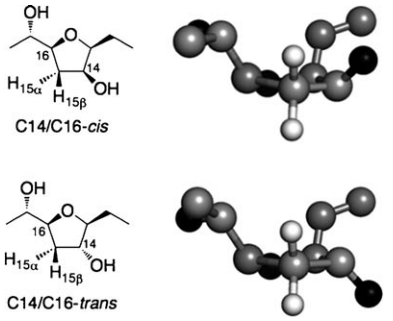
[a] Overlapping chemical shifts did not allow for an absolute assignment of C8 in analogue **L**.

Table 7. Difference in chemical shift of H15α and H15β for analogues **A**–**L** (NMR spectra recorded in $CDCl_3$).

H	C14–C16 *trans*						C14–C16 *cis*					
	A	**B**	**C**	**D**	**E**	**L**	**F**	**G**	**H**	**I**	**J**	**K**
H15α	2.00	2.02	2.12	2.03	2.10	2.02	2.41	2.37	2.48	2.18	2.36	2.41
H15β	1.83	1.81	1.84	1.83	1.95	1.93	1.82	1.87	1.79	1.63	1.87	1.92
$\Delta\delta$	0.17	0.21	0.28	0.20	0.15	0.09	0.59	0.50	0.69	0.55	0.49	0.49

ubstituted THF ring, the trisubstituted THF ring exhibits the same dependence of chemical shifts for *erythro* (downfield proton and upfield carbon) and *threo* (upfield proton and downfield carbon) isomers. The only difference is in the magnitude of chemical shifts for each isomer as compared to the disubstituted THF ring systems. As listed in Table 6, the chemical shift of H17 in the C16–C17 *erythro* isomers is larger than 3.90 ppm, while the C17 chemical shifts are less than 72.0 ppm. On the other hand, C16–C17 *threo* isomers have H17 chemical shifts below 3.55 ppm and C17 chemical shifts above 73.5 ppm. The latter observations suggest that Born's rule could also be applied for the relative stereochemical assignment of trisubstituted THF systems. Nonetheless, care must be applied since only the trend and not the absolute chemical shifts follow Born's rule. This necessitates the investigation of both *threo* and *erythro* analogues in order to obtain the chemical shift characteristics for different ring systems. The reported H17–C17 chemical shifts for mucoxin are 3.53 and 73.7 ppm, respectively, suggesting a *threo* relationship for C16–C17.

3) ***C14–C16 relationship***: Careful analysis of the data in Table 4 revealed a unique trend in the chemical shift difference of the two C15 methylene protons as a function of the C14–C16 stereochemical relationship. Table 7 illustrates this more succinctly by listing the chemical shifts and the $\Delta\delta$ values for each isomer. In all examples, C14–C16 *trans* isomers exhibit $\Delta\delta$ smaller than 0.30 ppm for H15α and H15β. On the other hand, the isomers that have *cis* C14–C16 relationship produce spectra with larger chemical shift disparity for H15α and H15β ($\Delta\delta$ is larger than 0.45 ppm). The reported $\Delta\delta$ for mucoxin is 0.44 ppm, thus suggesting a *cis* stereochemical relationship of C14–C16 in the natural product. This observation can be accounted for by considering the different anisotropic environment experienced by H15α and H15β in



Scheme 4. Energy minimized structures of C14–C16 *cis* and *trans* model compounds (Spartan 2008, PM3). The hydrogen atoms are omitted for clarity except for H15α and H15β. The larger environmental discrepancy in the *cis*-isomer leads to the larger chemical shift difference between H15α and H15β.

both the *cis* and *trans* ring systems. As depicted in Scheme 4, the C14–C16-*trans* isomer leads to a more balanced distribution of co-parallel C–C bonds (major factor that leads to diamagnetic anisotropical deshielding in saturated ring systems) with respect to the pseudo axially and pseudo equatorially disposed hydrogen atoms on C15. On the other hand, the C14–C16-*cis* isomer places the two hydrogen atoms in a more structurally segregated arrangement, whereby H15α enjoys larger deshielding as the result of the diamagnetic anisotropical effect of the substituents on the THF ring.

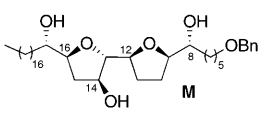
4) ***C12–C13 relationship***: The final discernable trend was observed for the splitting of H13 as a function of the relative configuration of C12–C13. As illustrated in Table 8, 12,13-*threo* isomers yield a dd splitting pattern (or a triplet with identical J_1 and J_2) for H13 with two similar coupling constants in the range of 2.4–3.9 Hz. On the other hand, the *erythro* isomers exhibit dd signals with a large J_1 (greater than 6.5 Hz) and a small J_2 (less than 3.5 Hz). The only exception to this rule was observed with analogue **I**, which exhibits a dd with a large and small coupling for H13. Considering the molecular modeling analysis presented in Table 2 on a similar system, it is not surprising that exceptions to the rule are observed, since the coupling constant is highly dependent on the dihedral angle. A number of mitigating factors control the overall favored population of an ensemble of rotameric possibilities, and as such, analogue **I** seems to defy the general trend that would dictate two small *J* values. Nevertheless, since most of the analogues fit the classification as depicted in Table 8, we proceeded to include H13 coupling as a defining characteristic for elucidation of mucoxin's structure, keeping in mind that the relative configuration of H13 might have to be probed further if a structural match to the available spectral data for mucoxin was not found. The observed coupling of H13 in mucoxin is a triplet with $J_1 \approx J_2 \approx$ 3.0 Hz, thus suggesting a *threo* relationship for C12–C13.

Table 8. The splitting pattern and the coupling constant of H13 for analogues **A–L** (NMR spectra recorded in $CDCl_3$).

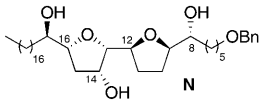
H13	*threo*								*erythro*			
	A	**B**	**C**	**E**	**G**	**H**	**I**[a]	**K**	**D**	**F**	**L**	**J**
splitting	t	dd	t	t	dd	dd	dd	dd	dd	dd	dd	dd
J [Hz]	3.3	3.4 3.9	3.9	3.5	2.3 2.9	2.4 3.9	2.7 6.9	3.4 4.8	3.1 7.2	3.4 6.8	2.9 7.3	3.3 7.2

[a] The data for analogue **I** does not fit the trend observed for the rest of the *threo* isomers. The source of this discrepancy is discussed in the text.

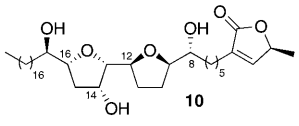
Table 9. ^1H and ^{13}C NMR comparison of *ttcTTT* and *cccTTT* truncated analogues and *cccTTT* full length acetogenin with naturally isolated mucoxin (NMR spectra recorded in $CDCl_3$).



ttcTTT



cccTTT



cccTTT

	ttcTTT **M** (truncated)		*cccTTT* **N** (truncated)		*cccTTT* (**10**)	
C–H	Δδ H	Δδ C	Δδ H	Δδ C	Δδ H	Δδ C
8	−0.05	+0.9	0.00	−0.1	0.00	+0.1
9	−0.09	−0.8	−0.02	+0.1	0.00	+0.1
10	−0.07, −0.10	–	0.00, 0.00	–	+0.02, +0.01	–
11	0.04, −0.10	–	0.00, 0.00	–	+0.02, +0.01	–
12	−0.31	+0.8	0.00	0.0	0.00	0.0
13	+0.17	+5.1	+0.03	+0.2	+0.04	+0.2
14	−0.05	+1.2	−0.01	+0.1	0.00	+0.1
15	−0.07, +0.09	−0.1	0.00, 0.00	+0.8[a]	+0.02, 0.00	+0.8[a]
16	+0.04	+1.7	0.00	+0.2	+0.01	+0.2
17	−0.01	+1.0	0.00	+0.1	+0.01	−0.2

[a] The reported deviation is based on tabulated data from the original isolation and characterization report, however, close inspection of the original NMR spectrum suggests that the listed data for C15 was transcribed incorrectly by ~1 ppm, and in actuality the deviation between the NMR spectrum of *cccTTT* and the original data is less than 0.2 ppm.

Considering the four conclusions derived above through analysis of the truncated analogues of mucoxin: C8–C9 *threo*, C16–C17 *threo*, C14–C16 *cis*, C12–C13 *threo*, the field of isomeric possibilities is narrowed from 64 total diastereomeric choices to only 4. These isomers are italicized in Figure 1. As luck would have it, two of the four possibilities (**H** and **K**) were synthesized as part of the 12 diastereomers chosen for spectral analysis, thus further narrowing the field to the remaining 2 isomers (*ttcTTT* **M** and *cccTTT* **N**, see Figure 1, bold and italicized). Both of the latter truncated isomers were synthesized (see Supporting In-

formation for synthetic scheme), and gratifyingly, the *cccTTT* analogue **N** was found to be a perfect match (Table 9). Further verification of this was obtained via the synthesis of the full-length *cccTTT* molecule (**10**), which produced identical ^{1}H and ^{13}C NMR spectra as compared to reported mucoxin. It is noteworthy that upon spectroscopic analysis of the additional two truncated structures *ttcTTT* (**M**) and *cccTTT* (**N**), the four trends derived from the original 12 diastereomers also fit these structures.

In the final analysis, our results indicate that the original *trans* stereochemical relationship for both rings (C9–C12 and C13–C16) is the source of error in the structure determination (*trans* stereochemistry of the ring substituents was assumed from lack of NOE). This clearly demonstrates the fallibility of using negative NOE results to assign relative stereochemistries, especially in five-membered rings (pseudo axial and pseudo equatorial disposition of substituents) if both *cis* and *trans* isomers are not available for comparative analysis. These problems are not isolated in structure determination, but also pose a challenge to synthetic organic chemists in assigning stereochemistry of synthetic products. The data also point to the fact that empirical results can be of great value in stereochemical assignments since they can be used as a guide and/or secondary verification for structural elucidations.

Conclusion

In summary, we have demonstrated a practical approach towards solving stereochemical misassignments for molecules that contain multiple chiral centers. In this "minimalist" approach, the initial goal is to generate spectroscopic data that can correlate structural relationships with observable trends. The choice of test structures is critical so that erroneous trends are avoided as a result of having a compendium of different stereochemical relationships besides the pair that is being probed. In this manner, we were able to use 12 of 64 possible diastereomers to derive four trends, which reduced the field of possibilities to four isomers. The spectroscopic data for one of the four isomers matched the reported data for mucoxin.

Acknowledgements

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