Nitroso Diels-Alder (NDA) Reaction as an Efficient Tool For the Functionalization of Diene-Containing Natural Products

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Nitroso Diels-Alder (NDA) Reaction as an Efficient Tool for the Functionalization of Diene-Containing Natural Products

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Natural products, nitroso Diels-Alder, 2-nitrosopyridine, drug discovery, chemical diversity.

Abstract

This review describes the use of nitroso Diels-Alder reactions for the functionalization of complex diene-containing natural products in order to generate libraries of compounds with potential biological activity. The application of this methodology to the structural modification of a series of natural products (thebaine, steroidal dienes, rapamycin, leucomycin, colchicine, isocolchicine and piperine) is discussed using relevant examples from the literature from 1973 onwards. The biological activity of the resulting compounds is also discussed. Additional comments are provided that evaluate the methodology as a useful tool in organic, bioorganic and medicinal chemistry.

1. Introduction.

Chemical substances derived from animals, plants and microbes have been used to treat human diseases since the dawn of medicine.[1-8] Natural products research, especially that of secondary metabolites derived from plants and microorganisms, has provided molecules that have revolutionized medicine and improved the quality of life. Relevant examples are antibiotics (e.g. penicillin, cephalosporin C, tetracycline, erythromycin, streptomycin and vancomycin), antiparasitics (e.g. avermectin), antimalarials (e.g. quinine and artemisin), lipid control agents (e.g. lovostatin and analogs), immunosuppressants for organ transplants (e.g. cyclosporine, FK-506, rapamycin) and anticancer drugs (e.g. taxol, doxorubicin). Furthermore, the World Health Organization (WHO) has estimated that plant-derived traditional medicines still play an important role in the healthcare of 65% of world's population.[9]

The fundamental role that nature plays as a source of lead compounds for drug discovery has been well documented in the literature.[10-13] Newman and Cragg published a series of three reviews in which they analyzed the sources of drugs approved for the treatment of human diseases.[14-16] Their study highlights the dominant role that natural products have played in drug discovery, especially in the areas of cancer and infectious diseases. In the area of cancer therapeutics alone, over the timeframe from 1940 to 2007, out of 155 small molecules, 73% were other than synthetic with 47% being actually either natural products or directly derived therefrom.[16] Thirteen natural product related drugs were approved from 2005 to 2007, five of which represented the first members of new classes of drugs.[17] In 2002, Proudfoot reported that 8 out of 29 small molecule drugs launched in 2000 were derived from natural products or hormones.[18]

Despite the positive results, during the past 15 years, research in natural products has been declining[19-22] and the pharmaceutical industry has focused increasing resources on other drug discovery methods and, in particular, on high throughput screening (HTS) of combinatorial chemistry libraries.[23-24] The rapid generation of libraries consisting of millions of synthetic compounds via combinatorial chemistry, followed by their screening by HTS, was expected to result in the rapid and cost-efficient discovery of several new drugs for all therapeutic areas with a consequent increase in profits. However, the expected surge in productivity has not materialized

and the output of newly launched drugs has fallen.[25-27] The hit rate of combinatorial libraries proved to be particularly low:[28] the number of new active substances (NAS), also known as New Chemical Entities (NCEs) hit a 20 year low of 37 in 2001 and it is still declining. Moreover, the relatively low hit rate of these libraries is often accompanied by a higher potential for side effects, since the structures of these molecules are relatively simple.

For these reasons, renewed interest has been directed to natural product research.[29] Medicinal chemistry research has shifted to the synthesis of libraries of compounds based on natural product-like scaffolds followed by the screening against a variety of different receptors. DTS (Diverted Total Synthesis)[30-34] and DOS (Diversity Oriented Synthesis)[35-39] are two examples of this approach to drug discovery. Large compound libraries based on natural product cores have been developed using combinatorial techniques.[40-41]

Natural products have been defined as "privileged structures"[42-43] in terms of their ability to be useful templates for the syntheses of novel biologically active molecules. Their complex three dimensional structure and stereochemical pattern[44] have been evolutionarily selected by nature to interact with proteins (enzymes or receptors) or other macromolecules such as nucleic acids and carbohydrates.[45-46] Moreover, since natural products are often produced and hosted in a living organism, their "pharmacoviability" or "drug-likeness" is greater than that of synthetic molecules.[46]

On the other hand, it is true that natural products did not undergo evolutionary selection to serve as human therapeutics. Therefore, they often do not possess the potency, selectivity, and pharmacokinetic traits which are desired in clinically useful drugs. As a consequence, the elaboration or simplification of natural product structures is often required in order to tailor and enhance their potency, selectivity and pharmacokinetic properties. However, since complex natural products often contain multiple functional groups with similar reactivity, many transformations that might be used for optimization cannot be accomplished due to selectivity issues and lack of compatibility with other functional groups.[46] Numerous examples of functionalization of natural products are reported in the literature and often they are limited and do not result in the generation of evolvable libraries of compounds for structure-activity relationship studies.

The nitroso Diels-Alder (NDA) reaction has been used as a powerful synthetic tool in the formation of heterocycles by direct incorporation of a 1,4-amino-oxo group.[47-49] Moreover, the mild reaction conditions, atom economy, absence of by-products and compatibility with a large variety of functional groups make NDA a powerful tool for the functionalization of diene-containing natural products. In particular, the NDA reaction introduces an oxazine moiety which not only rigidifies the structure of the natural product, altering its conformation, but also constitutes a handle for further functionalization (e.g. reductive cleavage of the N-O bond and/or further functionalization of the alkene or allylic functionalities).

The aim of this review is to display the potential of the nitroso Diels-Alder reaction as a methodology for the selective functionalization of complex diene-containing natural products, under mild conditions. A selection of representative natural products is presented and their elaboration through nitroso Diels-Alder chemistry is discussed using examples selected from the literature that have been published in the period of time from 1973 to 2011.

The presentation order emphasizes the evolution from early studies, which demonstrated the generation and transient nature of acyl nitroso moieties, to the testing of the ability of nitroso agents to be used chemically in the presence of sensitive functionalities and the practical applications, as well as scope and limitations, related to non-traditional diversification of complex natural products. Brief background information is given for each natural product along with a critical summary of changes in activity induced by the nitroso derivatization strategy, and in some cases, subsequent elaboration of the nitroso adducts.

2. Thebaine

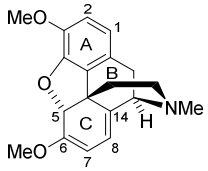


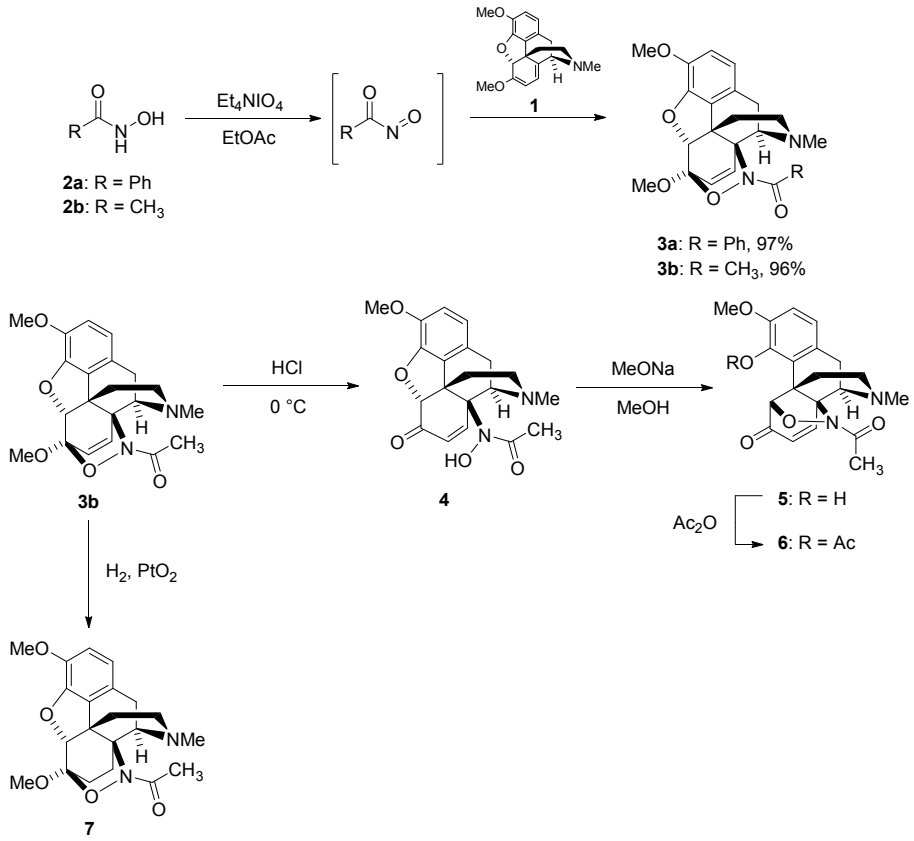
Fig. 1 Thebaine.

Thebaine is a metabolite of morphine that is found in small quantities in opium extracts and it has often been used as the starting material in the syntheses of opium alkaloids. Because of its toxicity it cannot be used as an

analgesic itself,[50] therefore numerous SAR studies have been reported and efforts have been directed toward the syntheses of related molecules with favorable analgesic activity but less side effects, especially addiction.

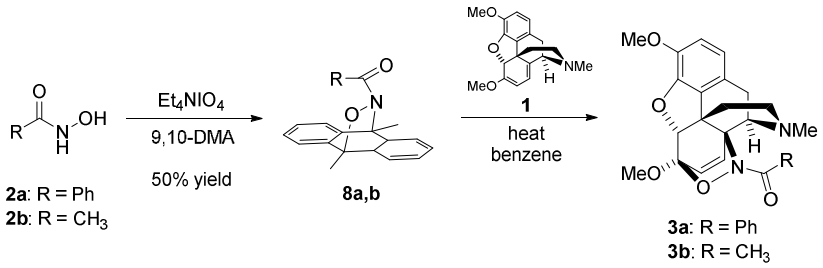
One characteristic of the thebaine skeleton is the presence of a conjugated diene in the C ring. Since it has been demonstrated that β-functionalization of the C14 position can lead to compounds with enhanced analgesic activity, several groups used this structural feature as a handle for the functionalization of the carbon skeleton through a Diels-Alder cycloaddition reaction with different dienophiles.[51-78] The early introduction of a two carbon bridge across the C ring and the resulting enhancement of the structure rigidity was envisaged to modify the interactions of the molecule with its receptors and therefore influence its biological effects. Moreover, the cycloadducts would be amenable to further functionalization. An important contribution to the field came from Bentley and co-workers who studied the cycloaddition of thebaine with a variety dienophiles and observed that the dienophile addition occurred exclusively at the less hindered *β*-face of the natural product to afford 6,14-*endo*-etheno derivatives.[51-56] The resulting compounds were then further functionalized to give derivatives with interesting biological activities.[69-78]

The first example of functionalization of the thebaine skeleton using a nitroso Diels-Alder reaction dates back to 1973 when Kirby used it as a trap for acyl nitroso compounds that were generated *in situ* through the oxidation of hydroxamic acids or by the thermal dissociation of their 9,10-dimethylanthracene (9,10-DMA) cycloadducts.[79] The aim of these studies was to furnish evidence for the existence of *C*-nitrosocarbonyl compounds which are fleeting intermediates[80] that cannot be isolated but nevertheless display high dienophilic character. The separate oxidation of hydroxamic acids **2a** and **2b** in the presence of thebaine afforded Diels-Alder cycloadducts **3a** and **3b** in 97% and 96% yield, respectively (**Scheme 1**). Further functionalization of compound **3b** was then performed in order to confirm its structure. Acid catalyzed hydrolysis at 0 °C afforded enone **4** which, upon treatment with sodium methoxide in methanol, cyclized to give phenol **5**. Catalytic hydrogenation of **3b** over PtO_2 gave the corresponding 7,8-dihydro-derivative **7**.



Scheme 1. Nitroso Diels-Alder reaction of thebaine with acyl nitroso compounds.

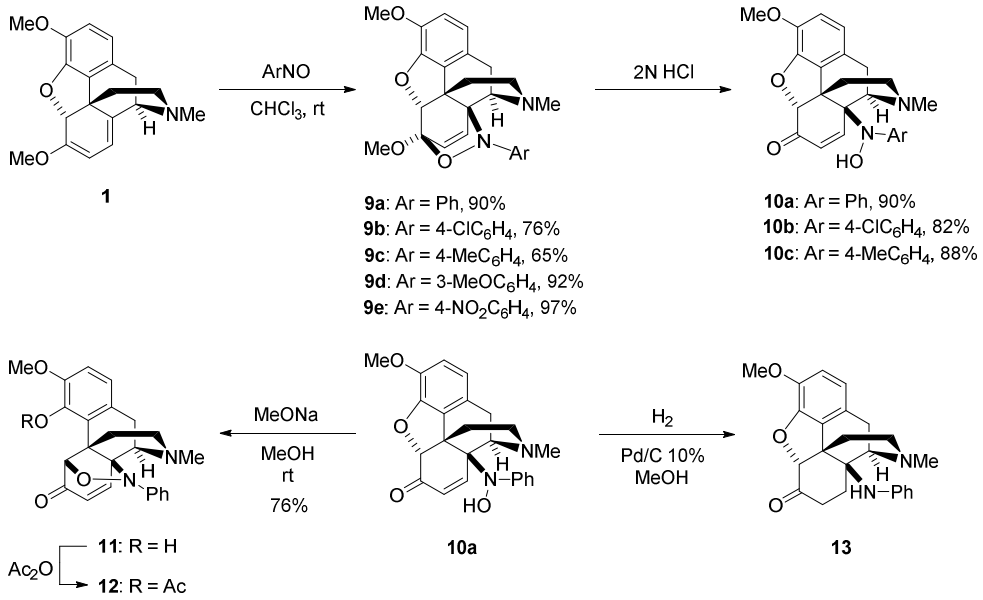
In order to obtain further evidence for the existence of the fleeting intermediates nitrosocarbonylmethane and nitrosocarbonylbenzene, cycloadducts **8a** and **8b** were prepared by the oxidation of hydroxamic acids **2a** and **2b** in the presence of 9,10-dimethylanthracene (9,10-DMA). Treatment of these cycloadducts with thebaine in hot benzene induced a retro Diels-Alder reaction and intermolecular transfer of the nitrosocarbonyl species to give compounds **3a** and **3b** (**Scheme 2**).



Scheme 2. Intermolecular transfer of acyl nitroso compounds to thebaine.

Further studies by Kirby *et al*. involved the reaction of thebaine with nitrosoarenes.[81] Treatment of thebaine **1** with a series of substituted nitrosobenzenes in chloroform at room temperature afforded the corresponding cycloadducts **9a-e** in moderate to high yields (**Scheme 3**). Products **9a-c** where then hydrolyzed under acidic

conditions to afford enones **10a-c**. Enone **10a** (Ar = Ph) was then further elaborated in order to confirm its structure. These early studies not only demonstrated the compatibility of nitroso cycloaddition chemistry with natural products, but also that the initial adducts could be further manipulated. The results indicated that nitroso cycloadducts could be very versatile evolvable scaffolds. However, the biological activity of the resulting compounds was not investigated.



Scheme 3. Nitroso Diels-Alder reaction of thebaine with nitrosoarenes.

The cycloadducts of thebaine with certain nitrosoarenes were found to undergo to retro Diels-Alder reaction at room temperature and the rate of dissociation depended on the electronic nature and the position of the substituent on the benzene ring. In particular, the presence of an electron-withdrawing substituent in the position *para* to the nitroso group slowed down the reaction while an electron-donating group increased its rate. The percentages of dissociation of the different cycloadducts as a 0.5 M solution in $CDCl_3$ at 35 °C were found to be as follows: Ar = 4-NO_2Ph (0), 4-ClPh (0), 3-MeOPh (0), Ph (10), 3-MePh (15), 4-MePh (35), 4-MeOPh (45), 4-Me_2NPh (100). These results suggested potential limits to the scope of nitroso cycloadditions for natural product scaffold modification.

In 1997, elaborating on his previous NDA reaction, Kirby *et al*. reported the syntheses of the acylamino and alkylamino codeinones **14** and **15** which were of particular interest because of their potent analgesic activity.[82] For example, 14*β*-pentylaminomorphinone (pentamorphone, **Fig. 2**) has proved to be an effective analgesic in

humans with tolerable side effects in a dose range of 0.12-0.24 μg/Kg.[83a] In addition, in the mouse hot-plate test, pentamorphone showed 1872 times the potency of morphine and 4 times that of fentanyl.[83b]

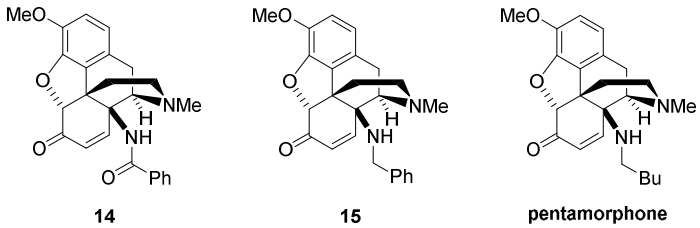
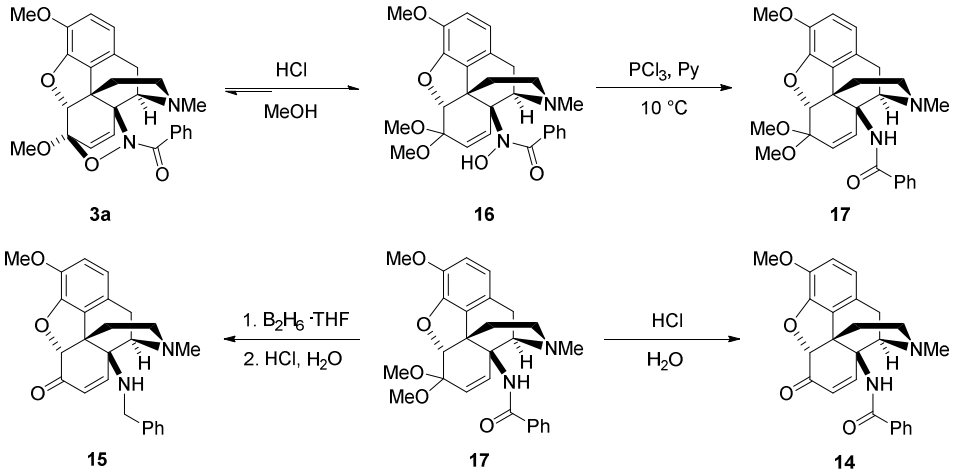


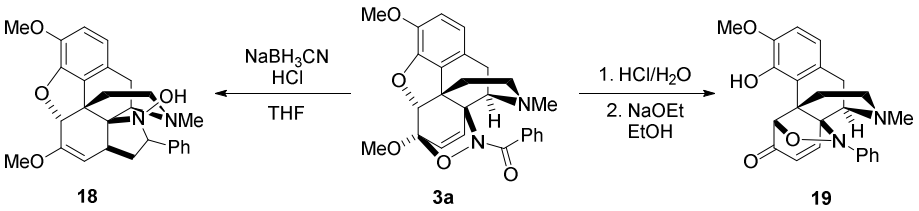
Fig. 2 Acylamino and alkylamino codeinones.

The Diels-Alder reaction of thebaine with the acyl nitroso compound generated *in situ* by the oxidation of *N*-hydroxybenzamide, more commonly referred to as benzohydroxamic acid, afforded cycloadduct **3a** which was elaborated to a series of compounds with potential analgesic activity (**Scheme 4**). Treatment of **3a** with dry methanolic hydrogen chloride at 0 °C gave dimethyl ketal **16**. Deoxygenation of compound **16** was induced upon its treatment with phosphorus trichloride in pyridine and hydrolysis of the resulting compound **17** with methanolic hydrochloric acid afforded codeinone **14**. Reduction of the amide group in compound **17**, followed by acidic hydrolysis, afforded cycloadduct **15**.



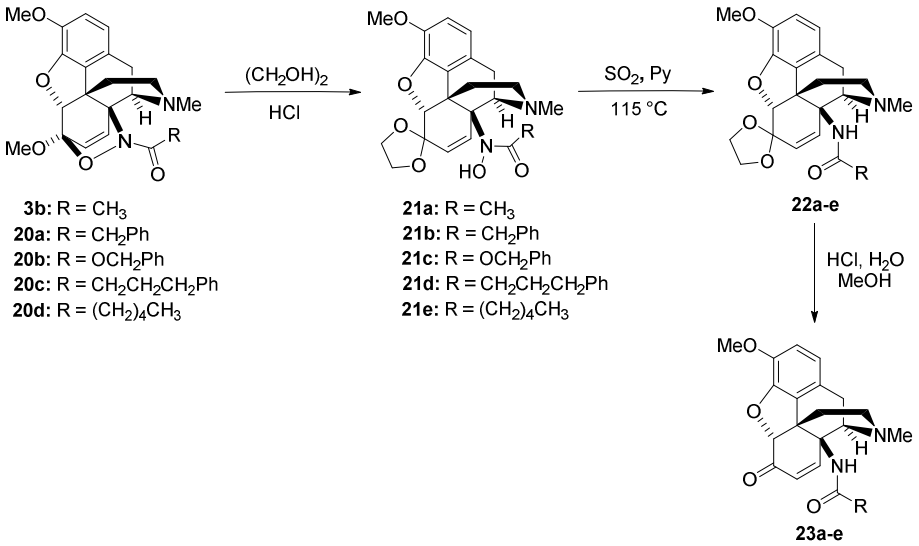
Scheme 4. Syntheses of acylamino and alkylamino codeinones **14** and **15**.

Cycloadduct **3a** was also elaborated into the bridged compounds **18** and **19** (**Scheme 5**). Treatment of **3a** with $NaBH_3CN$, under acidic conditions, afforded compound **18** while acidic hydrolysis of **3a**, followed by cyclization under basic conditions, afforded bridged compound **19** instead.



Scheme 5. Synthetic elaboration of cycloadduct **3a**.

The syntheses of cycloadducts **23a-e** (**Scheme 6**), with R groups other than the benzyl group, required a modification of the synthetic route. In particular, a cyclic acetal was used instead of the dimethyl acetal and the deoxygenation reaction was performed with a modified procedure, using SO_2 in pyridine.



3b: R = CH_3
20a: R = CH_2Ph
20b: R = OCH_2Ph
20c: R = $CH_2CH_2CH_2Ph$
20d: R = $(CH_2)_4CH_3$

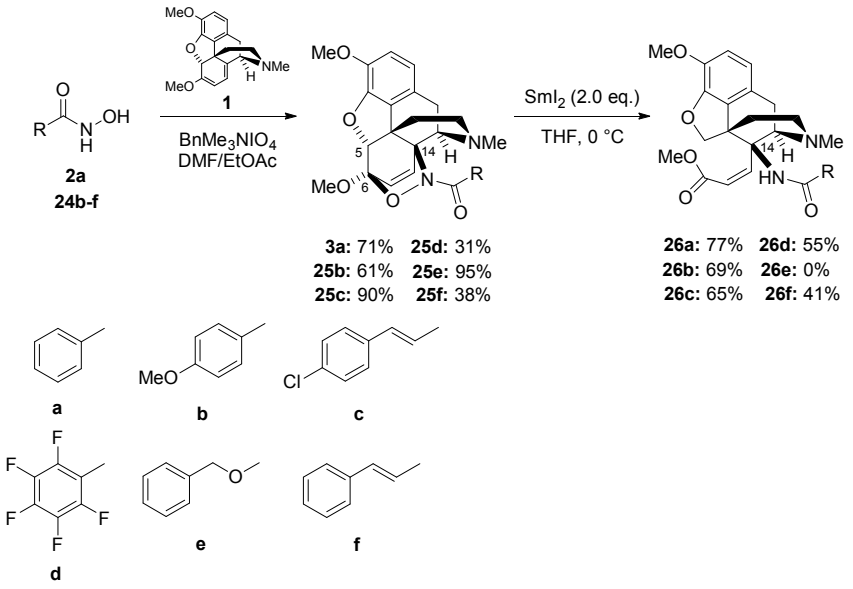
21a: R = CH_3
21b: R = CH_2Ph
21c: R = OCH_2Ph
21d: R = $CH_2CH_2CH_2Ph$
21e: R = $(CH_2)_4CH_3$

22a-e

23a-e

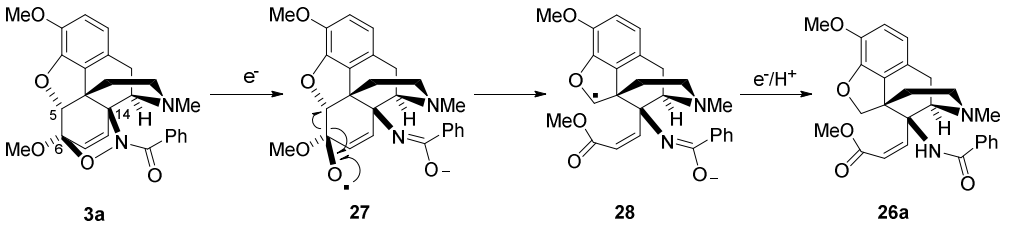
Scheme 6. Syntheses of acylamino codeinones **23a-e**.

In 2006, Sheldrake *et al*. reported a novel functionalization of the NDA-derivative of the thebaine skeleton in which the C ring was opened.[84] The Diels-Alder reactions of thebaine **1** with a series of acyl nitroso dienophiles, generated *in situ* by the oxidation of hydroxamic acids **2a** and **24b-f**, afforded cycloadducts **3a** and **25b-f** in moderate to good yields (**Scheme 7**). Treatment of these cycloadducts with two equivalents of samarium(II) iodide afforded a series of unexpected products **26a-f**, in which the C5-C6 bond was cleaved. This allylic transformation was possible only when the amide carbonyl group was conjugated to an aromatic ring either directly or vinylogously. In the absence of conjugation, the ring cleavage product was not observed.



Scheme 7. Formation of unexpected products from the reaction of NDA cycloadducts of thebaine with samarium(II) iodide.

The mechanism which was hypothesized for the reaction is shown in **Scheme 8**. An electron transfer from SmI_2 to cycloadduct **3a** would give a radical anion **27** which would undergo a second electron transfer, followed by protonation, to give the observed product **26a**.

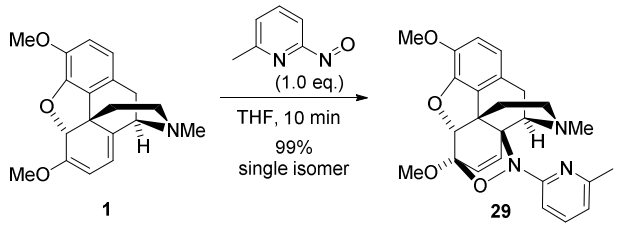


Scheme 8. Proposed mechanism for the reaction of cycloadduct **3a** with SmI_2.

These studies of thebaine derivatives (**Schemes 4**-**7**) again demonstrate the evolvable nature of initial nitroso cycloadducts.

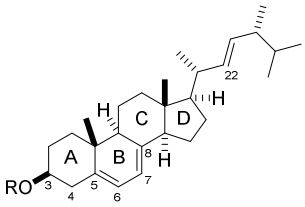
In 2007, Miller *et al*. reported a remarkably efficient functionalization of a series of diene-containing natural products using a hetero Diels-Alder reaction with pyridylnitroso compounds as dienophiles.[85] Substituted 2-nitrosopyridines were the dienophiles of choice since they display the ideal combination of reactivity and stability compared to that of aryl and acyl nitroso species. The Diels-Alder reaction of thebaine with 6-methyl-2-nitrosopyridine in THF afforded cycloadduct **29** in 99% yield, as a single isomer (**Scheme 9**). The configuration of this compound was assigned through X-ray diffraction analysis. The regio- and

stereoselectivity of the reaction was consistent with previous studies on the cycloaddition of thebaine with symmetrical dienophiles.[86] The biological activity of cycloadduct **29** was not investigated.



Scheme 9. NDA reaction of thebaine with 6-methyl-2-nitrosopyridine.

3. Steroidal dienes



30a R = H, Ergosterol
30b R = Ac, Ergosterol acetate

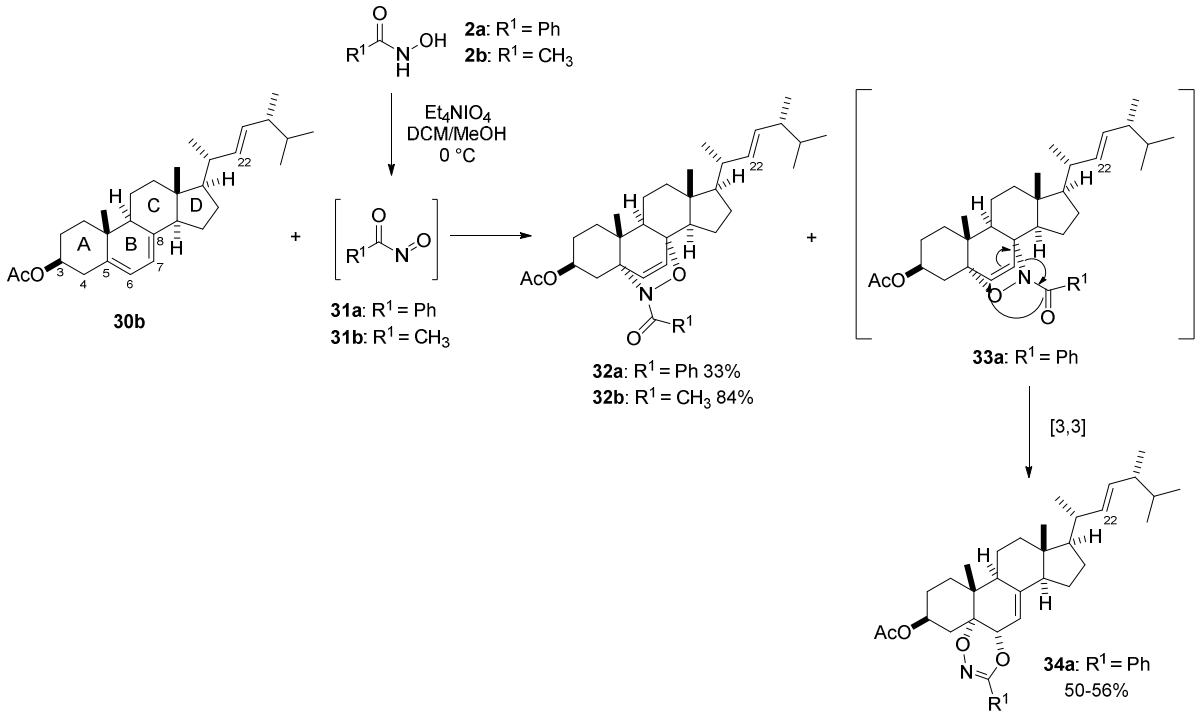
Fig. 3 Ergosterol and ergosterol acetate.

Ergosterol (ergosta-5,7,22-trien-3-β-ol) **30a** (**Fig. 3**) is a component of yeast and fungal cell membranes and it is biologically relevant as a precursor of vitamin D_2.[87] This natural product has been used over the years as a template for the design of new molecules with a variety of biological activities.[88-89] Numerous reports have appeared in the literature about the syntheses of steroidal compounds with a modified carbon skeleton in which one or more carbon atoms have been replaced by heteroatoms. The resulting modified molecules often displayed new interesting biological profiles. For example, azasteroids show a range of activities such as inhibition of cholesterol biosynthesis, antifertility activity and 5α-reductase inhibition.[90-95]

A structural feature of ergosterol is the presence of a diene moiety in the B ring that could be reacted with an appropriate dienophile in a Diels-Alder reaction. In particular, the use of a nitroso compound as the dienophile would allow the introduction into the carbon skeleton of a N-O bond that would represent a handle for further functionalization.

The first report on the functionalization of steroidal dienes using a nitroso Diels-Alder reaction dates back to 1977 as part of Kirby's studies on the reactivity of nitrosocarbonyl compounds.[96] The [4+2] cycloaddition of ergosterol and ergosterol acetate with nitrosocarbonyl compounds, generated *in situ* through the oxidation of hydroxamic acids, was used in order to provide further evidence for the existence of these transient intermediates.

The reaction of ergosterol acetate **30b** with nitrosocarbonylmethane **31b** gave cycloadduct **32b** as the only product in 84% yield (**Scheme 10**). When nitrosocarbonylbenzene **31a** was used instead, compound **32a** was obtained in 33% yield alongside another unexpected product **34a**, in 50-56% isolated yield. The same experiments were conducted using ergosterol **30a** as the diene and a similar distribution of products was obtained.

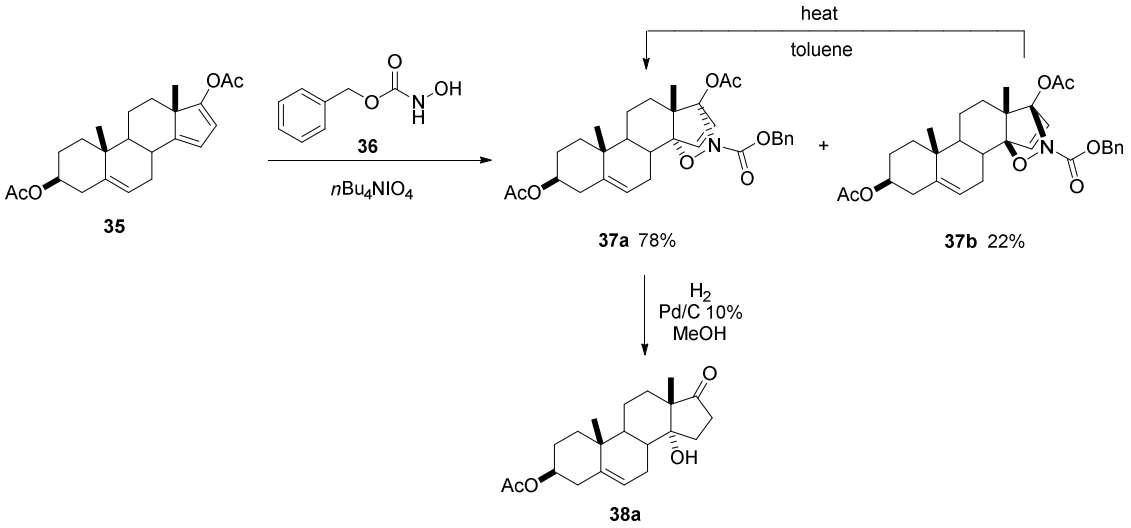


Scheme 10. Reaction of ergosterol acetate **30b** with acyl nitroso compounds.

Further mechanistic studies revealed that compound **34a** was generated via a [3,3]-sigmatropic rearrangement of the isomer **33a**. These studies dealt only with the mechanistic aspects of the nitroso Diels-Alder reaction and the biological activity of the newly formed cycloadducts was not investigated.

In 1989, Neef *et al.* used the cycloaddition of a steroidal diene **35** with an acyl nitroso compound as a

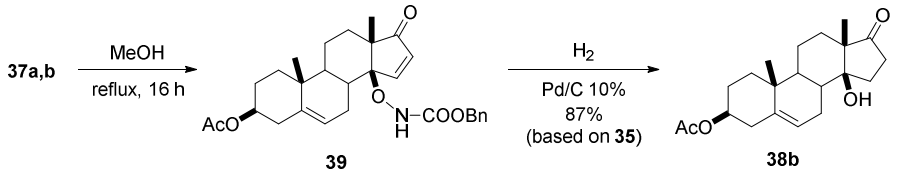
convenient methodology for the introduction of a 14-hydroxy functionality on the carbon skeleton (**Scheme 11**).[97] Benzyl nitrosoformate was generated *in situ* by the oxidation of benzyl-*N*-hydroxycarbamate **36** in the presence of the dienol acetate **35**. A mixture of isomers **37a**,**b** was obtained, with the α-adduct **37a** as the major component. This result was in contrast with literature precedents in which the cycloaddition of dienes similar to **35** with various dienophiles afforded the *β*-face adduct as the exclusive product.[98-99]



Scheme 11. Synthesis of 14-*α*-hydroxy androstane **38a**.

Since it was observed that heating of compound **37b** in toluene afforded a 9:1 mixture of the two cycloadducts **37a**,**b** (**37a** as the major product), the yield of compound **37a** was further increased (89%) using a separation-re-equilibration sequence. Finally catalytic hydrogenation on **37a** afforded the desired 14*α*-hydroxy androstane **38a**.

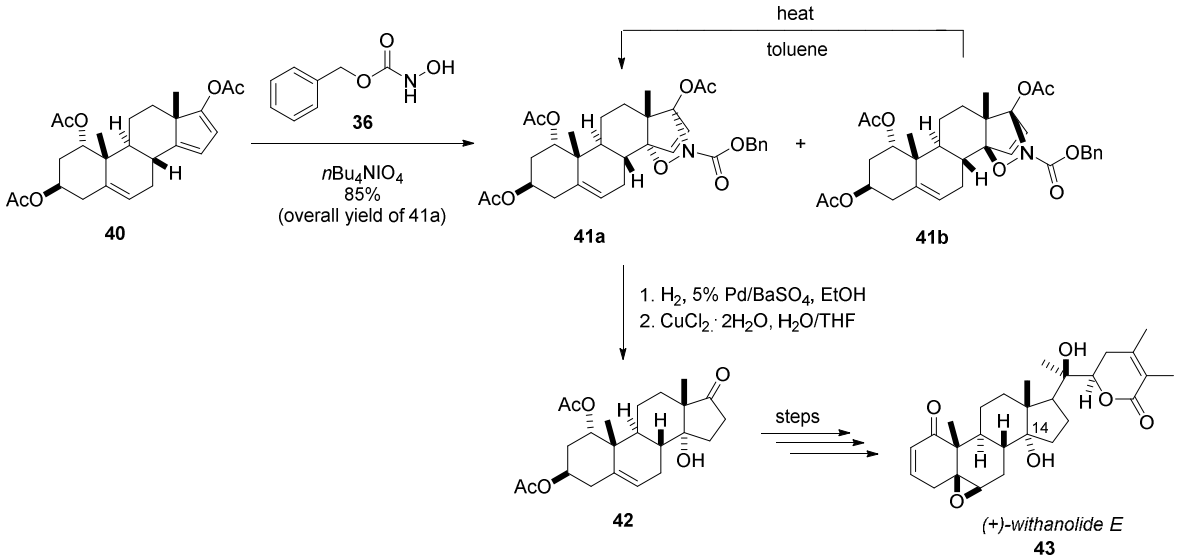
Alternatively, the synthesis of 14*β*-hydroxy androstane **38b** was obtained by heating of the crude mixture **37a**,**b** in methanol, followed by hydrogenation of the resulting 14*β*-oximino substituted enone **39** (**Scheme 12**).



Scheme 12. Synthesis of 14-*β*-hydroxy androstane **38b**.

A similar strategy for the introduction of an *α*-hydroxyl group at the C14 position of a steroidal diene was used by Grieco *et al*.[100] in 1991 in the context of the total synthesis of the natural product (+)-withanolide E

43[101-107] (**Scheme 13**). The reaction between dienol acetate **40** and benzyl nitrosoformate, generated *in situ* by the oxidation of benzyl-*N*-hydroxycarbamate **36** with tetrabutylammonium periodate, afforded isomeric cycloadducts **41a**,**b** as a 2:1 mixture, in nearly quantitative yield.

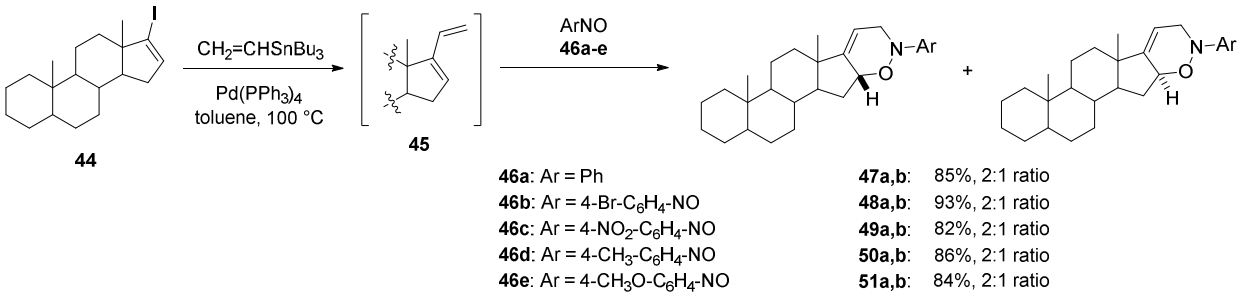


Scheme 13. Synthesis of (+)-withanolide E.

The minor isomer could be converted into the other upon heating in toluene. The resulting adduct **41a** was subjected to catalytic hydrogenation, followed by further transformation, to afford intermediate **42** which was then elaborated to final compound **43** in several steps. This study nicely demonstrates that not only can nitroso chemistry be used to modify natural products, but also for syntheses of rare natural products from others that are more available.

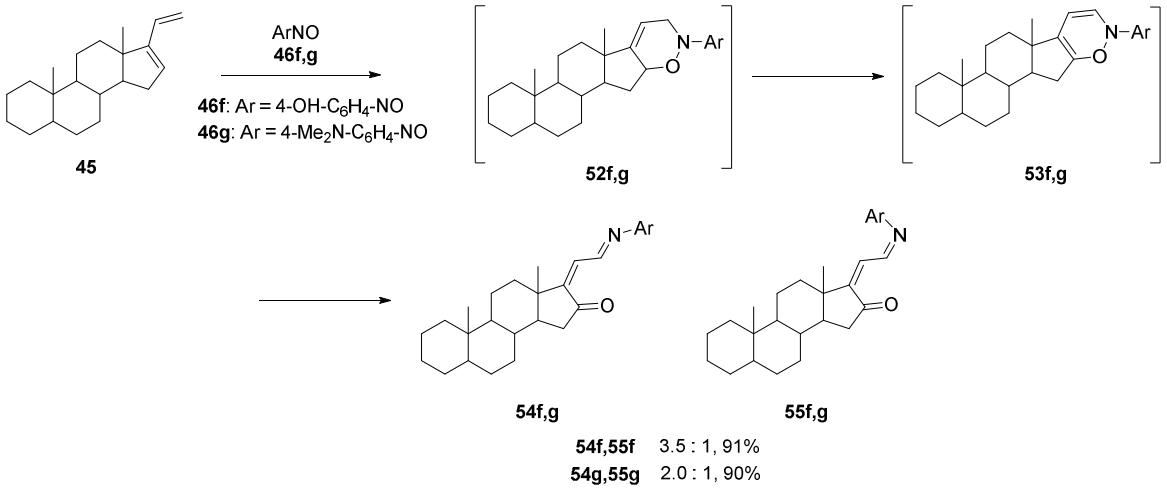
In 1999, Földes *et al*. reported the syntheses of steroids possessing a 3,6-dihydro-1,2-oxazine E-ring using a nitroso Diels-Alder reaction as the key step (**Scheme 14**).[108] Diene **45**, obtained by the Stille coupling between 17-iodo-androst-16-ene **44** and vinyltributylstannane, underwent a Diels-Alder reaction with a series of aryl nitroso compounds **46a-e**. The reaction with nitrosobenzene displayed complete regioselectivity and afforded the diastereoisomeric cycloadducts **47** as a 2:1 mixture (85% yield). The isomers **47a** and **47b** were obtained through transition states in which nitrosobenzene approached the steroid from the α- or β-face of the diene respectively. The approach from the β-side was disfavored by the steric hindrance of the 18-CH_3. It was also noted that the presence of a Lewis acid in the reaction mixture increased the amount of the 16-β-hydrogen

derivative but did not affect the rate of the reaction. Coordination of the Lewis acid with the dienophile was thought to hinder the already disfavored approach of the dienophile to the *β*-face of the diene. Similar regio- and stereoselectivity was obtained when different 4-substituted nitrosoarenes were used as dienophiles (**Scheme 14**).



Scheme 14. Syntheses of steroids possessing a 3,6-dihydro-1,2-oxazine E ring.

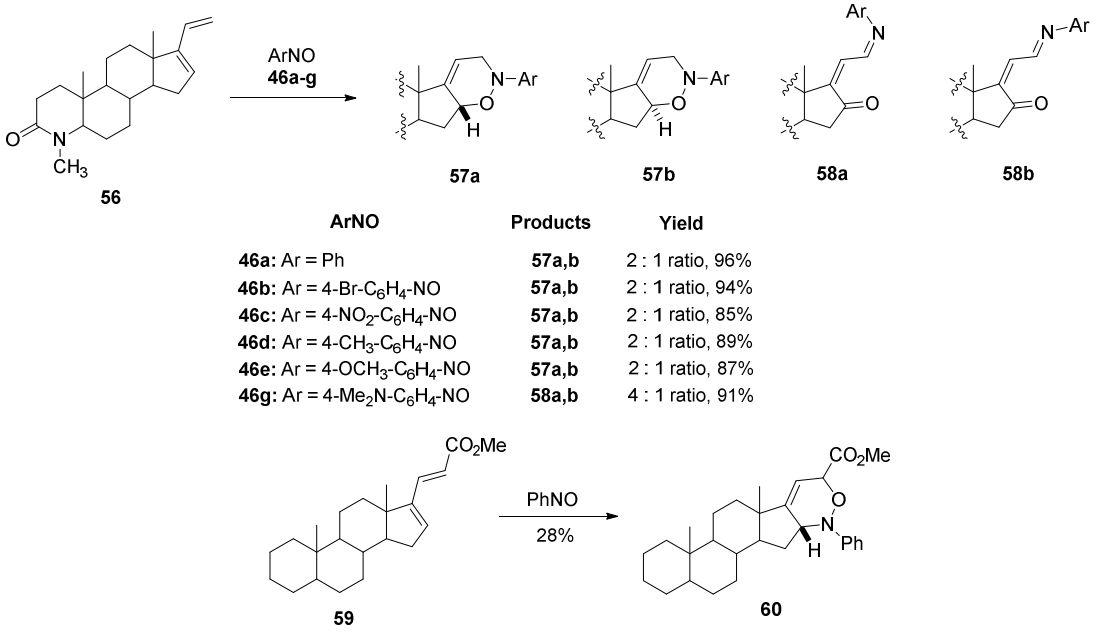
However, the same reaction of diene **45** with substituted nitrosoaromatics with highly negative Hammett substituent-constants (*p*-OH-ArNO, *p*-NMe_2-ArNO) afforded unexpected products (**54f**,**g** and **55f**,**g**), derived from dehydrogenation and rearrangement of cycloadducts **52f**,**g** (**Scheme 15**).



Scheme 15. Formation of unexpected products from the NDA reaction of diene **45** with arylnitroso compounds **46f**,**g**.

The effect of the structure of the steroidal skeleton on the regio- and stereoselectivity of the reaction was also studied. 17-Iodo-4-methyl-4-aza-androst-16-en-3-one **56** underwent a cycloaddition reaction with nitrosobenzene **46a** to afford the stereoisomeric cycloadducts **57a**,**b** in good yield (**Scheme 16**). Similar results were obtained using other *para*-substituted nitrosoarenes **46b**-**e**. Also, as previously observed, the reaction with

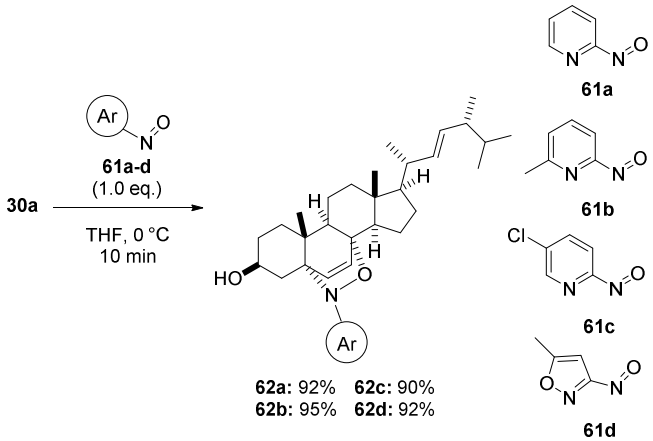
nitrosoarene **46g** led to the formation of rearranged products **58a**,**b**.



Scheme 16. NDA reaction of steroidal dienes **56** and **59** with nitrosoaromatics.

However, the reaction of nitrosobenzene with steroidal diene **59**, which contains an electron-withdrawing substituent, afforded cycloadduct **60** as a single isomer. The observed change in regioselectivity and decrease in the reaction rate were explained by the effect of the electron-withdrawing substituent on the HOMO-LUMO energy difference of the reactants and also by the change in the relative size of the coefficients of the atomic orbitals at C16 and C21 positions of the steroidal diene.

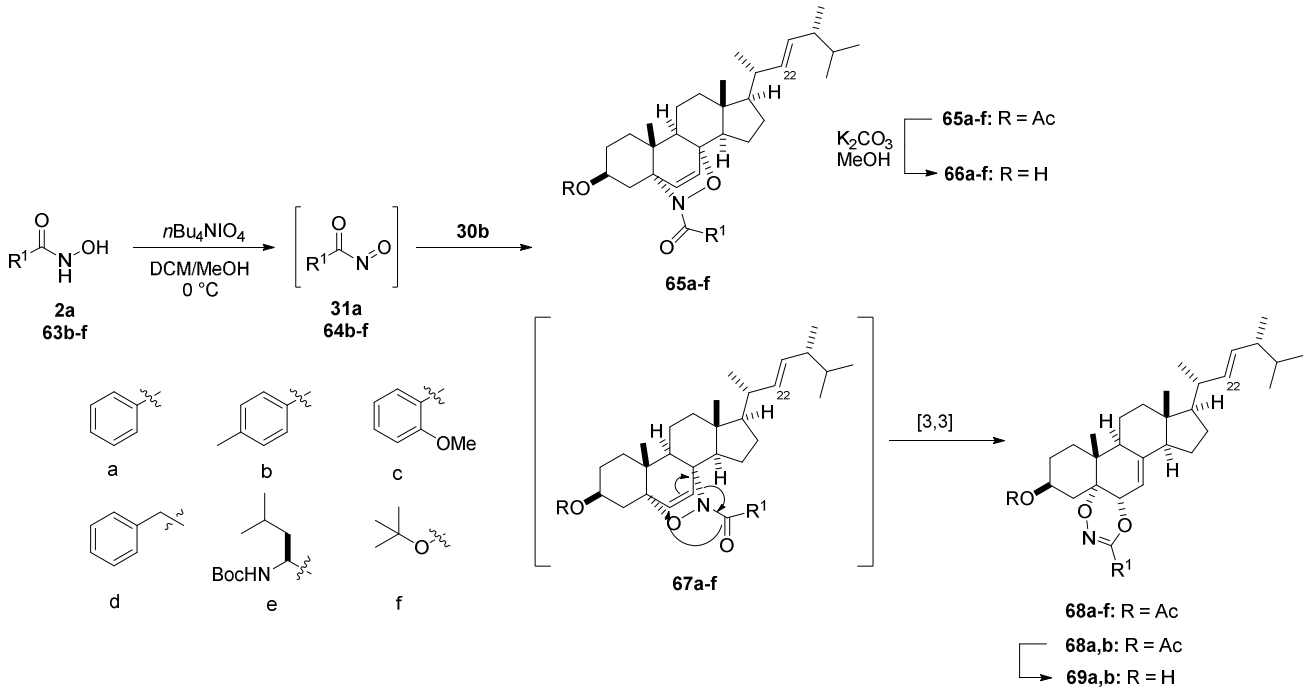
In 2007, Miller and co-workers reported the functionalization of ergosterol **30a** using an iminonitroso Diels-Alder reaction with nitrosopyridines **61a-c** and 5-methyl-3-nitrosoisoxazole **61d** as the dienophiles (**Scheme 17**).[85]



Scheme 17. Iminonitroso Diels-Alder reaction of ergosterol with nitrosopyridines.

All the reactions proceeded in 10 min to afford the corresponding cycloadducts **62a-d** in more than 90% yield (the structure of cycloadducts **62a** and **62d** was confirmed by X-ray crystallography).

These positive results encouraged further studies on the functionalization of ergosterol and ergosterol acetate using this methodology. Thus, in 2009, Miller and co-workers reported the syntheses of novel C5 and C8 disubstituted sterol analogs using nitroso Diels-Alder reactions of ergosterol acetate with acyl- and iminonitroso agents.[109] The oxidation of hydroxamic acids **2a** and **63b-f** to the transient acyl nitroso dienophiles was performed in the presence of ergosterol acetate (**Scheme 18**). The corresponding 5α-*N*-8α-*O*-adducts **65a-f** were obtained in various yields (**Table 1**). The reaction of ergosterol acetate **31b** with nitrosocarbonylbenzene **31a** afforded oxazine **68a** as the major product (54%). This was in agreement with previous work by Kirby on the functionalization of ergosterol and its acetate by their reaction with acyl nitroso compounds.[96] Also in this case the formation of the product was explained through a [3,3]-sigmatropic rearrangement of cycloadduct **67a**. Reaction of ergosterol with the acyl nitroso compound derived from the oxidation of 4-methylbenzohydroxamic acid **63b** gave, as the major product, oxazine **68b** as a mixture with adduct **67b**. In all the other cases, cycloadduct **65** was obtained in high yield, as the only product.

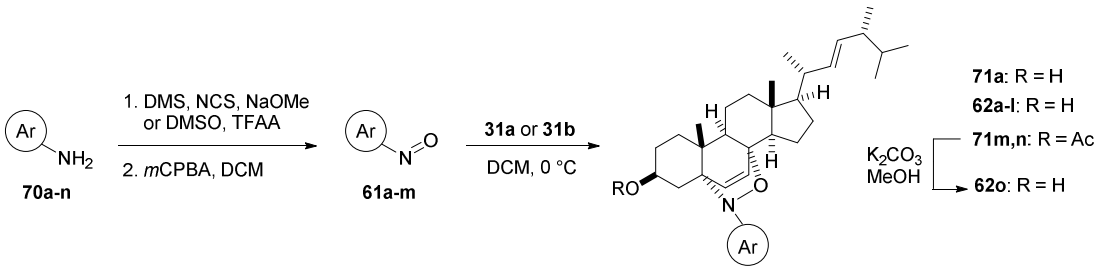


Scheme 18. Reaction of ergosterol acetate with acyl nitroso compounds.

Entry	Dienophile	Product
1	**31a**	**65a** 29%; **68a** 54%
2	**64b**	**65b** 12%; **68b** 63%[a]
3	**64c**	**65c** 74%; **68c** 0%[b]
4	**64d**	**65d** 95%; **68d** 0%[b]
5	**64e**	**65e** 93%; **68e** 0%[b]
6	**64f**	**65f** 93%; **68f** 0%[b]

Table 1. [a]As a nonseparable mixture of **68b** and **67b**. [b] Not detected.

The reaction of ergosterol and its acetate with a series of iminonitroso agents **61a-m**, obtained in two steps from the corresponding amino heterocyclic precursors,[110-111] was also examined (**Scheme 19**).



Scheme 19. Reaction of ergosterol and its acetate with iminonitroso species.

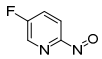
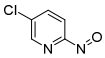
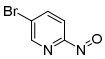
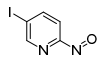
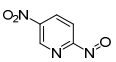
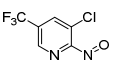
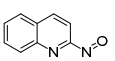
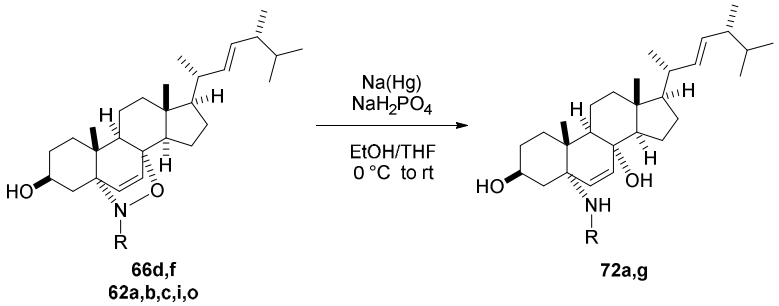
Entry	Dienophile		Product	Yield (%)
1	**46a**		**71a**	-
2[a]	**61a**		**62a**	95
3[a]	**61b**		**62b**	92
4[a]	**61d**		**62d**	92
5[a]	**61e**		**62e**	87
6[a]	**61c**		**62c**	90
7[a]	**61f**		**62f**	88
8[a]	**61g**		**62g**	82
9[a]	**61h**		**62h**	82
10[a]	**61i**		**62i**	84
11[a]	**61l**		**62l**	81
12[b]	**61m**		**71m + 71n**	95 (8.5:1 ratio)

Table 2. [a.] 1.2 eq. of nitroso compound was used. [b.] Ergosterol acetate **30b** was used.

The NDA reactions between ergosterol **31a** and the iminonitroso species were complete in 30 min, at 0 °C,

and afforded the 5α-*O*-8α-*N*-adducts **62a-l** as the sole products (**Table 2**). Only the reaction with nitroso species **61m** with ergosterol acetate afforded both the regioisomeric products, **71m,n**, in an 8.5:1 ratio. The major isomer, the configuration of which was determined by X-ray crystallography, was then deacetylated to give compound **62o**.

In order to demonstrate that the ergosterol nitroso cycloadducts are evolvable scaffolds which can be further functionalized to give a variety of new compounds, the cleavage of the N-O bond in these compounds was investigated (**Scheme 20**).



Scheme 20. Reductive cleavage of the N-O bond in the ergosterol nitroso cycloadducts.

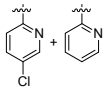
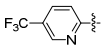
Entry	Adduct	Product	R	Yield (%)
1	**62b**	**72a**		82
2	**66d**	**72b**		26
3	**66f**	**72c**		Trace
4	**62a**	**72d**		61
5	**62c**	**72e + 72d**		60 (1:3)
6	**62i**	**72f**		59
7	**62o**	**72g**		63

Table 3.

The resulting 1,4-amino alcohols **72a-g** were evaluated for their biological activity. They showed no significant antibacterial activity; however, they displayed some inhibitory activity against the fungal strain *Sporobolomyces salmonicolor*, whereas ergosterol and its nitroso cycloadducts did not show such activity. More importantly, these compounds displayed inhibitory activity of PC-3 (prostate cancer) and MCF-7 (breast cancer) cell lines at micromolar concentrations (**Table 4**). Ergosterol acylnitroso-Diels-Alder adducts **62** also displayed

interesting growth inhibitory activity against PC-3 and MCF-7 cancer cell lines whereas the parent compound ergosterol did not.

		% inhibition at 20 μM		IC_{50} (μM)	
Entry	**Compd**	**PC-3**	**MCF-7**	**PC-3**	**MCF-7**
1	**31a**	10	15	-	-
2	**65a**	64	97	-	14
3	**65b**	40	97	16	14
4	**65c**	94	97	11	6
5	**65d**	76	96	12	14
6	**65e**	57	95	12	8
7	**65f**	73	89	17	20
8	**62b**	<10	15	-	-
9	**62a**	15	29	-	-
10	**62d**	76	100	-	15
11	**75c,e,g,h**	<10	<10	-	-
12	**62i**	22	90	-	17
13	**62l**	15	<10	-	-
14	**71m**	15	29	-	-
15	**62o**	8	20	-	-
16	**72b**	94	94	9	6.5
17	**72a**	100	100	6	2
18	**72d**	100	100	6	3.5
19	**72e**	100	100	7	3
20	**72f**	100	100	10	2.5

Table 4. Results of anticancer screening. Trichostatin A was used as the positive control (MCF-7, IC_{50} = 16 nM; PC-3, IC_{50} = 160 nM).

4. Rapamycin

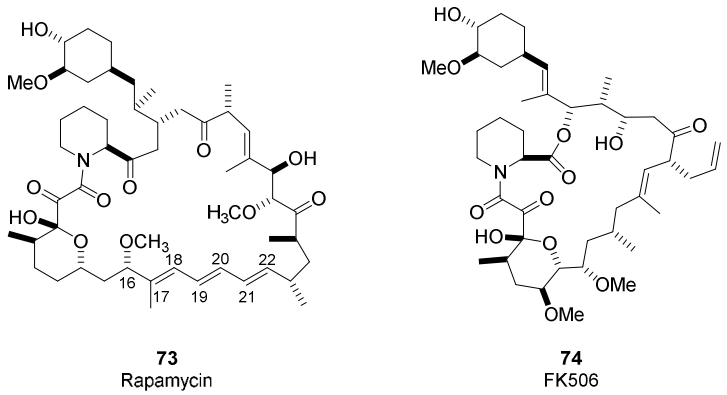


Fig. 4 Rapamycin and FK506.

Rapamycin **73** (**Fig. 4**) was isolated in 1975 from the species *Streptomyces hygroscopicus*.[112-114] It was first

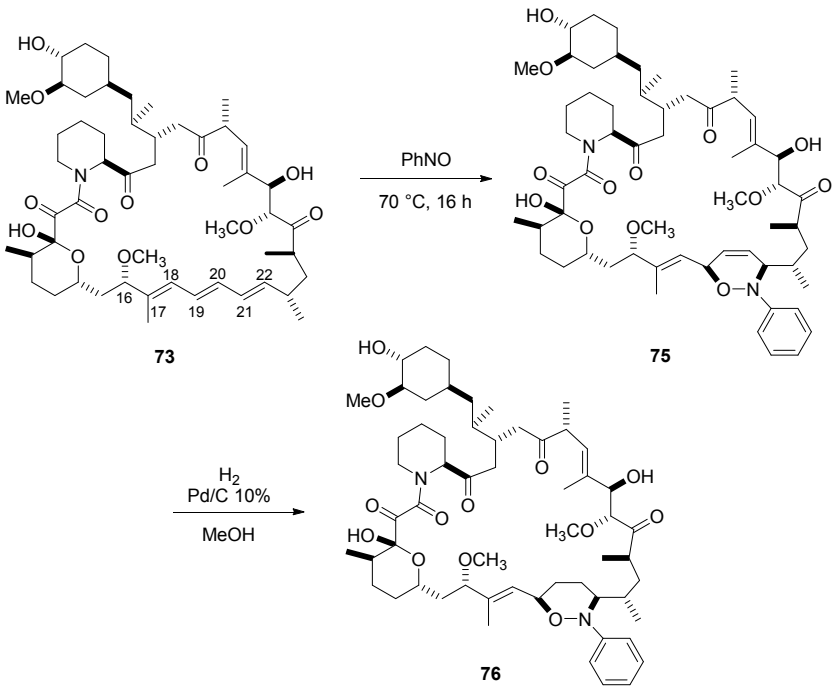
described as an antifungal agent but in the 1990's it attracted much attention for its potent immunosuppressant activity.[115]

Rapamycin and the structurally related FK506 **74** (**Fig. 4**) bind with high affinity to the immunophilin FKBP12 although they have different modes of action, suppressing T-cell activation at different stages.[114d,e] Both are also inhibitors of the peptidyl prolyl *cis-trans* isomerase activity inherent to FKBP proteins. Numerous studies have focused on the structure of the rapamycin-FKBP12 complex[116] as well as its role in the inhibition of the signal transduction pathways which lead to the activation of T lymphocytes. The structure of rapamycin can be divided into two regions: a binding domain, which binds to a hydrophobic cavity of the protein FKBP12, and an effector domain which determines the immunosuppressant activity of the molecule.

Analogs of rapamycin in which different parts of the molecule have been modified were synthesized by different groups in order to study the effects on the biological activity.[117-120] In particular, modification of the triene unit, which belongs to the effector domain, has been performed in order to obtain rapamycin analogs with similar binding affinity for the protein FKBP12 but varied immunosuppressive activities.[117-119] However, the complex array of functional groups in rapamycin considerably limit the number of transformations that can be carried out on this molecule. For example, rapamycin is incompatible with basic reagents[121-123] and strong mineral acids.[124] The Diels-Alder reaction, and in particular the nitroso Diels-Alder reaction on the triene unit, would therefore constitute a possible tool for the functionalization of rapamycin under mild conditions. For example, the hetero Diels-Alder reaction of rapamycin with 4-phenyl-1,2,4-triazoline-3,5-dione involving the C19-C22 diene partner has been reported.[118] In their studies on the acid catalyzed functionalization of rapamycin, Caufield *et al*. reported the elimination reaction of the C16 methoxy group, induced by BF_3 etherate, to give a tetraene which underwent a cycloaddition reaction with *N*-methyltriazolinedione.[117a]

An example of a Diels-Alder reaction of rapamycin with an aryl nitroso compound came from the work of Graziani *et al*.[125] Reaction with nitrosobenzene afforded oxazine **75** (**Scheme 21**), in 41% yield, as the only regioisomer and catalytic hydrogenation afforded compound **76** in which only the double bond in the oxazine ring was reduced. Previously inaccessible compounds **75** and **76**, as immunophilin ligands, showed potent

neurotrophic activities in cortical neuronal cultures, efficacy in a rodent model for ischemic stroke, and significantly reduced immunosuppressive activity.



Scheme 21. NDA reaction of rapamycin with nitrosobenzene and synthetic elaboration of the resulting cycloadduct.

5. Leucomycin

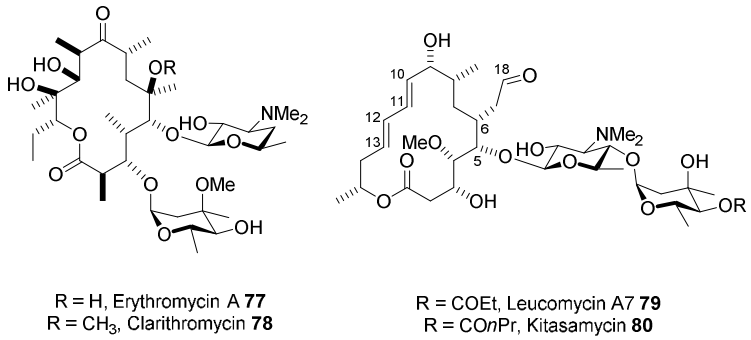


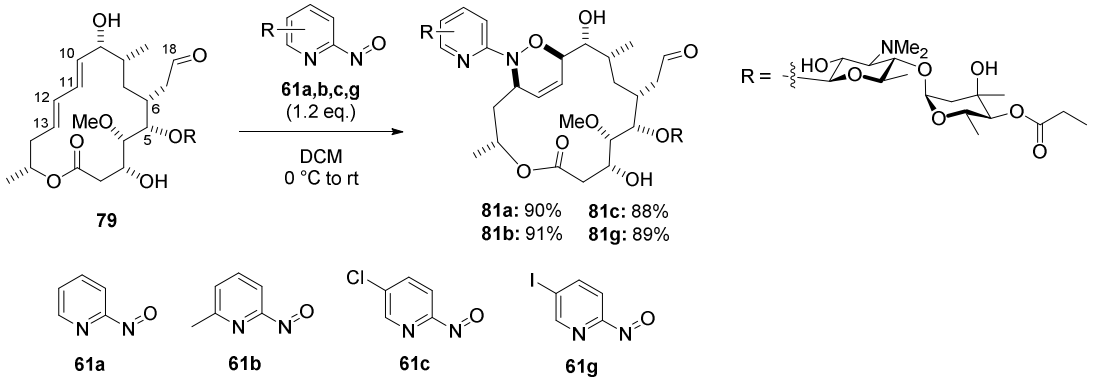
Fig. 5. Erythromycin A, clarithromycin, leucomycin A7, and kitasamycin.

Macrolide antibiotics have been used for many years to treat bacterial infections.[126-127] Their mode of action involves inhibition of the bacterial protein biosynthesis process which is mediated by binding with ribosomal *t*RNA.[128] Unfortunately, the spread of strains of bacteria resistant to these and other antibiotics has become a major concern in the last decade and therefore the development of new antibiotics is becoming more and more urgent.[129-131] Modification of naturally occurring antibiotics would not only allow tuning of their

pharmacological profile, but it would also offer an insight into the structure-activity relationship of these molecules. In order to overcome bacterial resistance through the syntheses of more potent compounds, it is important to study the mechanism of action of the antibiotic as well as the contribution of the different functional groups to its activity.

Leucomycin A7 **79** (**Fig. 5**) is an antibiotic of the macrolide group which is characterized by a 16-membered lactone ring. It was isolated in 1953 by Hata *et al*. from the fermentation broth of *Streptomyces kitasatoensis*.[132] Later, Omura *et al*. conducted an extensive investigation on the structure and antimicrobial activity of several components of the leucomycin complex, leucomycins A1-9, U and V.[133-139] Different groups reported structural modifications to members of the leucomycin family in order to identify the moieties responsible for binding to the microbial ribosomes and therefore antibacterial activity.[140-148] Examples of modification and derivatization of the 16-membered macrolide antibiotics, in particular leucomycin and its analogs, have been reported in the literature. However, the structural changes consisted mostly in the acetylation or oxidation of the hydroxyl groups or the reduction of the aldehyde functionality and only a few examples dealt with the modification of the diene unit.

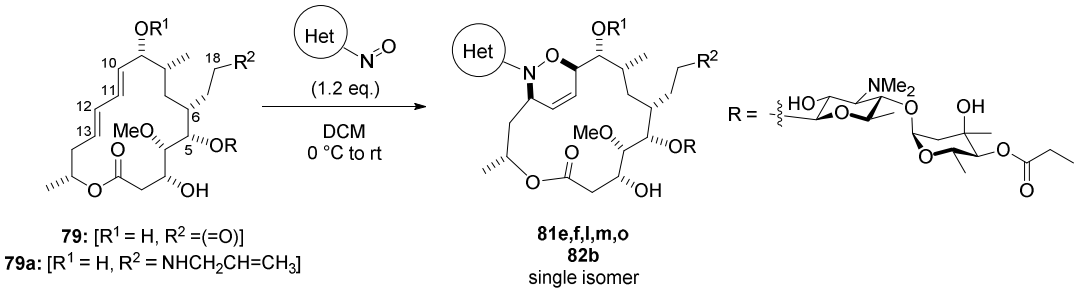
In 2007, Miller and co-workers reported, in the context of work on the functionalization of complex diene-containing natural products using an iminonitroso Diels-Alder reaction, the reaction of leucomycin A7 with a series of substituted nitrosopyridines[85] (**Scheme 22**). The corresponding cycloadducts **81** were obtained in high yield, with complete regio- and stereoselectivity.



Scheme 22. NDA reaction of leucomycin A7 with a series of nitrosopyridines.

This result encouraged further exploration of this methodology, since it allowed introduction of an oxazine ring, which not only enhances the strain and induces conformational changes in the molecule, but also represents a handle for further functionalization (e.g. reductive cleavage of the N-O bond). The interest was directed towards the creation of an extended library of analogs and the study of their biological activity.[149]

As shown in **Table 5**, nitroso Diels-Alder reactions between leucomycin A7 and a series of pyridylnitroso compounds afforded the cycloaddition products with complete regio- and stereoselectivity (the structures were confirmed by extensive 1D and 2D NMR analysis). The yields were generally high, with the exception of compounds **81m** and **81o**. This was likely due to the instability of the nitroso compounds **61m** and **61o** and the sensitivity of leucomycin towards oxidative conditions.



Scheme 23.

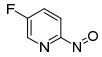
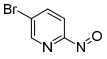
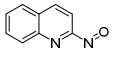
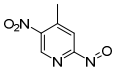
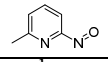
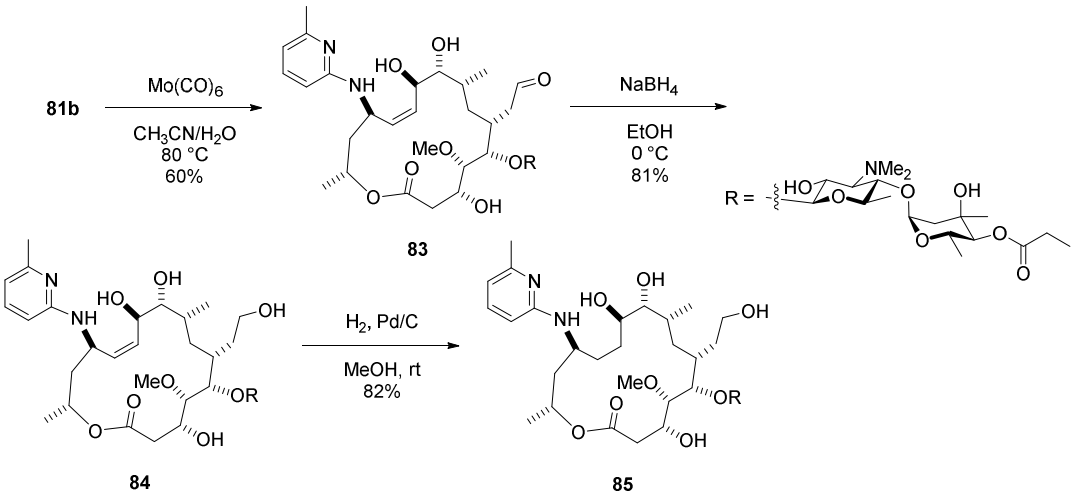
Entry	Leucomycin	Nitroso		Adduct	Yield (%)
1	**79**	**61d**		-	60[a]
2	**79**	**61e**		**81e**	90
3	**79**	**61f**		**81f**	86
4	**79**	**61l**		**81l**	78
5[b]	**79**	**61m**		**81m**	13
6[b]	**79**	**61o**		**81o**	26
10	**79a**	**61b**		**82b**	88

Table 5. [a.] Multiple isomeric adducts were determined by ^{1}H NMR and LC/MS. [b.] *in situ* trapping required.

Further elaboration of cycloadduct **81b**, which was chosen as a model substrate, was then accomplished in order to obtain further structural diversification (**Scheme 24**). Reductive cleavage of the N-O bond afforded compound **83** in which 1,4-amino alcohol substituents were incorporated in the structure. $NaBH_4$-mediated reduction of the aldehyde group in **83** afforded compound **84** in good yield and catalytic hydrogenation afforded

the final compound **85**.



Scheme 24. Structural modification of NDA cycloadduct **81b**.

All compounds were tested for their *in vitro* antibacterial activity against various strains of Gram-positive and Gram-negative bacteria using agar diffusion assays and they all showed activity similar to that of the parent compound with the exception of derivatives **84** and **85** which proved to be inactive. This was further evidence for the importance of the aldehyde functionality at the C18 position of leucomycin for the antibacterial activity. The importance of this aldehyde substituent in several other 16-membered macrolide antibiotics in the inhibition of protein biosynthesis, including for example carbomycin A and spiramycin, was also reported by Steitz *et al.*[150]

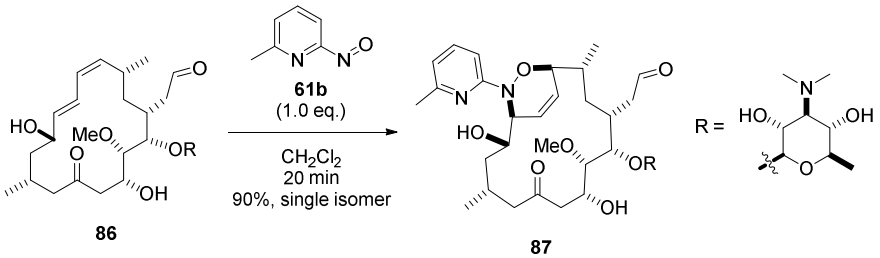
More remarkable was the study of the antiproliferative and cytotoxic activity of these cycloadducts on a series of cancer cell lines [L-929 (mouse fibroblasts), K-562 (human leukemia), PC-3 (prostate cancer), MCF-7 (breast cancer) and HeLa cell line (human cervix carcinoma)]. Although leucomycin itself did not show any activity in these assays, its NDA cycloadducts showed moderate antiproliferative activity in L-929 and K-562 cell lines and moderate cytotoxic activity in HeLa, PC-3 and MCF-7 cell lines (**Table 6**).

Entry	Compound	L-929	K-562	HeLa	PC-3	MCF-7
		GI_{50}/μM	GI_{50}/μM	CC_{50}/μM	IC_{50}/μM	IC_{50}/μM
1	**79**	>66	>66	>66	>50	>50
2	**81b**	31	34	44	20	14
3	**81o**	9.1	36.3	33.5	Nd	Nd
4	**81m**	>100	>100	>100	Nd	Nd
5	**81a**	>100	>100	>100	>100	>100
6	**81e**	nd	nd	nd	30	18
7	**81c**	15.6	15	23.5	16	11
8	**81g**	30	20	40	10	6
9	**81l**	nd	nd	nd	8	6
10	**82b**	18	8.3	37	Nd	Nd
11	**83**	>50	>50	>50	50	>50
12	**84**	nd	nd	nd	40	>50
13	**85**	nd	nd	nd	20	42

Table 6. Antiproliferative activity and cytotoxicity of leucomycin derivatives. (Nd. Not determined)

It is worth noting the promising activity of compound **81l**, which inhibited both PC-3 and MCF-7 cancer cells growth at micromolar concentrations, and also the influence of the substituent on the pyridine ring on activity. The N-O bond reduced analogs **83**, **84** and **85** were found to be relatively inactive against PC-3 and MCF-7 cells, suggesting that the incorporated substituted rigid N-O heterocycle might be essential for antiproliferative and cytotoxic activity.

The same methodology was also used for the functionalization of another antibiotic with a structure similar to that of leucomycin A7: isoforocidin **86**.[85] The reaction with 6-methyl-2-nitrosopyridine in dichloromethane afforded the HDA cycloadduct **87** in 90% yield, as a single isomer (yield and purity were determined by ^{1}H NMR of the crude reaction mixture) (**Scheme 25**). However, the biological activity of the modified natural product was not investigated.



Scheme 25. NDA reaction of isoforocidin with 6-methyl-2-nitrosopyridine.

6. Colchicine and Isocolchicine

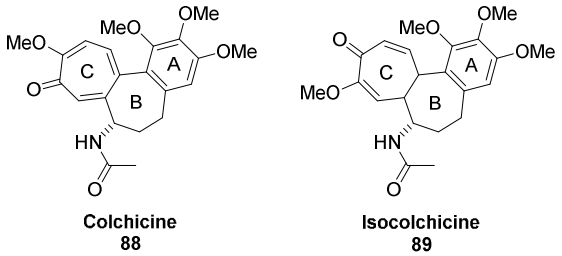
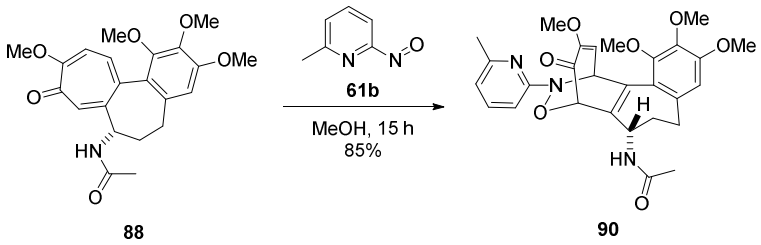


Fig. 6. Colchicine and isocolchicine.

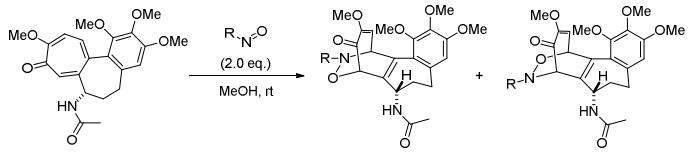
Natural colchicine **88** is the major alkaloid isolated from the plant *Colchicum autumnale L.* and its main biological activity consists in mitosis inhibition.[151] In particular, it binds to the protein tubulin inhibiting microtubule (MT) polymerization, a process necessary for mitosis. Since cancer cells are characterized by an increased rate of mitosis, they are in principle more vulnerable to this drug than healthy cells. However, the use of colchicine as a clinical agent in cancer therapies is precluded by its toxicity and its narrow therapeutic window.[152] Nevertheless, its biological activity is still promising and numerous studies have been reported on the structure-activity relationship of this compound. For example, it has been shown that the trimethoxy benzene ring (A) and the methoxy tropone ring (C) are structural features essential for activity.[153] Several modifications of the structure of colchicine and isocolchicine (the inactive analog) have been performed, in order to obtain potential new drugs with a more favorable biological profile.[154] Ring C is characterized by two facially differentiated diene moieties and this characteristic has been used to functionalize the molecule at this position through Diels-Alder reactions. Reactions with several hetero- and carbo-dienophiles has been reported[154b] but the biological activity of the resulting cycloadducts was not investigated.

In an early report, Miller and co-workers described the functionalization of the C ring of colchicine via a hetero Diels-Alder reaction with a pyridylnitroso compound as the dienophile (**Scheme 26**).[85] Colchicine **88** reversibly reacted with **61b** at room temperature to give cycloadduct **90** with high regio- and stereoselectivity (although two isomers were observed in a > 7:1 *ratio* based on LC/MS and ^{1}H NMR of the crude reaction mixture, only one isomer was isolated after silica gel chromatography).



Scheme 26. NDA reaction of colchicine with 6-methyl-2-nitrosopyridine.

This first positive result initiated a more systematic study on the application of iminonitroso Diels-Alder reactions to the syntheses of novel cycloadducts with potential biological activity.[154c] Aryl-, acyl- and pyridylnitroso compounds were used as the dienophiles but only the pyridylnitroso compounds afforded optimal results, because of their ideal combination of stability and reactivity (**Table 7**).



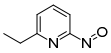
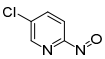
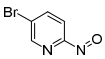
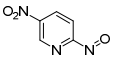
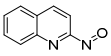
Entry	Nitroso compound		Product	Ratio[a]	Yield (%)
1	**46a**		**90a**		<10[a]
2	**32a**		**-**[b]	-	0
3	**61b**		**90b**	-	82
4	**61a**		**90c+91c**	3:1	78
5	**61n**		**-**	-	0
6	**61q**		**90d+91d**	6:1	61
7	**61p**		**90e**	-	62
8	**61l**		**90f+91f**	7:1	75
9	**61c**		**90g+91g**	7:1	78
10	**61f**		**90h+91h**	6:1	74
11	**61h**		**90i+91i**	7:1	73
12	**61e**		**90l+91l**	8:1	72
13	**61d**		**90m+91m**	2:1	80

Table 7. [a.] Determined by ^{1}H NMR analysis of the crude mixture. [b.] *in situ* oxidation-trapping required.

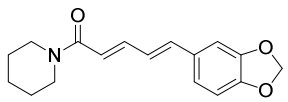
In each case the cycloaddition reaction furnished the two regioisomeric *endo* products, in high yield and with moderate selectivity. The structures of the major isomers were determined through 1D and 2D NMR studies. Only in the case of 3-methylnitrosopyridine, no product was obtained and this was probably due to the negative steric effect of a substituent in the 3-position.

In vitro cytotoxicity assays on cycloadducts **90b-m** were performed using PC-3 and MCF-7 cell lines: most of the analogs exhibited similar activity to that of colchicine itself (**Table 8**). It was also proved that the adduct of colchicine with 6-methylnitrosopyridine underwent a retro Diels-Alder reaction at 37 °C. Thus, the cycloadducts of colchicine would probably serve as prodrugs and their varying stabilities would account for their different biological activity. The colchicine nitroso adducts were also subjected to microtubule polymerization assays and their activity was compared to that of reference compounds, including colchicine itself, nocodazole (microtubule destabilizer) and paclitaxel (microtubule stabilizer). All the colchicine analogs showed a decreased inhibitory activity towards microtubule polymerization. This indicated that the introduction of the oxazine ring in the colchicine skeleton and the consequent enhancement of the structure rigidity resulted in modification of the interaction with the protein tubulin.

Entry	Compd.	IC_{50}, PC-3 (nM)	IC_{50}, MCF-7 (nM)
1	Colchicine **88**	20	12
2	**90c**	25	20
3	**90b**	28	17
4	**90d**	14	10
5	**91e**	23	22
6	**91f**	15.6	15
7	**91g**	10	15.6
8	**91h**	24	20
9	**91i**	250	230
10	**90m**	250	190
11	**91m**	245	250

Table 8. Cytotoxic activity against PC-3 and MCF-7 cell lines for colchicine nitroso adducts. Adducts **90f-i** were tested with a small amount of regioisomeric adducts **91f-i** present.

7. Piperine



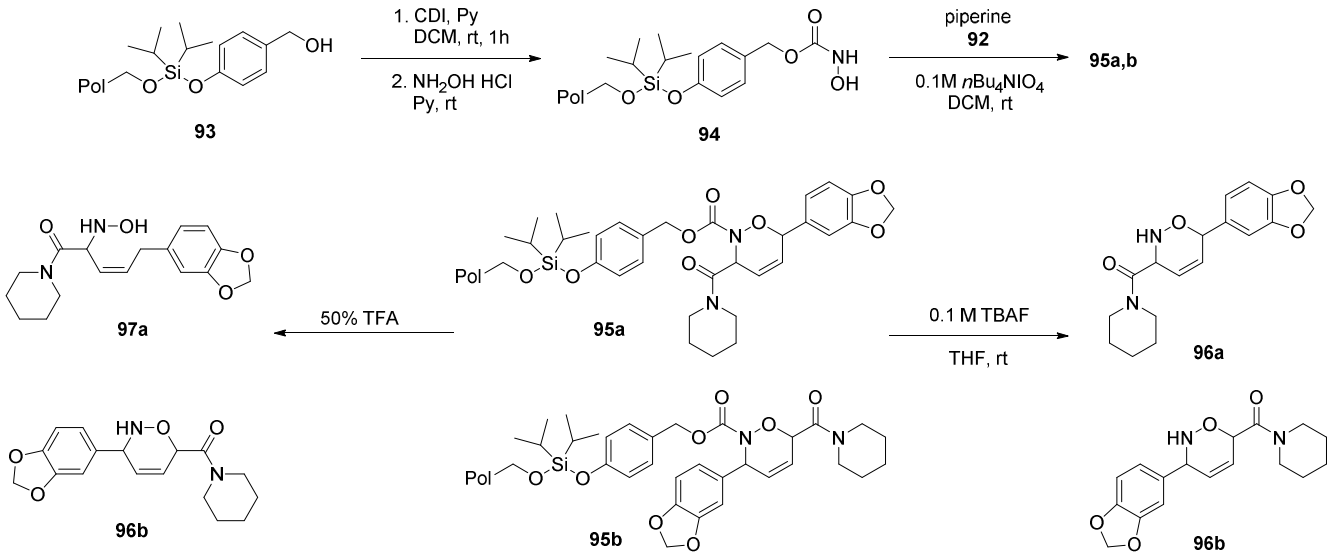
92
Piperine

Fig. 7. Piperine.

Piperine **92** (**Fig. 7**) is found as a major component in the Asian vine *Piper nigrum* and it is responsable for the spicy flavor of pepper. It has been used in traditional medicine and it displays a wide range of biological activities such as inhibition of human P-glycoprotein CYP3A4[155] and other enzymes important in drug metabolism,[156] stimulation of melanocyte proliferation[157] and enhancement of the bioavailability of drugs.[158] Piperine presents an electronically mixed diene, containing both an electron-withdrawing acyl group and an electron-rich aromatic ring, which can undergo nitroso Diels-Alder reactions with acyl- and aryl nitroso compounds in order to form a series of derivatives with varied biological activities.

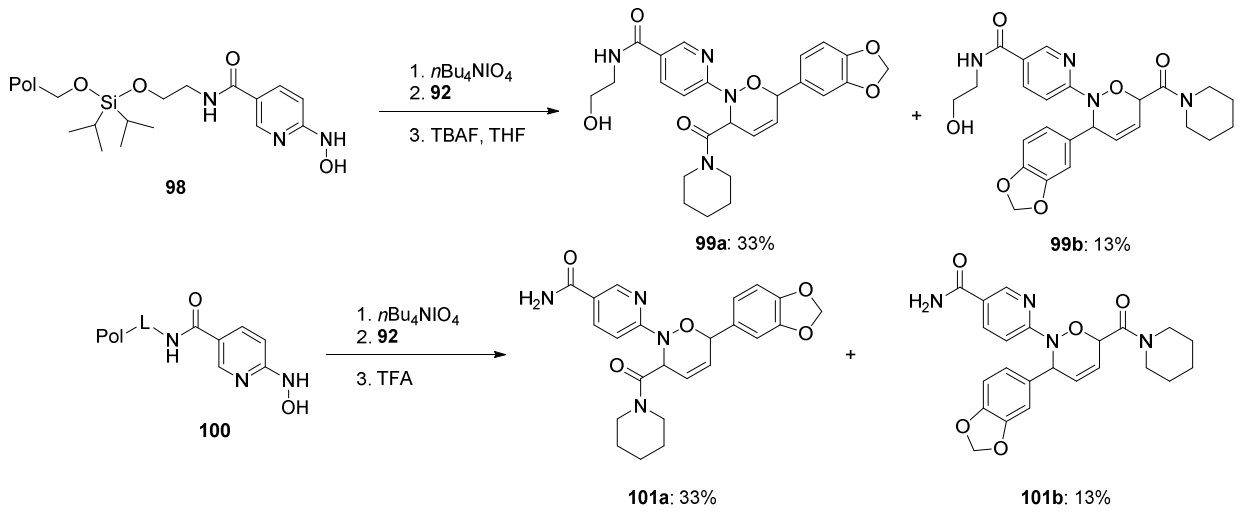
Acyl- and aryl nitroso polymer supported NDA reactions have been described in the literature.[159-163] However, few examples of the application of this methodology to the functionalization of natural products have been reported. In particular, Krchňák *et al*. reported the reaction of piperine with a series of polymer supported nitroso dienophiles to afford a variety of novel cycloadducts which were subjected to further functionalization in order to obtain a library of heterocycles with potential biological activity.[164]

The synthesis of N-H oxazines **96a**,**b** is displayed in **Scheme 27**. Oxidation of polymer supported hydroxamic acid **94** in the presence of piperine gave the NDA products **95a**,**b**. The use of a silyloxy-based linker allowed cleavage by the mild reagent tetrabutylammonium fluoride (TBAF) which afforded the N-O oxazines **96a**,**b** as a 7:3 mixture (13% yield). However, when the NDA adducts were released from the resin using trifluoroacetic acid (TFA), in the presence of the cation scavenger triethylsilane (TES), isomer **96b** was obtained alongside a different product **97a** which derived from the acid-induced rearrangement of cycloadduct **95a** (cleavage of the C-O bond with formation of a benzylic cation which was quenched by TES).



Scheme 27. NDA reaction of piperine on solid phase.

The NDA reaction of piperine with pyridylnitroso dienophiles, on solid phase and in solution, was also studied. The pyridylnitroso dienophiles were first immobilized on solid support using either a silyloxy linker or via the acylation of Rink resin to give precursors **98** and **100** (**Scheme 28**).

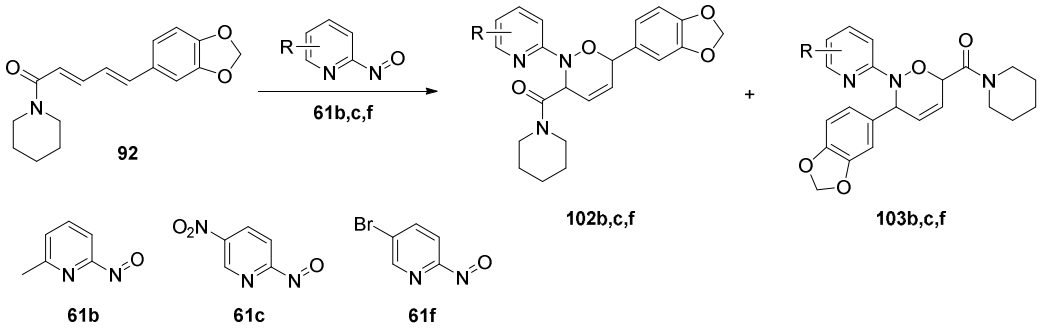


Scheme 28. NDA reaction of piperine with pyridylnitroso dienophiles on solid phase.

Oxidation of **98**, followed by reaction with piperine afforded, after cleavage with TBAF, two regioisomers **99a**,**b** in a 7:3 ratio. Analogously, oxidation of **100** in the presence of piperine, followed by cleavage with TFA, afforded the expected mixture of regioisomers **101a**,**b** in similar yields.

The same NDA reaction was then performed with both of the components in solution (**Scheme 29**). The reaction of piperine with 6-methyl-2-nitrosopyridine afforded cycloadducts **102b** and **103b**, as a 2:1 mixture of

regioisomers. The regioselectivity of the reaction could be improved by the use of a copper catalyst, $[(MeCN)_4Cu(I)PF_6]$[165] (**Table 9**). The reaction of piperine with nitroso compounds **61c** and **61f** gave similar results.

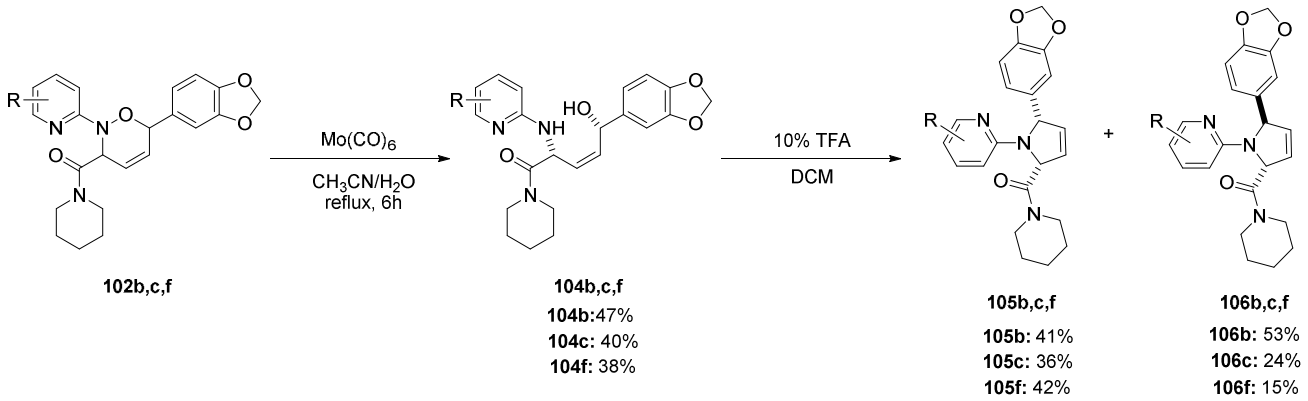


Scheme 29. NDA reaction of piperine and pyridylnitroso compounds in solution.

Entry	Nitroso	Cu catalyst	Products	
1	**61b**	-	**102b**:**103b** (86%)	2:1
2	**61b**	0.20 eq.	**102b**:**103b** (86%)	4:1
3	**61b**	0.50 eq.	**102b**:**103b**	8:1
4	**61b**	0.75 eq.	**102b**:**103b**	12:1
5	**61b**	1.0 eq.	**102b**:**103b**	16:1
6	**61c**	-	**102c**:**103c** (73%)	2:1
7	**61f**	-	**102f**:**103f** (64%)	2:1

Table 9. Reagents and conditions: $(MeCN)_4Cu(I)PF_6$, nitrosopyridine (1 eq.), rt, 30 min then piperine (1 eq.), rt, 16 h.

Further synthetic elaboration of cycloadducts **102b**,**c**,**f** was then investigated (**Scheme 30**). Treatment of the cycloadducts with $Mo(CO)_6$ in CH_3CN/H_2O mixture, afforded the N-O reduced products **104b**,**c**,**f** which upon treatment with 10% trifluoroacetic acid (TFA) rearranged to the corresponding diastereoisomeric dihydropyrroles **105b**,**c**,**f** and **106b**,**c**,**f**. These results again demonstrate that products generated from NDA reactions are versatile, evolvable scaffolds.



Scheme 30. Synthetic elaboration of the NDA cycloadducts **102b**,**c**,**f**.

8. Miscellaneous

In 2011, Krchňák *et al.* reported the use of solid supported nitroso dienophiles for the modification and isolation of a single diene-containing natural product from crude extracts.[166] In particular, the sequestration experiments were carried out with Lampong Black peppercorn. Its crude dichloromethane extract was exposed to 6-nitrosonicotinic acid attached to a Rink resin and the products were released upon treatment with TFA. The major product was expected to be the NDA cycloadduct of the nitroso species with either piperine **92** or piperettine **107**, two of the major components of peppercorn (**Fig. 8**).

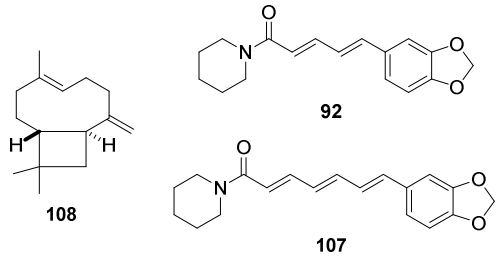
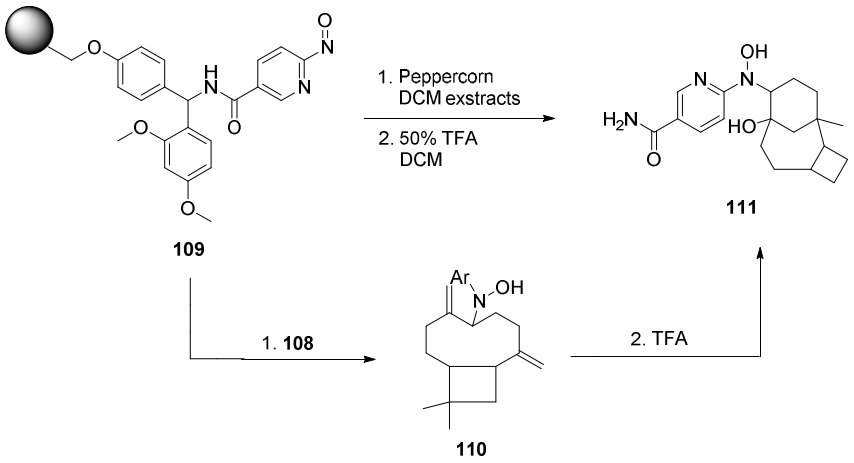


Fig. 8. Piperine, piperettine and *β*-caryophyllene.

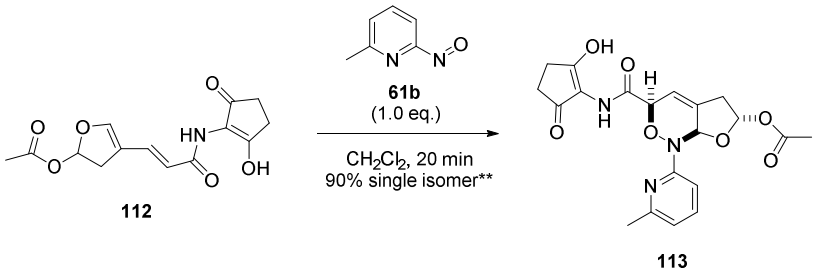
However, the major product was found to be the cyclobutane-containing tricyclic compound **111** (**Scheme 31**). Further studies revealed that the actual product was derived from the ene reaction of the nitroso species with another component of the peppercorn extract: β-caryophyllene **108**, a sesquiterpene containing two isolated double bonds. The ene reaction, which occurred at the C5 position, was followed by rearrangement induced by treatment with TFA.



Scheme 31.

This result is of particular interest since the solid-supported nitroso species reacted with exquisite selectivity with only one of the several diene- and ene-containing natural products which were present in the peppercorn extract. Therefore, this methodology could be potentially useful for the isolation and modification of single natural products from natural extracts.

Reductiomycin[166-170] **112** is a metabolite of the bacteria *Streptomyces xanthochromogenus* and displays a wide range of biological activities, including antitumoral, antibiotic, antifungal and antiviral activity. Miller and co-workers[85] reported the NDA reaction of reductiomycin with 6-methyl-2-nitrosopyridine which afforded the cycloadduct **113** as a single isomer, in 90% yield (**Scheme 32**). Since the absolute configuration of reductiomycin was still in question,[167,168] the relative stereochemistry of the product was assigned based of NOE correlations from ROESY experiments. The biological activity of this compound was not investigated because of its instability.



Scheme 32. NDA reaction of reductiomycin **112** with 6-methyl-2-nitrosopyridine **61b**. ** yield and purity were determined by ^{1}H NMR of the crude reaction mixture.

9. Conclusion

We have herein described the application of the nitroso Diels-Alder reaction to the efficient functionalization of complex diene-containing natural products, under mild conditions. This methodology proves to be particularly useful for Modular Enhancement of Nature's Diversity (MEND). In particular, it allows the generation of versatile and evolvable scaffolds which can be further functionalized in order to obtain natural products derived libraries of novel compounds with modified biological activity.

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