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8-Aminobicyclo[3.2.1]octanes: synthesis and anti-viral activity

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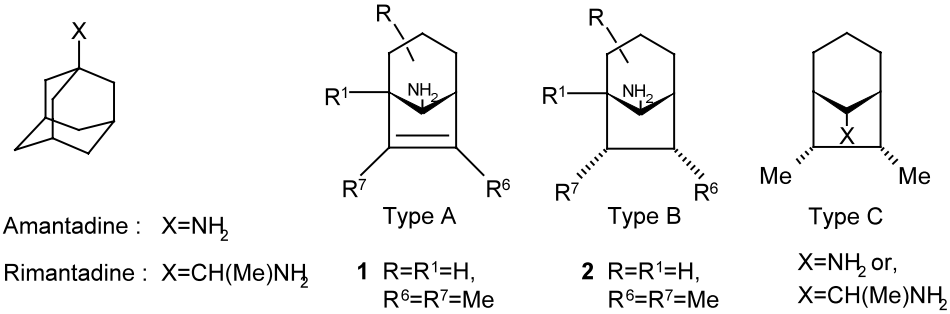
Abstract—A series of 8-chlorobicyclo[3.2.1]oct-6-enes has been prepared from cyclohexenyl chlorides and simple alkynes via a one-step [3+2] cycloaddition methodology, and then converted into the corresponding bicyclic amines. Against influenza-A virus a number of these showed in vitro activity, and amine (**2**) was comparable to amantadine in vivo. Good activity against influenza-B virus was less common, and none of the amines showed high potency against both viruses. Amines (**20**) and (**23**) showed significant activity versus respiratory syncytial virus. © 2001 Elsevier Science Ltd. All rights reserved.

The discovery of the therapeutic and prophylactic properties of amantadine nearly 40 years ago led to immense interest in the synthesis and testing of lipophilic, bulky amines as potential anti-influenza agents,[1] despite the fact that nothing was known then about the biological target of these compounds.[2] Moreover, although amantadine and its analogue rimantadine have had some success as influenza therapies, they have a number of drawbacks, notably CNS side-effects and a lack of activity against clinically relevant strains of influenza-B virus.[1]

Previous study of structure–activity relationships (SAR) in the amantadine series revealed that introduction of polar functionality beyond the basic amino group and/or addition of bulky ligands to the adamantane framework generally led to loss of activity.[1,3] Moreover, these SAR features extended to other amino substituted bridged bicyclic systems, such as bicyclo[2.2.2]octanes[4] and bicyclo[2.2.1]heptanes.[5] However, the synthetic routes to these analogues were often tedious, and were not always amenable to variation in substituents. The discovery[6] of a direct [3+2] cycloaddition route to 8-chlorobicyclo[3.2.1]oct-6-enes gave us the opportunity to synthesise a range of derived bicyclic amines, typified by the generic structures A and B. Our broad objective was to find compounds that were orally active against both influenza-A and influenza-B, the clinically important influenza viruses. This report outlines the synthesis of compounds of types A and B, as well as two compounds of type C, and describes their anti-viral properties against influenza-A and -B, and respiratory syncytial virus (RSV).

Given the simple SAR already established for amantadine and its congeners, our first target was to prepare and test a few highly symmetrical bicyclo[3.2.1] amines which were roughly the same size, shape and polarity as amantadine, and compounds **1** and **2** were chosen as representatives of types A and B, respectively. Of these, compound **2** was particularly striking because the two methyl groups at C-6 and C-7 are tucked under the bridge and ensure an amantadine-like shape, all com-



Amantadine : X=NH_2

Rimantadine : X=CH(Me)NH_2

1 R=R^1=H, R^6=R^7=Me

2 R=R^1=H, R^6=R^7=Me

X=NH_2 or, X=CH(Me)NH_2

Keywords: allyl cation; [3+2] cycloaddition; 8-aminobicyclo[3.2.1]octanes and 6-enes; anti-viral activity versus influenza-A and -B; respiratory syncytial virus.

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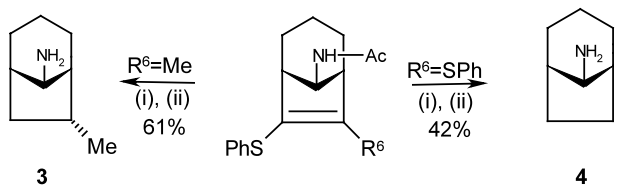
pounds have the C-8 bridge arbitrarily depicted as 'up'. We were therefore, highly encouraged when **1** was found to have an IC_{50} of 12 μM, and its saturated analogue **2** had an IC_{50} of 1–3 μM against influenza-A in vitro. On this basis, we embarked on a programme of synthesis of analogues of **1** and **2**.

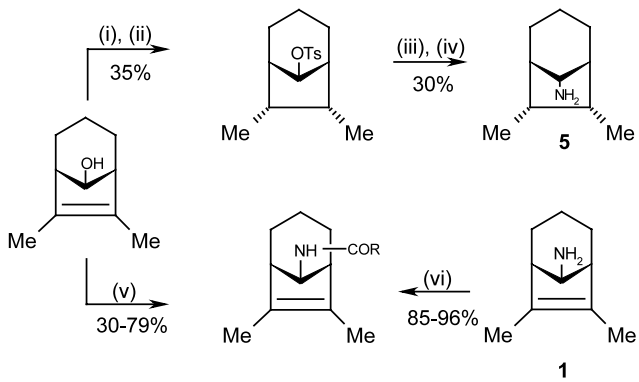
The general synthesis of type A and type B amines is outlined in Scheme 1. The [3+2] cycloaddition (the term indicates a structural feature, and has no mechanistic implications) was achieved using zinc chloride, in accord with the original conditions[6] and the adduct yields were generally in the moderate to good range (55–80%). When there is the possibility of forming two regiomeric cycloadducts, appropriate choice of R^6 and R^7 (e.g. H and Ph; or Me and Ph; or Me and SPh, respectively) results in only one isomer being observed. A further feature of the cycloadducts is that the bridge chlorine is always oriented *exo* to the double bond, i.e. it lies over the cyclohexane ring in the adduct. This has been observed consistently[6,7] and been commented upon elsewhere.[8] Moreover, provided that the 6–7 double bond is still in place when the bridge chlorine is replaced, nucleophilic substitution reactions of the chlorine at the bridge all result in retention of configuration, so that in the sequence C8-Cl→C8-N_3→C8-NH_2, as in Scheme 1, the stereochemistry of the azide and amino groups is assured. The relative stereochemistry at the bridge is readily assigned from the vicinal coupling constant of about 5 Hz between the bridge methine proton and those at C-1 and C-5.[7,9] For our type B targets, the double bond was finally removed using catalytic hydrogenation, which always occurred from the same face as the bridge, so that groups R^6 and R^7 were placed *endo* in the cyclopentane ring. It was this consideration which led to **2** as the preferred target for early synthesis.

The above issues were common to all of the bicyclo[3.2.1]octanes or oct-6-enes, but the functional group manipulations were otherwise routine and not worthy of detailed comment, apart from the few examples discussed below. After **1** and **2** had been identified as leads, there was a clear need to make analogues in which one or both of the methyl groups was removed, and this was achieved using reductive de-sulphurisation to yield compounds **3** and **4**, as in Scheme 2. One or more phenylsulphenyl groups in the alkyne improves the [3+2] cycloaddition yield noticeably, and reductive removal is also efficient, such that this route to **3** and **4** is preferred to routes using ethyne or propyne as the alkyne partner. Another obvious target was **5**, the C-8 epimer of the amine **2**. As shown in Scheme 3, and explained above, inversion at C-8 could only be achieved after removal of the C-6 to C-7 double bond, and this was followed by tosylate displacement by azide ion to give the inverted ($^3J = 1.5$ Hz for C8-H) azide (IR 2090 cm^{-1}). The starting material for this sequence, made by hydrolysis of the corresponding chloride,[7] was also used in the preparation of bridge amides via the Ritter reaction, see also Scheme 3. Other amides were made by direct acylation of the amine **1**.

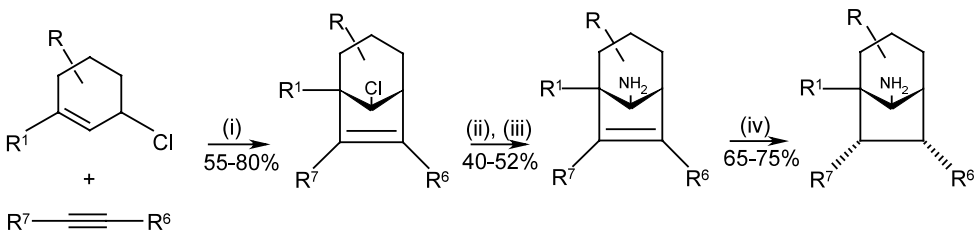
The same alcohol was also the starting material for the synthesis of rimantadine-like analogues of **2**, in which the basic amino group is moved out from the bridge by one carbon. As illustrated for amine **6** in Scheme 4, the chain extension was achieved by an Emmons–Horner-like sequence,[10] followed by hydrolysis of the intermediate enol–ethers under acidic conditions. The final product, **6**, was isolated and tested as a diastereoisomeric mixture.



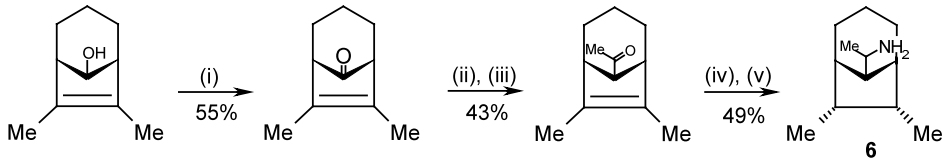
Scheme 2. *Reagents and conditions*: (i) W-2 Raney nickel, EtOH (reflux); (ii) HCl, H_2O, EtOH (reflux).



Scheme 3. *Reagents and conditions*: (i) 5% Pd–C, H_2 (50 atm), EtOH (50°C); (ii) *p*-toluenesulphonyl chloride, pyridine (40–60°C); (iii) NaN_3, HMPA (80°C); (iv) LAH, Et_2O (−10–22°C); (v) 57% H_2SO_4, RCN, $CHCl_3$ (0–22°C); (vi) $(RCO)_2O$, DMAP, pyridine.



Scheme 1. *Reagents and conditions*: (i) $ZnCl_2$, CH_2Cl_2; (ii) $ZnCl_2$, NaN_3; (iii) LAH, Et_2O (−10 to 22°C); (iv) Pd–C, H_2, AcOH.



Scheme 4. *Reagents and conditions*: (i) pyridinium chlorochromate, CH_2Cl_2; (ii) $Ph_2P(O)CH(Me)OMe$, LDA, THF; (iii) HCl, MeOH; (iv) NH_2OH, HCl, pyridine, EtOH (reflux); (v) PtO_2, H_2 (50 atm), AcOH (50°C).

All compounds were initially screened for activity against influenza-A using a standard plaque inhibition assay. Active compounds[11] were then assayed quantitatively by plaque reduction and an IC_{50} was assigned, as shown in the Table 1. As is general with anti-viral assays, any effects of compounds on host cell (usually MDCK cells) growth were also monitored, in order that anti-viral activity was not confused with host cell toxicity. With respect to anti-influenza activity, it was quickly confirmed that the established SAR, referred to above, also applied to the bicyclo[3.2.1]octanes. For example, in the B series, placement of a tertiary OH group at C-7 (see entry 8) caused complete loss of activity. Moreover, for both type A and B (entries 9 and 10, respectively), a simple replacement of the 6-Me by 6-Ph abolished activity. Similarly, introduction of a

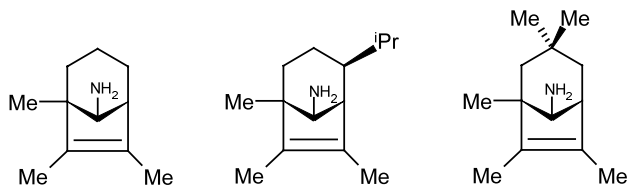
Table 1. Anti-viral activity of bicyclo[3.2.1]octane amines against enveloped viruses

Compd. no.	Structure type[a]	Structure details[b]										Anti-viral activity[c]			Toxicity rating[d]
		R^1	R^{2a}	R^{2e}	R^{3a}	R^{3e}	R^{4a}	R^{4e}	R^6	R^7	R^8	Flu A	Flu B	RSV	
1	A	H	H	H	H	H	H	H	Me	Me	NH_2	12.5	I	I	N
2	B	H	H	H	H	H	H	H	Me	Me	NH_2	1.0–3.0	I	I	N
3	B	H	H	H	H	H	H	H	H	Me	NH_2	4.0	55	>100	N
4	B	H	H	H	H	H	H	H	H	H	NH_2	30.1	68	>100	N
5	C	H	H	H	H	H	H	H	Me	Me	NH_2	5.3	20.0	34.0	N
6	B	H	H	H	H	H	H	H	Me	Me	$CH(Me)NH_2$	2.25	>50	I	S
7	C	H	H	H	H	H	H	H	Me	Me	$CH(Me)NH_2$	1.2	–	–	T
8	B	H	H	H	H	H	H	H	H		NH_2	>100	I	I	N
9	A	H	H	H	H	H	H	H	Ph	Me	NH_2	>25	I	I	N
10	B	H	H	H	H	H	H	H	Ph	Me	NH_2	>25	I	I	N
11	B	H	H	H	H	H	H	H	Et	Me	NH_2	17.9	I	>100	N
12	A	H	H	H	H	H	H	H	Et	Et	NH_2	I	I	I	T
13	B	H	H	H	H	H	H	H	Et	Et	NH_2	I	I	I	T
14	B	Me	H	H	H	H	H	H	H	H	NH_2	>25	I	I	N
15	A	Me	H	H	H	H	H	H	Me	Me	NH_2	>25	12.4	>100	N
16	B	Me	H	H	H	H	H	H	Me	Me	NH_2	13.4	64.3	<25	S
17	B	Me	H	H	H	H	H	H	Me	Me	NH·Et	15.1	>100	>100	N
18	B	H	H	H	H	H	H	H	H	Me	NH·Et	10	61.9	>100	N
19	B	H	H	H	H	H	H	H	Me	Me	$NHCH_2Ph$	I	>50	>50	S
20	A	Me	H	H	Me	Me	H	H	Me	Me	NH_2	30.4	36	15.9	N
21	A	H	H	H	H	H	Me	Me	Me	Me	NH_2	>20	≅30	>20	N
22	A	H	H	H	H	H	^{i}Pr	H	Me	Me	NH_2	>20	≅15	>20	S
23	A	Me	H	H	H	H	^{i}Pr	H	Me	Me	NH_2	>20	>50	8.6	S
24	A	Me	H	H	H	H	^{i}Pr	H	Et	Et	NH_2	>20	I	>20	T
25	A	H	H	H	H	H	H	Me	Me	Me	NH_2	>20	≅20	>20	N
26	B	Me	H	H	H	H	^{i}Pr	H	Me	Me	NH_2	67.0	>50	>100	S
27	A	H	H	Me	H	H	^{i}Pr	H	Me	Me	NH_2	53	34	>100	S
28	A	H	Me	H	H	H	^{i}Pr	H	Me	Me	NH_2	92	37	58	S
29	A	Me	H	H	H	H	Me	Me	Me	Me	NH_2	53	>25	>12.5	T
30	A	Me	H	Me	H	H	^{i}Pr	H	Me	Me	NH_2	12	45.1	>25	T
31	B	H	^{i}Pr	H	H	H	H	H	Me	Me	NH_2	33	61.6	–	S
32	A	H	Me	H	H	H	H	^{i}Pr	Me	Me	NH_2	51	35	>100	T
33	B	H	H	H	H	^{i}Pr	H	H	Me	Me	NH_2	34.3	53	>25	S

[a] Where A, B and C are as in main text, and C has epimeric configuration at C-8.
[b] Where superscripts a and e refer to axial or equatorial orientation (respectively) of R on bicyclooctanes or octenes.
[c] IC_{50}, minimum inhibitory concentration (μM) required to reduce viral plaques by 50% (I = inactive).
[d] Qualitative assessment from plaque inhibition experiments (N = non toxic; S = slightly toxic; T = toxic).

6-Et group in type B (entry 11) resulted in a drop of about 10-fold, whilst a further identical substitution at C-7, in either series (entries 12 and 13), again abolished activity. In the same vein, substitution of Me for H at C-1 (entries 14–16) or Me_2 for H_2 at C-4 (entry 21) resulted in a significant reduction in potency. From these and other data in the Table 1, it became clear that the symmetry, size and shape of the lead compound **2** was close to optimal, at least for influenza-A. Even the C-8 epimeric amine (entry 5) showed a reduction in activity. We also looked at the effect of removing the Me groups from **2**, and found that loss of the C-6 Me (entry 3) was tolerated, but loss of both Me groups (entry 4) led to up to a thirty-fold drop in potency.[12] In the rimantadine-type compounds (entries 6 and 7), potency was close to that of **2**, but there was clear evidence of toxicity to the host.

Further screening was also undertaken against influenza-B, and against RSV, which is now recognised to be the agent responsible for a range of severe, flu-like conditions that frequently affect very young children.[13] Against influenza-B, we confirmed genuine activity ($IC_{50} \leq 20$ μM) in only two compounds, **15** and **5**. The RSV screen identified compounds **20** and **23** as the most potent inhibitors, with IC_{50}s of 8 and 16 μM, respectively, although **5** was a weaker inhibitor at 34 μM and had the distinction of being the only compound with an IC_{50} of below 40 μM against all three viruses. From **20** and **23**, it looks as if RSV is tolerant of substitution at C-1, C-3 and C-4, although further examples would be required to substantiate this. The bicyclic amides (see Scheme 3, but not detailed in the Table 1), were universally inactive, and often quite toxic. A small number of *N*-alkyl derivatives of both A and B series was made and these showed activity, provided that the amine remained basic, and that the alkyl group was small, e.g. *N*-ethyl (entries 17 and 18) and not *N*-benzyl (entry 19).



12 **23** **20**

The lead compounds from the in vitro influenza screens (entries 2 and 15, for flu-A and flu-B, respectively) were assessed in vivo, in standard mouse models. Compound **15** was found to be essentially inactive versus influenza-B/Lee. In tests run under the same in vivo conditions, compound **2** was equiactive orally with amantadine against influenza-A/Sweden, influenza-A/Bell and influenza-A/Okuda, and inactive against two amantadine resistant strains of the latter two viruses. These data are suggestive that **2** and amantadine have a similar mode of action and potency, a situation more recently described for BL1743.[14] Cross-resistance with amantadine is a serious flaw in a potential anti-flu drug and is sufficient to deter further development of lead compounds, such as **2**.[1b]

[3+2] Cycloaddition procedure—preparation of 8-chloro-6,7-dimethylbicyclo[3.2.1]oct-6-ene: A solution of 3-chlorocyclohexene (2.0 g, 17 mmol) in dry dichloromethane (10 ml) was added dropwise to a stirred suspension of zinc chloride (3.5 g, 25.73 mmol) in a solution of but-2-yne (1.6 ml, 20.4 mmol) in dry dichloromethane (50 ml), at 0°C under nitrogen. After 1 h, the reaction mixture was allowed to warm up to room temperature and stirred for a further 3 h. Water (50 ml) was cautiously added and the aqueous layer extracted with chloroform (3×20 ml). The combined organic extracts were washed with water, sodium hydrogen carbonate solution and dried ($MgSO_4$). After filtering the dried extracts and evaporating the solvent, the crude product was purified by flash column chromatography, eluting with light petroleum (bp 40–60°C), to yield 8-chloro-6,7-dimethylbicyclo[3.2.1]oct-6-ene (1.72 g, 58.9%) as a light yellow oil. Key data included ^{1}H NMR ($CDCl_3$): δ 1.54 (bs, C-6 and C-7 Me), and 4.17 (t, $J=5$ Hz, 1H, C*H*Cl) ppm. IR (neat) showed strong, diagnostic band[15] for the chlorocyclopentene at 830 cm^{-1}.

General procedure for exchange at C-8—preparation of 8-azido-6,7-dimethylbicyclo[3.2.1]oct-6-ene: Sodium azide (7.54 g, 0.116 mol) and reagent grade anhydrous zinc chloride (11.8 g, 0.087 mol) were added in sequence to a stirred solution of 8-chlorobicyclo[3.2.1]oct-6-ene (10.02 g, 0.058 mol) in dry dichloromethane (150 ml) under nitrogen at room temperature, and the suspension stirred for a further seven days. Water (500 ml) was then added and the organic phase was separated, before being combined with chloroform (3×100 ml) washings of the aqueous phase. The combined organic extracts were then dried ($MgSO_4$) and filtered, and the solvents evaporated on a rotary evaporator. After careful flash chromatography, using light petroleum (bp 40–60°C), 8-azidobicyclo[3.2.1]oct-6-ene (6.6 g, 63%) was isolated as a clear colourless oil. Key data included ^{1}H NMR ($CDCl_3$): δ 3.82 (t, $J=5$ Hz, 1H, C*H*N_3) ppm. IR (neat) showed a very strong azide absorption at 2095 cm^{-1}.

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