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Side-Chain Homologation of Nodulisporic Acid: Synthesis of Potent New Dienyl Derivatives

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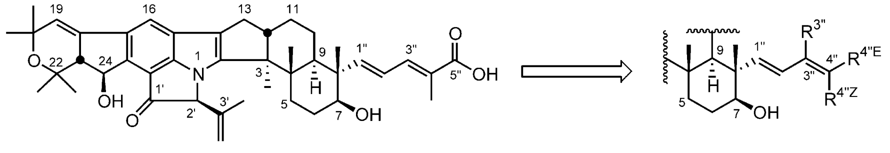
Abstract—A series of new, diene-modified nodulisporic acid analogues (**2**) bearing diverse functionality at the 3″- and 4″-sites was efficiently prepared from the 3″-aldehyde **3**. Biological evaluation of these synthetic nodulisporic acid analogues for systemic flea efficacy identified potent compounds and further clarified the structural requirements for ectoparasite activity. © 2002 Elsevier Science Ltd. All rights reserved.

The discovery[1,2] of the fungal metabolite nodulisporic acid A (NsA A, **1**) and its potent insecticidal activity,[3] including systemic activity against fleas fed drug-treated bovine blood,[4] was recently revealed by Merck scientists. The intriguing molecular architecture and promising biological activity profile of this new class of indole diterpenes also inspired imaginative efforts directed towards the total synthesis of **1**.[5] NsA A exerts its striking insecticidal activity by selectively modulating an invertebrate specific glutamate-gated chloride ion channel for which no mammalian homologue exists.[6] A medicinal chemistry program was initiated to delineate NsA's pharmacophore; this research identified new, side-chain modified nodulisporamides with enhanced efficacy against blood-sucking ectoparasites.[7,8] NsA A and several related nodulisporamides were subsequently shown to be safe and effective in a dog model.[4,9] In this model, the *N*-methyl-*N*-ethyl-nodulisporamide derivative conferred 14 days protection against fleas following a single oral dose.[9] In continuation of our drug discovery program, new NsA dienyl derivatives **2** were prepared wherein all structural modifications were restricted to the terminal 3″,4″-olefin region. These efforts led to the discovery of novel nodulisporamides and nodulisporates with significant potency in a flea membrane feeding assay and permitted the biological consequences of the introduction of diverse functionality at the 3″ and 4″ positions to be further elucidated.

Clearly, olefination reactions of our key intermediate, the side-chain truncated 3″-aldehyde **3**,[7,10] represents an appropriate strategy for the synthesis of the substituted



Nodulisporic Acid A (**1**)

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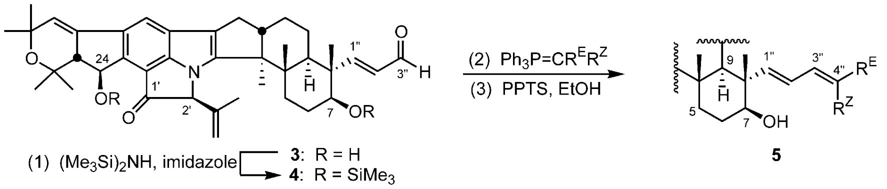
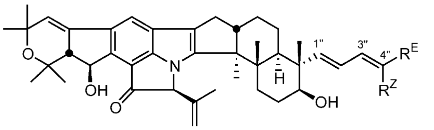


Scheme 1.

dienes **5** (Scheme 1). The Wittig, Horner–Emmons, and Knoevenagel reactions are classical methods for the stereoselective generation of either *cis*- or *trans*-olefins under conditions suitably mild for use with acid- and base-sensitive natural products such as NsA. We previously disclosed[10] a high-yielding, single-step conversion of **1** to **3**, prepared via $KMnO_4$-mediated oxidative degradation. An extensive series of mono- and disubstituted dienes was prepared, focusing primarily on carbonyl-containing targets; illustrative examples are presented in Tables 1 and 2.

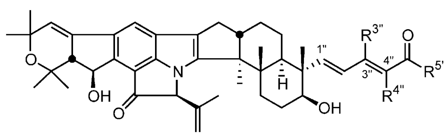
Both hydroxyls of aldehyde **3** were silylated[10] using hexamethyldisilazane in near quantitative yield to generate **4**. Reaction of bis-protected **4** with methylenetriphenylphosphorane, the simplest Wittig reagent, gave the terminal olefin **10** in very low yields (10%), accompanied by significant epimerization at 2′ and overreaction at the 1′-carbonyl. Yields of this 4″-unsubstituted diene were superior when **4** was instead treated with the Petasis reagent, $(Cp)_2TiMe_2$,[11] in THF-toluene at 70 °C for 2 h (65%). Chain extension of unprotected **3** using unsubstituted, stabilized Wittig ($Ph_3P{=}CHCO_2R$ or $Ph_3P{=}CHC(O)R$, toluene, 4–12 h, 26–43%) or Horner–Emmons reagents {$(MeO)_2P(O)CH_2CO_2R$, $(EtO)_2P(O)CH(F)CO_2Et$ or $[(EtO)_2P(O)]_2O$, LDA, THF, −78 °C → 25 °C, 1–4 h, 45–65%} proceeded smoothly to generate the desired dienes **5** where $R^Z = H$ or F, in which the more stable *E*-configuration predominated. Unwanted side reactions again occurred competitively at the 1′-carbonyl, producing biologically uninteresting 1′-desoxo products (data not shown) to appreciable extents (5–30%). Use of less nucleophilic, α-substituted Wittig reagents[12] [$Ph_3P{=}C(R)CO_2R$, benzene, 4–12 h, 70–80%] or bis-silylated aldehyde **4** generally eliminated the undesired secondary olefinations at 1′, forming the desired dienyl products, again with the *E* configuration predominating in comparable yields. Use of alternate solvents, excess olefinating reagents or elevated temperatures led to increasing quantities of

Table 1. 4″-Monosubstituted NsA derivatives[15,16]



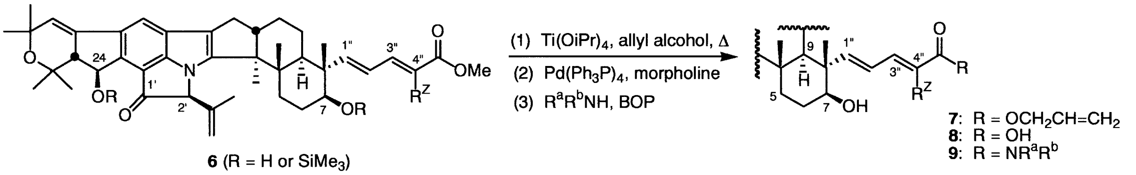
Compd	R^E group	R^Z group	Flea (ppm)
1	CO_2H	Me	1
10	H	H	10
11	$C(O)CH_3$	H	10
12	CO_2H	H	~10
13	CO_2Et	H	1
14	$CO_2CH_2CH{=}CH_2$	H	~1
15	CO_2tBu	H	10
16	CO_2CH_2Ph	H	10
17	$C(O)NH_2$	H	~1
18	H	$C(O)NH_2$	10
19	C(O)NH-Et	H	1
20	C(O)NH-*t*Bu	H	1
21	$C(O)NHCH_2Ph$	H	~10
22	$C(O)NMe_2$	H	~0.1
23	C(O)N(Me)Et	H	~1
24	C(O)(*N*-1-pyrrolidinyl)	H	1
25	$P(O)(OEt)_2$	H	10
26	C(O)NH-Et	F	~1
27	C(O)NH-*t*Bu	F	~1
28	$C(O)NMe_2$	CN	1
29	C(O)(*N*-1-pyrrolidinyl)	CN	10
30	$C(O)NMe_2$	C(O)Me	1
31	2-Benzimidazolyl	CN	~10

Table 2. New 4″- or 3″-disubstituted NsA analogues[15,16]



Compd	$R^{3''}$	$R^{4''}$	$R^{5''}$	Flea (ppm)
32	H	OMe	OH	~10
33	H	OMe	NH_2	~10
34	H	OMe	NH-Et	~10
35	H	OMe	NH-*t*Bu	~1
36[a]	H	OMe	NH-*t*Bu	>10
37	H	Et	OH	10
38	H	Et	NH_2	~1
39	H	Et	NH-Et	1
40	H	Et	NH-*t*Bu	1
41	H	Et	NMe_2	1
42	H	Et	*N*-1-pyrrolidinyl	~10
43	H	*n*Pr	OH	>10
44	H	*n*Pr	NH_2	10
45	H	*n*Pr	NH-Et	1
46[b]	H	*n*Pr	NH-Et	10
47	H	*n*Pr	NH-*t*Bu	~10
48	H	$CH_2CH{=}CH_2$	OMe	10
49	H	$CH_2CH{=}CH_2$	NH_2	~1
50	H	$CH_2CH{=}CH_2$	NH-Et	1
51	H	$CH_2CH{=}CH_2$	NH-*t*Bu	10
52	H	$CH_2CH{=}CH_2$	NMe_2	~1
53	H	*n*Bu	NH-Et	~1
59	H	*n*Bu	NH-*t*Bu	10
60	Me	H	OH	~10
61	Me	H	NH-Et	~10
62	Me	H	NH-*i*Pr	1
63	Me	H	NH-*c*Pr	1
64	Me	H	NH-*t*Bu	~10

[a] 23,24-dehydro.
[b] 2′-*epi*.

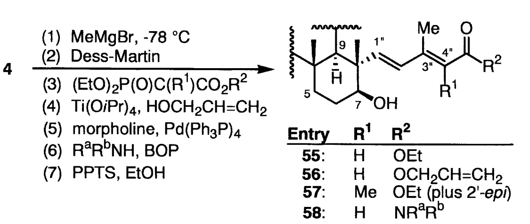


Scheme 2.

2′-*epi* products. The 7- and 24-OTMS groups were efficiently cleaved by treatment with PPTS in either EtOH or MeOH, essentially quantitatively. Alternatively, Knoevenagel condensations using **3** under standard conditions (CH_2Cl_2, 12 h, 0.5 equiv piperidinium acetate) with appropriate nucleophiles [$RO_2CCH_2C(O)R$, $R(O)CCH_2CN$, $ArCH_2CN$, etc.] produced **28**–**31** in adequate yields (29–60%).

In the case of **6** (Scheme 2), where R^Z (or R^E) was CO_2Me or CO_2Et, saponification to liberate the corresponding carboxylic acid was not viable; instead, epimerization at 2′ or dehydration at 24 rapidly occurred. Rather, transesterification to the corresponding allyl ester was accomplished by heating **6** at temperatures between 80 and 120 °C, depending on the specific derivative, in neat allyl alcohol in the presence of one equivalent of $Ti(OiPr)_4$.[13] This remarkable process generated the desired allyl esters (**7**), often in near quantitative yields, without concomitant deleterious eliminations at 24, epimerization at 2′ or diene *E*–*Z* olefin isomerizations. The allyl esters thus obtained were readily deprotected[14] using catalytic $Pd(Ph_3P)_4$ and stoichiometric $n Bu_3SnH$ to yield the requisite carboxylic acids **8**, which were cleanly converted into a series of amides (**9**) mediated by BOP with an appropriate amine nucleophile.

The 4″-methyl group of NsA A also was shifted to the 3″-position and, in conjunction with this methyl group shift, the corresponding 3″,4″-dimethyl homologues were prepared. The synthesis of these new 3″-modified derivatives is illustrated in Scheme 3 and representative examples (e.g., **60**–**64**) may be found in Table 2. Treatment of silylated aldehyde **4** with methyl Grignard in Et_2O at low temperature yielded the desired 3″-alcohol as a separable diastereotopic mixture (~1/1 ratio, 85% conversion). Little over-addition of the Grignard reagent at 1′ was noted, nor did significant epimerization at 2′ occur using these conditions. These intermediate alcohols were immediately subjected to Dess–Martin reagent mediated allylic oxidation to produce the corresponding 3″-methyl ketone **54** (70% yield, not shown). Although stabilized Wittig reagents completely failed to react with **54**, Horner–Emmons olefination proceeded slowly [$(MeO)_2P(O)CH_2CO_2Et$, NaH, THF, 60 °C, 3 h] forming the desired 3″-monomethylated dienoate **55**. This coupling reaction required the use of DMF as a cosolvent in THF (1/1 ratio); yields were poor (35%) and extensive epimerization at 2′ was observed. While an identical protocol was employed to synthesize the 3″,4″-dimethyl adduct **57**, due to steric congestion, this olefination was even more recalcitrant than the previous one, requiring longer reaction times (100 °C, 18 h) with progressively poorer yields (12%), generating **57** with essentially no *E*/*Z* selectivity (~1/1) and predominantly as its undesired, biologically uninteresting 2′-epimer. Transesterification of **55** to allyl ester **56** proceeded smoothly (90%); this ester was readily converted to its respective acid (85% from **55**) and amide derivatives (**58**) as described previously. Transesterification of **57** under identical conditions led only to degradation products.



Scheme 3.

Biological evaluation of the newly prepared nodulisporic acid dienyl derivatives was accomplished using a membrane feeding assay wherein adult fleas ingest drug-treated bovine blood.[4] The selected examples presented in Tables 1 and 2 serve to illustrate specific points and trends regarding systemic flea activity as comparatively minor structural substitutions at the 4″-position of newly prepared nodulisporic acid derivatives exerted profound effects on the observed biological activity and several previously noted[7] observations were recapitulated. Specifically, epimerization at 2′ (**45** vs **46**) or elimination of the 24-hydroxyl (**35** vs **36**) sharply decreased flea activity. New NsA analogues with the *E* configuration were more efficacious than those with the *Z* configuration, as clearly illustrated with the 4″-carboxamides **17** (~1 ppm) and **18** (10 ppm). The unsubstituted diene **10** was less potent than **1** against fleas, as were all 4″-ketones and most 4″-carboxylic acids, particularly those acids bearing bulkier 4″-substituents (**12** vs **32**, **37**, **43**) or the more lipophilic 4″-ketones (compounds not shown). Indeed, only the smallest of the ketones prepared, the 4″-methyl ketone **11**, had any flea activity at the highest routine screening level of 10 ppm. Dienoates with $R^Z = H$ bearing small ester groups retained good flea activity in the flea assay; progressive increases in ester steric bulk from ethyl to *t*Bu or benzyl promptly decreased flea efficacy (e.g., **13**–**16**) and dienoates bearing 4″-functionality other than hydrogen were similarly less active (i.e., **48**). Substitution of the phosphonate group $P(O)(OEt)_2$ (**25**) as a surrogate for ethyl ester **13** only served to decrease flea potency.

Fortunately, however, many dieneamides, including both those with and without an additional $4''$-alkyl substituent, exhibited good systemic activity against fleas. This was particularly true for nodulisporamides synthesized using small 1 or 2° amines (**38**–**42**) while decreased efficacy was measured for those with more lipophilic amides ($PhCH_2NH$, **21**). Tertiary amide derivative **22**, where $R^E = C(O)NMe_2$, was the most potent evaluated (~0.1 ppm), while flea activity was diminished somewhat for additional amides prepared from larger 2° amines such as **23** or **24**.

The biological consequences of introducing additional $4''$-substituents (R^Z) to the dieneamide's bioactivity were unambiguous: as their steric bulk increased, a compensatory decrease in amide lipophilicity was integral to retaining good flea activity. For example, for nodulisporamides with modest sized $4''$-substituents such as methoxy or ethyl, good flea activity was observed (i.e., **35** and **38**–**41**) for the smaller amides, but larger ones, such as pyrrolidinyl derivative **42**, exhibited decreased activity in direct contrast to their unsubstituted brethren. Upon further increasing bulk of the $4''$-group to *n*-propyl, allyl or *n*-butyl, only those amides bearing smaller groups such as ethyl (**45**, **50**, or **53**) exhibited good activity, with sharply attenuated flea efficacy noted for *t*Bu. Introduction of fluorine at $4''$ (**26**–**27**) served only to attenuate flea activity somewhat relative to the H-substituted analogues **19** and **20**. Shifting to the $3''$-Me-$4''$-H series, several of the smallest amides, such as NHMe or NH*c*Pr, had reasonable activity while activity rapidly diminished as amide steric bulk increased. None of the $3'',4''$-dimethyl NsA derivatives tested were highly active against fleas.

In summary, a series of dienyl-modified NsA analogues bearing diverse functionality at the $3''$ and $4''$ positions were efficiently synthesized from a common intermediate in good yields. The introduction of new functionality at these sites led to the discovery of new NsA derivatives with potent, systemic activity against fleas in vitro and further clarified the structural requirements for efficacy of this class of compounds.

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16. Active = ≥80% flea kill; partially active = 51–79% flea kill; inactive = ≤50% fleas killed.