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First synthesis of nitrosporeusines, alkaloids with multiple biological activities

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ABSTRACT

Synthesis of nitrosporeusines A and B, thioester-bearing alkaloids from the Arctic *Streptomyces nitrosporeus* with exceptional biological activity is disclosed for the first time. In addition, we have prepared another biologically important natural product, maleimycin, in optically pure form using a gram-scale enzymatic resolution method.

One of the dreadful diseases rapidly spreading across the globe is influenza commonly referred to as 'the flu'. Influenza spreads around the world in mostly seasonal epidemics, with an annual attack rate estimated at 5–10% in adults and 20–30% in children. This accounts to about 3–5 million cases of severe illness and about 250,000–500,000 deaths per annum.[1] The currently existing drugs in the market to treat influenza viruses are increasingly becoming ineffective due to constant resistance being developed by viruses[2] and hence the discovery of new inhibitors with a novel mode of action is necessary.[3] The natural products have also been used for the identification of anti-viral compounds with novel scaffolds so as to overcome the menace of drug resistance.[4] One such natural product family, with very good inhibitory activities against the H1N1 virus, is the nitrosporeusines, reported by Lin and co-workers,[5] attracted our attention (Fig. 1). According to this Letter, chemical examination from the sediments of the Arctic Chukchi Sea actinomycete *Streptomyces nitrosporeus* resulted in the isolation of two alkaloids, named as nitrosporeusines A (**1**) and B (**2**), with an unprecedented skeleton containing benzenecarbo-thiocyclopenta[*c*]pyrrole-1,3-dione. Both the compounds **1** and **2** are reported to have shown very impressive multiple biological activities (Fig. 1).[5,6] In the light of the extensive and exceptional

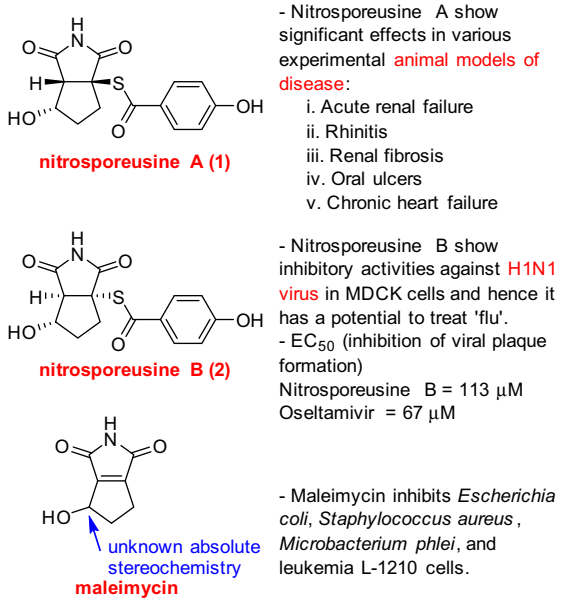

Figure 1. Structures of natural products along with their biological activities.

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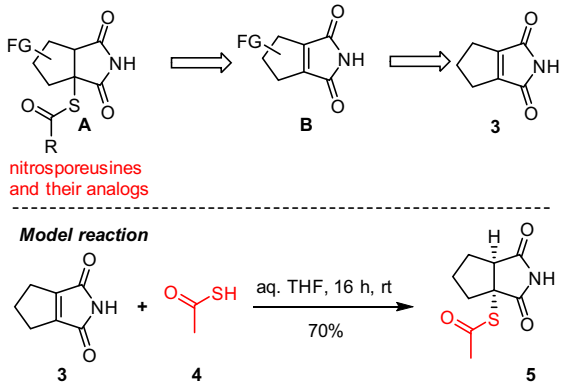
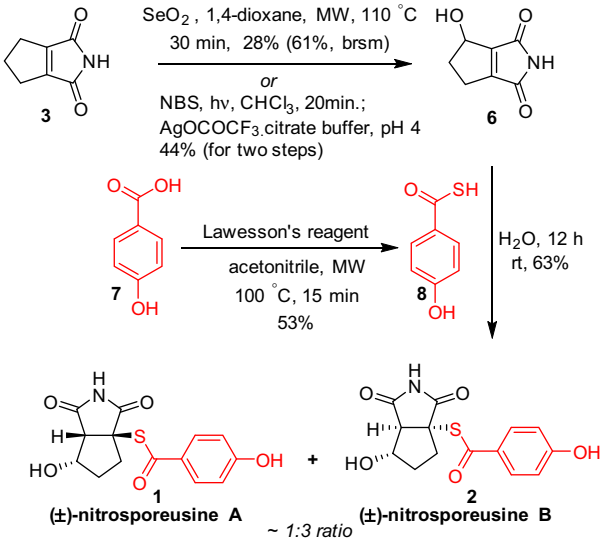


Scheme 1. Retrosynthesis and a model reaction.

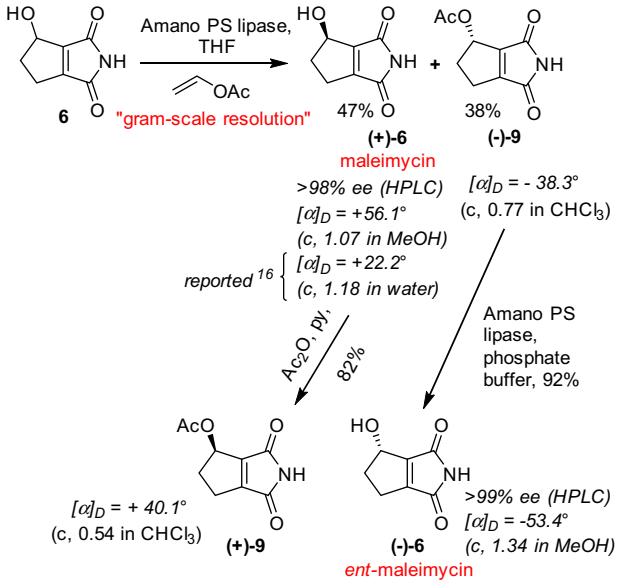
multiple medical applications of nitrosporeusines,[7] there is a dire need to increase the availability of the natural products in sufficient quantities by way of chemical synthesis, so as to facilitate further research activity.

Retrosynthetically, the target natural products and their analogs **A** are imagined from the functionalized maleimide derivatives **B**. The known compound **3**[8] could be functionalized to obtain the desired **B** using standard transformations known in the literature. To begin with, we have explored the Michael reaction on 5,6-dihydrocyclopenta[*c*]pyrrole-1,3(2*H*,4*H*)-dione **3** with a commercially available thioacetic acid **4**.[9] As expected, the reaction went smoothly to yield the desired Michael adduct **5** and a more pleasing outcome was that the reaction could be carried out in water at room temperature (Scheme 1).

With the success of model reaction, we began efforts toward actual natural products. Compound **6**, previously known in the literature,[8] was prepared using allylic oxidation on **3** with the help of SeO_2 under microwave conditions in moderate yields.[10] Alternatively, the same compound can be prepared using the known protocol (bromination, trifluoroacetylation followed by hydrolysis) in overall 44% yield.[8] The other partner, thioacid **8**, was synthesized from the corresponding *p*-hydroxybenzoic acid **7** using Lawesson's reagent under microwave conditions.[11] Having both partners in hand, compounds **6** and **8** were mixed in water and stirred at

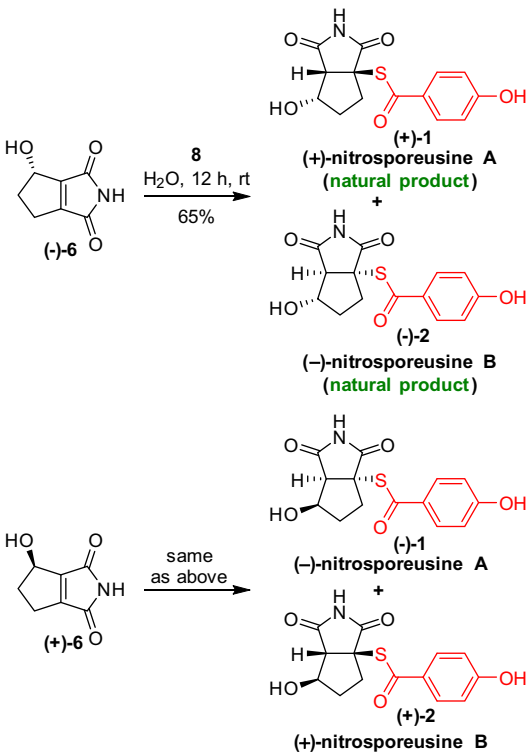


Scheme 2. Synthesis of racemic nitrosporeusines A and B.



Scheme 3. Enzymatic resolution toward maleimycins.

room temperature to furnish the desired racemic nitrosporeusines A (**1**) and B (**2**) in ~1:3 ratio, respectively (Scheme 2). Both the compounds were cleanly separated on a silica gel column as pure compounds. The spectral data (IR, ^{1}H NMR, ^{13}C NMR and HRMS) of the synthesized compounds **1** and **2** were compared with those of isolated nitrosporeusines[5] and found that they are identical in all respects. The next task was to make these compounds in optically pure form. For this purpose, we needed compound **6** in enantiopure form which was not reported earlier in the literature.[12] We have considered a few options to make this desired compound, and after a few attempts, we were successful in enzymatic resolution of



Scheme 4. Synthesis of enantiopure nitrosporeusines.

racemic alcohol **6**.[13] The Amano PS lipase mediated transesterification using vinylacetate in THF produced enantiopure alcohol (+)-**6** and corresponding acetate (−)-**9** in almost equal quantities. The enantiomeric excess (>98% ee) was determined by using the chiral HPLC method.[14] Also, we have cross checked the enantiopurity by converting (−)-**9** to (−)-**6** and (+)-**6** to (+)-**9** (Scheme 3).[15] It is worth highlighting that the biologically active natural product maleimycin (+)-**6** and its enantiomer were prepared in enantiopure form for the first time in good scale.

Maleimycin was reported to have shown interesting antibacterial activities like the inhibition of *Escherichia coli*, *Staphylococcus aureus,* and *Mycobacterium phlei* and anticancer activities in leukemia L-1210 cells.[16] Now that we have made this interesting molecule and its enantiomer available through chemical synthesis, they can be used for further biological evaluation. In addition, we have assigned the absolute configuration (indirectly) to the natural maleimycin (+)-**6** as (*R*)-configuration, since we have synthesized the natural nitrosporeusines of known absolute stereochemistry starting from (−)-**6**. Maleimycin (+)-**6** synthesized above was taken toward the natural products in a way similar to that followed for racemic compounds. To our surprise, we isolated the unnatural enantiomers of the reported nitrosporeusines A and B.[17] So, to obtain the desired natural nitrosporeusines, compound (−)-**6** was subjected to Michael addition with *p*-hydroxy thiobenzoic acid **8** to give nitrosporeusines A and B. The spectral data and optical rotation of synthetically prepared nitrosporeusines A (**1**) and B (**2**) were in agreement with the spectral data and optical rotation values reported by Lin and co-workers[5,18] Thus, we have prepared all the four enantiomers of nitrosporeusines (Scheme 4). As the absolute configuration of the hydroxyl group in the two natural products is known, we could indirectly assign their absolute stereochemistry of (−)-maleimycin as *S*-configuration and that of (+)-maleimycin as *R*-configuration, which were previously unknown.[17] Although nothing much is known about the biogenesis of nitrosporeusines, based on our findings, it can be surmised that natural (+)-maleimycin may not be the biogenetic precursor for the natural nitrosporeusines A and B.

The highlights of the present disclosure include (i) the first chemical synthesis of nitrosporeusines A and B in both racemic and enantiopure forms, (ii) scalable route under mild and green reaction conditions, (iii) gram-scale enzymatic resolution to access optically pure materials, (iv) synthesis of maleimycin in both enantiomeric forms and (v) determination of absolute stereochemistry of maleimycin. As the natural products are showing multiple interesting biological activities, it is expected that the analogs of nitrosporeusines will be highly useful. Efforts along these lines are underway and it will be the subject of future publications.

Acknowledgments

We thank the CSIR, New Delhi for the support through XII Five Year Plan programs: CSC0108 (ORGIN) and CSC0109 (NICE); Dr. Yanping Yan (Shantani Proteome Analytics Pvt. Ltd, Pune) for her help in translating Chinese patents; Professor Subrata Ghosh, Indian Association for the Cultivation of Science, Kolkata for helpful discussions in preparing starting materials for the project. SCP thanks the UGC, GRJ and VBG thank the CSIR, for the award of research fellowships.

Supplementary data

Supplementary data (Detailed experimental procedures, data comparison tables of synthetic and natural nitrosporeusines characterization data, and copies of NMR spectra for all the new compounds are provided.) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.01.143.

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15. Optical rotation data obtained for both the acetates were in opposite sign with same magnitude. (−)-**9**: $[\alpha]_D$ −38.3 (*c* 0.77 in $CHCl_3$); (+)-**9**: $[\alpha]_D$ +40.1 (*c* 0.54 in $CHCl_3$).
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17. We anticipated that absolute stereochemistry of natural (+)-maleimycin and natural nitrosporeusines will be the same. However, we have isolated unnatural (−)-nitrosporeusines from natural (+)-maleimycin and natural isomers of nitrosporeusines from unnatural enantiomer (−)-maleimycin.
18. In the case of (−)-nitrosporeusine B, we have observed some discrepancy in the magnitude of optical rotation. We have recorded $[\alpha]_D$ −121.8 (*c* 0.61 in MeOH) and the reported value for the same is $[\alpha]_D$ −59.3, (*c* 0.1 in MeOH).