On the total synthesis of terpenes containing quaternary stereocenters
Buter, Jeffrey

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*** CHAPTER 2 ***

‡ Synthesis and Analysis of Mycoketide: A Test of NMR Predictions for Saturated Oligoisoprenoid Stereoisomers‡

ABSTRACT: This chapter describes the asymmetric synthesis and NMR analysis of the side chain of β-mannosyl phosphomycoketide, a natural product from Mycobacterium tuberculosis. Mycoketide, containing a 1,5-methyl ramification with the five stereocenters exhibiting an all-syn relationship, was subjected to NMR analysis and compared to predicted spectra of all possible diastereoisomers. The resolution of empirical NMR data was enhanced by Traficante processing which allowed detailed analysis of the 1,5-methyl array. Comparison of the predicted and actual NMR spectra of mycoketide allowed us to unambiguously assess the relative stereochemistry and isomeric purity.

Part of this chapter has been published:

The work in this chapter is the result of a collaboration with the Curran laboratory (Pittsburgh University) who performed the NMR-studies of the provided synthetic material.
CHAPTER 2

2.1 Introduction

β-Mannosyl phosphomycoketides (MPMs) 1 and 2 (Figure 1a) are potent T-cell antigens produced by *Mycobacterium avium* and *Mycobacterium tuberculosis*.\(^1\) The MPMs are composed of β-D-mannose and a long, aliphatic chain linked together as a phosphate diester. In the MPM biosynthesis, the polyketide side chain is formed from a fatty acid precursor by repetitive incorporation of malonyl and methyl malonyl CoA followed by full reduction. Although MPM’s side chains have a polyketide origin, a large part of the chain \(3c\) (C2−C21) bears methyl groups on every fourth carbon and therefore resembles a saturated oligoisoprene. In polymer terminology, such repeat units are also called alternating propylene/ethylene co-oligos or 1-methyltetramethylene oligomers.\(^3\) Rigorously assigning the configuration of the five stereocenters of such oligomers is difficult.

**Figure 1.** Structures of β-mannosyl phosphomycoketide and its side chains.

Crich *et al.* intentionally synthesized a mixture of MPMs 1 (R = n\(_{C4}H_{9}\)) with complete stereocontrol of the mannosyl anomic center but with all possible configurations of the methyl-branched stereocenters.\(^3\) Analysis of this mixture of 32 stereoisomers confirmed the constitution of 1. Our laboratory more recently synthesized a pentamethylheptacosan-1-ol side chain with all five methyl-branched stereocenters in the (S)-configuration (all-(S)-3c, Figure 1b) and coupled this to β-mannose phosphate to make MPM 2 (R = n\(_{C6}H_{13}\)).\(^4\) This sample was assayed against Crich’s mixture and a
natural product sample and exhibited activity similar to that of the natural isolate.\[5\] The stereorandom mixture was found to be considerably less active. The five stereocenters in the side chains of 1 and 2 are biosynthesized in an iterative way by the polyketide synthase pks12 and therefore presumably have the same configuration.\[1,6\] This means that the 1,5-relative configurations of the methyl-branched stereocenters are syn. Of the two remaining configurations, all stereocenters (\(R\)) or all (\(S\)), the results of the bioassays support the all-(\(S\)) assignment of 2.\[4a,5\] In addition, the crystal structure of all-(\(S\))-MPM 2 bound to CD1c has been reported recently.\[7\] However, assignment of the absolute configuration as all-(\(S\)) contradicts a predictive model by Leadlay and co-workers, who investigated the biochemical introduction of chiral methyl groups by polyketide synthases.\[8\] Therefore, spectroscopic or chemical means to assess the side chain configurations is needed both to confirm the structure of the natural product and to assay structures and purities of synthetic samples.\[9\] Recently the Curran laboratory synthesized all four diastereomers of the truncated MPM side chain model 4,8,12-trimethylnonadecanol 3a (Figure 1c).\[10\] The methyl regions of both the \(^1\)H- and \(^{13}\)C-NMR spectra of the four isomers exhibited small but reliable differences depending on whether the nearest neighbor methyl groups were syn or anti. We used the data to predict the \(^1\)H- and \(^{13}\)C-NMR spectra of all stereoisomers of higher saturated oligoisoprenoids including 3b (8 isomers) and 3c (16 isomers). Unfortunately, it is not possible to test these predictions retrospectively with published spectra because special conditions for processing are required to enhance resolution.

In this chapter we report the synthesis of a new sample of all-(\(S\))-3c. Analysis of resolution-enhanced NMR spectra show that the sample is predominately all-(\(S\)) but also that the isomer ratio is lower than expected. Further analysis of a lower homologue prepared from synthetic intermediates suggests that the impurities arose not because an asymmetric synthesis step was compromised, but instead because of a late-stage partial epimerization. Just as the predicted spectra help to analyze the experimental spectra, the reverse is also true. The new chemical shift values obtained from the experimental spectra help to refine and expand the NMR model.

### 2.2 Synthesis of the β-mannosyl phosphomycoketide side chain

To facilitate the NMR studies envisioned, we needed to synthesize a new sample of 3c as no synthetic material from the previous endeavor was available.\[4a\] The synthesis started with the construction of three key intermediates, chiral building blocks 9, 14, and 20 (Scheme 1) and relied heavily on the asymmetric conjugate addition strategy developed by the Feringa laboratory. Consecutive, asymmetric Cu-catalyzed Michael addition of Me\(_2\)Zn to diene 5 using phosphoramidite L1 and its enantiomer \(\text{ent-L1}\) respectively, provided after quenching with TMSCl silyl enol ether 7. An ozonolysis with oxidative work-up was performed and subjected to a Fischer esterification to provide acyclic methyl ester 8 (64% yield over the 4 steps).\[11a\] At this stage the asymmetric induction was analyzed to exceed a diastereomeric and enantiomeric excess.
CHAPTER 2

of 98%\(^{[11b]}\). Oxidation of 8 with TPAP afforded our first asymmetric building block 9 in 96% yield. Compound 8 was also used in the synthesis of sulfone 14, our second asymmetric building block. Its construction started with silyl protection of the free alcohol in 8. Reduction of the methyl ester was followed by a Mitsunobu reaction with thiotetrazole 12 to afford sulfide 13. The sulfur atom was then oxidized affording 14 in 72% yield over the four steps.

Scheme 1. Asymmetric synthesis of the mycoketide side chain building blocks 9, 14, and 20.

The final building block to be crafted before unification was sulfone 20 bearing one stereocenter. After synthesis of α,β-unsaturated 16 the methyl group was introduced...
Synthesis and Analysis of Mycoketide

using an asymmetric conjugate addition of MeMgBr, catalyzed by copper/Josiphos L2. Compound 17 was obtained with 89% ee in 94% yield. Full reduction of the thioester with LiAlH₄ and subsequently the Mitsunobu/oxidation sequence furnished asymmetric building block 20.

Scheme 2. Unification of the building blocks in the synthesis of mycoketide all-(S)-3c.

With the desired building blocks in hand the unification was performed to construct the mycoketide side chain all-syn-3c (Scheme 2). A Julia-Kocienski[12] coupling between aldehyde 9 and sulfone 14 was performed to construct alkene 21. The ester was reduced to the alcohol (22), which was converted into a good leaving group, by means of a tosylation, to then introduce the n-pentyl group. Compound 24 was liberated from its protecting group to give after a Ley-Griffith oxidation aldehyde 26. Another Julia-
CHAPTER 2

Kocienski olefination, this time with chiral building block 20, was used to install the final stereogenic methyl unit. Dialkene 27 was thus obtained which after global hydrogenation and hydrogenolysis gave the desired all-syn mycoketide 3c. Additionally we also constructed late-stage derivative 28, which is a phytanyl-type oligoisoprenoid with a silyl-protected hydroxy group on the left end and four repeating stereocenters. Compound 28 was isomerically pure (vide infra) so by implication all the reactions leading to 27 occurred with high stereoselectivity, and the so-formed stereocenters were retained with fidelity.

2.3 NMR analysis of β-mannosyl phosphomycoketide side chain 3c

To assess the structure and isomeric purity of 3c, the observed resonances in the methyl regions of both its 1H- and 13C-NMR spectra were compared with recent predictions. The basis for predicting the resonances of any isomer of 3c (n = 3) from the observed resonances of 3a (n = 1) is shown in figure 2. Briefly, we divide the spectrum into three parts, the left-end, the middle, and the right-end, and then add in resonances of the methyl branches from the model set (all isomers of 3a) that have the appropriate stereochemical relationship to match those in 3c. End Me groups can be syn or anti, while middle ones can be syn/syn, anti/anti, or anti/syn (or syn/anti).

<table>
<thead>
<tr>
<th>Me</th>
<th>Stereochemistry</th>
<th>13C</th>
<th>1H</th>
</tr>
</thead>
<tbody>
<tr>
<td>left</td>
<td>anti</td>
<td>19.61</td>
<td>0.872</td>
</tr>
<tr>
<td>middle</td>
<td>syn/anti</td>
<td>19.73</td>
<td>0.842</td>
</tr>
<tr>
<td>right</td>
<td>anti</td>
<td>19.70</td>
<td>0.839</td>
</tr>
</tbody>
</table>

Figure 2. Basis for predictions of NMR spectra of all-syn-3c and its stereoisomers.

To predict a spectrum of the all-syn isomer of 3c (all-(S)-3c or all-(R)-3c), we simply take the spectrum of all-syn 3a and multiply the middle resonances by three (because all three middle Me groups have syn/syn relationships), as shown in figure 2a. Figure 2b lists the remaining resonances from the complete model set. These are not used for all-syn-3c but are needed to predict spectra of its stereoisomers.

Standard spectra of all-syn-3c were first recorded in the usual way at 400 MHz. These compared favorably to the spectra of the prior sample[64] but as expected did not provide information about isomer identity or purity. New sets of spectra were then recorded at 700 MHz for 1H and 175 MHz for 13C, and the data sets were processed by the Traficante algorithm[13] for resolution enhancement.

In its essence the Traficante algorithm is an apodization (“changing the shape of a mathematical function”) which deemphasizes the tail of the free induction decay (FID). The use of the “transform of reverse added FIDs” (TRAF) algorithm allows the
enhancement of resolution of the recorded NMR spectrum with little to no decrease in signal-to-noise ratio. As exemplified in figure 3, a dramatic increase of spectral detail can be obtained for the Traficante algorithm treated FID (black spectrum) compared to a regular processed FID (red spectrum). As we will also show for all-syn-3c (vide infra) the increased resolution allowed the assessment of stereochemical purity for synthetic all-syn-3a.\textsuperscript{[10]}

Figure 3. A direct comparison of the methyl region in the \textsuperscript{1}H-NMR spectrum of synthetic 3a\textsuperscript{[10]} showing the regular (red) vs. Traficante algorithm (black) processed spectrum. Notice the dramatic increase in spectral resolution allowing detailed analysis (Reproduced from reference 10b).

Figure 4a,b shows the methyl regions of the \textsuperscript{13}C- and \textsuperscript{1}H-NMR spectra of all-syn-3c, both predicted (bottom spectrum) and actual (top spectrum). Importantly, the use of Traficante processing renders the spectra of such samples interpretable for the first time! The predicted \textsuperscript{13}C-NMR spectrum of all-syn-3c (Figure 4a, bottom) shows three resonances: the left-end Me resonates at 19.67 ppm, while the right-end Me resonates at 19.77 ppm. The predicted chemical shift of the three middle methyl groups (on C8, C12, C16) is 19.79 ppm, with a relative intensity of three.
CHAPTER 2

Figure 4. Comparison of the predicted and the experimental NMR spectra of the branched methyl group region for all-syn-3c. (a) actual (top) and predicted (bottom) $^{13}$C-NMR spectra of 3c. (b) actual (top) and predicted (bottom) $^1$H-NMR spectra of 3c.

The experimentally obtained $^{13}$C-NMR spectrum (Figure 4a, top) is at first glance much more complex. There are 10 resonances grouped in two sets of five with a ratio of about 70/30. Thus, the sample is not isomerically pure. Three of the five resonances of the major set (19.79, 19.77, and 19.67 ppm) match perfectly (<10 ppb difference) to the predicted resonances. The two most downfield major resonances at 19.80 and 19.81 ppm are slightly downfield from their predicted location at 19.79 ppm. We compared the major set of resonances in the experimental spectrum of all-syn-3c to the predicted spectra of all 16 isomers of 3c, and none matches as well as the spectrum of the all-syn-isomer. Thus, we conclude that the five major resonances in the actual spectrum in figure 4a belong to all-syn-3c. This means that the predicted spectrum in figure 4a is only partially correct. Because the model 3a is too simple (it has only one middle unit), it cannot anticipate the very small differences in chemical shifts of the three “repeating” methyl groups in the middle of all-syn-3c. Indeed, given their chemical and stereochemical similarity, it is remarkable that separate resonances for these methyl groups are observed. The methyl regions of the predicted and experimental $^1$H-NMR spectra of all-syn-3c are shown in figure 4b. The predicted spectrum is again simpler than the actual one. Now there is an additional resonance; the triplet at 0.882 ppm is the terminal methyl group (C29 on the far right end). This triplet has the same predicted chemical shift in all of the isomers, so its value is not diagnostic. This leaves three
major doublets in a ratio of 1:3:1 for the five branched Me groups of the major isomers. The smaller ones at 0.874 and 0.840 ppm are the left and right-end methyl groups. As in the predicted $^{13}$C-NMR spectrum, the three middle methyl groups overlap, now at 0.844 ppm. The major resonances of the spectrum match the predictions of the all-syn isomer closely. Again, the match is clearly the best among all 16 predicted spectra.[10] The experimental $^1$H-NMR spectrum again has a minor set of resonances; at least three minor doublets can be seen in the 1D spectrum. To find the other two minor resonances and to correlate all the resonances, we conducted a $^1$H-$^{13}$C COSY experiment. Figure 5 shows two slices of the resulting spectrum: plot (a) is a higher slice that shows only the cross resonances of the five major resonances, while plot (b) is a lower slice that shows both sets, but only the five minor cross resonances are highlighted.

![Figure 5. The branched methyl region of the $^1$H-$^{13}$C COSY of all-syn 3c. (a) a higher slice that shows only major cross resonances. (b) a lower slice with minor cross resonances highlighted.](image)

All of the major resonances correlate as predicted by the model in figure 2a, thus reinforcing the assignment of the major component of 3c as all-syn. The reason that the largest doublet at 0.844 ppm in the $^1$H-NMR spectrum also appears to be further resolved into three resonances is revealed. The right shoulder comes from a minor isomer, correlated at 19.73 ppm in the $^{13}$C spectrum, while the left shoulder comes from one of the middle carbons of the major isomer correlated at 19.81 ppm. The large center part correlates to the other two middle methyl groups in the major isomer at 19.80 and 19.79 ppm. So in the $^1$H-NMR spectrum, the prediction that three middle methyl groups of all-syn-3c would coincide is again wrong, but only just. This time, two resonances coincide, while the third is a smidge downfield (~1 ppb), appearing as a shoulder on the other two.

In contrast to the clear situation with the major set of five resonances, assignment of the minor set of five resonances is not straightforward. Four of the $^{13}$C and $^1$H resonances correspond well to other resonances predicted by the model (see unused resonances in
CHAPTER 2

Figure 2b): 19.73/0.842 ppm is a middle methyl group with a syn/anti (or anti/syn relationship) to its neighbors; 19.70/0.839 ppm is the right methyl group with an anti neighbor; 19.66/0.841 ppm is a middle methyl group with an anti/anti relationship; and 19.61/0.872 ppm is left methyl group with an anti neighbor. There is also a minor signal at 19.68 ppm in the $^{13}$C-NMR that is not in the model set. This signal is only 10 ppb downfield from model signal 19.67, a left methyl group with a syn neighbor. However, if this assignment were correct, then that $^{13}$C signal should correlate to a $^1$H resonance at 0.874 ppm. Instead, it correlates to a resonance at 0.842 ppm.

In short, the major isomer in the new sample of 3c is clearly the all-syn isomer. However, because of the simplicity of the model (it provides only seven resonances for all resonances in all possible isomers), we cannot yet assign the minor resonances. If they come from a single isomer, then one of the resonances (19.68 ppm) is poorly predicted in the $^{13}$C-NMR spectrum. If they come from a mixture of isomers, then there must be other minor resonances under the major resonances in varying ratios, so direct interpretation is not easy.

2.4 NMR analysis of $\beta$-mannosyl phosphomycoketide side chain

We next conducted a series of experiments trying to localize the stage of the synthesis where the minor isomer(s) originated. Small samples of key intermediates saved during the synthesis, especially the sample of 24 (Scheme 1), proved very helpful. This alkene could not be analyzed directly for stereochemical integrity because it already contains E/Z isomers from the Julia−Kocienski reaction. So about 2 mg of 24 was carefully hydrogenated (Pt, H$_2$, CH$_2$Cl$_2$/MeOH) to make a sample of 28 whose spectra were recorded as above.

The predicted spectrum of all-syn-3b was used for comparison, and the structures of 3b and 28 are compared in figure 6. Notice that while the right-end and middle fragments of 28 and all-syn-3b are the same, the left-end fragment is different. Sample 28 has two carbon atoms less than model 3b at the left end, and its hydroxy group is protected with a TBDPS (tert-butyldiphenylsilyl) ether. Clearly the predicted left-end resonances for all-syn-3b cannot be applied to the left end of 28.

![Diagram](image)

Figure 6. Predicted spectra of all-syn-3b are used to interpret the actual spectra of 28. Only the middle and right-end predictions can be used because the left ends are different.
The methyl region of the experimental $^1$H-NMR spectrum of 28 is shown in figure 7 (left spectrum). There is only one set of resonances: four doublets and one triplet (the terminal methyl group on the end of the chain, C21). The resonances were assigned by 2D-NMR experiments, and these assignments are collected in table 1. Likewise, the $^{13}$C-NMR spectrum showed one set of resonances (right spectrum in Figure 7 and Table 1). Clearly this sample is a single isomer. To assign the configuration of 28, we compared the predicted methyl resonances of all-syn 28 with the actual resonances, as summarized in table 1. The observed resonances for the left-end methyl group (Me$^{22}$) appear at 0.917 ppm in the $^1$H-NMR spectrum and 16.99 ppm in the $^{13}$C-NMR spectrum. This pair of resonances has no equivalent in the model, but the values are not crucial to the stereochemical assignment because the chemical shifts of the next methyl group (Me$^{23}$) also contain the information about whether the relationship of the two is syn or anti.

Figure 7. Expansions of the methyl regions of the $^1$H- (left) and $^{13}$C-NMR spectra (right) of 28. The $^1$H-NMR expansion contains the terminal methyl group (C$^{21}$) resonance, the $^{13}$C-NMR spectrum does not.

Now compare (see table 1) the predicted and actual resonances for the remaining Me groups (Me$^{23}$, Me$^{24}$, and Me$^{25}$) in structure 28, starting at the right end of the molecule. In both the $^1$H- and the $^{13}$C-NMR spectra, the actual and predicted resonances for the right-end methyl group (Me$^{25}$) and its neighbor (Me$^{24}$) are spot on or almost spot on. At 0.830 ppm ($^1$H) and 19.76 ($^{13}$C) ppm, the actual resonances of the next methyl group in (Me$^{23}$) are slightly different from both Me$^{24}$ and the prediction. As above, this is again a deficiency of the model, which provides only a single middle resonance that is used to model both Me$^{23}$ and Me$^{24}$. It is sensible that the model is spot on for Me$^{24}$, which is further away from the left end (the part that differs between the model and the actual sample), and slightly off for Me$^{23}$, which is closer to the left end.

The values of the experimental $^1$H-NMR spectra of 28 in table 1 are much closer to the predicted values for the all-syn isomer of 3b than to any of the other seven isomers. Thus, both the relative configuration of the sample (all-syn) and the high level of purity ($\geq 95\%$) are confirmed.
CHAPTER 2

Table 1. Comparison of methyl resonances of the actual spectra of 28 with predicted resonances for the all-syn isomer of 28 derived from the predicted spectra of all-syn 3b: Data in ppm.

<table>
<thead>
<tr>
<th>Resonance</th>
<th>(^1H) obs.</th>
<th>(^1H) pred.</th>
<th>(^{13}C) obs.</th>
<th>(^{13}C) pred.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Me(^{22})</td>
<td>0.917</td>
<td>(a)</td>
<td>16.99</td>
<td>(a)</td>
</tr>
<tr>
<td>Me(^{23})</td>
<td>0.830</td>
<td>0.844</td>
<td>19.76</td>
<td>19.79</td>
</tr>
<tr>
<td>Me(^{24})</td>
<td>0.845</td>
<td>0.844</td>
<td>19.80</td>
<td>19.79</td>
</tr>
<tr>
<td>Me(^{25})</td>
<td>0.840</td>
<td>0.840</td>
<td>19.76</td>
<td>19.77</td>
</tr>
</tbody>
</table>

\(a\) The model does not predict the left-end resonance

\(b\) Middle resonance, see figure 2.

Finally, this exercise establishes a new left-end model compound with a CH\(_2\)OTBDPS group from which spectra of saturated isoprenoids and related reduced polyketides can be predicted. Compounds such as 28 terminating in CH\(_2\)OTBDPS on the left end will have a chemical shift of 0.830 and 19.76 ppm for the left-end methyl group (Me\(^{22}\)) if it is syn to the next methyl group (Me\(^{23}\)). On the basis of the established trends with 3a, we predict that Me\(^{23}\) will resonate a little further upfield in both the \(^1H\)- and \(^{13}C\)-NMR spectra if it is anti to Me\(^{23}\) (about 2 and 60 ppb, respectively, see Figure 2). From the chemical shifts in table 1, small upfield adjustments also need to be made for the resonances of Me\(^{23}\) in the syn/syn isomer to predict the spectra of isomers with this group syn/anti (or anti/syn) and anti/anti to Me\(^{22}\)/Me\(^{24}\). The magnitudes of the adjustments can again be estimated from the values for the analogous middle methyl groups in figure 2.

These new resonances can be added to the existing middle (used for Me\(^{24}\)) and right (used for Me\(^{25}\)) resonances in figure 2 to fill out predicted spectra for any isomer of 28 or a higher oligomer. Because the model 28 has four resonances, every isomer of 28 (or a higher oligomer) will also have four predicted resonances. This is an improvement over the use of 3a to model 3b and 3c, where two or three middle Me groups have the same predicted chemical shift in some isomers, even though in reality there are small differences.
2.5 Conclusions

In summary, for the first time it has been possible through NMR spectroscopy both to assess isomer purity and to assign relative configurations of saturated oligoisoprenoids such as \( 3c \). Predictions of the methyl regions of the \(^1\)H- and \(^{13}\)C-NMR spectra of all 16 stereoisomers of \( 3c \) enabled this analysis.\(^{[10]} \) The predicted \(^1\)H-NMR spectrum of all-syn-\( 3c \) is very close to the actual spectrum, but the predicted \(^{13}\)C-NMR spectrum proved to be too simple in one aspect: the three resonances from the middle methyl groups were predicted to coincide because they all have the syn/syn relationship, but they did not. However, the observed differences between predicted and actual spectra were small compared to the differences expected for other stereoisomers, so the value of the \(^{13}\)C-NMR predictions was not compromised.

The analysis of the synthetic sample of \( 3c \) showed that the all-(S) (all-syn) isomer was indeed the major component, present to the extent of about 70%. Unexpectedly, a minor stereoisomer component (or components) was present to the extent of about 30%. We cannot yet identify the minor component or pinpoint where it was introduced. However, by similar comparison of actual and predicted spectra, we could show that 28 (derived from a key synthetic intermediate 24 bearing four of the five stereocenters) is both pure (>90%) and has the expected all-(S) configuration. This narrows the source of the problem to the last few steps of the synthesis.

Perhaps most importantly, the values from the predicted spectra of compounds like \( 3c \) can now be leveraged to related compounds bearing different right or left ends. As an example, intermediate 28 with four stereocenters has a left end different from that of \( 3a-c \). Even though we only made one of the eight possible stereoisomers of 28, we can now combine the new data obtained for 28 with the data for 3a to assemble predicted spectra of the other seven isomers of 28 and its higher oligomers. This ability to directly analyze complex, saturated oligoisoprenoids is a powerful tool to clarify a heretofore cloudy situation with respect to stereoisomer structure and purity of synthetic and natural samples.

2.6 Discussion

The unfortunate loss of stereochemical purity during the course of the synthesis was an initial setback. This result however allowed us to show application of the method in assessment of stereochemical purity of synthetic MPM side chain \( 3c \). Shortly after our publication of the content within this chapter, Piccirilli and co-workers reported an alternative asymmetric synthesis of β-mannosyl phosphomycoketide.\(^{[15]} \) Aware of our NMR analysis method the authors confirmed the stereochemical purity of their synthesized C\(_{32}\)-mycoketide, showing the ease of use, and direct application of the methodology.

The stereopurity issues in our synthesis potentially raises questions about our previous synthesis of β-mannosyl phosphomycoketide in 2006.\(^{[4]} \) Despite the loss of stereochemical purity in the present case, we do not have doubts about our claims
regarding the overall stereochemical purity of β-mannosyl phosphomycoketide produced in 2006. The latter synthetic product was subjected to biological studies in which the material exhibited equal antigenic T-cell response to that of the natural isolate. In the same study a stereorandom mixture of MPMs was shown to be significantly less potent (~30 times less). Additionally it was reported that T-cell response was even dependent on the stereochemistry of the C4-methyl, where (S)-stereochemistry proved to be dramatically more active than the (R)-isomer. The study therefore indicates a strong influence of the lipid stereochemistry, more specifically the methyl ramification, on T-cell response. The biological evaluation of the 2006 produced natural product therefore indicates a high stereochemical purity of the synthetic material.

2.7 Studies on the stereochemistry of crenarchaeol (ongoing studies)

The use of Traficante-processed high-field NMR data, together with the produced set of predictions of chemical shifts was useful in the stereochemical assessment of the 1,5-methyl ramification of synthetic material. However, the potential of this method is far from fully exploited and we reasoned that this method might be helpful, that is without synthetic reference material, in the structural assignment of complex natural saturated oligoisoprenoids. We therefore set out to find a molecule of sufficient complexity, arriving at crenarchaeol (Figure 8).[16]

Figure 8. The molecular architecture of crenarchaeol.

Crenarchaeol is a complex membrane-spanning lipid found in Archaea, although in very low concentrations. The molecule exhibits an exquisite chemical architecture with an intriguing set of stereochemical features. Besides the obvious 1,5-methyl array, crenarchaeol also contains a 1,4-methyl relation, four cyclopentyl rings, a cyclohexyl ring, and two glycerol moieties, with a grand total of 22 stereocenters! The excitement we experienced observing its chemical structure provided us with the idea to investigate the stereochemical elements (relative stereochemistry) in crenarchaeol using Traficante processed high field NMR spectra in combination with predicted spectra of all possible stereoisomers. Although there is no substantiated doubt about the proposed structure of crenarchaeol (Figure 8),[16] there are several reasons to, with the current state of equipment and techniques, repeat this structure elucidation. 1) Some assumptions about the stereochemistry had to be made at that time as the
spectrum was largely but not fully resolved. 2) at several points, stereochemistry was inferred in analogy with other, related, compounds but not independently established. We therefore believe that crenarchaeol is a beautiful test case of the methodology. In this investigation we hope to gain more insight into several key questions. 1) Are we able to successfully assign the relative stereochemistry of crenarchaeol beyond reasonable doubt using this method? 2) Do the predictions match with data empirically obtained? 3) Is the Traficante processing in combination with high field NMR (750 MHz to 900 MHz) able to successfully resolve all stereochemical elements, even those of stereochemically similar methyl groups? 4) Will the data invoke revision of the previous assigned stereochemistry?

Concurrent with writing this dissertation we managed to obtain 5 mg of >95% pure crenarchaeol. This was the result of a three months isolation procedure performed in the Schouten laboratory at the NIOZ institute (Royal Netherlands Institute for Sea Research). The natural isolate was analyzed with 600 MHz and 900 MHz NMR providing us with $^1$H, $^{13}$C, COSY, NOESY, TOCSY, HMQC, HMBC, and DEPT spectra. Momentarily the extensive data set is carefully scrutinized in collaboration with the Curran laboratory (Pittsburgh University) to independently assign the relative stereochemistry of crenarchaeol.

2.8 Experimentals

General information on NMR experiments: NMR spectra were recorded on a 700 MHz spectrometer using deuterated chloroform spiked with 1% tetramethylsilane (TMS), unless otherwise indicated. The signals are given as in parts per million (δ, ppm) and were determined relative to the proton and carbon resonance of TMS. For the resolution-enhanced spectra of 3c and 28, data were collected and processed as described in D. D. Traficante, G. A. Nemeth, J. Magn. Reson. 1987, 71, 237.

Copies of predicted $^1$H and $^{13}$C-NMR spectra of all stereoisomers of both 3b and 3c (48 spectra total) are found in the Supporting Information associated with E. A. Yeh, E. Kumli, K. Damodaran, D. P. Curran, J. Am. Chem. Soc. 2013, 135, 1577.


General information on the synthesis efforts: For a detailed discussion and experimental procedure for the asymmetric total synthesis of the mycoketide side chain 3c we like to refer to the 2005 PhD dissertation of Ruben P. van Summeren, Total synthesis of enantiopure biomolecules: on mycolactones,
CHAPTER 2

saturated isoprenoid building blocks and β-mannosyl phosphomycoketides. As an alternative source references [4a], [11a] and [11b] can be consulted.

2.9 References


Synthesis and Analysis of Mycoketide

