

## Brief Articles

## Potential Nicotinic Acetylcholine Receptor Ligands from 2,4-Methanoproline Derivatives

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Potential nicotinic acetylcholine receptor (nAChR) ligands have been synthesized in which a methylisoxazole substituent is attached to the 1-position (**26**) of the 2-azabicyclo[2.1.1]hexane ring system or separated by “spacer” atoms (**21** and **23**). With ABT-594 as a model, a range of pyridine heterocycles have been attached to the 1-position via a  $-\text{CH}_2\text{O}-$  spacer (**11**, **14**, and **6**). The biological evaluation of target compounds showed there was no binding affinity at the  $\alpha 4\beta 2$  and  $\alpha 3\beta 4$  nAChR subtypes.

## Introduction

We recently described the conversion of derivatives of the nonproteinogenic amino acid 2,4-methanoproline (2-azabicyclo[2.1.1]hexane-1-carboxylic acid)<sup>1–3</sup> into potential nicotinic acetylcholine receptor<sup>a</sup> (nAChR) ligands having heterocycles attached to the 1-position of the 2-azabicyclo[2.1.1]hexane system via a methylene “spacer” (**1**).<sup>4,5</sup> We now describe variants in which heterocycles are directly attached to the 1-position (**2**) and examples in which the heterocycle is separated by two spacer units (**3**, **4**, and **5**, Figure 1).

Various methods available for the construction of the 2-azabicyclo[2.1.1]hexane system exist<sup>2,6–12</sup> and functionality has been studied at different positions around the bicyclic system.<sup>7,13–20</sup> We<sup>4,5,21</sup> used a [2 + 2] photocycloaddition<sup>22–24</sup> strategy, as this allows incorporation of a carboxylic ester substituent at the bridgehead ( $\text{C}_1$ ) position. This opens the way for further functionalization at  $\text{C}_1$ , leading to the formation of epibatidine ((4*S*,6*R*)-6-(6-chloro-3-pyridyl)-7-azabicyclo[2.2.1]heptane) analogues<sup>25–28</sup> in the search for high affinity and subtype selectivity for nAChRs (Figure 2).<sup>29–32</sup> We are aware of no other examples of the incorporation of heterocycles into this azabicyclic system as potential nAChR ligands.

There has been much recent interest concerning 3-pyridyl ether analogues of various azabicyclic systems.<sup>33–37</sup> Variants synthesized at Abbott Laboratories,<sup>34,38,39</sup> include ABT-594 (5-[(2*R*)-azetidin-2-yl]methoxy)-2-chloropyridine, a first-generation nAChR agonist.<sup>35</sup> During clinical trials, this compound was shown to have analgesic properties with improved separation of antinociceptive effects and nicotinic side effects. The chloro substituent and the azetidine ring were found to be important structural elements for potent analgesic activity. ABT-594 was found to have high affinity for nAChRs, although it was 180-fold less potent than (±)-epibatidine in activating peripheral skeletal muscle-type nAChRs. In designing potential ABT-594

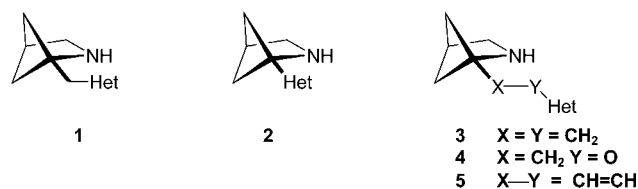


Figure 1. Target 2,4-methanoproline derivatives.

variants, we expected the rigid 2-azabicyclo[2.1.1]hexane system to provide a framework for maintaining the structural requirements and orientation necessary for activity at the nAChR. We hoped that this rigid, strained azabicyclic system might emulate the azetidine ring of ABT-594 and that attachment of a chloropyridyl heterocycle at the  $\text{C}_1$  position, linked by a  $-\text{CH}_2\text{O}-$  spacer (as in **6**), would reproduce the key  $\text{pyr}-\text{O}-\text{CH}_2-\text{CHR}-\text{NHR}$  moiety in ABT-594 (Figure 3). It was also anticipated that it would give extra flexibility once in the receptor, as opposed to traditional compounds that have the heterocycles directly attached to the bicycle. Certainly, according to simple molecular modeling studies, the ideal internitrogen distance for the nAChR pharmacophore may be attained.

More recent work has exploited the bioisosteric replacement the chloropyridyl heterocycle of (±)-epibatidine by the methylisoxazole group to form (±)-epiboxidine ((1*R*,4*S*,6*S*)-6-(3-methyl-5-isoxazolyl)-7-azabicyclo[2.2.1]heptane).<sup>40</sup> Epiboxidine is a potent nAChR agonist with antinociceptive activity and binds to ganglionic  $\alpha 4\beta 2$  subtype receptors. More importantly, (±)-epiboxidine was 20-fold less toxic than epibatidine, demonstrating that it is possible to synthesize potent compounds with lowered toxicity. There have been reports of the construction of epiboxidine analogues<sup>41–45</sup> of various azabicyclic systems including those from  $\alpha,\beta$ -unsaturated esters.<sup>46</sup> There have been no recent reports of functional group interconversion at the  $\text{C}_1$  position of the 2-azabicyclo[2.1.1] system other than our own,<sup>4,5</sup> and we now add to the range with examples in which the heterocycle is attached by alkenyl and ethano linkages.

## Discussion

As described previously,<sup>4,5</sup> we have utilized the [2 + 2] photocycloaddition approach established by Pirrung<sup>22</sup> and Clardy<sup>23</sup> to synthesize the key precursor **7** (Figure 3).

In the approach to analogues based on structure **4**, the *N*-benzoyl compound **7** was converted into the *N*-Cbz alcohol

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<sup>a</sup> Abbreviations: nAChR, nicotinic acetylcholine receptor; Het, heterocycle; Cbz, benzyloxycarbonyl; DEAD, diethyl azodicarboxylate; TPP, triphenylphosphine; THF, tetrahydrofuran; TMSI, iodotrimethylsilane; DCM, dichloromethane; Boc, *t*-butoxycarbonyl; DMSO, dimethylsulfoxide; TEA, triethylamine; BuLi, *t*-butyllithium; HEK, human embryonic kidney; BCA, bicinchoninic acid; SPA, scintillation proximity assay; WGA, wheat germ agglutinin; PVT, polyvinyltoluene.

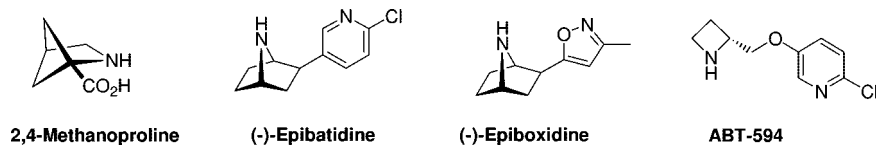


Figure 2. 2,4-Methanoproline and selected current nAChR agonists.

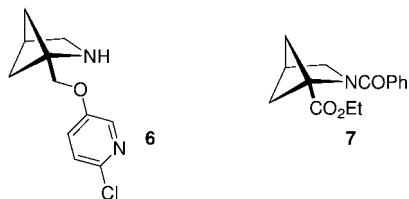


Figure 3. ABT-594 analogue 6 and key precursor compound 7.

8 as described previously,<sup>4,5</sup> and this was, in turn, reacted with the appropriate phenol (9) under Mitsunobu conditions<sup>47</sup> (Scheme 1).

Unlike previous reports concerning the formation of ABT-594 analogues,<sup>34,36,37</sup> we found it necessary to heat the reaction mixture to reflux conditions to achieve coupling. The primary alcohol group in 8 is adjacent to the bridgehead position and it is likely that steric hindrance leads to the need for harsher conditions and also the modest yields (22–27%) obtained. We were pleasantly surprised to be able to achieve ready displacement at the 1-methylene position. Following the successful attachment of a phenoxy substituent, similar reaction conditions were used to attach hydroxy-pyridine derivative (12, 15) to give 13 and 16 in similar yields. A simple deprotection step gave the respective products 11, 14, and 6 in good yield (81–87%); compound 6 is the desired azabicyclic analogue of ABT-594.

The literature<sup>40–46</sup> provides a wide array of epibatidine analogues containing the methylisoxazole heterocycle; the most potent of these is epiboxidine. Our initial aim was to synthesize 2-azabicyclo[2.1.1]hexane epiboxidine analogues having the methylisoxazole attached by a bis-methylene chain. The synthesis of these compounds would allow us to investigate the effects of an increase of the carbon chain length on binding to the nAChR. The *N*-Boc alcohol 17 was oxidized to the aldehyde 18 using Swern conditions in 97% yield (Scheme 2).

Wittig methodology<sup>48–50</sup> was then employed to form the  $\alpha,\beta$ -unsaturated ester 19 (24% yield). The  $\alpha,\beta$ -unsaturated methylisoxazole 20 was then constructed using the method of Daly and co-workers<sup>41</sup> in 30% yield. Deprotection of the Boc group with 3 M hydrochloric acid gave the desired product 21 in 97% yield. The <sup>1</sup>H NMR spectrum confirmed the construction of the methylisoxazole heterocycle in 20. The singlet at  $\delta$  2.21 corresponds to the methyl group, and the singlet at  $\delta$  5.95 is the olefinic proton in the ring system. The alkene protons at  $\delta$  6.28 and 6.46 showed mutual coupling of 16 Hz, confirming the trans stereochemistry. The double bond of 20 was selectively reduced using potassium azodicarboxylate to give the saturated methylisoxazole 22 in 34% yield. Removal of the Boc group gave the product 23 in a 99% yield (Scheme 3).

Next we aimed to construct the methylisoxazole heterocycle attached directly at the C<sub>1</sub> position of the 2-azabicyclo[2.1.1]hexane system (type 2). Other groups<sup>46,51</sup> have incorporated heterocycles directly at the bridgehead position of other azabicyclic systems in an attempt to create nAChR ligands. The methylisoxazole synthesis was attempted on the bridgehead ethyl ester 7 in the hope of making 24 (Scheme 4).

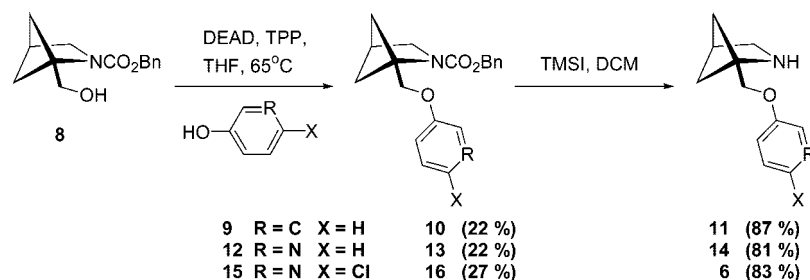
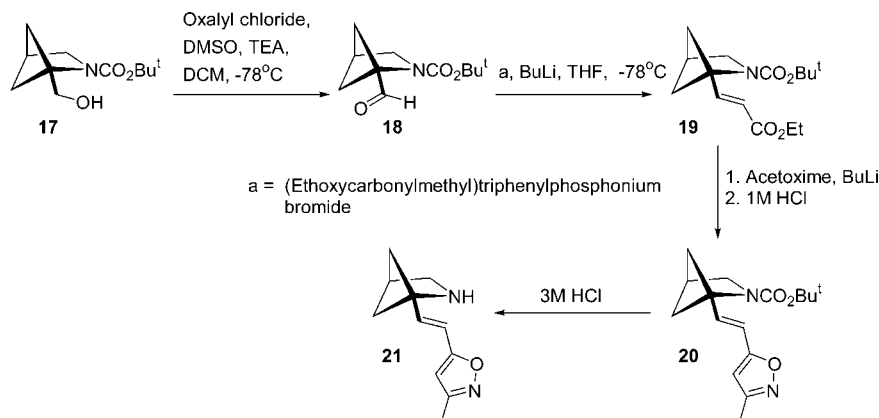
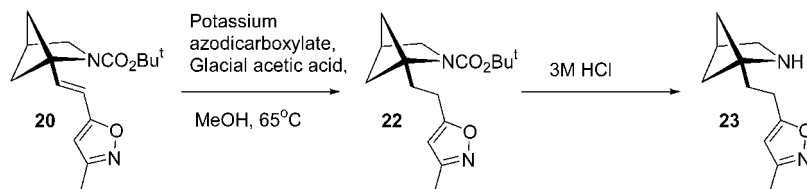
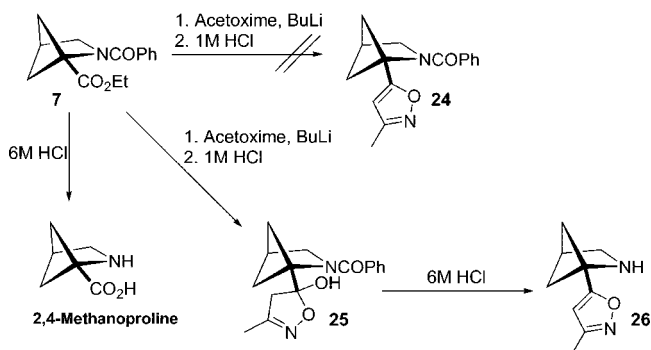
Bizarrely, we isolated the novel intermediate 25 (61% yield, based on recovered starting material) instead; such a nonaro-

matized intermediate has not previously been reported during methylisoxazole synthesis. The formation of 25 was confirmed by X-ray crystallography, which indicated hydrogen bonding between the carbonyl-oxygen and -hydrogen from the alcohol. This stabilization is thought to be the reason for the interesting isolation of 25. *N*-Deprotection of 25 with 6 M hydrochloric acid led to concomitant elimination of water, achieving conversion into the target 26. Daly and co-workers<sup>41</sup> have reported the isolation of a minor byproduct while constructing the methylisoxazole ring, but there are no reports of compounds akin to 25.

**Biological Evaluation.** The *p*-toluenesulfonate salts of racemic ABT-594 analogues 11, 14, and 6 and hydrochloride salts of racemic epiboxidine analogues 21, 23, and 26 were evaluated for binding to the high affinity nicotine binding site in human brain (at the  $\alpha 4\beta 2$  and  $\alpha 3\beta 4$  nAChR subtypes). Unexpectedly, for all of the compounds, the *K<sub>i</sub>* values could not be recorded even at a concentration of 1000 nM, showing that there was no significant binding at the nAChR subtypes. **Membrane preparation.** Cell pastes from large-scale production of HEK-293 cells expressing cloned human  $\alpha 4\beta 2$  or  $\alpha 3\beta 4$  nAChR were homogenized in 4 volumes of buffer (50 mM Tris-HCl, 150 mM NaCl, and 5 mM KCl, pH 7.4). The homogenate was centrifuged twice (40000g, 10 min, 4 °C) and the pellets resuspended in 4 volumes of Tris-HCl buffer after the first spin and 8 volumes after the second spin. The resuspended homogenate was centrifuged (100g, 10 min, 4 °C) and the supernatant kept and recentrifuged (40000g, 20 min, 4 °C). The pellet was resuspended in Tris-HCl buffer supplemented with 10% w/v sucrose. The membrane preparation was stored in 1 mL aliquots at –80 °C until required. The protein concentration of the membrane preparation was determined using a BCA protein assay reagent kit. **Nicotinic receptor radioligand binding scintillation proximity assay (SPA).** SPA radioligand binding assays were performed in 96-well plates in a final volume of 250  $\mu$ L Tris-HCl buffer (50 mM Tris-HCl, 150 mM NaCl, 5 mM KCl, pH 7.4) using the following conditions: [<sup>3</sup>H]epibatidine (53 Ci/mmol; Amersham)  $\alpha 4\beta 2$  = 1 nM,  $\alpha 3\beta 4$  = 2 nM; WGA-coated PVT SPA beads (Amersham)  $\alpha 4\beta 2$  = 1 mg/well,  $\alpha 3\beta 4$  = 1.5 mg/well; membrane protein = 30  $\mu$ g/well for both assay types. Nonspecific binding (<10% for both assay types) was determined using 10  $\mu$ M epibatidine. Reactions were allowed to equilibrate for 2–4 h at room temperature prior to reading on a Trilux Scintillation counter (Perkin-Elmer). Data were analyzed using a standard 4-parameter logistic equation (Multicalc, Perkin-Elmer) to provide IC<sub>50</sub> values that were converted to *K<sub>i</sub>* values using the Cheng–Prusoff equation.<sup>52</sup>

## Conclusion

In conclusion, we have been successful in the synthesis of a range of nAChR ligands with either the pyridyl ether moiety or the methylisoxazole heterocycle attached to the 2-azabicyclo[2.1.1]hexane. The Mitsunobu reaction has been effectively performed in order to synthesize a range of ABT-594 analogues; 11, 14, and 6. Methylisoxazole compounds have also been synthesized on the 2-azabicyclo[2.1.1]hexane framework, with

**Scheme 1.** Synthesis of 1-Substituted 2-Azabicyclo[2.1.1]hexane Derivatives**Scheme 2.** Synthesis of Methylisoxazole Compound **21****Scheme 3.** Synthesis of Methylisoxazole Compound **23****Scheme 4.** Attachment of a Methylisoxazole Substituent to C<sub>1</sub>

differing number of carbon atoms in the chain: **21** ( $n = 2$  alkene), **23** ( $n = 2$  alkane), and **26** ( $n = 0$ ). It is perhaps surprising that none of these compounds show binding despite the very different conformational opportunities open to them, as shown by modeling studies. On the route to constructing **26**, we have reported a novel intermediate, apparently stabilized by H-bonding via a seven-membered ring. This work, together with our earlier studies,<sup>4</sup> has led to the synthesis of a wide range of heterocycle-substituted derivatives of the 2-azabicyclo[2.1.1]-hexane ring system. The incorporation of other heterocycles at the C<sub>7</sub> position of this bicyclic ring system (the CH<sub>2</sub> attached

to C<sub>1</sub>) is currently under investigation in an attempt to widen the range of potential nAChR ligands.

**Experimental Section**

NMR spectra were recorded at 300 MHz using a Bruker DPX 300 spectrometer and at 400 MHz using a Bruker DRX 400 spectrometer. Chemical shifts are expressed in ppm ( $\delta$ ) relative to an internal standard tetramethylsilane (TMS). Signal characteristics are described using standard abbreviations: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), bs (broad singlet), AB (AB-system). Signals in <sup>13</sup>C NMR were determined by DEPT experiments. Signals were assigned with assistance of <sup>1</sup>H–<sup>1</sup>H COSY and <sup>1</sup>H–<sup>13</sup>C COSY spectra. Routine mass spectra were measured on a Micromass Quattro LC triple quadrupole spectrometer and were obtained using ionization by electrospray. Accurate mass measurements were measured on a Kratos Concept 1H sector mass spectrometer and were obtained using ionization by fast atom bombardment. Mass spectra were determined in units of mass relative to charge ( $m/z$ ). IR spectra were recorded on a Perkin-Elmer 298 FT spectrometer. Band intensities are described using standard abbreviations: s (strong), m (medium), w (weak), br (broad). Flash chromatography was carried out using silica gel (60) manufactured by Fisher. Thin layer chromatography was conducted on standard commercial aluminum sheets precoated with 0.2 mm layer of silica gel (Merck Kieselgel 60-254). All the target compounds were unsuitable for elemental analysis as they were obtained as volatile and viscous oils or



unstable salts. Purification by HPLC or other chromatographic methods caused the target compounds to decompose, so were not used.

**1-Phenoxymethyl-2-azabicyclo[2.1.1]hexane (11).** **10** (110 mg, 0.34 mmol) was dissolved in dry dichloromethane (3 mL) and stirred under a nitrogen atmosphere. TMSI (242  $\mu$ L, 1.70 mmol) was added and stirred for 7 min, followed by hydrofluoroboric acid–diethyl ether complex (51  $\mu$ L, 0.68 mmol), stirring for a further 6 min. The reaction mixture was quenched with water (200  $\mu$ L) and the solvent removed under reduced pressure. Water (3 mL) was added and the solution washed with petroleum ether (bp 40–60 °C) (3  $\times$  1 mL). The solution was neutralized with solid potassium carbonate and the product extracted with dichloromethane (6  $\times$  5 mL). The organic layer was dried with anhydrous magnesium sulfate, filtered, and the solvent removed under reduced pressure to yield **11** (56 mg, 0.29 mmol, 87% yield) as an orange oil.  $\delta_{\text{H}}$  (300 MHz,  $\text{CDCl}_3$ ): 1.36 (dd,  $J = 4.4$ , 1.4 Hz, 2 H,  $\text{H}_{5\text{s}}$ ,  $\text{H}_{6\text{s}}$ ), 1.71 (m, 2 H,  $\text{H}_{5\text{a}}$ ,  $\text{H}_{6\text{a}}$ ), 2.74 (m, 1 H,  $\text{H}_4$ ), 3.00 (s, 2 H,  $\text{H}_{3\text{x}}$ ,  $\text{H}_{3\text{n}}$ ), 4.12 (s, 2 H,  $\text{CH}_2\text{O}$ ), 6.82–6.89 (m, 3 H, Ph), 7.17–7.23 (m, 2 H, Ph).  $\delta_{\text{C}}$  (75.5 MHz,  $\text{CDCl}_3$ ): 37.69 ( $\text{C}_4$ ), 40.72 ( $\text{C}_5$ ,  $\text{C}_6$ ), 48.85 ( $\text{C}_3$ ), 68.45 ( $\text{CH}_2\text{O}$ ), 68.77 ( $\text{C}_1$ ), 114.55, 120.86, 129.44 (5  $\times$  aryl), 158.87 (aryl C–O).  $\nu_{\text{max}}$  ( $\text{CH}_2\text{Cl}_2$ ): 2880w (C–H), 1590w (Ph), 1490w (Ph), 1050w  $\text{cm}^{-1}$ .  $m/z$ : 190 ( $\text{MH}^+$ ).  $\text{C}_{12}\text{H}_{16}\text{NO}$  [ $\text{MH}^+$ ] requires  $m/z$  190.12311; observed 190.12319.

**1-(Pyridin-3-yloxymethyl)-2-azabicyclo[2.1.1]hexane (14).** **13** (101 mg, 0.31 mmol) was dissolved in dry dichloromethane (6 mL) and stirred under a nitrogen atmosphere. TMSI (222  $\mu$ L, 1.56 mmol) was added and stirred for 7 min, followed by hydrofluoroboric acid–diethyl ether complex (46  $\mu$ L, 0.62 mmol), stirring for a further 6 min. The reaction mixture was quenched with water (200  $\mu$ L) and the solvent removed under reduced pressure. Water (5 mL) was added and the solution washed with petroleum ether (bp 40–60 °C) (3  $\times$  3 mL). The solution was neutralized with solid potassium carbonate and the product extracted with dichloromethane (5  $\times$  10 mL). The organic layer was dried with anhydrous magnesium sulfate, filtered, and the solvent removed under reduced pressure to yield **14** (48 mg, 0.25 mmol, 81% yield) as a yellow oil.  $\delta_{\text{H}}$  (300 MHz,  $\text{CDCl}_3$ ): 1.37 (dd,  $J = 4.4$ , 2.0 Hz, 2 H,  $\text{H}_{5\text{s}}$ ,  $\text{H}_{6\text{s}}$ ), 1.72 (m, 2 H,  $\text{H}_{5\text{a}}$ ,  $\text{H}_{6\text{a}}$ ), 2.77 (m, 1 H,  $\text{H}_4$ ), 3.01 (s, 2 H,  $\text{H}_{3\text{x}}$ ,  $\text{H}_{3\text{n}}$ ), 4.16 (s, 2 H,  $\text{CH}_2\text{O}$ ), 7.14 (m, 2 H, heterocycle), 8.14 (m, 1 H, heterocycle), 8.24 (m, 1 H, heterocycle).  $\delta_{\text{C}}$  (75.5 MHz,  $\text{CDCl}_3$ ): 37.77 ( $\text{C}_4$ ), 40.65 ( $\text{C}_5$ ,  $\text{C}_6$ ), 48.73 ( $\text{C}_3$ ), 68.32 ( $\text{CH}_2\text{O}$ ), 68.89 ( $\text{C}_1$ ), 120.90, 123.71, 138.07, 142.14 (4  $\times$  heterocycle), 154.95 (heterocycle C–O).  $\nu_{\text{max}}$  ( $\text{CH}_2\text{Cl}_2$ ): 3000s (C–H), 2950s (C–H), 1420s, 1160s, 900s, 690br  $\text{cm}^{-1}$ .  $m/z$ : 191 ( $\text{MH}^+$ ).  $\text{C}_{11}\text{H}_{15}\text{N}_2\text{O}$  [ $\text{MH}^+$ ] requires  $m/z$  191.11844; observed 191.11840.

**1-(6-Chloro-pyridin-3-yloxymethyl)-2-azabicyclo[2.1.1]hexane (6).** **16** (57 mg, 0.16 mmol) was dissolved in dry dichloromethane (4 mL) and stirred under a nitrogen atmosphere. TMSI (113  $\mu$ L, 0.79 mmol) was added and stirred for 7 min, followed by hydrofluoroboric acid–diethyl ether complex (24  $\mu$ L, 0.31 mmol), stirring for a further 6 min. The reaction mixture was quenched with water (100  $\mu$ L) and the solvent removed under reduced pressure. Water (10 mL) was added and the solution washed with petroleum ether (bp 40–60 °C) (5  $\times$  5 mL). The solution was neutralized with solid potassium carbonate and the product extracted with dichloromethane (5  $\times$  10 mL). The organic layer was dried with anhydrous magnesium sulfate, filtered, and the solvent removed under reduced pressure to yield **6** (30 mg, 0.13 mmol, 83% yield) as a bright-yellow oil.  $\delta_{\text{H}}$  (300 MHz,  $\text{CDCl}_3$ ): 1.41 (dd,  $J = 4.5$ , 1.6 Hz, 2 H,  $\text{H}_{5\text{s}}$ ,  $\text{H}_{6\text{s}}$ ), 1.74 (m, 2 H,  $\text{H}_{5\text{a}}$ ,  $\text{H}_{6\text{a}}$ ), 2.79 (m, 1 H,  $\text{H}_4$ ), 3.05 (s, 2 H,  $\text{H}_{3\text{x}}$ ,  $\text{H}_{3\text{n}}$ ), 4.18 (s, 2 H,  $\text{CH}_2\text{O}$ ), 7.15 (m, 2 H, heterocycle), 8.00 (m, 1 H, heterocycle).  $\delta_{\text{C}}$  (75.5 MHz,  $\text{CDCl}_3$ ): 37.68 ( $\text{C}_4$ ), 40.59 ( $\text{C}_5$ ,  $\text{C}_6$ ), 48.76 ( $\text{C}_3$ ), 68.48 ( $\text{CH}_2\text{O}$ ), 69.10 ( $\text{C}_1$ ), 124.39, 124.92, 136.77 (3  $\times$  heterocycle), 142.76 (heterocycle C–Cl), 154.19 (heterocycle C–O).  $\nu_{\text{max}}$  ( $\text{CH}_2\text{Cl}_2$ ): 3020s (C–H), 2990s (C–H), 2300m, 1420s, 1260s, 900s, 750br (C–Cl)  $\text{cm}^{-1}$ .  $m/z$ : 225 ( $\text{MH}^+$ ).  $\text{C}_{11}\text{H}_{14}\text{N}_2\text{OCl}$  [ $\text{MH}^+$ ] requires  $m/z$  225.07944; observed 225.07947.

**1-[2-(3-Methyl-isoxazol-5-yl)-vinyl]-2-azabicyclo[2.1.1]hexane (21).** **20** (5 mg, 0.02 mmol) was stirred in a solution of 3 M HCl (2 mL) made in situ (dry ethanol (0.43 mL), dry ethyl acetate (1.2 mL), and acetyl chloride (0.36 mL)) at 0 °C for 1 h. The reaction mixture was washed with diethyl ether (3  $\times$  0.5 mL) and then evaporated to dryness to give the hydrochloride salt of **21** (4 mg, 0.02 mmol, 97% yield).  $\delta_{\text{H}}$  (300 MHz,  $\text{D}_2\text{O}$ ): 1.95–2.16 (m, 4 H,  $\text{H}_{5\text{s}}$ ,  $\text{H}_{6\text{s}}$ ,  $\text{H}_{5\text{a}}$ ,  $\text{H}_{6\text{a}}$ ), 2.04 (s, 3 H,  $\text{CH}_3$ ), 2.32 (m, 1 H,  $\text{H}_4$ ), 2.90 (m, 2 H,  $\text{H}_{3\text{x}}$ ,  $\text{H}_{3\text{n}}$ ), 6.08 (s, 1 H, =CH), 6.28 (d,  $J = 16.0$  Hz, 1 H,  $\text{HC}=\text{CH}-\text{CO}_2\text{Et}$ ), 6.46 (d,  $J = 16.0$  Hz, 1 H,  $\text{HC}=\text{CH}-\text{CO}_2\text{Et}$ ).  $\delta_{\text{C}}$  (75.5 MHz,  $\text{D}_2\text{O}$ ): 10.35 ( $\text{CH}_3$ ), 25.69 ( $\text{C}_4$ ), 37.76 ( $\text{C}_5$ ,  $\text{C}_6$ ), 44.24 ( $\text{C}_3$ ), 102.95 (=CH), 113.04 ( $\text{C}_8$ ), 141.20 ( $\text{C}_7$ ).  $m/z$ : 191 ( $\text{MH}^+$ ).  $\text{C}_{11}\text{H}_{19}\text{N}_2\text{O}$  [ $\text{MH}^+$ ] requires  $m/z$  191.17851; observed 191.17847.

**1-[2-(3-Methyl-isoxazol-5-yl)-ethyl]-2-azabicyclo[2.1.1]hexane (23).** **22** (10 mg, 0.02 mmol) was stirred in a solution of 3 M HCl (2 mL) made in situ (dry ethanol (0.43 mL), dry ethyl acetate (1.2 mL), and acetyl chloride (0.36 mL)) at 0 °C for 1 h. The reaction mixture was washed with diethyl ether (3  $\times$  0.5 mL) and then evaporated to dryness to give the hydrochloride salt of **23** (8 mg, 0.02 mmol, 99% yield).  $\delta_{\text{H}}$  (300 MHz,  $\text{D}_2\text{O}$ ): 1.49 (dd,  $J = 6.1$ , 2.2 Hz, 2 H,  $\text{H}_{5\text{s}}$ ,  $\text{H}_{6\text{s}}$ ), 1.85 (m, 2 H,  $\text{H}_{5\text{a}}$ ,  $\text{H}_{6\text{a}}$ ), 2.10 (s, 3 H,  $\text{CH}_3$ ), 2.42 (t,  $J = 7.8$  Hz, 2 H,  $\text{H}_7$ ), 2.73 (m, 1 H,  $\text{H}_4$ ), 2.78 (t,  $J = 7.8$  Hz, 2 H,  $\text{H}_8$ ), 3.24 (s, 2 H,  $\text{H}_{3\text{x}}$ ,  $\text{H}_{3\text{n}}$ ), 6.01 (s, 1 H, =CH).  $m/z$ : 193 ( $\text{MH}^+$ ).  $\text{C}_{11}\text{H}_{17}\text{N}_2\text{O}$  [ $\text{MH}^+$ ] requires  $m/z$  193.13409; observed 193.13407.

**1-(3-Methyl-isoxazole-5-yl)-2-azabicyclo[2.1.1]hexane (26).** **25** (20 mg, 0.07 mmol) was stirred in 6 M HCl (10 mL) and heated to reflux for 48 h. The reaction mixture was washed with diethyl ether (3  $\times$  0.5 mL) and then evaporated to dryness to give the hydrochloride salt of **26** (14 mg, 0.07 mmol, 99% yield).  $\delta_{\text{H}}$  (300 MHz,  $\text{D}_2\text{O}$ ): 1.84–1.98, 2.08–2.26 (m, 4 H,  $\text{H}_{5\text{s}}$ ,  $\text{H}_{6\text{s}}$ ,  $\text{H}_{5\text{a}}$ ,  $\text{H}_{6\text{a}}$ ), 1.97 (s, 3 H,  $\text{CH}_3$ ), 2.47 (m, 1 H,  $\text{H}_4$ ), 2.83 (m, 2 H,  $\text{H}_{3\text{x}}$ ,  $\text{H}_{3\text{n}}$ ), 6.04 (s, 1 H, =CH).  $\delta_{\text{C}}$  (75.5 MHz,  $\text{D}_2\text{O}$ ): 10.39 ( $\text{CH}_3$ ), 25.40 ( $\text{C}_4$ ), 38.99 ( $\text{C}_5$ ,  $\text{C}_6$ ), 44.45 ( $\text{C}_3$ ), 68.82 ( $\text{C}_1$ ), 101.56 (=CH), 155.81 ( $\text{C}_7$ ).  $m/z$ : 165 ( $\text{MH}^+$ ).  $\text{C}_9\text{H}_{12}\text{N}_2\text{O}$  [ $\text{MH}^+$ ] requires  $m/z$  165.48922; observed 165.48910.

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**Supporting Information Available:** Experimental details, spectroscopic characterization of all new compounds, and crystal structure data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References

- (1) Bell, E. A.; Qureshi, M. Y.; Pryce, R. J.; Janzen, D. H.; Lemke, R.; Clardy, J. 2,4-Methanoproline (2-Carboxy-2,4-methanopyrrolidine) and 2,4-Methanoglutamic Acid (1-Amino-1,3-dicarboxycyclobutane) in Seeds of *Ateleia herbert smithii* (Leguminosae). *J. Am. Chem. Soc.* **1980**, *102*, 1409–1412.
- (2) Rammeloo, T.; Stevens, C. A New and Short Method for the Synthesis of 2,4-Methanoproline. *J. Chem. Soc., Chem. Commun.* **2002**, 250–251.
- (3) Stevens, C. V.; Smagghe, G.; Rammeloo, T.; Kimpe, N. D. Insect Repellent/Antifeedant Activity of 2,4-Methanoproline and Derivatives Against a Leaf- and Seed Feeding Pest Insect. *J. Agric. Food Chem.* **2005**, *53*, 1945–1948.
- (4) Malpass, J. R.; Patel, A. B.; Davies, J. W.; Fulford, S. Y. Modification of 1-Substituents in the 2-Azabicyclo[2.1.1]hexane Ring System; Approaches to Potential Nicotinic Acetylcholine Receptor Ligands from 2,4-Methanoproline Derivatives. *J. Org. Chem.* **2003**, *68*, 9348–9355.
- (5) Patel, A. B. Nicotinic Acetylcholine Receptor Ligands from 2,4-Methanoproline Derivatives. Ph.D. Dissertation. University of Leicester: Leicester, UK, 2005; 148 pp.
- (6) Paquette, L. A.; Allen, G. R.; Broadhurst, M. J. Addition Reactions of the Unipartulate Electrophile Chlorosulfonyl Isocyanate to Highly Strained Bicyclic Hydrocarbons. *J. Am. Chem. Soc.* **1971**, *93*, 4503–4508.
- (7) Krow, G. R.; Cannon, K. C. Azabicyclo[2.1.1]hexanes. *Heterocycles* **2004**, *62*, 877–898.
- (8) Tamura, Y.; Ishibashi, H.; Hirai, M.; Kita, Y.; Ikeda, M. Photochemical Syntheses of 2-Aza- and 2-Oxabicyclo[2.1.1]hexane Ring Systems. *J. Org. Chem.* **1975**, *40*, 2702–2710.

- (9) Rammeloo, T.; Stevens, C. V.; Kimpe, N. D. Synthesis of 2,4-Methanoproline Analogues via an Addition–Intramolecular Substitution Sequence. *J. Org. Chem.* **2002**, *67*, 6509–6513.
- (10) Stevens, C.; Kimpe, N. D. A New Entry into 2-Azabicyclo[2.1.1]hexanes via 3-(Chloromethyl)cyclobutanone. *J. Org. Chem.* **1996**, *61*, 2174–2178.
- (11) Lescop, C.; Mevellec, L.; Huet, F. A New Synthesis of 2-Azabicyclo[2.1.1]hexanes. *J. Org. Chem.* **2001**, *66*, 4187–4193.
- (12) Amii, H.; Ichihara, Y.; Nakagawa, T.; Kobayashi, T.; Uneyama, K. Unusual Reactions of *N*-Allylic Difluoroamines under Thermal Conditions. *J. Chem. Soc., Chem. Commun.* **2003**, 2902–2903.
- (13) Krow, G. R.; Lee, Y. B.; Lester, W. S.; Liu, N.; Yuan, J.; Duo, J.; Herzon, S. B.; Nguyen, Y.; Zacharias, D. 2-Azabicyclo[2.1.1]hexanes. 2. Substituent Effects on the Bromine-Mediated Rearrangement of 2-Azabicyclo[2.2.0]hex-5-enes. *J. Org. Chem.* **2001**, *66*, 1805–1810.
- (14) Krow, G. R.; Lee, Y. B.; Lester, W. S.; Christian, H.; Shaw, D. A.; Yuan, J. A Novel Synthesis of 2-Azabicyclo[2.1.1]hexane from Pyridine. *J. Org. Chem.* **1998**, *63*, 8558–8560.
- (15) Krow, G. R.; Lester, W. S.; Liu, N.; Yuan, J.; Hiller, A.; Duo, J.; Herzon, S. B.; Nguyen, Y.; Cannon, K. Synthesis of Novel 2-Azabicyclo[2.2.0]- and [2.1.1]hexanols. *J. Org. Chem.* **2001**, *66*, 1811–1817.
- (16) Krow, G. R.; Yuan, J.; Lin, G.; Sonnet, P. E. The Rearrangement Route to 3-CH<sub>2</sub>X-2-azabicyclo[2.1.1]hexanes. Substituent Control of Neighboring Group Participation. *Org. Lett.* **2002**, *4*, 1259–1262.
- (17) Krow, G. R.; Lester, W. S.; Lin, G.; Fang, Y. Chlorosulfonyl Isocyanate Reactions with *N*-(Alkoxy-carbonyl)-2-azabicyclo[2.2.0]hex-5-enes. Regiospecific Two-Atom Insertion Pathways. *J. Org. Chem.* **2003**, *68*, 1626–1629.
- (18) Krow, G. R.; Lin, G.; Rapolu, D.; Fang, Y.; Lester, W. S.; Herzon, S. B.; Sonnet, P. E. The Rearrangement Route to 2-Azabicyclo[2.1.1]hexanes. Solvent and Electrophile Control of Neighboring Group Participation. *J. Org. Chem.* **2003**, *68*, 5292–5299.
- (19) Krow, G. R.; Lin, G.; Fang, Y.; Sonnet, P. E. Second-Chance Rearrangement Route to Novel 5(6)-syn,anti-Difunctional 2-Azabicyclo[2.1.1]hexanes. *Org. Lett.* **2003**, *5*, 2739–2741.
- (20) Krow, G. R.; Lin, G.; Moore, K. P.; Thomas, A. M.; Debrosse, C.; Ross, C. W.; Ramjit, H. G. Novel Selectfluor and Deoxo-Fluor-Mediated Rearrangements. New 5(6)-Methyl and Phenyl Methanopyrrolidine Alcohols and Fluorides. *Org. Lett.* **2004**, *6*, 1669–1672.
- (21) Davies, J. W.; Malpass, J. R.; Walker, M. P. Formal Dyotropic Rearrangements of *N*-Chloroamines Catalysed by Alumina. *J. Chem. Soc., Chem. Commun.* **1985**, 686–687.
- (22) Pirrung, M. C. Total Synthesis of 2,4-Methanoproline. *Tetrahedron Lett.* **1980**, *21*, 4577–4578.
- (23) Clardy, J.; Hughes, P.; Martin, M. Synthesis of 2,4-Methanoproline. *Tetrahedron Lett.* **1980**, *21*, 4579–4580.
- (24) Clardy, J.; Hughes, P. Total Synthesis of Cyclobutane Amino Acids from *Atelia herbert smithii*. *J. Org. Chem.* **1988**, *53*, 4793–4796.
- (25) Carroll, F. I. Epibatidine Structure–Activity Relationships. *Biol. Med. Chem. Lett.* **2004**, *14*, 1889–1896.
- (26) Carroll, F. I.; Liang, F.; Navarro, H. A.; Brieady, L. E.; Abraham, P.; Damaj, M. I.; Martin, B. R. Synthesis, Nicotinic Acetylcholine Receptor Binding, and Antinociceptive Properties of 2-*exo*-2-(2'-Substituted 5'-pyridinyl)-7-azabicyclo[2.2.1]heptanes. Epibatidine Analogues. *J. Med. Chem.* **2001**, *44*, 2229–2237.
- (27) Carroll, F. I.; Lee, J. R.; Navarro, H. A.; Brieady, L. E.; Abraham, P.; Damaj, M. I.; Martin, B. R. Synthesis, Nicotinic Acetylcholine Receptor Binding, and Antinociceptive Properties of 2-*exo*-2-(2'-Substituted 3'-phenyl-5'-pyridinyl)-7-azabicyclo[2.2.1]heptanes. Novel Nicotinic Antagonist. *J. Med. Chem.* **2001**, *44*, 4039–4041.
- (28) Carroll, F. I.; Ma, W.; Yokota, Y.; Lee, J. R.; Brieady, L. E.; Navarro, H. A.; Damaj, M. I.; Martin, B. R. Synthesis, Nicotinic Acetylcholine Receptor Binding, and Antinociceptive Properties of 3'-Substituted Deschloroepibatidine Analogues. Novel Nicotinic Antagonists. *J. Med. Chem.* **2005**, *48*, 1221–1228.
- (29) Spande, T. F.; Garraffo, H. M.; Edwards, M. W.; Yeh, H. J. C.; Pannell, L.; Daly, J. W. Epibatidine: a novel (chloropyridyl) azabicycloheptane with potent analgesic activity from an Ecuadorian Poison Frog. *J. Am. Chem. Soc.* **1992**, *114*, 3475–3478.
- (30) Dani, J. A.; De Biasi, M.; Liang, Y.; Peterson, J.; Zhang, L.; Zhang, T.; Zhou, F. M. Potential Applications of Nicotinic Ligands in the Laboratory and Clinic. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1837–1839.
- (31) Astles, P. C.; Baker, S. R.; Boot, J. R.; Broad, L. M.; Dell, C. P.; Keenan, M. Recent Progress in the Development of Subtype Selective Nicotinic Acetylcholine Receptor Ligands. *Curr. Drug Targets CNS Neurol. Disord.* **2002**, *4*, 337–348.
- (32) Decker, M. W.; Rueter, L. E.; Bitner, R. S. Nicotinic Acetylcholine Receptor Agonists: A Potential New Class of Analgesics. *Curr. Top. Med. Chem.* **2004**, *4*, 369–384.
- (33) Carreras, J.; Avenoza, A.; Busto, J. H.; Peregrina, J. M. Synthesis of Azabicyclo[2.2*n*]alkane Systems as Analogues of 3-[1-Methyl-2-(*S*)-pyrrolidinyl-methoxy]pyridine (A-84543). *J. Org. Chem.* **2007**, *3112*–3115.
- (34) Abreo, M. A.; Lin, N. H.; Garvey, D. S.; Gunn, D. E.; Hettinger, A. M.; Wasicak, J. T.; Pavlik, P. A.; Martin, Y. C.; Donnelly-Roberts, D. L.; erson, D. J.; Sullivan, J. P.; Williams, M.; Arneric, S. P.; Holladay, M. W. Novel 3-Pyridyl Ethers with Subnanomolar Affinity for Central Neuronal Nicotinic Acetylcholine Receptors. *J. Med. Chem.* **1996**, *39*, 817–825.
- (35) Jain, K. K. Modulators of Nicotinic Acetylcholine Receptors as Analgesics. *Curr. Opin. Investig. Drugs* **2004**, *5*, 76–81.
- (36) Cheng, J.; Izenwasser, S.; Wade, D.; Trudell, M. L. Synthesis and Nicotinic Acetylcholine Receptor Binding Affinity of *exo*- and *endo*-2-(pyridinylloxymethyl)-7-azabicyclo[2.2.1]heptanes. *Med. Chem. Res.* **2001**, *10*, 356–365.
- (37) Krow, G. R.; Yuan, J.; Fang, Y.; Meyer, M. D.; Anderson, D. J.; Campbell, J. E.; Carroll, P. J. Synthesis of 3- and 5-*endo*-(6-Chloro-3-pyridoxy)-methyl-2-azabicyclo[2.2.0]hexane and 3-*endo*-(6-Chloro-3-pyridoxy)-methyl-2-azabicyclo[2.2.0]hex-5-ene. ABT-594 Analogs. *Tetrahedron* **2000**, *56*, 9227–9232.
- (38) Holladay, M. W.; Dart, M. J.; Lynch, J. K. Neuronal Nicotinic Acetylcholine Receptors as Targets for Drug Discovery. *J. Med. Chem.* **1997**, *40*, 4169–4194.
- (39) Holladay, M. W.; Bai, H.; Li, Y.; Lin, N. H.; Daanen, J. F.; Ryther, K. B.; Wasicak, J. T.; Kincaid, J. F.; He, Y.; Hettinger, A. M.; Huang, P.; erson, D. J.; Bannon, A. W.; Buckley, M. J.; Campbell, J. E.; Roberts, D. L. D.; Gunther, K. L.; Kim, D. J. B.; Kuntzweiler, T. A.; Sullivan, J. P.; Decker, M. W.; Arneric, P. A. Structure–Activity Studies Related to ABT-594, a Potent Nonopioid Analgesic Agent: Effect of Pyridine and Azetidine Ring Substitutions on Nicotinic Acetylcholine Receptor Binding Affinity and Analgesic Activity in Mice. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2797–2802.
- (40) Badio, B.; Garraffo, H. M.; Plummer, C. V.; Padgett, W. L.; Daly, J. W. Synthesis and Nicotinic Activity of Epiboxidine: An Isoxazole Analogue of Epibatidine. *Eur. J. Pharmacol.* **1997**, *321*, 189–194.
- (41) Fitch, R. W.; Pei, X.-F.; Kaneko, Y.; Gupta, T.; Shi, D.; Federova, I.; Daly, J. W. Homoeipiboxindines: Further Potent Agonists for Nicotinic Receptors. *Bioorg. Med. Chem.* **2004**, *12*, 179–190.
- (42) Cheng, J.; Izenwasser, S.; Zhang, C.; Zhang, S.; Wade, D.; Trudell, M. L. Synthesis and Nicotinic Acetylcholine Receptor Binding Affinities of 2- and 3-Isoxazolyl-8-azabicyclo[3.2.1]octanes. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1775–1778.
- (43) Garvey, D. S.; Wasicak, J. T.; Decker, M. W.; Brioni, J. D.; Buckely, M. J.; Sullivan, J. P.; Carrera, G. M.; Holladay, M. W.; Arneric, S. P.; Williams, M. Novel Isoxazoles which Interact with Brain Cholinergic Channel Receptors Have Intrinsic Cognitive Enhancing and Anxiolytic Activities. *J. Med. Chem.* **1994**, *37*, 1055–1059.
- (44) Elliot, R. L.; Kopecka, H.; Lin, N.; He, Y.; Garvey, D. S. A Short, Efficient Synthesis of the Novel Cholinergic Channel Activator, ABT 418, from L-Proline. *Synthesis* **1995**, 772–774.
- (45) Avenoza, A.; Busto, J. H.; Cativiela, C.; Peregrina, J. M. Synthesis of 7-Azabicyclo[2.2.1]heptane Derivatives via Bridgehead Radicals. *Tetrahedron* **2002**, *58*, 1193–1197.
- (46) Silva, N. M.; Tributino, J. L. M.; Miranda, A. L. P.; Barreiro, E. J.; Fraga, C. A. M. New Isoxazole Derivatives Designed as Nicotinic Acetylcholine Receptor Ligand Candidates. *Eur. J. Med. Chem.* **2002**, *37*, 163–170.
- (47) Paquette, L. A. *Organic Reactions*, Volume 42; John Wiley & Sons: New York, 1992; pp 1–692.
- (48) Grison, C.; Geneve, S.; Coutrot, P. Enantioselective Synthesis of  $\alpha,\beta$ -Unsaturated  $\gamma$ - and  $\delta$ -Lactams. *Tetrahedron Lett.* **2001**, *42*, 3831–3834.
- (49) Kao, C.; Chern, J. A Novel Strategy for the Synthesis of Benzofuran Skeleton Neolignans: Application to Ailanthoidol, XH-14, and Obovatene. *J. Org. Chem.* **2002**, *67*, 6772–6787.
- (50) Sato, H.; Sakoh, H.; Hashihayata, T.; Imamura, H.; Ohtake, N.; Shimizu, A.; Sugimoto, Y.; Sakuraba, S.; Bamba, R.; Yamada, K.; Hashizume, T.; Morishima, H. Structure–Activity Relationships of 1 $\beta$ -Methyl-2-(5-phenylpyrrolidin-3-ylthio)carbapenems. *Bioorg. Med. Chem.* **2002**, *10*, 1595–1610.
- (51) Piotrowski, D. W. A Concise Route to Novel 1-Aryl and 1-Pyridyl-2-azabicyclo[2.1.1]hexanes. *Synlett.* **1999**, *22*, 1091–1093.
- (52) Cheng, Y.; Prusoff, W. H. Relationship Between the Inhibition Constant ( $[K_i]$ ) and the Concentration of Inhibitor which causes 50% Inhibition ( $[IC_{50}]$ ) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.