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Nodulisporic Acid Side-Chain Modifications: Access to the 2″, 3″, 4″, and 6″ Registers

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Abstract—Efficient routes to access the 2″, 3″, 4″, and 6″ registers of the nodulisporic acid (NsA) side chain are disclosed. A mild one-carbon, $Ph_2C{=}NCH_2C{\equiv}N$ mediated homologation of NsA's 3″-aldehyde permitted access to the 4″-register. Curtius reaction of NsA's 3″-acid yielded the corresponding 2″-aldehyde **4** from which the unnatural $\Delta^{2'',3''}$-olefin isomer **2b** was obtained. In addition, Arndt–Eistert reactions of the parent NsA permitted a one-carbon homologation to the 6″ register. These efforts identified new analogues with significant flea activity and illustrated the biological significance of unsaturation at the 1″,2″ register.

Merck scientists recently disclosed[1] the structure of a novel indole diterpene, nodulisporic acid A (NsA A, **1**), one member of a new family of fungal metabolites.[2] This structurally intriguing natural product exhibits significant insecticidal activity, both in vitro and in vivo.[1,3] These properties have inspired creative approaches towards the total synthesis of **1**.[4] NsA has been delineated into so called 'permissive' and 'non-permissive' regions. Synthetic manipulations of NsA's 'permissive' side chain identified modifications which improved flea potency in vitro (up to 100-fold),[5] including certain nodulisporamides exhibiting up to 14 days complete control of fleas on dogs following a single oral dose while lacking mammalian toxicity.[6] Further synthetic efforts produced new dienyl derivatives substituted at the 3″ and 4″ positions[7] in addition to a series of heterocyclic 3″,4″-olefin surrogates.[8] Integral to continued investigations, ready access to multiple oxidation states of the remaining side-chain registers (e.g., 2″, 3″, 4″ and 6″) of NsA was required. In this communication, the efficient preparation of **2–5** (where $R = OR^a$, NR^aR^b, H, or alkyl) from **1** is described. These efforts produced new analogues exhibiting useful flea activity in vitro. NsA analogues **2a** and **2b**, for instance, which differ only by their sites of unsaturation (either 1″,2″-dehydro as in **1** or its unnatural $\Delta^{2'',3''}$-regioisomer) allowed evaluation of olefin location on flea efficacy. In addition, aldehydes at these three newly accessible positions served as useful intermediates to novel structures.

The initial synthetic design to access **2a** originally appeared straightforward: react an appropriate Wittig reagent with silylated 3″-aldehyde **7a** or **7b**, thereby generating a hydrolytically labile enol ether or its equivalent, a classical route to accomplish a one carbon homologation (Scheme 1). Bis-protected **7a** was readily available in a two-step sequence from NsA A.[9] Wittig olefinations under standard conditions (**7a** or **7b**, $Ph_3P{=}CR^1R^2$, THF, $-78{\rightarrow}0\,^\circ C$) produced **8–11** in good yields (50–85%). These reactions, as well as others described herein, typically were performed on scales of 25–200 mg. Unfortunately, under no circumstances could these new NsA derivatives be successfully transformed either into the desired 4″-aldehyde or 4″-ester as only extensive degradation resulted. Alternatively, converting the 5″-carboxylic acid **12** to the corresponding acyl azide (DPPA, Et_3N, CH_2Cl_2) followed by Curtius rearrangement (PhMe, 80 °C, 3 h) yielded vinyl isocyanate **13** (80%

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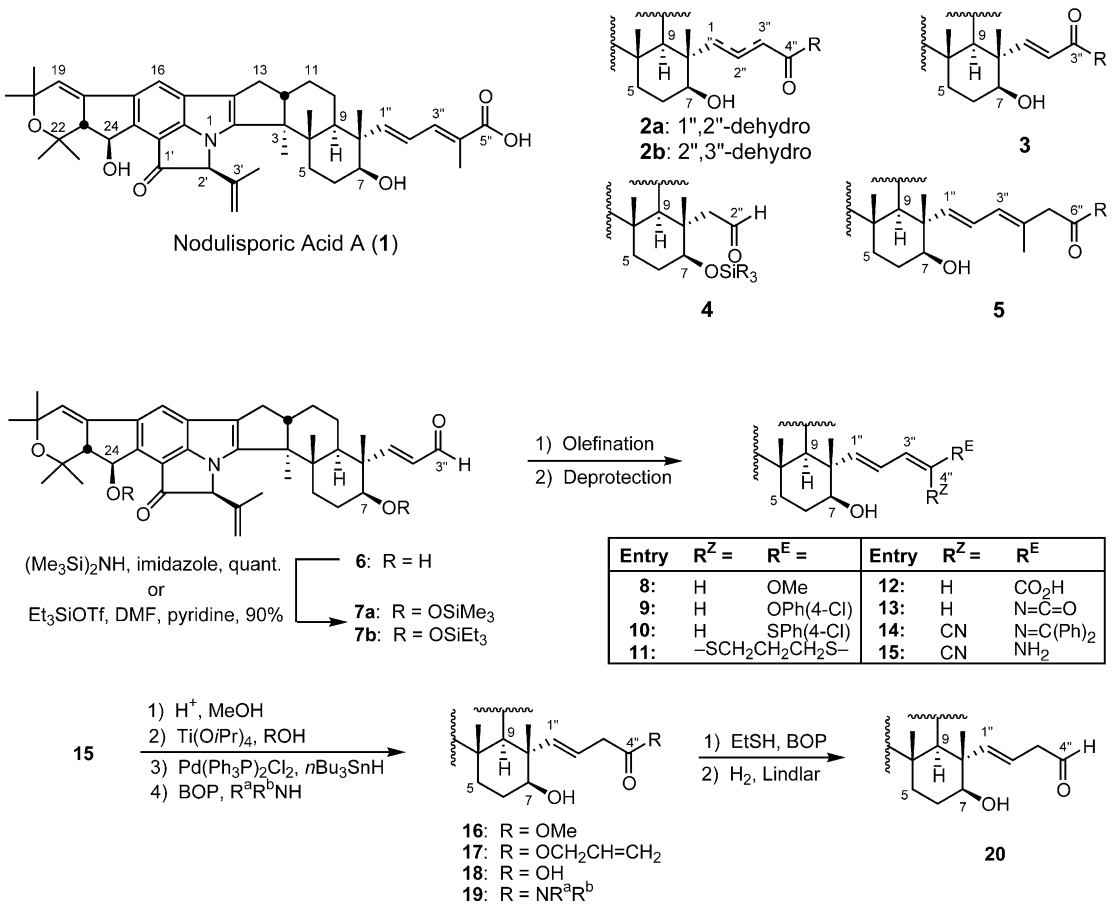


Scheme 1.

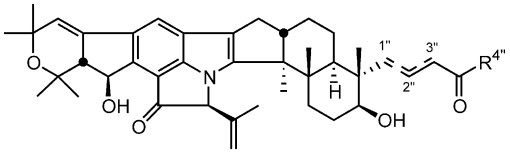
for two steps). Hydrolysis of **13**, however, again failed to produce the requisite 4″-aldehyde, even though this precise sequence successfully produced the 2″-aldehyde **4** in high yields (vide infra).

Ultimately, the successful synthesis of 4″-ester **16** occurred through recourse to condensation adduct **14**. Prepared by reacting **7a** with the lithium enolate of $N{\equiv}CCH_2N{=}CPh_2$[10] (THF, −78→0 °C), the labile imine **14** was unstable to purification but could be gently hydrolyzed to produce the chromatographically stable enamine **15** (65% for two steps).[11] Further acid catalyzed hydrolysis of this enamine in MeOH produced the long elusive 4″-methyl ester **16**, presumably via the intermediacy of a 4″-acyl nitrile in 60–80% yield along with variable quantities of an undesired 23,24-dehydrated NsA byproduct. Ester saponification to procure the corresponding carboxylic acid was not viable, as these conditions would result in biologically uninteresting 23,24-dehydro or 2′-epimeric NsA adducts. Instead, transesterification of **16** mediated by stoichiometric $Ti(OiPr)_4$[12] in neat allyl alcohol at 80 °C produced allyl ester **17**, remarkably in near quantitative yield without concomitant 24-dehydration or 2′-epimerization. Liberation of the target carboxylic acid **18** was readily accomplished by palladium-catalyzed allyl group deprotection using $n Bu_3SnH$.[13] This resultant acid was then converted into the corresponding amide derivatives **19** using standard, BOP-mediated coupling reactions[5] and the representative derivatives thus prepared are tabulated below (Table 1). In addition, carboxylic acid **18** was further elaborated to the related 4″-aldehyde **20** via its ethyl thioester intermediate which was promptly reduced using Lindlar's catalyst. *Rapid* degradation of this 4″-aldehyde occurred upon purification or exposure to air, although under an inert atmosphere its stability was enhanced.

A complementary approach to the synthesis of the 4″-methyl ester **16** also was developed and is illustrated in Scheme 2. The 3″-aldehyde derivative **7a** was selectively reduced with 9-BBN in excellent yield (80%) and the resultant alcohol acylated, producing allylic acetate **21** and carbonates **22–23**. Treatment of **21–23** with a suitable palladium catalyst in MeOH under a carbon monoxide atmosphere produced the desired 4″-methyl ester **16**, which could be further elaborated as previously noted. Yields of this carbonomethylation, however, remained modest, as the reaction failed to go to completion. The effect of various catalysts, CO pressures, extended reaction times and elevated temperatures were surveyed, but ultimately $Pd(OAc)_2$ (0.1 equiv), dppp (0.1 equiv) in a mixture of MeOH and DMF (1:1) at 1 atm gave the best yields (~20%), along with recovered starting material (~80%).

Successful synthesis of the regioisomeric, $\Delta^{2'',3''}$-unsaturated NsA derivatives **2b** required an alternative, six-step protocol (Scheme 3). The TES-protected 3″-carboxylic acid **24** was prepared from **7b** as previously described.[9]

Table 1. Unsaturated 4″-nodulisporic acid derivatives[14,15]



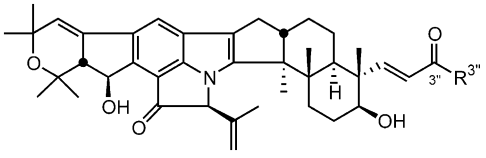
Compd	Unsaturation	$R^{4''}$ group	Flea (ppm)
1	(Nodulisporic Acid A)		1
16	1″,2″-Dehydro	OMe	~1
18	1″,2″-Dehydro	OH	
20	1″,2″-Dehydro	H	10
28	1″,2″-Dehydro	Me	1
29	1″,2″-Dehydro	NH-Et	~10
30	1″,2″-Dehydro	NH-*c*Pr	~1
31	1″,2″-Dehydro	NH-*t*Bu	~1
32	1″,2″-Dehydro	$NHCH_2$(2-furfuryl)	~10
33	1″,2″-Dehydro	$NHCH_2Ph$	~10
34	1″,2″-Dehydro	NHPh	~1
35	1″,2″-Dehydro	NMe_2	10
36	1″,2″-Dehydro	N(Me)*n*Pr	~1
37	1″,2″-Dehydro	*N*-1-morpholinyl	~1
38	2″,3″-Dehydro	Me	~10
39	2″,3″-Dehydro	OH	
40	2″,3″-Dehydro	OMe	~10
41	2″,3″-Dehydro	NH-Et	~10
42	2″,3″-Dehydro	NH-*c*Pr	>10
43	2″,3″-Dehydro	NMe_2	~10

This acid was quantitatively converted to the chromatographically stable acyl azide **25** using diphenylphosphoryl azide. Thermolysis of **25** induced a Curtius rearrangement, generating **26**, again in near quantitative yield. Although isolation of the intermediate vinyl isocyanate was viable, for expediency, following removal of reaction volatiles in vacuo, **26** was immediately subjected to acid-catalyzed hydrolysis, yielding the requisite protected 2″-aldehyde **27** (80%). With this key NsA intermediate in hand, attention turned to chain elongation with stabilized Wittig reagents. Aldehyde **27** reacted readily with these Wittig reagents only at elevated temperatures; at ambient temperatures, virtually no olefination occurred. For the case of **2b** where OR^a is $-OCH_2CH{=}CH_2$, allyl group removal as previously described proceeded smoothly. Subsequent coupling of this $\Delta^{2'',3''}$-unsaturated carboxylic acid **39** with diverse amines using BOP under standard conditions[5] produced the representative NsA amides **41–43** in high yields subsequent to TES removal. These conditions also served to generate the 3″-amides **44–50** from the precursor acid **24** (Table 2).

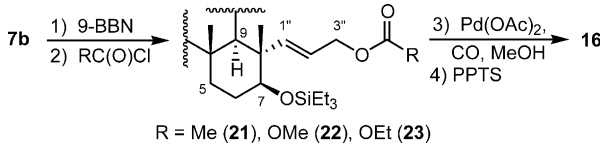
The 6″-carboxylic acid **5** (R = OH) was synthesized via Arndt–Eistert chain homologation of NsA A (**1**). Reacting **1** with $MeSO_2Cl$ yielded the corresponding mixed anhyride of **1** which was promptly exposed to diazomethane. Rearrangement of the resultant, chromatographically stable α-diazoketone, mediated by $PhCO_2Ag$ in MeOH, yielded the desired 6″-methyl ester in good yield. This 6″-ester could be converted into the corresponding 6″-acid (**5**, R = OH) following transesterification using allyl alcohol and deprotection. From this 6″-acid, the desired 6″-aldehyde and 6″-amides were prepared as discussed above. Uniformly, these compounds exhibited attenuated efficacy (≥10 ppm) in the flea in vitro assay (data not shown).

All new NsA derivatives were evaluated for systemic activity using a membrane feeding assay wherein adult fleas ingest drug-treated bovine blood.[3,5] In this assay,

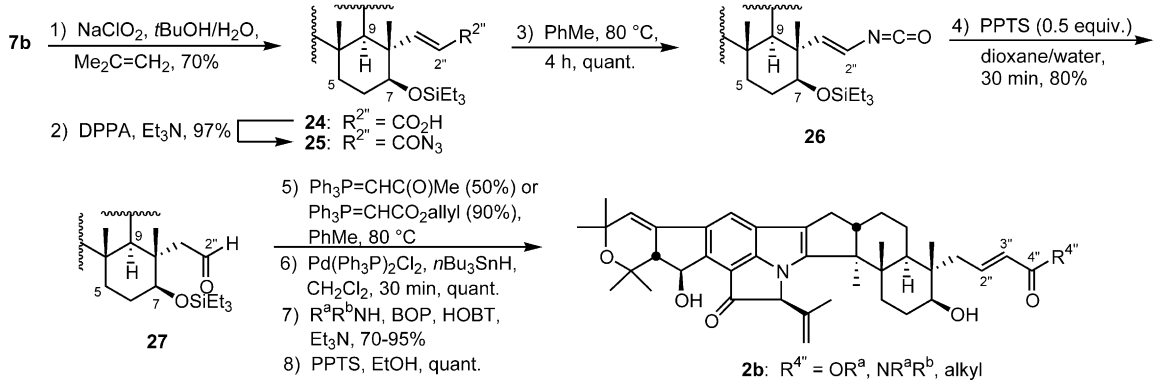
Table 2. 3″-Nodulisporamide derivatives[14,15]



Compd	$R^{3''}$ Group	Flea (ppm)
44	NH-Me	~10
45	NH-*n*Pr	>10
46	$NHCH_2Ph$	~10
47	$NHCMe_2$(2-pyridyl)	~1
48	NMe_2	>10
49	*N*-1-[4-(2-pyridyl)piperizinyl)	~1
50	$N(Me)CH_2CH{=}CH_2$	>10



Scheme 2.



Scheme 3.

nodulisporic acid A is fully active at the 1 ppm level. While only one of the newly prepared derivatives from either series exhibited full efficacy against fleas at this screening level, close inspection of the data for all NsA analogues in Table 1 served to reveal several trends. First, although the 4″-methyl ketone **28** was equipotent to **1**, only methyl ester **16** and a limited subset of nodulisporamides of intermediate lipophilicity (e.g., **30**, **31**, **34**, **36**, and **37**) displayed comparable ectoparasite activity. Small and larger 2° amides at 4″ (NHEt, $NHCH_2Ar$, etc.) were 10-fold less potent, as were smaller 3° amides, such as **35** (NMe_2). Interestingly, somewhat larger 3° nodulisporamides [e.g., **36** (N(Me)nPr) and **37** (*N*-1-morpholinyl)] retained their activity against fleas. Flea efficacy results obtained for the $\Delta^{2'',3''}$-dehydro-NsA series **38**–**43** clearly established the biological significance of unsaturation in register with that of the parent acid **1**: these regioisomeric derivatives were uniformly less active against fleas than their natural counterparts. Similarly, the 3″- and 6″-nodulisporamides exhibited diminished biological activity, with the exception of larger 3″-nodulisporamides such as **47** and **50**.

In summary, efficient protocols to prepare new side-chain modified nodulisporic acid analogues modified at the 2″, 3″, 4″ and 6″ side-chain registers were developed. Derivatives were synthesized differing in side-chain olefin substitution patterns, demonstrating the requirement for the natural unsaturation pattern for the most potent flea efficacy. While no derivatives with flea activity superior to that of the parent acid **1** were discovered, numerous analogues in these new structure classes with comparable activity were identified. In addition, several of the newly synthesized NsA analogues may serve as useful intermediates for the preparation of additional novel structures.

Acknowledgements

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14. All new nodulisporic acid analogues were characterized by ^1H NMR and mass spectrometry.
15. Active: $\geq$80% flea kill; partially active ($\sim$): 51–79% flea kill; inactive: $\leq$50% flea kill.