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# Synthesis of Nodulisporic Acid 2″-Oxazoles and 2″-Thiazoles

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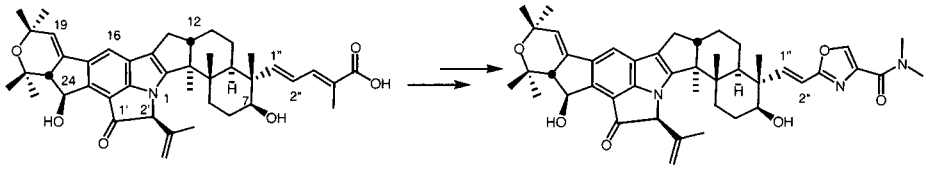
## ABSTRACT




**The semisynthetic conversion of nodulisporic acid A (1) into a set of three heterocyclic side chain derivatives provided compounds, highlighted by 6, with an improved spectrum of ectoparasiticidal activity and pharmacokinetic profile relative to the natural product.**

A number of factors have led to significant changes in the companion animal veterinary drug market over the past 5 years. One of these, the altered perception of dogs and cats as family members, is reflected in the way people commonly apply human standards of medicine to the care of their pets. In addition, the growing prevalence of tick-borne Lyme disease has become a serious human health concern that is directly impacted by indoor/outdoor pets. As a consequence, veterinary drugs with adulticidal efficacy against fleas and ticks on companion animals have received much attention in the popular press and have created a substantial consumer market for flea and tick control.[1,2]

Recently isolated at Merck, nodulisporic acid A (**1**) represents a new member within a class of structurally novel indole diterpene natural products produced by endophytic fungi.[3] A number of structurally related natural products such as janthitrem E (**2**)[4] and penitrem D (**3**)[5] shown in Figure 1 are known tremorgens, and their neurotoxicity has been ascribed to the presence of an angular hydroxyl moiety at C9 (nodulisporic acid numbering). Among other structural dissimilarities between nodulisporic acid A and these tremorgens, **1** lacks substitution at C9 and no overt toxicity has been noted.

A unique characteristic of **1** is that it modulates a subset of the invertebrate-specific glutamate-gated chloride ion channels targeted by ivermectin.[6] This inherent ion channel selectivity suggests that **1** may be dosed in dogs and cats at higher levels than viable with ivermectin, conferring increased antiparasitic activity with an improved therapeutic

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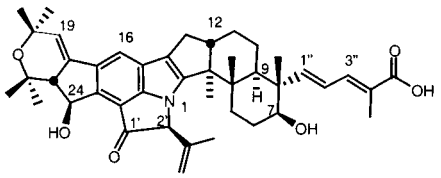
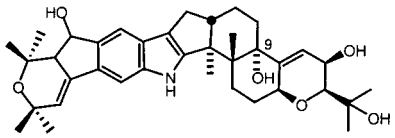
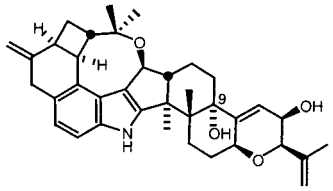


Nodulisporic Acid A (**1**)



Janthitrem E (**2**)



Penitrem D (**3**)

**Figure 1.** Indole diterpene natural products.

index. Recently, Shoop and co-workers have reported that **1** exhibits potent ectoparasiticidal properties and systemically controls flea infestations on dogs after oral dosing at 15 mg/kg absent of mammalian toxicity.[7] However, this efficacy had modest duration of action due to a shortened half-life of **1** in the dog (Table 1). Initial structure−activity studies

**Table 1.** Functional, in Vivo, and Pharmacokinetic Data for Nodulisporic Acid A (**1**) and Derivatives **4**−**7**

| | compound | | | | |
|---|---|---|---|---|---|
| | **1** | **4** | **5** | **6** | **7** |
| flea membrane (ppm)[a] | 1 | ∼0.1 | 10 | 1 | 1 |
| tick/mouse (mg/kg)[b] | >2.5 | 2.5 | ≤2.5 | ∼0.6 | ∼1.2 |
| flea/dog (weeks)[a] | ∼0.5[d] | <1[e] | <2[f] | ∼2[f] | |
| tick/dog (weeks)[b] | | | <2[f] | ∼4[f] | <2[f] |
| log *D*[c] | 5.3 | 6.4 | 7.1 | 6.2 | 6.2 |
| $t_{1/2}$/dog (days) | 0.8[f] | 3.2[f] | 6.1[f] | 2.8[f] | |

[a] *Ctenocephalides felis.* [b] *Dermacentor variabilis.* [c] RPHPLC (C18) pH 7.3, 65:35 MeOH:buffer. [d] Single po dose at 15 mg/kg. [e] Single po dose at 2.5 mg/kg. [f] Single po dose at 10 mg/kg.

demonstrated that simple nodulisporamides (such as **4**) conferred a 10-to-100-fold increase in antiflea efficacy with an extended duration of action in dogs (Table 1).[8,9]

Further studies targeted compounds with an improved balance of antiflea, antitick, and pharmacokinetic properties, resulting in the evolution of more sophisticated side chain designs. One approach was to delineate the effect of heterocycle insertion into the diene of the dienamide side chain of **4** (Figure 2). Oxazoles such as **5** and **6**, or thiazoles

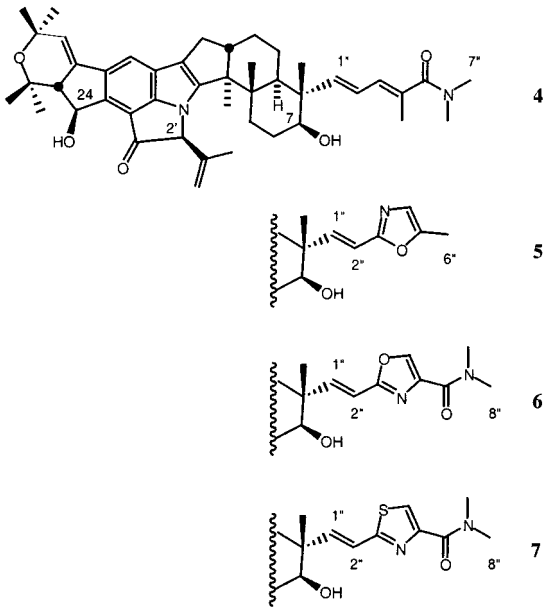


**Figure 2.** Nodulisporic acid 2″-oxazoles and 2″-thiazoles.

typified by **7**, were designed on the basis of their modification of side chain polarity and approximation of side chain length, relative to **4**.

Access to these heterocyclic hybrids required mild synthetic routes that were free of base-induced 2′-epimerization, acid-induced 23,24-dehydration, base-mediated elimination of 24-silyloxy derivatives, 1′-ketone reduction, and 2,14-indole oxidative cleavage. Recent reports by Wipf, Williams, and Charette have described new methods to construct these ring systems under mild conditions. Fortunately, these methodologies were quite conducive to the syntheses of **5**, **6**, **7**, and related derivatives, resulting in several well-balanced analogues of the natural product (**1**).

Methyl oxazole **5** was efficiently prepared via the method reported by Wipf.[10] For example, 7,24-protected 3″-carboxylic acid **8** was generated from **1** in three steps,[11,8a] and the subsequent $\beta$-hydroxy amide was oxidized and cyclodehydrated to form **5** after 7,24-silyl ether deprotection (Scheme 1). Six alkyl-substituted oxazoles other than **5** were

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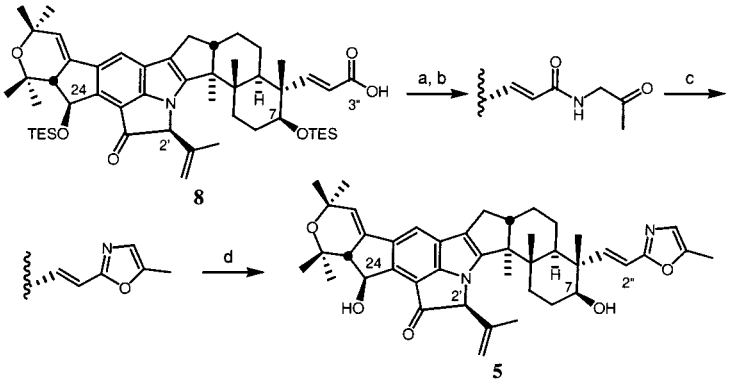
**Scheme 1**[a]



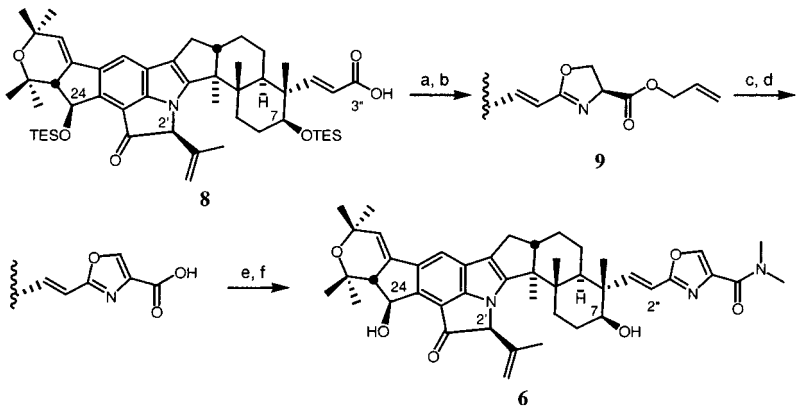

[a] Reagents and conditions: (a) 10 equiv of 1-amino-2-propanol, 1.1 equiv of BOP, 2 equiv of HOBt, 0.03 M $CH_2Cl_2$, 0 → 23 °C, 3 h, 92%; (b) 3 equiv of Dess−Martin periodinane, 10 equiv of pyridine, 0.01 M $CH_2Cl_2$, 0 °C, 45 min → 23 °C, 2 h, 80%; (c) 3 equiv of $(BrCl_2C)_2$, 3 equiv of $Ph_3P$, 8 equiv of *i*-$Pr_2NEt$, 0.01 M $CH_2Cl_2$, 0 °C, 45 min → 23 °C, 5 h, 60%; (d) 2.2 equiv of PPTS, 0.01 M EtOH, 23 °C, 16 h, 83%.

prepared in this manner from **8**. While methyl oxazole **5** displayed decreased flea activity, the tick activity and half-life of **5** were improved relative to **1** and **4**, setting a good precedent for the study of additional oxazole derivatives (Table 1).

To this end, intermediate **8** was efficiently converted to oxazoline allyl ester **9** following Wipf's protocol of the dehydration of an intermediate $\beta$-hydroxy amide derived from serine allyl ester (Scheme 2).[12] The following oxidation of **9** to the desired oxazole proved, however, to be problematic.[13,14] Ultimately, a timely report by Williams, describing a new methodology for heterocycle oxidation,[15] enabled a high-yielding synthesis of the requisite oxazole absent of any 2′-epimerization. After allyl ester deprotection, 18 carboxamido oxazoles were generated and desilylated at C7,24 to provide analogues exemplified by **6**. While equipotent to **1** in intrinsic activity, dimethylamido oxazole **6** proved to be a superior compound in both flea and tick efficacy in vivo with an improved pharmacokinetic profile, showing good duration of action in the dog (Table 1).

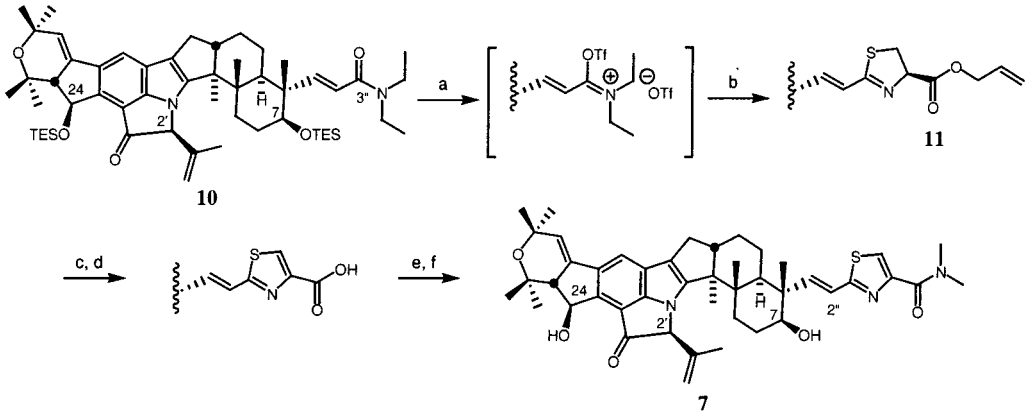
With this promising result of **6** in hand, attention was turned to the comparison of related carboxamido thiazoles. Methodology recently reported by Charette[16] was applied successfully to the synthesis of thiazoline **11** (Scheme 3). The use of buffered low temperature conditions smoothly provided **11** via the addition of cysteine allyl ester to the $Tf_2O$-activated amide **10**, absent of any competing side reactions at C2′, C24, or C2,14.[17] In a manner similar to the synthesis of **6**, thiazoline **11** was subsequently converted to 14 carboxamido thiazoles represented by **7**. The biological profile of thiazole **7** was ultimately inferior relative to that of oxazole **6** (Table 1).

**Scheme 2**[a]




[a] Reagents and conditions: (a) 2 equiv of serine hydrochloride allyl ester, 1.1 equiv of BOP, 2 equiv of HOBt, 6 equiv of *i*-$Pr_2NEt$, 0.03 M $CH_2Cl_2$, 0 → 23 °C, 3 h, 90%; (b) 1.2 equiv of Burgess reagent (in 5 portions), 4 Å sieves, 0.04 M dioxane, 23 °C, 2 h → 50 °C, 2 h, 75%; (c) 1.2 equiv of $BrCCl_3$, 1.2 equiv of DBU, 0.03 M $CH_2Cl_2$, 0 °C, 12 h, 80%; (d) 3% $Pd(PPh_3)_2Cl_2$, 1.4 equiv of $Bu_3SnH$, 0.03 M $CH_2Cl_2$, 0 °C, 10 min, 99%; (e) 10 equiv of 2.0 M $Me_2NH$−THF, 1.2 equiv of BOP, 2 equiv of HOBt, 0.03 M $CH_2Cl_2$, 0 → 23 °C, 10 h, 80%; (f) 2.2 equiv of PPTS, 0.03 M EtOH, 23 °C, 12 h, 85%.

**Scheme 3**[a]




[a] Reagents and conditions: (a) 1.8 equiv of triflic anhydride, 10 equiv of pyridine, 0.04 M $CH_2Cl_2$, −78 °C, 20 min → 0 °C, 4 h; (b) 3 equiv of cysteine allyl ester, −78 → 23 °C, 14 h, 90%; (c) 1.4 equiv of $BrCCl_3$, 1.4 equiv of DBU, 0.08 M $CH_2Cl_2$, 0 °C, 12 h, 95%; (d) 3% $Pd(PPh_3)_2Cl_2$, 1.3 equiv of $Bu_3SnH$, 0.02 M $CH_2Cl_2$, 0 °C, 10 min, 99%; (e) 10 equiv of 2.0 M $Me_2NH$−THF, 1.2 equiv of BOP, 2 equiv of HOBt, 0.01 M $CH_2Cl_2$, 0 → 23 °C, 10 h, 90%; (f) 3 equiv of PPTS, 0.02 M EtOH, 23 °C, 12 h, 76%.

The synthetic routes described in this Letter demonstrate the utility of the highlighted Wipf, Williams, and Charette methodologies in complex natural product synthesis and medicinal chemistry. Testament to this mild yet robust chemistry is the gram-scale generation of intermediates en route to **5**, **6**, and **7**, which in turn have been consistently prepared in high yield and in excess of 0.5 g each. Heterocyclic derivatives **5**, **6**, and **7** were evaluated in vivo, identifying **6** as a well-balanced compound with an overall improvement over **1** and **4** for oral flea and tick control in companion animals.

Generous supplies of nodulisporic acid A were provided by Dr. John Ondeyka of Merck Research Laboratories, Merck & Co., Inc., Rahway, NJ.

**Supporting Information Available:** Compounds **5**, **6**, and **7** were characterized by $^1$H NMR and LCMS. This material is available free of charge via the Internet at http://pubs.acs.org.

OL016651X

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(14) Selenoxide elimination was not possible due to exclusive *O*-selenation of **9** which could not be suppressed. Evans, D. A.; Gage, J. R.; Leighton, J. L. *J. Am. Chem. Soc.* **1992**, *114*, 9434.

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(16) Charette, A. B.; Chua, P. *J. Org. Chem.* **1998**, *63*, 908.

(17) The use of fresh aminothiol, prepared immediately prior to its addition to the activated diethylamide **10**, resulted in a cleaner preparation and higher yields of **11**. In practice, commercially available (Novabiochem) *N*-BOC-*S*-trityl-L-cysteine was converted to its allyl ester (1.2 equiv of $CsHCO_3$, 5 equiv of allyl bromide, 0.3 M DMF, 0 °C, 1 h, 80%) followed by trityl group removal to form the isolable and storable silver thiolate (1.2 equiv of $AgNO_3$, 1.2 equiv of pyridine, 0.04M 1:1 MeOH−EtOAc, 23 °C, 24 h, 99%) which was thoroughly dried under vacuum. The *N*-BOC-*S*-Ag salt of cysteine allyl ester is stable at 0 °C for several months. This silver salt was converted to the free thiol (0.05 M EtOAc, excess $H_2S_{(g)}$, 23 °C, 3 min, 95%) and then to the free amine (degassed 0.2 M 20% TFA−$CH_2Cl_2$, 0 °C, 4 h, 80%) to provide the requisite cysteine allyl ester.