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## Semisynthetic Studies on the Manzamine Alkaloids<sup>#</sup>

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### Abstract

Chemical transformation studies were conducted on (–)-8-hydroxymanzamine A (**1**), (–)-manzamine F (**2**), manzamine A (**3**), and (+)-8-hydroxymanzamine A (**4**), isolated from Indo-Pacific *Acanthostrongylophora* sponges.<sup>1</sup> Thirteen new semisynthetic manzamine derivatives, including four<sup>34,35</sup> manzamines (**5**, **6**, **8**, and **9**) and the unprecedented manzamine derivative **17**, are reported. The potent in vitro activities of the obtained semisynthetic manzamines against activated brain microglia and the AIDS opportunistic infection pathogen *Mycobacterium tuberculosis* are presented.

The manzamines are complex polycyclic marine-derived alkaloids first reported by Higa and co-workers in 1986 from the Okinawan sponge genus *Haliclona*.<sup>2</sup> Manzamines possess a fused and bridged tetra- or pentacyclic ring system, which is attached to a β-carboline moiety. Since the first report of manzamine A, several manzamine-type alkaloids have been reported from 16 sponge species belonging to the Chalinidae, Niphatidae, Petrosidae, Thorectidae, and Irciniidae families.<sup>3</sup> These include *Acanthostrongylophora*, *Amphimedon*, *Cribrochalina*, *Haliclona*, *Hyrtilos*, *Ircinia*, *Pachypellina*, *Pellina*, *Petrosia*, *Prianos*, *Reniera*, and *Xestospongia* sponge species.<sup>1,3–6</sup> Manzamines exhibit a diverse range of bioactivities including cytotoxicity<sup>2</sup> and insecticidal,<sup>7</sup> antibacterial,<sup>8</sup> and antileishmaniasis effects.<sup>9</sup> Manzamines also show potent activity against HIV-1<sup>10</sup> and several AIDS opportunistic

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**Supporting Information Available:** Schemes S1 and S2, plausible mechanisms of formation for compounds **6**, **9**, and **17**, are available free of charge via the Internet at <http://pubs.acs.org>.

infection (OI) pathogens, e.g., *Cryptosporidium parvum*,<sup>1,3</sup> *Toxoplasma gondii*,<sup>1,3</sup> and *Mycobacterium tuberculosis*,<sup>1,3,9-12</sup> as well as the potential curative activity against malaria in animal models.<sup>1</sup> Anti-inflammatory activity of manzamines A–F was also recently reported.<sup>13-15</sup>

In 2001, the antipodes of 8-hydroxymanzamine A (**1**) and manzamine F (**2**) were reported. This was based on comparison of <sup>1</sup>H and <sup>13</sup>C NMR, optical rotation, TLC, and melting point data for compound **1** and authentic (+)-8-hydroxymanzamine A free bases (both separately and mixed).<sup>1</sup> The antipodes of compounds **1** and **2** revealed identical physical properties to **1** and **2** themselves with the exception of opposite optical rotation values. *ent*-8-Hydroxymanzamine A exhibited improved activity against the P-388 murine lymphocytic leukemia cell line with an IC<sub>50</sub> of 0.25 µg/mL, when compared with the previously published value of >20 µg/mL for the (+)-compound, examples further supporting the occurrence of an enantiomeric pair.<sup>1</sup> Manzamine A (**3**) production by a putative symbiotic actinobacterium, *Micromonospora* sp., supports the possibility of the occurrence of manzamine antipodes due to the wide diversity of microbial enzymes that could be involved in the biosynthesis and later biotransformation of these alkaloids.<sup>16</sup>

Before the achievement of the total synthesis of manzamine A (**3**),<sup>17</sup> numerous efficient synthetic strategies were conducted to accomplish this goal including the synthesis of manzamine C, the simplest member of the manzamines.<sup>5</sup> Herein, we report the first semisynthetic study of the manzamines, which provides important information regarding the chemistry of these alkaloids. The potential importance of the manzamines as antituberculosis and anti-inflammatory leads is also highlighted.

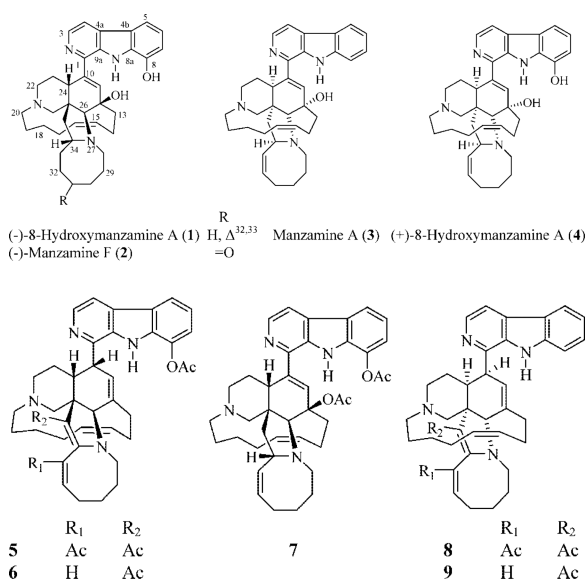
## Results and Discussion

The lipophilic extract of different collections of undescribed Indo-Pacific *Acanthostrongylophora* sponges (order Haplosclerida) afforded manzamines **1–4** in relatively high yields (1.24, 0.055, 0.66, and 1.24%, respectively).<sup>1,12</sup>

In an attempt to better understand the structure–activity relationship of the manzamine alkaloids, several chemical reactions were performed in parallel starting with **1–4**. Reaction of **1** with acetic anhydride in the presence of excess sodium acetate at 80 °C, for 30 min under nitrogen, afforded the minor new <sup>34,35</sup> products **5** and **6** (9.8% and 1.5%, respectively) as well as 8,12-di-*O*-acetyl-8-hydroxymanzamine A (**7**) as a major product (24%). The same reaction with manzamine A (**3**) afforded **8** (7.6%) along with the major manzamine analogue **9** (40%).

The IR spectrum of **5** indicated the presence of an acetate ester and α,β-unsaturated ketones. The HRFTMS suggested the molecular formula of C<sub>42</sub>H<sub>49</sub>N<sub>4</sub>O<sub>4</sub>. The <sup>1</sup>H and <sup>13</sup>C NMR data displayed close similarities to **1**. The methyl carbon resonating at δ 21.6 and correlated to the methyl proton singlet at δ 2.56 was assigned as the 8-*O*-acetate methyl. This methyl proton singlet showed a <sup>2</sup>*J*-HMBC correlation to the quaternary ester carbonyl carbon at δ 169.5. The proton double-doublets resonating at δ 7.14 correlated to the olefinic methine carbon at δ 144.3 and was assigned to H-32. This proton doublet of doublets displayed COSY

coupling to H<sub>2</sub>-31. Proton H-32 showed a <sup>3</sup>*J*-HMBC correlation to the quaternary carbon at δ 161.8 (C-34) and the ketone carbon at δ 195.0 (C-33 acetyl ketone). The methyl carbon resonating at δ 27.1, which correlated to the methyl singlet at δ 2.21, was assigned as the C-33 acetyl methyl. The latter signal displayed a <sup>2</sup>*J*-HMBC correlation to the ketone carbon at δ 195.0 and a <sup>3</sup>*J*-HMBC correlation to the quaternary olefinic carbon at δ 135.2 (C-33). The assignment of the <sup>34,35</sup> was based on the <sup>3</sup>*J*-HMBC correlations with the H-26 proton singlet at δ 4.88 and the <sup>3</sup>*J*-HMBC correlation between H<sub>2</sub>-36 and C-35, in addition to the disappearance of the H<sub>2</sub>-35 and H-34 proton signals, as compared to **1**. Protons H<sub>2</sub>-28 also showed a <sup>3</sup>*J*-HMBC correlation to the quaternary C-34 resonance. The location of the C-35 methyl ketone substituent was indicated from the <sup>3</sup>*J*-HMBC correlation of the methyl signal at δ 2.12 to the quaternary olefinic carbon at δ 114.9 (C-35). The latter methyl singlet also showed a <sup>2</sup>*J*-HMBC correlation to the ketone carbon resonating at δ 192.7. The β-orientation of H-10 was proposed on the basis of its NOESY correlation with β-H-24. Final confirmation of the structure **5** was accomplished through a <sup>1</sup>H-<sup>15</sup>N-GHMBC experiment, in which the quaternary nitrogen N-2 resonating at δ 295.9 was correlated to H-3 and H-4, and the protonated N-9 resonating at δ 128.7 correlated to the proton singlet at δ 10.45. The quaternary nitrogen resonating at δ 36.4 (N-27) displayed a <sup>3</sup>*J*-correlation with H<sub>2</sub>-29, with nitrogen N-21 not observed.



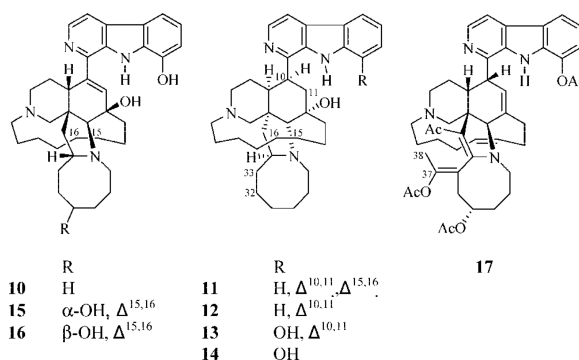
The MS and NMR data of **6** indicated a close structure similarity to **5** except in the lack of one acetyl group. The olefinic proton doublet resonating at δ 6.44 (10.7 Hz) was assigned to H-33, as it showed COSY coupling with the olefinic proton multiplet resonating at δ 5.18 (H-32).

The IR spectrum of **7** displayed two ester absorption bands. The HRFTMS and <sup>1</sup>H and <sup>13</sup>C NMR data of **7** suggested that it is *ent*-8,12-di-*O*-acetyl-8-hydroxymanzamine A. The methyl singlet resonating at δ 2.53 was assigned to the 8-*O*-acetate methyl. This signal showed a <sup>2</sup>*J*-HMBC correlation to the acetate carbonyl at δ 169.5. Similarly, the methyl

singlet resonating at  $\delta$  2.17 showed a  $^2J$ -HMBC correlation to the acetate carbonyl at  $\delta$  171.1 and was assigned to an 8-*O*-acetate group.

The HRFTMS and NMR data of **8** and **9** indicated their similarity to **5** and **6**, respectively, having a manzamine A skeleton instead of that of (-)-8-hydroxymanzamine A. The methyl proton singlets resonating at  $\delta$  2.19 and 2.21 in **8** and **9**, respectively, were assigned to the 35-acetyl methyl groups. Each of these methyl singlets showed a  $^2J$ -HMBC correlation to the methyl ketone carbon resonating at  $\delta$  192.6 and 196.8 in **8** and **9**, respectively. They also displayed a  $^3J$ -HMBC correlation to the quaternary olefinic carbon resonating at  $\delta$  114.7 and 114.5 (C-35) in **8** and **9**, respectively. Similar correlations were observed for the C-33 acetyl methyl group ( $\delta$  2.27) in **8**. The proton doublet resonating at  $\delta$  6.44, which was assigned to H-33 in **9**, showed COSY coupling to the olefinic proton multiplet at  $\delta$  5.23 (H-32).

Reduction of **1** in absolute ethanol using hydrazine hydrate in the presence of Pd/carbon for 12 h at room temperature afforded the major product **10** (19%). The same reaction starting with **3** using 18 h reflux afforded **11** (12.8%) and **12** (5.4%). Similarly, reduction of **4** using a 34 h reflux afforded **13** (14.5%) and **14** (3.4%). The order of reduction is as expected, being more favorable for the disubstituted  $^{32,33}$ , followed by the  $^{15,16}$  system, and finally for the trisubstituted  $^{10,11}$ .



The HRFTMS data of **10–14** indicated reduced derivatives of **1**, **3**, or **4**. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data suggested that **10** is (-)-15,16,32,33-tetrahydro-8-hydroxymanzamine A, since the olefinic proton and carbon signals C-15, 16, 32, and 33 were replaced by upfield aliphatic signals. Similarly, **11** and **12** were found to be 32,33-dihydro- and 15,16,32,33-tetrahydromanzamine A, respectively. Likewise, the identity of **14** was assigned as 10,11,15,16,32,33-hexahydro-8-hydroxymanzamine A.

Reduction of **2** using sodium borohydride in anhydrous tetrahydrofuran at room temperature afforded **15** (27.5%) and **16** (6%). The HRFTMS of **15** and **16** displayed molecular ion peaks  $[\text{M} + \text{H}]^+$  at  $m/z$  583.3600 and 583.3615, respectively, suggesting the same molecular formula,  $\text{C}_{36}\text{H}_{47}\text{N}_4\text{O}_3$ , and 16 degrees of unsaturation. The broad proton singlets resonating at  $\delta$  3.77 and 3.86, respectively, were assigned to the new oxygenated H-31 in **15** and **16**. The  $\beta$ -orientation of H-31 in **15** was attributed on the basis of comparison of its NOESY correlation with the  $\beta$ -oriented H-34. Compound **16** must then be the C-31 epimer of **15**, since the only spectroscopic difference between these compounds was evident at C-31.

Reaction of the semisynthetic product **15** with (Ac)<sub>2</sub>O/pyridine at 100 °C for 12 h afforded **17** (56%). The HRFTMS data of **17** displayed a molecular ion peak [M + H]<sup>+</sup> at *m/z* 775.4271, suggesting the molecular formula C<sub>46</sub>H<sub>55</sub>N<sub>4</sub>O<sub>7</sub> and 22 unsaturation units. The IR spectrum of **17** showed the presence of both acetate esters and an α,β-unsaturated ketone functionality. The <sup>1</sup>H and <sup>13</sup>C NMR data of **17** demonstrated the presence of three *O*-acetates, one methyl ketone, and one methyl group located on a tetrasubstituted double bond. The methyl singlet resonating at δ 2.58 was <sup>2</sup>*J*-HMBC correlated to the acetate carbonyl at δ 169.8 and assigned to an 8-*O*-acetate unit. In turn, the methyl singlet resonating at δ 2.07 that showed a <sup>2</sup>*J*-HMBC correlation to the acetate carbonyl at δ 170.3 was assigned as 31-*O*-acetate. The <sup>34,35</sup> formation was confirmed through the <sup>3</sup>*J*-HMBC correlation between the H-26 proton singlet and the quaternary olefinic carbons at δ 163.9 (C-34) and 111.7 (C-35), in addition to the <sup>3</sup>*J*-HMBC correlations of H<sub>2</sub>-28 with C-34. The methyl singlet resonating at δ 2.29, which exhibited a <sup>2</sup>*J*-HMBC correlation to the ketone at δ 192.0 and a <sup>3</sup>*J*-HMBC correlation to C-35, was assigned as the 35-acetyl functionality. The methyl singlet resonating at δ 1.85 that correlated to the methyl carbon at δ 16.8 was assigned to C-38, on the basis of its <sup>2</sup>*J*- and <sup>3</sup>*J*-HMBC correlations to the quaternary oxygenated olefinic carbons resonating at δ 148.1 (C-37) and 116.9 (C-33). The assignment of the system <sup>33,37</sup> rather than <sup>32,37</sup> was based on the COSY correlation between H-31 and H<sub>2</sub>-32, the <sup>3</sup>*J*-HMBC correlation between H<sub>2</sub>-32 and C-30, C-34, and C-37, and the <sup>2</sup>*J*-HMBC correlation of H<sub>2</sub>-32 to C-33. The only remaining acetate methyl singlet resonated at δ 2.21 and showed a <sup>2</sup>*J*-HMBC correlation with the acetate carbonyl resonating at δ 168.8; it was assigned as the C-37-*O*-acetate. This was further supported by the downfield resonance of C-37 (δ 148.1) due to the additional oxygenation. The configuration at C-31 was assigned similar to the parent compound **15**.

Plausible mechanistic schemes of the generation of compounds **5–7**, **9**, and **17** are proposed in Scheme 1 as well as Schemes S1 and S2 found in the Supporting Information. It is noteworthy that a stereospecific hydride transfer with reductive elimination of water occurred via the C-10–11–12 allylic alcohol system. On the basis of manzamine A (**3**), which produced only compounds with an α-H at C-10, and (–)-8-hydroxymanzamine A (**1**), producing only compounds with a β-H at C-10, it is presumed that the incorporation of a hydrogen atom at C-10 might well occur by an intramolecular H atom transfer, or one controlled by the substrate in some other way. To examine the feasibility of such an unusual process occurring by intramolecular H atom transfer, a molecular modeling study was performed. Careful examination of the manzamine A (**4**) model showed a distance of 5.30 Å between the C-34–H and the C-10/C-9 double bond (Figure 1). This hydrogen was close enough to produce an intramolecular H transfer, but the distance was not optimal for such a process. Also, hydrogen bonds between N-9–H---(C-12)O–H---N-27 anchoring the aromatic heterocycle almost perpendicular to the bicyclic A,B-ring system could be proposed. To produce such a transfer, the OH must be lost, generating a carbocation intermediate. This could occur via protonation of the acetylated intermediate such as **7** (OAc-8, -12 from **1**; and OAc-12 from **3**) or by protonation of the free hydroxyl group, leading to loss of either acetic acid or water and the generation of an allylic carbonium ion. The carbocation intermediate **1a** would rearrange by hydride transfer through a bridge transition state to produce intermediate **1b**. This is evident from the modeled structure of manzamine **3** that supplied an

approach of C-34–H to C-10 from 5.14 Å, whereas the allylic carbocation **1a** showed this distance as 3.43 Å due to the conformational change associated with the loss of OH (Scheme 1, Figure 2). This loss produced an alteration of the B ring from an almost planar ring to a boat conformation in which the hydrogen more closely approached the double bond, facilitating the transfer. The rearrangement could now proceed by a transannular 1,5-hydride shift, transferring the hydride to C-10 while shifting the electrons to generate the <sup>8,9</sup> double bond. Similar transannular 1,5-hydride shifts have been observed,<sup>18,19</sup> where the molecular orbital demand requires a migrating σ-bond to be perpendicular to the plane of the sp<sup>2</sup> carbon. An examination of the lowest unoccupied molecular orbital (LUMO) of this cation **1a** showed that the hydrogen atom directed to the p-orbital over C-10 was in accordance with the orbital demand for this type of rearrangement. Therefore, the carbocation intermediate **1b** was modeled showing no significant conformational change, but this cation was 37.4 kcal/mol more stable than **1a**.<sup>20</sup> This was undoubtedly due to resonance stabilization of the C-34 carbocation via the adjacent (α) nitrogen as an iminium ion and the release of the unfavorable boat conformation of the B ring to a more energetically favorable twist-boat conformation (Figure 3).

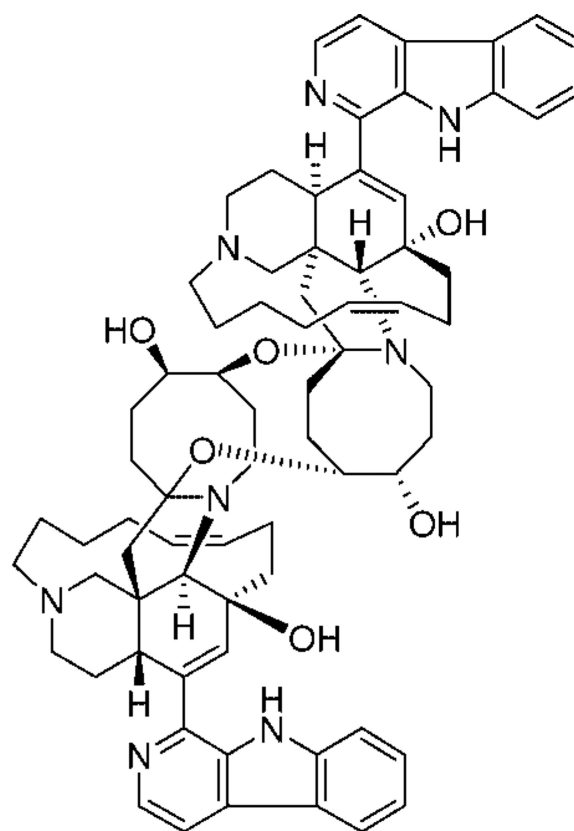
With the formation of the iminium ion **1b** stereospecifically rationalized by molecular modeling, incorporation of acetate units into the intermediate was examined next (Scheme 1). Iminium ions generated in the presence of base are known to undergo elimination to a neutral enamine or, in this case, a dienamine, **5a**. Reaction of the nucleophilic enamine **5a** with the electrophile acetic anhydride can then proceed via attack of the C-33–34 double bond upon the electrophilic carbonyl carbon in an accepted mechanistic fashion for reactions of enamines with electrophiles. With acetate now present at C-33 and an iminium regenerated between the N and C-34, the system **5b** is ideally suited to undergo deprotonation to give **5c**. To envisage formation of the product **5**, one must now place another unsaturated acetate unit at C-35. This may be achieved rationally by protonation–deprotonation to equilibrate **5c** with **5f** via the intermediates **5d** and **5e**. Then, **5f** would be suited to undergo another acetylation at C-35 via attack of the C-34–35 double bond on acetic anhydride. As before, the resultant iminium ion **5g** now simply undergoes deprotonation to furnish the isolated product **5**.

To explain the formation of **6** (Scheme S1, Supporting Information), **1b** could be deprotonated to afford the intermediate enamine **6a**. Monoacetylation of **6a** would give the iminium ion **6b**, which deprotonates to furnish the observed product **6**. By analogy to the formation of **5** and **6** (not shown), the enantiomeric manzamine **3** would lead to products **8** and **9**.

Formation of the remaining semisynthetic manzamine **17** from its precursor **15** is shown in Scheme S2, Supporting Information. After acetylation of the sterically accessible C-31 hydroxyl group to give **15a**, loss of water to give the allylic carbonium **15b** is consistent with Scheme 1, as is the intramolecular H atom transfer to afford **15c**. Enamine formation (**15d**) results from deprotonation of **15c** and would lead to attack on acetic anhydride as before to give **15e**. This iminium ion–acetate could undergo enamine formation again to the available C-35 position by loss of a C-35 H, affording **15f**. Attack on acetic anhydride again

leads to an iminium ion, **15g**, that then undergoes deprotonation to give **15h**. Finally, enolization of **15h** favorably places another double bond in conjugation with the enone system at C-34–35 via deprotonation of the acidic proton at C-33 by pyridine. Acetylation of the enolate affords the observed reaction product **17**.

The enormous potential of the marine environment to provide new antituberculous leads has been documented.<sup>21</sup> The in vitro activity of natural and semisynthetic manzamines against *M. tuberculosis* (H37Rv) was evaluated in the present investigation. Most manzamines induced 95–99% inhibition of *M. tuberculosis* with MIC values of <12.5 µg/mL, except for manzamines **9**, **16**, and *neo*-kauluamine (**18**).<sup>1</sup> Rifampin, the positive drug control, induced 100% inhibition at 0.5 µg/mL. (+)-8-Hydroxymanzamine A induced 100% inhibition with a MIC of <6.25 µg/mL, suggesting the precedence of the (+)-over the (–)-enantiomer for TB activity. While **15** induced 100% inhibition with a MIC of <6.25 µg/mL, its C-31 epimer (manzamine **16**) was less active (61% inhibition). *neo*-Kauluamine (**18**), the second known manzamine dimer with two β-oriented ether bonds bridging both monomers at C-31/C-34' and C-30'/C-34 carbons of the azacyclooctane site, in addition to two free α- and β-hydroxyl groups at C-30 and C-31', respectively,<sup>1</sup> also displayed less activity (44% inhibition). This suggests that α-hydroxylation at C-31 maintains activity, while β-hydroxylation at this position reduces activity. Reduction of the <sup>10,11</sup>, <sup>15,16</sup>, and <sup>32,33</sup> systems has no effect on the activity, as suggested by the activities of semisynthetic manzamines **10**, **11**, and **13**. Substitution at C-33 improves the activity, as indicated by the higher bioactivity of **5** as compared to that of **6** and **9**.



*neo*-Kauluamine (**18**)

Neuroinflammatory conditions appear to involve release of the eicosanoid thromboxane B<sub>2</sub> (TXB<sub>2</sub>) and the free radical superoxide anion (O<sub>2</sub><sup>-</sup>) by activated brain microglia BMΦ.<sup>22</sup> *Escherichia coli* lipopolysaccharide-activated rat neonatal microglia represent a convenient in vitro model to study natural products that could potentially inhibit production of neurotoxic TXB<sub>2</sub> and O<sub>2</sub><sup>-</sup>.<sup>23</sup> Using this model, we have investigated the effect of natural and semi-synthetic manzamines on the release of BMΦ O<sub>2</sub><sup>-</sup> and TXB<sub>2</sub>. Manzamines **2**, **4–13**, and **15–17** did not inhibit BMΦ O<sub>2</sub><sup>-</sup> and TXB<sub>2</sub> release even at 30 μM, the highest concentration tested. However, (-)-8-hydroxymanzamine A (**1**), manzamine A (**3**), and tetrahydro-8-hydroxymanzamine A (**14**) inhibited TXB<sub>2</sub> (IC<sub>50</sub> 0.25, <0.1, and 1.97 μM, respectively), but in contrast, did not affect O<sub>2</sub><sup>-</sup>. Furthermore, because in vitro toxicity by these manzamines, determined as lactate dehydrogenase release, was observed to be minimal, the current data appear to support the potential of **1**, **3**, and **14** as lead compounds for the development of novel anti-inflammatory agents to modulate activated brain microglia.<sup>13–15</sup>

On the basis of the results reported herein and the absence of in vivo toxicity from previous reports, the manzamine alkaloids are viable antituberculosis and antineuroinflammatory leads.<sup>1</sup> β-Hydroxylation at position C-31 of the azacyclooctane ring significantly reduces the resultant activity, as opposed to α-hydroxylation, which has little effect. Double-bond systems<sup>10,11, 15,16, and 32,33</sup> are not essential for the antimycobacterial activity.



Substitution at C-33 was shown to enhance activity, as indicated by the high potency of manzamine analogue **5**.

## Experimental Section

### General Experimental Procedures

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Optical rotations were measured at room temperature with a JASCO DIP-370 digital polarimeter. UV spectra were acquired using a Perkin-Elmer Lambda 3B UV/vis spectrophotometer. The IR spectra were recorded on an ATI Mattson Genesis Series FTIR spectrophotometer. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded in  $\text{CDCl}_3$  on Bruker DRX- or AMX-NMR spectrometers operating at 400 or 500 MHz for  $^1\text{H}$  NMR and 100 or 125 MHz for  $^{13}\text{C}$  NMR. The HRMS were measured using a Bioapex FTMS with electrospray ionization. TLC was carried out on precoated silica gel G<sub>254</sub> or aluminum oxide ALOX-100 UV<sub>254</sub> 500  $\mu\text{m}$  (E. Merck), with the following developing systems: *n*-hexanes/acetone (65–35) or cyclohexane/EtOAc/ $\text{NH}_4\text{OH}$  (100:50:0.1) or C<sub>18</sub>-reversed phase plates, 200  $\mu\text{m}$  using MeOH/ $\text{H}_2\text{O}$ / $\text{NH}_4\text{OH}$  (80:20:0.1). For column chromatography, Si gel 60 (40  $\mu\text{m}$ ) was used.

### Biological Material

The sponge was collected in November 1994, in Manado Bay, Sulawesi, Indonesia, from a depth of 20 m.<sup>1,9</sup> It is irregularly massive with a rough surface, the texture is tough and crumbly, and the colors in life are maroon externally and yellow internally. The skeleton is very irregular and composed of small, round meshes set in irregular curving fascicles. The spicules are irregularly curved strongyles, 80–140  $\mu\text{m}$ .<sup>1,9</sup> The sponge was identified as a species of the genus *Acanthostrongylophora* (order Haplosclerida, family Petrosiidae) by Michelle Kelly of the National Institute of Water and Atmospheric Research Ltd., Auckland, New Zealand. A voucher specimen designated BMNH 1997.11.11.9 is deposited at the Natural History Museum, London, UK.<sup>1,9</sup>

### $^{15}\text{N}$ NMR Spectroscopy

Inverse-detected  $^{15}\text{N}$  NMR spectra were recorded using a 500 MHz NMR spectrometer equipped with a 3 mm inverse-detection gradient probe. A gradient HMBC pulse sequence with 1 ms Gaussian Z-axis gradient pulses (70:30:50) was used. Referencing of the indirectly detected  $^{15}\text{N}$  dimension was accomplished using nitromethane as an external standard. A GHMBC experiment was performed with nitromethane, and the  $^{15}\text{N}$  correlation was calibrated to 380.2 ppm. This same calibration value was then used for the manzamines. The acquisition time was 12 to 24 h for each compound.

### Computational Methods

A full conformational search with no symmetry constraints using hybrid MonteCarlo simulations was done using MOE, or Molecular Operating Environment. Many conformations were generated, minimized, and compared to each other to discard duplicates. Energetically and conformationally, most representative conformations were selected and minimized at the semiempirical level using the AM1 force field as implemented on

HyperChem 4.5 using a conjugate gradient algorithm (Polak-Riviere) with a gradient of 0.01. Then, global minima were used as initial conformations to minimize the structure at the *ab initio* level, using HF and 3-21G basis sets as implemented in Gaussian 98.<sup>20</sup> The nature of all the minima at semiempirical and *ab initio* levels was established by a complete vibrational analysis of each conformation. Final edition of the pictures was performed using Viewer Pro 4.0 (Accelrys).

### Reaction of Acetic Anhydride/NaOAc with 1 and 3

A solution of 100 mg of **1** or **3** in 2 mL of acetic anhydride was added to 30 mg of anhydrous NaOAc under nitrogen. Each reaction mixture was stirred for 30 min at 80 °C. The reaction mixture was brought to room temperature, brine solution (10 mL) was then added, and the solution was made alkaline by aqueous NH<sub>4</sub>OH (1.5 mL). Each reaction mixture was extracted with CHCl<sub>3</sub> (2 × 15 mL). The organic layer was washed with H<sub>2</sub>O (2 × 20 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and evaporated under reduced pressure. The reaction residue of **1** (90 mg) was fractionated on Si gel 60 (10 g) using *n*-hexanes/EtOAc, gradient elution, followed by preparative TLC on Si gel G<sub>254</sub>, using cyclohexane/EtOAc/NH<sub>4</sub>OH (100/50/0.1) to afford **5** (9.8 mg, *R<sub>f</sub>* 0.25), **6** (1.5 mg, *R<sub>f</sub>* 0.36), and **7** (24.0 mg, *R<sub>f</sub>* 0.88). The reaction residue of **3** was chromatographed on a Si gel 60 column (20 g), by isocratic elution with *n*-hexanes/acetone (80/20) as solvent system, to afford **8** (7.6 mg, *R<sub>f</sub>* 0.26) and **9** (40.0 mg, *R<sub>f</sub>* 0.41).

### Compound 5

yellowish powder from EtOH; mp 128–130 °C; [ $\alpha$ ]<sub>D</sub><sup>25</sup> –14.0 (*c* 0.10, CHCl<sub>3</sub>); UV  $\lambda_{\max}$  (log  $\epsilon$ ) (MeOH) 267 (2.17), 368 (2.24), 400 (2.12) nm; IR  $\nu_{\max}$  (CHCl<sub>3</sub>) 3631–3260 (NH), 3011–2838, 1730, 1702 (C=O) 1690, 1563, 1472, 1421, 1232, 1017 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; HRFTMS *m/z* 673.3714 (calcd for C<sub>42</sub>H<sub>49</sub>N<sub>4</sub>O<sub>4</sub>, 673.3754 [M + H]<sup>+</sup>).

### Compound 6

yellowish powder from EtOH; mp 134 °C; [ $\alpha$ ]<sub>D</sub><sup>25</sup> –26.0 (*c* 0.12, CHCl<sub>3</sub>); UV  $\lambda_{\max}$  (log  $\epsilon$ ) (MeOH) 267 (2.26), 356 (2.20), 368 (2.32) nm; IR  $\nu_{\max}$  (CHCl<sub>3</sub>) 3631–3260 (NH), 3011–2838, 1729, 1702 (C=O) 1633, 1503, 1472, 1236, 1011 cm<sup>-1</sup>; <sup>1</sup>H NMR CDCl<sub>3</sub>, 400 MHz,  $\delta$  8.44 (1H, d, *J* = 5.6 Hz, H-3); 7.81 (1H, d, *J* = 5.6 Hz, H-4); 7.95, d (1H, d, *J* = 7.7 Hz, H-5); 7.21 (1H, dd, *J* = 7.7 and 7.6 Hz, H-6); 7.29 (1H, d, *J* = 7.6 Hz, H-7); 4.61 (1H, brs, H-10); 6.34 (1H, s, H-11); 5.48 (1H, m, H-15); 5.39 (1H, m, H-16); 2.71 (1H, m, H-20a); 2.46 (1H, m, H-20b); 2.72 (2H, m, 2.30, H<sub>2</sub>-22); 3.52 (1H, m, H-24); 4.25 (1H, s, H-26); 4.20 (1H, m, H-28a); 4.09 (1H, brd *J* = 13.5 Hz, H-28b); 5.18 (1H, m, H-32); 6.44 (1H, d, *J* = 10.7 Hz, H-33); 2.56 (3H, s, H<sub>3</sub>-8-*O*-acetate); 2.15 (3H, s, H<sub>3</sub>-35-acetyl); HRFTMS *m/z* 631.3619 (calcd for C<sub>40</sub>H<sub>47</sub>N<sub>4</sub>O<sub>3</sub>, 631.3648 [M + H]<sup>+</sup>).

### (–)-8,12-Di-*O*-acetyl-8-hydroxymanzamine A (7)

yellowish-green powder (EtOH); mp 168–170 °C; [ $\alpha$ ]<sub>D</sub><sup>25</sup> –22.0 (*c* 0.10, CHCl<sub>3</sub>); UV  $\lambda_{\max}$  (log  $\epsilon$ ) (MeOH) 266 (2.35), 356 (2.29), 368 (2.42) nm; IR  $\nu_{\max}$  (CHCl<sub>3</sub>) 3458 (NH), 3100–2930, 1740, 1714 (C=O) 1660, 1637, 1561, 1466, 1240, 1013 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; HRFTMS *m/z* 649.3678 (calcd for C<sub>40</sub>H<sub>49</sub>N<sub>4</sub>O<sub>4</sub>, 649.3754 [M + H]<sup>+</sup>).

**Compound 8**

yellowish powder (EtOH); mp 136–138 °C;  $[\alpha]_{\text{D}}^{25} +21.0$  (*c* 0.09, CHCl<sub>3</sub>); UV  $\lambda_{\text{max}}$  (log  $\epsilon$ ) (MeOH) 266 (2.61), 356 (2.65), 367 (2.75) nm; IR  $\nu_{\text{max}}$  (CHCl<sub>3</sub>) 3640–3250 (NH), 3010–2840, 1725 (C=O) 1660, 1620, 1500, 1460, 1365, 1020 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 2; HRFTMS *m/z* 615.3658 (calcd for C<sub>40</sub>H<sub>47</sub>N<sub>4</sub>O<sub>2</sub>, 615.3699 [M + H]<sup>+</sup>).

**Compound 9**

yellowish powder (EtOH); mp 130–132 °C;  $[\alpha]_{\text{D}}^{25} +13.0$  (*c* 0.10, CHCl<sub>3</sub>); UV  $\lambda_{\text{max}}$  (log  $\epsilon$ ) (MeOH) 267 (2.65), 355 (2.60), 368 (2.73) nm; IR  $\nu_{\text{max}}$  (CHCl<sub>3</sub>) 3631–3260 (NH), 3011–2838, 1729 (C=O) 1660, 1626, 1504, 1466, 1364, 1013 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 2; HRFTMS *m/z* 573.3571 (calcd for C<sub>38</sub>H<sub>45</sub>N<sub>4</sub>O, 573.3593 [M + H]<sup>+</sup>).

**Diimide Reduction of 1 and 3**

To a solution of 50 mg of **1** in absolute EtOH (5 mL) was added 10 mg of Pd on carbon. Hydrazine hydrate (0.5 mL) was gradually added to the mixture with stirring, over a 30 min period. After the addition was complete, the reaction mixture was refluxed for 12 h until TLC indicated a complete reaction. The reaction mixture was filtered while hot through Celite, treated with brine solution (5 mL), alkalized with NH<sub>4</sub>OH to pH 9, and extracted with CHCl<sub>3</sub> (2 × 10 mL). The organic layer was washed with H<sub>2</sub>O (2 × 10 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and evaporated under vacuum. Preparative TLC of the residue on Si gel G<sub>254</sub> plates using cyclohexane/EtOAc/NH<sub>4</sub>OH (100:50:0.1) afforded **10** (9.5 mg, *R<sub>f</sub>* 0.51). The same reaction was repeated starting with 50 mg of **3** or 100 mg of **4**. Final purification of the reaction residue of **3** was conducted using preparative Si gel G<sub>254</sub> TLC, using *n*-hexanes/acetone (70:30), to afford **11** (6.4 mg, *R<sub>f</sub>* 0.48) and **12** (2.7 mg, *R<sub>f</sub>* 0.53). Similarly, preparative TLC of the reaction residue of **4**, using *n*-hexanes/acetone (65:35), afforded **13** (14.5 mg, *R<sub>f</sub>* 0.42) and **14** (3.4 mg, *R<sub>f</sub>* 0.18).

**15,16,32,33-Tetrahydro-8-hydroxymanzamine A (10)**

yellowish-brown powder (EtOH); mp 162–164 °C dec;  $[\alpha]_{\text{D}}^{25} -41.0$  (*c* 0.10, CHCl<sub>3</sub>); UV  $\lambda_{\text{max}}$  (log  $\epsilon$ ) (MeOH) 267 (1.98), 355 (1.92), 368 (2.04) nm; IR  $\nu_{\text{max}}$  (CHCl<sub>3</sub>) 3620–3230 (NH and OH), 3110–2854, 1637, 1561, 1468, 1364, 1019 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 3; HRFTMS *m/z* 569.3844 (calcd for C<sub>36</sub>H<sub>49</sub>N<sub>4</sub>O<sub>2</sub>, 569.3856 [M + H]<sup>+</sup>).

**32,33-Dihydromanzamine A (11)**

colorless powder (EtOH); mp 131–134 °C;  $[\alpha]_{\text{D}}^{25} +51.0$  (*c* 0.24, CHCl<sub>3</sub>); UV  $\lambda_{\text{max}}$  (log  $\epsilon$ ) (MeOH) 265 (2.00), 353 (1.80), 366 (2.01) nm; IR  $\nu_{\text{max}}$  (CHCl<sub>3</sub>) 3500–3100 (NH and OH), 2926, 2854, 1658, 1625, 1564, 1460, 1360 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 3; HRFTMS *m/z* 551.3752 (calcd for C<sub>36</sub>H<sub>47</sub>N<sub>4</sub>O, 551.3750 [M + H]<sup>+</sup>).

**15,16,32,33-Tetrahydromanzamine A (12)**

colorless powder (EtOH); mp 145–147 °C;  $[\alpha]_{\text{D}}^{25} +41.0$  (*c* 0.05, CHCl<sub>3</sub>); UV  $\lambda_{\text{max}}$  (log  $\epsilon$ ) (MeOH) 262 (1.99), 350 (1.77), 365 (2.00) nm; IR  $\nu_{\text{max}}$  (CHCl<sub>3</sub>) 3500–3100 (NH and OH), 2920, 2850, 1625, 1560, 1450, 1355 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  9.46 (1H, brs, NH), 8.41 (1H, d, *J* = 5.0 Hz, H-3); 7.83 (1H, d, *J* = 5.1 Hz, H-4); 8.09 (1H, d, *J* = 7.9 Hz,

H-5); 7.26 (1H, dd,  $J = 7.9, 7.6$  Hz, H-6); 7.53 (1H, dd,  $J = 7.6, 7.4$  Hz, H-7); 7.58 (1H, d,  $J = 7.4$  Hz, H-8); 6.66 (1H, s, H-11); 2.97 (1H, m, H-20a); 2.45 (1H, m, H-20b); 2.93 (1H, m, H-22a); 2.28 (1H, m, H-22b); 3.09 (1H, m, H-24); 3.68 (1H, s, H-26); 3.38 (1H, dd,  $J = 13.4, 12.7$  Hz, H-28a); 2.42 (1H, dd,  $J = 12.5, 4.9$  Hz, H-28b); 3.11 (1H, m, H-34); 2.67 (1H, brd,  $J = 11.1$  Hz, H-36a); 2.19 (1H, d,  $J = 11.4$  Hz, H-36b); HRFTMS  $m/z$  553.3902 (calcd for  $C_{36}H_{49}N_4O$ , 553.3906 [M + H]<sup>+</sup>).

#### 15,16,32,33-Tetrahydro-8-hydroxymanzamine A (13)

colorless powder (EtOH); mp 122–124 °C;  $[\alpha]_D^{25} +7.0$  ( $c$  0.4,  $CHCl_3$ ); UV  $\lambda_{max}$  (log  $\epsilon$ ) (MeOH) 264 (1.94), 351 (1.70), 363 (1.99) nm; IR  $\nu_{max}$  ( $CHCl_3$ ) 3475, 3196 (NH and OH), 2929, 2850, 1633, 1593, 1562, 1460, 1160  $cm^{-1}$ ; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 4; HRFTMS  $m/z$  569.3838 (calcd for  $C_{36}H_{49}N_4O_2$ , 569.3856 [M + H]<sup>+</sup>).

#### 10,11,15,16,32,33-Hexahydro-8-hydroxymanzamine A (14)

colorless powder (EtOH); mp 162–164 °C dec;  $[\alpha]_D^{25} +17.0$  ( $c$  0.10,  $CHCl_3$ ); UV  $\lambda_{max}$  (log  $\epsilon$ ) (MeOH) 265 (1.88), 353 (1.90), 364 (2.00) nm; IR  $\nu_{max}$  ( $CHCl_3$ ) 3475, 3200 (NH and OH), 3110–2850, 1550, 1460, 1360, 1100  $cm^{-1}$ ; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 4; HRFTMS  $m/z$  571.4009 (calcd for  $C_{36}H_{51}N_4O_2$ , 571.4012 [M + H]<sup>+</sup>).

#### Reduction of 2 with NaBH<sub>4</sub>

To a solution of 20 mg of **2** in anhydrous THF was added 2 mg of NaBH<sub>4</sub>. The reaction was stirred at room temperature for 9 h until TLC indicated a complete reaction. Then, brine solution was added (10 mL), and the mixture was alkalized by NH<sub>4</sub>OH to pH 9 and extracted with  $CHCl_3$  (2 × 10 mL). The organic layer was washed by H<sub>2</sub>O (2 × 10 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and evaporated under vacuum. Preparative TLC of the residue on Si gel G<sub>254</sub>, using cyclohexane/EtOAc/NH<sub>4</sub>OH (100: 50:0.1) afforded **15** (5.5 mg,  $R_f$  0.36) and **16** (1.2 mg,  $R_f$  0.30).

#### 31 $\beta$ -Hydroxy-32,33-dihydro-8-hydroxymanzamine A (15)

yellowish powder (EtOH); mp 134–136 °C;  $[\alpha]_D^{25} -26.0$  ( $c$  0.10,  $CHCl_3$ ); UV  $\lambda_{max}$  (log  $\epsilon$ ) (MeOH) 267 (2.26), 368 (2.33), 389 (2.21) nm; IR  $\nu_{max}$  ( $CHCl_3$ ) 3650–3250 (NH and OH), 3100–2820, 1636, 1559, 1466, 1013  $cm^{-1}$ ; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 5; HRFTMS  $m/z$  583.3600 (calcd for  $C_{36}H_{47}N_4O_3$ , 583.3648 [M + H]<sup>+</sup>).

#### 31 $\alpha$ -Hydroxy-32,33-dihydro-8-hydroxymanzamine A (16)

yellowish powder (EtOH); mp 140–142 °C,  $[\alpha]_D^{25} -34.0$  ( $c$  0.10,  $CHCl_3$ ); UV  $\lambda_{max}$  (log  $\epsilon$ ) (MeOH) 267 (2.94), 356 (2.88), 368 (3.01) nm; IR  $\nu_{max}$  ( $CHCl_3$ ) 3650–3250 (NH and OH), 3100–2800, 1636, 1561, 1459, 1015  $cm^{-1}$ ; <sup>1</sup>H NMR ( $CDCl_3$ , 400 MHz)  $\delta$  8.35 (1H, d,  $J = 5.6$  Hz, H-3); 7.82 (1H, d,  $J = 5.6$  Hz); 7.59 (1H, d,  $J = d$  7.7 Hz, H-5); 7.13 (1H, dd,  $J = 7.7, 7.6$  Hz, H-6); 7.06 (1H, d,  $J = 7.6$  Hz, H-7); 6.88 (1H, s, H-11); 5.60 (1H, m, H-15); 5.54 (1H, m, H-16); 2.61 (1H, m, H-20a); 2.41 (1H, m, H-20b); 2.59 (1H, m, H-22a); 2.32 (1H, m, H-22b); 3.08 (1H, m, H-24); 3.86 (1H, s, H-26); 3.48 (1H, m, H-28a); 2.86 (1H, m, H-28b); 3.86 (1H, brs, H-31); 4.22 (1H, brs, H-34); HRFTMS  $m/z$  583.3615 (calcd for  $C_{36}H_{47}N_4O_3$ , 583.3648 [M + H]<sup>+</sup>).

### Acetylation of Manzamine 15

To a solution of 5 mg of **15** in 2.5 mL of acetic anhydride was added 1.0 mL of pyridine. The reaction mixture was stirred at 100 °C for 12 h. About 5 mL of a saturated NaCl solution was added, and the mixture was alkalized by NH<sub>4</sub>OH to pH 9 and extracted with CHCl<sub>3</sub> (2 × 10 mL). The organic layer was washed by H<sub>2</sub>O (2 × 10 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and evaporated under a vacuum. Preparative TLC of the residue on Si gel G<sub>254</sub>, using cyclohexane/EtOAc/NH<sub>4</sub>OH (100:50:0.1), afforded **17** (2.8 mg, *R<sub>f</sub>* 0.69).

### Compound 17

yellowish powder (EtOH); mp 118 °C; [α]<sub>D</sub><sup>25</sup> -48.0 (*c* 0.12, CHCl<sub>3</sub>); UV λ<sub>max</sub> (log ε) (MeOH) 266 (2.68), 355 (2.63), 368 (2.76) nm; IR ν<sub>max</sub> (CHCl<sub>3</sub>) 3412 (NH), 3100–2820, 1753, 1730, 1690 (C=O), 1638, 1568, 1473, 1213, 1011 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 5; HRFTMS *m/z* 775.4271 (calcd for C<sub>46</sub>H<sub>55</sub>N<sub>4</sub>O<sub>7</sub>, 775.4231 [M + H]<sup>+</sup>).

### Antituberculosis Assays

Compounds were tested in the microplate Alamar Blue assay as previously published.<sup>24</sup>

### Anti-inflammatory Assays

Rat neonatal microglia (2 × 10<sup>5</sup> cells/24-well cell culture clusters) were stimulated with *Escherichia coli* lipopolysaccharide (LPS) (0.3 ng/mL) in 1 mL of Dulbecco's modified Eagle medium, with 10% fetal bovine serum, penicillin, and streptomycin, for 17 h in a humidified 5% CO<sub>2</sub> incubator at 37 °C.<sup>23</sup> The medium was then removed, and the microglia cells were washed with warm (37 °C) Hanks' balanced salt solution (HBSS) and then incubated with manzamines **1–17** (0.1–10 μM) or vehicle (DMSO) for 15 min prior to stimulation with phorbol 12-myristate 13-acetate (PMA) (1 μM). All experimental treatments were run in triplicate and in a final volume of 1 mL. The cells were stimulated for 70 min with PMA, after which, HBSS was aspirated, and O<sub>2</sub><sup>-</sup>, TXB<sub>2</sub>, and LDH release were determined, as described elsewhere.<sup>23</sup>

### Supplementary Material

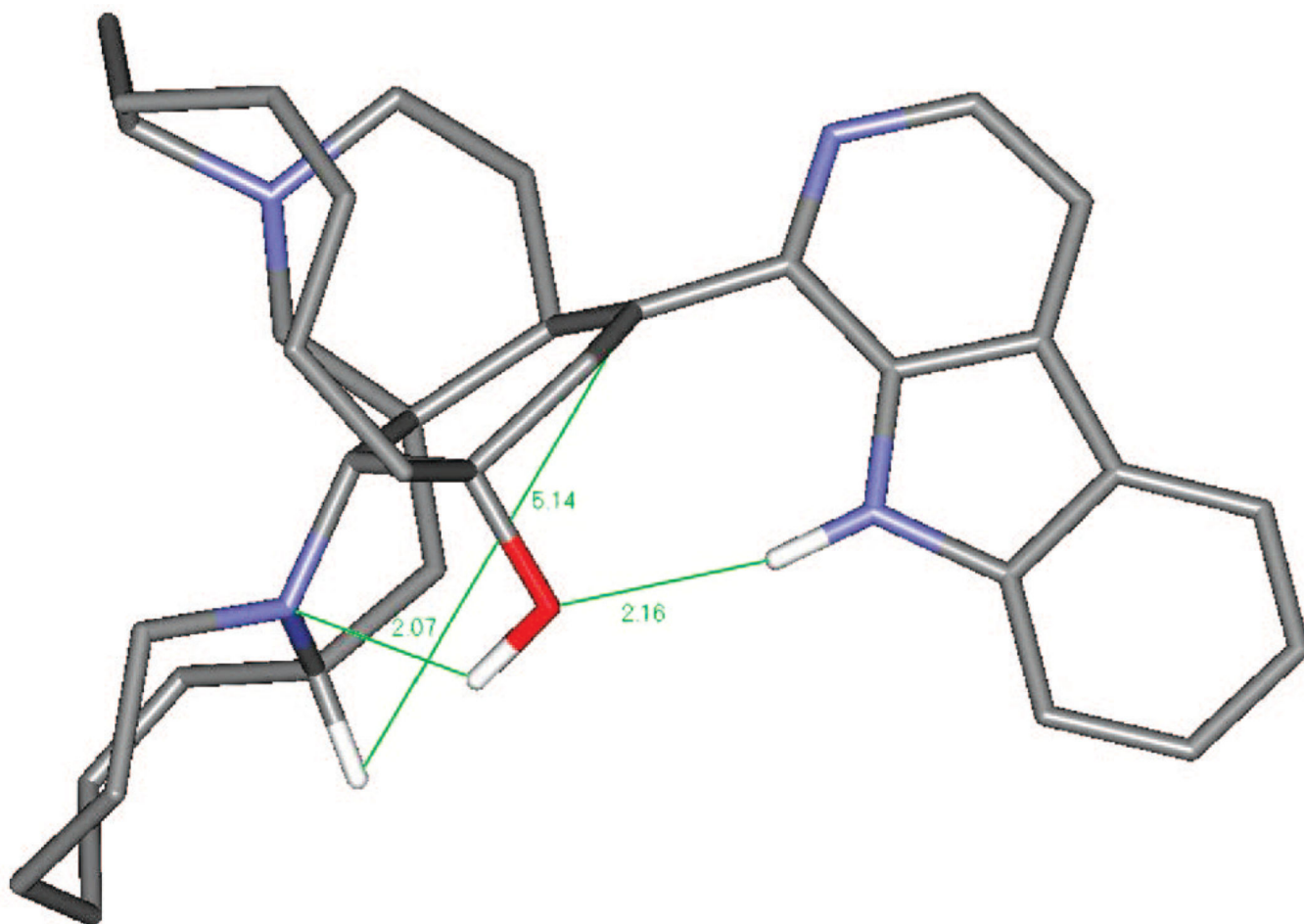
Refer to Web version on PubMed Central for supplementary material.

### Acknowledgments

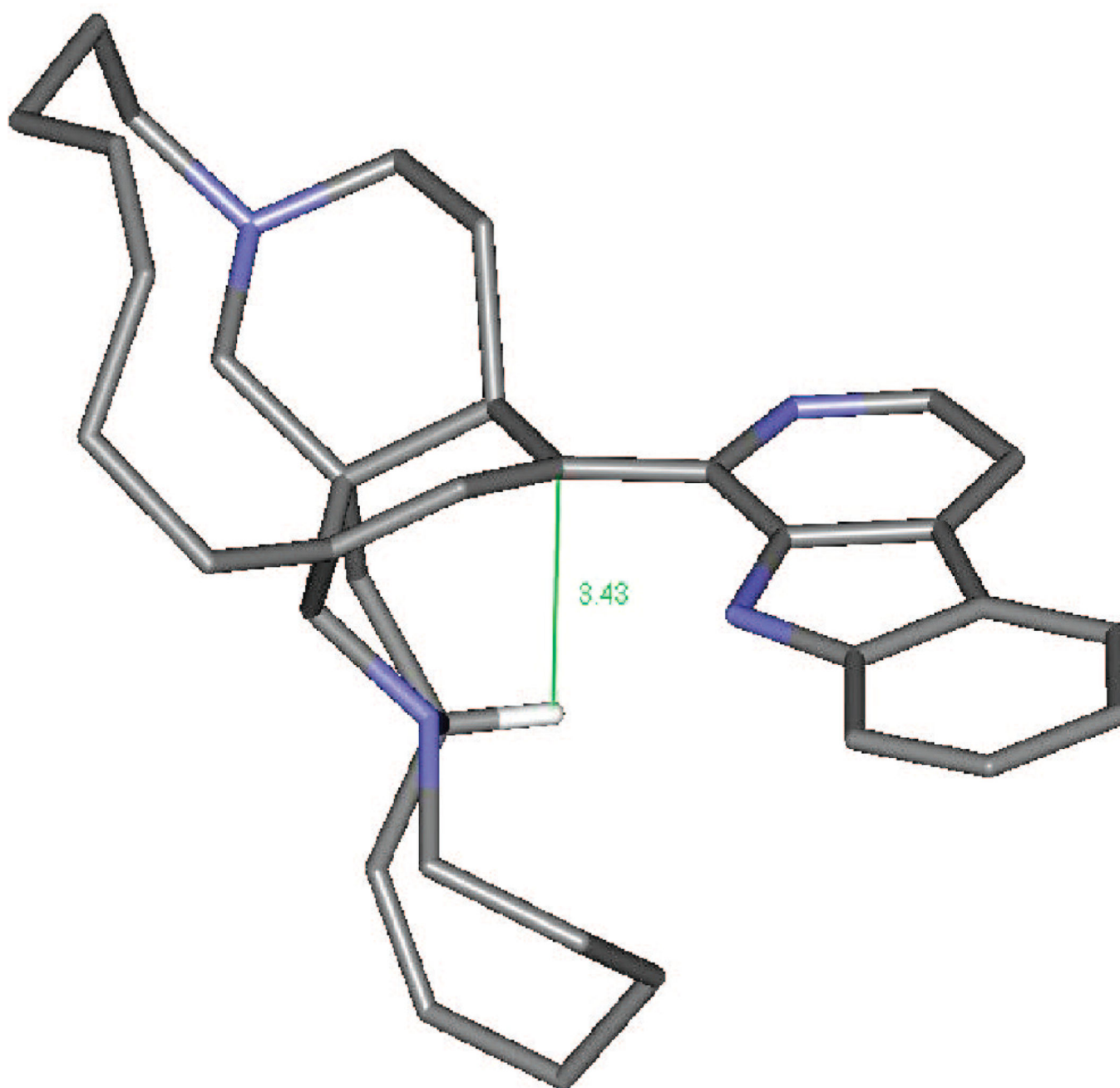
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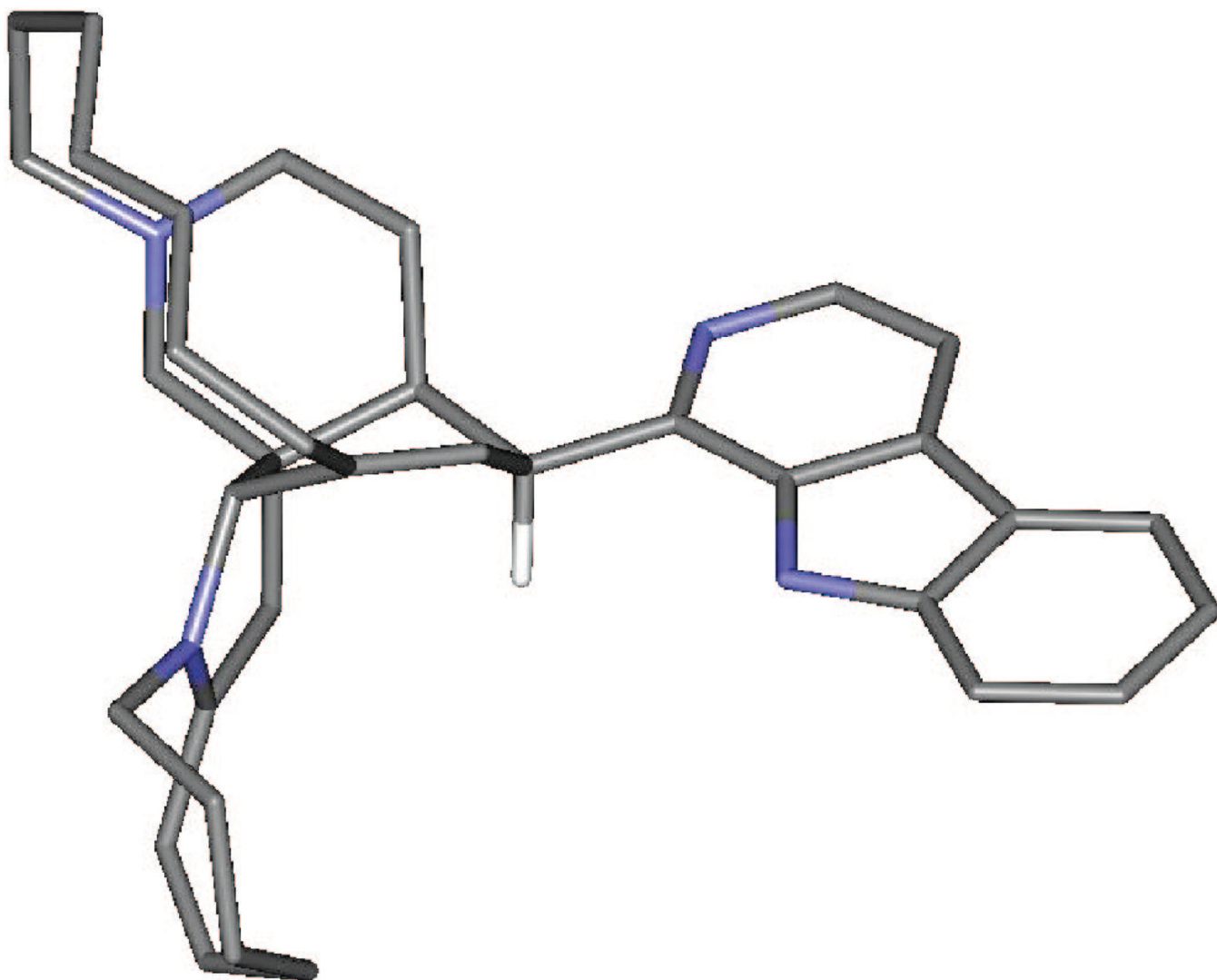


**Figure 1.** Manzamine A (**3**) global minimum energy by 3–21G *ab initio* methods. Note the green hydrogen bonds between heterocycle 9-NH, the 12-OH lone pair, and the OH with the tertiary 27-N lone pair.

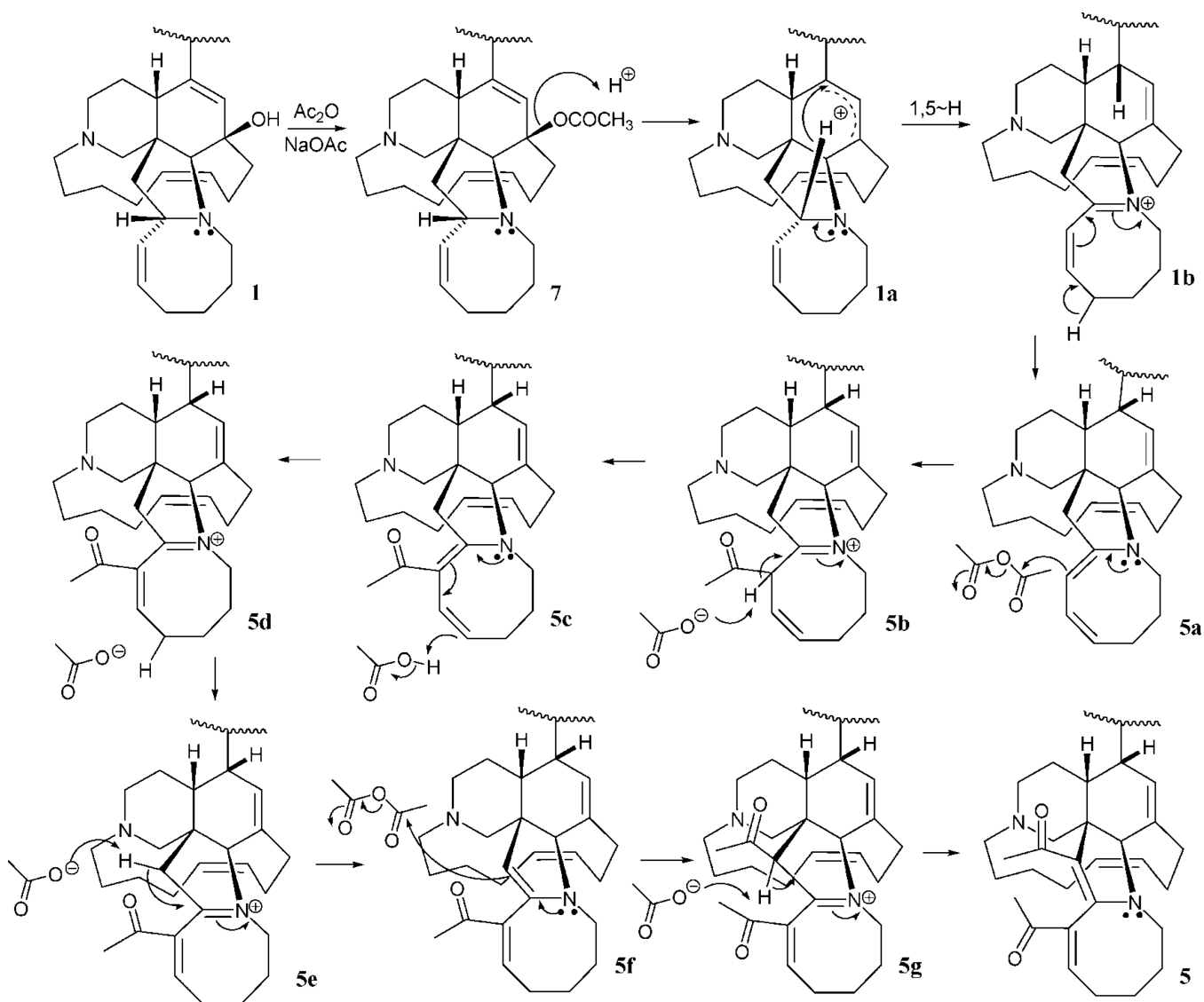


**Figure 2.** 3–21G-calculated manzamine-derived allylic carbonium ion **3a** showing a pretransition state transannular H-34 to C-10 distance of 3.43 Å. Of interest, the H-34 to C-10 distance in Figure 1 (manzamine A, **3**) is 5.14 Å, the shortened distance arising as a result of conformational transmission.





**Figure 3.** Intermediate **3b** after transannular 1,5-H transfer from H-34 to C-10 cation, calculated by *ab initio* methods at the 3-21G level. This stable iminium ion (over 35 kcal/M more stable than **3a**) can lose a proton to generate different nucleophilic enamines, as shown in Scheme 1.



**Scheme 1.**  
Plausible Mechanism of Formation of Compounds 5 and 7

Table 1

<sup>13</sup>C and <sup>1</sup>H NMR Data of Compounds **5** and **7**<sup>a</sup>

position	5		7	
	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$
1	146.5, s		146.3, s	-
3	137.9, d	8.41, d (5.3)	138.5, d	8.38, d (5.6)
4	113.0, d	7.81, d (5.3)	113.5, d	7.82, d (5.6)
4a	128.3, s		128.9, s	
4b	124.2, s		124.4, s	
5	118.9, d	7.96, d (7.5)	119.1, d	7.97, d (7.8)
6	119.5, d	7.14, dd (7.6, 7.5)	119.5, d	7.23, dd (7.8, 7.6)
7	120.2, d	7.30, d (7.6)	120.2, d	7.29, d (7.6)
8	136.5, s		136.7, s	
8a	133.3, s		133.2, s	
9a	134.6, s		133.9, s	
10	40.9, d	4.41, brs	144.0, s	
11	125.8, d	6.60, s	135.0, d	6.50, s
12	138.7, s		84.0, s	
13	34.3, t	2.65, m 2.40, m	40.3, t	1.79, m 1.68, m
14	21.6, t	2.12, m	21.5, t	2.21, m 1.80, m
15	130.6, d	5.43, m	127.4, d	5.56, m
16	131.2, d	5.35, m	133.1, d	5.50, m
17	23.7, t	1.89, m 1.73, m	25.6, t	1.65, m
18	24.7, t	1.38, m	27.0, t	1.42, m 1.30, m
19	27.7, t	1.92, m	26.7, t	1.40, m 1.32, m
20	59.6, t	2.62, m 2.51, m	53.1, t	2.60, m 2.43, m
22	51.2, t	2.71, m 1.98, m	49.4, t	2.83, m 1.95, m
23	28.0, t	2.73, m 2.41, m	34.2, t	2.81, m
24	38.7, d	3.96, m	41.7, d	2.44, m
25	52.2, s		47.7, s	
26	64.3, d	4.88, s	72.3, d	3.50, s
28	45.6, t	3.85, dd (14.7, 4.7) 3.34, dd (14.7, 10.8)	50.5, t	3.14, dd (11.9, 8.7) 2.99, dd (11.8, 4.7)
29	24.8, t	1.38, m	25.7	1.76, m
30	28.4, t	1.88, m 1.60, m	27.7, t	2.15, m 2.05, m
31	29.5, t	2.15, m	31.7, t	2.42, m
32	144.3, d	7.14, dd (8.5, 7.0)	132.8, d	5.84, dd (7.7, 7.6)
33	135.2, s		131.1, d	5.21, m
34	161.8, s		53.7, d	3.99, dd (8.3, 8.2)
35	114.9, s		44.8, t	2.42, m 1.49, d (12.9)
36	62.7, t	2.62, d (10.9) 2.51, d (10.9)	68.6, t	2.76, d (11.5) 2.30, m

position	5		7	
	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$
OAc-8	169.5, s 21.6, q	2.56, s	169.5, s 21.4, q	2.53, s
OAc-12			171.1, s 23.2, q	2.17, s
Ac-33	195.0, s 27.1, q	2.21, s		
Ac-35	192.7, s 29.3, q	2.12, s		

<sup>a</sup>In CDCl<sub>3</sub>, 400 or 500 MHz for <sup>1</sup>H and 100 or 125 MHz for <sup>13</sup>C NMR. Carbon multiplicities were determined by DEPT135° experiments.

s = quaternary, d = methine, t = methylene, q = methyl carbons. Coupling constants (*J*) are in Hz.

Table 2

 $^{13}\text{C}$  and  $^1\text{H}$  NMR Data of Compounds **8** and **9**<sup>a</sup>

position	8		9	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$
1	146.9, s		147.0, s	
3	137.5, d	8.42, d (5.5)	138.0, d	8.43, d (5.6)
4	112.8, d	7.85, d (5.5)	113.3, d	7.87, d (5.6)
4a	127.9, s		129.5, s	
4b	121.4, s		121.8, s	
5	121.2, d	8.11, d (7.5)	122.0, d	8.10, d (7.7)
6	119.3, d	7.25, dd (7.6, 7.5)	121.5, d	7.26, dd (7.7, 7.4)
7	127.6, d	7.55, dd (8.0, 7.8)	128.9, d	7.54, dd (7.7, 7.4)
8	112.4, d	7.71, d (8.0)	112.5, d	7.63, d (7.7)
8a	140.5, s		139.8, s	
9a	134.5, s		134.8, s	
10	41.2, d	4.49, brs	40.1, d	4.63, brs
11	126.2, d	6.62, s	128.2, d	6.40, s
12	138.6, s		140.1, s	
13	35.0, t	2.78, m 1.88, m	32.0, t	2.66, m 2.40, m
14	29.6, t	2.78, m	21.1, t	2.10, m
15	131.1, d	5.38, m	130.4, d	5.48, m
16	130.6, d	5.51, m	131.2, d	5.39, m
17	27.7, t	2.45, m 2.32, m	24.0, t	1.88, m
18	25.0, t	1.42, m	24.7, t	1.40, m
19	28.3, t	1.84, m	27.5, t	1.92, m
20	59.6, t	2.45, m	59.0, t	2.70, m 2.47, m
22	51.9, t	2.78, m 2.02, m	51.0, t	2.71, m 2.00, m
23	24.2, t	1.79, m 0.88, m	28.0, t	2.20, m
24	39.2, d	3.98, m	36.9, d	3.62, m
25	52.6, s		52.0, s	
26	64.9, d	4.89, s	66.0, d	4.27, s
28	45.7, t	3.83, dd (15.0, 4.0) 3.35, dd (14.5, 11.0)	45.5, t	4.05, brd (17.7) 3.49, brd (17.7)
29	28.9, t	1.91, m 1.66, m	24.6, t	1.38, m
30	23.2, t	2.03, m 1.48, m	29.7, t	1.88, m 1.60, m
31	30.7, t	2.55, m 2.20, m	30.1, t	2.72, m 2.40, m
32	144.3, d	7.16, dd (8.5, 7.0)	128.5, d	5.19, m
33	135.1, s		135.5, d	6.46, d (10.7)
34	161.9, s		155.1, s	
35	114.7, s		114.5, s	
36	62.8, t	2.63, d (10.9) 2.55, d (10.9)	62.0, t	2.98, d (11.9) 2.69, d (11.9)

position	8		9	
	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$
Ac-33	194.7, s 27.4, q	2.27, s		
Ac-35	192.6, s 30.1, q	2.19, s	197.0, s 29.8, q	2.19, s

<sup>a</sup>In CDCl<sub>3</sub>, 400 or 500 MHz for <sup>1</sup>H and 100 or 125 MHz for <sup>13</sup>C NMR. Carbon multiplicities were determined by DEPT 135° experiments.

s = quaternary, d = methine, t = methylene, q = methyl carbons. Coupling constants (*J*) are in Hz.

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**Table 3**<sup>13</sup>C and <sup>1</sup>H NMR Data of Compounds **10** and **11**<sup>a</sup>

position	<b>10</b>		<b>11</b>	
	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$
1	142.8, s		144.8, s	
3	138.1, d	8.40, d (5.6)	138.6, d	8.64, d (5.0)
4	113.8, d	7.78, d (5.6)	113.6, d	7.83, d (5.1)
4a	130.0, s		129.6, s	
4b	123.2, s		121.8, s	
5	112.5, d	7.51, d (7.7)	122.0, d	8.09, d (7.7)
6	120.7, d	7.06, dd (7.7, 7.6)	120.0, d	7.26, dd (7.6, 7.5)
7	112.4, d	6.98, d (7.6)	128.3, d	7.53, dd (7.6, 7.5)
8	144.0, s		112.0, d	7.58, d (7.5)
8a	130.4, s		141.8, s	
9a	133.3, s		133.5, s	
10	139.5, s		144.2, s	
11	136.5, d	6.77, s	137.3, d	6.61, s
12	69.1, s		68.7, s	
13	37.6, t	2.10, m	41.2, t	2.02, m 1.83, m
14	20.3, t	1.65, m	21.3, t	2.30, m 2.21, m
15	23.7, t	1.45, m	128.5, d	5.65, m
16	25.5, t	1.70, m	132.7, d	5.54, m
17	27.1, t	1.55, m	25.8, t	2.57, m 1.57, m
18	24.6, t	1.50, m	26.9, t	1.70, m
19	26.0, t	1.62, m, 1.40, m	25.6, t	1.72, m
20	58.9, t	2.45, dd (12.2, 5.3) 2.32, m	53.1, t	2.68, m 2.43, m
22	52.3, t	2.97, m 2.34, m	49.9, t	2.80, m 1.99, m
23	32.7, t	2.05, m 1.71, m	33.8, t	2.30, m
24	41.1, d	3.29, m	42.0, d	3.14, m
25	45.9, s		46.0, s	
26	81.5, d	3.76, s	81.3, d	3.78, s
28	45.6, t	3.44, m 2.97, m	52.7, t	3.38, m 2.95, m
29	29.7, t	1.70, m	25.9, t	1.80, m 1.65, m
30	25.7, t	2.03, m 1.70, m	28.5, <i>b</i> <sub>t</sub>	1.55, m
31	28.6, t	1.58, m	25.4, <i>b</i> <sub>t</sub>	1.77, m
32	25.2, t	1.70, m	28.9, <i>b</i> <sub>t</sub>	2.15, m 1.55, m
33	30.5, t	1.72, m	29.9, t	1.65, m
34	63.8, d	3.05, m	63.6, d	3.11, m
35	51.8, t	2.40, m	49.3, t	1.85, m 1.56, m
36	69.5, t	2.64, d (11.3) 2.21, d (11.3)	69.2, t	2.62, brd (11.9) 2.30, d (12.0)

<sup>a</sup>In CDCl<sub>3</sub>, 400 MHz for <sup>1</sup>H, 100 MHz for <sup>13</sup>C NMR. Carbon multiplicities were determined by DEPT 135° experiments.

s = quaternary, d = methine, t = methylene, q = methyl carbons. Coupling constants (*J*) are in Hz.

<sup>b</sup>Interchangeable in the same column.

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Table 4

<sup>13</sup>C and <sup>1</sup>H NMR Data of Compounds **13** and **14**<sup>a</sup>

position	13		14	
	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$
1	142.9, s		142.5, s	
3	138.2, d	8.40, d (5.2)	138.3, d	8.31, d (5.2)
4	114.0, d	7.78, d (5.2)	113.5, d	7.81, d (5.2)
4a	130.2, s		129.9, s	
4b	123.4, s		123.1, s	
5	112.6, d	7.56, d (7.7)	113.2, d	7.58, d (7.6)
6	121.0, d	7.03, dd (7.7, 7.5)	121.2, d	7.12, dd (7.7, 7.6)
7	112.4, d	6.96, d (7.5)	112.2, d	7.05, d (7.6)
8	144.0, s		143.3	
8a	130.8, s		133.4, s	
9a	133.6, s		134.0, s	
10	140.7, s		32.5, d	3.01, m
11	137.7, d	6.78, s	30.6, t	1.50, m
12	69.2, s		69.3, s	
13	37.9, t	2.07, m	30.1, t	1.55, m
14	20.6, t	1.60, m	20.3, t	1.45, m
15	23.8, t	1.42, m	24.3, t <sup>b</sup>	1.46, m
16	25.4, t	1.73, m	25.0, t <sup>b</sup>	1.55, m
17	27.3, t	1.58, m	24.9, t <sup>b</sup>	1.57, m
18	25.7, t	1.55, m	26.3, t	1.66, m
19	26.3, t	1.64, m, 1.43, m	25.4, t	1.70, m
20	59.1, t	2.45, dd (12.0, 5.3) 2.32, m	59.3, t	2.58, m 2.25, m
22	52.5, t	2.97, m 2.34, m	51.3, t	3.11, m 2.03, m
23	33.2, t	2.05, m 1.74, m	33.6, t	2.34, m 1.67, m
24	41.2, d	3.36, m	38.1, d	1.85, m
25	46.8, s		45.5, s	
26	81.4, d	3.76, s	78.5, d	4.93, s
28	45.8, t	3.44, m 2.97, m	50.1, t	4.65, brd (14.9) 4.24, m
29	29.9, t	1.70, m	27.3, t	1.70, m
30	25.9, t	2.05, m 1.75, m	29.2, t	2.02, m 1.65, m
31	28.7, t <sup>b</sup>	1.58, m	28.8, t <sup>c</sup>	1.50, m
32	25.2, t <sup>b</sup>	1.75, m	26.0, t <sup>c</sup>	1.70, m
33	30.8, t	1.72, m	29.2, t	1.75, m
34	64.3, d	2.99, m	63.0, d	2.99, m
35	52.0, t	2.40, m	48.2, t	2.60, m
36	69.6, t	2.62, d (11.2)	67.9, t	2.52, d (11.3)

position	13		14	
	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$
	2.18, d (11.2)		2.20, m	

<sup>a</sup>In CDCl<sub>3</sub>, 400 MHz for <sup>1</sup>H, 100 MHz for <sup>13</sup>C NMR. Carbon multiplicities were determined by DEPT 135° experiments.

s = quaternary, d = methine, t = methylene, q = methyl carbons. Coupling constants (*J*) are in Hz.

<sup>b</sup>Interchangeable in the same column.

<sup>c</sup>Interchangeable in the same column.

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Table 5

<sup>13</sup>C and <sup>1</sup>H NMR Data of Compounds **15** and **17**<sup>a</sup>

position	15		17	
	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$
1	142.5, s		146.7, s	
3	137.2, d	8.37, d (5.6)	137.9, d	8.39, d (5.6)
4	113.7, d	7.83, d (5.6)	112.9, d	7.79, d (5.6)
4a	128.9, s		128.2, s	
4b	123.1, s		124.1, s	
5	113.5, d	7.59, d (7.7)	118.9, d	7.97, d (7.8)
6	120.9, d	7.13, dd (7.7, 7.6)	119.4, d	7.23, dd (7.8, 7.6)
7	112.2, d	7.04, d (7.6)	120.1, d	7.29, d (7.6)
8	143.3		136.6, s	
8a	133.4, s		133.3, s	
9a	134.1, s		134.7, s	
10	137.9, s		40.8, d	4.36, brs
11	127.9, d	6.81, s	126.0, d	6.53, s
12	68.3, s		138.4, s	
13	36.5, t	2.20, m	34.4, t	2.52, m 2.37, m
14	21.3, t	2.15, m	28.1, t	2.42, 2H m
15	130.1, d	5.46, m	130.5, d	5.47, m
16	132.3, d	5.39, m	131.4, d	5.35, m
17	24.9, t	1.57, m	24.7, t	1.37, 2H m
18	26.5, t	1.66, m	27.8, t	2.70, m 2.42, m
19	25.4, t	1.60, m	27.3, t	1.95, 2H m
20	52.7, t	2.88, m 2.41, m	59.4, t	2.39, m 2.30, m
22	49.6, t	2.87, m 2.59, m	51.2, t	2.89, 2H m
23	32.6, t	2.44, m 2.15, m	23.6, t	1.68, 2H m
24	40.1, d	3.16, m	37.9, d	
25	45.5, s		52.9, s	
26	81.5, d	3.88, s	63.3, d	4.83, s
28	41.1, t	3.15, m 2.99, m	45.8, t	3.77, 2H m
29	25.3, t	1.60, m	29.7, t	1.81, 2H m
30	29.1, t	2.12, m 1.65, m	30.4, t	1.89, 2H m
31	64.0, d	3.77, brs	71.7, d	4.91, ddd (11.9, 8.2, 3.6)
32	31.1, t	2.15, m 1.55, m	36.9, t	3.04, dd (14.1, 8.2) 2.41, m
33	29.4, t	1.75, m	116.9, s	
34	64.0, d	4.24, brs	163.9, s	
35	36.5, t	2.30, m 2.15, m	111.7, s	
36	68.9, t	2.68, d (11.9) 2.35, d (11.9)	62.1, t	2.62, d (11.9) 2.51, d (11.9)

position	15		17	
	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$
37			148.1, s	
38			16.8, q	1.85, s
OAc-8			169.8, s	21.7, q
			21.7, q	2.58, s
OAc-31			170.3, s	20.7, q
			20.7, q	2.07, s
Ac-35			192.0, s	27.3, q
			27.3, q	2.29, s
OAc-37			168.8, s	21.2, q
			21.2, q	2.21, s

<sup>a</sup>In CDCl<sub>3</sub>, 400 or 500 MHz for <sup>1</sup>H and 100 or 125 MHz for <sup>13</sup>C NMR. Carbon multiplicities were determined by DEPT 135° experiments.

s = quaternary, d = methine, t = methylene, q = methyl carbons. Coupling constants (*J*) are in Hz.

**Table 6**

In Vitro Inhibitory Activity and Minimum Inhibitory Concentrations (MIC) of Natural and Semisynthetic Manzamines against *M. tuberculosis* (H37Rv)

compound	MIC ( $\mu\text{g/mL}$ )	% inhibition
1	0.91	100
2	<12.5	99
3	1.56	99
5	<6.25	98
6	<6.25	95
7	>6.25	96
8	NT <sup>a</sup>	NT <sup>a</sup>
9	>6.25	58
10	<6.25	99
11	7.41	99
12	NT <sup>a</sup>	NT <sup>a</sup>
13	1.84	98
14	NT <sup>a</sup>	NT <sup>a</sup>
15	<6.25	100
16	>6.25	61
17	<6.25	99
18 <sup>1</sup>	>12.5	44
rifampin	0.5	100

<sup>a</sup>NT = not tested.