

Development of a Scalable Synthesis of a Common Eastern Tricyclic Lactone for Construction of the Nodulisporic Acids

Amos B. Smith, III,* László Kürti, Akin H. Davulcu, and Young Shin Cho

Department of Chemistry, Monell Chemical Senses Center and Laboratory for Research on the Structure of Matter, University of Pennsylvania, Philadelphia, Pennsylvania 19104, U.S.A.

Abstract:

A scalable, second-generation synthesis of the densely functionalized eastern tricyclic lactone (+)-6, a common intermediate, for construction of the nodulisporic acids has been achieved. Modifications to the first-generation route now permit access to (+)-6 in 17 steps with an overall 16.5% yield. Key carbon–carbon bond constructions include a Kirk–Petrov (phenylthio)-methylation, a $\text{Sc}(\text{OTf})_3$ -catalyzed hydroxymethylation, a Stille carbonylation, and a Koga three-component, conjugate addition–alkylation sequence.

Introduction

(+)-Nodulisporic acid A (**1**), the most complex member of the nodulisporane class of indole diterpenoids, was first isolated by Ondeyka and co-workers at the Merck Research Laboratories as part of an extensive screening program to identify structurally unique, biologically active natural products (Figure 1).¹ The nodulisporic acids comprise a family of potent insecticides particularly effective against flea and tick infestations in dogs and cats.² Nodulisporic acid A [(+)-**1**] was found to be the most potent nodulisporane, displaying a 10-fold greater systemic adulticidal efficacy than ivermectin against the *Ctenocephalides felis* (common flea), a commercially relevant flea target.

The closely related nodulisporic acids B, D, and F (cf. **2**, **3**, and **4**) proved to be less active by 5 to >100-fold. Importantly, (+)-nodulisporic acid A (**1**) displays no apparent toxicity to the host animal while effectively killing fleas subsequent to their ingestion of a blood meal. The mode of action of the nodulisporic acids involves modulation of the invertebrate-specific, glutamate-gated, chloride ion channels not present in mammals.³ However, extensive biological evaluation revealed that, although (+)-**1** possessed good in vivo and in vitro activity, the stability and pharmacokinetic profile were not optimal. A medicinal chemistry campaign, in conjunction with a chemical mutagenesis program, was

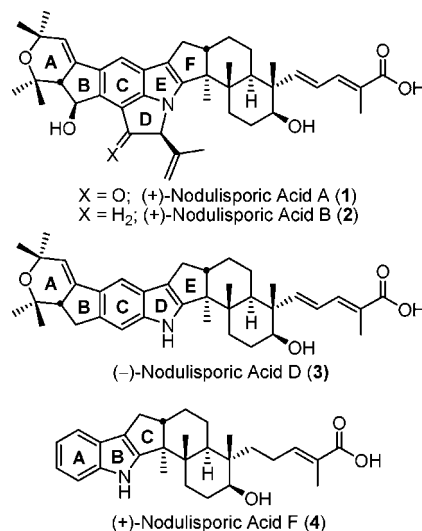


Figure 1. Representative nodulisporic acid congeners.

thus launched at Merck & Co. to develop (+)-**1** as a novel anti-flea therapeutic agent for companion animals. To date over 1000 synthetic analogues have been prepared.^{4,5}

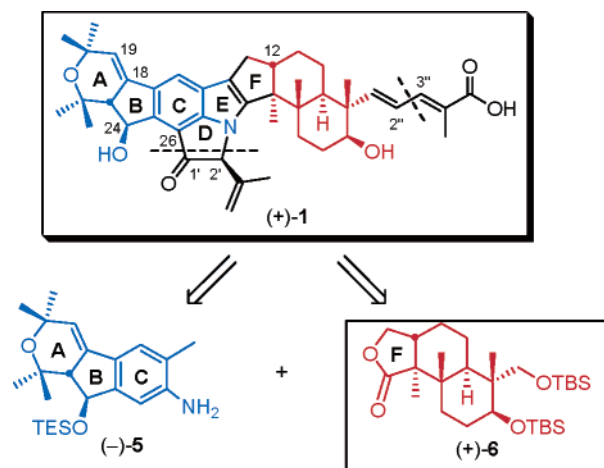
Given our long-standing interest in the total synthesis of indole diterpenoids, in conjunction with the architectural complexity and remarkable antiparasitic activity of (+)-nodulisporic acid A (**1**), we initiated a synthetic program to devise a modular synthetic strategy that would permit construction not only of the parent nodulisporic acid A [(+)-**1**] but also a number of analogues not readily accessible by chemical modification of the naturally occurring indole diterpenoids (i.e., the nodulisporanes). At the outset of this program, the cornerstone of our modular synthetic strategy was envisioned to involve the indole synthetic protocol^{6,7} developed in our laboratory and successfully employed to construct a number of related indole diterpenoid natural products, including penitrem D⁸ and 21-isopentenylpaxilline.⁹ From the retrosynthetic perspective (Scheme 1), application

* To whom correspondence should be addressed. E-mail: smithab@sas.upenn.edu.

- (1) Ondeyka, J. G.; Helms, G. L.; Hensens, O. D.; Goetz, M. A.; Zink, D. L.; Tsiouras, A.; Shoop, W. L.; Slayton, L.; Dombrowski, A. W.; Polishook, J. D.; Ostlind, D. A.; Tsou, N. N.; Ball, R. G.; Singh, S. B. *J. Am. Chem. Soc.* **1997**, *119*, 8809–8816.
- (2) Sings, H.; Singh, S. In *The Alkaloids: Chemistry and Biology*; Cordell, G. A., Ed.; Elsevier Academic Press: San Diego, CA, United States, 2003; Vol. 60, pp 51–163.
- (3) Ludmerer, S. W.; Warren, V. A.; Williams, B. S.; Zheng, Y.; Hunt, D. C.; Ayer, M. B.; Wallace, M. A.; Chaudhary, A. G.; Egan, M. A.; Meinke, P. T.; Dean, D. C.; Garcia, M. L.; Cully, D. F.; Smith, M. M. *Biochemistry (Mosc.)* **2002**, *41*, 6548–6560.

- (4) Chakravarty, P. K.; Shih, T. L.; Colletti, S. L.; Ayer, M. B.; Snedden, C.; Kuo, H.; Tyagarajan, S.; Gregory, L.; Zakson-Aiken, M.; Shoop, W. L.; Schmatz, D. M.; Wyvratt, M.; Fisher, M. H.; Meinke, P. T. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 147–150.
- (5) Ok, D.; Li, C.; Shih, T. L.; Salva, S.; Ayer, M. B.; Colletti, S. L.; Chakravarty, P. K.; Wyvratt, M. J.; Fisher, M. H.; Gregory, L.; Zakson-Aiken, M.; Shoop, W. L.; Schmatz, D. M.; Meinke, P. T. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1751–1754.
- (6) Smith, A. B., III; Visnick, M.; Haseltine, J. N.; Sprengeler, P. A. *Tetrahedron* **1986**, *42*, 2957–2969.
- (7) Smith, A. B., III; Visnick, M. *Tetrahedron Lett.* **1985**, *26*, 3757–3760.
- (8) Smith, A. B., III; Kanoh, N.; Ishiyama, H.; Hartz, R. A. *J. Am. Chem. Soc.* **2000**, *122*, 11254–11255.
- (9) Smith, A. B., III; Cui, H. *Org. Lett.* **2003**, *5*, 587–590.

Scheme 1. Retrosynthetic analysis of (+)-nodulisporic acid A (1)



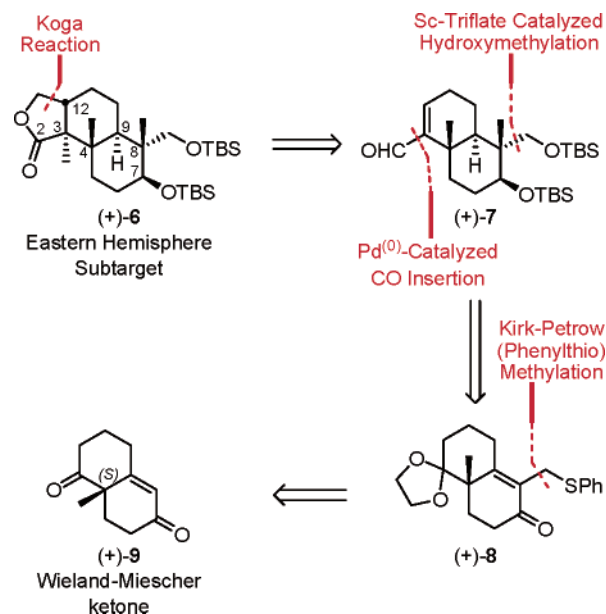
of our indole retron, after removal of ring D from (+)-1 leads to a heptacyclic core that would arise from the union of western and eastern hemisphere subtargets (–)-5 and (+)-6, respectively. That all of the nodulisporic acids possess the identical stereogenicity in the eastern hemisphere suggested that (+)-6 would serve as a viable common building block for this class of indole diterpenoids.

In 2001, we disclosed a first-generation 17-step synthesis of (+)-6 that proceeded in 9% overall yield.¹⁰ Several transformations in the original sequence, however, limited the efficiency, which in turn did not permit ready scale-up. Thus, before we could contemplate a serious campaign to construct the nodulisporic acids and derivatives thereof, a scalable synthesis of (+)-6 was required. Described herein is a modified synthetic sequence now capable of providing multigram quantities (ca. 5–10 g) of the requisite advanced eastern lactone (+)-6.

Results and Discussion

Tricyclic lactone (+)-6 possesses a number of synthetic challenges, given the array of functionality including the following: the *trans*-fused lactone at C(3) and C(12) (nodulisporic acid numbering), the *trans*-fused decalin with vicinal quaternary stereocenters at C(3) and C(4), and the quaternary stereocenter at C(8). To access this array of stereogenicity, our first-generation synthesis employed to good advantage the following carbon–carbon bond construction steps beginning with (+)-Wieland–Miescher ketone (9): a Kirk–Petrov (phenylthio)methylation¹¹ and a diastereoselective Sc(OTf)₃-catalyzed hydroxymethylation to construct the C(8) quaternary stereocenter,¹² a Birch reduction¹³ directed by the C(4) quaternary stereocenter to introduce the *trans*-fused decalin system, and a Stille carbonylation followed by a Koga alkylation to generate the C(3) and C(12) stereocenters in a tandem fashion.^{14,15}

Scheme 2. Retrosynthetic analysis of (+)-6



Construction of lactone (+)-6 began with the preparation of enantiomerically pure (>95% ee) Wieland–Miescher ketone (+)-9, employing the well-known L-proline-catalyzed Robinson annulation.^{16–18} Chemoselective protection of the saturated carbonyl as the ethylene ketal (+)-10 was achieved via the conditions of Demnitz et al. (Scheme 3).¹⁹ In our first-generation approach the transketalization protocol²⁰ required an impractically long reaction time (cf. 1 week); purification also required a time-consuming flash chromatographic separation. The Demnitz transformation which called for the treatment of (+)-9 with a full equivalent of *p*-toluenesulfonic acid in ethylene glycol scaled well, allowing for the conversion of 50-g batches of (+)-9 in ca. 20–25 min. Importantly, crystallization from hexanes/Et₂O (20:1) furnished (+)-10 in 89–91% yield, without the need for chromatographic purification. Small amounts of the bis-ketal product and unreacted Wieland–Miescher ketone remained in solution during the crystallization even at low temperatures. In practice, 215 g of (+)-10 can be prepared in 1 day. That a highly selective reaction was combined with an efficient crystallization protocol enabled us to increase considerably the material throughput.

One-carbon homologation of (+)-10 at the α -carbon was next achieved via the Kirk–Petrov protocol.¹¹ This transformation is inherently slow (4 days), and as such, the thiophenol employed in slight excess undergoes partial air oxidation to give Ph₂S₂ that coelutes with (+)-8, making purification in our first-generation sequence difficult. Implementation of a protective Ar atmosphere to exclude air completely prevented formation of Ph₂S₂. Purification of (+)-8 could thus be achieved by base extraction of PhSH and crystallization; a total of ca. 260 g was prepared.

(10) Smith, A. B., III; Cho, Y. S.; Ishiyama, H. *Org. Lett.* **2001**, 3, 3971–3974.

(11) Kirk, D. N.; Petrov, V. J. *Chem. Soc.* **1962**, 1091–1096.

(12) Kobayashi, S. *Chem. Lett.* **1991**, 2187–2190.

(13) Stork, G.; Rosen, P.; Goldman, N.; Coombs, R. V.; Tsuji, J. *J. Am. Chem. Soc.* **1965**, 87, 275–286.

(14) Hashimoto, S. I.; Yamada, S. I.; Koga, K. *Chem. Pharm. Bull.* **1979**, 27, 771–782.

(15) Kogen, H.; Tomioka, K.; Hashimoto, S.; Koga, K. *Tetrahedron* **1981**, 37, 3951–3956.

(16) Buchschacher, P.; Fuerst, A. *Org. Synth.* **1985**, 63, 37–43.

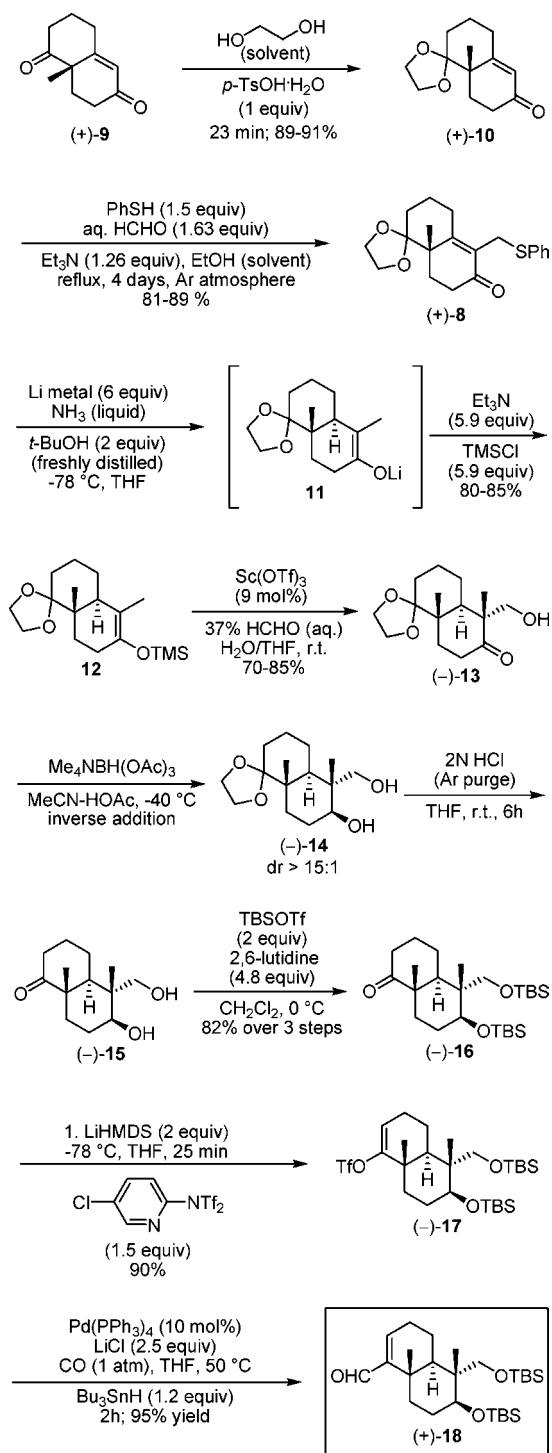
(17) Harada, N.; Sugioka, T.; Uda, H.; Kuriki, T. *Synthesis* **1990**, 53–56.

(18) Gutzwiller, J.; Buchschacher, P.; Fuerst, A. *Synthesis* **1977**, 167–168.

(19) Ciceri, P.; Demnitz, F. W. J. *Tetrahedron Lett.* **1997**, 38, 389–390.

(20) Bauduin, G.; Pietrasanta, Y. *Tetrahedron* **1973**, 29, 4225–4231.

Scheme 3. Preparation of aldehyde (+)-18



The *trans*-decalin stereochemistry required for (+)-6 was next installed by Birch reduction of (+)-8, employing the intermediacy of lithium enolate **11**, the latter captured as the corresponding trimethylsilyl enol ether **12** (Scheme 3). Hydroxymethylation was then achieved under aqueous conditions, employing Sc(OTf)_3 to furnish β -hydroxyketone, (-)-**13**, possessing the correct configuration at the C(8) quaternary center. In our first-generation synthesis this reaction sequence proved problematic, particularly when the Birch reduction was conducted on intermediate scale (5–10 g). Extensive experimentation revealed several issues,

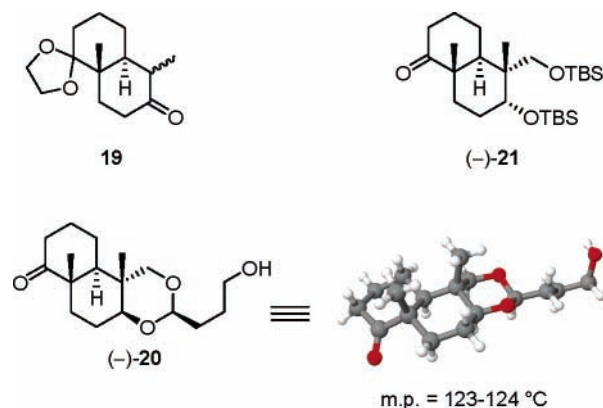


Figure 2. Side- and byproducts.

along with several corresponding solutions: (1) given that lithium metal dissolves slowly in liquid ammonia, considerable time was required to generate the dissolved metal solution (ca. 1 h); (2) the *t*-BuOH must be freshly distilled prior to use²¹ to diminish the formation of ketone **19** (Figure 2); (3) efficient capture of the lithium enolate (**11**) required the use of freshly distilled TMSCl and Et_3N ; and (4) the presence of residual thiophenol upon workup was found to inhibit the subsequent Sc(OTf)_3 -promoted hydroxymethylation. Pleasingly, the residual thiophenol could be readily removed without silyl ether hydrolysis via passage of the product through a pad of basic alumina. Thus by employing standard academic glassware, 25-g batches of (-)-**13** could be readily prepared. Quenching the excess lithium metal was achieved with isoprene. With these improvements, the two-step preparation of (-)-**13** could be readily accomplished in moderate to good yield (56–72%).

The three-step conversion of (-)-**13** to (-)-**16** (Scheme 3) was next performed without need for purification of (-)-**14** and (-)-**15**. To this end diastereoselective hydride reduction of β -hydroxyketone (-)-**13** was achieved with tetramethylammonium triacetoxyborohydride, a reagent introduced by Gribble^{22,23} and further developed by Evans and co-workers.²⁴ When the reducing agent was added to a cold (-40°C) solution of (-)-**13**, an inseparable mixture of diastereomeric 1,3-diols (5.5:1) was formed, favoring (-)-**14**. The lack of stereoselectivity may have been due to significant heat evolution above -40°C . At these temperatures, the reduction was less selective. This problem becomes worse as the scale is increased. However, when the reaction was conducted via inverse addition of the substrate (-)-**13** to a cooled (-40°C) solution of the reducing agent, the diastereoselectivity improved dramatically to 15:1. Presumably, with inverse addition (i.e., addition to a slurry of the reducing agent at -40°C) control of the temperature is easier. Prior to this modification, the minor diastereomer could only be removed at the stage of the bis-TBS ether (-)-**21**. Removal of the dioxolane protecting group was also problematic in the first-generation synthesis,

(21) With this change the formation of **19** was decreased to 5% from as high as 40% in the first-generation procedure.

(22) Gribble, G. W.; Nutaitis, C. F. *Tetrahedron Lett.* **1983**, 24, 4287.

(23) Gribble, G. W.; Ferguson, D. C. *Chem. Commun.* **1975**, 535.

(24) Evans, D. A.; Chapman, K. T. *Tetrahedron Lett.* **1986**, 27, 5939–5942.

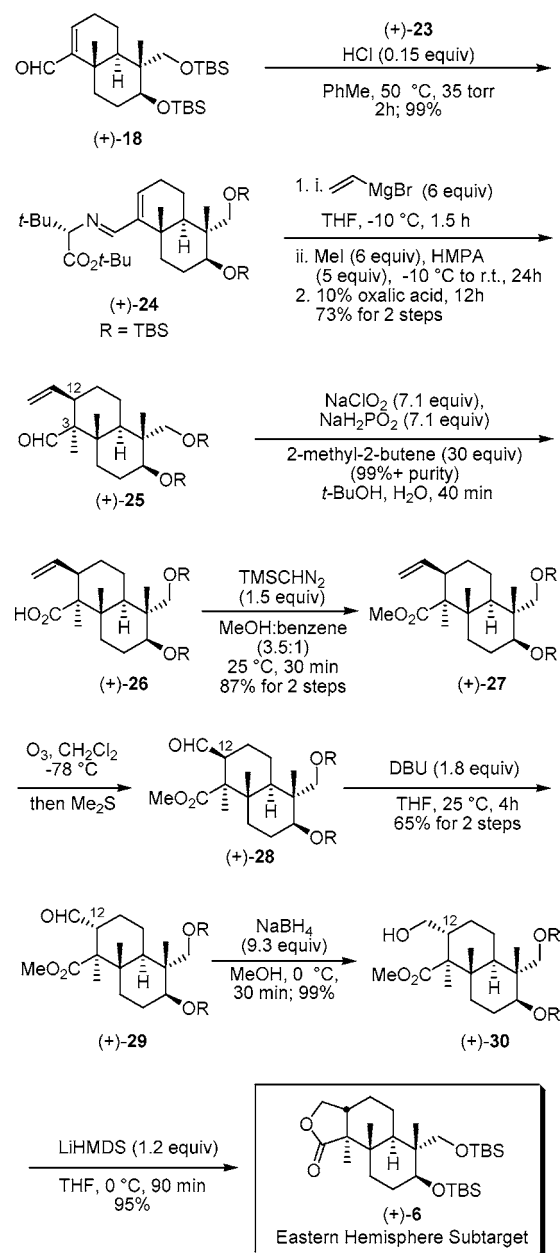
given that the lengthy reaction time (6 h) permitted the THF to undergo partial oxidation in the aqueous 2 N HCl solution, leading to up to 10% of acetal (–)-**20**. A simple purge of the aqueous HCl solution with Ar prior to the hydrolysis completely eliminated formation of (–)-**20**. An improvement in the bis-TBS protection of (–)-**15** was also introduced. Reduction in the amount of TBSOTf from 2.8 to 2.0 equiv led to a much cleaner reaction, thereby simplifying chromatographic purification of (–)-**16**. Presumably the strong Lewis acidity of TBSOTf results in some decomposition of the substrate and/or product. With these improvements, the three-step sequence now proceeds in 82% overall yield, requiring only chromatographic purification of ketone (–)-**16**.

Having installed four of the requisite six stereogenic centers in (+)-**6**, we turned to the installation of the γ,δ -unsaturated aldehyde functionality, that would set the remaining two centers (Scheme 3). Treatment of ketone (–)-**16** with 2 equiv of LiHMDS at –78 °C, followed by capture of the resulting lithium enolate with the Comins' reagent,²⁵ furnished enol triflate (–)-**17** in excellent yield after purification by flash chromatography. On large scale, the subsequent Stille Pd-catalyzed carbonylation²⁶ initially proved problematic due to the quality of the Pd(PPh₃)₄. Best results were obtained with Pd(PPh₃)₄ purchased from Strem Chemical Co. Purging the reaction mixture with carbon monoxide prior to the addition of the catalyst also permitted both a lower catalyst load (cf. from 30 to 10 mol %) and a shorter reaction time (4 vs 2 h) compared to the first-generation protocol. Under these conditions, carbonylation could be conveniently achieved on 10 g scale; a total of 31 g of (+)-**18** was prepared.

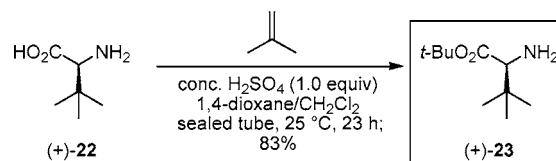
With large quantities of the vinyl aldehyde (+)-**18** in hand, the stage was now set for the tandem introduction of the remaining two stereocenters [C(3) and C(12)] employing the Koga reaction.^{14,15} Based on the first-generation sequence, we faced two serious problems. First, the required chiral auxiliary (+)-**23** was available in only moderate yield, requiring an impractically long period to produce (ca. 4 weeks). Second, the reaction time required to prepare aldimine (+)-**24** was excessively long. After extensive experimentation, both procedures were markedly improved (Scheme 4 and Scheme 5). First, preparation of the chiral amine auxiliary (+)-**23** (Scheme 5) was accelerated by loading the components at a low temperature (–40 °C) and running the reaction at room temperature in a sealed tube to prevent loss of the volatile isobutylene; under these conditions consumption of the starting material was complete in only 23 h. Preparation of (+)-**23** (ca. 30 g) could thus be achieved in a just a few days.

Turning to the preparation of the α,β -unsaturated imine (+)-**24**, the first generation protocol called for the use of 4 Å molecular sieves as the dehydrating agent. Although a high yield of (+)-**24** was obtained, the reaction time was

Scheme 4. Preparation of tricyclic lactone (+)-**6**



Scheme 5. Preparation of chiral auxiliary (+)-**23**



unacceptably long (ca. 33 days), affording a 10:1 mixture of product and unreacted aldehyde (+)-**18**. Initially, we experimented with azeotropic removal of the water with benzene via the Dean–Stark tactic. Under these conditions (80 °C), decomposition of (+)-**18** proved to be a significant problem. Gratifyingly, by adding 15 mol % of the HCl salt of (+)-**23** and using toluene as solvent, followed by successive vacuum azeotropic distillation, we obtained imine (+)-**24** in near quantitative yield (Scheme 4). Purification was then best achieved via flash column chromatography

(25) Comins, D. L.; Dehghani, A. *Tetrahedron Lett.* **1992**, 33, 6299–6302.
 (26) (a) Scott, W. J.; Crisp, G. T.; Stille, J. K. *J. Am. Chem. Soc.* **1984**, 106, 4630–4632. (b) Cacchi, S.; Morera, E.; Ortari, G. *Tetrahedron Lett.* **1985**, 26, 1109–1112.

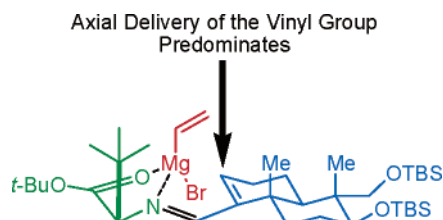


Figure 3. Explanation of the observed stereochemistry in (+)-**25**.

using basic alumina. The alternative use of buffered silica gel resulted in modest hydrolysis ($\sim 5\text{--}10\%$) of the imine.

Turning to the Koga alkylation,^{14,15} which entails a tandem 1,4-addition followed by in situ alkylation, imine (+)-**24** was treated with vinylmagnesium bromide to furnish an intermediate magnesium aza-enolate that was then captured with excess MeI in the presence of HMPA. Removal of the chiral auxiliary was next achieved in high yield by using oxalic acid. In the first-generation synthesis, citric acid had been employed. The reaction proved slow (3 days) and suffered from the formation of an unidentified byproduct. By replacing the citric acid ($\text{p}K_{\text{a}} = 3.15$) with the more acidic oxalic acid ($\text{p}K_{\text{a}} = 1.23$), the rate of imine hydrolysis increased significantly to furnish cleanly (+)-**25** in 12 h without formation of side products.

The stereochemical outcome of the Koga alkylation can be understood employing the model illustrated in Figure 3. Axial delivery of the vinyl group and subsequent axial alkylation with methyl iodide are the direct result of the stereoelectronic preference for axial delivery and enolate-like alkylations.

Optimization of the Koga reaction required considerable effort. The composition and origin of the vinylmagnesium bromide reagent are critical factors. Freshly prepared vinylmagnesium bromide works considerably better than aged reagent. Nonetheless, on occasion even freshly prepared reagent affords a poor yield. As a result, we recommend a small-scale (100 mg) test reaction with the vinylmagnesium bromide at hand before committing to larger scale.

Pinnick oxidation of aldehyde (+)-**25** next furnished carboxylic acid (+)-**26**.²⁷ Particularly important here is the use of distilled 2-methyl-2-butene and not the technical grade (90%) reagent. Methyl ester formation is then achieved without purification by treating (+)-**26** with a slight excess of (trimethylsilyl)diazomethane; the yield of (+)-**27** was 83% for the two steps.²⁸

With ester (+)-**27** in hand, the vinyl group was next converted to the aldehyde moiety via ozonolysis, employing reduction of the ozonide with dimethylsulfide or PPh_3 , followed by treatment with DBU in THF to effect epimerization; aldehyde (+)-**29** was produced in a combined yield of 65% for the two steps. Due to the hindered nature of the vinyl group in (+)-**27** 3 days were required to reduce the ozonide; fortunately, the reaction progress can be monitored easily by TLC.

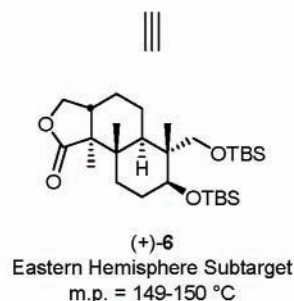
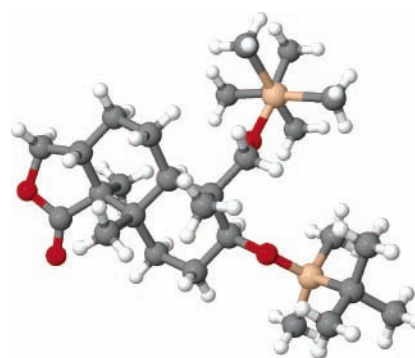


Figure 4. Single-crystal X-ray image of (+)-**6**. (Generated from the PDB file using Jmol Viewer, version 10).

While the four steps to convert aldehyde (+)-**25** to ester (+)-**29** did not require major modification, vis-à-vis the first-generation synthetic sequence, significant changes were made to the final two steps, that is the conversion of aldehyde (+)-**29** to lactone (+)-**6**, the eastern hemisphere subtarget. First, lowering the temperature of the chemoselective NaBH_4 reduction of the C(12) aldehyde moiety in (+)-**29** to $0\text{ }^\circ\text{C}$ furnished the corresponding hydroxyester (+)-**30** in near quantitative yield, without over-reduction of the ester as was observed at higher temperatures in the original procedure. Second, the acid-promoted lactonization was abandoned in favor of a strong base protocol, thereby eliminating the modest decomposition, presumably arising via loss of the TBS groups in both the starting material and the lactone. Best results involved use of LiHMDS at $0\text{ }^\circ\text{C}$. These conditions permitted facile lactonization to furnish the eastern hemisphere lactone (+)-**6** in 95% yield after chromatographic purification. The structure of (+)-**6** was confirmed by single-crystal X-ray analysis (Figure 4).

Conclusion

A scalable synthesis of the common eastern hemisphere lactone (+)-**6** for construction of the nodulisporic acids has been achieved. The improvements in the second-generation sequence, compared to the first-generation route, entailed elimination of eight chromatographic purifications, the modification and amplification of several reaction conditions, and significant reduction in the overall reaction times for several transformations. The second-generation synthesis now proceeds with an overall yield of 16.5%, corresponding to an average of 90% yield for each step. Importantly, the efficiency of the sequence will now enable us to proceed with our synthetic campaign to construct the nodulisporic acids.

(27) Bal, B. S.; Childers, W. E., Jr.; Pinnick, H. W. *Tetrahedron* **1981**, *37*, 2091–2096.

(28) Hashimoto, N.; Aoyama, T.; Shioiri, T. *Chem. Pharm. Bull.* **1981**, *29*, 1475–1478.

Acknowledgment

Financial support was provided by the National Institutes of Health (National Institute of General Medical Sciences) through Grant GM-29028 and by the Bristol-Myers Squibb Pharmaceutical Research Institute (for stipend support of A.H.D.). We are also grateful to Eli Lilly and Company for the graduate fellowship to L.K. and to Dr. P.T. Meinke and the Merck Research Laboratories for generous financial support of this research program. Finally, we thank Dr. G. Furst, Dr. R. K. Kohli, and Dr. P. Carroll for assistance in

obtaining NMR spectra, high-resolution mass spectra, and X-ray crystallographic data, respectively.

Supporting Information Available

Experimental details and selected spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Received for review October 4, 2006.

OP060204L