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Novel 3-(2-Adamantyl)pyrrolidines with Potent Activity Against Influenza A Virus—Identification of Aminoadamantane Derivatives Bearing Two Pharmacophoric Amine Groups

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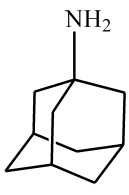
Abstract—The 3-(2-adamantyl)pyrrolidines **8a–g**, **14** were synthesized and evaluated for activity against influenza A virus. The parent N–H compound **14** was several times more active than amantadine against H_2N_2 and H_3N_2 influenza A virus. The combined use of NMR spectroscopy and computational chemistry showed that the conformation around the pyrrolidine–adamantyl carbon–carbon bond is *trans* and the pyrrolidine heterocycle has an envelope conformation with C-2 out of the plane of the other ring atoms. *N*-Dialkylaminoethyl substitution of compound **14** resulted in the potent diamine analogues **8e**,**f**,**g**. Interestingly, their lactam amine precursors were also active. Compounds **8e**,**f**,**g** are the first adamantane derivatives, bearing two amine groups, reported to be active against influenza A virus.

Introduction

Influenza A is a major respiratory tract disease affecting millions of people each year. Influenza A is characterized by the abrupt onset of constitutional and respiratory signs and symptoms (e.g., fever, myalgia, headache, severe malaise, nonproductive cough, sore throat and rhinitis).[1] However, in some persons the infection can cause, for example pulmonary or cardiac disease or lead to secondary bacterial pneumonia or primary viral pneumonia. Epidemics of influenza occur during the winter months nearly every year and are responsible for an average of approximately 20,000 deaths per year in the United States.[2,3] Influenza A viruses have the ability to undergo changes by the mechanisms of antigenic drift and shift and new evolving strains can be a serious threat to the human population.[4] Thus, pandemic



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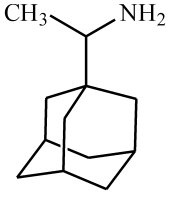

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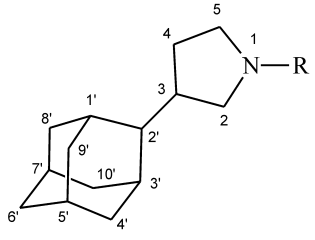

14: R=H
8a: R=CH_3
8b: R=C_2H_5
8c: R=*n*-C_3H_7
8d: R=*n*-C_4H_9
8e: R=$CH_2CH_2NMe_2$
8f: R=$CH_2CH_2NEt_2$
8g: R=CH_2CH_2N 

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influenza A viruses appeared in 1918 ('Spanish' H_1N_1), 1957 ('Asian' H_2N_2) and 1968 ('Hong Kong' H_3N_2). Given that influenza shifts occur every 20–30 years and that a new lethal variant appeared in Hong Kong in 1997, the danger of future influenza A pandemics is real.

Amantadine **1** and rimantadine **2** are anti-influenza A drugs that inhibit virus replication at micromolar concentrations.[5,6] Many aminoadamantanes active against influenza A virus were synthesized in our laboratory during the past 5 years.[6,7] Of these compounds, the most potent ones bear an amino group substitution at the C-2 position of the adamantane nucleus. In order to further examine the stereoelectronic requirements for optimal activity we report here the synthesis of the 3-(2-adamantyl)pyrrolidines **14** and **8a**–**g** and their anti-influenza A virus activity evaluation. The biological activity of the lactam amine precursors of diamines **8e** and **8f** was also examined.

Results and Discussion

Chemistry

The synthetic route followed for the preparation of the novel compounds is illustrated in Scheme 1. *N*-Alkyl 2-pyrrolidinones **4a**–**g** were prepared through *N*-alkylation of 2-pyrrolidinone **3** and used as starting materials for the preparation of the target compounds **8a**–**g**. Alcohols **5a**–**g** were synthesized by the reaction of 2-oxo-3-pyrrolidinyl lithium with 2-adamantanone. Subsequent dehydration and catalytic hydrogenation of the indermediate methylene lactams **6a**–**g** afforded the 3-(2-adamantyl)-2-pyrrolidinones **7a**–**g**. Reduction of lactams **7a**–**g** with $LiAlH_4$ yielded compounds **8a**–**g**.

The parent N–H pyrrolidine **14** was synthesized using the *N*-trimethylsilyl 2-pyrrolidinone **9** as starting material (Scheme 2). Lithiation of lactam **9** and subsequent reaction with 2-adamantanone afforded alcohol **10**. Compound **10** was treated with thionylchloride and ethanolic potassium hydroxide solution to give methylenelactam **12**. Catalytic hydrogenation and subsequent $LiAlH_4$ reduction resulted in the pyrrolidine **14**.[8]

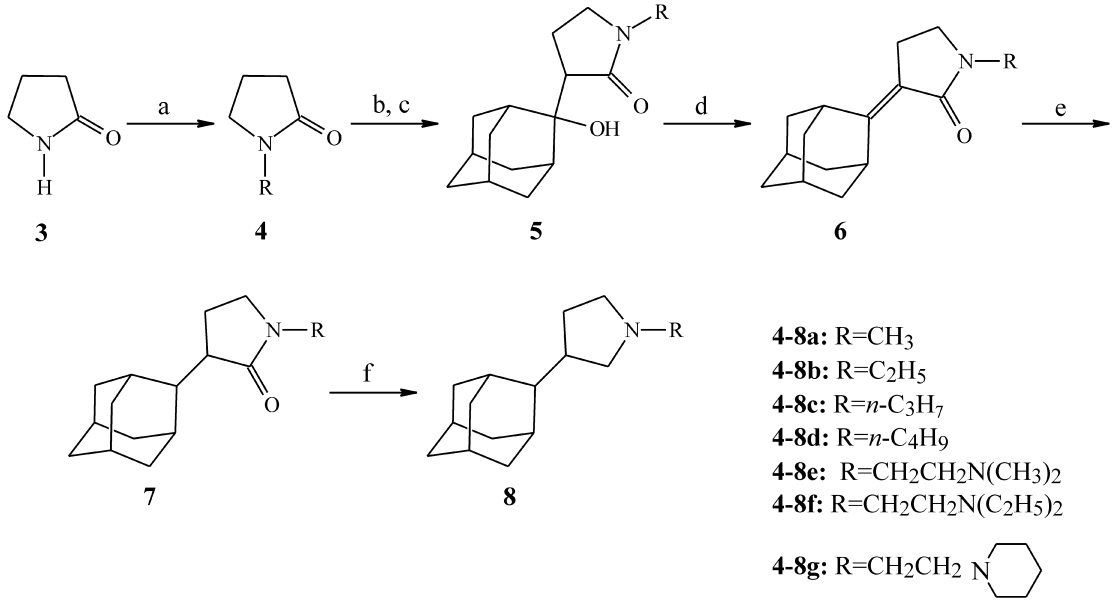
The preparation of the parent pyrrolidine **14** through demethylation of *N*-methyl derivative **8a** failed to succeed. Although the reaction of *N*-methylpyrrolidine **8a** with 2,2,2-trichloroethyl chloroformate gave the corresponding carbamate, treatment of the latter with Zn/AcOH failed to produce the N–H derivative as was expected from literature data.[9] Instead the *N*-methyl derivative **8a** was identified to be the reaction product.

The above described routes can be considered as general for the 3-substitution of γ-lactams and pyrrolidines with cycloalkyl groups.

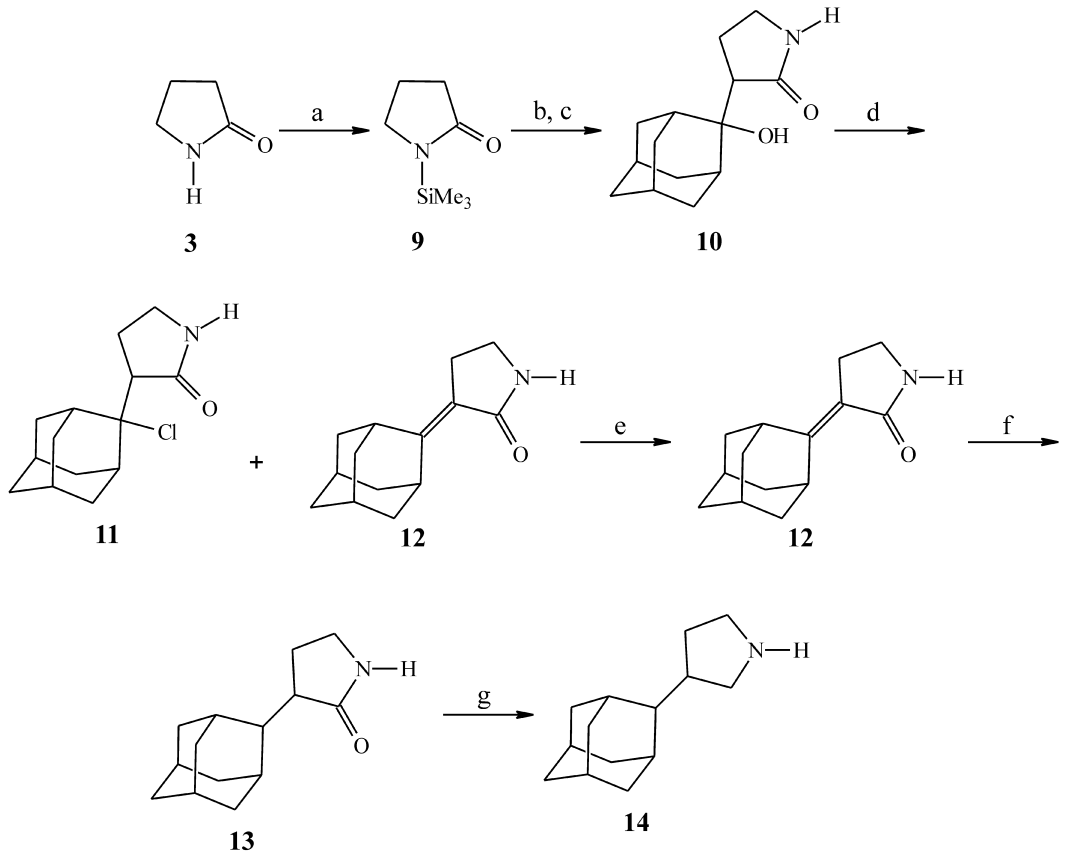
Antiviral activity evaluation

The cytotoxicity and activity of the new aminoadamantane heterocycles **7e**,**f**, **8a**–**g** and **14** were examined against influenza A virus strains H_2N_2 and H_3N_2 according to previously reported methods (Table 1).[10]

From the MIC_{50} and MCC_{50} values presented in Table 1, it appears that, except for compounds **8b**–**d**, all the others were active against influenza virus A strains. Whereas *N*-alkylation of the parent amine **14** caused a dramatic reduction in antiviral activity, *N*-dialkylaminoethyl substitution led to active analogues. The activity of compounds **14**, **8e**,**g** was 2–7 times higher than that of amantadine, while compounds **14** and **8e** had a



Scheme 1. Reagents: (a) NaH, RX, DMF or benzene, 60–70 °C (23–58%); (b) LDA, −70 °C; (c) adamantanone, THF, −80 °C and then H^+/H_2O, 0 °C (73–95%); (d) TsOH, benzene, reflux (79–96%); (e) H_2, PtO_2, ethanol, 40 lb/in^2, rt quant; (f) $LiAlH_4$, THF, 20–25 h reflux (72–89%).



Scheme 2. Reagents: (a) NaH, Me_3SiCl, benzene, rt, 12 h (67%); (b) LDA, −70 °C; (c) adamantanone, THF, −80 °C and then H^+/H_2O, 0 °C (95%); (d) $SOCl_2$, $CHCl_3$, reflux, 3 h; (e) CH_3ONa/CH_3OH, reflux (53% from **10**); (f) H_2, PtO_2, EtOH, 40 lb/in^2 (quant); (g) $LiAlH_4$, THF, 15 h reflux (68%).

Table 1. Anti-influenza A virus activity and cytotoxicity of aminoadamantane derivatives **7e**,**f**, **8a**–**g** and **14**[a] in MDCK cells[b]

Compound	MIC_{50}[c] (μM)		MCC_{50}[d] (μM)
	Influenza virus A H_2N_2[b]	Influenza virus A H_3N_2[b]	
7e	14.7	14.7	122.4
7f	11.1	11.1	92.0
8a	18.8	18.8	≥156.4
8b	>29.7	>29.7	148.2
8c	>28.2	>28.2	140.9
8d	>26.9	>26.9	134.3
8e	0.38	9.4	78.7
8f	8.9	>14.9	74.5
8g	1.7	0.35	14.6
14	0.60	5.0	124.4
Amantadine	2.6	12.8	>533.3
Ribavirin	614.8	122.9	≥1024.6

All data represent mean values for at least two separate experiments.
[a]Aminoadamantanes **7e**,**f** **8a**–**g** and **14** were tested as hydrochlorides or fumarate salts.
[b]Abbreviations and strains used: MDCK, Madin–Darby canine kidney cells, human epithelial cells; influenza A H_2N_2 (A2 Japan/305/57), influenza A H_3N_2 (X31).
[c]Minimum inhibitory concentration or concentration required to reduce virus-induced cytopathogenicity by 50%.
[d]Minimum cytotoxic concentration or concentration required to cause a microscopically detectable alteration of normal cell morphology.

selectivity index ~ 210. In the past, it has been reported that *N*-aminoethyl substitution of amantadine resulted in inactive analogues.[11] Thus, the potency of compounds **8e**,**g** bearing two amine groups is interesting since these compounds are the first examples of active aminoadamantanes bearing two amine groups. The specific activity of compounds **8e**,**g** can be exerted by three pharmocophores, that is adamantane and two amine groups. It is interesting to note that the lactam amines **7e**,**f** were also active anti-influenza A compounds, albeit less potent than the diamines **8e**–**g**.

Conformational analysis

Recently, we have been involved in the investigation of the conformational properties of potent aminoadamantanes with the future goal a deeper understanding of their SAR relationship. In this work a combination of computational chemistry and NMR spectroscopy was used in order to explore the conformational preferences[12] of the parent pyrrolidine **14** in its protonated form.[13]

The molecular dynamics simulation and grid scan search analysis around the C3–C2′ bond revealed many low energy conformers for the protonated form of compound **14**.[13] The conformers obtained differed in the pyrrolidine conformation,[14] adamantyl orientation and C3–C2′ bond conformations. Thus, the pyrrolidine heterocycle can adopt an envelope conformation with any atom out of the plane of the four other atoms, the 2-adamantyl group can be axial or equatorial, while the conformation around the C3–C2′ bond can be *trans* or *gauche*.

However, conformers **A**–**D** with an envelope E(2) pyrrolidine conformation, that is with the C-2 atom out of the plane of the other atoms, are most favored. The conformational descriptors of these conformers are summarized in Table 2. Conformers **A**, **B** and **C** with a 2-adamantyl group in the equatorial position have *trans*, *gauche*-(−) and *gauche*-(+) orientation around the C3–C2′ bond, while in conformer **D**, the 2-adamantyl group is axial and the C3–C2′ bond conformation is *trans* (Fig. 1). The molecular mechanics calculations suggest **A** to be the lowest in energy conformer.[14a] All the remaining conformers are higher in energy by more than 3.3 kcal mol^{-1} (Table 2) and are considered unpopulated at room temperature according to equation $\Delta G^{\circ} = -RT\ln K$.[14b] AM1 and MNDO calculations produce almost completely flat structures and failed to give significant information as has already been pointed out in recent studies on pyrrolidine structure.[14a,15] The E(2) structure of pyrrolidine is consistent with the triplet ($J = 10.4$ Hz) at 2.61 ppm assigned at the H-2 axial proton which must be *trans* to H-3 proton and the NOE correlation between H-2 and H-5 axial protons. Experimental information for the conformation around H3–C3–C2′–H2′ dihedral angle can be extracted from the adamantane H-2′ signal. However, this was not resolved for protonated **14** but appeared as a doublet ($J \sim 10.7$ Hz) at 1.41 ppm in the proton NMR spectrum of the free amine form. Thus, the conformation by rotation around the C3–C2′ bond must be *anti* for compound **14**.

The *anti* conformation by rotation around C3–C2′ bond can be explained on the basis of the more generalized situation found in 1,1,2,2-tetrasubstituted ethanes. The last molecules can adopt two low energy conformations, that is, the *anti*-staggered conformation **E** with two *gauche* interactions and the *gauche*-staggered conformation **F** or its enantiomer with three *gauche* interactions (Fig. 2). In fact, when the four substituents are methyl groups, as in 2,3-dimethylbutane, the two conformations

Table 2. Low energy conformers of parent pyrrolidine **14**. Relative energies (kcal mol^{-1}) were calculated using the MM+ force field[14]

Compound	Conformer	Conformer descriptors			Relative energy
		Conformation around C3–C2′ bond	2–Ad[a] group orientation	Pyrrolidine conformation	
14	**A**	*trans*	eq[b]	E(2)[c]	0.00
	B	*gauche*(−)	eq	E(2)	5.50
	C	*gauche*(+)	eq	E(2)	4.61
	D	*trans*	ax[d]	E(2)	3.32

[a]Ad, adamantyl.
[b]Equatorial.
[c]Envelope with C-2 out of plane.
[d]Axial.

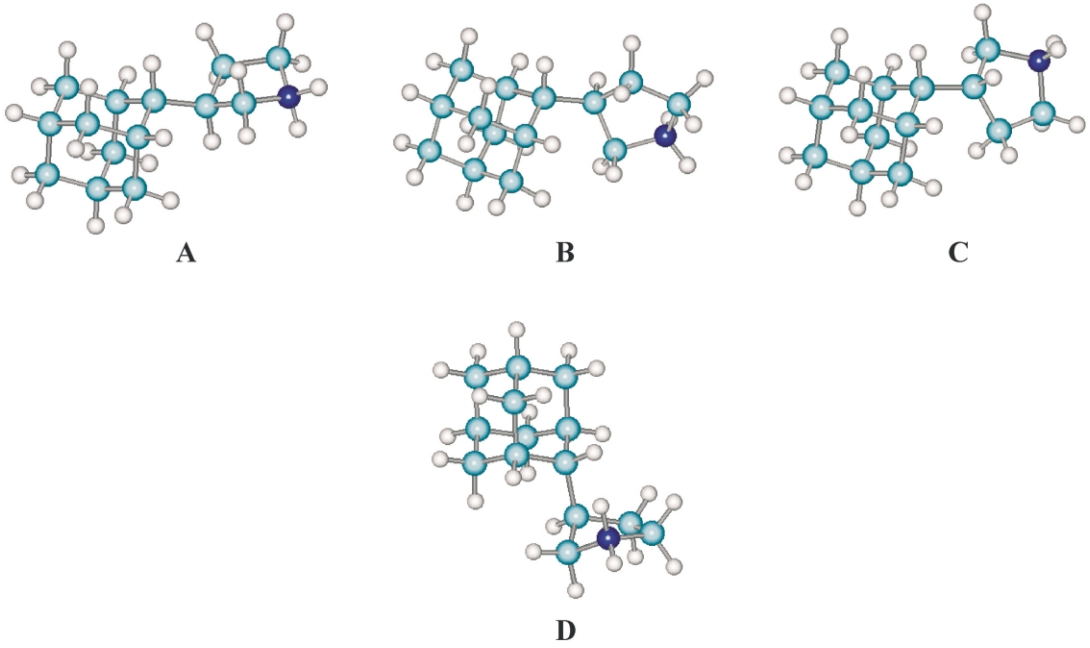


Figure 1. Low energy conformations **A**–**D** of the compound **14** as resulting from molecular mechanics calculations.

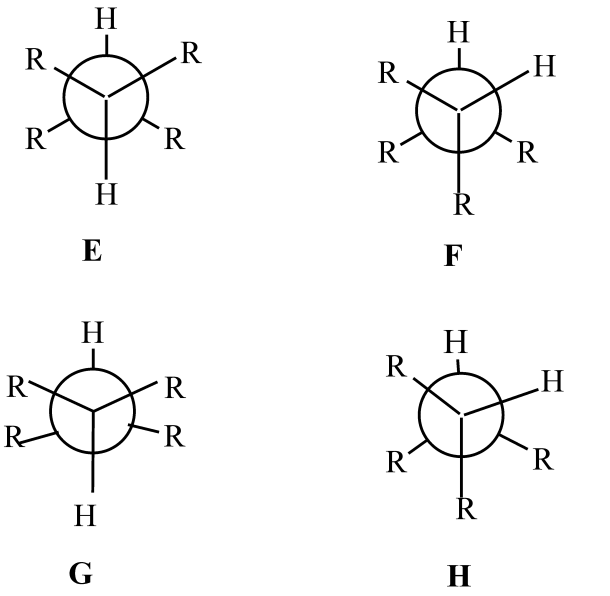


Figure 2. Newman structures to describe the conformational behavior of 1,1,2,2-tetrasubstituted ethanes.

are about equal in energy,[16] whereas for larger substituents, only the *gauche* conformations **F** and enantiomer are populated.[17]

The *anti* conformation **E** is less stable than the *gauche* **F** for large substituents because one of the favored ways of relieving strain at tertiary atoms such as $H–CR^1R^2R^3$ is by opening up of bond angles R–C–R. If this occurs in conformation **E**, *gauche*-substituents are forced nearer each other with no obvious possibility of relief, see structure **G**. If this occurs in conformation **F**, *gauche*-substituents are moved apart somewhat and steric interaction may be further eased by rotation about the central bond, see structure **H**. A contrasting example of a 1,1,2,2-tetrasubstituted bond is provided by the bond from the ring to the 2-adamantyl substituent in compound **14**. As pointed out above, the vicinal coupling constant of 10.4 Hz across this bond shows that it strongly prefers to adopt the *trans* conformation. Here bond angle opening is resisted by the adamantyl structure and the *gauche* conformers, calculated to be more than 4.6 kcal mol^{-1} higher in energy, are unpopulated.

N-Alkyl derivatives of the parent amine have the same pyrrolidine conformation and an equatorial *N*-substituent orientation. It seems that the dramatic decrease of activity observed for *N*-alkyl derivatives can be attributed simply to steric reasons.

Conclusion

This work reports the synthesis of a new series of potent aminoadamantanes. Pyrrolidines **14**, **8a**,**e**,**g** were found active against H_2N_2 and H_3N_2 strains of influenza virus A. Through a combination of NMR spectroscopy and molecular mechanics calculations an *anti* conformation around C3–C2′ rotor and an E(2) envelope conformation were identified for this series of compounds.

The fact that aminoadamantanes with a second amine group, that is, compounds **8e** and **8g**, have potent antiviral activity is unprecedented and surmises the presence of a corresponding binding site in the M2 transmembrane pore. Dialkylaminoethyl substitution of 1-adamantanamine reportedly led to inactive compounds.[11] Yet, *N*-dialkylaminoethyl substitution on 3-(2-adamantyl)pyrrolidines generates potent *anti*-influenza A virus activity.

It was also shown that lactam amine precursors of diamines are less active compounds. These findings suggest that certain stereoelectronic requirements must be fulfilled for aminoadamantanes to exert M2 ion channel blocking activity and demontrate *anti*-influenza A virus potency.[5]

Acknowledgements

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8. The ^1H and ^{13}C NMR spectra of free pyrrolidine **14** was assigned using COSY and CHCORR spectroscopy. ^1H NMR ($CDCl_3$, 200 MHz): δ 1.21–1.37 (m, 1H, 4-H), 1.38–1.44 (d,

1H, 2′-H), 1.48–1.99 (15H, adamantane–H, 4-H), 2.30–2.47 (m, 2H, 2, 3-H), 2.90–3.08 (m, 2H, 5-H), 3.10–3.20 (dd, 1H, 2-H), 4.03 (bs, 1H, N–H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 27.7, 28.1 (5′,7′-C), 30.6 (4-C), 31.0, 31.3 (1′,3′-C), 31.8 (4′,9′-C), 38.3 (6′-C), 39.1, 39.2 (8′,10′-C), 40.5 (3-C), 46.2 (5-C), 49.5 (2′-C), 51.0 (2-C). Spectra and elemental analysis of all the synthesized compounds were in accord with their structure.

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13. (a) Solutions of protonated compound **14** in DMSO-d_6 solution (fumarate salt) or $CDCl_3$ solution (TFA-d_1 salt) were studied. The ^{1}H and 2D COSY, HMQC and NOESYPH NMR spectra were run on a Bruker DRX 400 machine. (b) Aminoadamantanes have been considered to act biologically through their protonated form; see ref 5.

14. (a) Molecular mechanics were performed using the MM+ force field provided by Hyperchem. This force field is an extension of the MM2 force field. Molecular mechanics calculations give the most consistent results with experimental data for pyrrolidine conformers, see ref 15. An initial structure was constructed and minimized using conjugate gradient and Newton–Raphson algorithms and an energy gradient tolerance of 0.001 kcal mol^{-1} Å^{-1}. This structure was then subjected to grid scan search analysis using 10° steps. (b) According to equation $\Delta G^\circ = -RT\ln K$ the relative population **D/A** is 0.4:99.6 at 298 K (see Table 2).

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