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Perspectives from Nearly Five Decades of Total Synthesis of Natural Products and Their Analogues for Biology and Medicine

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Abstract

By definition total synthesis is the art and science of making the molecules of living Nature in the laboratory, and by extension, their analogues. Although obvious, its application to the synthesis of molecules for biology and medicine was not always the purpose of total synthesis. In recent years, however, the field has acquired momentum as its power to reach higher molecular complexity and diversity is increasing, and as the demand for rare bioactive natural products and their analogues is expanding due to their recognised potential to facilitate biology and drug discovery and development. Today this component of total synthesis endeavors is considered highly desirable, and could be part of interdisciplinary academic and/or industrial partnerships, providing further inspiration and momentum to the field. In this review we provide a brief historical background of the emergence of the field of total synthesis as it relates to making molecules for biology and medicine. We then discuss specific examples of this practice from our laboratories as they developed over the years. The review ends with a conclusion and future perspectives for natural products chemistry and its applications to biology and medicine and other added-value contributions to science and society.

Graphical Abstract

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Conflicts of interest

There are no conflicts to declare.

1. Introduction

The serendipitous preparation of urea, from silver cyanate (more accurately, following today's nomenclature, silver isocyanate)¹ and ammonium chloride, or lead cyanate and ammonia, respectively, by Friedrich Wöhler in 1828² had a profound impact on chemistry. It gave birth to organic synthesis and its flagship, total synthesis of natural products, of which urea was the first. It also helped shape the concept of isomerism,³ a fundamental principle and important feature of the molecular sciences. As the power of total synthesis improved, more complex natural products were replicated in the laboratory while the main purpose of the endeavour evolved to become the means for confirming the hypothetical structures of natural products.⁴ This purpose remained as the main objective of the continuously growing in power discipline of total synthesis until well in the twentieth century when R. B. Woodward emerged as the leader of the field in the 1940s and 1950s. With his important contributions he turned synthesis into an art, with one complex molecule after another falling as he was marching over them in glory, mesmerising his admirers with his aesthetically pleasing synthetic strategies. Then came E. J. Corey, who turned total synthesis into a systematic art and science through his logic-based approach by introducing the retrosynthetic analysis concept and method development as integral parts of total synthesis endeavours. Corey also demonstrated the crucial value of total synthesis to biology and medicine by synthesising scarce naturally occurring molecules, and other molecular structures hypothesised to be naturally occurring, thereby rendering them readily available for structural confirmation, elucidation, and biological investigation. His landmark accomplishments included a number of prostaglandins⁵⁻⁷ and leukotrienes,⁸⁻¹³ among others. By the 1970s, the seeds were in the ground for the field of making molecules for biology and medicine through total synthesis to emerge and gather momentum in the following decades. In the meantime, isolation chemists, both from academia and the pharmaceutical industry, were busy harvesting the molecules of Nature as potential drug candidates, a revolution that gathered strong momentum with the discovery of penicillin, and accelerated during the second World War as incentivised by the need to treat and save the lives of wounded soldiers in the field. The second half of the twentieth century was indeed a golden era for natural products isolation and total synthesis, not to mention the myriad drugs derived from or inspired by them that were approved for clinical use, with a good number of them, or their descendants, still in use today.

Then random combinatorial chemistry kicked in, to some perhaps an unfortunate occurrence for both natural products isolation and total synthesis. Based on assumptions, some believed that random combinatorial chemistry would provide all the drugs needed to treat every disease, and there was perhaps no need for rational drug design or natural products anymore. Although combinatorial chemistry had its positive impact in academia and the pharmaceutical industry, depending on how it was practiced, it left a wound and a void in terms of exploring Nature for more of its hidden treasures, molecules that perhaps, can teach and inspire us more about chemistry, biology and medicine than any other source of knowledge. A sign of hope, however, is on the horizon that can be translated into action, with total synthesis incorporating a biology and medicine component in its endeavours and, hopefully, re-establishment of the isolation of natural products as a priority that will surely

provide inspiration, reigniting and fuelling new momentum to the field. The community of total synthesis practitioners with this philosophy is on the move, both in terms of numbers and new initiatives as evidenced from the collection of the articles in this issue of *Natural Product Reports*.

The design, synthesis and biological evaluation of natural product analogues and other molecules for biology and medicine in academia was rather sporadic and rare in the 1970s. One of us (KCN), as a postdoctoral fellow in the Corey group at Harvard University, had the opportunity to be given one of the first projects in the lab to synthesise an envisioned stable analogue (9,11-diazo-PGH₂, Fig. 1C2) of the labile prostaglandin endoperoxide (PGH₂, Fig. 1C2), the biosynthetic precursor of prostaglandins from arachidonic acid.¹⁴ With teammate Dr Yoshi Machida, we completed its synthesis from PGF_{2α} in short order, and Professor Corey promptly dispatched a sample to Professor Bengt Samuelsson at the Karolinska Institute in Stockholm for biological evaluation. The results came back shortly thereafter, and for the first time I (KCN) felt the magical joy of biology intertwined with that of chemical synthesis. Our molecule turned out to be not only more stable but also more potent than its “parent” compound (PGH₂) with regards to its ability to induce platelet aggregation and release of serotonin when added to human platelet-rich plasma.¹⁴ It also demonstrated higher potency than the naturally occurring PGG₂ (15-hydroperoxy-PGH₂)¹⁴ in inducing muscle contraction in an isolated rabbit aorta strip assay. As a result of this exciting and inspirational experience, the idea of organic synthesis merged with biology and medicine was embodied within our research programs in total synthesis from the very beginning of my (KCN) independent career at the University of Pennsylvania (1976). Before I (KCN) was granted the privilege to join the Corey group (1973), my research as an undergraduate student with Roger Bolton at Bedford (now Royal Holloway) College London (1966–1969), as a graduate student with Franz Sondheimer and Peter Garratt (1969–1972) at University College London,¹⁵ and as a postdoctoral fellow with Thomas J. Katz at Columbia University (1972–1973)⁸ involved the synthesis of theoretically interesting molecules such as those shown in Fig. 1A,B and C1. My work (KCN) in the Corey group in the 1970s included method development (e.g., double activation macrolactonisation, Fig. 1C2) and total synthesis of complex natural products and their analogues (e.g., erythronolide B, and 9,11-diazo prostaglandin H₂, respectively, Fig. 1C2), an experience that anchored my interests within the field of total synthesis, and expansion of its periphery to include contributions to chemistry, biology and medicine.

The opportunity to practice this paradigm in our laboratories at the University of Pennsylvania, where KCN started his independent career in 1976, came quickly with the arrival on the scene of prostacyclin (PGI₂, **1**, Fig. 2A), a new prostaglandin reported by Vane and his group in 1977.¹⁶ Processing potent antithrombotic and vasodilatory properties, prostacyclin ignited excitement in the biology, chemistry and medicine communities and despite its short lifetime at ambient temperature, became in great demand from biologists and clinicians due to its potential in treating cardiovascular disorders. In order to address the demand from the biologists and pharmacologists we devised a semisynthesis of prostacyclin from PGF_{2α} and supplied it to the many of them who requested it for biological investigations. In parallel, we designed and synthesised a number of stable analogues of

prostacyclin, including its methylene (6,9-CH₂, carboprostacyclin, **2**, Fig. 2A)¹⁷ and sulphur [(5*Z*)-6,9-thiaprostacyclin, **3**, Fig. 2A]¹⁸ analogues, with the former proving stable, albeit somewhat less potent,¹⁷ and the latter demonstrating higher stability and equipotency to the natural product, both as antithrombotics and vasodilators.¹⁹

Inspired by the structure and important biological properties (i.e., vasoconstricting and thrombotic) of the highly labile thromboxane A₂ (TXA₂, **4**, Fig. 2B),²⁰ we also initiated a program directed towards the design, synthesis and biological evaluation of its carbocyclic analogues, pinane-thromboxane A₂²¹ (pinane TXA₂, **5**, Fig. 2B) and carbocyclic thromboxane A₂²² (CTA₂, **6**, Fig. 2B), the former exhibiting inhibitory activity against coronary artery contraction induced by stable PGH₂ analogues, and the latter demonstrating significant increase in coronary perfusion pressure in cat arteries.²³ Within the same program we also synthesised a number of designed analogues of the naturally occurring leukotrienes A₄ (inflammation inhibitor, **7**, Fig. 2C) and B₄ (chemotactic agent, **8**, Fig. 2C), namely, 5,6-cyclopropyl leukotriene A₄ (**9**, Fig. 2C)^{24,25} and 20-trifluoromethyl leukotriene B₄ (**10**, Fig. 2C),²⁶ respectively, the former acting as an inhibitor of the enzymatic production of leukotriene B₄.^{24,25}

In the 1980s, a new family of eicosanoids was discovered in the Karolinska Institute by the Samuelsson group.^{27,28} Derived from metabolism of arachidonic acid as facilitated by lipoxygenase enzymes, and isolated in minute amounts, these new naturally occurring compounds were named lipoxins. Although suspected, their structures could not be fully determined or confirmed, and their biological properties and physiological role remained speculative until larger quantities of them became available from our laboratories. Our collaborative work on the lipoxins with Charles Serhan (one of Samuelsson's co-workers on the lipoxin project at the Karolinska, and later an independent investigator at Harvard) was intended to make these compounds readily available for structural determination and biological investigation purposes. Thus, we synthesised all possible variations of the suspected lipoxins A₄ and B₄ structures (i.e., **11–17** and **18–24**, respectively, Fig. 3A),^{29,30} comparison of which with the enzymatically produced compounds revealed their molecular structures (i.e., **11** for lipoxin A₄, and **18** for lipoxin B₄, Fig. 3A), including their absolute configurations. We also synthesised, through similar strategies, the corresponding lipoxins A₅ (**25**, **26**) and B₅ (**27**, **28**),³¹ suspected to be derived biosynthetically from eicosapentaenoic acid, and confirmed their structures (i.e., **25** and **27**, respectively, Fig. 3B) by comparison with the enzymatically derived materials. In addition to confirming their molecular structures, rendering these scarce molecules readily available through chemical synthesis allowed their thorough biological evaluation and elucidated their action as modulators of human physiology and pathophysiology, thereby providing understanding of their role and opening opportunities for further research and drug discovery and development.^{32–43}

Our expanded collaborative studies in the eicosanoid field resulted in several additional contributions that facilitated biological investigations. Thus, besides synthesising leukotriene B₄ (**8**, Fig. 2C) and several of its analogues, we also synthesised various mono-hydroxyeicosatetraenoic acids (HETEs)^{44–48} and a number of their analogues as well as the 15-hydroxy 5,6-epoxytetraene hypothesised biosynthetic precursor (i.e., **29**, Fig. 3C) of

lipoxins A₄ (**11**) and B₄ (**18**, Fig. 3A).⁴⁹ The latter precursor was shown to be enzymatically convertible to lipoxins A₄ and B₄ (**11** and **18**, Fig. 3A), thereby confirming its biochemical role.⁵⁰ In addition, we synthesised the 5,6-methano counterpart (i.e., **30**, Fig. 3C);⁴⁹ this stable analogue was shown to inhibit the enzymatic conversion of the corresponding epoxide biogenetic precursor (i.e., **29**, Fig. 3C) of lipoxins A₄ and B₄.²⁵ These contributions of total synthesis to biology, and potentially medicine, are exemplary for the field in general. Many more are out there in the literature and, no doubt, myriad others may emerge in the future as rare natural products are unearthed and taken on by synthetic chemists as challenges and opportunities.

2. Miscellaneous Strategies for Natural Product Analogue Construction, Including Compound Libraries

We would be remiss if we do not mention briefly a number of exemplary projects that led to a rich harvest of molecules for biology and medicine during the 1990s and early 2000s in our group at The Scripps Research Institute. These programs (e.g., calicheamicin γ_1^I ,⁵¹ Taxol⁵²⁻⁵⁶) involved, in addition to specific analogue construction, the systematic design and synthesis, through either parallel solution or combinatorial solid phase methods, of analogue libraries of numerous natural products, including the antitumor agents epothilones A and B⁵⁷⁻⁷⁶ and sarcodictyins,^{77,78} the antibiotic of last resort vancomycin,⁷⁹⁻⁸⁵ the bioactive benzopyran-type natural products,⁸⁶⁻⁹¹ and the antibiotic psammaplin A.^{92,93} Several lead compounds were discovered and optimised from these studies that employed specially designed and synthesised resins and MicroKans⁹⁴ or NanoKans⁸⁹ equipped with encoding systems readable by radiofrequency (chips) or light (laser). The Kans themselves consisted of a reactor vessel of differing size with an optically readable laser-etched grid or containing a radiofrequency tag for identification purposes and a suitable resin. This allowed for automated computer-guided pooling. Ranging from tens to thousands of compounds, these compound libraries were prepared on milligram scale per compound so as to allow full structural characterisation of each member as discrete and of high purity compounds. These efforts yielded a number of impressively bioactive compounds (more often than not, possessing higher potencies than their parent naturally occurring molecules) that emerged as powerful biological tools, with some becoming, or considered as, drug candidates. More details of these investigations can be found in the original publications given above and in our 2005 review.^{95,96}

Among the most important molecules synthesised was the calicheamicin γ_1^I (**31**, Fig. 4A) analogue, calicheamicin θ_1^I (**32**, Fig. 4A),⁹⁷ that proved more potent against a number of cancer cell lines than its parent compound, calicheamicin γ^{II} (**31**), and was attached, in collaboration with the Reisfeld group at Scripps, to an antibody targeting a specific cancer cell line to produce an antibody–drug conjugate (ADC).⁹⁸ Antibody–drug conjugates, still a rather new paradigm for the treatment of cancer, consist of a cytotoxic payload attached through a molecular chain (the chemical linker) to an antibody. The entire assembly binds selectively to the surface receptor of the target cancer cell, is then internalized through endocytosis, and eventually releases its payload within the latter where it exerts its cytotoxic action, thus killing the cancer cell. The anti-GD₂ murine mAb 14G2a–calicheamicin θ_1^I

ADC (**33**, Fig. 4A) exhibited impressive growth suppression and dissemination of liver metastases in a syngeneic model of murine neuroblastoma.⁹⁸ Published in 1998, this ADC served as a harbinger to Mylotarg, the first ADC to be approved by the FDA in 2000 as a targeted cancer therapy.⁹⁹⁻¹⁰²

From the numerous Taxol (**34**, Fig. 4B) analogues we designed and synthesised, we highlight here the Taxol 2'-methyl pyridinium acetate (Taxol-2'-MPA, **35**, Fig. 4B)⁵³ and the fluorescein fluorophore taxoid (**36**, Fig. 4B),⁵⁶ the former showing high antitumor potencies and higher than Taxol solubility for improved delivery purposes,¹⁰³ the second serving as a biological tool employed for imaging purposes probing the destination of Taxol upon administration to cells, animal or human.

The epothilones [e.g., A (**37**) and B (**38**), Fig. 5A] provided a long-lasting platform for synthetic and biological investigations since the mid-1990s. Investigations within this family of naturally occurring compounds led to not only the syntheses of numerous designed analogues (e.g., **39–42**, Fig. 5A) but also to the discovery of important structure–activity relationships (SARs) within this class of compounds⁵⁷ (more about epothilones below, see section 3.11).

Similar studies within the sarcodictyin (e.g., **43**, **44**, Fig. 5B)^{77,78,104,105} and eleutherobin¹⁰⁴⁻¹⁰⁶ family of marine natural products employing solution and solid phase synthetic strategies led to a plethora of analogues, from which **45**, **46** and **47** were identified among the most potent against a number of cancer cell lines as indicated in Fig. 5B.⁷⁸

Our vancomycin (**48**, Fig. 5C) total synthesis campaign⁷⁹⁻⁸² included combinatorial synthesis of monomeric and dimeric vancomycin analogues^{84,85} made possible by several synthetic strategies. These included the so-called “target accelerated” combinatorial synthesis⁸³ in which a library of building blocks was allowed to react in the presence of the vancomycin binding target domain (L-Lys-D-Ala-D-Ala), with the most “potent” combinations of building blocks expected to first assemble onto the L-Lys-D-Ala-D-Ala fragment and then couple through covalent bond formation as the favoured products. These studies resulted in numerous potent vancomycin analogues such as the ones displayed, together with their impressive antibacterial MIC values, in Fig. 5C (e.g., **49** and **50**).

Comprising a large number of natural products (general structure **51**, Fig. 5D) and exhibiting an impressive range of biological activities, the benzopyran family of compounds attracted our attention as a worthy class of molecules for chemical synthesis and biological investigations. Thus, an analogue library of over 10000 compounds⁸⁹ was designed and synthesised, in single digit milligram scale for structural elucidation and biological screening, through combinatorial chemistry using MacroKans⁹⁴ and NanoKans⁸⁹ with radiofrequency or light-read encoding and robotic manipulation. From this large library, and through several collaborations with biologists, we identified a number of lead compounds whose optimisation led to more potent agents than their parent compounds. Among them antibacterial agent **52** (Fig. 5D, MIC=2 μ M against *MRSA*),⁹¹ ubiquinone oxidoreductase inhibitor **53** (Fig. 5D, IC₅₀=18 nM),¹⁰⁷ and fexaramine (**54**, Fig. 5D), a farnesyl X receptor (FXR) agonist (EC₅₀=25 nM),¹⁰⁸ the latter facilitating structural biology studies as a

crystalline FXR-ligand complex revealing the structure of the receptor and the binding side of the ligand.¹⁰⁹ These efforts provided important biological tools that served well biology, and possibly, medicine upon pending further optimisation and development.

A designed combinatorial analogue library of the naturally occurring antibiotic psammaplin A (**55**, Fig. 5E) was synthesised and screened for antibacterial activity, leading to the eventual establishment of SARs and the discovery of highly potent antibacterial agents, examples of which are depicted in Fig. 5E (i.e., **56**: MIC=0.09 µg/mL; **57**: MIC=0.11 µg/mL; **58**: MIC=0.28 µg/mL, and **59**: MIC=0.11 µg/mL).^{92,93}

Requiring the development of sophisticated solution and/or solid phase technologies, and often coupled with robotic systems, these rationally designed combinatorial/optimisation studies collectively demonstrated the power of these automated strategies for making valuable molecules for biology and medicine as evidenced from numerous lead and optimised compounds discovered. Robotics in organic synthesis are currently on the move and are expected to become a popular means to replace human hands in the laboratory.

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3. Selected Total Synthesis Endeavours

3.1 Abyssomicin and *atrop*-Abyssomicin

The intriguing molecular structure and potent antibacterial activities reported for abyssomicin C (**60**, Fig. 6) in 2004^{115,116} prompted our interest as a synthetic challenge. Our total synthesis^{117,118} of this fascinating molecule, and its congeners *atrop*-abyssomicin C (**61**, Fig. 6) and abyssomicin D (structure not shown),¹¹⁹ turned out to be even more intriguing than the initial discovery of abyssomicin C itself^{115,116} in that it revealed the existence of two atropisomers of the originally reported structure. Separated chromatographically, these distinct isomers exhibited different NMR spectral data and were distinguished and defined by X-ray crystallographic analysis.^{117,118} Furthermore, *atrop*-abyssomicin (**61**, Fig. 6B) exhibited even higher antibacterial potencies than those of the originally reported abyssomicin C (**60**, Fig. 6B). To their credit, the discoverers of abyssomicin C made, later on, the discovery of *atrop*-abyssomicin C from the same producing species, marine-derived actinomycete strain *Verrucosipora*.¹²⁰ Our synthetic strategy towards the abyssomicins as derived from the retrosynthetic analysis depicted in Fig. 6A, defined building blocks **62**, to be obtained from Weinreb amide **63**,¹²¹ and the readily available lactone **64**, in their enantiopure forms as the key starting materials for the projected total synthesis. The total synthesis of abyssomicin C (**60**) and *atrop*-abyssomicin C (**61**) proceeded as summarised in Fig. 6B. Thus, Weinreb amide **63** was converted to phenylthiol hydroxydiene **65** by standard chemistry (i.e., displacement with PhSCH₂Li and asymmetric reduction, 71% yield over two steps). The latter was reacted with methyl acrylate (**67**) in a controlled Diels–Alder reaction in the presence of MeMgBr and 2-(pyrrolidine-1-yl) phenol (**66**, acting as an auxiliary ligand) to afford desired product **69**, through transient assembly **68**, in 80% yield. This intermediate was then converted to tricyclic system **62** through a six-step sequence and 58% overall yield as indicated in Fig. 6B. Exposure of lactone **64** to *t*-BuLi followed by reaction of the resulting anion with

tricyclic lactone **62** furnished the corresponding ketone, whose protection and further elaboration afforded bis-terminal olefin **71**, via intermediate **70**, as shown in Fig. 6B. Ring closure metathesis within **71** under the influence of precatalyst **72** followed by oxidation of the resulting cyclic alcohol furnished protected abyssomicin precursor **73**. Both abyssomicin C (**60**) and its atropoisomer, *atrop*-abyssomicin C (**61**), were generated upon removal of the dithiane protecting group $[\text{PhI}(\text{OCOCF}_3)_2]$ and equilibration of the two products under acidic conditions, as shown in Fig. 6B.

Employing our developed synthetic strategies and tactics,^{119,122} we synthesised a series of designed analogues of abyssomicins¹¹⁸ **60** and **61**, including those exhibited in Fig. 6C {i.e., **74** (acetylated abyssomicin C); **75** (dithiane protected *atrop*-abyssomicin C); **76** and **77** [(7*S*)- and (7*R*)-epimers of hydroxy dithiane abyssomicin C, respectively]}. Biological evaluation of these compounds revealed slight potency superiority (against MSRA, see Fig. 6C) of *atrop*-abyssomicin C (**61**, MIC=15 μM) over abyssomicin (**60**, MIC=20 μM), loss of some activity upon acetylation of the hydroxyl group of *atrop*-abyssomicin (analogue **74**), and significant loss of potency upon reduction of the enone carbonyl group of the molecule (analogues **76**, >500 μM ; and **77**, MIC >500 μM) as depicted in Fig. 6C. Finally, it is worth noting that the type of isomerism detected in abyssomicins C (**60**) and *atrop*-abyssomicin C (**61**) is rare and speaks volumes of the power and value of total synthesis for structural elucidations, even in these days of supreme analytical techniques and instrumentation.

3.2 Biyouyanagins A and B

Isolated from a *Hypericum* genus (Clusiaceae) (i.e., *H. Chinense*), biyouyanagin A (**78**, Fig. 7A) exhibited interesting biological properties, including anti-HIV activity and inhibition of lipopolysaccharide-induced cytokine production, implying potential treatments of AIDS and inflammation, respectively.¹²³ Attracted by its relevance to biology and medicine and its novel molecular structure, we initiated a program directed towards its total synthesis, despite the ambiguity of its C24-stereochemical configuration. Inspired by the proposed hypothesis for the biosynthesis of biyouyanagin A (**78**), we devised a synthetic strategy involving a photo-induced [2+2] cycloaddition to fuse the two advanced building blocks **81** (naturally occurring zingiberene or its antipode, *ent*-zingiberene) and **79** (naturally occurring hyperolactone C), generated by retrosynthetic rupture of the cyclobutane ring and traced back to citronellal [**82**, either (*S*) or (*R*)], and (*S*)-malic acid (**80**), respectively, as shown retrosynthetically in Fig. 7A. At this point we realised, using manual molecular modelling, that the [2+2] cycloaddition required to cast the reported structure was disfavoured on steric congestion grounds, leading to doubts as to the correctness of the configurations of the stereocentres around the cyclobutane moiety of the molecule (i.e., **78**). Nevertheless, we proceeded with implementation of the devised strategy, leaving the last word for the final step to the experiment. Fig. 7B summarises the total synthesis of biyouyanagin A (**78**), and as it turned out, biyouyanagin B (**89**) and biyouyanagin C (**90**, see Fig. 7B for structures). Thus, (*S*)-malic acid (**80**) was elaborated to lactone **83** through a five-step sequence, and the latter was converted to acetylenic hydroxy lactone **84** by oxidation followed by addition of the acetylene unit, as shown in Fig. 7B. Hydroxy acetylene lactone **84** was then coached, via an aesthetically pleasing and remarkably efficient cascade of reactions initiated and sustained with $\text{Pd}(\text{PPh}_3)_4$ cat. in the presence of CO, CO₂ and Et₃N, to afford the desired

bicyclic precursor **85**, whose conversion to the targeted hyperolactone C (**79**) took only three steps, as summarised in Fig. 7B. The other required fragment, *ent*-zingiberene (**81**), was constructed from (*R*)-citronellal (**82**) as depicted in Fig. 7B. Thus, reaction of **82** with methyl vinyl ketone in the presence of proline-derived catalyst **86**, co-catalyst **87**, KOH, and *n*-Bu₄NOH cat. furnished cyclohexenone **88** (via an enamine-mediated reaction of citronellal with methyl vinyl ketone). The latter was finally transformed to the desired *ent*-zingiberene (**81**) through a two-step process, as depicted in Fig. 7B.

The much anticipated [2+2] cycloaddition of building blocks **79** and **81** induced by UV light proceeded as expected, and after careful isolation procedures yielded not only the initially targeted biyouyanagin A (**78**, 51% yield, Fig. 7B) but also, in lesser amounts, what turned out to be biyouyanagin B (**89**, 3% yield, Fig. 7B),¹²⁴ isolated from the same *Hypericum* genus and reported as a natural product in 2009,¹²⁵ four years after the report of its sibling biyouyanagin A.¹²³ A third compound named by us as biyouyanagin C (**90**, Fig. 7B) was isolated from the reaction mixture (in 2% yield) and fully characterised.¹²⁴ Formed by a [2+2] photo-cycloaddition of hyperolactone (**79**) to the endocyclic trisubstituted olefinic bond of *ent*-zingiberene (**81**), it will not be surprising if this synthesised biyouyanagin isomer (i.e., **90**) is found in Nature one day, especially if the biosynthesis of these class of molecules involves a photo-induced reaction without the aid of enzymes.

It should be noted that the final synthetic strategies summarised in Fig. 7B do not tell the entire story of the biyouyanagins A and B chase, which was much more adventurous and intriguing in its entirety. It started with biyouyanagin A (**78**), the first shrouded in a structural mystery member of the class to be reported,¹²³ and then included biyouyanagin B (**89**)¹²⁵ that turned out to be a second structural puzzle, even though we must have made it in the laboratory before it was discovered in Nature, but never isolated it due to the minimal amounts formed. It was only after we exhausted all possible combinations of zingiberene/hyperolactone structural isomers in our final photo-induced fusion, and because of an astute observation regarding the ¹H NMR signals of the various biyouyanagin stereoisomers around the cyclobutane ring, that we went back to our original synthesis of biyouyanagin A to search for, in a last and desperate attempt, and to possibly isolate the true structure of biyouyanagin B (**89**). And we did!

Thrilling as it was, the biyouyanagin campaign was not complete without the “dessert” of discovering molecules for biology and medicine. And so, a large number of biyouyanagin-type molecules were synthesised and, collaboratively, tested for their ability to neutralise the HI Virus, and for their activity against the LCMV (lymphocytic choriomeningitis mammarenavirus).¹²⁶ A select number of biyouyanagins [A (**78**), B (**89**), and C (**90**)] and their analogues (e.g., **91**, **92**, **93**, **94**), together with their relevant biological activities, are shown in Fig. 7C. Most striking among them is analogue **94** that exhibited superior activity against HIV (HIV-1HxB1 clone) than any of the many compounds we synthesised for biological screening, including the two naturally occurring, biyouyanagins A (**78**) and B (**89**) as shown in Fig. 7C.^{126,127}

3.3 Epidithio- and Bis(methylthio)diketopiperazines

The epidithio- and bis(methylthio)diketopiperazines are a diverse family of naturally occurring molecules endowed with a variety of disease relevant biological properties, including antiviral, antibacterial, antimalarial, and antitumor activities.¹²⁸ Members of this family of molecules include epicoccin G (**95**, Fig. 8A), 8,8'-*epi-ent*-rostratin B (**96**, the epimer of the enantiomer of the naturally occurring rostratin, Fig. 8A),¹²⁸ gliotoxin (**117**, Fig. 8D),¹²⁸ gliotoxin G (**118**, Fig. 8D),¹²⁸ emethallicin E (**119**, Fig. 8D)¹²⁸ and haematocin (**120**, Fig. 8D).¹²⁸ These natural products, and others like them, presented, in addition to assembling their often complex molecular scaffolds, the special challenge of installing the epidisulphide and bis(methylthio) structural motifs within their diketopiperazine scaffolds. Their scarcity from natural sources, their important biological properties, and the methodology void for installing their sulphur structural motifs served as the motivation for us to initiate a research program that combined strategy design, method development, and design, synthesis and biological evaluation of analogues thereof.

Fig. 8 summarises our investigations in this field starting with the retrosynthetic analysis of epicoccin G (**95**) and 8,8'-*epi-ent*-rostratin B (**96**, Fig. 8A) defining *N*-Boc-L-tyrosine-derived building block **98** as our starting material, via advanced intermediate pentacyclic diketopiperazine **97**, as indicated in Fig. 8A. As a prerequisite to our total synthesis, we first developed a new practical and efficient method for installing the epidithio- and bis(methylthio) structural motifs into their diketopiperazine frameworks (Fig. 8B). Thus, reaction of sulphur (S₈, **99**) with NaHMDS led mainly, via **100**, to tetrasulphide **101**, a reagent that reacted with diketopiperazine **102** in the presence of NaHMDS (enolate formation, **102**→**103**), via **104**, to afford tetrasulphide **105**, which was diverted selectively to produce either bis(methylthio) diketopiperazine **107** (NaBH₄; then MeI, 72% overall yield for the three steps from **101**+**102**) or epidisulphide **108** (NaBH₄; then NH₄Cl; then KI₃, 69% overall yield over the four steps from **102**) as depicted in Fig. 8B.

Armed with this practical and versatile sulphenylation method, we completed the total syntheses of both epicoccin G (**95**) and 8,8'-*epi-ent*-rostratin B (**96**) starting with building block **98**, the latter readily available from *N*-Boc-L-tyrosine, through intermediates **113** and 2,2'-*epi*-**113**, respectively, obtained as a separable mixture, as summarised in Fig. 8C. Intermediate **113** was diverted towards epicocin G (**95**), and 2,2'-*epi*-**113** was funnelled through a different pathway towards 8,8'-*epi-ent*-rostratin B (**95**), as depicted in Fig. 8C. Thus, building block **98** was converted to hydroxy methyl ester amine TFA salt **109** (five steps), and separately, to hydroxy acid Boc-protected amine **110** (five steps). Stepwise coupling through amide bond formations gave first intermediate amide **111** (intermolecular), and finally diketopiperazine **112** (intramolecular). The latter was elaborated, in two steps, to tetraene diketopiperazine scaffold **97**, whose sulphenylation and reduction/oxidation of the so-formed tetrasulphide furnished separable intermediates **113** (α C–S bonds) and 8,8'-*epi*-**113** (β C–S bonds). Processing this mixture to the next step towards epicoccin G (reduction with NaBH₄, followed by methylation of the resulting dithiol with MeI) furnished a mixture of the corresponding, chromatographically separable, bis(methylthio) products **114** and 2,2'-*epi*-**114**. Elaboration of the latter to the targeted natural product, epicoccin G (**95**), via intermediate bis-endoperoxide **115**, formed by a Kornblum–DeLaMare singlet

oxygen addition (O₂, TPP; then DBU),^{129,130} proceeded as summarised in Fig. 8C. A similar photo-induced oxygenation of pure 2,2'-*epi*-**113** resulted in the formation of the transient bis-endoperoxide **116** exposure of which to CuH(PPh₃)₆ followed by KI₃ addition led to 8,8'-*epi-ent*-rostratin B (**96**), as shown in Fig. 8C.

The developed synthetic strategies and technologies were employed to synthesise the naturally occurring epidithio diketopiperazine natural products gliotoxin (**117**), gliotoxin G (**118**), bis(methylthio) diketopiperazine emethallicin E (**119**), and epitetrathio diketopiperazine hematocin (**120**, see Fig. 8D for structures). A number of designed analogues were also synthesised, including **121**, 2,2'-*epi*-**121**, **122** and **123**, whose structures and selected biological activities are shown in Fig. 8E. It is noteworthy that some of these analogues demonstrated superior bioactivities to those exhibited by epicoccin G, and pirodavis [EC₅₀ (Poliovirus) = 1.58 μM (neutral red assay)],¹²⁸ the standard anti-poliovirus drug, used in these studies as a control.

Blending total synthesis with method development and analogue design, synthesis and biological evaluation, this strategy proved delightfully fruitful in that it resulted in a) the total synthesis of a multi-member family of natural products; b) the development of a practical and useful sulphenylation procedure; and c) the discovery of potent lead compounds for further studies and optimisation directed towards potential treatments of the poliovirus and malaria diseases.

3.4 Platensimycin

The report of the discovery of platensimycin (**124**, Fig. 9A and B) by Merck Sharp & Dohme scientists in 2006,¹³¹ precipitated intense interest among biologists and chemists alike, not the least because of its potent antibacterial activities against drug resistant pathogens and its novel mechanism of action (i.e. selective blockage of bacterial fatty acid biosynthesis through inhibition of the elongation-condensing enzyme FabF). For those reasons, and because of the novelty of platensimycin's molecular structure, we were prompted into a campaign to achieve its total synthesis and exploit the developed synthetic strategies and methods to construct novel analogues for biological evaluation studies. Our synthetic efforts towards this important target molecule yielded first a total synthesis of the racemic mixture, then an optimised asymmetric synthesis via two different approaches, and, finally, the synthesis of a number of its naturally occurring congeners (i.e., platencin,^{132,133} platensimycins B₁, B₂ and B₃)¹³⁴ and a series of designed analogues aiming to establish useful structure–activity relationships (SARs) within the resulting family of natural and designed platensimycins and related congeners.

Fig. 9A summarises our two asymmetric synthetic approaches to platensimycin (**124**) in retrosynthetic format. Thus, disconnection of the molecule's amide bond generated advanced aniline derivative **125** and fragment **127** (platensic acid,¹³⁵ naturally occurring). The former was traced back to the readily available 2-nitroresorcinol (**126**), while the latter was unfolded to bis-spiroenone aldehyde **128**. Serving as a common intermediate, enone aldehyde **128** was retro-diverted to the two planned asymmetric approaches, one (a) employing a catalytic asymmetric spirocyclisation/isomerisation to start with the easily

obtained ethoxy enone **129**, and the other (b) traced back to the readily available peptide fragment **130**, and following a chiral auxiliary method to achieve selective alkylation followed by a downstream dearomatisation reaction, as summarised in Fig. 9A. Fig. 9B summarises the two approaches for the enantioselective synthesis of spiroaldehyde **128** and its conversion to platensimycin (**124**) via the naturally occurring platensic acid (**127**). Thus, enone **129** (top right) was alkylated with TBS-ether bromide **131** and the resulting product was converted to spirosystem **132**, from which bis-enone **133** was generated in two simple steps. Upon considerable optimisation, it was found that substrate **133** could be converted, efficiently and enantioselectively, to the targeted spirocyclic aldehyde **128** through asymmetric cycloisomerisation as induced by $[\text{Rh}(\text{S})\text{-BINAP}]_2\text{SbF}_6$ catalyst¹³⁶⁻¹³⁸ (86% yield, >99% *ee*). The same intermediate was obtained from building block **130** equipped with the (*S,S*)-pseudoephedrine moiety, through the sequence shown in Fig. 9B (left). Thus, alkylation of **130** with benzyl bromide **134** furnished derivative **135**, in good yield and diastereomeric enrichment (87% yield, 85% *de*), from which the desired fragment **136** was obtained upon cleavage of the auxiliary and further elaboration (81% yield, four steps) as shown in Fig. 9B. The latter was converted to bis-enone **137** by $\text{PhI}(\text{OAc})_2$ -induced dearomatisation (68% yield), and thence to the desired bis-enone aldehyde **128** under acidic conditions. The so-obtained aldehyde **128** was transformed to tricyclic compound **138** through the intermediacy of the corresponding ketyl radical (SmI_2), and thence to the platencin scaffold **139** (46% overall yield) upon exposure to TfOH , as shown in Fig. 9B. Methylation of the latter followed by a second alkylation via enolate formation (KO^tBu) and a 1,4-conjugate addition to *tert*-butyl acrylate (**140**) furnished, after acid-induced cycloetherification and *tert*-butyl ester cleavage, platensic acid (**127**), in 84% yield over the three steps as shown in Fig. 9B. The other required building block, aniline derivative **125**, was prepared from 2-nitroresorcinol (**126**) through a five-step standard sequence and coupled with platensic acid (**127**) affording, upon methyl ester hydrolysis and MOM removal, platensimycin (**124**) in good overall yield, as depicted in Fig. 9B.

The synthetic strategies and methods developed in this program were applied to the total synthesis of several naturally occurring congeners of platensimycin, namely platensimycins B_1 (**141**), B_2 (**142**), and B_3 (**143**), platencin (**144**, see structures in Fig. 9C), platensic acid (**127**, Fig. 9B), methyl platensinoate (methyl ester of **127**), platensimide A, homoplatensimide A, and homoplatensimide methyl esters (structures not shown).¹³⁹

The same synthetic strategies and methods also provided a platform from which numerous designed analogues of platensimycin were synthesised.^{140,141} Biological evaluation of these analogues revealed a number of potent compounds with comparable potencies to those of the natural product against methicillin- and vancomycin-resistant bacterial strains. Clear and valuable structure–activity relationships (SARs) consistent with the X-ray crystallographic analysis picture of the binding of the platensimycin molecule to its receptor¹³¹ emerged from these studies^{140,141} that could guide further investigations in the field if they were needed. Among the most potent of the many synthesised platensimycin analogues were carbaplatensimycin (**145**),¹⁴¹ adamantaplatensimycin (**146**)¹⁴⁰ and analogue **147**¹⁴² (for structures and potencies, see Fig. 9D). This collaborative program with biologists enriched our knowledge of the binding mode of the platensimycin molecule to its biological target,

and provided strong guidance as to the design of analogues with high probability of possessing potent antibacterial properties as consistently reflected in the structures of not only the analogues synthesised but also those of the naturally occurring platensimycin congeners mentioned above (see Fig. 9).

3.5 Antibiotic CJ-16,264

Decorated with two distinct molecular domains, its lipophilic terpenoid-like fused bicyclic moiety and its heterocyclic structural motif, and possessing potent antibacterial properties, antibiotic CJ-16,264 [(+)-**148**, Fig. 10] attracted our attention as a target of opportunity for total synthesis with a blend of biology, and perhaps medicine, through analogue design, synthesis and biological evaluation. Our retrosynthetic analysis (Fig. 10A) demanded bicyclic iodo lactam **149** and bicyclic aldehyde **151** as the prerequisite building blocks for the proposed synthetic strategy, making their construction the first priority for our drive towards the targeted molecule. Bicyclic aldehyde **151** and tricyclic iodide **149** were traced back to (*S*)-citronellal (**152**) and known racemic bicyclic lactam carboxylic acid ($\pm$)-**150**,^{143,144} respectively, as indicated retrosynthetically in Fig. 10A. Tricyclic iodide **149** required an especially sophisticated resolution, albeit short, synthetic approach for its enantioselective construction. Thus, and as indicated in Fig. 10B, carboxylic acid (+)-**150** was preferentially trapped as an amide through kinetic resolution of its racemic form achieved by reaction with readily available amine (–)-**153** to afford lactam **154** [originating from the (+)-enantiomer of **150**] in good yield [leaving behind (–)-**150**]. Cyclisation of diastereoisomer **154** as facilitated by I(*sym*-collidine)₂ClO₄ then furnished the desired fragment (**149**, 47% yield, 73% brsm) as depicted in Fig. 10B. The construction of terpenoid aldehyde fragment **151** started from (*S*)-citronellal (**152**) and proceeded through intermediate hydroxy acid **155** and macrodiolide **157**, as summarised in Fig. 10B. Thermally induced double intramolecular Diels–Alder reaction within **157** afforded pentacyclic dimeric product **158** (88% yield). Double cleavage of the latter diolide, and standard elaboration of the resulting monomeric hydroxy acid gave, in good overall yield, the desired terpenoid aldehyde fragment, **151**, as depicted in Fig. 10B.

Antibiotic CJ-16,264 [(+)-**148**] was then assembled from building blocks **149** and **151** through a short path involving enolate generation from iodo lactam **149** (BET₃) and addition of the resulting species to the aldehyde partner [i.e., **151**] to furnish coupling product alcohol **159**, whose desilylation, followed by oxidation of the so-formed tertiary hydroxyl secondary alcohol (IBX or DMP), led to the targeted natural product antibiotic CJ-16,264 [(+)-**148**] in good overall yield as depicted in Fig. 10B.

The practical and efficient synthetic strategies and technologies developed resulted in the total synthesis of six diastereoisomers [(+)-**148**, (–)-**148**, (+)-**161**, (–)-**161**, (+)-**162** (Fig. 10C), and 1-*epi*-(–)-**148** (structure not shown)] of the originally reported, apparently wrong, structure of the CJ-16,264 molecule, from which (+)-**148** was proven to be the natural product's true structure. In addition, two collections of CJ-16,264 analogues were synthesised, one of racemic mixtures and the other of enantiopure compounds. Biological evaluation of these substances led to important structure–activity relationships (SARs) and the identification of several potent antibacterial agents, some rivalling the activities of the

natural product [(+)-**148**], despite their significantly simpler molecular structures [e.g., (+)-**163**, (–)-**164**, Fig. 10C]. It was also interesting to note that some of the synthesised racemic CJ-16,264 analogues (structures not shown) and enantiopure simplified analogues [e.g., **164**], CJ-16,264 diastereoisomers (–)-**161**, (+)-**161** and (+)-**162**, and the enantiomerically pure antipode [(–)-**148**] of the natural product exhibited comparable potencies (with the exception of some deviations with certain bacterial strains) to those of the natural product [(+)-**148**] (see Fig. 10C).

The CJ-16,264 total synthesis campaign demonstrated the value of total synthesis endeavours for discoveries and innovations, specifically in a) strategy design [i.e., the intramolecular Diels–Alder collapse of macrocyclic dimer **157** to two cleavable precursor units and their elaboration to the terpenoid aldehyde building block **151**]; b) method development [i.e., the efficient kinetic resolution of racemic **150** through amide formation with the enantiopure amine (–)-**153**]; c) the revision of the originally assigned structure of CJ-16,264; d) discovery of significantly simpler, and yet equipotent to the natural product, antibacterial agents; and e) the derivation of important and useful SARs within the CJ-16,264 family of natural and designed molecules.

3.6 Brevetoxin B and Maitotoxin

The ladder-like marine derived neurotoxins elicited considerable interest from chemists and biologists alike since brevetoxin B (**165**, Fig. 11A) appeared on the scene in 1981.¹⁴⁵ Since then, numerous new members were added to this class of marine natural products, with maitotoxin (**168**, Fig. 11B) being the most impressive with regards to a) molecular complexity and size (the largest non-polymeric natural product discovered as yet); b) potency as a neurotoxin (the most potent, non-proteinic, neurotoxin known to date); and c) synthetic challenge. Synthetic efforts towards ladder-like marine neurotoxins in our laboratories resulted in the total syntheses of hemibrevetoxin (1993, **175**, Fig. 11C),¹⁴⁶ brevetoxin B (1995, **165**, Fig. 11A)^{147,148} and brevetoxin A (1998, **176**, Fig. 11C).^{149–153} Our forays towards maitotoxin (**168**, Fig. 11B) led to confirmation of its originally assigned structure,¹⁵⁴ after it was challenged,¹⁵⁵ the total synthesis of several large domains of the molecule,^{156–161} and a number of new synthetic methods^{162,163} for the construction of its varying in size cyclic ether structural motifs, including the rings *P* and *B'* that remain to be constructed (designated in red, see structure **168**, Fig. 11B). Unfortunately, funding restrictions did not allow the completion of its total synthesis, although, in principle, it became feasible given the fact that all other 30 rings of the molecule were constructed as fused large fragments with their correct stereochemical configurations, and the methods to couple them and cast the remaining two rings were developed and tested successfully in our total syntheses of brevetoxins B (**165**)^{147,148} and A (**176**).^{149–153}

The brevetoxin B and maitotoxin campaigns gave us the opportunity to explore, collaboratively, their biological properties, propose binding modes of these fascinating neurotoxins to their receptors, and study structure–activity relationships of large segments of their molecular structures. Fig. 11 shows, in addition to the molecular structures of the naturally occurring ladder-like marine neurotoxins hemibrevetoxin (**175**), brevetoxins A (**176**) and B (**165**), and maitotoxin (**168**), the structures of a number of analogues and

segments (**166**, **167**, for brevetoxin B, Fig. 11A, and **169–174** for maitotoxin, Fig. 11B) of these molecules that we synthesised for biological investigations. Thus, synthesised truncated *AFGHIJK* brevetoxin B (**167**)¹⁶⁴ (missing the *BCDE* domain of the parent molecule) and desoxycarbonyl brevetoxin B (**166**)¹⁶⁵ (featuring a cyclic ether ring *A*, instead of the lactone ring *A* of the natural product) were investigated in collaborative efforts with biologists. Serving as a biological tool, the truncated version (i.e., **167**) of brevetoxin B (**165**) revealed significant loss of binding affinity, as compared to brevetoxin B, to its receptor ($IC_{50} = 15.02$ nM) (i.e., site 5 of the voltage-gated sodium channel), as well as a modified action behaviour with regards to its biological role.¹⁶⁵ The desoxybrevetoxin analogue **166** exhibited significantly weaker binding within the same receptor ($IC_{50} = 36.5$ nM) and lost its potency as a prolonging agent of the channel's open time duration.¹⁶⁵ These observations provided support for the previously proposed hypothesis of the “common pharmacophore” for brevetoxins B (**165**) and A (**176**), and their bioactive congeners, that envisioned it to be a “cigar shaped” structural motif of approximately 30 Å length, binding to its receptor site mainly through hydrophobic and non-polar solvation forces, with H-bonding of the ring *A* carbonyl group playing a possible role.^{165,166} Furthermore, these biological studies led to a better understanding of the role of the “cigar shape” of these molecules in their expression of their biological activities and provided further information on their precise mode and location of binding to their receptor.^{165,166}

The maitotoxin project also yielded opportunities for biological investigations of numerous, more or less, complex fragments prepared during the total synthesis campaign, and which were subjected by our biology collaborators to assays testing their ability to inhibit maitotoxin-induced $^{45}Ca^{2+}$ influx in rat C6 glioma cells.¹⁶¹ Fig. 11B displays a number of maitotoxin fragments with interesting capabilities to inhibit maitotoxin-elicited $^{45}Ca^{2+}$ influx in rat C6 glioma cells. Thus, fragment **169** (*QRSTUVWXYZA*) exhibited relatively strong inhibitory activity ($IC_{50}=3.2$ μM), while its bis-benzyl ether (**170**, rings *Q* and *W*) exhibited only modest activity ($IC_{50} \approx 30$ μM). Similarly, *QRSTUVWXYZA* fragment **171** demonstrated significant potencies in this assay ($IC_{50} = 5.4$ μM) with its bis-benzyl derivative **172** (rings *Q* and *W*) showing lower potency ($IC_{50} > 30$ μM). Apparently the two benzyl moieties at the same locations within the structures of **169** and **171** had a detrimental effect on the binding of these molecules to their receptor as revealed consistently in these two examples (the terminal *n*-propyl and allyl groups on ring *Q* are irrelevant to the binding of the molecule's polycyclic framework). On the other hand, compounds **173** and **174** (*F'E'D'C'* fragments) revealed the opposite effect, in that the TBDPS protecting group on **174** enhanced its potency ($IC_{50} = 2.3$ μM) as an inhibitor in this assay, as compared to that ($IC_{50} > 30$ μM) of its free hydroxyl counterpart (**173**). These observations may reflect the fact that the bulk and lipophilicity of the TBDPS moiety plays the role of an extension of the rather short lipophilic *C'D'E'F'* domain (i.e., **173**, **174**) of the natural product, thereby fulfilling, at least partly, the requirements of proper binding to the receptor.¹⁶¹

A number of these synthesised maitotoxin fragments were also tested against the NCI-60 DTP Human Tumor Cell Line Panel. Interestingly, fragment *QRSTUVWXYZA* (**170**, Fig. 11B), that showed the second best inhibitory activity in the maitotoxin-elicited $^{45}Ca^{2+}$ influx test (see above and Fig. 11B),¹⁶¹ demonstrated the highest growth inhibition (GI_{50}) values

against a number of cancer cell lines, including leukemia (cell line RPMI-8226: $GI_{50} = 2.15 \mu M$), non-small cell lung cancer (cell line HOP-92: $GI_{50} = 2.44 \mu M$), colon cancer (cell line HCT-116: $GI_{50} = 2.34 \mu M$), CNS cancer (cell line SF-539: $GI_{50} = 4.50 \mu M$), melanoma (cell line 5K-MEL-5: $GI_{50} = 2.13 \mu M$), ovarian cancer (cell line OVCAR-3: $GI_{50} = 384 \mu M$), renal cancer (cell line SN12C: $GI_{50} = 1.86 \mu M$), prostate cancer (cell line PC-3: $GI_{50} = 1.83 \mu M$) and breast cancer (cell line MDA-MB-468: $GI_{50} = 1.26 \mu M$).¹⁶¹

The maitotoxin campaign may have not been completed in terms of reaching its destination as yet, but the valuable knowledge and wisdom gathered thus far should be an inspiration as to what can be done by total synthesis. Thus, in addition to confirming maitotoxin's legendary molecular structure, the methods and strategies developed essentially assure the prospects of its total synthesis, given the opportunity. They also make credible the statement that "any molecule found in Nature can be replicated in the laboratory by chemical synthesis". That does not mean by any means that improvements in the field cannot be made – on the contrary, we still have a long way to go before we can reach the elegance and performance of Nature in this regard. In terms of the biological investigations and their suggestive findings enabled by the synthesised molecules in this program, one can only speculate as to their value and importance to biology and wonder of potential applications. Could one develop antidotes to combat poisoning from certain marine neurotoxins such as the brevetoxins and maitotoxin? And as in many other instances, could a cure for cancer emerge one day from molecules such as the maitotoxin inspiring fragments as killers of cancer cells? And perhaps as neurotoxins can these gifts from Nature teach us something about how our brain works? And can such knowledge help in discovering a treatment for Alzheimer disease and other neurological disorders? In answering such questions, we must not forget that most inventions, if not all, stand on fundamental research.

3.7 Viridicatumtoxin B

As one of the most structurally complex members of the tetracycline class of antibiotics, and because of its reported high potencies against drug resistant bacterial strains and the questionable assignment of its molecular structure (i.e., hydroxy epoxide structural motif), viridicatumtoxin B¹⁶⁷ (**177**, Fig. 12) tempted us into initiating a research program directed towards its total synthesis, one that also included full structural assignment and analogue design, synthesis and biological evaluation. Our first total synthesis¹⁶⁸ delivered racemic viridicatumtoxin B [(±)-**177**] and resulted in the revision of its structure to what we believed it to be in the first place, namely the one with the hydroxy ketone structural motif (**177**, Fig. 12), rather than its isomeric hydroxy epoxide moiety reported.¹⁶⁷ Our second, and enantioselective total synthesis of viridicatumtoxin B [(+)-**177**]¹⁶⁹ dictated the development of a practical method for the required asymmetric anthrone alkylation (i.e., anthrone **180** with bromide **178**, see Fig. 12A, retrosynthetic analysis). To this end we synthesised and screened a number of phase transfer catalysts and identified Corey's quinine-derived catalyst **181** (Fig. 12B)¹⁶⁹ as our lead for optimisation. Thus, from the numerous newly synthesised **181**-related compounds, we discovered a number of optimised catalysts for a general asymmetric alkylation of anthrones (for both enantiomers) and eventually applied catalyst **182** (Fig. 12B) and its quinidine-derived pseudoenantiomer (**192**, Fig. 12C) to the synthesis of both enantiomeric forms of viridicatumtoxin B [**182** for (+)-viridicatumtoxin B, that

turned out to be the natural form of the molecule, and **192** for (–)-viridicatumtoxin B, the antipode of the natural product], respectively.¹⁶⁹

Fig. 12B summarises the total synthesis of the naturally occurring viridicatumtoxin B [(+)-**177**]. Thus, alkylation of anthrone **180** with allylic bromide (*S*)-**178** in the presence of catalyst **182** followed by crystallisation of the alkylated product furnished substituted anthrone (6*R*,17*S*)-**183** (>99:1 *dr*). The latter underwent spirocyclisation upon exposure to BF₃·Et₂O leading to pentacyclic compound (6*R*,15*S*)-**184** (>99:1 *er*), whose sequential treatment with PIDA and CSA afforded (+)-**185** (via the corresponding keto dimethoxyketal). Coupling of (+)-**185** with heterocyclic phenyl ester **179** in the presence of KO^{*t*}-Bu and chiral catalyst **186** ensured the high stereoselectivity of the reaction that led to heptacyclic product **187** (via the corresponding dimethoxy enone), whose decarboxylative desilylation followed by hydroxylation with DMDO in the presence of Ni(acac)₂ cat. furnished hydroxylated advanced intermediate **188**, as shown in Fig. 12B. Reduction of the latter with NaCNBH₃ followed by further elaboration of the resulting product (**189**) led to hydroxy diketone **190**, whose further hydroxylation with Davis oxaziridine in the presence of KHMDS afforded dihydroxylated precursor **191** in good overall yield. Desilylation, oxidation and debenzoylation of the latter then furnished (+)-viridicatumtoxin B [(+)-**177**] as summarised in Fig. 12B.¹⁶⁹

Applying the developed synthetic strategies and methods we synthesised an array of (+)- and (–)-viridicatumtoxin B analogues. Biological evaluation of these compounds against *E. faecalis*, *E. faecium*, and *MRSA* revealed a number of potent antibacterial agents, including (+)-**193** (*E. faecalis*: MIC=0.5 µg/mL; *E. faecium*: MIC=2 µg/mL; *MRSA*: MIC=2 µg/mL), (–)-**193** (*E. faecalis*: MIC=1 µg/mL; *E. faecium*: MIC=1 µg/mL; *MRSA*: MIC=2 µg/mL), (+)-**194** (*E. faecalis*: MIC=1 µg/mL; *E. faecium*: MIC=1 µg/mL; *MRSA*: MIC= 2 µg/mL), and (–)-**194** (*E. faecalis*: MIC=1 µg/mL; *E. faecium*: 0.5 µg/mL; *MRSA*: MIC=2 µg/mL) (see Fig. 12C). It is worth pointing out that both enantiomers of viridicatumtoxin B (**177**) exhibited almost the same potencies in these assays [(+)-**177**: *E. faecalis*: MIC=2 µg/mL; *E. faecium*: MIC=2 µg/mL; *MRSA*: MIC= 4 µg/mL; (–)-**177** (structure not shown): *E. faecalis*: MIC=4 µg/mL; *E. faecium*: MIC=4 µg/mL; *MRSA*: MIC= 8 µg/mL], an interesting phenomenon we also observed in several other projects on antibacterial agents in our group.

The viridicatumtoxin campaign was rich in delivery and impact in that it resulted in contributions in a) strategy design; b) structural revision of the originally erroneous structural assignment of the molecule; c) method discovery and development that filled a void at the time; d) development of valuable structure–activity relationships (SARs); and e) identification of several potential drug candidates. It was, indeed, a rewarding and exemplary total synthesis endeavour with multiple discoveries that made it, like so many others, worth pursuing in our laboratories.

3.8 Prostaglandins J₃ and J₂

A rather latecomer and cousin of the previously known ¹²-prostaglandin J₂ (¹²-PGJ₂, **199**, Fig. 13B),^{170,171} ¹²-prostaglandin J₃ (¹²-PGJ₃, **195**, Fig. 13) was reported in the early 2010s as a naturally occurring secondary metabolite of eicosapentaenoic acid (EPA) and was

claimed to possess impressive antileukemic properties by eradicating leukaemia stem cells.^{172,173} This exciting report prompted us to undertake a program directed towards the total synthesis of ¹²-PGJ₃ (**195**) and its cousin molecule ¹²-PGJ₂ (**199**), and apply the developed strategies and technologies to construct designed analogues of them for the purposes of biological investigations. Fig. 12A and C summarise our optimised total synthesis of ¹²-PGJ₃ (**195**), which employed easily prepared key building blocks **196**, **197** and **198**, revealed by the retrosynthetic analysis as shown in Fig. 13A (strategy I). Their assembly proceeded as summarised in Fig. 13C. It involved first alkylation of **196** with **197** to afford **204** (and C8-epimer **203**, which is convertible to **204**) in good yield, then reductive cleavage of the menthol auxiliary group leading to enone **205**. Aldol reaction of the latter with aldehyde **198** furnished hydroxy enone **206**, from which ¹²-PGJ₃ (**195**) was generated through elimination, via the corresponding mesylate, and ester hydrolysis, as summarised in Fig. 13C.

Our streamlined total synthesis approach to ¹²-prostaglandin J₂ (¹²-PGJ₂, **199**, Fig. 13B) relied on a different synthetic route. Involving a similar synthetic strategy for the construction of its “lower” chain as that employed in the synthesis of ¹²-PGJ₃ (**195**), and an olefin metathesis tactic for the instalment of its “upper” chain, our approach to ¹²-PGJ₂ (**199**) is shown retrosynthetically in Fig. 13B (strategy II, building blocks **200**, **201**, **202**). Fig. 13D summarises the execution of this strategy starting with the readily available cyclopentenone **201**, whose aldol coupling with aldehyde **202** followed by mesylation/elimination of the resulting mixture of alcohols led to terminal olefin **207**. The latter was subjected to cross metathesis with terminal olefin **200**, in the presence of catalyst **208**, to afford protected ¹²-PGJ₂ precursor **209**, whose one-step global deprotection furnished ¹²-PGJ₂ (**199**) as depicted in Fig. 13D.

Aiming to investigate the biological properties of these secondary metabolites (i.e., ¹²-PGJ₃ and ¹²-PGJ₂) and their variations, we exploited our synthetic strategies to construct a series of designed analogues,¹⁷⁴⁻¹⁷⁶ including those shown in Fig. 13D (**210a** and **210b–210d**). Thus, exposing a number of the most potent PGJ₃- and PGJ₂-type synthesised compounds¹⁷⁴⁻¹⁷⁶ to standard Shiina macrolactonisation conditions, we prepared the corresponding 1,15-macrolactone analogues (**199**→**210a**, Fig. 13D), and, by using appropriate dilution conditions, their dimeric, trimeric and tetrameric counterparts (i.e., more concentrated, **199**→**210b–210d**, Fig. 13D).¹⁷⁵ Biological evaluation of these analogues led to interesting structure–activity relationships and the identification of a number of potent antitumor agents against a number of normal cancer cell lines (see Fig. 13E). Thus, depending on the cell line, the macrolactone and dimeric macrolactone analogues [e.g., **210a** (Fig. 13D and E), **210b** (Fig. 13D and E), **212**¹⁷⁴ (Fig. 13E)] were found to be more potent than their hydroxy acid precursors, with the dimeric analogues (e.g., **213**,¹⁷⁴ Fig. 13E) often, but not always, being somewhat more active than the monomers. Interestingly, and in contrast to their monomeric and dimeric macrolactones, the trimers (e.g., **210c**, Fig. 13D) and tetramers (e.g., **210d**, Fig. 13D) did not exhibit significant activities in those assays as compared to their precursor hydroxy acids or their monomeric cyclic counterparts. Tested at the NCI (National Cancer Institute, USA), analogue **212**¹⁷⁴ (Fig. 13E) demonstrated efficacy in treating cancer in mice. This program proved, once again, the importance of total

synthesis in rendering extremely rare natural products readily available, and synthesising novel analogues of them for extensive biological investigations, not to mention confirming their molecular structures and preparing the ground for further investigations.

3.9 Tubulysin H

*N*¹⁴-Desacetoxytubulysin H (**214**) is a member of a large family of tubulin binding, naturally occurring molecules endowed with antitumor properties.¹⁷⁷ Our research program in this area was directed towards the total synthesis of key members of the family and their designed analogues, aiming for high potencies as needed for antibody–drug conjugate (ADC) payloads.^{101,102} As an example, we describe here the total synthesis of *N*¹⁴-desacetoxytubulysin H (**214**, Fig. 14). Our retrosynthetic analysis (Fig. 14A) defined building blocks **218** (to be derived from fragments **219** and **220**), **215**, **216** and **217**. The execution of the derived synthetic strategy is summarised in Fig. 14B. Thus, a C–H activation-based coupling of aldehyde **219** with thiazole derivative **220** under the influence of $\text{PhI}(\text{OCOCF}_3)_2$ and TMSN_3 ^{178–181} furnished ketone **218** (top right, Fig. 14B), whose asymmetric reduction led to alcohol **221**. A four-step standard sequence from the latter afforded acetoxy carboxylic acid **222**, whose sequential peptide couplings with fragments **215**, **216** and **217** gave advanced precursor **224**, via **223**, as summarised in Fig. 14B. The latter was then converted to *N*¹⁴-desacetoxytubulysin H (**214**) in two simple steps as shown in Fig. 14B.^{182,183}

Employing the developed synthetic strategies and methods, a series of well over a hundred designed analogues were synthesised and biologically evaluated for their cytotoxicities against a number of cancer cell lines. Among the most potent were analogues **225**,¹⁸² **226**,¹⁸³ **227**¹⁸³ and **228**,¹⁸³ whose structures, together with their potencies against certain cell lines, are shown in Fig. 14C. As two of the most active tubulysins, compounds **227** and **228** formed complexes with microtubules, whose X-ray crystallographic analysis elucidated their binding modes which were consistent with a previous study employing tubulysin M.¹⁸⁴ These investigations led to a set of potential payloads to ADCs for targeted cancer therapies, provided a better understanding of the binding mode of tubulysins to their biological target, and expanded the established structure–activity relationships within the family.^{182,183}

3.10 Thailanstatins

With its novel molecular structure and spliceosome inhibitory properties, thailanstatin A (**229**, Fig. 15) provided another challenge and opportunity for us to explore yet another promising natural product as a lead compound for ADC payloads, or “drug alone” (or in combination with other drugs). Our total synthesis proceeded according to the synthetic strategy derived from the retrosynthetic analysis shown in Fig. 15A) revealing building blocks **230**, **231** and **233**, with **231** traced back to pyrone **232**, and **233** to hydroxy vinyl iodide **234**. The total synthesis of thailanstatin A (**229**) proceeded as summarised in Fig. 15B. Thus, pyrone **232** was reacted with building block **235** (I_2 ; then K_2CO_3) to afford ketone methyl ester **236**, whose standard elaboration over four steps led to vinyl iodide methyl ester **237**. The latter was converted to desired fragment, hydroxy epoxide **231**, through the four-step sequence shown in Fig. 15B. In parallel, vinyl iodide building block **234**, in the presence of allyl alcohol (**239**), was transformed to $\alpha,\beta,\gamma,\delta$ -unsaturated hydroxy

aldehyde **233**, as summarised in Fig. 15B, and thence to dihydropyran **240** through a three-step standard sequence. The latter was selectively reduced to tetrahydropyran system **241** as summarised in Fig. 15B. Intermediate **241** was converted to the corresponding aldehyde, and the latter was reacted with the Tebbe reagent to afford, after further elaboration and coupling with carboxylic acid **230**, amide **242**, whose cross metathesis (Grubbs II catalyst) with vinyl boronate fragment **243** furnished vinyl boronate building block **244**. The latter was joined through a Suzuki coupling with vinyl iodide **231** to furnish, after hydrolysis of the *tert*-butyl ester, the coveted thailanstatin A (**229**, Fig. 15B).

As part of this program an array of designed thailanstatin analogues were synthesised and tested for their antitumor properties, leading to optimisation beyond the potencies of previously known members of the family. Two of the most potent compounds synthesised thus far in this program, analogues **245** and **246**, are depicted in Fig. 15C together with their potencies against certain cell lines, which are significantly higher than those of thailanstatin A (see Fig. 15B and 15C), making them suitable as antibody–drug conjugate payloads intended for targeted cancer therapies.^{101,102}

3.11 Aziridinyl Epothilones

Beginning in 1996, our epothilone project continued through the ensuing period to the present time with numerous innovations and rewarding discoveries. Recently, we took advantage of the Ess–Kürti–Falck (EKF) aziridination reaction¹⁸⁵ to produce a series of 12,13-aziridinyl epothilone B analogues⁷⁶ whose biological evaluation revealed a number of highly active compounds (i.e., **247**, **248**, **249** and **250**, Fig. 16). Their short synthesis from epothilone B (**38**, see Fig. 5A) proceeded through a sequence involving ozonolysis (to cleave the side chain forming the corresponding methyl ketone), deoxygenation of the epoxide moiety (to reveal the trisubstituted olefinic bond), side chain attachment followed by EKF aziridination (to install selectively the aziridinyl structural motif), and optional placement of substituents on the N atom to generate the desired aziridinyl epothilone analogues. Fig. 16 depicts a number of highly potent analogues of this family of epothilones, together with their IC₅₀ values against several cancer cell lines (see also section 2 and Fig. 5A).⁷⁶ Such compounds or their decedents may find application as payloads for antibody–drug conjugates.

3.12 Disorazoles A₁ and B₁

The tubulin binding natural products disorazoles A₁ and B₁ (**251** and **252**, Fig. 17)¹⁸⁶ are the most potent members of this family of compounds. Our recent total synthesis¹⁸⁷ of these molecules provided opportunities to design, synthesise and biologically evaluate analogues of them, as well as to complete the structural assignment of the latter (disorazole B₁, **252**, Fig. 17) with regards to the epoxide moieties whose configurations were left undefined.¹⁸⁶ Among the analogues synthesised are the ones depicted, together with their potencies against certain cell lines, in Fig. 17 (i.e., cyclopropyl analogues **253** and **255**). It was of interest to observe the significant differences between the two diastereomeric bisepoxide structures **252** and **254** (Fig. 17) with regards to their biological activities, with the highly potent one (**252**) turning out to be the natural product and the essentially inactive one (**254**) relegated to an analogue, demonstrating the importance of the configuration of the two

epoxide structural motifs to biological activity. Both cyclopropyl analogues, however, of disorazole A₁ and B₁ (i.e., **253** and **255**, Fig. 17) turned out to be active,¹⁸⁸ making them worth investigating further as potential leads to antibody–drug conjugates and/or as drugs alone or in combination with other anti-cancer drugs.

3.13 Uncialamycin

The discovery of the marine natural product uncialamycin (**256**, Fig. 18) in 2005¹⁸⁹ elicited our interest as an ideal target molecule for total synthesis due to several reasons, including its challenging molecular structure, the fact that its structural assignment remained incomplete, leaving the relative stereochemistry of one of its stereocentres unassigned, and its phenomenal potencies as an antibiotic. Odd as it was, the disclosure of its isolation¹⁸⁹ did not include any DNA cleavage studies, nor was any mention of cytotoxicity assays with cancer cells, an occurrence that could be explained by the meagre amounts (300 µg) isolated from its natural source. The natural scarcity of uncialamycin (**256**) provided yet another and compelling reason for undertaking its replication in the laboratory as a prelude to further biological investigations. Our first total synthesis (delivering racemic material) of uncialamycin was achieved in 2007.¹⁹⁰ Our second, and enantioselective total synthesis,¹⁹¹ led to the molecule's full structural assignment and made possible further biological investigations in our laboratories that revealed its expected DNA single and double strand cleavage of duplex DNA and potent cytotoxicities against several cancer cell lines. Later on, and as a consequence of a new method we discovered for the synthesis of substituted anthraquinones, we developed an optimised, streamlined total synthesis of uncialamycin¹⁹² and applied it to the synthesis of numerous designed analogues for the purposes of discovering suitable payloads for antibody–drug conjugates.^{101,102,192,193}

Fig. 18 summarises our most recent and improved total synthesis of uncialamycin (**256**) and a number of analogues that exhibited higher potencies than their parent natural product.¹⁹² Retrosynthetic analysis of uncialamycin (**256**) as depicted in Fig. 18A defined building blocks **257** (to be derived from dichloride **258**), **259** and **260**. Thus, starting from 5-methoxy isatin (**260**), tricyclic intermediate **261** was constructed in five standard steps (65% yield), while dichloride **258** was converted to TIPS-enediyne **257** through a three-step sequence as indicated in Fig. 18B. Reaction of quinoline **261** with the acetylide generated from enediyne **257** and *i*-PrMgCl followed by quenching with Alloc chloride furnished, selectively, the corresponding Yamaguchi coupling product, whose elaboration as indicated in Fig. 18B led to hydroxy acetoxy epoxide **262**. Epoxide **262** was sequentially processed, via intermediates **263** and **264**, to stable methoxy quinone system **265**, as indicated in Fig. 18B. The latter served well as a coupling partner with cyanophthalide **259** in a Hauser–Kraus-type fusion/annulation to afford, upon further elaboration, uncialamycin (**256**) in enantiopure form and good overall yield as depicted in Fig. 18B. These synthetic strategies and technologies were applied to synthesise several naturally occurring congeners of uncialamycin (i.e., tiancimycins A and B, yangpumicin A)¹⁹⁴ and numerous analogues,¹⁹⁴ from which those exhibited in Fig. 18C (**266**, **267** and **268**) are among the most potent against certain cancer or cancer-like cell lines.¹⁹²

3.14 Shishijimicin A

Although reported in 2003,¹⁹⁵ shishijimicin A (**269**, Fig. 19) remained unexplored for more than a decade, despite its phenomenal cytotoxicity against certain cancer cell lines and, therefore, its potential as an antibody–drug conjugate payload. The reasons for this neglect most likely was its scarcity that made further, and well deserved, biological investigations virtually impossible. Prompted by the synthetic challenge posed by shishijimicin A and our desire to study its biological properties and those of analogues thereof, we initiated a synthetic program towards its total synthesis. Reported in 2015,¹⁹⁶ our first total synthesis of shishijimicin A was later refined and improved, and employed to construct several analogues¹⁹² as shown in Fig. 19. Thus, retrosynthetic analysis (Fig. 19A) led to enediyne fragment **270**, traced back to olefinic oxime **271**, and carbohydrate carboline fragment **272**, traced back to disaccharide ketoaldehyde **273** and serotonin hydrochloride (**74**). The streamlined total synthesis of shishijimicin A (**269**, Fig. 19)¹⁹² proceeded as summarised in Fig. 19B. Thus, oxidation of oxime **271** followed by spontaneous intramolecular [3+2] cycloaddition of the so formed olefin nitrile oxide (**275**) afforded bicyclic system **276**. Elaboration of the latter to the corresponding ketone, and addition of the acetylide generated from enediyne **277** as summarised in Fig. 19B, furnished intermediate **278**, whose further elaboration led to acetylenic aldehyde fragment **279**. The latter was exposed to LiHMDS in the presence of $\text{LaCl}_3 \cdot 2\text{LiCl}$ to induce double ring closure, first to form the 10-membered enediyne ring and thence the δ -lactone, leading to enediyne fragment **280** upon removal of the phthalide protecting group and elaboration of the resulting amino group to the desired carbamate, as shown in Fig. 19B. The latter was subsequently converted to the targeted thioacetate enediyne domain **270** through a standard four-step sequence, the latter ready to be coupled with carbohydrate-carboline domain **272**. Trichloroacetimidate donor **272** was synthesised from disaccharide keto aldehyde intermediate **273**¹⁹⁷ by fusion with serotonin hydrochloride salt (**274**) through the sequential exposure to AcOH and O_2 that led to intermediate **281**, whose standard four-step elaboration furnished the targeted glycosyl donor **272**, as summarised in Fig. 19B. Coupling of glycosyl donor **272** with carbohydrate acceptor **270** furnished thioacetate shishijimicin A precursor **282**, whose sequential conversion to methyltrisulfide protected precursor (**283**), and thence to shishijimicin A (**269**), is summarised in Fig. 19B.¹⁹⁷

Employing the developed synthetic strategies and methods a number of shishijimicin A analogues were synthesised, among which the most potent were the methyl disulfide **284** and the simpler shishijimicin A analogue **285**, the latter lacking the MeS and the OH groups on carbohydrate ring A. Their potencies against the MES SA, MES SA DX and HEK 293T cell lines are shown in Fig. 19C, together with those of synthetic shishijimicin A (**269**).¹⁹⁷ As can be seen, both these analogues turned out to be even more potent than the parent natural product, despite their less complex structures, especially true for analogue **285**. Most importantly, rendering shishijimicin A (**269**) readily available through our total synthesis put us in a position to study its DNA binding and cleaving modes, in collaboration with the Tianhu Li group at the Nanyang University in Singapore, demonstrating its intercalating properties and powerful double strand cuts of duplex DNA.¹⁹⁸

4. Conclusion and Future Perspectives

Introduced by Friedrich Wöhler in 1828 by accident, total synthesis and its more general version, organic synthesis, emerged from experimentation with inorganic substances [i.e., AgNCO (silver isocyanate, originally, and even today, called silver cyanate)¹ and NH₄Cl] that resulted in the formation of urea,² an organic substance and a natural product (see ①, Fig. 20). Recognising its potential power to replicate the molecules of Nature and others like them in the laboratory, and inspired further by Hermann Kolbe's intentional synthesis of acetic acid (1845),¹⁹⁹ synthetic chemists adopted the field of total synthesis and expanded it to include all organic molecules, natural or designed, by introducing organic synthesis as the name of the art and science of making organic molecules. Thus, from an unintended purpose, total synthesis assumed the burden (others will say the privilege or the pleasure!) as the science of confirming the hypothetical structures of natural products⁴ (see ②, Fig. 20), a purpose that, ironically, remains valid to this day despite our sophisticated analytical and spectroscopic instrumentation and techniques. In the 1950s and 1960s total synthesis gained much appreciation as an art and was valued for its elegance and aesthetically pleasing bond making and breaking manoeuvres, as demonstrated so elegantly by R. B. Woodward (see ③, Fig. 20). This artistic aspect of total synthesis continues to be admired today,²⁰⁰ and it is often accompanied by other advantages as is evident in cascade reactions in total synthesis²⁰¹⁻²⁰³ where the benefits extend beyond the beauty, translating into practicality, saving economic cost and labour, and providing environmental protection through green chemistry (Fig. 20).

From the 1960s onwards, total synthesis entered into yet another era, when logic and generality took their proper place in planning strategies towards the ever increasing, in numbers and complexity, natural products unearthed from the forests, the soil, and the oceans. Introduced and championed by E. J. Corey with his contributions to the theory and methodology of total synthesis (see ④, Fig. 20), this last period moved forward steadily to systematically include retrosynthetic analysis, targeting rare biologically active natural products, and applications of the developed synthetic strategies and methods to the synthesis of designed analogues for biological investigations. These developments within the field of total synthesis, coupled with the trends of interdisciplinary research, collaborations and partnerships²⁰⁴ in more recent times, led to the present-day paradigm of total synthesis endeavours as already adopted by many practitioners around the world (see ⑤, Fig. 20).

The ideal modern paradigm of total synthesis endeavours includes, although not all obligatory or limited to, the following components interwoven together harmoniously, while maintaining the fundamentals of the science and its advancement to higher levels of sophistication and performance.

1. The endeavour should preferably include the discovery and invention of new chemistry, usually in the form of novel synthetic strategies and new methods. Such discoveries and inventions contribute to the advancement of the field of total synthesis, and organic synthesis in general, for their own sake, an important principle from the science point of view.

2. The overall project of total synthesis should preferably include a biology and, potentially, medicine, either within the same laboratory or, more often than not, as part of a collaboration/partnership with other academic/industrial groups. Such arrangements could result in added-value to the endeavour and provide opportunities for discoveries and inventions, all the way from the fundamentals (basic research) to the translational (drug discovery and development). This added-value of the endeavour could attract substantial funding and may result in lucrative benefits to science and society in terms of knowledge, understanding of biology, and faster translation of the fundamentals to the applied. These principles apply beyond biology and medicine and into other beneficiary fields such as agrochemicals, cosmetics, materials science and nanotechnology once the roles of the targeted natural products are recognised or suspected. One such example is that of the ladderanes as demonstrated recently by Noah Burns and his colleagues.^{205,206}
3. Intended and/or unintended complete structural elucidations of natural products whose molecular structures were left incomplete for one reason or another are often added-value to total synthesis endeavours. From our experience involving almost 200 natural products total syntheses, at least 13% of them resulted in some sort of added value in terms of natural product structure elucidations, ranging from stereochemical configurations, relative or absolute, to synthesising naturally occurring molecules before their discovery from Nature, or prediction as naturally occurring molecules.²⁰⁷
4. We will be remiss if we do not mention the importance of total synthesis endeavours as platforms to educate and train young scientists in the art and science of making molecules for all intents and purposes. Being one of the most demanding endeavours in chemistry, total synthesis often presents what initially seem like intransigent problems beyond the limits of our knowledge and synthetic capabilities at the time. This is an opportunity “to challenge the challenge”, for it is only then that we can break the existing glass ceilings, for they are only temporary as it has been shown time and time again in the history of total synthesis and organic synthesis in general. It is during such challenging projects that students learn how to solve problems and develop immunity to disappointment and despair upon failing. Instead, resourcefulness, creativity and imagination, and hard work should come into play, with motivation and patience as supporting allies, to solve the problems often arising one after the other along the way.

It is gratifying to see that the new paradigm and philosophy of total synthesis (see ⑤, Fig. 20) is spreading around the world as the new model of practicing this fascinating and multifaceted discipline. Let us hope that funding agencies, and charities alike, recognise and appropriately honour the benefits to science and society derived from total synthesis endeavours, so as to provide the necessary support for the field to thrive in its newly and rapidly emerging form. For this to occur, isolation of natural products should also be rekindled as a means to provide the myriad treasure molecules of Nature waiting to be discovered, and inspire the new generation of practitioners of the art and science of total

synthesis to exploit their potential as opportunities to increase our knowledge in science and apply it to discover new medicines for people.

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Notes and references

1. Britton D and Dunitz JD, *Acta Crystallogr.*, 1965, 18, 424–428.
2. Wöhler F, *Ann. Phys.*, 1828, 88, 253–256.
3. Esteban S, *J. Chem. Educ.*, 2008, 85, 1201.
4. Hoffmann RW, *Classical Methods in Structure Elucidation of Natural Products*, Wiley-VHCA, Zürich, Switzerland, 2018.
5. Corey EJ, Weinshenker NM, Schaaf TK and Huber W, *J. Am. Chem. Soc.*, 1969, 91, 5675–5677. [PubMed: 5808505]
6. Corey EJ, Schaaf TK, Huber W, Koelliker U and Weinshenker NM, *J. Am. Chem. Soc.*, 1970, 92, 397–398. [PubMed: 5411057]
7. Corey EJ, *Ann. N. Y. Acad. Sci.*, 1971, 180, 24–37. [PubMed: 5286099]
8. Hammarström S, Samuelsson B, Clark DA, Goto G, Marfat A, Mioskowski C and Corey EJ, *Biochem. Biophys. Res. Commun.*, 1980, 92, 946–953. [PubMed: 6102463]
9. Lewis RA, Austen KF, Drazen JM, Clark DA, Marfat A and Corey EJ, *Proc. Natl. Acad. Sci. U.S.A.*, 1980, 77, 3710. [PubMed: 6106193]
10. Rådmark O, Malmsten C, Samuelsson B, Clark DA, Goto G, Marfat A and Corey EJ, *Biochem. Biophys. Res. Commun.*, 1980, 92, 954–961. [PubMed: 6244821]
11. Corey EJ, Marfat A, Goto G and Brion F, *J. Am. Chem. Soc.*, 1980, 102, 7984–7985.
12. Corey EJ, Pyne SG and Su W.-g., *Tetrahedron Lett.*, 1983, 24, 4883–4886.
13. Marfat A and Corey EJ, *Adv. Prostaglandin, Thromboxane, Leukotriene Res.*, 1985, 14, 155–228.
14. Corey EJ, Nicolaou KC, Machida Y, Malmsten CL and Samuelsson B, *Proc. Natl. Acad. Sci. U.S.A.*, 1975, 72, 3355–3358. [PubMed: 1059121]
15. See KCN's CV at: <https://nicolaou.rice.edu/classes.html#kcn>
16. Moncada S, Gryglewski R, Bunting S and Vane JR, *Nature*, 1976, 263, 663–665. [PubMed: 802670]
17. Nicolaou KC, Sipio WJ, Magolda RL, Seitz S and Barnette WE, *J. Chem. Soc., Chem. Commun.*, 1978, 1067–1068.
18. Nicolaou KC, Barnette WE and Magolda RL, *J. Am. Chem. Soc.*, 1981, 103, 3472–3480.
19. Nicolaou KC, Barnette WE, Gasic GP and Magolda RL, *J. Am. Chem. Soc.*, 1977, 99, 7736–7738. [PubMed: 334822]
20. Hamberg M, Svensson J and Samuelsson B, *Proc. Natl. Acad. Sci. U.S.A.*, 1975, 72, 2994–2998. [PubMed: 1059088]
21. Nicolaou KC, Magolda RL, Smith JB, Aharony D, Smith EF and Lefer AM, *Proc. Natl. Acad. Sci. U.S.A.*, 1979, 76, 2566–2570. [PubMed: 288046]
22. Lefer AM, Smith EF, Araki H, Smith JB, Aharony D, Claremon DA, Magolda RL and Nicolaou KC, *Proc. Natl. Acad. Sci. U.S.A.*, 1980, 77, 1706–1710. [PubMed: 6929517]
23. Smith III EF, Lefer AM and Nicolaou KC, *Am. J. Physiol. Heart Circ. Physiol.*, 1981, 240, H493–H497.
24. Koshishara Y, Murota S.-i., Petasis NA and Nicolaou KC, *FEBS Lett.*, 1982, 143, 13–16. [PubMed: 6811315]

25. Koshihara Y, Murota S and Nicolaou KC, *Adv. Prostaglandin, Thromboxane, Leukotriene Res.*, 1983, 11, 163–167.
26. Tsai BS, Keith RH, Villani-Price D, Haack RA, Bauer RF, Leonard R, Abe Y and Nicolaou KC, *Prostaglandins*, 1989, 37, 287–302. [PubMed: 2543037]
27. Serhan CN, Hamberg M and Samuelsson B, *Biochem. Biophys. Res. Commun.*, 1984, 118, 943–949. [PubMed: 6422933]
28. Serhan CN, Hamberg M and Samuelsson B, *Proc. Natl. Acad. Sci. U.S.A.*, 1984, 81, 5335–5339. [PubMed: 6089195]
29. Nicolaou KC, Veale CA, Webber SE and Katerinopoulos H, *J. Am. Chem. Soc.*, 1985, 107, 7515–7518.
30. Nicolaou KC and Webber SE, *Synthesis*, 1986, 1986, 453–461.
31. Nicolaou KC, Webber SE, Ramphal J and Abe Y, *Angew. Chem. Int. Ed. Engl.*, 1987, 26, 1019–1021.
32. Greenberg R, Antonaccio MJ and Steinbacher T, *Eur. J. Pharmacol.*, 1982, 80, 19–27. [PubMed: 6807699]
33. Ramstedt U, Serhan CN, Nicolaou KC, Webber SE, Wigzell H and Samuelsson B, *J. Immunol.*, 1987, 138, 266–270. [PubMed: 3782800]
34. Palmblad J, Gyllenhammar H, Ringertz B, Serhan CN, Samuelsson B and Nicolaou KC, *Biochem. Biophys. Res. Commun.*, 1987, 145, 168–175. [PubMed: 3593338]
35. Badr KF, Serhan CN, Nicolaou KC and Samuelsson B, *Biochem. Biophys. Res. Commun.*, 1987, 145, 408–414. [PubMed: 3593345]
36. Nicolaou KC and Petasis NA, in *Handbook of Eicosanoids, Prostaglandins and Related Lipids, Part B (Chemical and Biochemical Aspects)*, ed. Willis AL, CRC Press, Boca Raton, Florida, USA, 1987, pp. 1–18.
37. Dahlén S-E, Franzén L, Raud J, Wikström E, Björck T, Matsuda H, Westlund P, Puustinen T, Haeggström J, Samuelsson B, Serhan CN, Webber SE, Veale CA and Nicolaou KC, in *Lipoxins: Biosynthesis, Chemistry, and Biological Activities*, eds. Wong PYK and Serhan CN, Springer US, Boston, MA, 1988, pp. 107–130.
38. Lefer AM, Stahl GL, Lefer DJ, Brezinski ME, Nicolaou KC, Veale CA, Abe Y and Smith JB, *Proc. Natl. Acad. Sci. U.S.A.*, 1988, 85, 8340–8344. [PubMed: 3186729]
39. Stahl GL, Tsao P, Lefer AM, Ramphal JY and Nicolaou KC, *Eur. J. Pharmacol.*, 1989, 163, 55–60. [PubMed: 2545463]
40. Dahlén SE, Veale CA, Webber SE, Marron BE, Nicolaou KC and Serhan CN, *Agents Actions*, 1989, 26, 93–95. [PubMed: 2711954]
41. Brezinski ME, Gimbrone MA, Nicolaou KC and Serhan CN, *FEBS Lett.*, 1989, 245, 167–172. [PubMed: 2494071]
42. Hedqvist P, Raud J, Palmertz U, Haeggström J, Nicolaou KC and Dahlén S-E, *Acta Physiol. Scand.*, 1989, 137, 571–572. [PubMed: 2557732]
43. Nicolaou KC, Ramphal JY, Petasis NA and Serhan CN, *Angew. Chem. Int. Ed. Engl.*, 1991, 30, 1100–1116.
44. Nicolaou KC and Webber SE, *J. Am. Chem. Soc.*, 1984, 106, 5734–5736.
45. Nicolaou KC, Ladduwahetty T and Elisseou EM, *J. Chem. Soc., Chem. Commun.*, 1985, 1580–1581.
46. Nicolaou KC, Ladduwahetty T, Taffer IM and Zipkin RE, *Synthesis*, 1986, 1986, 344–347.
47. Nicolaou KC, Ramphal JY, Palazon JM and Spanevello RA, *Angew. Chem. Int. Ed. Engl.*, 1989, 28, 587–588.
48. Nicolaou KC, Ramphal JY and Abe Y, *Synthesis*, 1989, 1989, 898–901.
49. Nicolaou KC and Webber SE, *J. Chem. Soc., Chem. Commun.*, 1986, 1816–1817.
50. Puustinen T, Webber SE, Nicolaou KC, Haeggström J, Serhan CN and Samuelsson B, *FEBS Lett.*, 1986, 207, 127–132. [PubMed: 3770188]
51. Nicolaou KC, Hummel CW, Pitsinos EN, Nakada M, Smith AL, Shibayama K and Saimoto H, *J. Am. Chem. Soc.*, 1992, 114, 10082–10084.

52. Nicolaou KC, Riemer C, Kerr MA, Rideout DR and Wrasidlo WW, *Nature*, 1993, 364, 464–466. [PubMed: 8101355]
53. Nicolaou KC, Guy RK, Pitsinos EN and Wrasidlo W, *Angew. Chem. Int. Ed. Engl*, 1994, 33, 1583–1587.
54. Nicolaou KC, Couladouros EA, Nantermet PG, Renaud J, Guy RK and Wrasidlo W, *Angew. Chem. Int. Ed. Engl*, 1994, 33, 1581–1583.
55. Nicolaou KC, Nantermet PG, Ueno H and Guy RK, *J. Chem. Soc., Chem. Commun*, 1994, 295–296.
56. Guy RK, Scott ZA, Sloboda RD and Nicolaou KC, *Chem. Biol*, 1996, 3, 1021–1031. [PubMed: 9000007]
57. Nicolaou KC, Vourloumis D, Li T, Pastor J, Winssinger N, He Y, Ninkovic S, Sarabia F, Vallberg H, Roschangar F, King NP, Finlay MRV, Giannakakou P, Verdier-Pinard P and Hamel E, *Angew. Chem. Int. Ed. Engl*, 1997, 36, 2097–2103.
58. Nicolaou KC, He Y, Vourloumis D, Vallberg H, Roschangar F, Sarabia F, Ninkovic S, Yang Z and Trujillo JJ, *J. Am. Chem. Soc*, 1997, 119, 7960–7973.
59. Nicolaou KC, Ninkovic S, Ray VFinlay M, Sarabia F and Li T, *Chem. Commun*, 1997, 2343–2344.
60. Nicolaou KC, Vallberg H, King NP, Roschangar F, He Y, Vourloumis D and Nicolaou CG, *Chem. - Eur. J*, 1997, 3, 1957–1970.
61. Nicolaou KC, Sarabia F, Finlay MRV, Ninkovic S, King NP, Vourloumis D and He Y, *Chem. - Eur. J*, 1997, 3, 1971–1986.
62. Nicolaou KC, Sarabia F, Ninkovic S, Finlay MRV and Boddy CNC, *Angew. Chem. Int. Ed*, 1998, 37, 81–84.
63. Nicolaou KC, He Y, Roschangar F, King NP, Vourloumis D and Li T, *Angew. Chem. Int. Ed*, 1998, 37, 84–87.
64. Nicolaou KC, Finlay MRV, Ninkovic S and Sarabia F, *Tetrahedron*, 1998, 54, 7127–7166.
65. Nicolaou KC, Finlay MRV, Ninkovic S, King NP, He Y, Li T, Sarabia F and Vourloumis D, *Chem. Biol*, 1998, 5, 365–372. [PubMed: 9662505]
66. Nicolaou KC, Hepworth D, Ray VFinlay M, Paul King N, Werschkun B and Bigot A, *Chem. Commun*, 1999, 519–520.
67. Nicolaou KC, King NP, Finlay MRV, He Y, Roschangar F, Vourloumis D, Vallberg H, Sarabia F, Ninkovic S and Hepworth D, *Bioorg. Med. Chem*, 1999, 7, 665–697. [PubMed: 10400321]
68. Nicolaou KC, Hepworth D, King NP, Finlay MRV, Scarpelli R, Pereira MMA, Bollbuck B, Bigot A, Werschkun B and Winssinger N, *Chem. - Eur. J*, 2000, 6, 2783–2800. [PubMed: 10985727]
69. Nicolaou KC, Scarpelli R, Bollbuck B, Werschkun B, Pereira MMA, Wartmann M, Altmann KH, Zaharevitz D, Gussio R and Giannakakou P, *Chem. Biol*, 2000, 7, 593–599. [PubMed: 11048950]
70. Nicolaou KC, Namoto K, Li J, Ritzén A, Ulven T, Shoji M, Zaharevitz D, Gussio R, Sackett DL, Ward RD, Hensler A, Fojo T and Giannakakou P, *ChemBioChem*, 2001, 2, 69–75. [PubMed: 11828429]
71. Nicolaou KC, Namoto K, Ritzén A, Ulven T, Shoji M, Li J, D’Amico G, Liotta D, French CT, Wartmann M, Altmann K-H and Giannakakou P, *J. Am. Chem. Soc*, 2001, 123, 9313–9323. [PubMed: 11562214]
72. Nicolaou KC, Ritzén A, Namoto K, Buey RM, Díaz JF, Andreu JM, Wartmann M, Altmann K-H, O’Brate A and Giannakakou P, *Tetrahedron*, 2002, 58, 6413–6432.
73. Nicolaou KC, Sasmal PK, Rassias G, Reddy MV, Altmann K-H, Wartmann M, O’Brate A and Giannakakou P, *Angew. Chem. Int. Ed*, 2003, 42, 3515–3520.
74. Nicolaou KC, Pratt BA, Arseniyadis S, Wartmann M, O’Brate A and Giannakakou P, *ChemMedChem*, 2006, 1, 41–44. [PubMed: 16892332]
75. Nicolaou KC, Rhoades D, Wang Y, Totokotsopoulos S, Bai R and Hamel E, *ChemMedChem*, 2015, 10, 1974–1979. [PubMed: 26447977]
76. Nicolaou KC, Rhoades D, Wang Y, Bai R, Hamel E, Aujay M, Sandoval J and Gavriluk J, *J. Am. Chem. Soc*, 2017, 139, 7318–7334. [PubMed: 28513142]

77. Nicolaou KC, Kim S, Pfefferkorn J, Xu J, Ohshima T, Hosokawa S, Vourloumis D and Li T, *Angew. Chem. Int. Ed.*, 1998, 37, 1418–1421.
78. Nicolaou KC, Winssinger N, Vourloumis D, Ohshima T, Kim S, Pfefferkorn J, Xu JY and Li T, *J. Am. Chem. Soc.*, 1998, 120, 10814–10826.
79. Nicolaou KC, Natarajan S, Li H, Jain NF, Hughes R, Solomon ME, Ramanjulu JM, Boddy CNC and Takayanagi M, *Angew. Chem. Int. Ed.*, 1998, 37, 2708–2714.
80. Nicolaou KC, Jain NF, Natarajan S, Hughes R, Solomon ME, Li H, Ramanjulu JM, Takayanagi M, Koumbis AE and Bando T, *Angew. Chem. Int. Ed.*, 1998, 37, 2714–2716.
81. Nicolaou KC, Takayanagi M, Jain NF, Natarajan S, Koumbis AE, Bando T and Ramanjulu JM, *Angew. Chem. Int. Ed.*, 1998, 37, 2717–2719.
82. Nicolaou KC, Mitchell HJ, Jain NF, Winssinger N, Hughes R and Bando T, *Angew. Chem. Int. Ed.*, 1999, 38, 240–244.
83. Nicolaou KC, Hughes R, Cho SY, Winssinger N, Smethurst C, Labischinski H and Endermann R, *Angew. Chem. Int. Ed.*, 2000, 39, 3823–3828.
84. Nicolaou KC, Cho SY, Hughes R, Winssinger N, Smethurst C, Labischinski H and Endermann R, *Chem. - Eur. J.*, 2001, 7, 3798–3823. [PubMed: 11575782]
85. Nicolaou KC, Hughes R, Cho SY, Winssinger N, Labischinski H and Endermann R, *Chem. - Eur. J.*, 2001, 7, 3824–3843. [PubMed: 11575783]
86. Nicolaou KC, Pfefferkorn JA and Cao G-Q, *Angew. Chem. Int. Ed.*, 2000, 39, 734–739.
87. Nicolaou KC, Cao G-Q and Pfefferkorn JA, *Angew. Chem. Int. Ed.*, 2000, 39, 739–743.
88. Nicolaou KC, Pfefferkorn JA, Roecker AJ, Cao GQ, Barluenga S and Mitchell HJ, *J. Am. Chem. Soc.*, 2000, 122, 9939–9953.
89. Nicolaou KC, Pfefferkorn JA, Mitchell HJ, Roecker AJ, Barluenga S, Cao GQ, Affleck RL and Lillig JE, *J. Am. Chem. Soc.*, 2000, 122, 9954–9967.
90. Nicolaou KC, Pfefferkorn JA, Barluenga S, Mitchell HJ, Roecker AJ and Cao GQ, *J. Am. Chem. Soc.*, 2000, 122, 9968–9976.
91. Nicolaou KC, Roecker AJ, Barluenga S, Pfefferkorn JA and Cao G-Q, *ChemBioChem*, 2001, 2, 460–465. [PubMed: 11828478]
92. Nicolaou KC, Hughes R, Pfefferkorn JA, Barluenga S and Roecker AJ, *Chem. - Eur. J.*, 2001, 7, 4280–4295. [PubMed: 11686609]
93. Nicolaou KC, Hughes R, Pfefferkorn JA and Barluenga S, *Chem. - Eur. J.*, 2001, 7, 4296–4310. [PubMed: 11686610]
94. Xiao X-Y, Li R, Zhuang H, Ewing B, Karunaratne K, Lillig J, Brown R and Nicolaou KC, *Biotechnol. Bioeng.*, 2000, 71, 44–50. [PubMed: 10629535]
95. Nicolaou KC, *J. Org. Chem.*, 2005, 70, 7007–7027. [PubMed: 16122219]
96. Nicolaou KC, *J. Med. Chem.*, 2005, 48, 5613–5638. [PubMed: 16134928]
97. Nicolaou KC, Li T, Nakada M, Hummel CW, Hiatt A and Wrasidlo W, *Angew. Chem. Int. Ed. Engl.*, 1994, 33, 183–186.
98. Lode HN, Reisfeld RA, Handgretinger R, Nicolaou KC, Gaedicke G and Wrasidlo W, *Cancer Res.*, 1998, 58, 2925–2928. [PubMed: 9679947]
99. Hinman LM, Hamann PR, Wallace R, Menendez AT, Durr FE and Upešlacijs J, *Cancer Res.*, 1993, 53, 3336–3342. [PubMed: 8324745]
100. Hamann PR, Hinman LM, Hollander I, Beyer CF, Lindh D, Holcomb R, Hallett W, Tsou H-R, Upešlacijs J, Shochat D, Mountain A, Flowers DA and Bernstein I, *Bioconjugate Chem.*, 2002, 13, 47–58.
101. Nicolaou KC and Rigol S, *Acc. Chem. Res.*, 2019, 52, 127–139. [PubMed: 30575399]
102. Nicolaou KC and Rigol S, *Angew. Chem. Int. Ed.*, 2019, 58, 11206–11241.
103. Wrasidlo W, Gaedicke G, Guy RK, Renaud J, Pitsinos E, Nicolaou KC, Reisfeld RA and Lode HN, *Bioconjugate Chem.*, 2002, 13, 1093–1099.
104. Nicolaou KC, Xu JY, Kim S, Ohshima T, Hosokawa S and Pfefferkorn J, *J. Am. Chem. Soc.*, 1997, 119, 11353–11354.

105. Hamel E, Sackett DL, Vourloumis D and Nicolaou KC, *Biochemistry*, 1999, 38, 5490–5498. [PubMed: 10220336]
106. Nicolaou KC, Ohshima T, Hosokawa S, van Delft FL, Vourloumis D, Xu JY, Pfefferkorn J and Kim S, *J. Am. Chem. Soc.*, 1998, 120, 8674–8680.
107. Nicolaou KC, Pfefferkorn JA, Schuler F, Roecker AJ, Cao GQ and Casida JE, *Chem. Biol.*, 2000, 7, 979–992. [PubMed: 11137820]
108. Nicolaou KC, Evans RM, Roecker AJ, Hughes R, Downes M and Pfefferkorn JA, *Org. Biomol. Chem.*, 2003, 1, 908–920. [PubMed: 12929628]
109. Downes M, Verdecia MA, Roecker AJ, Hughes R, Hogenesch JB, Kast-Woelbern HR, Bowman ME, Ferrer J-L, Anisfeld AM, Edwards PA, Rosenfeld JM, Alvarez JGA, Noel JP, Nicolaou KC and Evans RM, *Mol. Cell*, 2003, 11, 1079–1092. [PubMed: 12718892]
110. Li J, Ballmer SG, Gillis EP, Fujii S, Schmidt MJ, Palazzolo AME, Lehmann JW, Morehouse GF and Burke MD, *Science*, 2015, 347, 1221–1226. [PubMed: 25766227]
111. Granda JM, Donina L, Dragone V, Long D-L and Cronin L, *Nature*, 2018, 559, 377–381. [PubMed: 30022133]
112. Granda JM, Donina L, Dragone V, Long D-L and Cronin L, *Nature*, 2018, 562, E26–E26. [PubMed: 30042506]
113. Granda JM, Donina L, Dragone V, Long D-L and Cronin L, *Nature*, 2019, 570, E67–E69. [PubMed: 31243376]
114. Sanderson K, *Nature*, 2019, 568, 577–579. [PubMed: 31015690]
115. Riedlinger J, Reicke A, Zähner H, Krismer B, Bull AT, Maldonado LA, Ward AC, Goodfellow M, Bister B, Bischoff D, Süßmuth RD and Fiedler HP, *J. Antibiot.*, 2004, 57, 271–279.
116. Bister B, Bischoff D, Ströbele M, Riedlinger J, Reicke A, Wolter F, Bull AT, Zähner H, Fiedler H-P and Süßmuth RD, *Angew. Chem. Int. Ed.*, 2004, 43, 2574–2576.
117. Nicolaou KC and Harrison ST, *Angew. Chem. Int. Ed.*, 2006, 45, 3256–3260.
118. Nicolaou KC and Harrison ST, *J. Am. Chem. Soc.*, 2007, 129, 429–440. [PubMed: 17212423]
119. Nicolaou KC and Harrison ST, *J. Am. Chem. Soc.*, 2007, 129, 429–440. [PubMed: 17212423]
120. Keller S, Nicholson G, Drahl C, Sorensen E, Fiedler H-P and Süßmuth RD, *J. Antibiot.*, 2007, 60, 391–394.
121. Tamejiro H, Bhaskar RG, Tatsuya M and Takeshi H, *Bull. Chem. Soc. Jpn.*, 1995, 68, 350–363.
122. Nicolaou KC, Harrison ST and Chen JS, *Synthesis*, 2009, 2009, 33–42. [PubMed: 20047014]
123. Tanaka N, Okasaka M, Ishimaru Y, Takaishi Y, Sato M, Okamoto M, Oshikawa T, Ahmed SU, Consentino LM and Lee K-H, *Org. Lett.*, 2005, 7, 2997–2999. [PubMed: 15987189]
124. Nicolaou KC, Sanchini S, Wu TR and Sarlah D, *Chem. - Eur. J.*, 2010, 16, 7678–7682. [PubMed: 20572192]
125. Tanaka N, Kashiwada Y, Kim SY, Hashida W, Sekiya M, Ikeshiro Y and Takaishi Y, *J. Nat. Prod.*, 2009, 72, 1447–1452. [PubMed: 19606850]
126. Nicolaou KC, Sanchini S, Sarlah D, Lu G, Wu TR, Nomura DK, Cravatt BF, Cubitt B, de la Torre JC, Hessel AJ and Burton DR, *Proc. Natl. Acad. Sci. U.S.A.*, 2011, 108, 6715–6720. [PubMed: 21245351]
127. Nicolaou KC, Wu TR, Sarlah D, Shaw DM, Rowcliffe E and Burton DR, *J. Am. Chem. Soc.*, 2008, 130, 11114–11121. [PubMed: 18646750]
128. Nicolaou KC, Lu M, Totokotsopoulos S, Heretsch P, Giguère D, Sun Y-P, Sarlah D, Nguyen TH, Wolf IC, Smee DF, Day CW, Bopp S and Winzeler EA, *J. Am. Chem. Soc.*, 2012, 134, 17320–17332. [PubMed: 22978674]
129. Kornblum N and DeLaMare HE, *J. Am. Chem. Soc.*, 1951, 73, 880–881.
130. Staben ST, Linghu X and Toste FD, *J. Am. Chem. Soc.*, 2006, 128, 12658–12659. [PubMed: 17002354]
131. Wang J, Soisson SM, Young K, Shoop W, Kodali S, Galgoci A, Painter R, Parthasarathy G, Tang YS, Cummings R, Ha S, Dorso K, Motyl M, Jayasuriya H, Ondeyka J, Herath K, Zhang C, Hernandez L, Allocco J, Basilio Á, Tormo JR, Genilloud O, Vicente F, Pelaez F, Colwell L, Lee SH, Michael B, Felcetto T, Gill C, Silver LL, Hermes JD, Bartizal K, Barrett J, Schmatz D, Becker JW, Cully D and Singh SB, *Nature*, 2006, 441, 358–361. [PubMed: 16710421]

132. Jayasuriya H, Herath KB, Zhang C, Zink DL, Basilio A, Genilloud O, Diez MT, Vicente F, Gonzalez I, Salazar O, Pelaez F, Cummings R, Ha S, Wang J and Singh SB, *Angew. Chem. Int. Ed.*, 2007, 46, 4684–4688.
133. Wang J, Kodali S, Lee SH, Galgoci A, Painter R, Dorso K, Racine F, Motyl M, Hernandez L, Tinney E, Colletti SL, Herath K, Cummings R, Salazar O, González I, Basilio A, Vicente F, Genilloud O, Pelaez F, Jayasuriya H, Young K, Cully DF and Singh SB, *Proc. Natl. Acad. Sci. U.S.A.*, 2007, 104, 7612–7616. [PubMed: 17456595]
134. Zhang C, Ondeyka J, Zink DL, Burgess B, Wang J and Singh SB, *Chem. Commun.*, 2008, 5034–5036.
135. Herath KB, Zhang C, Jayasuriya H, Ondeyka JG, Zink DL, Burgess B, Wang J and Singh SB, *Org. Lett.*, 2008, 10, 1699–1702. [PubMed: 18393511]
136. Lei A, Waldkirch JP, He M and Zhang X, *Angew. Chem. Int. Ed.*, 2002, 41, 4526–4529.
137. Lei A, He M, Wu S and Zhang X, *Angew. Chem. Int. Ed.*, 2002, 41, 3457–3460.
138. Lei A, He M and Zhang X, *J. Am. Chem. Soc.*, 2002, 124, 8198–8199. [PubMed: 12105894]
139. Nicolaou KC, Li A, Edmonds DJ, Tria GS and Ellery SP, *J. Am. Chem. Soc.*, 2009, 131, 16905–16918. [PubMed: 19874023]
140. Nicolaou KC, Lister T, Denton RM, Montero A and Edmonds DJ, *Angew. Chem. Int. Ed.*, 2007, 46, 4712–4714.
141. Nicolaou KC, Tang Y, Wang J, Stepan AF, Li A and Montero A, *J. Am. Chem. Soc.*, 2007, 129, 14850–14851. [PubMed: 17988130]
142. Nicolaou KC, Stepan AF, Lister T, Li A, Montero A, Tria GS, Turner CI, Tang Y, Wang J, Denton RM and Edmonds DJ, *J. Am. Chem. Soc.*, 2008, 130, 13110–13119. [PubMed: 18771264]
143. Lambert TH and Danishefsky SJ, *J. Am. Chem. Soc.*, 2006, 128, 426–427. [PubMed: 16402826]
144. de RM, Fröhlich Figueiredo R. and Christmann M, *Angew. Chem. Int. Ed.*, 2007, 46, 2883–2886.
145. Lin Y-Y, Risk M, Ray SM, Van Engen D, Clardy J, Golik J, James JC and Nakanishi K, *J. Am. Chem. Soc.*, 1981, 103, 6773–6775.
146. Nicolaou KC, Reddy KR, Skokotas G, Sato F and Xiao X-Y, *J. Am. Chem. Soc.*, 1992, 114, 7935–7936.
147. Nicolaou KC, Theodorakis EA, Rutjes FPJT, Tiebes J, Sato M, Untersteller E and Xiao X-Y, *J. Am. Chem. Soc.*, 1995, 117, 1171–1172.
148. Nicolaou KC, Rutjes FPJT, Theodorakis EA, Tiebes J, Sato M and Untersteller E, *J. Am. Chem. Soc.*, 1995, 117, 1173–1174.
149. Nicolaou KC, Yang Z, Shi G.-q., Gunzner JL, Agrios KA and Gartner P, *Nature*, 1998, 392, 264–269. [PubMed: 9521320]
150. Nicolaou KC, Bunnage ME, McGarry DG, Shi S, Somers PK, Wallace PA, Chu X-J, Agrios KA, Gunzner JL and Yang Z, *Chem. - Eur. J.*, 1999, 5, 599–617.
151. Nicolaou KC, Wallace PA, Shi S, Ouellette MA, Bunnage ME, Gunzner JL, Agrios KA, Shi G.-q., Gärtner P and Yang Z, *Chem. - Eur. J.*, 1999, 5, 618–627.
152. Nicolaou KC, Shi G.-q., Gunzner JL, Gärtner P, Wallace PA, Ouellette MA, Shi S, Bunnage ME, Agrios KA, Veale CA, Hwang C-K, Hutchinson J, Prasad CVC, Ogilvie WW and Yang Z, *Chem. - Eur. J.*, 1999, 5, 628–645.
153. Nicolaou KC, Gunzner JL, Shi G.-q., Agrios KA, Gärtner P and Yang Z, *Chem. - Eur. J.*, 1999, 5, 646–658.
154. Nicolaou KC and Frederick MO, *Angew. Chem. Int. Ed.*, 2007, 46, 5278–5282.
155. Gallimore AR and Spencer JB, *Angew. Chem. Int. Ed.*, 2006, 45, 4406–4413.
156. Nicolaou KC, Frederick MO, Burtoloso ACB, Denton RM, Rivas F, Cole KP, Aversa RJ, Gibe R, Umezawa T and Suzuki T, *J. Am. Chem. Soc.*, 2008, 130, 7466–7476. [PubMed: 18481856]
157. Nicolaou KC, Aversa RJ, Jin J and Rivas F, *J. Am. Chem. Soc.*, 2010, 132, 6855–6861. [PubMed: 20415445]
158. Nicolaou KC, Gelin CF, Seo JH, Huang Z and Umezawa T, *J. Am. Chem. Soc.*, 2010, 132, 9900–9907. [PubMed: 20666400]

159. Nicolaou KC, Baker TM and Nakamura T, J. Am. Chem. Soc, 2011, 133, 220–226. [PubMed: 21166430]
160. Nicolaou KC, Seo JH, Nakamura T and Aversa RJ, J. Am. Chem. Soc, 2011, 133, 214–219. [PubMed: 21166429]
161. Nicolaou KC, Heretsch P, Nakamura T, Rudo A, Murata M and Konoki K, J. Am. Chem. Soc, 2014, 136, 16444–16451. [PubMed: 25374117]
162. Nicolaou KC, Postema MHD and Claiborne CF, J. Am. Chem. Soc, 1996, 118, 1565–1566.
163. Nicolaou KC, Postema MHD, Yue EW and Nadin A, J. Am. Chem. Soc, 1996, 118, 10335–10336.
164. Nicolaou KC, Tiebes J, Theodorakis EA, Rutjes FPJT, Koide K, Sato M and Untersteller E, J. Am. Chem. Soc, 1994, 116, 9371–9372.
165. Gawley RE, Rein KS, Jeglitsch G, Adams DJ, Theodorakis EA, Tiebes J, Nicolaou KC and Baden DG, Chem. Biol, 1995, 2, 533–541. [PubMed: 9383457]
166. Rein KS, Baden DG and Gawley RE, J. Org. Chem, 1994, 59, 2101–2106.
167. Zheng C-J, Yu H-E, Kim E-H and Kim W-G, J. Antibiot, 2008, 61, 633–637.
168. Nicolaou KC, Nilewski C, Hale CRH, Ioannidou HA, ElMarrouni A and Koch LG, Angew. Chem. Int. Ed, 2013, 52, 8736–8741.
169. Nicolaou KC, Liu G, Beabout K, McCurry MD and Shamoo Y, J. Am. Chem. Soc, 2017, 139, 3736–3746. [PubMed: 28256128]
170. Fitzpatrick FA and Wynalda MA, J. Biol. Chem, 1983, 258, 11713–11718. [PubMed: 6578214]
171. Mahmud I, Smith DL, Whyte MA, Nelson JT, Cho D, Tokes LG, Alvarez R and Willis AL, Prostaglandins, Leukotrienes Med., 1984, 16, 131–146.
172. Hegde S, Kaushal N, Ravindra KC, Chiaro C, Hafer KT, Gandhi UH, Thompson JT, van den Heuvel JP, Kennett MJ, Hankey P, Paulson RF and Prabhu KS, Blood, 2011, 118, 6909–6919. [PubMed: 21967980]
173. Nicolaou KC, Heretsch P, ElMarrouni A, Hale CRH, Pulukuri KK, Kudva AK, Narayan V and Prabhu KS, Angew. Chem. Int. Ed, 2014, 53, 10443–10447.
174. Nicolaou KC, Pulukuri KK, Rigol S, Heretsch P, Yu R, Grove CI, Hale CRH, ElMarrouni A, Fetz V, Brönstrup M, Aujay M, Sandoval J and Gavrilyuk J, J. Am. Chem. Soc, 2016, 138, 6550–6560. [PubMed: 27171618]
175. Nicolaou KC, Pulukuri KK, Rigol S, Peitsinis Z, Yu R, Kishigami S, Cen N, Aujay M, Sandoval J, Zepeda N and Gavrilyuk J, J. Org. Chem, 2019, 84, 365–378. [PubMed: 30557504]
176. Nicolaou KC, Pulukuri KK, Rigol S, Peitsinis Z, Yu R, Kishigami S, Cen N, Aujay M, Sandoval J, Zepeda N and Gavrilyuk J, J. Org. Chem, 2019, 84, 2377–2378. [PubMed: 30715889]
177. Sasse F, Steinmetz H, Heil J, Höfle G and Reichenbach H, J. Antibiot, 2000, 53, 879–885.
178. Matcha K and Antonchick AP, Angew. Chem. Int. Ed, 2013, 52, 2082–2086.
179. Khemnar AB and Bhanage BM, Synlett, 2014, 25, 110–114.
180. Chatgililoglu C, Crich D, Komatsu M and Ryu I, Chem. Rev, 1999, 99, 1991–2070. [PubMed: 11849018]
181. Yeung CS and Dong VM, Chem. Rev, 2011, 111, 1215–1292. [PubMed: 21391561]
182. Nicolaou KC, Yin J, Mandal D, Erande RD, Klahn P, Jin M, Aujay M, Sandoval J, Gavrilyuk J and Vourloumis D, J. Am. Chem. Soc, 2016, 138, 1698–1708. [PubMed: 26829208]
183. Nicolaou KC, Erande RD, Yin J, Vourloumis D, Aujay M, Sandoval J, Munneke S and Gavrilyuk J, J. Am. Chem. Soc, 2018, 140, 3690–3711. [PubMed: 29381062]
184. Wang Y, Benz FW, Wu Y, Wang Q, Chen Y, Chen X, Li H, Zhang Y, Zhang R and Yang J, Mol. Pharmacol, 2016, 89, 233–242. [PubMed: 26660762]
185. Jat JL, Paudyal MP, Gao H, Xu Q-L, Yousufuddin M, Devarajan D, Ess DH, Kürti L and Falck JR, Science, 2014, 343, 61–65. [PubMed: 24385626]
186. Jansen R, Irschik H, Reichenbach H, Wray V and Höfle G, Liebigs Ann. Chem, 1994, 1994, 759–773.
187. Nicolaou KC, Bellavance G, Buchman M and Pulukuri KK, J. Am. Chem. Soc, 2017, 139, 15636–15639. [PubMed: 29064682]

188. Nicolaou KC, Buchman M, Bellavance G, Krieger J, Subramanian P and Pulukuri KK, *J. Org. Chem.*, 2018, 83, 12374–12389. [PubMed: 30277774]
189. Davies J, Wang H, Taylor T, Warabi K, Huang X-H and Andersen RJ, *Org. Lett.*, 2005, 7, 5233–5236. [PubMed: 16268546]
190. Nicolaou KC, Zhang H, Chen JS, Crawford JJ and Pasunoori L, *Angew. Chem. Int. Ed.*, 2007, 46, 4704–4707.
191. Nicolaou KC, Chen JS, Zhang H and Montero A, *Angew. Chem. Int. Ed.*, 2008, 47, 185–189.
192. Nicolaou KC, Wang Y, Lu M, Mandal D, Pattanayak MR, Yu R, Shah AA, Chen JS, Zhang H, Crawford JJ, Pasunoori L, Poudel YB, Chowdari NS, Pan C, Nazeer A, Gangwar S, Vite G and Pitsinos EN, *J. Am. Chem. Soc.*, 2016, 138, 8235–8246. [PubMed: 27266267]
193. Poudel YB, Rao C, Kotapati S, Deshpande M, Thevanayagam L, Pan C, Cardarelli J, Chowdari N, Kaspady M, Samikannu R, Kuppusamy P, Saravanakumar P, Arunachalam PN, Deshpande S, Rangan V, Rampulla R, Mathur A, Vite GD and Gangwar S, *Bioorg. Med. Chem. Lett.*, 2020, 30, 126782. [PubMed: 31767265]
194. Nicolaou KC, Das D, Lu Y, Rout S, Pitsinos EN, Lyssikatos J, Schammel A, Sandoval J, Hammond M, Aujay M and Gavrilyuk J, *J. Am. Chem. Soc.*, 2020, 142, DOI: 10.1021/jacs.9b12522.
195. Oku N, Matsunaga S and Fusetani N, *J. Am. Chem. Soc.*, 2003, 125, 2044–2045. [PubMed: 12590521]
196. Nicolaou KC, Lu Z, Li R, Woods JR and Sohn T.-i., *J. Am. Chem. Soc.*, 2015, 137, 8716–8719. [PubMed: 26133230]
197. Nicolaou KC, Li R, Lu Z, Pitsinos EN, Alemany LB, Aujay M, Lee C, Sandoval J and Gavrilyuk J, *J. Am. Chem. Soc.*, 2018, 140, 12120–12136. [PubMed: 30216054]
198. Zhang H, Li R, Ba S, Lu Z, Pitsinos EN, Li T and Nicolaou KC, *J. Am. Chem. Soc.*, 2019, 141, 7842–7852. [PubMed: 31050893]
199. Kolbe H, *Ann. Chem. Pharm.*, 1845, 54, 145–188.
200. Nicolaou KC, Vourloumis D, Winssinger N and Baran PS, *Angew. Chem. Int. Ed.*, 2000, 39, 44–122.
201. Nicolaou KC, Montagnon T and Snyder SA, *Chem. Commun.*, 2003, 551–564.
202. Nicolaou KC, Edmonds DJ and Bulger PG, *Angew. Chem. Int. Ed.*, 2006, 45, 7134–7186.
203. Nicolaou KC and Chen JS, *Chem. Soc. Rev.*, 2009, 38, 2993–3009. [PubMed: 19847336]
204. Nicolaou KC, *Angew. Chem. Int. Ed.*, 2014, 53, 4730.
205. Chen Z, Mercer JAM, Zhu X, Romaniuk JAH, Pfattner R, Cegelski L, Martinez TJ, Burns NZ and Xia Y, *Science*, 2017, 357, 475–479. [PubMed: 28774923]
206. Su JK, Feist JD, Yang J, Mercer JAM, Romaniuk JAH, Chen Z, Cegelski L, Burns NZ and Xia Y, *J. Am. Chem. Soc.*, 2018, 140, 12388–12391. [PubMed: 30229652]
207. Nicolaou KC, Rigol S and Yu R, *CCS Chemistry*, 2019, 1, 3–37.

Fig. 1.

Highlights of KCN's studies in the UK and USA. Predoctoral (**A**), doctoral (**B**), and postdoctoral (**C1** and **C2**). From benzene to 3-chloro-2,5,6-trimethylphenol at Bedford (now Royal Holloway) College London to cyclic bis-diallenes and bis-cumulenes at University College London, and from benzvalene and its derivatives at Columbia University, to erythronolide B, method development (double activation macrolactonisation), and analogues for biology and medicine (9,11-diazo- PGH_2) at Harvard University.

Fig. 2. Highlights of our work on prostacyclin, thromboxanes, and leukotrienes. **A.** Prostacyclin and analogues; **B.** Thromboxane A_2 and analogues; **C.** Leukotrienes A_4 and B_4 and analogues.

Fig. 3.
Highlights of our work on lipoxins. **A.** Lipoxins A₄ and B₄; **B.** Lipoxins A₅ and B₅; **C.** Biogenetic epoxide precursor of lipoxins A₄ and B₄ and its cyclopropyl analogue.

Fig. 4. Select highlights of designed natural product analogues synthesised (1990s–early 2000s). **A.** Calicheamicin γ_1^I , and calicheamicin θ_1^I and its antibody–drug conjugate; **B.** Taxol and analogues.

Fig. 5. Select highlights of designed natural product analogues synthesised (1990s–early 2000s). **A.** Epothilones A and B and analogues; **B.** Sarcodictyins A and B and analogues; **C.** Vancomycin and analogues; **D.** Benzopyran natural products and analogues; **E.** Psammaplin A and analogues.

Fig. 6. Highlights of the abyssomicin C project. **A.** Retrosynthetic analysis; **B.** Total synthesis of abyssomicin C (**60**) and atrop-abyssomicin C (**61**); **C.** Select designed and synthesised abyssomicin C analogues and their potencies against methicillin-resistant *S. aureus* (MRSA).

Fig. 7. Highlights of the biyouyanagins A and B project. **A.** Retrosynthetic analysis; **B.** Total synthesis of biyouyanagins A (**78**), B (**89**) and C (**90**); **C.** Molecular structures and cytotoxicity potencies of select designed and synthesised biyouyanagin analogues.

Fig. 9. Highlights of the platensimycin project. **A.** Retrosynthetic analysis; **B.** Total synthesis of platensimycin; **C.** Totally synthesised related natural products; **D.** Select designed and synthesised platensimycin analogues and their potencies against certain bacterial strains.

Fig. 10.

Highlights of the antibiotic CJ-16,264 project. **A.** Retrosynthetic analysis; **B.** Total synthesis of antibiotic CJ-16,264; **C.** Selected isomers and analogues of antibiotic CJ-16,264 and their potencies against certain bacterial strains.

Fig. 11.
Molecular structures and biological properties of select ladder-like marine neurotoxins and analogues thereof.

Fig. 13.

Highlights of the campaigns towards Δ^{12} -prostaglandin J₃ (PGJ₃) and J₂ (Δ^{12} -PGJ₂) and their analogues. **A.** and **B.** Retrosynthetic analyses I and II; **C.** Optimised synthetic route towards Δ^{12} -PGJ₃; **D.** Metathesis-based total synthesis of Δ^{12} -PGJ₂; **E.** Molecular structures and cytotoxicity potencies of designed and synthesised Δ^{12} -PGJ₂ and Δ^{12} -PGJ₃ analogues.

Fig. 14. Highlights of the *N*¹⁴-desacetoxytubulysin H project. **A.** Retrosynthetic analysis; **B.** Total synthesis of *N*¹⁴-desacetoxytubulysin H; **C.** Molecular structures and cytotoxicity potencies of select designed and synthesised tubulysin analogues.

Fig. 15.

Highlights of the thailanstatin A project. **A.** Retrosynthetic analysis; **B.** Total synthesis of thailanstatin A; **C.** Molecular structures and cytotoxicity potencies of select designed and synthesised thailanstatin A analogues.

Fig. 16.

Select designed synthesised aziridinyl epothilone B analogues.

Fig. 17.

Disorazoles A₁ and B₁ and select synthesised designed analogues.

Fig. 18.

Highlights of the uncialamycin project. **A.** Retrosynthetic analysis; **B.** Total synthesis of uncialamycin; **C.** Molecular structures and cytotoxicity potencies of select designed and synthesised uncialamycin analogues.

Fig. 19. Highlights of the shishijimicin A project. **A.** Retrosynthetic analysis; **B.** Total synthesis of shishijimicin A; **C.** Molecular structures and cytotoxicity potencies of select designed and synthesised shishijimicin A analogues.

Fig. 20.
Evolution of the impact of total synthesis for science and society.