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Selective Molecular Sequestration with Concurrent Natural Product Functionalization and Derivatization: From Crude Natural Product Extracts to a Single Natural Product Derivative in One Step

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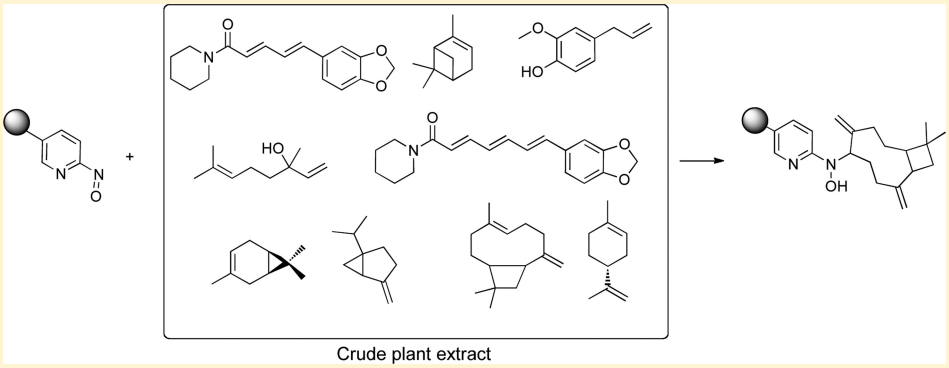
Supporting Information



ABSTRACT: A resin-bound nitroso compound sequestered a single unexpected component from crude plant seed extracts. Several plants, including *Piper nigrum*, *Eugenia caryophyllata*, and *Pimenta dioica*, were extracted with organic solvent in the presence of a nitroso-containing resin. The nitroso resin selectively sequestered a single compound, *β*-caryophyllene, via a chemo- and regioselective ene reaction. The ene product was released from the resin, and proper selection of the solid-phase linker and cleavage cocktail allowed concomitant further transformation of the primary ene product to a novel functionalized polycycle. Preliminary studies indicate that the new hydroxylamine-containing natural product derivatives have antibiotic activity.

Many current drugs originated from structural types created by nature.[1,2] A typical scenario of natural products-based drug discovery includes identification of the producing source, isolation of particular components, assessing their activities, and chemical transformation of natural products in order to improve pharmacological properties.

Nitroso derivatives are known to selectively react with compounds containing carbon−carbon double bonds: reactions of isolated alkenes with allylic C−H bonds form ene products,[3] reactions with conjugated dienes lead to hetero-Diels−Alder adducts.[4,5] We previously reported that the nitroso hetero-Diels−Alder (HDA) reaction is effective for simultaneous functionalization and derivatization of a number of structurally varied and highly functionalized diene-containing natural products to promote *M*odular *E*nhancement of *N*ature's *D*iversity (MEND) in solution[6] and on solid phase.[7−9] The HDA reaction with even relatively simple diene-containing natural products, like piperine, provided access to structurally diverse compounds, including structurally unrelated heterocycles.[10]

The relative reactivity of dienes with nitroso compounds extends over several orders of magnitude. For example, 1,3-cyclohexadiene is >100 times more reactive than 2,4-hexadiene.[8] Because of the high selectivity of nitroso compounds toward (di)enes and immense differences in reactivity among individual (di)enes, we decided to evaluate this reaction for direct and selective sequestration of individual components from crude extracts obtained from natural sources. Herein we report the use of a solid-phase nitroso agent for isolation and modification of a single natural product from crude extracts using a very efficient and operationally simple procedure.

To demonstrate the selective sequestration concept, we turned our attention to natural products from herbs and spices: their extracts contain complex mixtures of natural products including diene- and ene-containing species. The first experiments were carried out with extracts from black peppercorn (*Piper nigrum*). The main component (∼2−4% by weight, depending on the source and processing) of peppercorn is piperine (**1**, Figure 1), responsible for the pungent taste of pepper.[11] We have previously shown that piperine reacted with resin-bound nitroso dienophiles and provided access to a diverse set of derivatives including heterocyclic compounds.[8] Black peppercorn also contains piperettine (**2**), a vinylogous homologue of piperine.[11,12] The amount of piperettine is about

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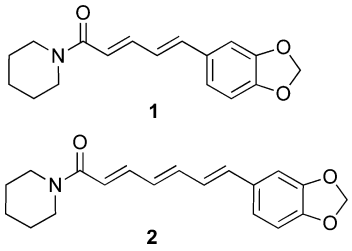


Figure 1. Structures of piperine (**1**) and piperettine (**2**).

15% of that of piperine; however, on the basis of our previous results, it was anticipated to be more reactive than piperine in the HDA reactions. Analysis of extracts from 10 peppercorns that originated from different geographical regions revealed insignificant variation in piperine and piperettine content (see the Supporting Information). The sequestration experiments were then arbitrarily carried out with Lampong Black peppercorn.

The crude dichloromethane extract of Lampong Black peppercorn was exposed to 6-nitrosonicotinic acid attached to a Rink amide resin (**3**). The resin was separated and product(s) released by treatment with a TFA-containing cleavage cocktail.[8,10] LCMS analysis revealed the presence of two major components: an alcohol and its TFA ester (the later eluting ester was then hydrolyzed to the alcohol). However, the MS spectrum did not correspond to the expected HDA adduct of either piperine or piperettine. Isolation and full characterization revealed that the product was a totally unrelated cyclobutane-containing tricyclic product (**4**) derived from a selective ene reaction (Scheme 1).

Black peppercorn contains essential oils, including potential ene-reactive terpenoids with the total amount of oil reportedly being 0.6 mg/g of pepper (0.06%).[13] However, none of the known components of pepper contained the carbon skeleton found in the isolated ene product. Pepper does contain one known cyclobutane-containing component, β-caryophyllene (**5**); however, its content may vary from 1 to 70%.[13] β-Caryophyllene is a sesquiterpene containing two isolated double bonds amenable to ene reactions, not cycloadditions, with nitroso compounds. On the basis of detailed structural analyses, a selective ene reaction occurred at the C5-carbon of β-caryophyllene, and subsequent treatment with TFA formed a bridged scaffold (Scheme 2). Acid-mediated rearrangement of β-caryophyllene is known.[11] The rearrangement was remarkably clean as no other side product was detected. The identity of the ene product was also confirmed by reacting authentic, commercially available β-caryophyllene with resin-bound nitroso species.

Despite the molar ratio of piperine/caryophyllene in pepper being ~150:1, the reaction of the nitroso resin with the crude extract afforded the β-caryophyllene product with exquisite selectivity (only traces of piperine HDA adduct were detected). Thus, the ene reaction of β-caryophyllene with the nitroso resin is >1000 times faster than the [4 + 2] cycloaddition reaction with the major component, piperine. In order to further evaluate the relative reactivity of β-caryophyllene with respect to dienes, we performed a competitive reaction of the nitroso agent **3** with equimolar amounts of β-caryophyllene (**5**) and cyclohexadiene (**9**), one of the most reactive dienes in nitroso cycloadditions.[7] As now expected, after cleavage from the resin, a product ratio of 1:4 in favor of the β-caryophyllene product (**4**) was obtained (Scheme 3).

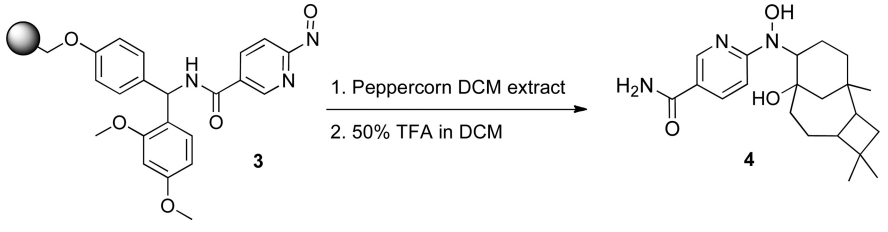
Although the rearrangement of the initially formed ene product produced a structurally very different and interesting scaffold, we anticipated that repetition of the experiments with a nitroso resin amenable to product release under nonacidic conditions would allow isolation of the ene product itself. Thus, a silicon-based linker (**11**), from our previous studies,[5,6] was used, and the ene product (**12**) was released by tetrabutylammonium fluoride (Scheme 4). We also demonstrated that the rearranged product (**13**) could be produced by TFA-mediated release. Thus, two different cleavage conditions provided access to two structurally diverse scaffolds from a single resin-bound precursor. Independently, product **13** was also prepared from resin **14** using the TFA cleavage cocktail.

While pyridylnitroso reagents, such as **11**, can be generated and separately reacted with enes and dienes, acylnitroso agents are unstable and typically are generated in situ by oxidation of their hydroxamic acid precursor. To avoid problems with oxidatively sensitive substrates, acylnitroso agents can be thermally regenerated from dimethylanthracene[14] or α-terpinene cycloadducts (**15**).[15] Indeed, exposure of β-caryophyllene to a resin-bound HDA α-terpinene adduct (**15**), even at 100 °C as required for the retro HDA reaction, produced the ene product cleanly (Scheme 5). Interestingly, after TFA-mediated release from the resin, we were able to isolate and characterize the rearranged product (**16**). However, the 1,2-oxazetidine ring-containing product (**16**) hydrolyzed in aqueous solutions and afforded **17**, analogous to compounds obtained from arylnitroso resins.

Similar reactions with a variety of peppercorns (see the Supporting Information) all yielded the β-caryophyllene ene product, although the amounts varied and the yields where reduced by ~50% when 24-h-old ground rather than freshly ground peppercorn was used. Reactions of crude extracts of freshly ground clove (*Eugenia caryophyllata*),[16] known to contain 1.4% of β-caryophyllene, and allspice (*Pimenta dioica*),[17] where the main essential oil is eugenol, also produced the β-caryophyllene ene reaction product.

The structure and stereochemistry (Figure 2) of all compounds was determined by measuring and analyzing 1D ^{1}H and ^{13}C NMR, and 2D homo- (gCOSY, TOCSY) and

Scheme 1. Reaction of Nitroso Resin with Peppercorn Extract



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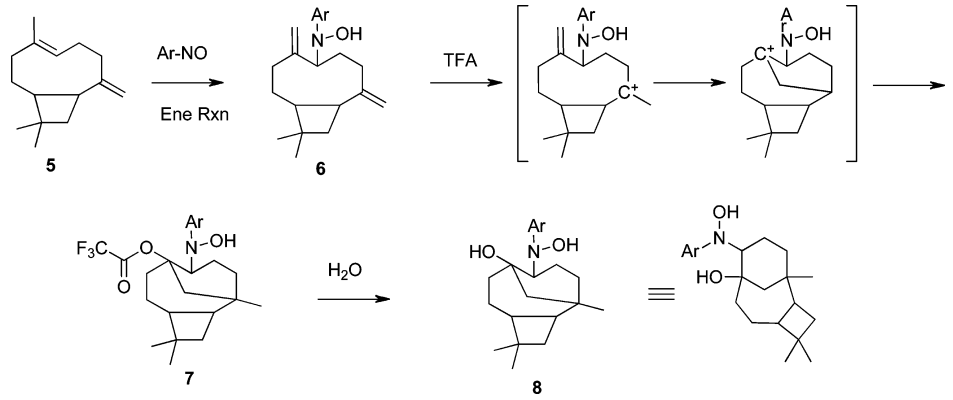
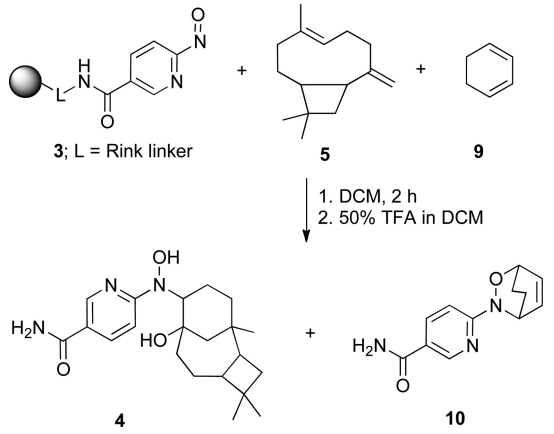
Scheme 2. Nitroso Ene Reaction and Acid-Mediated Rearrangement of the β-Caryophyllene Skeleton



Scheme 3. Evaluation of Relative Reactivity of Cyclohexadiene and β-Caryophyllene with a Resin-Bound Nitroso Agent



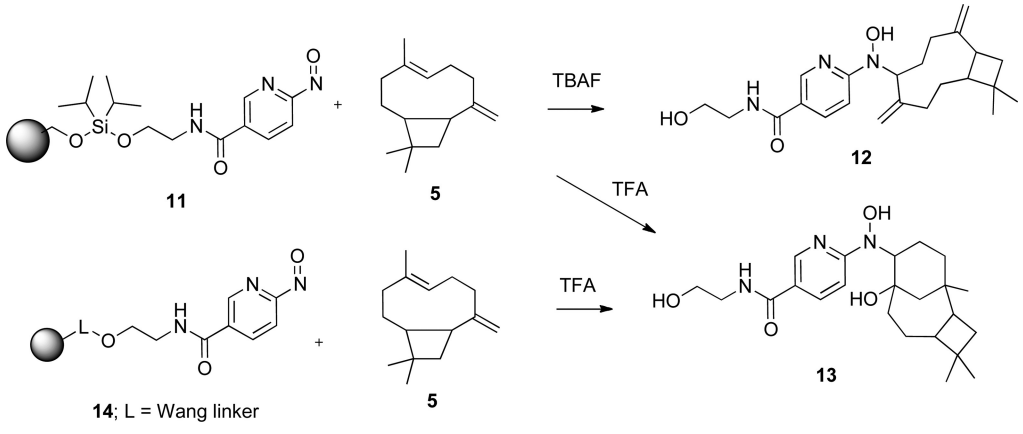
heteronuclear $^{1}H-^{13}C$ (gHSQC, gHMBC, gHSQC-TOCSY) spectra (Supporting Information).

Compounds **4**, **12**, **13**, **16**, and **17** were tested for their antibacterial activities against various strains of Gram-positive and Gram-negative bacteria as well as *Mycobacterium smegmatis* and *Mycobacterium vaccae*, using agar diffusion assays (Table 4, Supporting Information). In general, all compounds, except β-caryophyllene itself, exhibited moderate to good activity against the Gram-positive strains and slightly less against Gram-negative strains. All compounds had activity against mycobacteria, including *M. vaccae*, a common model for *M. tuberculosis*. The generally strong activity against *M. luteus* is notable since this pathogen is resistant to the broad-spectrum antibiotic, ciprofloxacin, the control antibiotic (Table 4, Supporting Information). Subsequently, we have determined that the nitroso chemistry can be broadly applied to ene substrates to generate libraries of antibiotics that are selectively active against cipro-resistant *M. luteus*.[19]

In conclusion, we demonstrated a very efficient and operationally simple procedure for sequestration and concurrent transformation of nitroso-reactive natural products. Upon exposure of solutions of complex mixtures from crude natural product extracts to nitroso resin under mild conditions, a single olefinic natural product, β-caryophyllene, present in black peppercorn only in 0.06%, was sequestered. Use of different cleavage conditions allowed transformation of the primary ene products to structurally diverse molecules. Preliminary screening indicated that the new derivatives have antibiotic activity. Overall, the process further demonstrates the utility of the MEND process by providing a direct method for enhancement of nature's diversity.

The process is also a form of molecular fishing. To portray the parallelism with traditional fishing, our fishing rod is a resin used for solid-phase synthesis, the bait is a nitroso species, the river/sea is a crude extract from a natural source and the fish is a (di)ene-containing natural product. In addition, we added processing, i.e., chemical transformation of the "caught" natural product.

Scheme 4. Product Diversification by Different Cleavage Conditions



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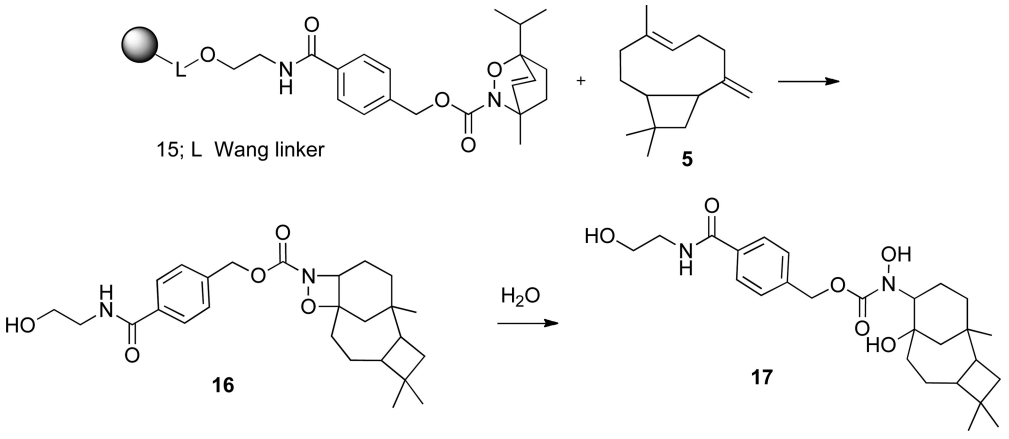
Scheme 5. Retro-HDA Reaction with β-Caryophyllene



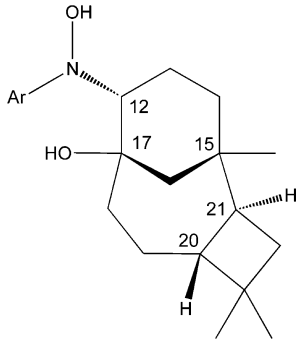


Figure 2. Stereochemistry of tricyclic skeleton.

EXPERIMENTAL SECTION

General information related to sources of materials, solvent purity, and generalized conditions is provided in the Supporting Information.

Analysis of Extracts from Various Peppercorns. Pepper, 3 g, was ground and extracted with 10 mL of DCM at ambient temperature overnight. The solution was filtered, evaporated, and lypholized. The quantity of piperine and piperettine was calculated from LC traces at 350 nm using piperine as a standard. The amounts of total material extracted and amount of piperine and piperettine obtained depended somewhat on the source and type of peppercorn (see Table 1, Supporting Information)

Nitroso Resins. Nitroso resins **3**, **11**, and **14** were prepared following our previously described protocol.[8] Resins were stored in the form of hydroxylamine derivatives and oxidized prior to their use.

Molecular Sequestration. Typically, nitroso resin **3**, 50 mg, and ground peppercorn were placed into a plastic reaction vessel for solid-phase synthesis, and 10 mL of dichloromethane (DCM) was added. The slurry was shaken vigorously for 1 h and transferred into a separation funnel, and the resin was separated from the ground pepper based on different density of the resin and ground pepper by adjusting the ratio of DCM (resin floats) and methanol (resin sinks). The resin was transferred into a plastic reaction vessel and washed 5 times with DCM.

Compound Isolation and Purification. Resin-bound compounds, 50 mg, in a fritted polypropylene reaction vessel were treated with 1 mL of 50% TFA in DCM for 1 h, unless stated otherwise. The TFA solution was collected. The resin was washed three times with 10% TFA, washes were collected, and the combined extracts were evaporated by a stream of nitrogen. Compounds synthesized on silicon linkers were cleaved by reaction with 1 mL of 0.1 M TBAF solution in THF for 30 min. The THF solution was collected, resin was washed three times with THF, washes were collected, and the combined extracts were evaporated by a stream of nitrogen. The crude product was purified by HPLC. Collected fractions were concentrated by a stream of nitrogen, frozen, and lyophilized. The yield was calculated with respect to linker loading and was found to vary considerably depending on the spice and whether it was freshly ground before the reaction (Table 2, Supporting Information).

Reaction with Authentic β-Caryophyllene. A solution of β-caryophyllene (1 mmol, 254 μL) in 3 mL of DCM was added to the nitroso resin **14**, 300 mg, and the slurry was shaken for 1 h. The resin was washed 5× with DCM. The product was cleaved from the resin by 10% TFA in DCM for 1 h, and the crude product was purified as described.

Relative Reactivity of β-Caryophyllene and 1,3-Cyclohexadiene. A solution of β-caryophyllene (0.5 mmol, 127 μL) and 1,3-cyclohexadiene (0.5 mmol, 50 μL) in 2 mL of DCM was added to the nitroso resin **3** (50 mg). The resin slurry was shaken for 2 h. The resin was washed 5× with DCM. The products were cleaved form the resin by 50% TFA in DCM, and the cleaved product was analyzed on LCMS. The relative ratio of products was calculated from the UV response at 230 nm. The ratio of HDA cyclohexane adduct/β-caryophyllene ene product was 1:4.2 (average from three experiments).

Retro-Hetero-Diels–Alder (HDA) Reaction. Polymer-supported HDA adduct with α-terpinene **15** (50 mg) was reacted with β-caryophyllene (0.5 mmol, 127 μL) in 1 mL of DMF at 100 °C overnight. The resin was washed 3× with DMF and 5× with DCM and treated with 50% TFA in DCM for 30 min. The crude reaction mixture contained two major components, **16** and **17**, which were isolated after HPLC purification. Compound **16** was not stable in aqueous solutions, and after isolation, it was lyophilized from benzene to remove residual water.

*6-[Hydroxy-N-(8-hydroxy-1,4,4-trimethyltricyclo[6.3.1.0^{2,5}]dodec-9-yl)amino]pyridine-3-carboxamide (**4**).* The yield from 50 mg of nitroso resin **3** was 5.2 mg (46%). Repetition with a different batch of resin **1** gave 5.7 mg (37%): HRMS (ESI) *m*/*z* calcd for $C_{21}H_{32}N_3O_3$ $[M + H]^+$ 374.2399, found 374.2469.

*6-[N-(10,10-Dimethyl-2,6-dimethylidenebicyclo[7.2.0]undecan-5-yl)hydroxyamino]-N-(2-hydroxyethyl)pyridine-3-carboxamide (**12**).* The yield was 4.9 mg (18%): HRMS (ESI) *m*/*z* calcd for $C_{23}H_{34}N_3O_3$ $[M + H]^+$ 400.2600, found 400.2595

*6-[Hydroxy-N-(8-hydroxy-1,4,4-trimethyltricyclo[6.3.1.0^{2,5}]-dodecan-9-yl)amino]-N-(2-hydroxyethyl)pyridine-3-carboxamide (**13**).* The yield from 300 mg of nitroso resin **14** was 33.5 mg (89%): HRMS (ESI) *m*/*z* calcd for $C_{23}H_{36}N_3O_4$ $[M + H]^+$ 418.2706, found 418.2700

*[4-[(2-Hydroxyethyl)carbamoyl]phenyl]methyl 5,5,8-Trimethyl-13-oxa-12-azatetracyclo- [6.5.1.0^{1,11}.0^{4,7}]tetradecane-12-carboxylate (**16**).* The yield was 8.3 mg (18%): HRMS (ESI) *m*/*z* calcd for $C_{26}H_{37}N_2O_5$ $[M + H]^+$ 457.2683, found 457.2697

*[4-[(2-Hydroxyethyl)carbamoyl]phenyl]methyl N-Hydroxy-N-(8-hydroxy-1,4,4-trimethyltricyclo[6.3.1.0^{2,5}]dodecan-9-yl)carbamate (**17**).* The yield was 2.0 mg (4%): HRMS (ESI) *m*/*z* calcd for $C_{26}H_{38}N_2NaO_6$ $[M + H]^+$ 497.2593, found 497.262.

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Proton connectivities were derived by examination of the gCOSY and TOCSY spectra. Signals of all carbons with directly attached protons were assigned using the gHSQC spectra. Finally, the gHMBC and gHSQC-TOCSY spectra were used to assign quaternary carbons and to check the correctness of the connectivities established by the interpretation of the other spectra. The stereochemistry at C-20 and C-21, originated from caryophyllene, is known,[18] C-12, C-15, and C-17 were determined by analyzing the 1D proton (magnitudes of the 1H-1H spin−spin coupling constants; J) and 2D ROESY spectra of compound **17**. Its six-membered-ring adopts a chair conformation as evident from the following facts. The equatorial position of the substituent originated from the nitroso species at C-12 is supported by the fact that the H_a-12 signal (δ 4.02) exhibits a large diaxial spin−spin coupling of 12.8 Hz and an axial−equatorial spin−spin coupling of 4.6 Hz with the H_a-13 (δ 2.32) and H_e-13 (δ 1.57) methylene proton resonances, respectively. Additionally, the 14,16-diequatorial protons are connected by four bonds in a W arrangement. Their corresponding signals show a mutual spin−spin coupling of 2.9 Hz. Moreover, the ROESY spectrum displays a crosspeak between the H_e-16 proton resonance (δ 1.86) and the H-20 signal (δ 1.69), which indicates that both the CH_3-26 (δ 0.84) at C-15 and OH group at C-17 are in the equatorial positions.

■ ASSOCIATED CONTENT

Supporting Information

Full experimental details, descriptions of biological assays, and copies of all ^{1}H NMR, ^{13}C NMR, and multidimensional NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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