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Iminonitroso Diels–Alder Reactions for Efficient Derivatization and Functionalization of Complex Diene-Containing Natural Products

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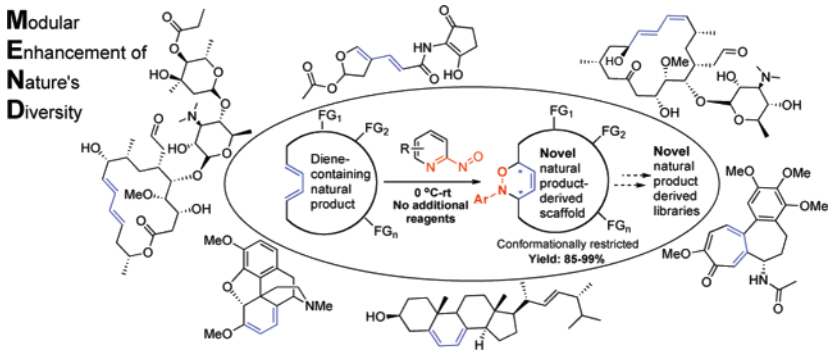
ABSTRACT



A remarkably efficient method for derivatization of complex diene-containing natural products by using stabilized iminonitroso Diels–Alder reactions is described. Turimycin H3, ergosterol, reductiomycin, isoforocidin, colchicine and thebaine were found to react with nitrosopyridines in a highly efficient regio- and stereoselective fashion. Preliminary bioactivity evaluations of turimycin cycloadducts are reported.

Although there have been impressive advances in synthetic, medicinal, and combinatorial chemistry, natural products and their derivatives remain a significant source of molecular diversity for drug discovery.[1] Diversification and functionalization of natural products or natural-product-like compounds are likely to continue to play important roles in this process because they serve not only as therapeutic leads but also as probes of complex biological processes.[2] However, the development of highly efficient and versatile natural product derivatization methods remains a challenge. Classical derivatization methods are often limited to standard modification of nucleophilic or electrophilic functional groups contained in natural products and other bioactive compounds. Many natural products contain multiple functional groups of the same or similar type so derivatization selectivity is problematic. Here we report our findings on the use of stabilized iminonitroso Diels−Alder reactions, particularly the pyridine nitroso Diels−Alder reaction, as a remarkably efficient method for derivatization of complex diene-containing natural products. This totally atom economical cycloaddition reaction produces conformationally restricted ana-

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(1) (a) Newman, D. J.; Cragg, G. M.; Holbeck, S.; Sausville, E. A. *Curr. Cancer Drug Targets* **2002**, *2*, 279−308. (b) Newman, D. J.; Cragg, G. M.; Snader, K. M. *Nat. Prod. Rep.* **2000**, *17*, 215−234. (c) Newman, D. J.; Cragg, G. M.; Snader, K. M. *J. Nat. Prod.* **2003**, *66*, 1022−1037. (d) Wilson, R. M.; Danishefsky, S. J. *J. Org. Chem.* **2007**, *72*, 4293−4305.

(2) (a) Boldi, A. M. *Curr. Opin. Chem. Biol* **2004**, *8*, 281−286. (b) Liao, Y.; Hu, Y.; Wu, J.; Zhu, Q.; Donovan, M.; Fathi, R.; Yang, Z. *Curr. Med. Chem.* **2003**, *10*, 2285−2316.

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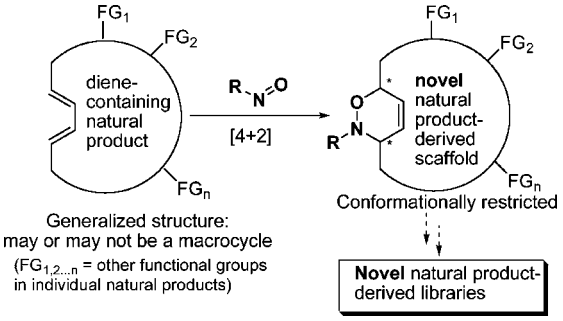
logues and introduces new functionality to rarely modified centers, diene or polyene components, of bioactive natural products in a highly regio- and stereoselective and chemically efficient fashion.

Nitroso Diels−Alder (NDA) reactions have been used as powerful synthetic tools in the formation of heterocycles, by direct incorporation of a 1,4-amino-oxo group.[3] We anticipated that, with the proper choice of nitroso dienophiles, NDA reactions with diene-containing natural products could selectively produce new conformationally restricted compounds with incorporation of N−O functionality, which might then serve as new evolvable scaffolds suitable for further elaboration to novel libraries (Scheme 1).

Scheme 1



In fact, some of the first applications of nitroso cycloadditions by Kirby and others involved reactions with natural products like thebaine[4] and ergosterol acetate.[5] However, other than a few related cases,[6] these remarkable reactions seldom have been exploited for derivatization of complex bioactive natural products. One major reason has been the lack of an ideal nitroso dienophile with an appropriate combination of reactivity and stability. For example, the generation of highly reactive and nonisolatable acylnitroso species **1** (Figure 1) was usually achieved by oxidation of

Figure 1. Structures of *C*-nitroso dienophiles.

the corresponding hydroxamic acids[3b] or thermal retro-Diels−Alder reactions,[7] which often caused compatibility problems with multiple functional groups in complex natural products. The goal we sought was to develop a facile nitroso Diels−Alder reaction that would not use additional reagents, catalysts, or extreme thermal conditions that often generate side products and necessitate extensive purification of products after the reaction.

Initial screening of nitroso reagents **1**, **2**, and **3a** (Figure 1) with the macrolide antibiotic turimycin H3 (**4**) (Figure 2)

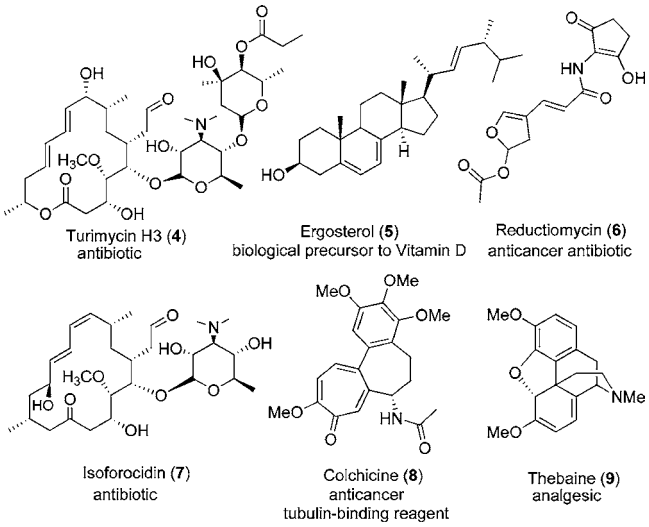


Figure 2. Structures of the selected diene-containing natural products.

showed that, while no reaction occurred upon treatment with nitrosobenzene (**2**), even at elevated temperatures, and reaction with a representative acylnitroso reagent **1**[8] was very low yielding (<5% based on LC/MS), the cycloaddition reaction of **4** and nitrosopyridine **3a** proceeded very smoothly and cleanly in CH_2Cl_2 at ambient temperature in 30 min (eq 1). The cycloadduct was isolated in 90% yield as a single isomer! Thus, the reaction was remarkably compatible with a tremendous array of sensitive functionality and stereogenic centers. Even more noteworthy was that, since there was no byproduct in this reaction, cycloadduct **10** was obtained in very pure form (>90%, ^{1}H NMR analysis, Supporting information) without any workup or purification. The complete regioselectivity of nitrosopyridine DA reactions with 2-substituted 1,3-cyclohexadienes was also observed by Yamamoto.[9] We envisioned that 2-nitrosopyridines, as stabilized forms of iminonitroso reagents, would constitute an ideal combination of reactivity and stability for reactions with complex natural products, relative to benzenenitroso and acylnitroso compounds, respectively.

(3) (a) Yamamoto, Y.; Yamamoto, H. *Eur. J. Org. Chem.* **2006**, 2031−2043. (b) Vogt, P. F.; Miller, M. J. *Tetrahedron* **1998**, *54*, 1317−1348.

(4) (a) Kirby, G. W.; Bentley, K. W.; Horsewood, P.; Singh, S. *J. Chem. Soc., Perkin Trans. 1* **1979**, 3064−3066. (b) Kirby, G. W.; Sweeny, J. G. *Chem. Commun.* **1973**, 704−705.

(5) Kirby, G. W.; Mackinnon, J. W. M. *J. Chem. Soc., Perkin Trans. 1* **1985**, 887−892.

(6) (a) Skoda-Foeldes, R.; Vandor, K.; Kollar, L.; Horvath, J.; Tuba, Z. *J. Org. Chem.* **1999**, *64*, 5921−5925. (b) Sheldrake, G. N.; Soissons, N. *J. Org. Chem.* **2006**, *71*, 789−791.

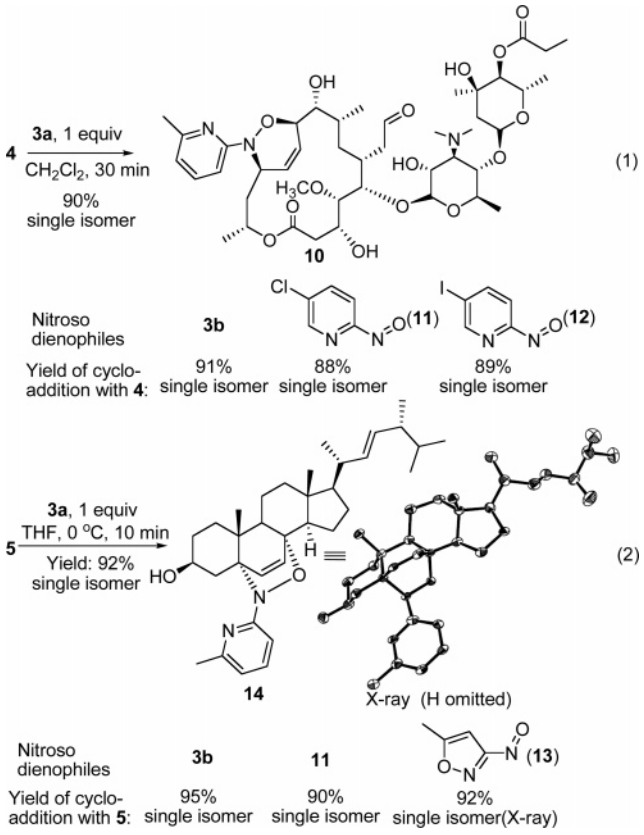
(7) Corrie, J. E. T.; Kirby, G. W.; Mackinnon, J. W. M. *J. Chem. Soc., Perkin Trans. 1* **1985**, 883−886.

(8) Acylnitroso **1** was generated in situ by thermal cycloreversion from the corresponding dimethylanthracene adduct.

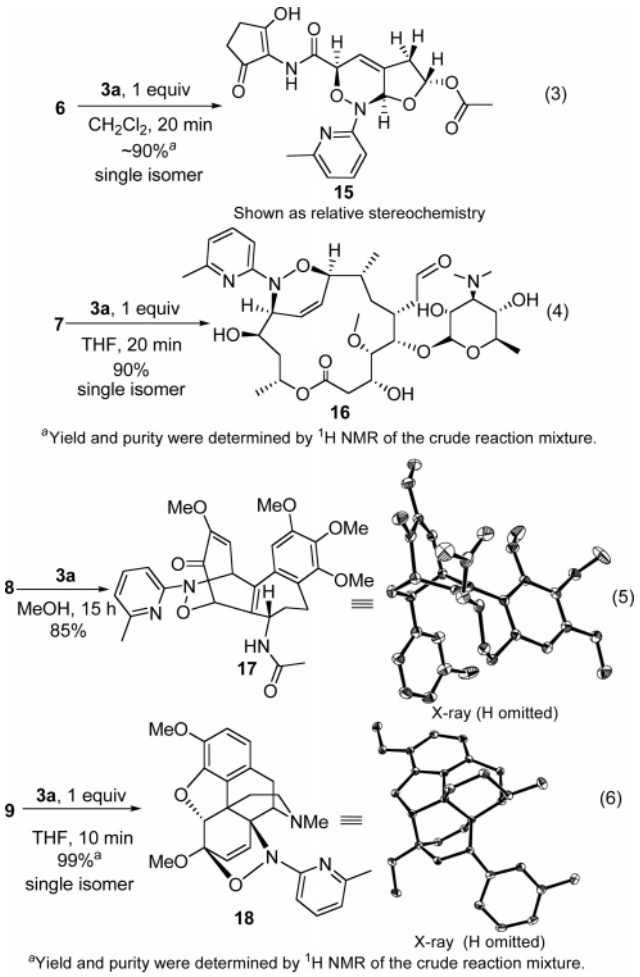
(9) Yamamoto, Y.; Yamamoto, H. *J. Am. Chem. Soc.* **2004**, *126*, 4128−4129.

We considered that early trials should include structurally and bioactively diverse natural products. Hence, six natural products (turimycin H3 **4**,[10] ergosterol **5**, reductiomycin **6**,[11] isoforocidin **7**,[12] colchicine **8**,[13] and thebaine **9**) were selected for our initial studies (Figure 2).

We performed the reaction of **4** separately with nitroso dienophiles **3b**, **11**, and **12**. We were pleased to find that the cycloadditions again proceeded to afford the corresponding oxazine cycloadducts as single isomers in ~90% yield (eq 1). The stereochemistry of cycloadduct **10** was established based on extensive 1D and 2D high-resolution NMR studies (Supporting Information). Next, ergosterol (**5**) was treated with nitrosopyridines **3a**, **3b**, and **11** at 0 °C in THF (eq 2). All of the reactions proceeded in 10 min to afford the corresponding cycloadducts as single isomers in more than 90% yield. The stereochemistry of cycloadduct **14** was revealed by X-ray diffraction studies. To explore an alternative form of stabilized iminonitroso species, 5-methyl-3-nitrosoisoxazole **13** was synthesized and treated with **5** to provide a 92% isolated yield of the cycloadduct as a single isomer that, based on X-ray crystallography, has the same regio- and stereochemistry as pyridine cycloadduct **14**.



Nitroso Diels−Alder reaction of reductiomycin **6**, which has an acyclic diene, was similarly effective and selective (eq 3). When reductiomycin was treated with **3** in THF at 0 °C, cycloadduct **15** was obtained as a single isomer in good purity (>90%, based on ^{1}H NMR analysis). While compound **15** was found to slowly decompose in solution phase, it was more stable in solid form. Since the absolute configuration of reductiomycin remains in question,[11] the relative stereochemistry of **15** was assigned mainly based on NOE correlations from a ROESY experiment (Supporting Information).



Related reactions of **3a** with the antibiotic isoforocidin (**7**), the tubulin-binding alkaloid colchicine (**8**), and the analgesic alkaloid thebaine (**9**) were investigated. Cycloaddition of isoforocidin proceeded smoothly in 20 min to give cycloadduct **16** in 90% yield as a single isomer (eq 4). On the other hand, colchicine (**8**) was found to reversibly react with **3a** at room temperature to regio- and stereoselectively provide cycloadduct **17** as a predominate isomer (eq 5, two isomers were observed in a >7:1 ratio based on LC/MS and ^{1}H NMR of the crude reaction mixture, while only one was isolated after silica gel chromatography). The NDA reaction of colchicine showed the same regio- and stereoselectivity relative to colchicine's framework as the previously reported Diels−Alder reactions with symmetrical dienophiles.[14] The stereochemistry of cycloadducts **16** and **17** was established

(10) Gebhardt, P.; Perner, A.; Graefe, U. *Chromatographia* **2004**, *60*, 229−234.

(11) (a) Cho, H.; Beale, J. M.; Graff, C.; Mocek, U.; Nakagawa, A.; Omura, S.; Floss, H. G. *J. Am. Chem. Soc.* **1993**, *115*, 12296−12304. (b) Donohue, J.; Smith, A. B., III; Carroll, P. J. *J. Crystallogr. Spectrosc. Res.* **1984**, *14*, 35−43.

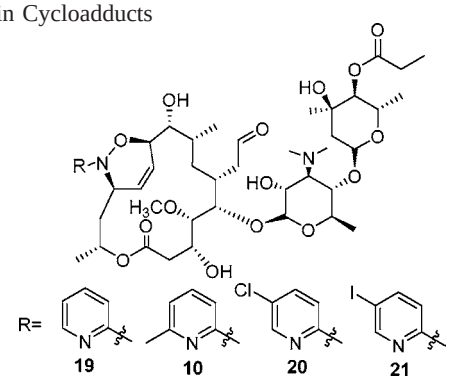
(12) Ramu, K.; Shringarpure, S.; Williamson, J. S. *Pharm. Res.* **1995**, *12*, 621−629.

(13) (a) Ravelli, R. B. G.; Gigant, B.; Curmi, P. A.; Jourdain, I.; Lachkar, S.; Sobel, A.; Knossow, M. *Nature* **2004**, *428*, 198−202. (b) Capraro, H. G.; Brossi, A. *Alkaloids* **1984**, *23*, 1−70.

by 2D high-resolution NMR studies (for **16**) and X-ray analysis of **17**, respectively. In the case of the NDA reaction of **3a** with thebaine (**9**), cycloadduct **18** was obtained after 10 min as a single isomer in quantitative yield based on ^{1}H NMR analysis of the crude reaction mixture. The configuration of cycloadduct **18** was unambiguously assigned based on X-ray diffraction analysis (eq 6).

Preliminary bioactivity evaluations of turimycin cycloadducts (**10**, **19**−**21**) showed an activity profile comparable to that of turimycin H3 (**4**) itself against a series of Gram-positive and Gram-negative bacteria, yeast, and fungi (Supporting information). Interestingly, cycloadducts **10**, **20**, and **21** showed moderate antiproliferative activity and cytotoxicity, while turimycin **(4)** was not active at all (Table 1).

Table 1. Antiproliferative Activity and Cytotoxicity of Turimycin Cycloadducts



compd no.	L-929 GI_{50} (μM)[a]	K-562 GI_{50} (μM)[a]	HeLa CC_{50} (μM)[a]	PC-3 IC_{50} (μM)[b]	MCF-7 IC_{50} (μM)[b]
4	>66	>66	>66	>50	>50
19	>100	>100	>100	>100	>100
10	31	34	44	20	14
20	15.6	15	23.5	16	11
21	30	20	40	10	6

[a] Assay performed at HKI (see Supporting Information for protocols). [b] Assay performed at UND (see Supporting Information for protocols).

This suggests that the nitroso heterocycle altered the activity profile of its parent natural product. Also noteworthy is the fact that the only difference among cycloadducts **10** and **19**−**21** is the substituent group in the pyridine ring, which changed the activity from none (**19**) to notable (**10**, **20**, and **21**). Further exploration of the effect of related functionalization and derivatization processes is in progress.

The excellent regio- and stereoselectivity of nitroso cycloadditions of all of the substrates examined here have clearly shown that 2-nitrosopyridines are very effective dienophiles with exquisite sensitivity to electronic and steric influences of complex dienes, which is probably derived from the asymmetric nature of 2-nitrosopyridines. On the other hand, the directing influences of interactive substituents on the natural dienes might also play a role for the reaction selectivity. Such effects have been noted in many heterofunctionalization reactions, such as the Henbest effect[15] in epoxidation of allylic alcohols. Additional studies with a broad array of diene-containing natural products will provide further indications of predictive trends with this chemistry. The advantages of this methodology include simple operation, mild reaction conditions, avoidance of the need of catalysts, excellent regio-, chemo-, and stereoselectivity, as well as superior chemical efficiency. In addition, in many cases, no byproducts are formed under the reaction conditions and cycloadducts were obtained in essentially pure form. We believe that the methodology presented herein provides a practical method to derivatize and functionalize complex diene-containing bioactive natural products at rarely modified diene motifs. Bringing together two molecular modules, an iminonitroso reagent and a diene-containing natural product, provides Modular Enhancement of Nature's Diversity (MEND). Further efforts to diversify the nitroso dienophile, to determine the scope of natural products, and to elaborate the cycloadducts, as well as evaluation of biological activity, are in progress and will be reported in due course.

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Supporting Information Available: Experimental procedures; structure elucidation of cycloadduct **10**, **15** and **16**; antimicrobial activity and antiproliferative and cytotoxicity assays protocols of turimycin H3 cycloadducts; crystallographic data of **14**, **S1**, **17**, and **18**; copies of ^{1}H NMR and ^{13}C NMR spectra of new compounds; copies of 2D-ROESY spectra of **10**, **15**, and **16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) Brecht, R.; Haenel, F.; Seitz, G.; Frenzen, G.; Pilz, A.; Massa, W.; Wocadlo, S. *Liebigs Ann./Recl.* **1997**, 851−857.

(15) (a) Henbest, H. B.; Wilson, R. A. L. *J. Chem. Soc.* **1959**, 1958−1965. (b) Hoveyda, A. H.; Evans, D. A.; Fu, G. C. *Chem. Rev.* **1993**, 1307−1370.