New methods for stereochemical determination of complex polyketides: configurational assignment of novel metabolites from myxobacteria

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Covering: 1999 up to the end of 2007

An overview on recently developed methods for the stereochemical determination of complex polyketides is given and NMR-spectroscopic, computational, biosynthetic and synthetic methods are discussed. These methods are presented in their applications to structurally novel polyketide classes from myxobacteria.

1 Introduction

Polyketides are structurally a very diverse family of natural products with an extremely broad range of biological activities and pharmacological properties.[1] Polyketide antibiotics, antifungals, cytostatics, antiparasitics and natural insecticides are in commercial use.[2] Biosynthetically, they are derived by iterative condensations of acetyl and propionyl subunits giving rise to diverse assemblies of methyl and hydroxyl-bearing stereogenic centers, enabling large numbers of stereochemical permutations. This diversity together with a high degree of spectral complexity and conformational flexibility renders the stereochemical assignment of polyketides a challenging task. Even nowadays, polyketide structures are frequently published 'flat',[3,4] despite the importance of stereochemistry for biological activity and the general significance of stereochemical knowledge to many other fields ranging from chemical physics, biochemistry to synthetic organic chemistry or catalysis.

Myxobacteria are a particularly rich source of novel polyketides. Over the last three decades, due to the pioneering work of the groups of Prof. Höfle and Prof. Reichenbach at the Gesellschaft für Biotechnologische Forschung (GBF, recently renamed to: Helmholtz-Zentrum für Infektionsforschung, HZI), an impressive number of structurally unique and biosynthetically diverse polyketides have been reported from these soil-living organisms. In total, they span a range of approximately 60 structurally new classes of polyketides and many structural variants thereof.[5,6] Many of these compounds are associated with very high biological activities, including antiproliferative, antibiotic, antifungal or antiplasmodial activities. On a molecular level, a variety of molecular targets are specifically addressed, including the cytoskeleton, nucleic acid polymerases, the respiratory chain, nuclear transport, microfilaments, and protein or fatty acid synthesis. This renders these compounds highly attractive targets for further development.

For configurational assignment of polyketides, a number of novel methods have recently been developed, which are based on spectroscopic, computational, biosynthetic and synthetic procedures.[7] Importantly, they have demonstrated their true usefulness in natural product assignments. Within this review, they will be discussed within their applications to polyketides of myxobacterial origin. This review covers all structurally novel classes of myxobacterial polyketides reported from 1999 up to the end of 2007.[5,6]

2 Novel polyketides from myxobacteria

Since 1999, 15 constitutionally novel polyketides have been reported from myxobacteria. Their structures are shown in Fig. 1. Chivosazol A (**11**) had previously been reported as a planar structure.[8] Its full three-dimensional structure has been elucidated within the time frame discussed within this review (up to 2007).[9] For most of the metabolites, full or partial configurations have been disclosed, and only for etnangien (**15**)[3] has no stereochemical information been published. Full details on the biosynthetic origin, the biological activity, the method of configurational assignment and synthetic approaches are given in Table 1. Notably, *Sorangium cellulosum* was the most productive myxobacterium, with tuscolid (**6**)/tuscoron,[10] spirangien (**8**),[11] chlorotonil (**12**),[12] etnangien (**15**)[3] and leupyrrin (**16**)[4] isolated from various strains of this microorganism. Of quite remarkable productivity was also *Chondromyces crocatus*. A single strain yielded crocacin (**1**),[13] ajudazol (**4**),[14] chondrochloren (**5**)[15] and

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thuggacin (**13**).[16] Various strains of *Archangium gephyra* gave the aurafurons (**7**),[17] the melithiazols (**2**),[25] archazolids (**10**)[42–45] and the cyrmenins (**14**).[50]

Most of these polyketides are associated with very potent biological activities. Particularly pronounced are antiproliferative activities, and apicularen A (**3**),[29,30] the archazolids (**10**),[30,43] cruentaren A (**9**)[38,39] chivosazol (**11**)[8] and the spirangiens (**7**)[11] inhibit the growth of various cancer cell lines in low nanomolar or even subnanomolar concentrations. Importantly, very specific targets have been identified, except for spirangien, which might be associated with a novel mode of action. High antifungal activity was observed for the crocacins (**1**)[13] and melithizols (**2**).[25] Particularly notably are also the thugaccins (**13**),[16] which are very effective against *Mycobacterium tuberculosis*, the causative agent of tuberculosis. No activity has been reported for the ajudazols (**4**),[14] tuscolid (**6**)[10] and chlorotonil (**12**)[12] in primary biological screenings for cytotoxic, antifungal or antibacterial potency, suggesting alternative targets.

3 NMR-based methods

For configurational assignment of these polyketides, NMR-based methods have been most important and widely used, in particular in cases where no X-ray data are available (Table 1).[7,53] This section will present the various procedures, in combination with key spectroscopic methods involved. They will include conventional techniques (NOESY-, 2D-*J*-resolved-, TOCSY- and homonuclear decoupling spectra), and more advanced techniques (HSQC-HECADE, residual dipolar couplings).

3.1 Conventional NOE-based approach

NOE (nuclear Overhauser) experiments continue to be the most fundamental and important technique for configurational assignment. They represent correlations through space rather than through bond correlations, as compared to a COSY spectrum. Particularly valuable are NOESY experiments, which include acquisition of all NOEs in a single experiment and the valuable option to analyse NOEs between protons that may be partially overlapped, which may not be selectively irradiated using the traditional 1D NOE experiment or 1D NOESY experiment. In general, protons in the close vicinity of an irradiated proton give more intense signals, and protons farther away give weaker peaks. The intensity also depends on the mixing time. By increasing the mixing time, signals between protons that are further apart may also be seen, allowing for a very detailed understanding of the spatial distance of essentially all protons in complex polyketides. An instructive example for the use of this method is shown in Fig. 2, the configurational assignment of apicularen A (**2**) by Jansen, Höfle *et al.*[29] It presents a highly cytotoxic V-ATPase inhibitor[30] from various *Chondromyces* species. Its unique structure contains a 12-membered oxygen-bridged bicyclic macrocyclic core with four stereogenic centres. Distinct NOE-correlations between H-13 and H-15, H-8a, H-14b, H-10b and H-12b indicated that these protons reside on the same face of the ring system, while NOEs from H-11 to H-9a and H-10a suggested that these protons are on the opposite side. In combination with a characteristic sequence of large coupling constants, indicating antiperiplanar relationships between the respective protons, this allowed assignment of the relative configuration of this polyketide. This was subsequently confirmed by X-ray crystallography, and the absolute configuration was assigned by Mosher ester analysis. The structure of apicularen was proven by four total syntheses by the groups of De Brabander,[31] Nicolaou,[32] Panek[33] and Maier.[34]

In a similar fashion, this conventional NOE-based approach may also be applicable to non-cyclic systems, in particular when they reside in one major conformation. An instructive example, likewise by Jansen, Höfle *et al.*, is given in Fig. 3, the assignment of the crocacins (**1**),[13] potent antifungal agents from *Chondromyces* species. From a stereochemical point of view, they are characterized by a central polypropionate unit with four contiguous stereogenic centres. As indicated in Fig. 3b, large homonuclear couplings between H-15 and H-16, H-17 and H-18 as well as H-19 and H-20 indicated antiperiplanar relationships between these protons, while *gauche* conformations for H-16–H-17 and H-18–H-19 were deduced from small scalar couplings. Notably, these small couplings alone do not allow discrimination between *gauche*$^-$ or *gauche*$^+$ situations for the respective protons. On the basis of distinct NOE data (Fig. 3a), however, crocacin A was expected to reside in the conformation as shown with the relative assignment depicted (Fig. 3a).

In a similar fashion to that described for apicularen and crocacin, the stereochemistries of ajudazol (**4**),[14] chondrochloren (**5**)[15] and tuscolid (**6**)[10] were fully or partially assigned.

Dirk Menche

Dirk Menche, born in 1972, studied chemistry and biochemistry at the University of Würzburg and received his diploma in 1998. During his PhD thesis under the guidance of Professor Gerhard Bringmann, he was involved in natural product isolation, structure determination and total synthesis. In 2002, he moved to the University of Cologne to work with Professor Albrecht Berkessel on the development of novel synthetic methodology, which he subsequently applied in the synthesis of marine macrolides in the group of Professor Ian Paterson at the University of Cambridge (2002–2004). In 2005, he received a Liebig Stipendium (Fonds der Chemischen Industrie) to start his independent research at the Helmholtz-Zentrum für Infektionsforschung (formerly Gesellschaft für Biotechnologische Forschung, GBF) in the former laboratories of Professor Gerhard Höfle in Braunschweig. In 2007, he finished his habilitation with Professor Markus Kalesse at the University of Hannover. Very recently, he became associate professor at the University of Heidelberg. His research interests are in the area of natural product chemistry, ranging from natural product isolation and structure elucidation to total synthesis as well as structure–activity studies and novel synthetic methodologies.

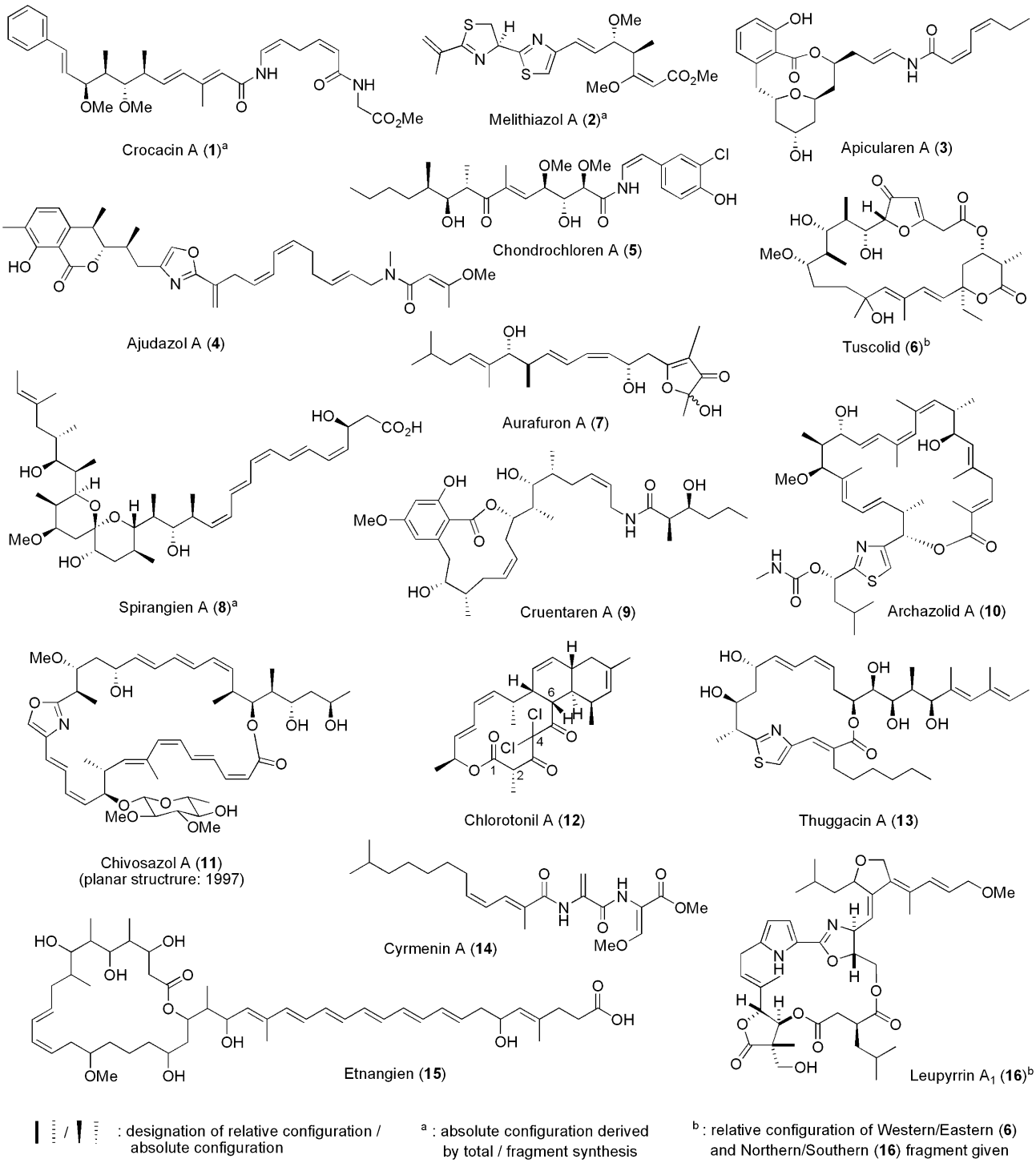


Fig. 1 Novel polyketides from myxobacteria reported between 1999 and 2007.

3.2 Murata's method of *J*-based configurational assignment

Such a *J*-based configurational method has recently been further extended by incorporation of proton–carbon coupling constants. This approach has been discussed in more general terms in a recent paper from Murata.[54] The general principle is outlined in Fig. 4. It relies on a detailed conformational analysis based on the respective $^{2/3}J_{C,H}$ and $^{3}J_{H,H}$ coupling constants. While vicinal homo- and heteronuclear constants follow similar Karplus-type equations (Fig. 3a), geminal carbon–proton coupling constants may also be able to provide conformational information. When an oxygen functionality on a carbon atom is *gauche* to its geminal proton, $^{2}J_{C,H}$ becomes large, and when it is *anti*, the value becomes small (Fig. 3a), which allows discrimination between

Table 1 Myxobacterial polyketides reported since 1999.[5,6,18]

Polyketide (year of assignment)	Source	Biological activity[a]	Method of configurational assignment[b]	Total synthesis
Crocacin A (**1**) –D (1999),[13c]	*Chondromyces crocatus*, *C. pediculatus*	Antifungal, low cytotoxicity (inhibition of eukaryotic respiratory chain)	NOE[c]	2000,[19] 2001,[20] 2002,[21] 2003,[22,23] 2005[24]
Melithiazol A (**2**) –N (1999)[25c]	*Melittangium lichenicola*, *Archangium gephyra*, *Myxococcus stipitatus*	Strongly antifungal (inhibition of NADH oxidation), cytotoxic	X-Ray[c]	2005,[26] 2006,[27] 2007[28]
Apicularen A (**3**) and B (2000)[29]	*Chondromyces apiculatus*, *C. pediculatus*, *C. lanuginosus*, *C. robustus*	Cytotoxic, inhibition of V-atpase[30]	X-Ray (Mosher ester)	2001,[31] 2002,[32] 2004,[33,34]
Ajudazol A (**4**) and B (2002)[14]	*Chondromyces crocatus*	Unknown	NOE	—[35]
Chondrochloren A (**5**) and B (2003)[15]	*Chondromyces crocatus*	Weak antibiotic activity	NOE, Mosher ester	—
Tuscolid (**6**), tuscoron A and B (2004)[10]	*Sorangium cellulosum*	Unknown	NOE	—
Aurafuron A (**7**) and B (2005)[17]	*Stigmatella aurantiaca*, *Archangium gephyra*	Moderately antifungal, weakly antibacterial, moderately cytotoxic	NMR, degradation	—
Spirangien A (**8**) and B (2005)[11c]	*Sorangium cellulosum*	Highly cytotoxic (unknown target)	X-Ray[c]	—[36,37]
Cruentaren A (**9**) and B (2006)[38]	*Byssovorax cruenta*	Highly cytotoxic, (F-atpase inhibition)[39]	X-Ray (Mosher ester, database-approach)	2007[40,41]
Archazolid A (**10**), B (2006),[42,43] C[44] and D (2007)[45]	*Archangium gephyra*, *Cystobacter violaceus*	Highly cytotoxic (V-atpase inhibition)[30]	Murata's method, modeling, Mosher ester	2007[46,47]
Chivosazol A (**11**) (2007)[9] (planar structure: 1997)[8]	*Sorangium cellulosum*	Highly cytotoxic	NMR, modeling, fragment synthesis, bio-informatics analysis	—[48]
Chlorotonil A (**12**) and B (2007)[12]	*Sorangium cellulosum*	Unknown	X-Ray	2007[49]
Thuggacin A (**13**), B and C (2008)[16]	*Chondromyces crocatus*	*Mycobacterium tuberculosis*	NMR, modelling derivatisations, bio-informatics analysis	—
Cyrmenin A (**14**) –B_2 (2003)[50,51]	*Cystobacter armeniaca*, *Archangium gephyra*	Antifungal (inhibition of NADH oxidation)	Achiral	—
Etnangien (**15**, 2008)[3]	*Sorangium cellulosum*	Antibacterial (RNA polymerase)	—	—
Leupyrrin A_1 (**16**) –D (2003)[4,52]	*Sorangium cellulosum*	Antifungal	NOE[4]	—

[a] Only most pronounced activity given. [b] Main methods given. [c] Absolute configuration assigned by total/fragment synthesis.[19,26,36]

gauche$^+$ and *gauche*$^-$ vicinal proton–proton conformations, something that is impossible by using $^3J_{H,H}$ coupling constants alone (see section 3.1).

As shown in Fig. 4b, these data can then be utilized for assigning the relative configuration of vicinal stereogenic centres. Among the six possible conformers possible for the 1,2-*syn* or 1,2-*anti* stereoisomer **17a** or **17b**, the four *gauche* conformers, A-1, A-2, B-1 and B-2, can be identified using the respective vicinal and geminal homo- and heteronuclear coupling constants. In the case of the H/H-*anti* and C/C-*gauche* conformations (A-3/B-3), the two configurations may be differentiated by NOE data. As shown in Fig. 3c, NOESY correlations from H-1 to H-4 and from H-X to H-OR would result in a 1,2-*syn* assignment (**17a**), while correlations from H-1 to H-OR and from H-X to H-4 would lead to a 1,2-*anti* assignment (**17b**), respectively. Using these criteria, all six conformers may be discriminated, with their relative configuration determined accordingly.

While originally developed for linear polyketides, this method may also be applied to macrolides.[7] One of the most recent applications in natural product structure elucidation, and an instructive example of the method in general, is the stereochemical determination of the archazolids (**10**), recently accomplished by us in cooperation with the group of Carlomagno.[42] These polyunsaturated polyketides, originally isolated from the myxobacterium *Archangium gephyra*,[43] are highly potent cytostatic agents which inhibit the growth of a wide range of human cancer cell lines in subnanomolar concentrations.[43] On a molecular level, they are a structurally novel type of particularly efficient (IC_{50} in the low nanomolar range) and specific inhibitors of V-ATPases.[30] Being composed of a 24-membered macrocyclic core with a thiazole side chain, they contain a characteristic sequence of eight methyl- and hydroxyl-bearing stereocentres of apparently polypropionate origin, which may be clustered in three subunits of vicinal stereogenic centres.

Application of Murata's *J*-based configurational assignment to each of these subunits requires precise determination of vicinal proton–proton and proton–carbon coupling constants. While H–H coupling constants may be determined from a simple ^{1}H

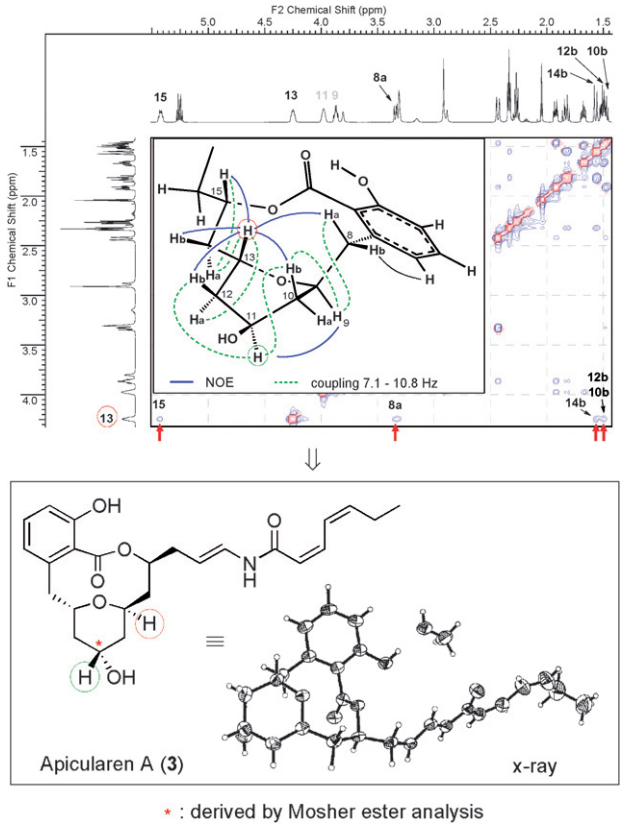


Fig. 2 Configurational assignment of apicularen A based on NOE-data and X-ray analysis.[29]

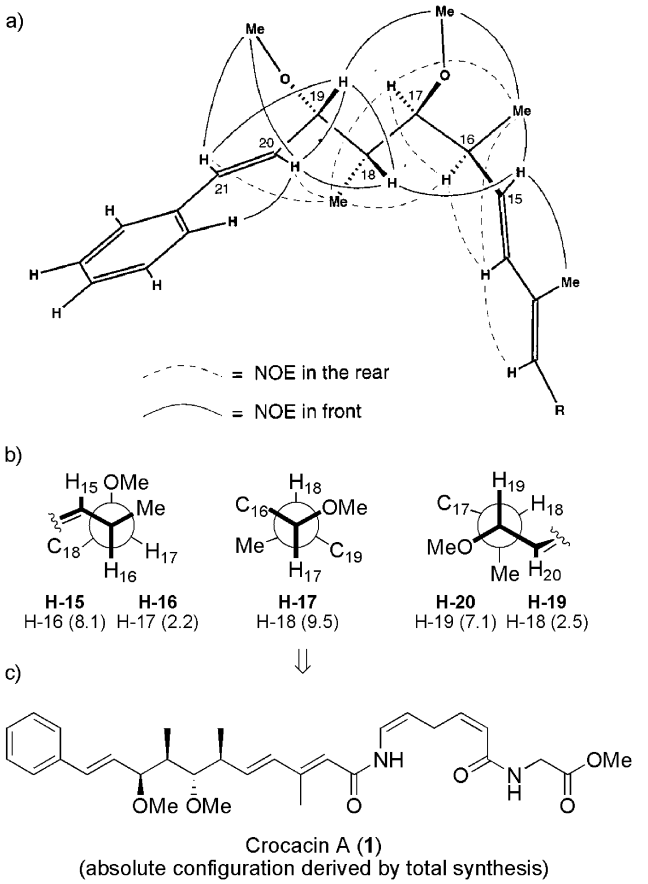


Fig. 3 Crocacin A (**1**): (a) Postulated conformation; (b) rotamers for the C-15–C-20 subunit, and (c) assigned configuration. Coupling constants ($^3J_{H,H}$/Hz) are in parentheses.[13]

NMR spectrum,[55] this may be difficult in cases when the ^{1}H NMR spectrum is crowded and signals are partially overlapping, which is usually the case in complex polyketides. As shown in Fig. 5, one such example is H-16 of archazolid A, which is overlapped by H-6′ and the resonance of the methyl-group at C-10.

A useful experiment to extract coupling constants in such systems involves use of 2D *J*-resolved spectra. As shown in Fig. 6a, they include a ^{1}H NMR spectrum on one axis and the coupling constants (in Hz) on the other axis. An alternative for determination of coupling constants in such systems employs homonuclear decoupling experiments, which involves irradiation of a specific proton resonance while acquiring a normal ^{1}H NMR spectrum. The result is a ^{1}H NMR spectrum in which the irradiated proton resonance is absent and all protons coupled to the irradiated proton are simplified by the absence of their *J* coupling to the irradiated proton. This experiment is particularly useful for determination of coupling constants of protons with methyl substituents. As shown in Fig. 6b, irradiation of the methyl resonance at C-16 simplifies the complex splitting pattern of the respective methine proton H-16, allowing for an accurate determination of vicinal coupling constants. One drawback of this method, however, is that for selective irradiation of one specific resonance, this signal must be separated from other peaks by ~0.1 ppm. A similarly attractive alternative involves use of a TOCSY (Total Correlation Spectroscopy) experiment. Here, correlations are only seen between protons within one spin system. A spin system consists of protons which are directly connected together by *J*-couplings, *i.e.* each member of the group must have at least one *J*-coupling with another member of the group.[56] For example, since there is no coupling from H-9 to H-11 and from H-17 to H-19, the 9 protons for the C-13 to C-17 fragment form one distinct spin system. As shown in Fig. 6c, if one member of this spin system (*e.g.* the H-17 proton) is irradiated, the TOCSY mixing sequence will transfer that magnetization first to its direct, vicinal coupling partners (H-16 and the CH_3 group) and then to *their* *J*-coupled partners (H-15) until all members of the spin system are excited. The corresponding spectrum will then only contain peaks belonging to that spin system. This technique dramatically simplifies complex spectra and allows delineation of coupling constants in crowded spectra for protons which are obscured by overlapping signals. In general, signal intensity correlates to the distance to the irradiated proton, *i.e.* protons in close vicinity usually give more intense peaks and protons farther away give weaker signals. Furthermore, in a similar fashion to a NOESY experiment (see above), signal intensity also depends on the mixing time. Thus, if short mixing times are chosen, only the irradiated proton and protons directly coupled to it will be observed, while extended mixing times will provide a spectrum of the entire spin system. This experiment is of similar sensitivity as a normal ^{1}H NMR spectrum. Additionally, it may

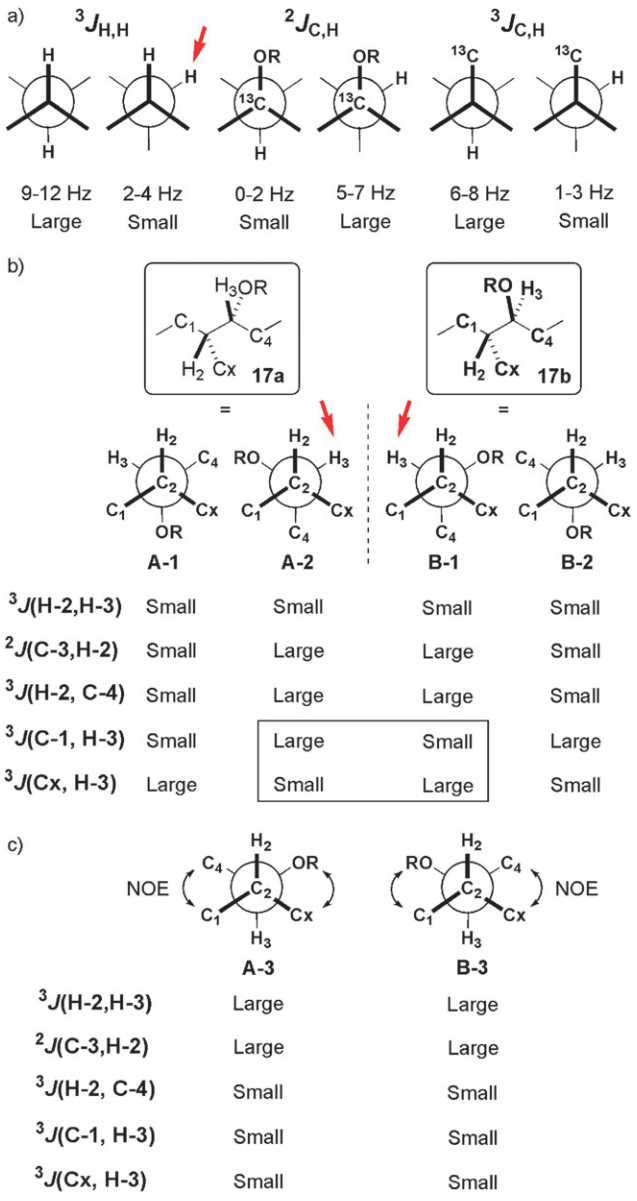


Fig. 4 Murata's method of *J*-based configurational assignment.[54]

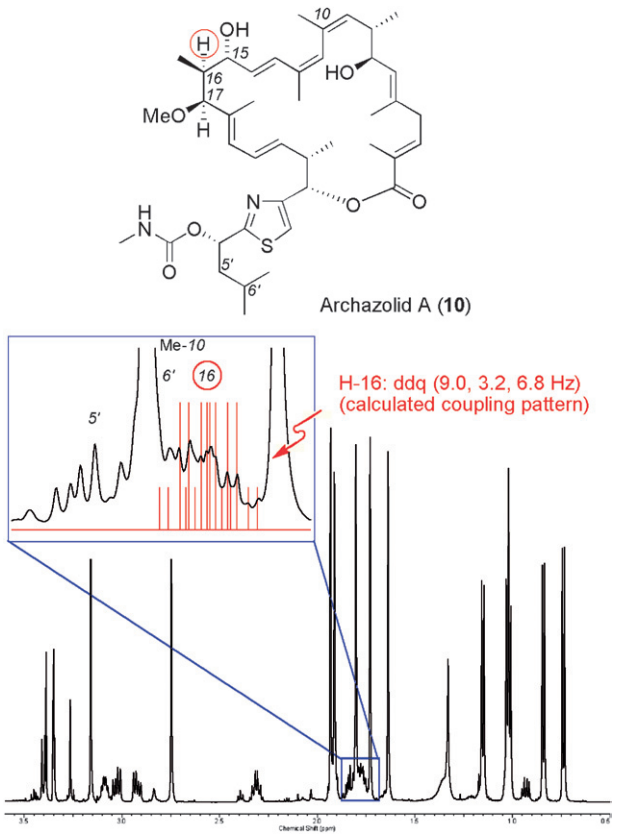


Fig. 5 Slice of the normal ^{1}H-NMR spectrum of archazolid A (**10**) and the calculated coupling pattern for H-16.

also be run as a 2D experiment (2D TOCSY). These techniques were utilized to unambiguously establish the splitting pattern and coupling constants of H-16 as given in Fig. 5.

As compared to determination of homonuclear coupling constants, detection of proton–carbon coupling constants is more challenging.[57] One technique to determine such coupling constants is an HSQC-HECADE experiment.[58] As schematically shown in Fig. 7, the resulting 2D spectrum is similar to an HMBC spectrum, where a ^{1}H NMR spectrum is on one axis and a ^{13}C NMR spectrum is on the other axis. However, all correlations are split into two separate resonances. The horizontal distance represents the $^{2/3}J_{CH}$ coupling constants between the corresponding proton and carbon signal (a, c, d, e), while the vertical distance (b) is due to the 1 bond coupling between the methylene proton and methylene carbon (typically 150 Hz). While this value is not important, it can be adjusted by any multiple of the one-bond coupling constants. The multiplication of this offset is useful to 'clean up' sections of a spectra where correlations are overlapping. As depicted by (c), 3-bond couplings generally have negative values, while 2-bond couplings generally have positive values (d, e). Deleterious artefacts of this experiment, which are often present, are co-occurring HMQC correlations which interfere with useful signals and are separated by $^1J_{CH}$ in both dimensions. Drawbacks of this technique include difficulties in detecting heteronuclear coupling constants between protons and carbons where these atoms originate from different spin systems, or where the coupling constants of the respective protons are very small (<2 Hz). These difficulties may be overcome through the use of an HSQMBC experiment; however, this experiment is less sensitive and requires more material.[57]

As shown in Fig. 8, one of the characteristics of our configurational assignment of the archazolids and Murata's method in general,[42] is the ready applicability to conformationally biased areas, such as the C-6–C-9 and the C-15–C-19 regions of the archazolids, while use of the method to conformationally more flexible areas, *e.g.* the C-20–C-23 region, is more precarious (*vide infra*). As shown in Fig. 8a for the north-western subunit, one of the advantages is the valuable option to confirm the stereochemical assignment obtained from analysis of homo- and hetereonuclear coupling constants by NOE data. For instance, the 15,16-*syn*-16,17-*anti*-relationship in the north-eastern region, as deduced by the respective coupling constants, was strongly supported by a characteristic sequence of NOESY correlations,

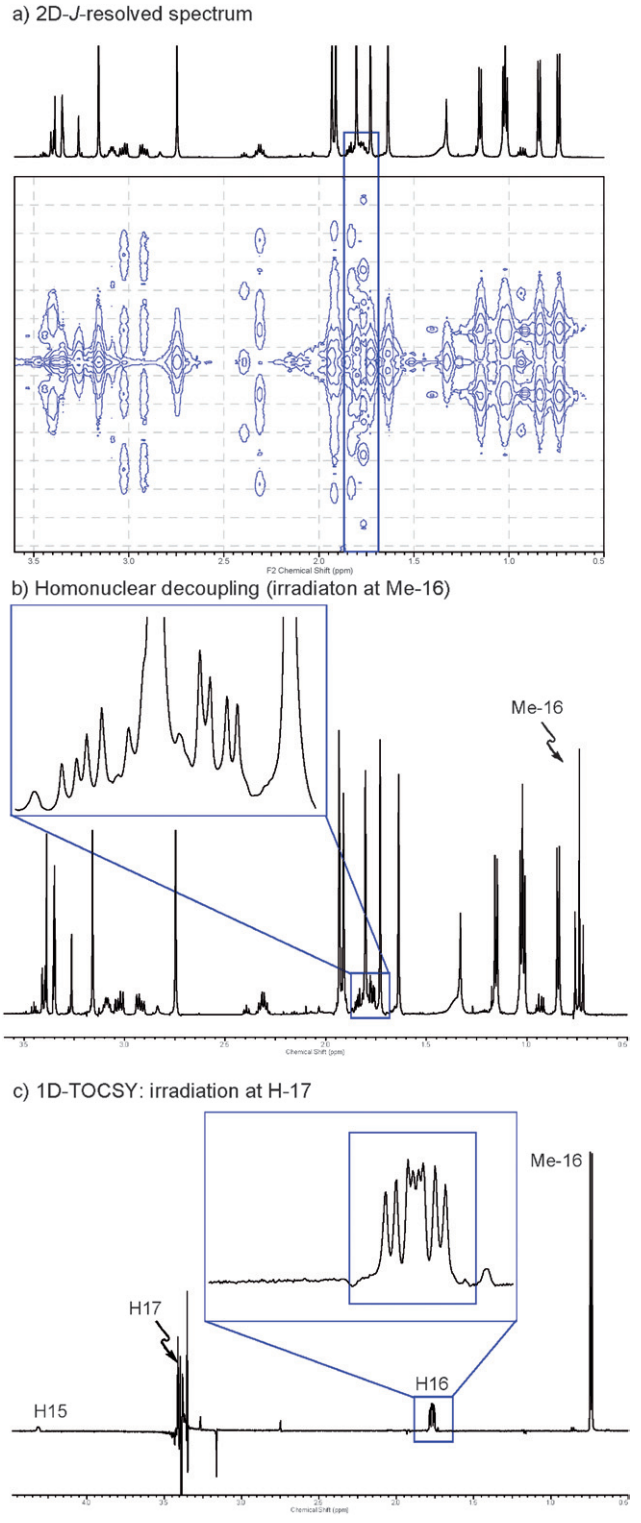


Fig. 6 ^{1}H NMR spectra of archazolid A: (a) Segment of the 2D *J*-resolved spectrum; (b) homonuclear decoupling: irradiation of the methyl resonance Me-16 resolves the methine proton H-16; (c) 1D TOCSY: irradiation of H-17 (3.40 ppm) results in a spectrum including H-15, H-16 and Me-16.

suggesting this subunit to reside in the conformation and configuration as depicted. Notably, NOE data are crucial for configurational assignment of vicinal stereogenic centres where

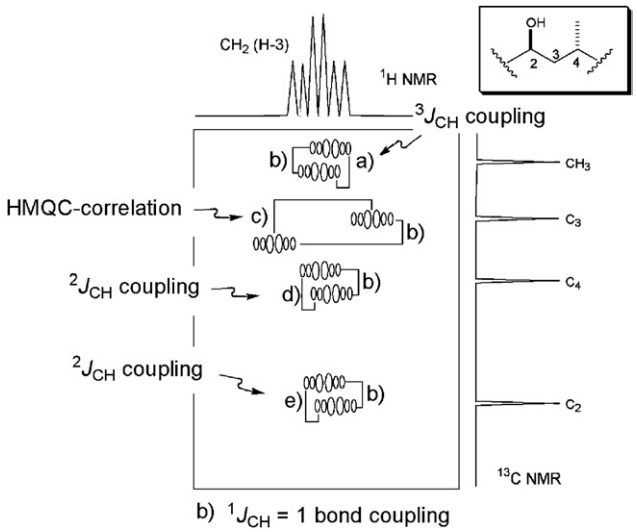


Fig. 7 Simple example of section from a HSQC-HECADE spectrum: (a) $^{3}J_{CH}$ = 3-bond coupling between the methylene proton and the methyl carbon; (b) $^{1}J_{CH}$ = 1-bond coupling between the methylene proton and methylene carbon (typically 150 Hz); (c) HMQC correlation; (d) $^{2}J_{CH}$ = 2-bond coupling between the methylene proton and the methine carbon (C-4); (e) $^{2}J_{CH}$ = 2-bond coupling between the methylene proton and the oxy-methine carbon (C-2).

the respective protons reside in antiperiplanar relationships, such as protons H-16 and H-17. In such cases, heteronuclear coupling constants alone do not enable stereochemical determination, since both the 16,17-*syn* and 16,17-*anti* stereoisomers would lead to similar sets of data (see also Fig. 4c). However, analysis of NOE data is usually straightforward, allowing for an unambiguous assignment of stereochemistry even in such cases. An application of Murata's method also to configurational flexible subunits is given in Fig. 8b. In such cases, $^{3}J_{H,H}$ and $^{2,3}J_{C,H}$ may attain intermediate values between respective *gauche* and/or *anti* isomers and respective rotamers with H/H-*anti* and *gauche* orientations have to be considered. In general, use of this method is only possible in cases where only two main conformers are to be considered and/or where a third rotamer comprises less than 10% of the population, which may be disregarded. Also, configurations with H/H *gauche* pairs cannot be identified. Such pairs, however, are very unlikely for thermodynamic reasons, as all substituents are gathered on one side.[54] In all other cases, the possible alternating pairs may be identified using $^{2,3}J_{C,H}$ and $^{3}J_{H,H}$ as the possible H/H-*anti*/*gauche* pairs of rotamers A-2/A-3, A-1/A-3, B-2/B-3 and B-1/B-3 by characteristic combinations of *J* values, thus allowing stereochemical determination. Using such an approach, an A-1/A-3 combination was deduced for the south-western subunit of the archazolids. Again the 22,23-*syn* assignment was confirmed by NOE data. The full stereostructure of the archazolids was subsequently solved by computational (see section 4) and chemical methods[42] and subsequently confirmed by total synthesis and residual dipolar couplings (see section 3.4).

While Murata's method may also be applied to proximal stereogenic centres, in cases where the two diastereotopic protons are resolved from each other, determination of the relationship between remote stereogenic centres, such as the three

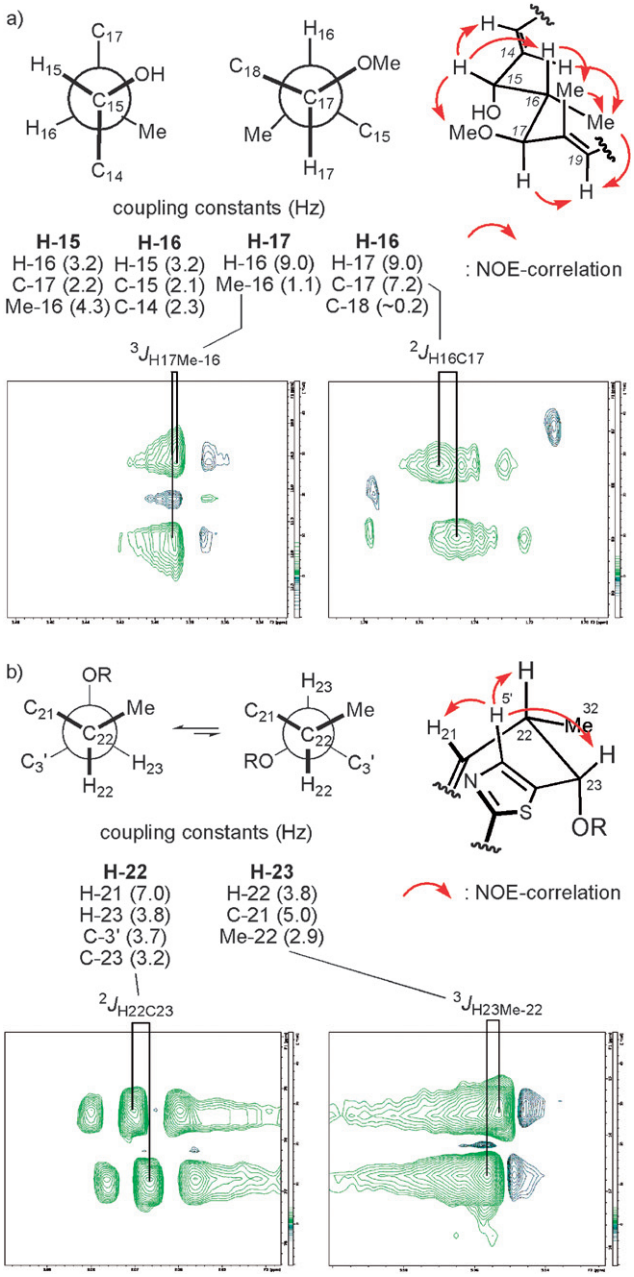


Fig. 8 Rotamers determined for (a) the C-13–C-19 subunit and (b) the C-20–C-23 subunit of archazolid A (**8**) with slices of the HSQC-HECADE spectrum.[42]

stereoclusters of archazolid, is usually not possible through *J*-based configuration analysis, requiring alternative approaches, such as more advanced NMR methods, molecular modelling or biosynthetic considerations (see below).

3.3 Kishi's NMR database method

As an alternative to the *J*-based method described above, the group of Kishi has evolved an NMR database approach for configurational assignment of neighbouring stereogenic centers.[59,60] It is a purely empirical procedure, which relies on the assumption that NMR-spectroscopic properties are inherent to the specific stereochemical pattern and independent from the rest of the molecule, and involves comparing observed chemical shifts of unknown vicinal or proximal centres with libraries of configurationally known model compounds

An instructive example of the viability of this method has recently been reported by Jundt and Höfle in their configurational determination of the cruentarens A (**9**, see Fig. 1) and B (**18**, Fig. 9), stereochemically identical but constitutionally isomeric undecaketides from *Byssovorax cruenta* containing a 12- and a 6-membered lactone.[38] Cruentaren A belongs to the most cytotoxic metabolites from myxobacteria, acting by selective inhibition of mitochondrial F-ATPase.[39] While configurational assignment of the western (9,10) and eastern (24,25) subunits relied on more conventional methods using Mosher ester analysis and GC-comparison with synthetic material, Kishi's procedure was applied to the central C-14 to C-19 stereotetrad. By using this method, an arithmetical elaboration of carbon chemical shifts of cruentaren B (**18**)

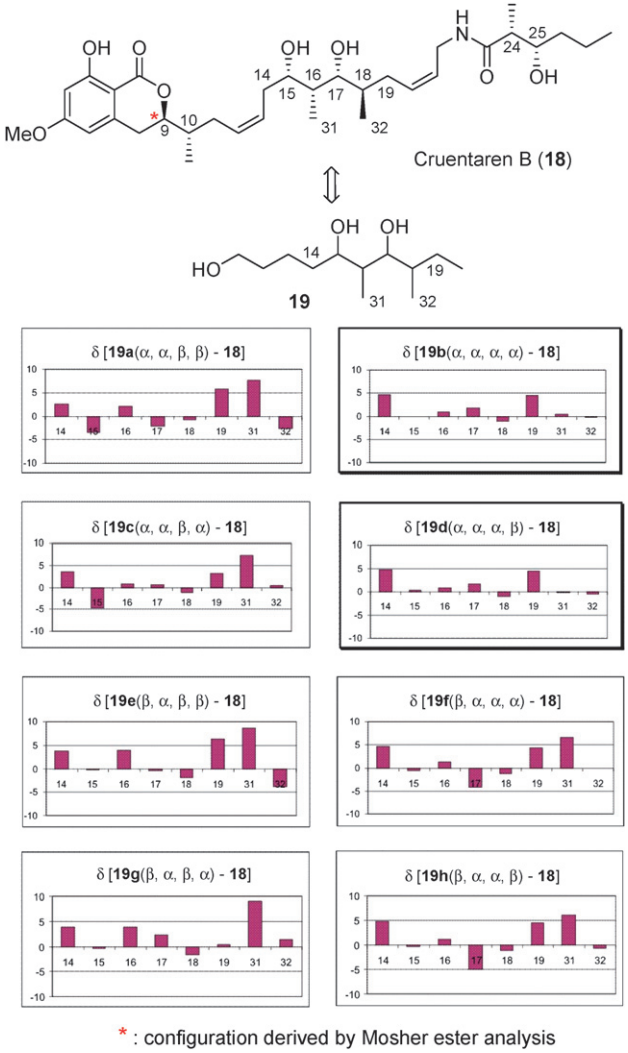


Fig. 9 Deviations from the average of the carbon chemical shifts of model compounds **19a–h** and adjusted values of cruentaren B (**18**). The *x* and *y* axes represent position number and $\Delta\delta$ in parts per million, respectively. α/β denote the relative configurations.[38]

relative to the eight diastereoisomers **19a–h** shown as NMR histogram plots, is expressed as individual deviations. In this way, the full chemical shift data set of compounds **19a–h** represents the database of this general structure **19**. As these data have been made available by the group of Kishi, they may be successfully used to determine the relative configuration of an unknown compound having a structure that encompasses a fragment like **19**, provided that this fragment is conformationally unconstrained, *i.e.* acyclic or part of a sufficiently large ring. Indeed, a highly complex and functionalized molecule can be considered, from a structural viewpoint, as the sum of independent subunits (stereoclusters) linked together. This assumption constitutes one of the key foundations for this approach. As a consequence, each subunit can be related to that of a model diastereoisomer with the same constitution. By simply comparing the carbon and proton (not shown) chemical shifts of the unknown stereocluster of cruentaren B (**18**) with those of **19a–h**, a configurational assignment may be made. As shown, a prediction of the (α,α,α,α) and (α,α,α,β) isomers **19b** and **19d** as the most possible configurations was obtained with high significance. Subsequently, the correct configuration was derived by detailed NMR-analysis of cyclic benzylidenacetal-derivatives and confirmed by X-ray analysis and Mosher ester derivatisation.[38] Ultimately, the structure of the cruentarens was proven by total syntheses of cruentaren A by Maier[40] and Fürstner.[41]

While this assignment represents the only application of this database approach in the area of myxobacterial polyketides, various other applications have been reported for polyketides of different biosynthetic origin.[61] Compared to Murata's method, the notable advantages of Kishi's procedure are its independence from conformational flexibility and use of conventional NMR techniques.

3.4 Residual dipolar couplings

While both Murata's *J*-based configurational analysis and Kishi's database approach may be successfully applied for the relative assignment of neighbouring stereogenic centres, determination of the relationship between remote stereogenic centres is usually not possible. A novel NMR-based approach for such cases has recently been explored by use of residual dipolar couplings (RDCs).[62] In contrast to conventional scalar couplings, they necessitate partial alignment of a molecule in solution, requiring anisotropic media. While this may be regarded as a drawback of the method, the potential of this approach is generally considered as enormous, in particular due to the distance dependence of these RDCs being much more sensitive than conventional NOE interactions (r^{-3} *vs.* r^{-6}), which allows a much more detailed analysis of long-range interactions. Originally discovered in the late 1960s,[63] they have found widespread use in biomacromolecular NMR spectroscopy. Applications to small molecules, however, had been much less advanced due to lack of suitable anisotropic media for organic solvents. However, this has been recently remedied and a number of such media have now been developed, which rely on anisotropic swelling of a polystyrene stick in a standard NMR tube.[64] First applications of RDCs to stereochemical issues involve assignment of diastereotopic protons[65] and elucidation of non-consecutive asymmetric centres in small and rigid organic molecules with relatively close stereocentres.[66] Very recently, also a first analysis of neighbouring stereocentres in more flexible molecule has been successfully addressed.[67]

The first application of RCDs for a structurally complex polyketide macrolide, archazolid A (**10**, see also section 3.2) has now been explored by the group of Carlomagno.[68] They dispersed a DMSO solution of this polyketide in an anisotropic polyacrylamide gel. In the resulting NMR spectra, the RCDs add to the corresponding scalar couplings ($J + D$) and may therefore be determined by simple subtraction of the spectra with and without the alignment medium. While RCDs may be conveniently measured, analysis of the data is much more challenging and involves determination of the alignment tensor as a measure of the degree of alignment of the molecule, which requires a reliable structural model. This was obtained for various stereoisomers of the archazolids by more conventional methods using NOE restraints. Fig. 10a shows the structure for the correct configuration of archazolid A (grey). This conformation and that of respective stereoisomers were then evaluated for their compatibility with an ensemble of measured $^1J_{CH}$ RCDs. The best agreement was observed for the correct configuration of this macrolide, as shown in Fig. 10b, which demonstrates the general usefulness of this highly innovative method for complex polyketides, both for vicinal as well as remote stereogenic centres. This analysis was then further refined by RCDs (green structure), leading to an even better agreement between measured and calculated data as shown in Fig. 10b, and thus further corroborating the usefulness of this procedure. Notably, this approach also confirms the relative assignment of the flexible 22,23-configuration, where application of Murata's method has been more challenging (see section 3.2).

In general, it is expected that RDCs will find further applications to other complex polyketides and will be particularly useful for the relative assignment of remote stereocentres as well as conformationally labile structures.

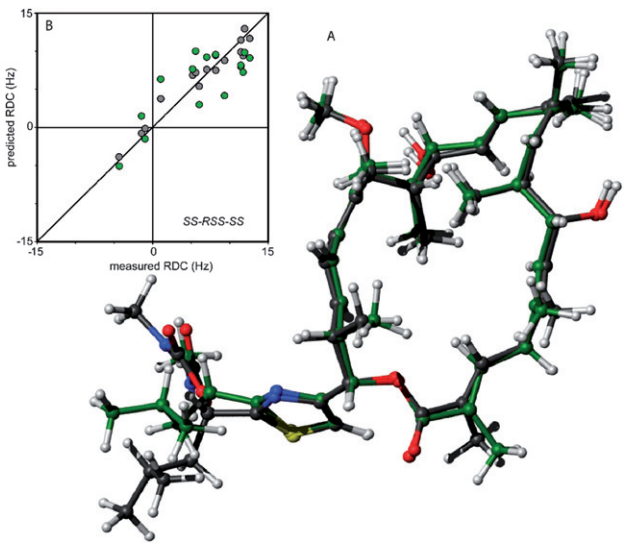


Fig. 10 Use of residual dipolar couplings (RCDs) for stereochemical determination of archazolid A (**10**): (A) structure before (green skeleton) and after (black skeleton) refinement by RCDs; (B) correlation of the theoretical *vs.* experimental RCDs of the conformation before (green dots) and after (grey dots) refinement.[68]

4 Molecular modelling

As an alternative to resolving stereochemical relationships of remote centres, molecular modelling has become a very popular choice and Monte Carlo searches have been amply used for such issues.[7] In some cases, useful information may already be obtained by analysis of the global minimum obtained by field calculations. Other substrates require conformational restraints and/or refinement by more high level calculations. In all cases, close comparisons between calculated and observed dihedral angles and transannular NOEs are required to validate the method.

An illustrative example for a recent application of a computational method is the stereochemical assignment of the macrocyclic core of the thugaccins.[16] These macrolide antibiotics from *Sorangium cellulosum* show pronounced activity against *Mycobacterium tuberculosis*, including clinical isolates, which renders them important lead structures for development of novel causative agents that target tuberculosis. The analysis of Jansen and Höfle was initiated by MM+ force field calculations of various stereoisomers of thuggacin A on the basis of conformational restraints based on dihedral angles and approximation of selected H–H distances by NOESY experiments. The structures thus obtained were then further refined by semiempirical calculations (PM3). As shown in Fig. 11, the lowest energy conformation found accounted for a number of critical NOESY correlations and resulted in a very good match between the calculated dihedral angles and correlation to a corresponding series of $^{3}J_{H,H}$ coupling constants. This assignment was further corroborated by an analogous approach for thuggacin B (not shown), a stereochemically identical natural isomer with a different ring size. Again, a very good correlation between calculated and observed data was obtained, which further confirms the validity of this assignment.

In the computational studies of archazolid A (**10**) in our group,[42] two families of conformers were predicted by Monte-Carlo searches using Macromodel[69] with the generalised Born/surface area (CB/SA) solvent model[70] using the MMFS force field, and restraints based on dihedral angles and approximation of selected H–H distances by NOESY experiments. The structures were then further refined by AM1. Notably, the calculated low energy conformation for the correct configuration, as shown, was found to be higher in energy than the global minimum as obtained from a *non*-restrained conformational search under the same conditions. In addition, defining a convincing relationship between the stereoclusters in the northern part and the southern part of the macrocycle and a correlation to the side chain was not possible by modelling alone, and called for more rigorous means for stereochemical proof. Finally, this was achieved by chemical derivatisations allowing for a convincing stereochemical proposal. Ultimately, the correct structure of the archazolids was proven by total syntheses from our group (archazolid A)[46] and that of Trauner (archazolid B).[47]

Chivosazol A (**11**) is a 31-membered macrolide from *Sorangium cellulosum*, originally isolated by the group of Höfle in the late 1990s.[8] The chivosazols form a class of (so far) eight derivatives, which are active against yeasts, filamentous fungi and are highly cytotoxic against mammalian cell cultures. One of the most striking features in the computational study on chivosazol A[9] by the group of Kalesse is that they started their Monte-Carlo searches not with the natural product itself but with a largely truncated derivative where all substituents except the 8-Me group were replaced by hydrogen atoms. In addition, formal ring opening at the macrocyclic O-ester bond (C30–O1) was

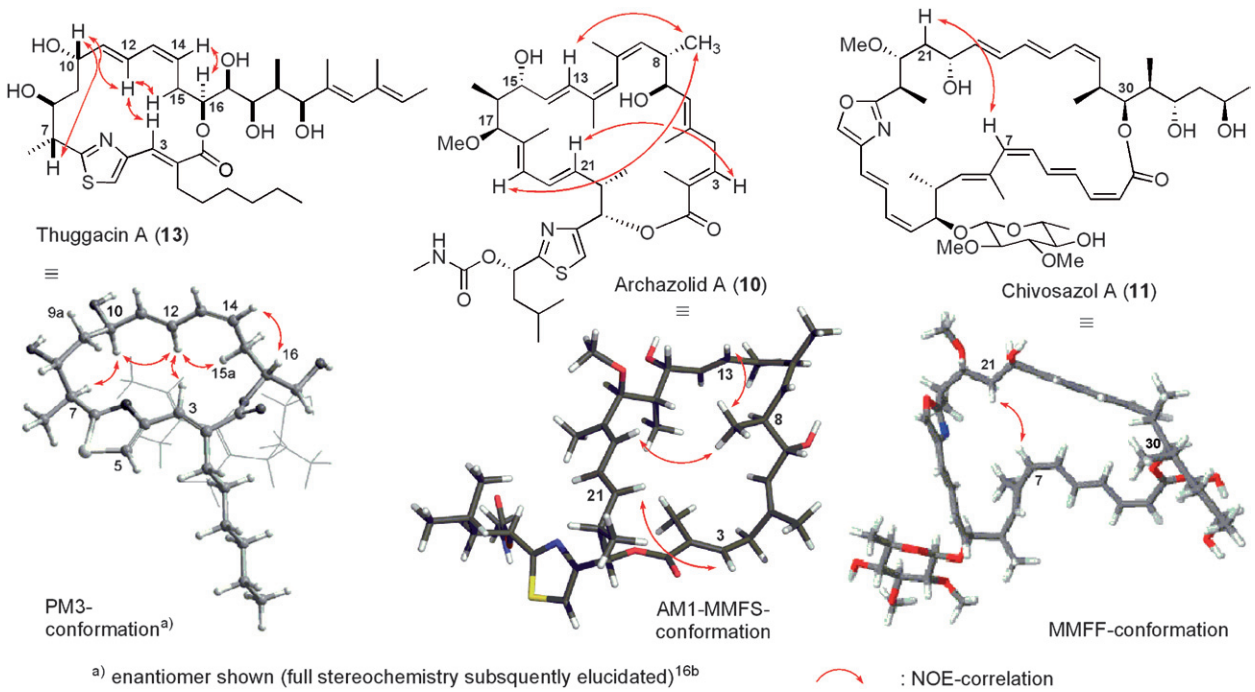


Fig. 11 Perspective drawing of low energy conformations of myxobacterial polyketides with selected transannular NOESY correlations.[16,42,9]

simulated and the calculated conformations were only accepted if the distance between H-7 and H-21 was less than 4 Å. The omitted substituents were then added afterwards according to vicinal proton–proton coupling constants and the structure thus obtained was again submitted to Monte-Carlo searches. Interestingly, the conformation before and after addition of the substituents remained largely unchanged. This conformation could explain key NOE-data and coupling constants. Subsequently, this structure was further validated by biosynthetic considerations (see section 5) and a synthesis of a side chain degradation fragment. In general, this innovative approach by starting with a simplified structure significantly simplifies the *in silico* method as no stereochemical permutations have to be considered in the beginning of the calculations. It remains to be seen whether this procedure is of general usefulness to conformationally less restricted macrocycles than the chivosazols.

5 Bioinformatics analysis

Biosynthetically, polyketides are derived by the condensation of acetyl and propionyl subunits. Mechanistically, this follows an iterative Claisen-type condensation process of ACP-bound malonyl- or methyl-malonyl-CoA, with a growing polypeptide on the current acyl carrier protein (ACP), giving rise to ACP-bound β-ketoesters of type **20**. As shown in Fig. 12, these intermediates may then be reduced by the action of NADH-dependent ketoreductases to furnish the respective β-hydroxyketones **21**, which may be obtained with either L- or D-configuration in a stereospecific manner, depending on two types of ketoreductases (KRs). Quite recently, the groups of Reid[71] and Caffrey[72] have analysed in detail the core region of these ketoreductases and proposed a mechanism for this stereospecificity. According to their model, the substrate may coordinate in two ways. They only differ in the rotation about the axis of the reduced carbonyl bond to orient the substrate differently in the two types of KRs, as shown in Fig. 12a, to give after reduction either the L- or D-configured products. As shown in Fig. 12b, on the basis of the active site arrangement of the tropinone reductase II from *Datura stramonium* with NADP and a substrate analogue, the authors propose that from the various amino acids involved, the presence of only one amino acid, an aspartate residue (N118) is critical for the orientation of the substrate and thus the facial bias of the reduction. Accordingly, when aspartate is present, this amino acid is believed to align with the putative catalytic lysine (K163) and tyrosine (Y159) residues, thus leading to the D-configured product. In its absence, however, the orientation of the substrates is rotated, thus giving the L-diastereomer.

By application of this model of Reid and Caffrey, the absolute stereochemistry of secondary alcohols in polyketides may therefore be determined in a fairly simple fashion by analysis of the presence of only one amino acid, depending on the accessibility of the biosynthetic gene cluster encoding the critical ketoreductases. Very recently, the very first application has been described by the groups of Kalesse and Müller in their assignment of chivosazol A.[9] The respective biosynthetic gene cluster was elucidated by the group of Müller and encodes a complex hybrid of polyketide synthases (PKS) and a nonribosomal peptide synthetase. As shown in Table 2, analysis of the KR core regions revealed the presence of an aspartic acid residue in KRs

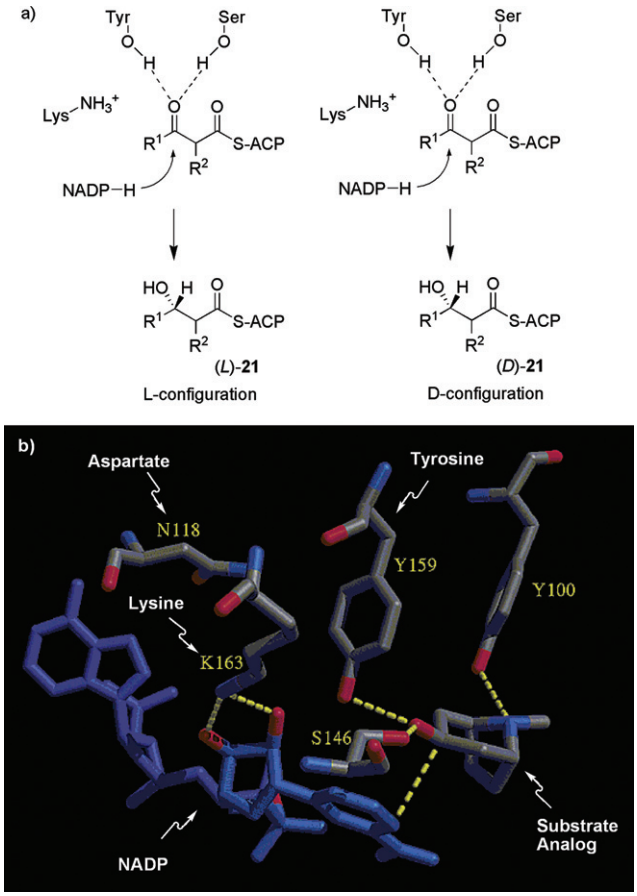


Fig. 12 Proposed model for the stereospecific reduction by modular polyketide synthase ketoreductase (PKS KR) domains. Depending on the orientation of the substrate in the two types of KRs, either the L- or D-configured product may be obtained (a). In the active site, the presence of Asn118 leads to carbon centres with D-configuration, or to in its absence to the L-configuration.[73]

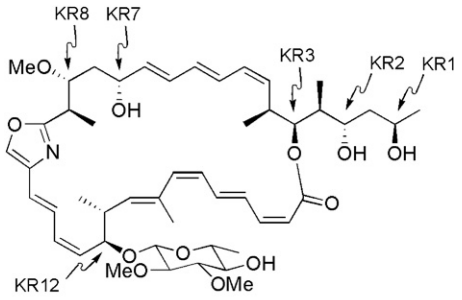
1, 3 and 8, suggesting a D-configuration at the respective hydroxyl-bearing centres, while its absence in KRs 2, 7 and 12 indicated an L-configuration at these centres.

Notably, this assignment was confirmed in a complementary and independent fashion by NMR methods, molecular modelling (see section 4) and chemical synthesis of a degradation product. All these methods consistently proposed the same configurations for the individual centres. This corroborates the general usefulness of this biosynthetic approach for configurational assignment in complex polyketides. Very recently, this approach has been also successfully applied to the assignment of the hydroxyl-bearing stereogenic centers of thuggacin (**13**).[16] It is expected that this bioinformatics approach will find further applications, as more and more information on the biosynthesis of these polyketides is unravelled.[74]

6 Synthetic methods

As shown in Table 1 (section 2), myxobacterial polyketides have attracted high interest from the synthetic community. This is particularly true when the full 3D structures have been disclosed, and so far total syntheses have been reported for six

Table 2 Ketoreductase core regions of the chivosazole biosynthesis with aspartate (D) residues in bold and the proposed configuration of the corresponding stereocentres in the product.[9]



Chivosazol A (**11**)

KR[a]	Central region	Configuration
KR1	AGVLR**D**GLCL	C34 (D)
KR2	ALSYQGAPLA	C32 (L)
KR3	ALRLE**D**RTID	C30 (D)
KR7	AGGTDATRIG	C22 (L)
KR8	AITLA**D**GLLA	C20 (D)
KR12	AFLFASEPLA	C11 (L)

[a] Reductions with KRs 4, 5, 6, 9 and 10 is followed by elimination of water, resulting in double bonds.

representatives, which were critical in assigning the absolute configuration of the crocacins (**1**),[19] melithiazols (**2**)[26] and spirangiens (**8**),[36] besides the general importance of unambiguously confirming the configurational assignment, in particular in cases where no X-ray data were available, such as for the archazolids (**10**).[46]

For stereochemical determination of myxobacterial polyketides, a number of conventional derivatisation methods have been used, namely Mosher ester analysis,[15,29,38,42] Rychnovsky's acetonide method[16b,38,75] and ozonolysis.[9]

A conceptionally new degradation protocol has been reported by the group of Höfle. It relies on selective cleavage of the least hindered double bond by cross-metathesis, in a complementary fashion to conventional ozonolysis. As shown in Fig. 13 for cruentaren A (**9**, see also section 3.3)[38] this involves treatment of the natural product with ethylene and a ruthenium catalyst, and results in a selective cleavage at the sterically least hindered olefin, namely the C-20–C-21 bond. No attack at the more hindered C-12–C-13 double bond was observed, demonstrating the very high selectiveness of this approach. The very mild reaction conditions and the valuable option to start from the natural product itself without protecting groups are further advantages of this method. Another advantage is the access to less lipophilic products, such as **22**, which are more prone to crystallization. Indeed, this derivative gave crystals suitable for X-ray analysis, allowing for unambiguous assignment of the relative configuration of the macrocyclic core of cruentaren A.

In an analogous fashion, selective cleavage of apicularen[76] and spirangien[11] were also accomplished by the group of Höfle. In the latter case, again a crystalline degradation product suitable for X-ray analysis was obtained, which was critical in assigning the

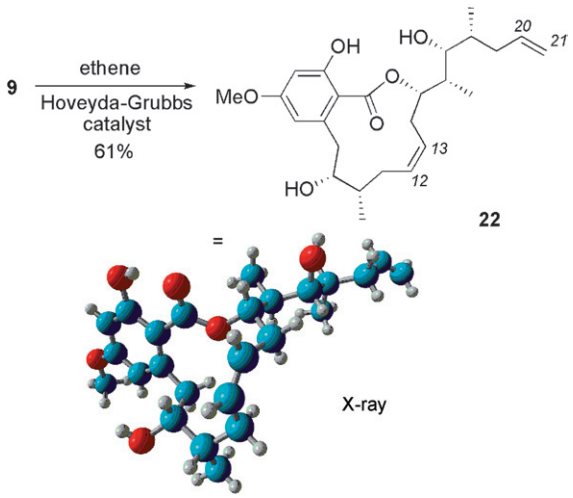


Fig. 13 Selective degradation of cruentaren A (**9**) by olefin cross-metathesis in the presence of ethylene to give crystalline 21,22-seco-cruentaren (**22**).[38]

stereochemistry of this cytotoxic polyketide. It is expected that this very mild novel degradation approach will find further applications.

7 Concluding remarks

In summary, the state of art for stereochemical determination of complex polyketides has considerably advanced within the last few years, and a variety of efficient and truly applicable methods have been developed, allowing for a reliable configurational assignment even in cases where no X-ray data are available. NMR-based methods are particularly valuable for determination of vicinal and proximal stereogenic centres by Murata's *J*-based configurational method or Kishi's database approach. For determination of the relationship between remote stereogenic centres, *in silico* approaches have been amply used. However, additional means for stereochemical proof are often required. As an alternative to chemical synthesis, the use of residual dipolar couplings has recently been explored, which may resolve such issues. In a complementary fashion, amino acid alignments using the model of Reid and Caffrey have recently been introduced for assignment of secondary alcohols. It is expected that the combination of these different and (in particular) independent options will further advance and speed up the challenging feat of stereochemical determination of complex polyketides and will be critical for further advancing these natural products in general.

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