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Covalent modification of biological targets with natural products through Paal–Knorr pyrrole formation

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Abstract

Natural products and endogenous metabolites engage specific targets within tissues and cells through complex mechanisms. This review examines the extent to which natural systems have adopted the Paal–Knorr reaction to engage nucleophilic amine groups within biological targets. Current understanding of this mode of reactivity is limited by only a few examples of this reaction in a biological context. This highlight is intended to stimulate the scientific community to identify potential research directions and applications of the Paal–Knorr reaction in native and engineered biological systems.

1 Introduction

1.1 Modification of biological molecules with chemical agents

In nature, proteins are often post-translationally modified at reactive functional groups within their side chains.[1] Common modifications, such as acylation, phosphorylation and alkylation, often play critical roles through engaging signal transduction cascades and discerning between different states of activity. These modifications play quintessential roles in nearly all facets of life. It is no surprise that secondary metabolites have been evolved to specifically target residues that undergo posttranslational modification.

Having been extensively reviewed,[2] many of the key players in the chemical biological modification of proteins include Michael addition and acylation or alkylation of residues such as Cys, His, Lys and Ser. Here one can impart molecular specificity through stereochemical and/or reactivity based control. Akin to reaction design, the chemical biologist is now experiencing the advance of tools like the Staudinger ligation[3] or Huisgen-based click chemistry,[4] which can be modulated to fit one's exact biological needs. The same is true for other types of facile chemical processes such as thiol–ene,[5] Diels–Alder,[6] or photo-click chemistry.[7] These reactions have played a fundamental role in bridging the gap between chemical and biological studies.

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Lessons learned from understanding this reactivity have enabled the development of chemical biological methods, such as activity-based protein profiling (ABPP).[8] They have also engaged new tools for medicinal chemistry[9] and shed new light on the complex modes and mechanisms of action of natural products.[10]

As our knowledge expands, one begins to wonder what percentage of the chemists' toolbox of name reactions bear homologs that have been advanced through complex evolutionary processes and endowed natural products with intriguing modes of reactivity. In this review, we examine the scope in which natural systems use 1,4-dicarbonyl moieties to deliver pyrrole products through the Paal–Knorr reaction.[11]

1.2 The Paal–Knorr reaction

Pyrroles are a common subunit in natural products and synthetic compounds.[12] Among many methods utilized to construct this heterocyclic ring, the classical Paal–Knorr pyrrole synthesis, reported independently by Paal[13] and Knorr[14] in 1884, stands out as a single-step operation often carried out without advanced reagents. The reaction involves the condensation of a primary amine with 1,4-dicarbonyl **1** in the presence of an acid catalyst to give pyrrole **5** through the intermediary hemiaminals **2**, **3** and **4** (Scheme 1).

As the Paal–Knorr reaction proceeds under mild conditions often at room temperature with the use of a mild acid, it is the method of choice for sensitive functionality. This has been well documented through a variety of synthetic applications, including the total synthesis of natural products, as illustrated by the Trost–Doherty synthesis of roseophilin (**6** to **7**, Scheme 2).[15]

In addition, the Paal–Knorr reaction has been applied to large-scale processes. One example is in the commercial ton-quantity preparation of an anti-cholesterolemic drug, atorvastatin (Lipitor), as illustrated by the condensation of diketone **8** and amine **9** to afford pyrrole **10** (Scheme 3).[16]

1.3 The role of the Paal–Knorr reaction in the neurotoxicity of hexane

The Paal–Knorr reaction is believed to be involved in *n*-hexane-induced axonal atrophy in the central nervous system.[17] Research over the past 30 years points to 2,5-hexanedione (Scheme 4) as a neurotoxic *n*-hexane metabolite. Experiments show that 2,5-hexanedione undergoes a selective Paal–Knorr reaction with lysine residues of axonal cytoskeleton proteins, forming 2,5-dimethylpyrrole adducts within specific regions of neurofilaments (Scheme 4).[18] This pyrrole-forming reaction is now considered to be the first step in 2,5-hexanedione-induced neuropathy. Indeed, derivatives with substituents that accelerate pyrrole formation, such as 3,4-dimethyl-2,5-hexanedione are even more potent neurotoxicants.[17] In contrast, 3,3-dimethyl-2,5-hexanedione, which cannot form a pyrrole, is non-neurotoxic.[17]

Additional studies show that after pyrrole formation, the resulting pyrrole adducts undergo progressive oxidative protein crosslinking, which can be inter- or intramolecular, depending on the adduct site.[18] These could be minimized in the absence of air or the presence of antioxidants.[19] It is believed that the conversion of positively charged lysine residues to

neutral hydrophobic pyrroles should lead to changes in many neurofilament characteristics, such as water solubility, electrostatic potential and/or three-dimensional structure. These chemically modified neurofilament proteins should not be capable of proper interaction with the cytoskeletal network, leading to the development of axonal atrophy.[17]

2 Paal–Knorr modifications of biological targets with natural products

2.1 Ophiobolin sesterterpenes

Ophiobolins are a group of sesterterpenoid fungal metabolites produced by phytopathogenic fungi that attack rice, maize and sorghum and produce brown spot lesions.[20] These characteristic lesions can also be produced by applying drops containing ophiobolins on the leaves of plants demonstrating the role of these metabolites in disease progression. Ophiobolin A (Fig. 1) was the first member of this family of over 25 metabolites, isolated independently by teams led by Orsenigo[21] and Nakamura.[22]

Although most of the early research on the biological activity of ophiobolins was aimed at their toxicity to plants, recently a number of these sesterterpenes have been investigated as potential anticancer leads. In particular, ophiobolin A (Fig. 1) has been shown to induce a type of cell death known as paraptosis in glioblastoma cells.[23] Paraptosis is a recently discovered form of non-apoptotic cell death, which is distinguished from apoptosis by the absence of apoptotic morphology, DNA fragmentation or caspase activation.[24] Glioblastoma cells are characterized by resistance to apoptosis, which is largely responsible for the low effectiveness of classical chemotherapeutic approaches, most of which are based on apoptosis induction. Thus, ophiobolin A is an agent with a novel mode of action (MOA) capable of overcoming apoptosis resistance in glioblastoma.[23]

A key biologically active element in ophiobolins is the C5, C21-dicarbonyl functionality that is critical for antitumour activity (Fig. 1). Indeed, natural ophiobolins and synthetic analogues that are devoid of this moiety are inactive, such as **11** (Fig. 1). In contrast, changes in the tetrahydrofuran and C18, C19-olefin fragments, such as those found in ophiobolin K or synthetic analogue **12** (Fig. 1), are tolerated.[25,26]

While conducting structure–activity relationship (SAR) studies through the derivatization of ophiobolin A, our group found that this natural product undergoes a smooth Paal–Knorr reaction with primary amines such as benzylamine to form pyrrole adduct **13** (Scheme 5). The reaction takes place at room temperature in the presence of a mild acid catalyst. This led us to propose that ophiobolin A undergoes Paal–Knorr pyrrole formation with a primary amino group on its intracellular target, such as a Lys residue, as illustrated by **14** (Scheme 5). Although the reaction was less efficient with *N*-α-acetyl-L-lysine in phosphate buffer at pH 7.4, our proposed explanation was based on the idea that in a relatively hydrophobic pocket of its target containing an H-bond donating residue the conditions would be more similar to those used for the preparation of **13**.[26]

This initial hypothesis was confirmed by a team led by O'Shea, who found that ophiobolin A reacts with the primary amino group of phosphatidylethanolamine (PE) to form a pyrrole-containing covalent adduct, **15** (Scheme 6).[27] To detect the formation of the pyrrole, they

utilized phospholipase D (PLD) to release the modified ethanolamine head group through hydrolysing the synthetically prepared PE-ophiobolin A covalent adduct **15** to give **16**. They followed this through PLD-mediated digestion of the total cellular phospholipid from HEK293T and HCT-116 cells treated with ophiobolin A and detected **16** with LC-MS/MS. The investigators proposed that this covalent adduct leads to cell death through the destabilization of cellular lipid bilayers. In support of this idea, they used artificial liposomes with a variable content of PE and showed that ophiobolin A induces membrane leakiness. They were able to directly correlate this effect with the PE content.

2.2 Sesquiterpene 1,4-dialdehydes

Polygodial (Fig. 2) and many related bicyclic sesquiterpenes containing an unsaturated 1,4-dialdehyde functionality are pungent components of plants often used as spices in various culinary traditions.[28] Early biological investigations of these dialdehydes focused on their hot taste and antifeedant activity, both of which were theorized to arise from their covalent interaction with taste receptors. Importantly, the biological properties of sesquiterpene 1,4-dialdehydes appeared to depend on the configuration of the aldehyde group at the C9 position.[29] Thus, polygodial tastes hot to the human tongue and possesses potent antifeedant activity as demonstrated in fish and African armyworms. In contrast, 9-epipolygodial is tasteless and devoid of antifeedant activity.[29] Similarly, saccalutal is hot tasting, while 9-episaccalutal is tasteless.[29]

A team led by Sodano proposed that these natural products undergo the Paal–Knorr pyrrole formation with an amino group of a taste receptor to yield adducts such as **17** and **18** (Scheme 7). They proposed that the ability to form the pyrrole ring depends on the configuration at C9 or, more precisely, the distance between the two carbonyls.[30] Indeed, ^{1}H NMR monitoring of the reaction with methylamine revealed that polygodial, displaying a shorter distance between its carbonyls, was reactive, whereas 9-epipolygodial with a longer distance was not.[30]

Another instructive example arises from synthetic analogues of polygodial (Scheme 8).[30] Here, the change of stereochemistry at the ring junction places the distance between the nitrogen of the Schiff base and the carbonyl in hypothetical intermediate **20a** at 2.3 A, whereas this distance in the corresponding intermediate **20b** that would result from C9-epimeric **19b** is 3.2 A. As a result, isomer **19a** displayed Paal–Knorr reactivity similar to that of polygodial and is hot tasting, whereas **19b** was unreactive toward methylamine under biomimetic conditions and tasteless.

Because the produced pyrroles are unstable and not isolable, the Paal–Knorr reactions of sesquiterpene 1,4-dialdehydes have been studied primarily through ^{1}H NMR monitoring. Thus, in the above example, dialdehydes **19a** and **19b** dissolved in CD_3CN were added to a solution of Na_2DPO_4 in D_2O and a ^{1}H NMR spectrum was taken 10 minutes after the addition of CH_3NH_2. In the reaction of **19a**, the observed signals were consistent with the presence of **21a** and **23** in a 1:3 ratio with *ca.* 50% of the starting materials remaining unreacted. After 2 h, the spectrum contained signals only due to **23**. In contrast, **19b** was found to be unchanged even 2 days after the addition of methylamine.[30]

Recent studies aimed at the identification of intracellular receptors for these sesquiterpenes revealed that polygodial, in a manner similar to the active component of chili peppers capsaicin, targets the transient receptor potential vanilloid 1 receptor (TRPV1),[31] a temperature sensitive ion channel with a preference for Ca^{2+} ions. Unfortunately, the manner in which polygodial inhibits TRPV1 and whether covalent pyrrole adducts are involved remains to be elucidated.[32]

Encouraged by the fact that TRPV1 is also up-regulated by human cancer cells and thus appears to be a new target for cancer therapy, our laboratories initiated studies of polygodial and its analogues as potential anti-tumour agents.[32,33] To obtain firm evidence of Paal–Knorr reactivity in these compounds, we attempted to isolate such pyrrole adducts (Scheme 9). Our proposed explanation for the lack of stability of the produced pyrroles was their electron-rich character and, thus, high propensity toward oxidation. Indeed, the increase in stability was observed when the nitrogen substituent was changed from benzyl in **24a** to phenyl in **24b**. Finally, **24c**, in which the nitrogen substituent was a highly electron-withdrawing *p*-nitrophenyl, was a stable isolable pyrrole-containing compound that was fully characterized.[32]

Our recent studies also led to the discovery of α,β-unsaturated polygodial derivatives, such as **25** (Scheme 10). These agents are capable of overcoming drug resistance in glioblastoma, melanoma and other cancer cells serving as models for tumours typically associated with dismal prognoses.[33] It is noteworthy that such derivatives have a pyrrole-forming reactivity of their own, producing stable adducts, such as **28** *via* **26** and **27**, when reacted with primary amines. Efforts are now underway to understand the role of this chemistry in cancer and identify their protein targets.

2.3 Rearranged spongian diterpenes

Rearranged spongian diterpenes are a group of diverse marine natural products derived from skeletal rearrangements and oxidations of a spongian diterpene precursor resulting in the production of, for example, aplyviolene and macfarlandin E (structures shown in Scheme 11).[34] These terpenes are isolated from sponges and dorid nudibranchs, the latter of which appear to acquire these molecules from sponges as part of their chemical defence mechanism. Among the different types of biological activities displayed by these natural products, a team led by Overman showed that macfarlandin E induced a novel Golgi morphological phenotype characterized by ribbon fragmentation and maintenance of the resulting Golgi apparatus fragments in the pericentriolar region (Fig. 3) as well as the blockage of protein transport from the Golgi apparatus to plasma membranes.[35,36] This activity was retained by the structurally simplified analogue **30**, indicating that the dioxabicyclic lactone fragment is the key part of the pharmacophore.

Further studies demonstrated that simplified analogues **29** and **30** undergo the Paal–Knorr reaction when treated with benzylamine (Scheme 11).[36] Specifically, it was shown that **29** (R = H) produced pyrrole **33** in high yield in its reaction with 5 equivalents of benzylamine in aq. DMSO, while **30** (R = OAc) yielded pyrrole **36** when treated with 2.5 equivalents of perdeutero-benzylamine in THF-d_8. It is clear that under these conditions both Paal–Knorr reactions proceed through dialdehydes **31** and **32**. The formation of **36** from **30**, in which an

amino group replaced the acetate, can be explained by a Gramine-type rearrangement involving intermediate **35**.

These investigators further showed that both **29** and **30** undergo pyrrole formation with Lys residues using hen egg white lysozyme (HEWL) as a model protein (Fig. 4a).[36] Using ESI-MS analysis, they observed an increase in *m*/*z* of 164 in phosphate buffer (pH 7) when treated with **29**, clearly corresponding to the product of a pyrrole-forming reaction of one lysine residue with **29** (Fig. 4c).

Treatment of HEWL protein with **30**, which proved to be more effective, led to an adduct with +180 *m*/*z*, evidently incorporating a hydroxyl at the benzylic position (Fig. 4b). The modified lysozymes were subjected to trypsin digestion followed by MALDI-MS/MS, revealing covalent modification of the most surface-accessible Lys residues, K33 and K97.[36] This investigation provided evidence for the Paal–Knorr modification of an as yet elusive target. They also demonstrated that pro-drug like motifs can be used to trigger Paal–Knorr reactivity and associated cellular activity.

3 Paal–Knorr modifications of biological targets with endogenous metabolites

The next section explores the role which the Paal–Knorr reaction plays in natural biological processes. The first example focuses on a family of phospholipid esters called isolevuglandins. The second study examines the role of the Paal–Knorr reaction in the activity of dopamine-derived dicatecholaldehydes. Here, we outline recent evidence that indicates that the Paal–Knorr reaction occurs naturally within mammalian systems.

3.1 Isolevuglandins

The isolevuglandins (isoLGs) are a family of phospholipid esters incorporating a γ-ketoaldehyde functionality. They are generated through the free radical oxidation of arachidonyl phospholipids, such as 1-palmitoyl-2-arachidonyl-phosphatidylcholine (PAPC, Scheme 12).[37] For example, isoPGH$_2$-PC and iso[4]PGH$_2$-PC, endoperoxide intermediates, spontaneously rearrange to isoLGE$_2$, containing a prostanoid side chain, and iso[4]LGE$_2$, incorporating a non-prostanoid side chain. IsoLGs are highly reactive intermediates and have never been detected *in vivo*. Presumably, they react covalently with primary amines within seconds,[38] for example with ethanol-amine phospholipids and/or Lys residues in proteins.[39] Indeed, they have orders of magnitude higher reactivity in forming covalent adducts than other lipid peroxidation products, such as malondialdehyde.[40]

There is considerable evidence that their covalent reactions with primary amines produce pyrroles in the Paal–Knorr manner, and often undergo oxidative dimerization forming crosslinks (*e.g.* **39** and **40** in Scheme 13). For example, exposure of large concentrations of isoLGs to ovalbumin was demonstrated to produce protein–protein crosslinks.[41] In addition, DNA–protein crosslinking was also observed when cells in culture were treated with isoLGs. This reaction occurred more readily than that with other oxidative lipid-derived electrophiles, such as malondialdehyde.[42]

These pyrrole-forming reactions have important biological consequences. Thus, preferential adduction of isoLGs to Cyp27A1 at a specific lysine residue blocks its ability to metabolize cholesterol leading to its accumulation and resulting in retinal pathology.[43] In the human eye, iso-LG-modified calpain-1 accumulates in the trabecular meshwork and loses its enzymatic activity.[40] Also, protein crosslinking by iso-LGs may interfere with aqueous outflow through the trabecular meshwork within the eye resulting in increased intraocular pressure in primary open angle glaucoma.[40] There is also evidence that a subfamily of iso-LGs produced through the cyclooxygenase pathway, such as LGE_2 and LGD_2, may promote aggregation of the β-amyloid peptide and contribute to Alzheimer's disease.[40]

The reaction of iso[4]LGE_2 with *N*-Ac-Gly-Lys-OMe was studied using MALDI-TOF spectroscopy by a team led by Salomon.[40] The crude reaction product mixture produced ions at *m*/*z* 576, 592, 608, 853 and 1149, which were attributed to protonated pyrrole, lactam, hydroxylactam, aminal crosslink and bispyrrole, respectively, as well as adducts resulting from the loss of water from these products. The separation of this mixture using reverse phase HPLC with LC-ESI monitoring gave fractions corresponding to lactam, aminal crosslink and bispyrrole eluting in the order of decreasing polarity.

A MALDI-TOF MS spectrum of the bispyrrole-containing HPLC fraction is shown in Fig. 5. In addition to the bispyrrole **43** observed as *m*/*z* 1149, the other prominent peaks at 1147 and 1145, derived from the loss of 2H and 4H, presumably corresponded to the mono- and diketo derivatives **42** and **41** formed through the oxidation of allylic hydroxyl groups. Additional peaks likely resulted from further oxidations of the bispyrrole; the amounts of these products were higher when the reaction was conducted over 15 days compared with 4 days. These and additional studies by the Salomon laboratory provided experimental evidence for the formation IsoLG-derived pyrrole–pyrrole crosslinks and demonstrated that the pyrrole–pyrrole adducts can undergo further oxidations to result in polyoxygenated crosslinked species.

3.2 Dopamine-derived dicatecholaldehyde

Parkinson's disease is characterized by the debilitating loss of motor control that is associated with the death of dopaminergic neurons in the substantia nigra and the appearance of proteinaceous aggregates called Lewy bodies, primarily composed of the neuronal protein α-synuclein.[44] The cause of Parkinson's disease is generally unknown and has led to significant research effort and vigorous debate.[44] The "catecholaldehyde hypothesis" suggests the involvement of an endogenous toxin that is unique to dopamine-producing cells.[45] Of these, 3,4-dihydroxyphenylacetaldehyde (DOPAL, Fig. 6), an intermediate in dopamine metabolism, appears to be the likeliest source of toxicity. Extensive experimental evidence of DOPAL's involvement in Parkinson's disease suggests its ability to covalently crosslink α-synuclein and play a role in Parkinson's etiology.[46]

In a recent study, a team led by Levine and Bax reacted DOPAL with Ac-α-synuclein and characterized the reaction products with LC-MS.[47] To the investigators' surprise, instead of the expected increase in mass of +134 Da due to the Schiff base formation between DOPAL and a lysine residue,[48] the modified protein showed a clear and homogeneous molecular ion peak corresponding to an increase in mass of +266. Because α-synuclein contains 15 lysine

residues, they continued their study of this unexpected covalent modification in a simplified system with *N*-Ac-Lys. In a manner similar to the experiments with the protein, they observed a steady accumulation of a product with an *m*/*z* +266 mass increase over *N*-Ac-Lys. The NMR investigation of the obtained product DCPL (Fig. 6) led to the elucidation of its structure (Scheme 14), incorporating a pyrrole ring that clearly formed from two molecules of DOPAL and one *N*-Ac-Lys. Reaction monitoring using NMR showed that the concentration of DCPL increased linearly over the first 2.5 h and reached a maximum value after 5 h. This was followed by a plateau in DCPL formation, presumably resulting from its further reactions, such as oxidative degradation and further reactions with DOPAL, among other possibilities.[47]

The obtained mechanistic data were consistent with two possible reaction pathways. In one of them (path A, Scheme 14), it is the oxidation of DOPAL itself into quinone methide **44** that allows for the Michael addition of another molecule of DOPAL to give Paal–Knorr precursor dialdehyde **45**. In the other possible pathway (path B, Scheme 14), the initially formed imine **46** undergoes oxidation to quinone methide **47**, which turns the molecule into a Michael acceptor that reacts with another molecule of DOPAL to produce Paal–Knorr precursor **48**. The two pathways converge on intermediate **48**, which ultimately terminates in the formation of DCPL.

Overall, the study described the formation of DCPL, containing electron-rich pyrrole and catechol rings. The presence of these groups suggests the potential to undergo further oxidation and polymerization, and therein provides a mechanistic explanation of α-synuclein oligomerization in dopaminergic neurons.

4 Outlook

As the development of Chemical Biology advances into new reaction types, we learn how relevant the understanding of the intersection between chemical reactions and biological experimentation is. For one, we realize that tools observed in the flask often have direct correlations with applications in biological systems. Heralding efforts involving the advance of name reactions, such as Huisgen or Diels–Alder cycloadditions, into click chemistry,[49] offer a foundation for the discovery and advance of new chemical biological tools.

The above examples demonstrate the occurance of the Paal–Knorr reaction in biological systems with important implications in mammalian metabolism and toxicology. It’s utility arises from the lack of requirement for special catalysts or reagents. It is a highly facile process, often requiring only modest pH adjustment to operate at room temperature. Furthermore, it is no surprise that natural products such as ophibiolin A, aplyviolene, macfarlandin E or polygodial utilize such reactivity to engage specific target proteins. While the diversity and selectivity of this reaction is not yet defined, we look forward to the expansion of Paal–Knorr agents.

Recently, we demonstrated the potential for these Paal–Knorr agents through the development of an immunoaffinity fluorescent (IAF) probe, **51**, from ophibiolin A.[50] As shown in Scheme 15, we were able to append an alkyne linker in **49** and use it to attach a

IAF azide tag, **50**, to prepare the corresponding IAF-labelled probe **51** in two steps. We then used this probe to demonstrate a facile means to image natural products using STORM microscopy.

As depicted in Fig. 7, one can see that the application of these materials for STORM imaging (Fig. 7c) provided a profoundly improved resolution over conventional methods (Fig. 7b). In the image in Fig. 7c, probe **51** was localized in thick filamentous structures, corresponding to the endoplasmic reticulum (ER). This study demonstrated the potential for further conjugation of Paal–Knorr reagents and begins to suggest a facile means to deliver covalently reactive probes for cellular applications.

It is our hope that the studies of the Paal–Knorr reactions of natural products with biological targets described herein will stimulate the scientific community to identify potential research directions and applications of the Paal–Knorr labelling method. Indeed, the Paal–Knorr reaction is a facile, selective and highly viable tool ready to be advanced into the chemical biologists' toolkit.

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Biographies

Alexander Kornienko was born in the small Arctic town of Vorkuta, Russia in 1971. He studied at Mendeleev University in Moscow and then moved to the United States, where he received his Ph.D. in synthetic organic chemistry at Tufts University. After a two-year postdoctoral fellowship at the University of Montreal, working on the synthesis of novel aminoglycoside antibiotics, he started his independent career at New Mexico Tech in 2001 and then moved to Texas State University in 2012, where he is now Professor of Chemistry. Throughout his career he has published 88 refereed research papers primarily dealing with natural product-based cancer drug discovery.

James J. La Clair was born in the Summer of Love, July 1967. He received his Ph.D. in Chemistry from SUNY Buffalo under the tutelage of Prof. Peter T. Lansbury. His studies continued through a postdoctoral fellowship with Prof. Gilbert Stork at Columbia University. He then undertook a position at the Scripps Research Institute. In 1999, he went independent in order to focus his time on research. This has included research positions at UC San Diego and the Salk Institute, among other collaborative endeavours including the Xenobe Research

Institute. To date, he has authored over 99 peer-reviewed publications, primarily focused on key aspects of the structure, function and preparation of natural products.

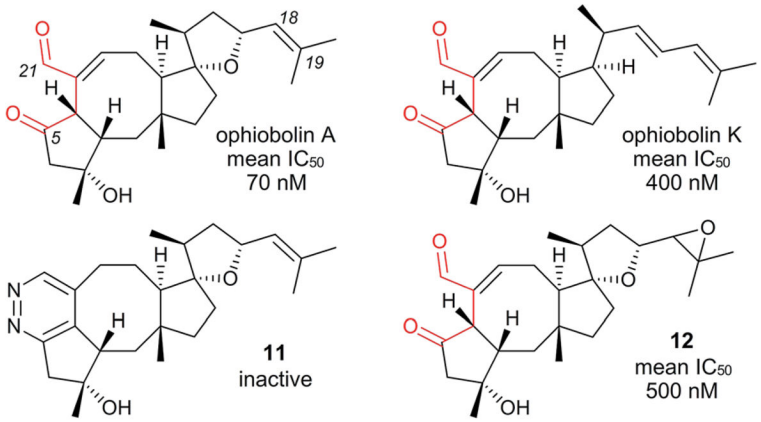


Fig. 1.

The criticality of C5, C21-dicarbonyl for the antitumour activity of natural ophiobolins and their synthetic analogues.

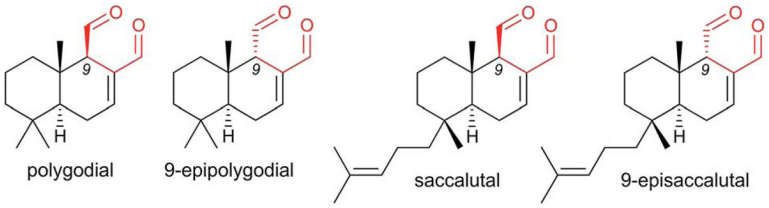


Fig. 2.
Chemical structures of exemplary sesquiterpene 1,4-dialdehydes.

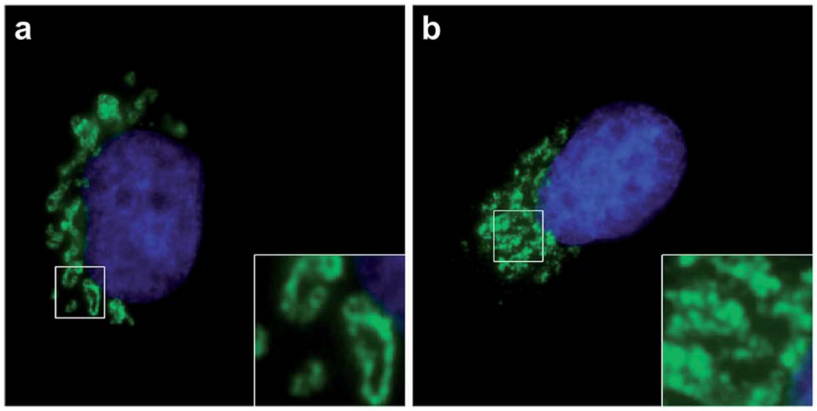


Fig. 3.

Golgi-apparatus-modifying properties of macfarlandin E. NRK cells were treated with (a) control (DMSO) or (b) 45 μM macfarlandin E for 1 h at 37 °C. After treatment, the cells were fixed and stained for the Golgi (green) using a fluorescently labeled antibody against man-nosidase II (green) and the nucleus (blue) with Hoechst 33342 (blue). The areas in the white boxes are enlarged (insets) to show the details of Golgi organization following each treatment.

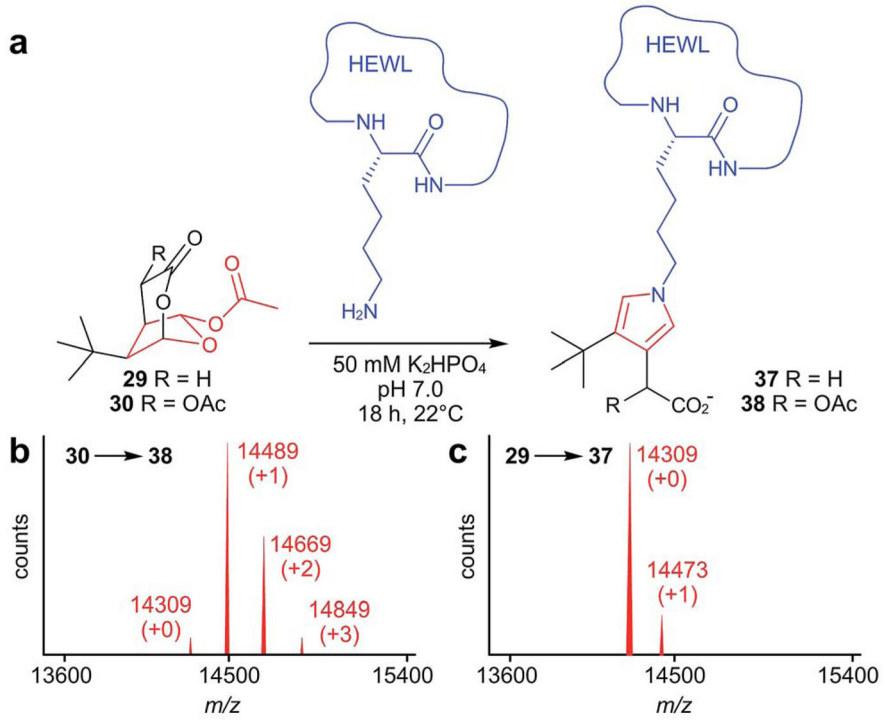


Fig. 4.

Protein labelling. (a) Modification of HEWL protein with **29** or **30** led to the formation of pyrrole adducts **37** and **38**, respectively. ESI-MS spectra reconstructed from charge ladders denoting the distribution of alkylated products obtained from (b) **30** and (c) **29**.

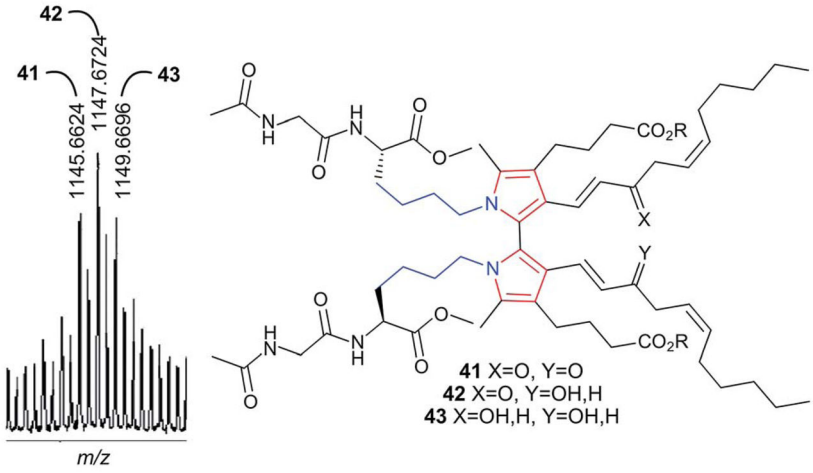


Fig. 5.

MALDI-TOF MS spectrum of an HPLC fraction containing bispyrrole products **41–43** from the reaction of *N*-acetyl-glycyl-lysine methyl ester with iso[4]LGE$_2$ with air.

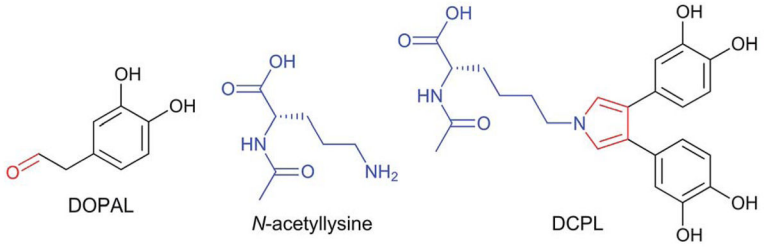


Fig. 6.
Structures of DOPAL, *N*-acetyllysine (*N*-Ac-Lys) and DCPL.

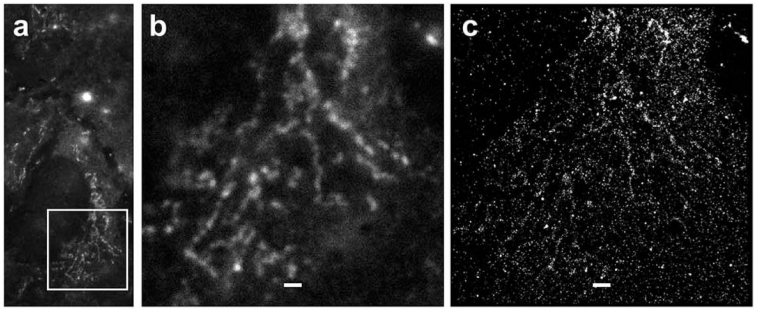
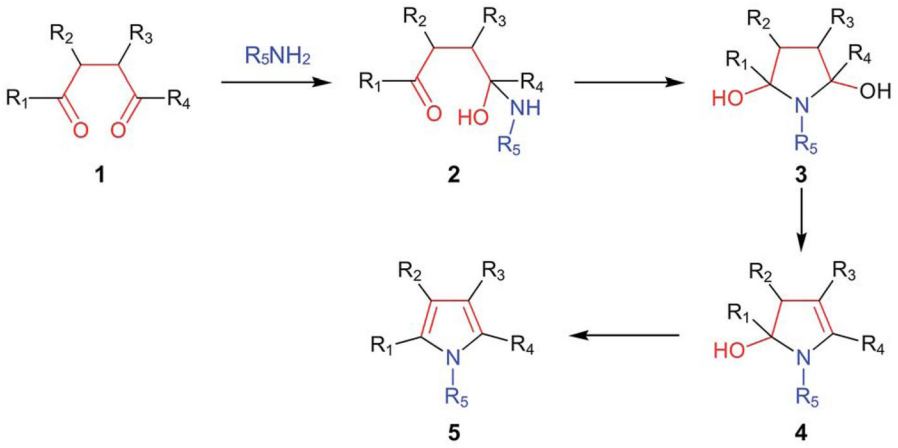


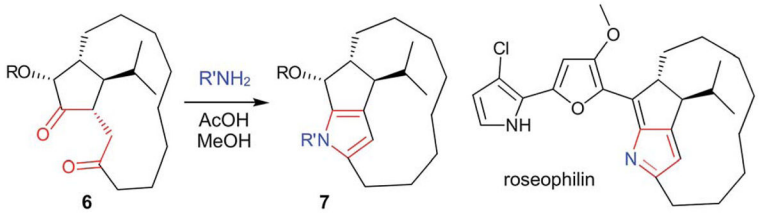
Fig. 7.

Super-resolution imaging of ophiobolin probe **51**. U2OS cells cultured in an 18 mm plate at 10^6 cells per cm^2 were treated with a 5 μL drop of 50 μM **51** and incubated for 1 h, fixed and stained with 80 μM Alexa 647-conjugated anti-IAF TF35 mAb for STORM imaging. (a) An image depicting the area of interest as shown by a white box. (b) An epifluorescence image within the box in (a). (c) A STORM image obtained from the same region in (b). Scale bars denote 10 μm.



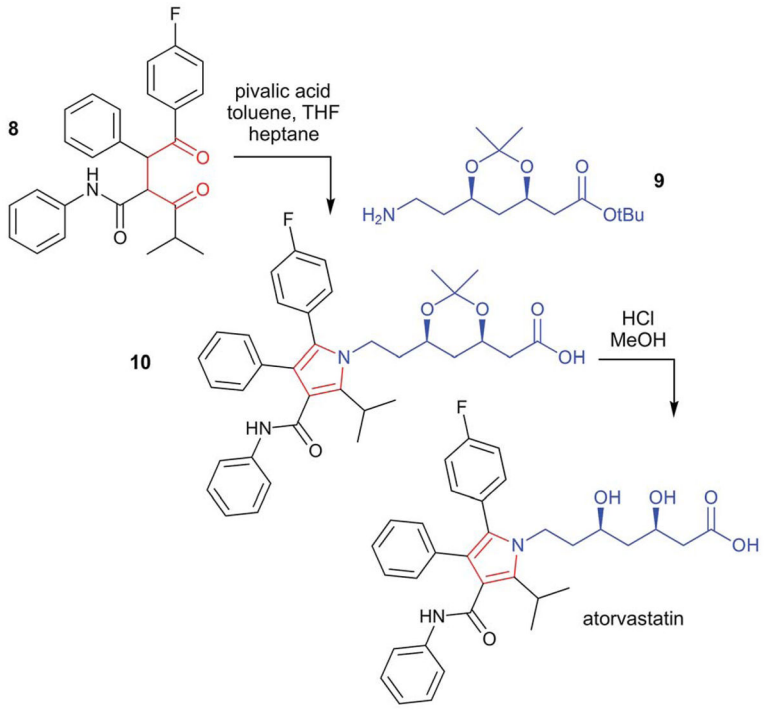
Scheme 1.

The mechanism of the Paal–Knorr reaction. Diketone **1** (red) condenses with an amine (blue) to generate hemiaminals **2–4**. Facile double dehydration of **4** affords aromatic pyrrole **5**.



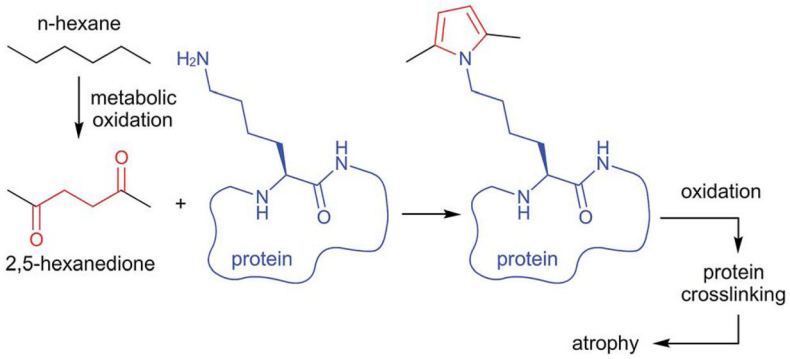
Scheme 2.

Trost–Doherty's application of the Paal–Knorr reaction in the synthesis of roseophilin. The diketone and amine groups are coloured in red and blue, respectively.



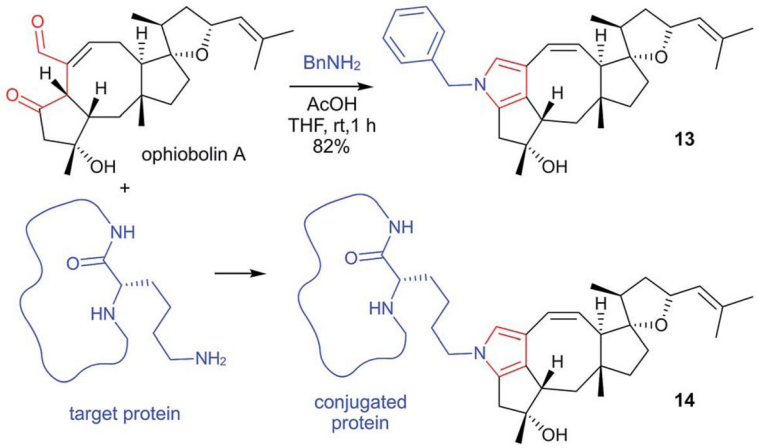
Scheme 3.

Commercial synthesis of atorvastatin (Lipitor). The Paal–Knorr reaction plays a central role in the condensation of diketone **8** with amine **9** to afford pyrrole **10**. Atorvastatin is then delivered through acetal deprotection.



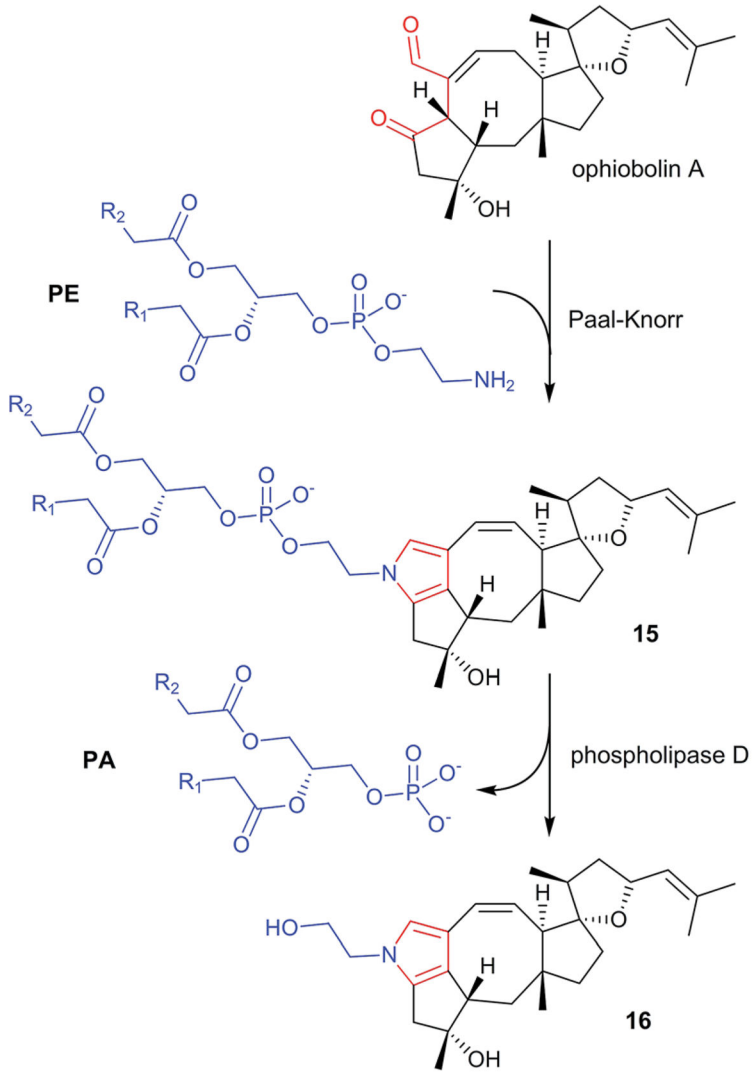
Scheme 4.

Metabolic conversion of *n*-hexane to 2,5-hexanedione enables Paal–Knorr condensations to occur at lysine residues within proteins resulting in the corresponding 2,5-dimethylpyrrole-conjugated proteins. In neuronal cells, further oxidation and protein cross-linking lead to axonal atrophy.



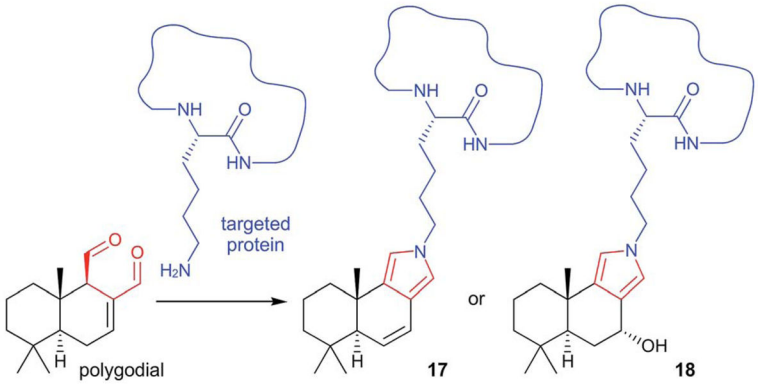
Scheme 5.

Proposed Paal–Knorr modification of target proteins by ophiobolin A and chemical demonstration of its feasibility.



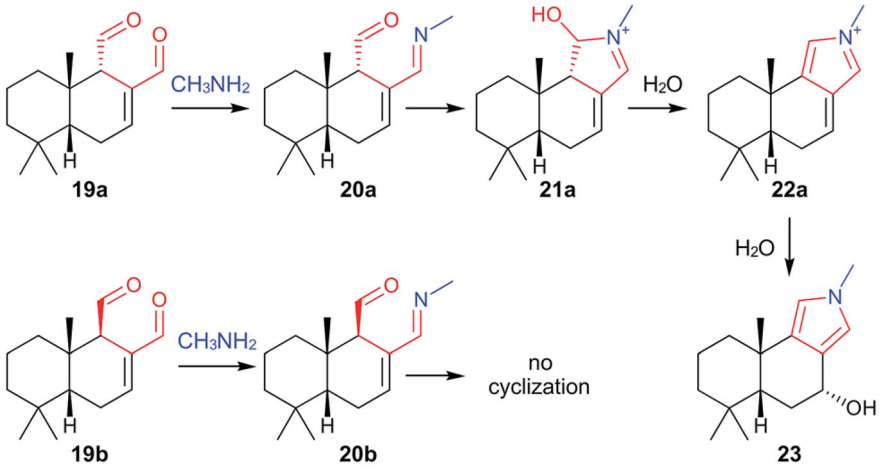
Scheme 6.

Reaction of ophiobolin A with PE and cleavage of the covalent adduct **15** with phospholipase (PLD) to produce pyrrole **16**.



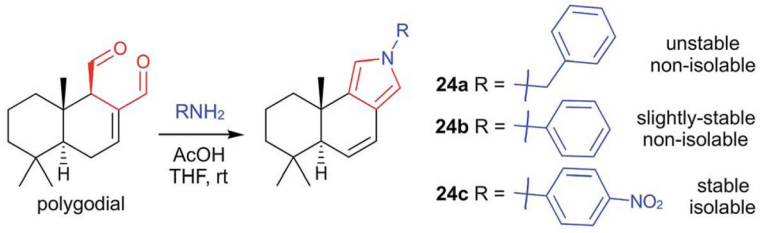
Scheme 7.

Proposed Paal–Knorr reaction on polygodial results in two possible pyrrole products, **17** and **18**.



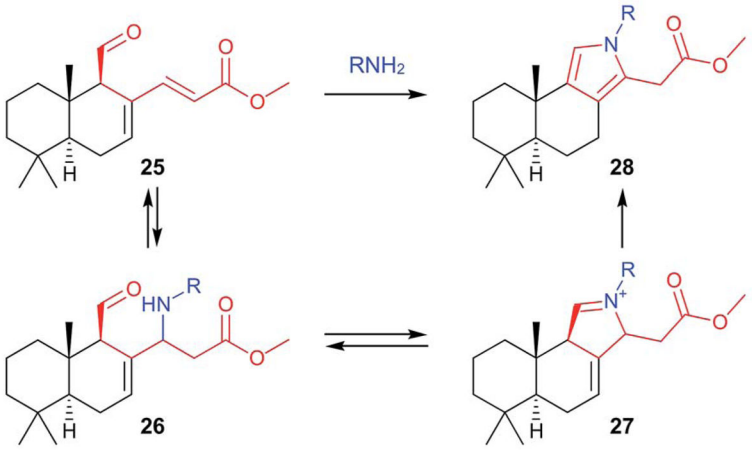
Scheme 8.

Addition of methylamine to *cis*-fused decalin analogue **19a** results in the facile conversion to **23** *via* **20a–22a**. Comparable reactivity with the other aldehyde isomer **19b** did not lead to the observed products.



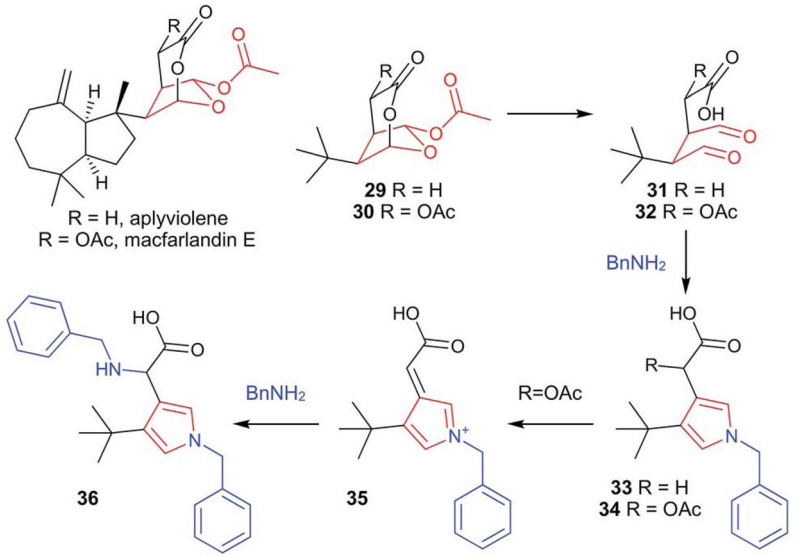
Scheme 9.

Exploration of the Paal–Knorr reaction on polygodial illustrates that the stability of the resulting pyrrole adducts can be increased by using electron-withdrawing substituents on the nitrogen. The use of *p*-nitrophenylamine led to stable and isolable product **24c**.



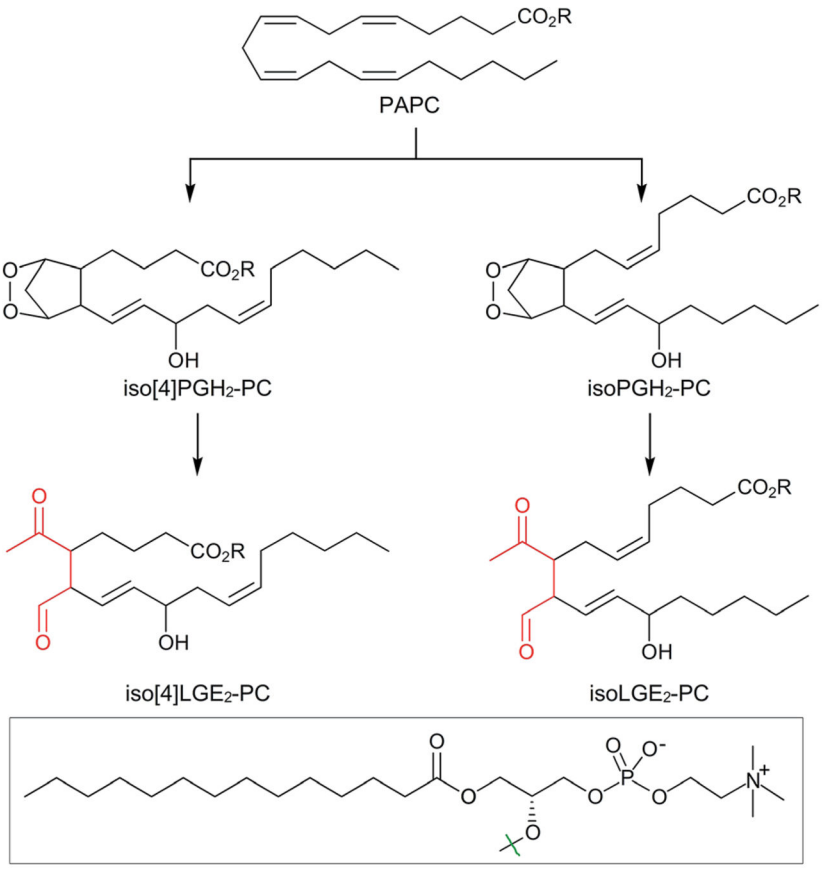
Scheme 10.

Recent studies have shown that comparable trapping can also occur between an α,β-unsaturated ester and an aldehyde.



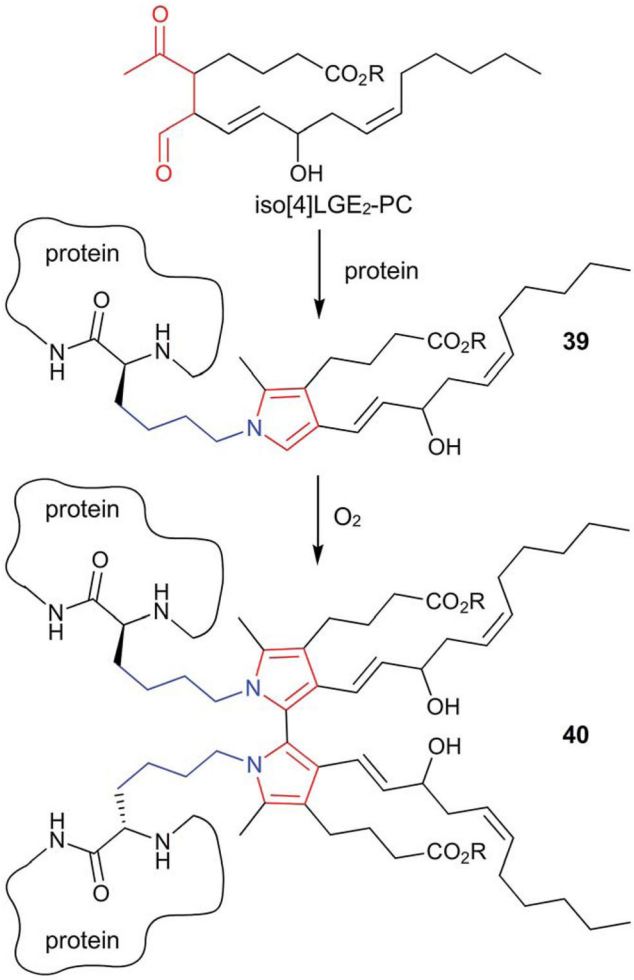
Scheme 11.

Structures of aplyviolene and macfarlandin E and their analogues **29–30**. Treatment of **29** with benzylamine leads to the formation of pyrrole **33**. In the case of **30**, pyrrole **34** loses acetate to give **35**, which in turn undergoes a second addition of benzylamine to afford pyrrole **36**.



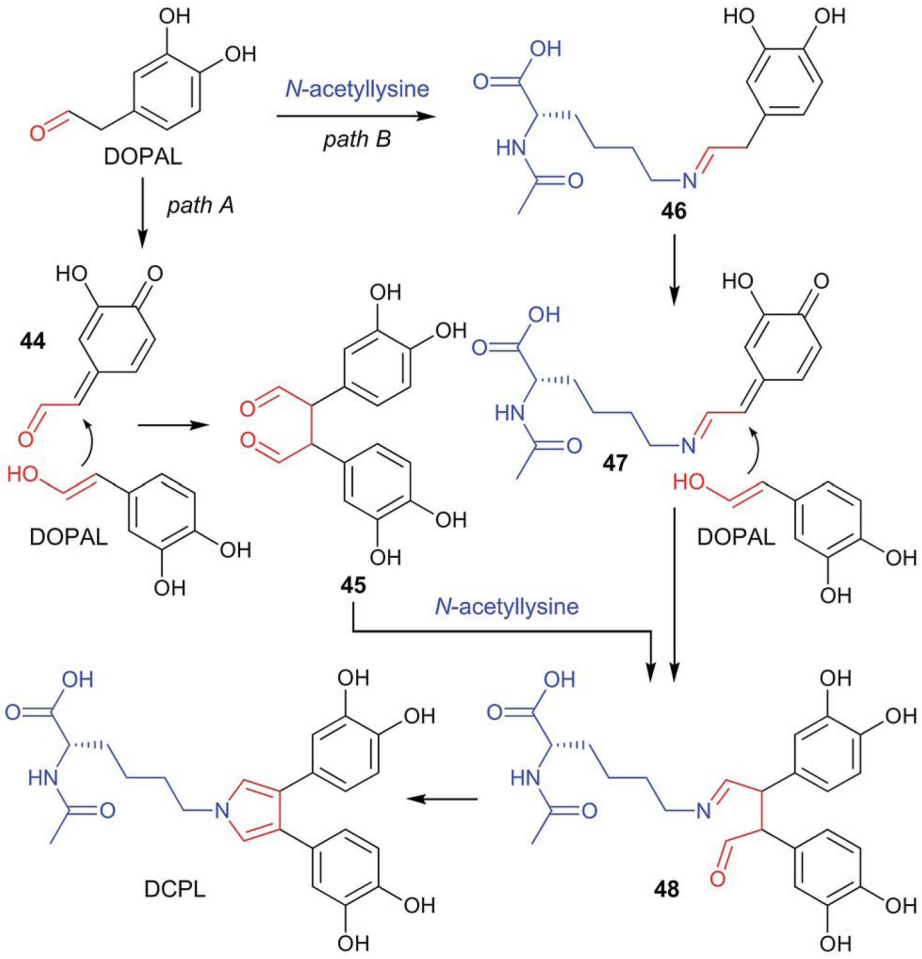
Scheme 12.

Structure of 1-palmitoyl-2-arachidonyl-phosphatidyl-choline (PAPC) and its conversion to iso[4]LGE_2-PC and isoLGE$_2$-PC. The R group is depicted in the box with the point of connection denoted by the wavy line (green).



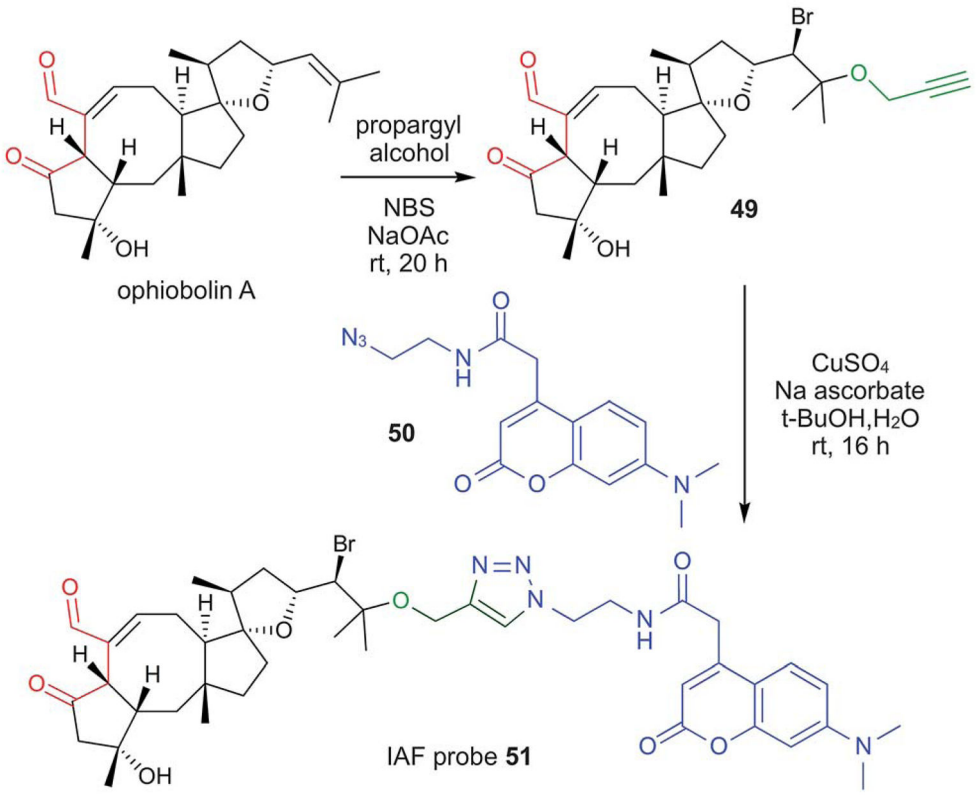
Scheme 13.

Reaction of iso[4]LGE_2-PC with lysine residues within proteins leads to Paal–Knorr pyrrole adduct **39**, which dimerizes to **40** upon oxidation with air.



Scheme 14.

Proposed mechanisms for the formation DCPL from the reaction of DOPAL with *N*-acetyllysine. The first option, A, arises through the oxidation of DOPAL to **44** followed by dimerization to afford dialdehyde **45**. Subsequent Paal–Knorr reaction with *N*-acetyllysine delivers DCPL. The second option, B, occurs through sequential formation of imine **46** from DOPAL and *N*-acetyllysine, followed by the corresponding imine-based Paal–Knorr reaction *via* **48**.



Scheme 15.

A schematic representation of the development of an immunoaffinity fluorescent (IAF) probe **51** from ophiobolin A.