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The synthesis and nicotinic binding activity of (±)-epiquinamide and (±)-*C*(1)-*epi*epiquinamide

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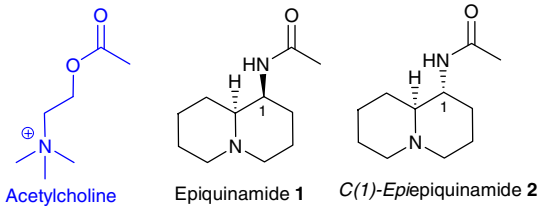
Abstract—The synthesis of (±)-epiquinamide **1** and (±)-*C*(1)-*epi*epiquinamide **2** based on the use of a Curtius rearrangement to introduce the C(1) amino residue is reported. In a competition binding assay for [^{3}H]epibatidine binding to rat brain membranes neither (±)-**1** nor (±)-**2** showed any significant level of nicotinic activity.

Agonists and partial agonists acting at nicotinic acetylcholine receptors (nAChR) are currently of much interest,[1] and the recent FDA approval of Pfizer's varenicline, a smoking cessation agent based on cytisine, serves to underscore the therapeutic potential that nicotinic ligands possess.[2] In addition to cytisine, other naturally occurring ligands, such as nicotine,[3a] epibatidine[3b] and anatoxin-a,[3c] have contributed greatly to the evolution of nicotinic pharmacology, and new ligands offering alternative or enhanced profiles are very attractive.

Recently Daly and co-workers[4] reported the isolation of epiquinamide **1** from the skin extracts of the Ecuadorian poison dart frog *Epipedobates tricolor*. Epiquinamide **1** was reported to have modest activity, measured as a change in membrane potential,[5] in cells expressing various nAChR subtypes, with highest activity noted in SH-SY5Y cells and K-177 cells expressing human α4β2 nAChR. Epiquinamide represents the first example of a new class of nicotinic ligand and although the absolute configuration of **1** has not been established, clearly **1** incorporates within its structure a motif that closely resembles acetylcholine itself.



The first synthesis of epiquinamide was reported in 2005 by Blaauw and co-workers.[6a] This was an asymmetric route leading to the (1*S*, 9a*S*)-(+)-**1**, but no biological data for this single enantiomer were reported. The ability to measure the optical rotation of natural **1**, which would have aided correlation of Blaauw's synthetic product to the natural product, was presumably limited by the small amount (a total of 240 μg of **1**[4]) that was isolated. More recently, Huang[6b] has reported an entry to the enantiomeric series, that is, (1*R*, 9a*R*)-(−)-**1**, but again no biological data for this enantiomer were reported.

In this paper, we report a quite different synthetic approach to (±)-**1** that is also readily adapted to the construction of the C(1) epimer of **1**, *C*(1)-*epi*epiquinamide **2**.[7] We also report the results of an independent assessment of the nicotinic profile of both **1** and **2** based on a competition binding assay using [^{3}H]epibatidine.

Keywords: Epiquinamide; *Epi*epiquinamide; Nicotinic agonist.
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The synthesis of (±)-epiquinamide is outlined in Scheme 1, with the key intermediate being the quinolizidine based ester **5**.[8]

Methyl *N*-Boc homopipecolate **3** underwent a diastereoselective Michael reaction with diethyl methylenemalonate to give adduct **4** in 71% yield. *N*-Boc deprotection was straightforward, and the lactamisation and decarboxylation steps were both achieved under the same Krapcho conditions to give the key ester intermediate **5** as a single diastereomer in 57% yield from **4**. Ester hydrolysis under acidic conditions (in order to avoid epimerisation, see below) provided acid **6** which was subjected to Curtius rearrangement to introduce the C(1) amino moiety. This was best achieved by trapping[9] the intermediate isocyanate with 9-fluorenylmethanol (9-FM) to give the target Curtius product **7** in Fmoc-protected form in 76% overall yield from ester **5**. The presence of the Fmoc group enabled facile purification and characterization of **7**, which was also readily deprotected under standard conditions to give **8**. Lactam reduction of **8** was carried out using $LiAlH_4$ and the crude product was acetylated immediately. Purification by chromatography gave racemic epiquinamide (±)-**1** in 70% yield (for two steps) from **8** and the overall route was achieved in 8 steps and in 16.5% overall yield from **3**.

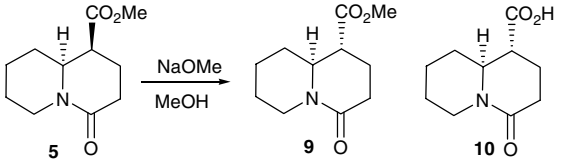
We were able to compare our synthetic material with spectroscopic data reported by both Daly[4] and Blaauw.[6a] Direct comparison of our ^1H NMR spectra with those reproduced by the Blaauw group in their publication confirmed the identity of our material with that reported earlier.[6a]

The bioactive conformation of epiquinamide remains to be defined, but the conformation of a ligand will determine how the key pharmacophore elements are presented to a receptor. As a result, structural variants of **1** which orientate differently these essential components offer a good opportunity to explore receptor space and define more accurately the nature of the ligand–receptor interaction. Accordingly, we have also pursued as the corresponding C(1) epimer of **1**, *C*(1)-*epi*epiquinamide **2**, with the strategy outlined in Scheme 1 being readily adapted to the synthesis of this novel diastereomer.

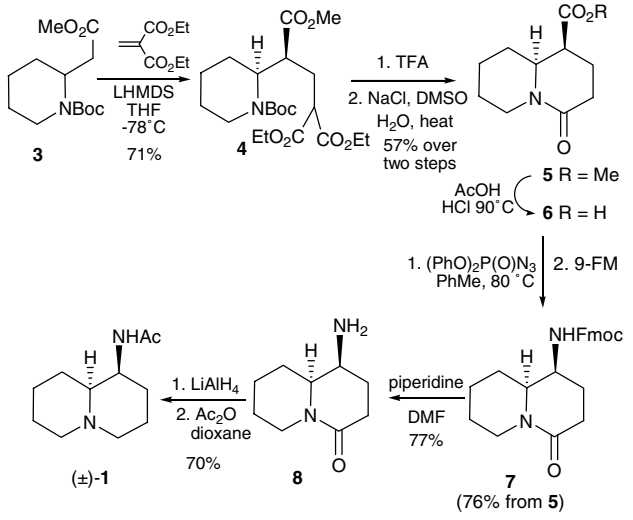
When the lactamisation step leading to ester **5** was carried out under prolonged reaction times, we did observe formation of small amounts of the diastereomeric ester **9**. For this reason, the use of a hydroxide-mediated hydrolysis of **5**–**6** has to be done with some care, and use of mild acidic hydrolysis conditions is significantly more reliable and is preferred. That isomer **9** is the thermodynamically more stable was readily verified by treatment of **5** with a base at room temperature under equilibrating conditions which led to complete epimerisation and formation of ester **9**[8] (Scheme 2).[10] Ester hydrolysis was, however, facile and varying amounts of the corresponding epimeric carboxylic acid **10**—the intermediate needed for the synthesis of **2**—were always isolated in this process.

This epimerisation process was readily applied on a synthetically useful scale. This was best done by combining together the equilibration and ester hydrolysis steps in a single pot which gave the target acid **10** in 57% yield. The application of this process to the synthesis of racemic *C*(1)-*epi*epiquinamide **2** is shown in Scheme 3. This sequence parallels that for epiquinamide **1**, and (±)-**2** was obtained in 20.5% overall yield from ester **5**.[11]

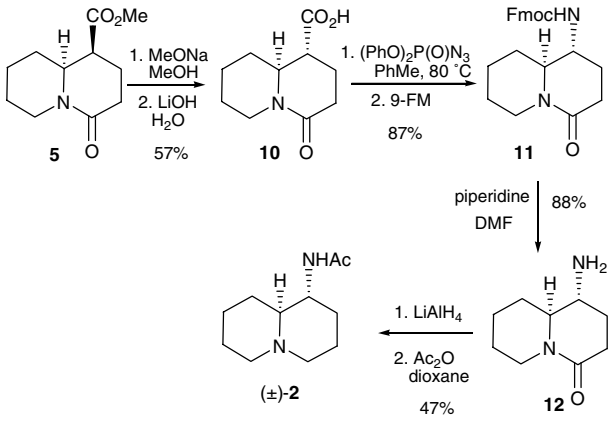
We have evaluated both racemic epiquinamide **1** and racemic *C*(1)-*epi*epiquinamide **2** in a competition binding assay. This employed rat brain P2 membranes with [^{3}H]epibatidine as the reference ligand that labels mainly α4β2-containing nAChR in this tissue preparation.[12] Using ligand concentrations of **1** and **2** of 10^{-4} to



Scheme 2. Base-mediated epimerisation/hydrolysis of ester **5**.



Scheme 1. Synthesis of (±)-epiquinamide **1**.



Scheme 3. Synthesis of (±)-*C*(1)-*epi*epiquinamide **2**.

10^{-11} M, neither epiquinamide **1** nor *C*(1)-*epi*epiquinamide **2** showed any significant inhibition of [^{3}H]epibatidine binding, in contrast to epibatidine itself that inhibited radioligand binding with an $IC_{50} \sim 10^{-10}$ M (Fig. 1).

Although this assay does not distinguish agonist from competitive antagonist binding to nAChR, it is more sensitive in detecting agonist interactions than functional assays (because it reflects binding to the high affinity desensitised state of the nAChR). Daly and co-workers[4] had reported epiquinamide **1** had weak agonist activity ($EC_{50} < 50$ μM) in a functional assay based on membrane potential fluorescence,[5] although in the absence of reported evidence that the activity of **1** can be blocked by nAChR antagonists, it is possible that it reflects a non-specific membrane interaction. This group also employed cell lines expressing human receptor subtypes; hence there are several differences associated with the assay that we have used. Alternatively, the presence of a minor but potent contaminant in the material isolated from this natural source remains a possibility to explain the observed activity, as the isolation of **1** was reported[4] to give material that was '>90% pure.'

In summary, the synthesis of epiquinamide **1** and its diastereomer, *C*(1)-*epi*epiquinamide **2**, which also contains the same acetylcholine-like motif[13] as present in **1**, has been described. Neither (±)-**1** nor (±)-**2** displays a useful level of nicotinic affinity as measured in a competition binding assay (based on rat brain P2 membranes) for α4β2-containing nAChR subtypes, and this report highlights the need to confirm the structure of and elucidate more fully the nicotinic profile of **1**. Synthetic material, which is available on a larger scale and more amenable to purification and now available in both enantiomeric series,[6a,b] will play an important role in achieving this goal.

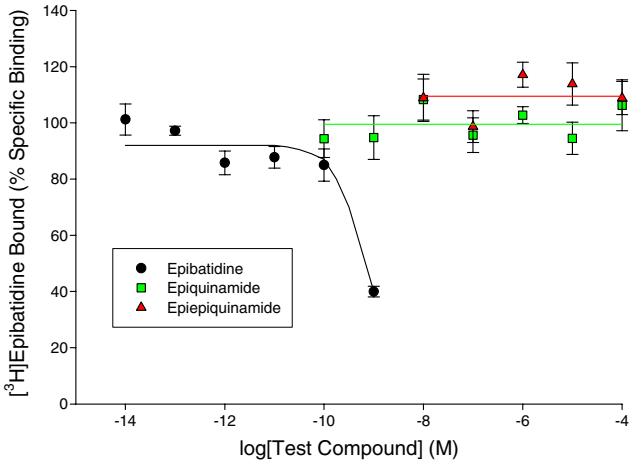


Figure 1. Competition for [^{3}H]epibatidine binding sites by epiquinamide **1** and *C*(1)-*epi*epiquinamide **2**. Data points are the mean ± SEM from three independent experiments. A representative curve for competition by epibatidine, used as a positive control and examined in parallel, is included for comparison.

Acknowledgments

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Supplementary data

Copies of ^{1}H and ^{13}C NMR spectra for both racemic epiquinamide **1** and racemic *C*(1)-*epi*epiquinamide **2** are available as supplementary data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.05.100.

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7. We focused initially on the synthesis of racemic **1** and **2** because of (i) the absolute configuration of natural **1** is unknown (ii) **2** is novel and was of unknown activity, and (iii) the possibility that biological activity may reside in only one enantiomer of either or both of **1** and **2**.
8. Bicyclic ester **5** has also been used as an intermediate for the synthesis of the lupin alkaloid thermopsine, and the relative stereochemistry of **5** was established by X-ray crystallographic analysis: Gray, D.; Gallagher, T. *Angew. Chem. Int. Ed.* **2006**, *45*, 2419, The synthesis of ester **5** in enantiomerically pure form has now been achieved but in light of the biological data reported here, it has not yet been applied to an asymmetric synthesis of epiquinamide **1**.
9. For the use of 9-FM to aid isolation and purification of Curtius rearrangement products, see: Spino, C.; Gobdout, C. *J. Am. Chem. Soc.* **2003**, *125*, 11207.
10. We have described an alternative entry to the quinolizidine based ester **9**, which does not rely on a base-mediated equilibration of **5**, as an intermediate in the synthesis of anagyrine.[8] Other routes to esters **5** and **9** and related compounds have been reported by others and these are detailed in Ref. 8.

11. The name epiquinamide relates to the natural source (*Epipedobates tricolor*) of **1**, and in order to avoid any confusion we have consistently used *C*(1)-*epi*epiquinamide to refer to diastereomer **2**. Characterization data for epiquinamide **1**: R_f 0.28 ($CHCl_3$/MeOH/NH_3 = 30:1:1%). Mp 90–95 °C. IR (cm^{-1}) (neat): 3229, 2933, 1637, 1544, 1288. ^1H NMR (400 MHz, $CDCl_3$): δ 5.21 (1H, br s, NH), 3.94–3.91 (1H, m, H-1), 2.80–2.74 (2H, m), 2.01 (3H, s, Me), 1.97–1.93 (3H, m), 1.89–1.83 (1H, m, H-2a), 1.77–1.58 (3H, m), 1.54–1.23 (6H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.5 (CO), 64.3 (C-10), 56.7 (C-4, 6, signals overlapping), 48.1 (C-1), 29.6 (C-2), 29.0 (C-9), 25.6 (C-7), 24.0 (C-8), 23.4 (Me), 20.6 (C-3). HRMS: calcd for $[M+H]^+$ $C_{11}H_{21}N_2O$, 197.1648; found, 197.1656. Characterization data for *C*(1)-*epi*epiquinamide **2**: R_f 0.15($CHCl_3$/MeOH/NH_3 = 30:1:1%). Mp 165–167 °C. IR (cm^{-1}) (neat): 3289, 2926, 1640, 1555, 1121. ^1H NMR (400 MHz, $CDCl_3$): δ 5.21 (1H, br s, NH), 3.76–3.68 (1H, m, H-1), 2.85 (1H, br d, J = 12.2 Hz), 2.77 (1H, br d, J = 11.5 Hz), 1.97 (3H, s, Me), 2.04–1.93 (3H, m), 1.83–1.49 (7H, m), 1.31–1.07 (3H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.3 (CO), 67.5 (C-10), 56.5 (CH_2), 55.8 (CH_2), 51.1 (C-1), 32.2 (C-9), 29.0 (CH_2), 25.5 (CH_2), 24.4 (CH_2), 23.9 (CH_2), 23.6 (Me). HRMS: calcd for $[M+H]^+$ $C_{11}H_{21}N_2O$, 197.1648; found, 197.1656. Copies of ^1H and ^{13}C NMR data for **1** and **2** are available as Supplementary data.
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13. The effect of amide variants of acetylcholine on activity at nAChR has been studied: Barlow, R. B.; Bremner, J. B.; Soh, K. S. *Br. J. Pharmacol.* **1978**, *62*, 39, Replacement of the ester moiety of acetylcholine by an amide, by analogy to epiquinamide, reduces activity on guinea-pig ileum (which corresponds to α3-containing neuronal nAChR) over 1000-fold and on frog rectus (i.e., muscle nAChR) over 50-fold.