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MOSHER'S AMIDE-BASED ASSIGNMENT OF THE ABSOLUTE CONFIGURATION OF PHANTASMIDINE

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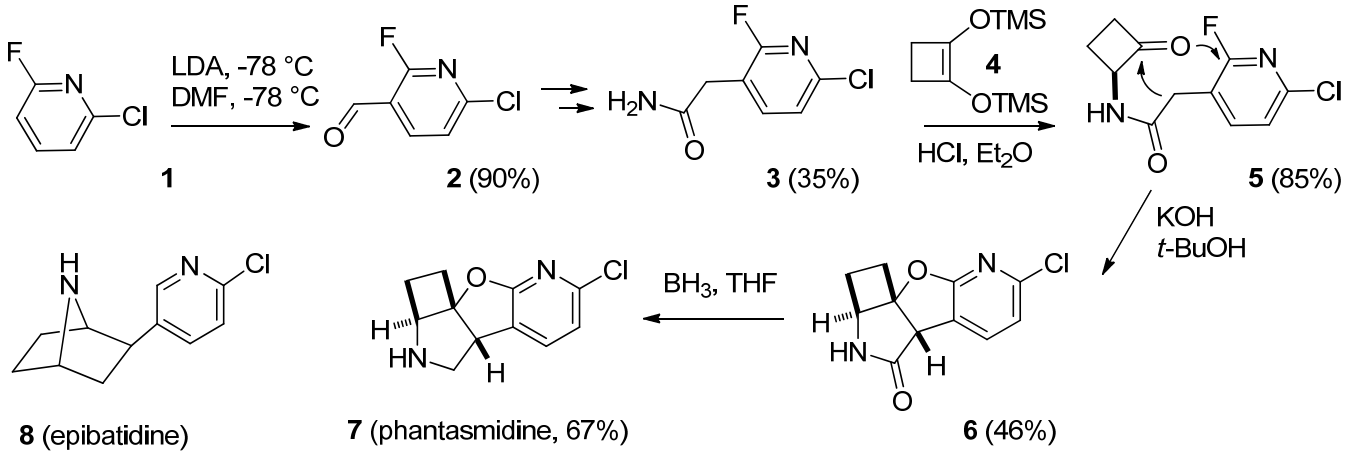
Abstract – The absolute configuration of phantasmidine was assigned by analysis of the two conformers of the two Mosher amides. The expected shielding of the protons adjacent to the nitrogen and large long range shielding (~1 ppm) of the cyclobutane methylene protons in **16major** and the pyridine protons of **16minor** by the phenyl group of the Mosher amide allows the assignment of absolute configuration as shown in Schemes 4 and 5.

Dedicated to Professor Victor Snieckus on the occasion of his 77th birthday

In 1992 Daly isolated the much studied epibatidine (**8**) from the skin of the Ecuadorian poison frog *Epipedobates anthonyi*.[1] In 2010 Fitch, Daly and co-workers isolated the minor, rigid, tetracyclic congener phantasmidine (**7**) from the same frog.[2] The structure of phantasmidine was tentatively assigned spectroscopically from HPLC-purified **7** and the derived acetamide **17** with a total sample size of only <20 μg. We recently reported a short and efficient synthesis of racemic phantasmidine (**7**) that confirmed the structure assignment.[3] Deprotonation of 2-fluoro-6-chloropyridine (**1**) at the 3-position with LDA and trapping of the resulting carbanion with DMF provided **2**, which was elaborated to primary amide **3**. Coupling of primary amide **3** with protected acyloin **4** gave racemic secondary amide **5**, which was treated with potassium hydroxide in *t*-butanol to effect an intramolecular aldol reaction and nucleophilic aromatic displacement to afford tetracyclic pyrrolidinone **6**, which was reduced with borane to give racemic phantasmidine (**7**) in 8 steps and 8% overall yield (see Scheme 1).

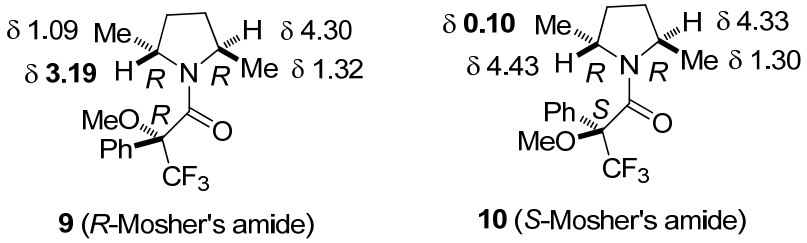
The coupling of amide **3** with **4** gave racemic **5**, making this route unsuitable for the synthesis of enantiomerically pure phantasmidine. Fortunately, the two enantiomers were very readily separated by chiral HPLC on a Chiralcel OJ-H column with retention times of 29 and 44 minutes, making both enantiomers readily available for biological investigation.[3] The optical rotation of the faster eluting enantiomer (+)-phantasmidine is $[\alpha]^{25}_{D}$ +70 (c 0.17, CH_2Cl_2) and that of the slower eluting enantiomer

(-)-phantasmidine is $[\alpha]^{25}_{D}$ -77 (c 0.17, CH_2Cl_2). The two enantiomers of epibatidine (**8**) have very similar shapes and very similar biological activity.[1] We expected that there would be a significant difference in the biological activity of the two enantiomers of the much more rigid tetracyclic congener phantasmidine making it important to assign the absolute configuration, which we report here.



Scheme 1. Synthesis of (±)-phantasmidine (**7**)

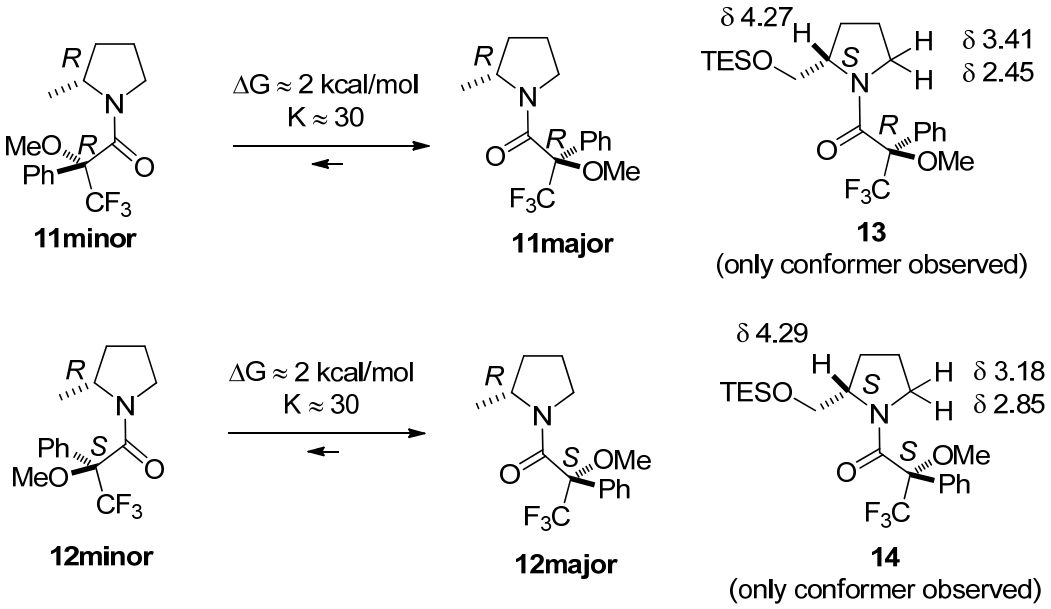
Although (±)-phantasmidine is highly crystalline, we were unable to obtain suitable crystals of the pure enantiomers obtained by chiral HPLC. Initial attempts at resolving larger quantities and forming a crystalline salt suitable for X-ray crystallography by addition of 0.5 equivalent of various enantiomerically pure acids to racemic phantasmidine were also unsuccessful. We then turned our attention to the use of Mosher's amides as developed by Hoye, who prepared the diastereomeric Mosher's amides **9** and **10** from (2*R*,5*R*)-2,5-dimethylpyrrolidine and showed that the ^{1}H NMR spectra are remarkably different as indicated in Scheme 2.[4-6] Conformational studies indicated that the predominant conformer has the trifluoromethyl group syn to the carbonyl group as shown. The assignment was facilitated by the C_2-symmetry of (2*R*,5*R*)-2,5-dimethylpyrrolidine which results in only a single amide conformer. One methyl group, presumably that adjacent to the carbonyl group absorbs at δ 1.30 and δ 1.32 in the two diastereomers. As expected, the methyl group shielded by the phenyl group in **10** (δ 0.10) is shifted upfield by 0.99 ppm from the methyl group adjacent to the methoxy group in **9** (δ 1.09). Similarly, the methine



Scheme 2. NMR absorptions for the Mosher's amides of (2*R*,5*R*)-2,5-dimethylpyrrolidine

hydrogen in **9** shielded by the phenyl group (δ 3.19) is shifted upfield by 1.24 ppm from the methine hydrogen adjacent to the methoxy group in **10** (δ 4.43).

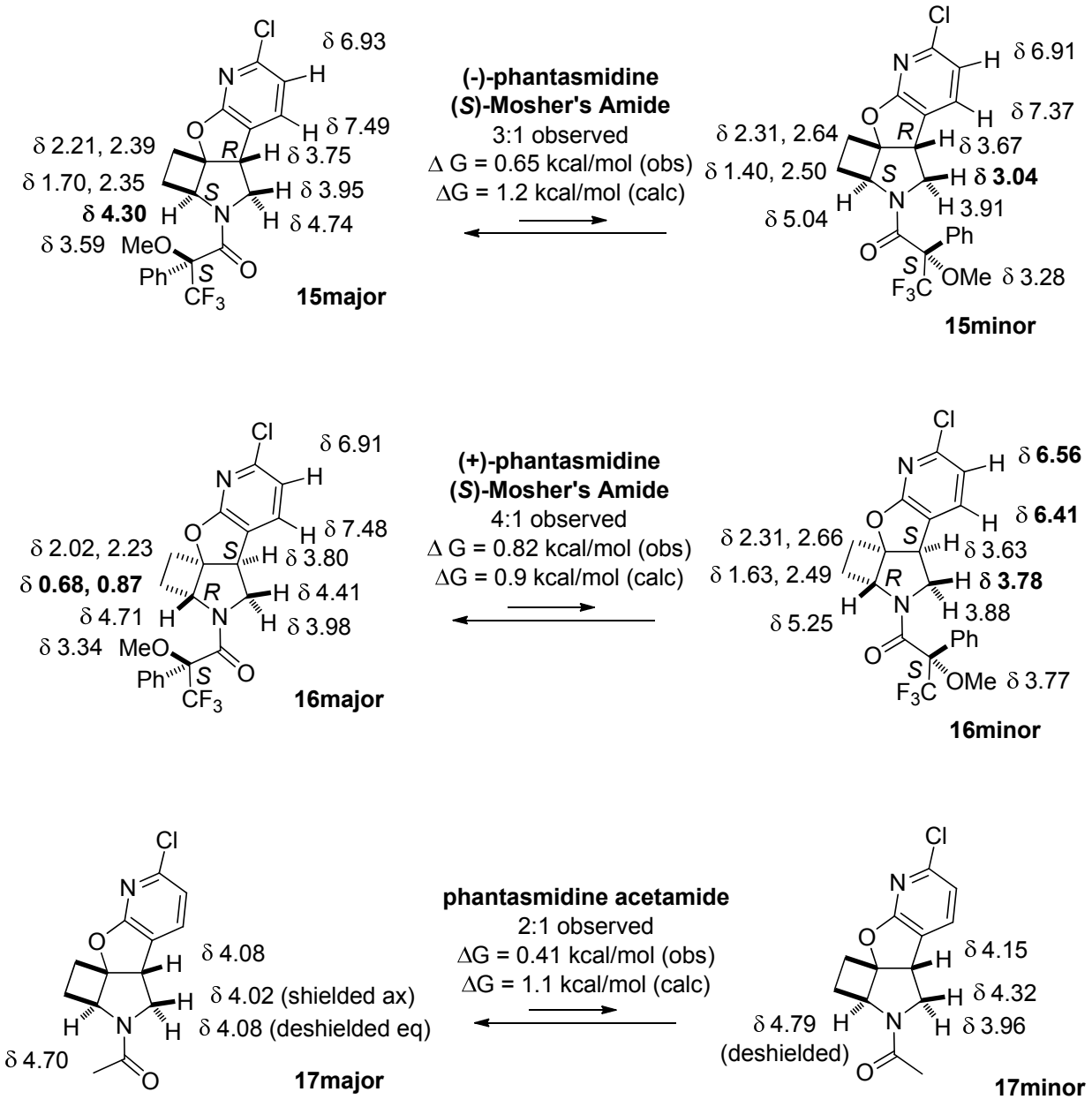
We had some concerns regarding the application of this procedure to phantasmidine. (2*R*,5*R*)-2,5-Dimethylpyrrolidine is C_2-symmetric so that there is only one amide conformer of **9** and **10**. The Mosher's amides of phantasmidine **15** and **16** will exist in two amide conformers on the NMR time scale, as does *N*-acetylphantasmidine (**17**),[2] making the assignment of absolute configuration more difficult. MMX calculations by PCMODEL 8.0[7] indicated that the major amide conformers **11major** and **12major** of the unsymmetrical (*R*)-2-methylpyrrolidine (*R*)-Mosher's amides **11** and (*S*)-Mosher's amide **12** with the large Ph(OMe)CF_3C group adjacent to the smaller methylene carbon are about 2 kcal/mol more stable than the minor amide conformers **11minor** and **12minor** with the large Ph(OMe)CF_3C group adjacent to the large methyl-substituted methine carbon (see Scheme 3). As expected, only one conformer corresponding to **11major** and **12major** was observed by ^{1}H NMR spectroscopy for both diastereomers of the closely related triethylsilyloxymethylpyrrolidine Mosher's amides **13** and **14**.[4c] The methine protons of **13** and **14** on C-2 have almost identical chemical shifts (Δδ = 0.02) whereas the methylene protons shifted significantly (Δδ = 0.23 and -0.41). This suggested that in both cases the carbonyl group is adjacent to the methyl-substituted methine proton whereas the major shielding factor, the phenyl group, is adjacent to the methylene group. Other 2-alkylpyrrolidine Mosher's amide spectroscopic data support this analysis.[5]



Scheme 3. Conformational analysis and spectra of 2-alkylpyrrolidine Mosher's amides

To our surprise, MMX calculations by PCMODEL 8.0 suggested that the large Ph(OMe)CF_3C group is adjacent to the cyclobutane-substituted methine carbon in the major amide conformers (more stable by 0.9-1.2 kcal/mol) of phantasmidine Mosher's amides **15major** and **16major** rather than the methylene

carbon as in **11major** and **12major** (see Scheme 4). Similar calculations indicate that the methyl group of phantasmidine acetamide (**17**) also prefers to be adjacent to the cyclobutane-substituted methine carbon by 1.1 kcal/mol. These calculations suggest that the methylene group of the cyclobutane fused to the pyrrolidine is smaller than a proton, whereas the C-2 methyl group of a pyrrolidine is obviously much larger than a proton. Apparently, the ~90° bond angles in the cyclobutane move the cyclobutane methylene carbon away from the nitrogen so that it is effectively smaller than a proton.



Scheme 4. Conformational analysis and spectra of phantasmidine Mosher's amides

Acylation of readily available synthetic (±)-phantasmidine (**7**) with (*R*)-Mosher's acid chloride (prepared from the (*S*)-Mosher's acid)[8] gave a mixture of diastereomers **15** and **16**. The ^{1}H NMR spectrum was

complex with two amide conformers for each diastereomer. We were able to assign the peaks for each of the four amide conformers from the COSY spectrum of the mixture. Acylation of 0.5 mg of (-)-phantasmidine and 0.5 mg of (+)-phantasmidine with *R*-Mosher's acid chloride[8] in $CDCl_3$ gave the corresponding (-)-phantasmidine *S*-Mosher's amide **15** and (+)-phantasmidine *S*-Mosher's amide **16**, respectively. The crude products were washed with 5% aq. HCl and used for spectroscopic analysis without further purification. The ^{1}H NMR spectrum of the purified mixture of diastereomers **15** and **16** was used to exclude impurities due to unreacted Mosher's acid or Mosher's acid chloride.

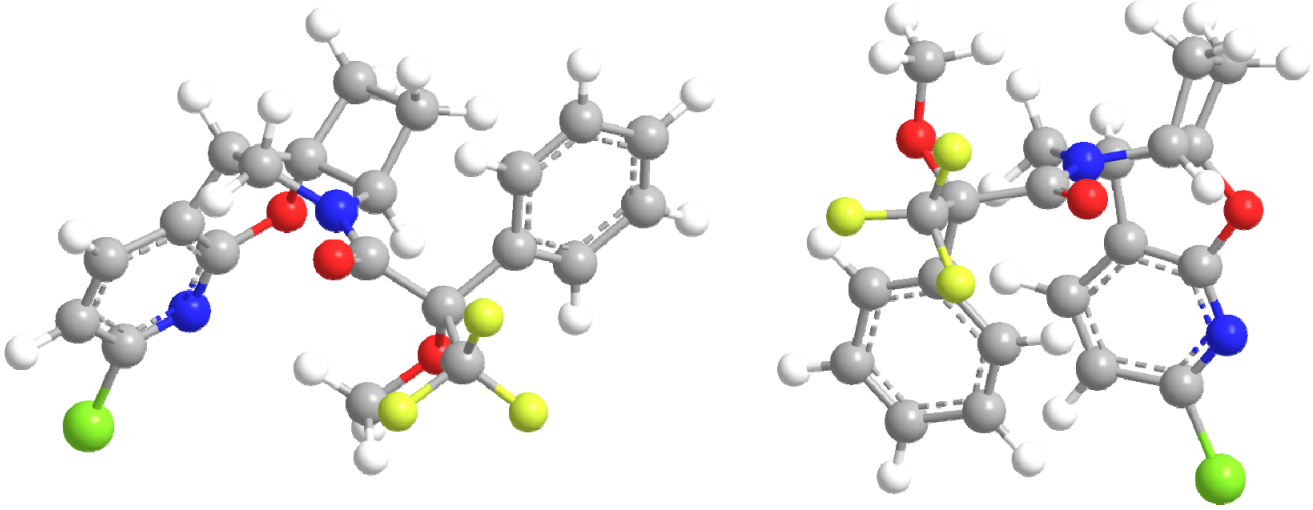
A 3:1 mixture of amide conformers was observed for (-)-phantasmidine *S*-Mosher's amide **15**. The methine hydrogen adjacent to the phenyl group in **15major** (δ 4.30) is shifted upfield by 0.74 ppm from the methine hydrogen in **15minor** (δ 5.04). The methylene hydrogen adjacent to the phenyl group in **15minor** (δ 3.04) is shifted upfield by 0.91 ppm from the methylene hydrogen in **15major** (3.95). These shifts allow us to assign the absolute configuration as shown and indicate that, as calculated, the methylene carbon is larger than the cyclobutane-substituted methine carbon, although the observed ΔG (0.65 kcal/mol) is smaller than the calculated ΔG (1.2 kcal/mol)).

A 4:1 mixture of amide conformers was observed for (+)-phantasmidine *S*-Mosher's amide **16**. The methylene hydrogens of the cyclobutane adjacent to the phenyl group in **16major** (δ 0.68, δ 0.87) are shifted upfield by 0.95 and 1.62 ppm from those in **16minor** (δ 1.63, δ 2.49). The pyrrolidine methylene proton and two aryl protons that are adjacent to the phenyl group in **16minor** (δ 3.78, δ 6.41, and δ 6.56) are also shifted upfield significantly (0.63, 1.07, and 0.35 ppm) from those in **16major** (δ 4.41, δ 7.48, and δ 6.91). These shifts allow us to assign the absolute configuration as shown and indicate that, as calculated, the methylene carbon is larger than the cyclobutane-substituted methine carbon, although the observed ΔG (0.82 kcal/mol) is smaller than the calculated ΔG (0.9 kcal/mol).

The exceptionally large Δδ values established the absolute configuration of (-)-phantasmidine as shown in **15** and (+)-phantasmidine as shown in **16**. The remarkably large shielding of the cyclobutane methylene group of **16major** and the pyridine protons of **16minor** by the Mosher's amide phenyl group is consistent with the MMX-minimized structures shown in Figure. 1. The close agreement of calculations and experiment on the relative amide conformer energies provides further support for this assignment.

Phantasmidine acetamide (**17**) showed a 2:1 mixture of amide conformers in the ^{1}H NMR spectrum whose conformations were assigned from NOE data.[2] The methine proton (δ 4.79) adjacent to the carbonyl group in the minor amide conformer **17minor** is deshielded by the carbonyl group and shifted downfield 0.07 ppm from the methine proton adjacent to the methyl group in the major amide conformer **17major** (δ 4.70). The methylene protons adjacent to the nitrogen were assigned based on the cis vicinal coupling constant (7.0-8.4 Hz, 20-30°) being larger than the trans vicinal coupling constant (3.2 Hz, 95-110°). The

pseudoequatorial methylene hydrogen is in the deshielding cone of the carbonyl group and is shifted downfield to δ 4.08 in the major amide conformer from δ 3.96 in the minor amide conformer. The pseudoaxial methylene hydrogen is in the shielding cone of the carbonyl group and is shifted upfield to δ 4.02 from δ 4.32 in the minor amide conformer. The 2:1 mixture of amide conformers indicates that **17major** is more stable by 0.41 kcal/mol, which is somewhat smaller than the calculated value of 1.1 kcal/mol, but does again indicate that the methylene group is larger than the cyclobutane-substituted methine carbon.

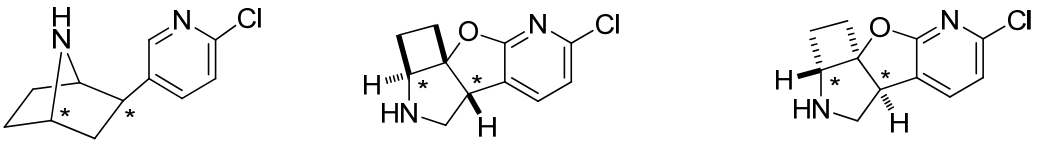


(a) (b)

Figure 1. (a) MMX-minimized structure of **16major** showing shielding (δ 0.68 and 0.87) of the cyclobutane methylene group by the phenyl group. (b) MMX-minimized structure of **16minor** showing shielding (δ 6.41 and 6.56) of the pyridine hydrogens by the phenyl group.

Both enantiomers of epibatidine have comparable biological activity.[1] The absolute configuration of epibatidine was established as shown in **8** by chiral HPLC comparison with synthetic material of known absolute configuration.[9] The biosynthetic pathways to epibatidine and phantasmidine are not known nor is it clear how they are biosynthetically related. The slower eluting (-)-phantasmidine ((-)-**7**) has the same absolute configuration as natural epibatidine at the asterisked carbons (see Scheme 5). We are unable to determine the absolute configuration of natural phantasmidine because a sample is no longer available. Surprisingly, preliminary results indicate that faster eluting (+)-phantasmidine ((+)-**7**) is considerably more biologically active than slower eluting (-)-phantasmidine.[10] We believe that the assignment of the absolute configuration of phantasmidine based on the Mosher's amides is secure because of the magnitude of the chemical shift differences and we were carful to deal with the formal change that occurs when (*S*)-Mosher's acid is converted to the (*R*)-Mosher's chloride and back to the (*S*)-Mosher's amide. This raises several possibilities that can't be distinguished between at this time. Nature could make epibatidine and (+)-phantasmidine with opposite absolute configuration at the asterisked carbons, nature could make the

less biologically active (-)- or racemic phantasmidine, or the absolute configuration of epibatidine could be misassigned.



8 (epibatidine) (-)-**7** ((-)-phantasmidine) (+)-**7** ((+)-phantasmidine)

Scheme 5. Structures of natural epibatidine (**8**), less biologically active (-)-phantasmidine and more biologically active (+)-phantasmidine

In conclusion, the absolute configuration of phantasmidine was assigned by MMX analysis of the two conformations of Mosher amides **15** and **16** and their ^{1}H NMR spectra. The expected shielding of the protons adjacent to the nitrogen and large long range shielding (~1 ppm) of the cyclobutane methylene protons in **16major** and the pyridine protons of **16minor** by the phenyl group of the Mosher amide allows the assignment of absolute configuration as shown in Schemes 4 and 5.

EXPERIMENTAL

Racemic Phantasmidine (*S*)-Mosher's Amide (15 and 16). A mixture of (*S*)-Mosher's acid (8.5 mg, 36 μmol) in 1 mL of hexanes was treated with 2 μL of DMF and then 10 μL (14.8 mg, 116 μmol) of oxalyl chloride at 25 °C. The reaction was kept at 25 °C for 30 min and filtered. The filtrate was concentrated to give crude (*R*)-(-)-Mosher's acid chloride as colorless oil, which was used directly without further purification.

A solution of racemic phantasmidine (**7**, 4 mg, 18 μmol) and diisopropylethylamine (30 μL, 170 μmol) in 0.2 mL of anhydrous CH_2Cl_2 was treated with a solution of the crude (*R*)-(-)-Mosher's acid chloride in 0.5 mL of anhydrous CH_2Cl_2 at 25 °C under N_2. The reaction was stirred at 25 °C for 1 h and quenched with 2 mL of 5% aqueous Na_2CO_3. The resulting mixture was extracted with CH_2Cl_2 (3 mL × 3) and the combined organic extracts were dried ($MgSO_4$) and concentrated. Flash chromatography of the residue on silica gel (CH_2Cl_2 to 100:1 CH_2Cl_2/MeOH) gave 6.1 mg (77%) of a 1:1 mixture of (±)-phantasmidine (*S*)-Mosher's amide diastereomers **15** and **16**. One diastereomer exists as a 3:1 mixture of amide conformers and the other as a 4:1 mixture.

(*S*)-Mosher's Amide of the Slower Eluting (-)-Phantasmidine Enantiomer (15). The identical procedure using ~0.5 mg of (-)-phantasmidine gave a mixture of **15major** and **15minor** whose ^{1}H NMR spectrum showed a 3:1 mixture of amide conformers. The spectral data described below were obtained by analysis of the spectrum of this crude diastereomer and the spectrum of the purified mixture of both

diastereomers described above.

Partial data not including the phenyl protons for **15major**: ^{1}H NMR δ 7.49 (dd, 1, J = 7.6, 1.2), 6.93 (d, 1, J = 7.6), 4.74 (dd, 1, J = 12.8, 1.2), 4.30 (dd, 1, J = 8.4, 6.8), 3.95 (dd, 1, J = 12.8, 7.2), 3.75 (br d, 1, J = 7.2), 3.59 (q, 3, $J_{H,F}$ = 1.8, OMe), 2.42-2.32 (m, 2), 2.21 (br dd, 1, J = 12, 10.4), 1.74-1.66 (m, 1).

Partial data not including the phenyl protons for **15minor**: ^{1}H NMR δ 7.37 (presumed d, 1, assigned from COSY spectrum), 6.91 (d, 1, J = 7.6), 5.04 (dd, 1, J = 7.6, 7.6), 3.91 (dd, 1, J = 12.8, 2,1), 3.67 (br d, 1, J = 7.6), 3.28 (q, 3, $J_{H,F}$ = 1.8, OMe), 3.04 (dd, 1, J = 12.8, 7.6), 2.64 (br ddd, 1, J = 12, 10, 10), 2.54-2.46 (m, 1), 2.35-2.27 (m, 1), 1.44-1.36 (m, 1).

(*S*)-Mosher's Amide of the Faster Eluting (+)-Phantasmidine Enantiomer (16). A solution of (+)-phantasmidine (~0.5 mg, 2.2 μmol) and dry pyridine (1 μL, 12.5 μmol) in anhydrous 100 μL of $CDCl_3$ was treated with (*R*)-(-)-Mosher's acid chloride (6.4 μmol, prepared as described above from the (*S*)-(-)-Mosher's Acid) in 100 μL of $CDCl_3$ at 25 °C under N_2. The reaction was kept at 25 °C for 1 h and diluted with 0.3 mL of dry $CDCl_3$. The entire $CDCl_3$ solution was transferred to an NMR tube for spectroscopic analysis. The ^{1}H NMR spectrum of this diastereomer showed a 4:1 mixture of Mosher's amide conformers **16major** and **16minor**. The spectral data described below were obtained by analysis of the spectrum of this crude diastereomer and the spectrum of the purified mixture of both diastereomers described above.

Partial data not including the phenyl protons for **16major**: ^{1}H NMR δ 7.48 (dd, 1, J = 7.6, 1.2), 6.91 (d, 1, J = 7.6), 4.71 (dd, 1, J = 7.6, 7.2), 4.41 (dd, 1, J = 13.2, 1.7), 3.98 (dd, 1, J = 13.2, 8.0), 3.80 (br d, 1, J = 8.0), 3.34 (q, 3, $J_{H,F}$ =1.8, OMe), 2.23 (ddd, 1, J = 12.8, 11.2, 10.4), 2.02 (ddd, 1, J = 12.8, 9.2, 4), 0.91-0.83 (m, 1), 0.73-0.63 (m, 1).

Partial data not including the phenyl protons for **16minor**: ^{1}H NMR δ 6.56 (d, 1, J = 7.6), 6.41 (dd, 1, J = 7.6, 1.2), 5.25 (dd, 1, J = 7.6, 7.2), 3.88 (dd, 1, J = 12.8, 7.6), 3.78 (br d, 1, J = 12.8), 3.77 (q, 3, $J_{H,F}$ = 1.8, OMe), 3.63 (br d, 1, J = 7.6), 2.66 (ddd, 1, J = 12.8, 11.2, 10.4), 2.54-2.45 (m, 1), 2.35-2.27 (m, 1), 1.67-1.59 (m, 1).

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