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Two-step synthesis and biological evaluation of calyxamines A and B

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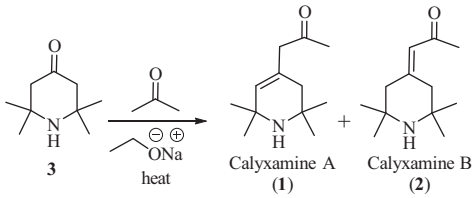
ABSTRACT

A two-step synthesis of naturally occurring alkaloids calyxamines A and B featuring a tandem Mannich–aldol condensation reaction under solvent free conditions, and their inhibitory activity against acetylcholinesterase (AChE) is reported.

Calyxamines A (**1**) and B (**2**) are naturally occurring alkaloids isolated from the marine sponge *Calyx podatypa* by Rodríguez et al. in 1997.[1] Their structural determination was performed by applying NMR and X-ray methods to the corresponding trifluoroacetate salt of the calyxamine A. Two years later, Cóbar and Pinto reported that calyxamines A and B possess modest antimicrobial activity against *Staphylococcus aureus* and *Mycobacterium smegmatis*.[2] The biosynthetic proposal for **1** and **2** envisions the formation of piperidone **3** from the condensation of NH_3 and 4 equiv of acetone (or biosynthetic equivalents) via the corresponding imine intermediate.[1] Experimentally, it seems that the best way to prepare calyxamines A and B is starting from the condensation of piperidone **3** with acetone, especially because the preparation of **3** is well-documented.[3] In this sense, the single reported synthesis of calyxamines A and B involves the condensation reaction between the 2,2,6,6-tetramethyl-4-piperidone **3** and acetone under basic conditions (Scheme 1).[2]

On this basis, we decided to develop a more rapid and efficient protocol for the synthesis of calyxamines A and B, and thus to obtain sufficient amount for biological assays. Our proposal involves the preparation of the imine intermediate **4** from the condensation reaction between NH_4Cl and acetone; and then, the preparation of piperidone **3** could be obtained from a Mannich condensation with another equivalent of acetone followed by aldol condensation of **3** with acetone, as well. We planned to conduct both condensations



Scheme 1. Synthesis of calyxamines A and B starting from piperidone **3**.

in a two-step sequential process (Scheme 2). Additionally, we envisioned that both condensation reactions might be catalyzed by commercially available diamines, and as the acetone is used for both condensations, we anticipated that the reactions could be conducted under free-solvent conditions.

It has demonstrated the efficacy of the chiral diamines in the enantioselective Mannich condensation;[4] however, because the calyxamines A and B are not chiral compounds, there was no need for using optically pure amines. Thus, the imine **4** was quantitatively prepared from the reaction of acetone with ammonium chloride,[5] followed by the use of ethyldiamine **5** (10% mol) as organocatalyst in the Mannich reaction, wherein the acetone is used as the enamine generator. The reaction was maintained in vigorous stirring for 24 h expecting to obtain piperidone **3**, in the first instance; however, we only observed the formation of piperidone **3** as a minor by-product. Interestingly, the main observed products were the calyxamines A (**1**) and B (**2**) in an equimolar ratio (Scheme 3).[6] By running a blank reaction we proved that the

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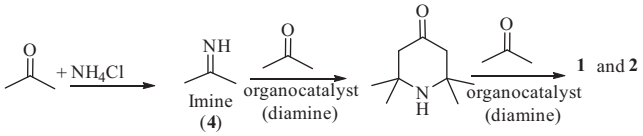
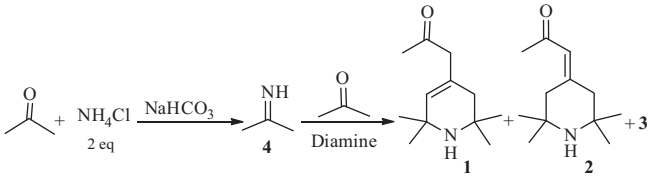
diamine **5** is the actual organocatalyst. Apparently, diamine **5** not only catalyzes the Mannich condensation, but also the cross-aldol condensation of the incipient piperidone **3** with acetone.[7] Diamines **6** and **7** were also tested as organocatalyst for the tandem Mannich/aldol condensation reaction obtaining lower yield for the case of diamine **7**, and only traces when cyclohexane diamine **6** was used (Scheme 3). It is worth to mention that this efficient two-step protocol for the synthesis of calyxamines A and B was successfully conducted under solvent free conditions.

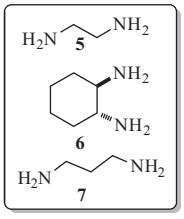
Having established an efficient protocol for the synthesis of calyxamines A and B, we proceed to test them on the inhibition of acetylcholinesterase.[8] The hydrolysis of the no fluorescent indoxyl acetate produces a highly fluorescent material for measuring cholinesterase activity. In the presence of AChE inhibitors a decrease in the reaction rate of AChE is expected to quantitatively assess the inhibition capacity of **1** and **2**. Figures 1a and 1b show their pronounced inhibition activities after 5 min incubation with AChE.[9]

Although many natural products have been reported as inhibitors of AChE (e.g., compounds in Table 1), the inhibition achieved by calyxamine B is comparable with that exhibited by natural phenolic compounds such as ferulic acid,[10] but less effective than triterpenoidal alkaloids from Buxushyrcana: tiliroside, 3-methoxy quercetin, quercitrin, quercetin and dehydroevodiamine,[11] and also some flavonoids from Artocarpusnobilis.[12]



Scheme 2. Our sequential proposal route for the synthesis of calyxamines A and B.





Entry	Diamine	Ratio 1:2	Yield %
a	**5**	1:1	50
b	**6**	-	-
c	**7**	1:1	35

Scheme 3. Two step-synthesis of calyxamines A and B.

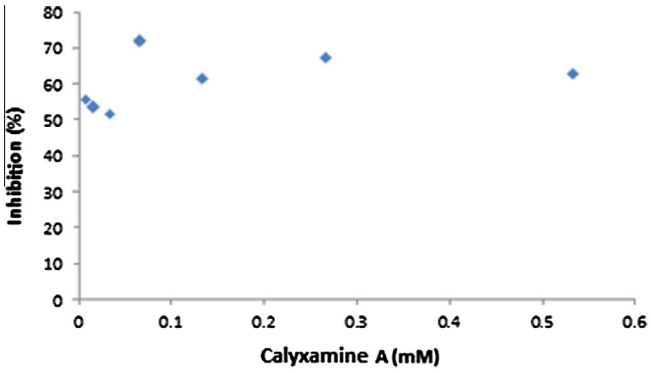


Figure 1a. Calyxamine A inhibited 50% of the AChE from 8 to 0.5 μM.

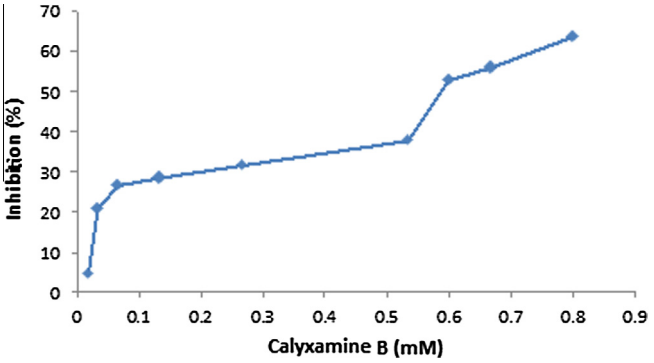


Figure 1b. Calyxamine B in a concentration-dependent manner reaching 50% inhibition around 0.6 mM.

Table 1
The inhibitory activities on AChE

Compound	IC_{50}[a] (μg/mL)
Calyxamine B	117
Ferulicacid	200–500
Quercetin	5.9
Tiliroside	13.97
Quercetrin	29.99
Artoblidxanthone	32.5
Artoninse	42.5

[a] The values indicate 50% AChE inhibitory effect and are the means of triplicate data.

In conclusion, we have developed a highly efficient two-step solvent free protocol for the synthesis of calyxamines A and B. The calyxamine B showed binding capacity to AChE comparable to those natural inhibitors of AChE.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.10.022. These data include MOL files and InChiKeys of the most important compounds described in this article.

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5. *Preparation of imine **4**:* A 25 mL flask was charged with acetone (1.25, 17 mmol), ammonium chloride (0.909 g, 17 mmol) and water (1 mL). Then the mixture was heated to 60 °C maintaining the mixture under constant stirring, then $NaHCO_3$ was added portion wise until bubbling ceases, at that point there is formation of a large amount of NaCl. The precipitate was filtered off and the formed imine is used without purification in the next reaction.
Blank reaction: The imine **4** was dissolved in 2 mL of acetone and kept stirring for 48 h, the mixture remained colorless all the time, then was concentrated

under reduced pressure leaving the flask empty after evaporation. Indicating that ethylenediamine is essential to carry out the reaction.

6. *Synthesis of 2,2,6,6-tetramethyl-4-piperidone (**3**) and calyxamines A (**1**) and B (**2**):* Imine **4** and EDA (0.1136 mL, 1.7 mmol) were dissolved in acetone (2 mL) at room temperature. The reaction turned, form colorless to red wine, and was maintained at room temperature for 24 h, where at this time, the major observed product was the 2,2,6,6-tetramethyl-4-piperidone **3**; however by allowing to stir the reaction mixture for another 24 h, the major observed products were the calyxamine A and B. The reaction mixture was concentrated under reduced pressure to afford 1.45 g of crude reddish brown solid (approx. 55% of acetone transformed). The crude product was purified by flash chromatography using dichloromethane (DCM) as eluent; to obtain 0.0122 g of piperidone **3** (2%), then the mobile phase was changed to a mixture of DCM/acetone 3:1; to yield 0.073 g of calyxamine A (12%) as a white crystalline solid that sublimes at 225–226 °C; finally calyxamine B (0.044 g, 0.23 mmol, 7%) of a crystalline solid mp 205–210 °C, and finally 0.522 g of a mixture of both calyxamines that we could not separate.
*2,2,6,6-Tetramethyl-4-piperidone (**3**):* ^{1}H NMR δ (ppm) 1.25 (s, 12H), 2.28 (s, 4H). ^{13}C NMR δ (ppm) 30.8, 31.9, 54.05, 55.31, 203.5. ESI-HRMS: *m*/*z* calcd for $C_9H_{17}NO$: 155.1310, found 155.1315.
*1-(2,2,6,6-Tetramethyl-1,2,3,6-tetrahydropyridin-4-yl)propan-2-one, calixamine A (**1**):* ^{1}H NMR δ (ppm) 1.18 (s, 6H), 1.23 (s, 6H), 1.83 (s, 2H), 2.15 (s, 3H), 3.07 (s, 2H), 5.49 (s, 1H). ^{13}C NMR δ (ppm) 29.8, 31.0, 40.6, 49.9, 51.6, 52.9, 126.9, 133.0, 207.0. ESI-HRMS: *m*/*z* calcd for $C_{12}H_{21}NO$: 195.1623, found 195.1593.
*1-(2,2,6,6-Tetramethylpiperidin-4-ylidene)propan-2-one, calyxamine B (**2**):* ^{1}H NMR δ (ppm) 1.62 (s, 6H), 1.63 (s, 6H), 2.2 (s, 4H), 3.1 (s, 3H), 6.2 (s, 1H). ^{13}C NMR δ (ppm) 27.6, 32.0, 38.4, 46.7, 58.9, 59.6, 127.0, 148.4, 198.2. ESI-HRMS: *m*/*z* calcd for $C_{12}H_{21}NO$: 195.1623, found 195.1617.

7. Similar version of this tandem Mannich–aldol condensation has been reported by Chan and Yip: Feng, L.; Xu, L.; Lam, K.; Zhou, Z.; Yip, C.-W.; Chan, A. S. C. *Tetrahedron Lett.* **2005**, *46*, 8685–8689.
8. Acetylcholinesterase from electrophorus electricus (electric eel), type VI-S, and indoxyl acetate from Sigma–Aldrich.
9. *Acetylcholinesterase activity:* Acetylcholin esterase (AchE) activity was determined by a fluorescence method measuring the indoxyl acetate hydrolysis. The 3 mL-reaction mixture contained 1.25 mM indoxyl acetate and 2 Ache units in phosphate buffer 60 mM pH 7. The reaction progress was followed by the increase in the fluorescence emission at 470 nm with an excitation of 395 nm.
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