Azaspirovesamicols—Regioselective Synthesis and Crystal Structure Analysis of a Novel Class of Vesamicol Analogues as Potential Ligands for the Vesicular Acetylcholine Transporter

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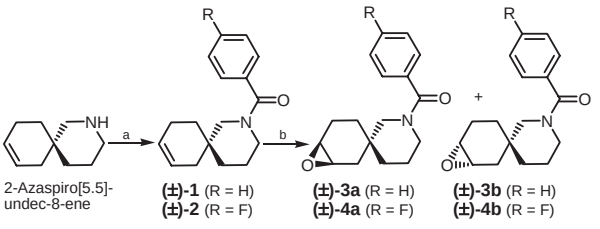
This report describes the high regioselectivity of nucleophilic epoxide ring-opening reactions which resulted in two of four possible regioisomers of *N*-benzoyl- (**5a** and **5b**) and *N*-fluorobenzoylazaspirovesamicol derivatives (**6a** and **6b**), respectively. Based on structural information obtained from X-ray crystal structure analyses of **5a** and **5b** the mode of epoxide ring-opening is discussed.

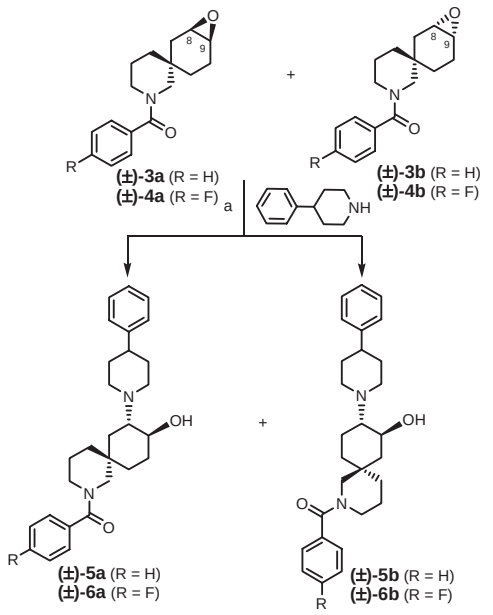
The drug vesamicol [2-(4-phenylpiperidin-1-yl)cyclohexanol] binds with high affinity to an allosteric binding site of the vesicular acetylcholine transporter (VAChT).[1] Suitable radioligands for the VAChT provide an opportunity to visualize cholinergic deficits in brain using PET (positron emission tomography), which is of importance for the diagnosis of neurodegenerative disorders. Since the VAChT only tolerates vesamicol-like structures,[2] all known ligands are based on the vesamicol skeleton. However, for many of them a low selectivity or other causes prevented their clinical application. Therefore, there is a need for further evaluation of VAChT ligands with improved properties. In this study, we report the synthesis and structures of azaspirovesamicols, a novel class of vesamicol analogues. Radiolabeled with ^{18}F, these compounds could be potential PET radioligands for the VAChT.

The four new vesamicol analogues **5a**–**6b** and their required epoxide precursors **3a**–**4b** were synthesized as outlined in Schemes 1 and 2.[11]

2-Azaspiro[5,5]undec-8-ene was prepared according to the synthetic route described by Liebowitz et al.[3] Subsequent benzoylation and fluorobenzoylation, respectively, yielded in **1** and **2**. These amides were epoxidized with ethyl chloroformate and hydrogen peroxide to give a mixture of *syn*/*anti* epoxides **3a** and **3b**[4] and **4a** and **4b**. Using this epoxidation method, the isomers were formed at an averaged ratio of 65:35 (*anti*-**3a**:*syn*-**3b** and *anti*-**4a**:*syn*-**4b**, resp.) as determined by HPLC.[11]



Scheme 1. Synthesis of *syn*/*anti* epoxide precursors **3a**–**4b**. (a) Benzoyl and 4-fluorobenzoyl chloride, resp./$NaHCO_3$, (b) H_2O_2/ethyl chloroformate/Na_2HPO_4.



Scheme 2. Synthesis of azaspirovesamicols **5a**–**6b**. (a) Ethanol at 75 °C for 5 days.

Because of their similar R_f values, they could not be separated via flash chromatography on silica gel. The synthesis of **5a** and **5b** was accomplished by nucleophilic ring-opening reaction of the epoxide mixture **3a**/**3b** with 4-phenylpiperidine in ethanol at 75 °C.

In theory, the nucleophilic attack of an amine can proceed on the two positions, C8 and C9, of *syn*/*anti* epoxide and finally should result in the formation of four isomers. However, only two compounds, **5a** and **5b**, were obtained in an averaged ratio of 66:34 (**5a**:**5b**). These two isomers could be separated in a moderate yield via fractionated crystallization from an ethanolic solution. Therefore, preparative HPLC separation of **5a** and **5b** as well as of the epoxide precursors **3a** and **3b** was unnecessary.

Precise determination of molecular structures of **5a** and **5b** was accomplished by X-ray structure analysis (Figure 2).[5] On the basis of the structural information it is possible to disclose the mode of epoxide ring-opening. Nucleophilic attack of 4-phenylpiperidine on C8 of the *anti* epoxide **3a** leads to the regioisomer **5a**. In contrast, regioisomer **5b** was formed by nucleophilic attack on C9 of the *syn* epoxide **3b**, exclusively. Therefore, we can conclude that the formation of these two isomers is strongly favored. Furthermore, we observed a correlation of the ratios of the regioisomers **5a**:**5b** (66:34) and the precursors

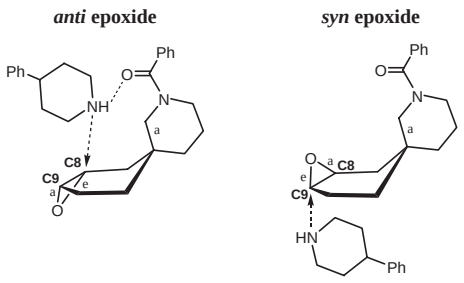


Figure 1.

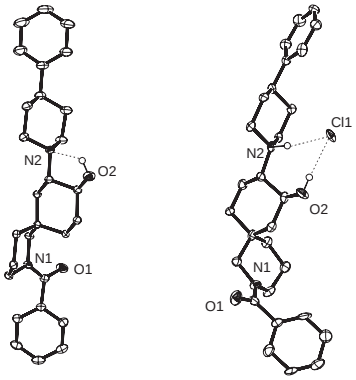


Figure 2. ORTEP drawings of the molecular structures of **5a** (left) and **5b** (right). Hydrogen atoms other than O–H and N–H have been omitted for clarity.

3a:**3b** (65:35). Hence, under reaction conditions described above, the amine does not attack preferred the *anti* or *syn* epoxide. As expected, reaction of 4-fluorobenzoyl substituted *syn*/*anti* epoxides **4a** and **4b** (*anti*-**4a**:*syn*-**4b** = 65:35) with 4-phenylpiperidine also resulted in the formation of two isomers in an averaged ratio of 63:37 (**6a**:**6b**).

The high regiocontrol of epoxide ring-opening is remarkable and was also observed for the comparable spiro[1,3]-dioxalane-2,3′-[7]oxabicyclo[4,1,0]-heptane reported by Cheng et al.[6] and Matzanke et al.[7] However, the authors did not discuss possible reasons for this selectivity. On closer examination of the three-dimensional structure of one of the *anti* epoxide conformers and supposing a trans diaxial transition state,[8] the following explanation seems to be plausible: By formation of hydrogen bonds between the benzoyl oxygen and the proton of the amine, the nitrogen is sterically closer to C8 than to C9 (Figure 1). This could also apply for the dioxalane compounds mentioned above, but not for the *syn* epoxide.

Due to the syn orientation, the benzoyl oxygen and the epoxide oxygen are located on the same side of the molecule, hence the amine has to converge from the other side. In this case, we assume a conformer in which the nitrogen atom of the aza ring is arranged in the unexpected axial position again, because the nucleophilic attack has to proceed on C9 in order to accomplish a trans diaxial transition state. However, we could not find an explanation for the preferred existence of this conformer. If we consider a diequatorial cleavage, the three-dimensional structure of the correspondingly conformer shows clearly, that sterical factors do not exist, which would force the unfavored diequatorial attack on C9 (often described in literature).[8,9] To gain deeper insight into these reaction mechanisms, detailed computational investigations as well as additional synthetic work are in process.

The X-ray crystal structures[5] of **5a** and **5b** are shown in Figure 2. All saturated six-membered rings have chair conformations. Both the hydroxy and the 4-phenylpiperidine substituents are in equatorial positions with respect to the central cyclohexane ring. Compound **5a** shows an intramolecular O-H···N hydrogen bond between the hydroxy group and the amine nitrogen atom. Both the hydroxy group and the amine N–H group of **5b** are hydrogen bonded to a chloride anion. The crystal structure of **5b** has two independent molecules. These molecules only differ in the relative orientation of the phenyl group attached to the piperidine ring.

In conclusion, we have started the development of a novel class of vesamicol analogues as ligands for the vesicular acetylcholine transporter. Four azaspirovesamicol derivatives (**5a**–**6b**) were synthesized by reaction of *syn*/*anti* epoxides (**3a**–**4b**) with 4-phenylpiperidine. These nucleophilic ring-opening reactions were found to proceed in a highly regioselective manner. The molecular structures of the regioisomers **5a** and **5b** were determined by X-ray structure analysis. The next step in this project will be to synthesize further azaspirovesamicol derivatives bearing different fluoro-substituted groups, in respect of future ^{18}F labeling. Furthermore, the binding affinity and selectivity to the VAChT of the described compounds will be determined.

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5 The structures were determined by direct methods and refined by least-squares against all measured F^2 values.[10] **5a**: $C_{28}H_{36}N_2O_2$, M_r = 432.59, triclinic, $P\bar{1}$ (no. 2), a = 6.1634(9), b = 9.970(3), c = 19.717(3) Å, α = 86.432(12), β = 84.113(11), γ = 76.328(19)°, V = 1170.2(4) Å^3, Z = 2, T = 149 K, D_{calcd} = 1.228 g cm^{-3}, μ = 0.077 mm^{-1}, 15658 refections measured, 6911 unique reflections, R_{int} = 0.024, 294 refined parameters, $R_1(F)$ [$I > 2\sigma(I)$] = 0.043, GOF = 1.05; **5b**: $C_{28}H_{37}N_2O_2^+$ Cl^-, M_r = 469.05, orthorhombic, $Pca2_1$ (no. 29), a = 14.439(4), b = 8.8908(18), c = 39.195(8) Å, V = 5032(2) Å^3, Z = 8, T = 157 K, D_{calcd} = 1.238 g cm^{-3}, μ = 0.179 mm^{-1}, 43010 measured reflections, 11251 unique reflections, R_{int} = 0.140, 525 refined parameters, $R_1(F)$ [$I > 2\sigma(I)$] = 0.082, GOF = 1.05. Crystallographic data reported in this manuscript have been deposited with Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 624382 (**5a**) and CCDC 624383 (**5b**). Copies of the data can be obtained free of charge via www.ccdc.cam.ac.uk./conts/retrieving.html.
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