## Syntheses and Biological Activity Studies of Novel Sterol Analogs from Nitroso Diels—Alder Reactions of Ergosterol

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## ABSTRACT



A series of novel sterol analogs was prepared using nitroso Diels-Alder reactions with ergosterol. Most cycloaddition reactions proceeded in an excellent regio- and stereoselective fashion. Further N-O bond cleavage of cycloadducts generated compounds with biological activity in PC-3 and MCF-7 cancer cell lines.

Natural products and their derivatives represent the most prolific source of molecular diversity in drug discovery.<sup>1</sup> Functional-group transformation of natural products constitutes one of the main avenues for generating pharmacologically relevant compounds with altered and sometimes improved biological properties. Typical chemical derivatizations of natural products are often limited to standard modification of nucleophilic or electrophilic functional groups. Since many natural products contain multiple functional groups of the same or similar type, derivatization selectivity is often problematic. Hence, new derivatization methods are still needed. Our effort in this area has involved nitroso Diels-Alder (NDA) reactions as efficient methods for derivatization and functionalization of diene-containing natural products. We and others<sup>2</sup> have demonstrated that many complex diene-containing natural products readily undergo nitroso cycloadditions, generating 1-amino-4- hydroxy-2-ene heterocycle scaffolds with high regio- and stereoselectivity. Ergosterol (Figure 1), a natural sterol containing a conjugated diene, was





chosen for further exploration of this method for modular enhancement of Nature's diversity (MEND).<sup>2a</sup> Ergosterol **1a** (ergosta-5,7,22-trien-3 $\beta$ -ol) is biologically relevant as a precursor (a provitamin) to Vitamin D<sub>2</sub><sup>3</sup> and an integral component of fungal cell membranes. The presence of ergosterol in fungal cell membranes coupled with its

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absence in animal cell membranes makes it a useful target for antifungal drugs.<sup>4</sup> Several groups have shown that sterols with a heteroatom substituent at C-24 or C-25 are effective antifungal compounds.<sup>5</sup> Nitroso Diels–Alder reactions with ergosterol acetate **1b** were reported by Kirby et al.;<sup>2d,e</sup> however, this method was never exploited as a tool for derivatization of sterol compounds and the dienophiles used were often limited to transient aroyl nitroso species. Herein we report the syntheses of novel C5 and C8 disubstituted sterol analogs using nitroso Diels–Alder reactions of ergosterol, and its acetate, with acyl- and iminonitroso agents, and wish to demonstrate the utility of this methodology in natrual product derivatization by evaluation of their biological activity.

A series of hydroxamic acids  $2\mathbf{a}-\mathbf{e}$  was obtained by reaction of the corresponding methyl esters with hydroxylamine;<sup>6</sup> *N*-hydroxycarbamate **2f** was prepared from direct acylation of hydroxylamine with di-*tert*-butyl dicarbonate. Oxidation of compounds  $2\mathbf{a}-\mathbf{f}$  to acyl nitroso dienophiles  $3\mathbf{a}-\mathbf{f}$  with tetrabutylammonium periodate in the presence of ergosterol acetate **1b** gave the  $5\alpha$ -*N*- $8\alpha$ -*O*-adducts  $4\mathbf{a}-\mathbf{f}$ in various yields (Scheme 1, Table 1). The stereoisomeric

Table 1. Result	s of	Acylnitroso	Diels-Alder	Reactions
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entry	compd	adduct	yield (%)	dioxazine	yield (%)
1	2a	4a	29	6a	54
2	<b>2b</b>	<b>4b</b>	12	<b>6</b> b	$63^a$
3	<b>2c</b>	<b>4c</b>	74	6c	$0^b$
4	<b>2d</b>	<b>4d</b>	95	6d	$0^b$
5	<b>2e</b>	<b>4e</b>	93	<b>6e</b>	$0^b$
6	<b>2f</b>	<b>4f</b>	93	<b>6f</b>	$0^b$
<sup><i>a</i></sup> As a nonseparable mixture of dioxazine <b>6b</b> and $5\alpha$ - <i>O</i> - $8\alpha$ - <i>N</i> -adduct <b>5b</b> <sup><i>b</i></sup> Not detected					

configuration of 4a-f was assigned by <sup>1</sup>H NMR studies based on literature analysis of 4a.<sup>2d</sup> In the case of benzohydroxamic acid 2a (entry 1, Table 1), similar to Kirby's observation,<sup>2d</sup> dioxazine **6a** was isolated as the major product in 54% yield, with adduct **4a** as the minor product in 29% yield. Compound **6a** was formed as a result of [3, 3] sigmatropic rearrangement of  $5\alpha$ -O-8 $\alpha$ - *N*-adduct **5a**, a much more sterically congested isomer compared to adduct **4a**.<sup>2d</sup> Reaction with 4-methylbenzohydroxamic acid **2b** gave a similar result, although dioxazine **6b** was only isolated as a mixture with adduct **5b** (entry 2). In contrast, oxidation of 2-methoxybenzohydroxamic acid **2c**, phenylacetohydroxamic acid **2d**, L-leucine-derived hydroxamic acid **2e** and *tert*-butyl *N*-hydroxycarbamate **2f** gave **4c**-**f** as the sole products in good to excellent yields (entry 3–6). Further K<sub>2</sub>CO<sub>3</sub> mediated deacetylation of **4a**-**f**, **6a** gave compounds **7a**-**f** and **8a** in 90% average isolated yields.

A nitroso Diels–Alder reaction with ergosterol **1a** was also investigated using nitrosobenzene **8** as a representative aryl nitroso dienophile (entry 1, Scheme 2). The NDA reaction occurred based on TLC and <sup>1</sup>H NMR analysis; however, adduct **11** was not able to be isolated in pure form by chromatography. Additionally, cycloadditions with various iminonitroso agents **10**, mainly 2-nitrosopyridine derivatives, were examined (Scheme 2). Those nitroso compounds were prepared from aminoheterocyclic precursors **9** in a two-step sequence (*N*, *N*-dimethyl sulfilimine intermediate formation, followed by oxidation using *m*-CPBA).<sup>7</sup> Most of the

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iminonitroso compounds could be isolated and stored in pure form, except for 10k. The NDA reactions between ergosterol 1a and iminonitroso species 10a-j usually were complete within 30 min at 0 °C. In each case, the cycloadducts 11a-j were obtained as single  $5\alpha O-8\alpha N$ -adducts in good to excellent yields after column chromatography (Scheme 2). The regio- and stereochemistry of the adducts was assigned based on our previous report and NMR studies.<sup>2a</sup> The instability of nitroso agent 10k required its immediate use, so it was trapped in situ with ergosterol acetate 1b. Interestingly, both regioisomers 11k and 11l were obtained in a 8.5:1 ratio in 95% combined yield (entry 12, Scheme 2). The major adduct **11k** has the same stereochemistry as cycloadducts **11a**-j, based on X-ray crystallography (Figure 2). Further deacetylation of 11k generated compound 11m. Clearly, 2-nitrosopyridines 10, as stabilized forms of iminonitroso reagents, constitute an ideal combination of reactivity and stability for NDA reactions with ergosterol, relative to benzenenitroso 8 and acylnitroso moieties 3, respectively

We next set out to demonstrate the synthetic utility of ergosterol nitroso cycloadducts through N-O bond cleavage, a method for generating 1,4-amino alcohols suitable for



Figure 2. X-ray of cycloadduct 11k with ergosterol acetate 1b.

further diversification. Adduct **11a** was chosen as model for optimizing reaction conditions. Reactions with  $SmI_2/THF$ ,<sup>8</sup> Zn/CH<sub>3</sub>COOH, Mo(CO)<sub>6</sub>,<sup>9</sup> etc. were attempted; however, all of these conditions failed to yield desired product **12a** (Supporting Information). Detailed screenings showed that Na amalgam<sup>10</sup> successfully reduced the N–O bond of **11a** to give **12a** in 82% yield (entry 1, Scheme 3). Then, a series



of ergosterol nitroso adducts was subjected to the optimized Na(Hg) conditions. While low transformation was found with acylnitroso adducts **7d** and **7f** (entries 2–3), moderate yields were achieved for pyridinylnitroso adducts. Not surprisingly, these conditions also reduced the -Cl substituent (entries 5–6).

Ergosterol peroxide  $13^{11}$  is a natural sterol derivative that possesses a variety of biological properties including immunosuppressive and antitumor activity. Simply removing the *N*-Boc group of cycloadduct **7f** afforded a quick access

<sup>(8)</sup> Revuelta, J.; Cicchi, S.; Brandi, A. Tetrahedron. Lett. 2004, 45, 8375.

to compound **14** as an isoelectronic analog of ergosterol peroxide **13** (Scheme 4).



Broad antibacterial testing of these novel sterol analogs using the argar diffusion method revealed no significant activity against Gram-positive or Gram-negative organisms. However, N-O cleaved analogs had their largest zone of inhibition against the fungal strain, Sporobolomyces salmonicolor, whereas ergosterol, 1a, and adducts did not (Supporting Information). More interestingly, N-O reduced compounds 12, 12a-b,e,h,j, along with ergosterol acylnitroso adducts 7a-f, showed growth inhibitory activity in MCF-7 (breast cancer) and PC-3 (prostate cancer) tumor cell assays, while parent erogsterol, 1a, and most pyridinylnitroso adducts were relatively inactive (entries 1, 9, 10, 12, 14-16, Table 2). All N-O reduced analogs reached the low micromolar range of inhibition against both PC-3 and MCF-7 cells (entries 17-22). In general, all active compounds showed significantly improved inhibitory activity against the MCF-7 cell line. These biological assays clearly suggest that the nitroso heterocycle changed the biological activity profile of its parent natural product.

In summary, we have reported that nitroso Diels-Alder reactions between ergosterol (or ergostrol acetate) and 2-nitrosopyrdines as well as acylnitroso agents proceeded with excellent regio- and stereoselectivity. A new class of sterol analogs that show encouraging anticancer activity was generated through N-O bond cleavage of the cycloadducts. The method presented herein provides a novel and efficient way to derivatize diene-containing natural products. Efforts to determine the exact mechanism

Table 2	. Results	of Anticancer	Screenings <sup>a</sup>
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		% inhibition at 20 $\mu\mathrm{M}$		$\rm IC_{50}~(\mu M)$	
entry	compd	PC-3	MCF-7	PC-3	MCF-7
1	1a	10	15	_	_
2	7a	64	97	_	14
3	7b	40	97	16	14
4	7e	94	97	11	6
5	7d	76	96	12	14
6	7e	57	95	12	8
7	<b>7f</b>	73	89	17	20
8	14	48	82	>20	>20
9	11a	<10	15	_	_
10	11b	15	29	_	_
11	11c	76	100	_	15
12	11d-e, g-h	<10	<10	_	_
13	11i	22	90	_	17
14	11j	15	<10	_	_
15	11k	15	29	_	_
16	11m	8	20	_	_
17	12	94	94	9	6.5
18	12a	100	100	6	2
19	12b	100	100	6	3.5
20	12e	100	100	7	3
21	12h	100	100	10	2.5
22	12j	100	100	4	2.5
<sup><i>a</i></sup> Tri	chostatin A was	used as the	positive control	(MCF-7	$IC_{50} = 16$

nM, PC-3 IC<sub>50</sub> = 160 nM).

of anticancer action and further diversification of ergosterol adducts are in progress.

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**Supporting Information Available:** Full experimental procedures, characterization data and copies of <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra, protocols of antibacterial and anticancer assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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