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A new synthesis and preliminary evaluation of some analogues of mecamylamine – a compound with anti-addiction properties.

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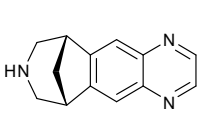
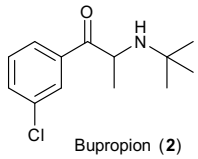
Abstract: A new synthesis of mecamylamine – a known anti-hypertensive drug with anti-addictive properties is described. The new route allowed access to two novel analogues whose activity at two nicotinic acetylcholine receptor subtypes was assessed.

Addiction is a devastating condition that can result in a dramatic loss of life quality for the addict and those closely associated with them. Furthermore it is a considerable driver of crime and causes significant burden on the state. Addiction to illicit substances including heroin and cocaine and to legal substances nicotine, alcohol and some prescription medications are well known.[1] Behavioural addictions such as those related to gambling have also been documented.[1,2] In each addiction, the underlying cause is thought to be the highjacking of the reward pathway in the CNS which culminates in the release of the neurotransmitter dopamine (DA) in the nucleus accumbens (NAc), thus producing a feeling of reward.[3]

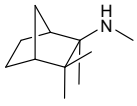
Currently, the only pharmacological agents available to treat addiction are related to addiction to nicotine. The WHO estimates that addiction to nicotine and the use of cigarettes as a delivery device is responsible for 6 million deaths per year globally with *ca.* one in two smokers dying prematurely of a smoking-related illness.[4] One of the attributes of addiction is that the addict will continue with behaviour that is known to be detrimental to their health and this is observed in the number of smokers at any given time who would like to quit, but are unable to do so.[1] The act of smoking cessation, or breaking the cycle of addiction associated with any addictive substance, is notoriously difficult; resulting in an initial period of withdrawal and the continuing problem of remaining abstinent for life. In addition to the use of nicotine replacement therapy (gum, lozenges and sprays) two products are currently available to assist in smoking cessation. Varenicline (**1**- Chantix® (US) and Champix® (Europe), Pfizer) is a nicotinic acetylcholine α4β2 partial agonist and α7 agonist, [5] based on cytisine which has itself been used in some jurisdictions to assist smoking cessation.[6] Bupropion (**2**- Zyban®, GSK) is a repurposed anti-depressant, a noradrenaline/dopamine reuptake inhibitor and α3β4 nicotinic acetylcholine (nAChR) antagonist (Figure 1).[7] Clinical trials have indicated that after 12 months smokers using varenicline were a little more than twice as likely to be abstinent (22-23%) compared to a pharmacologically unassisted placebo attempt (8-10%). Varenicline had slightly higher quit-rates than those associated with bupropion (15-16%) and varenicline was slightly higher than nicotine replacement therapy (NRT) alone.[6] Unfortunately, neither of these treatments is universally successful and in 2009 the FDA required manufacturers of varenicline and bupropion to add boxed warnings related to neuropsychiatric disturbances.[8] However, these treatments do highlight the potential for the exploitation of nAChRs via the ventral tegmental area (VTA)-NAc axis for the treatment of addictive disorders. However, there is clearly a need for improved pharmacological assistance to facilitate cessation and long term abstinence from addictive agents and/or behaviours.



Varenecline (**1**)



Bupropion (**2**)



Mecamylamine (**3**)

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Figure 1 – Possible treatments to assist smoking cessation

Another compound that has stimulated interest in the area and is of direct relevance to the work described herein is the general nAChR antagonist mecamylamine (**3**). Mecamylamine (MA) was initially developed as a treatment for hypertension and was sold as Inversine® by Merck for many years until the development of β-blockers which were more effective and have an improved side-effect profile.[9] More recently, interest in mecamylamine has been reinvigorated by observations that it has anti-addictive properties against not only nicotine but other drugs of abuse.[10] For example, clinical trials have shown that smokers are more likely to quit and more likely to be abstinent after 12 months when assisted by MA in combination with NRT - a remarkable 40% remained abstinent after 12 months.[11] MA has been shown to reduce cue-induced craving in human cocaine addicts.[12] In human clinical studies MA was shown to diminish the euphoric and stimulant effects of ethanol and was also found to reduce the self-reported desire to consume ethanol in healthy male and female non-smoking social drinkers.[13] The significance of this is enhanced by the fact that, in addition to the well known health consequences of excessive alcohol consumption, there is growing evidence of a direct link between alcohol intake and some cancers.[14] MA has been shown to reduce self-administration (a model for addiction) of a variety of drugs of abuse such as ethanol, opiates and stimulants in animal models.[15] There are also indications that varenicline may have some role to play in the treatment of cocaine addiction.[16]

It is currently unknown whether the general activity of MA at different nAChR subtypes is important for activity, or indeed whether fine-tuning the structure could produce a more effective compound by enhancing its activity at a specific receptor subtype; $\alpha3\beta4$[15(f)] and $\alpha4\beta2$[17] are currently considered prime candidates. Moreover, the generation of subtype-specific compounds can help elucidate the role of different subtypes within the CNS and offer insight into other disorders.[18] Surprisingly, despite the long history of MA the influence of stereochemistry on activity was only assessed in 2001.[19] Furthermore, MA seems to have effects in other disorders such as Tourette's Syndrome[20] and nAChR's have been postulated to have roles in many other neuropsychiatric disorders.[18]

In addition, MA is an attractive lead for drug development since the pharmacokinetics are well understood, bioavailability of MA is high and the compound is known to readily cross the blood-brain barrier.[10] MA has been used by millions of patients which indicates that serious side-effects are rare and are typically anti-cholinergic in nature *e.g.* dry mouth and constipation. Additionally, doses for anti-addictive action are considerably lower than those required for anti-hypertensive activity. The work described above indicates that the development of analogues merits further investigation.

The seminal SAR studies of MA have been completed by Corne,[21] Stone,[22] Wragg,[23] Herr[24] and Suchocki.[25] The key points, of particular relevance to this study, are summarised in Figure 2. More recent studies by Crookes have developed some interesting dimeric and trimeric systems.[26]

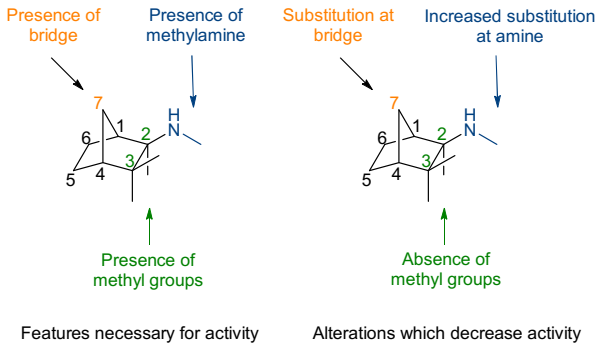
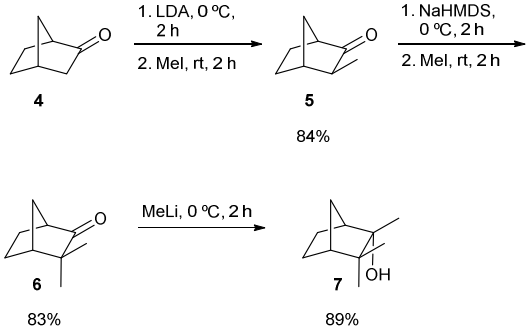


Figure 2 – Factors associated with the activity of mecamylamine

Analysis of the current SAR data highlights two significant points; increased steric bulk (for H to Me) at positions 2 and 3 (Figure 2) improves activity, and although the bridge is important, the relationship of the amine with the 1-carbon and 2-carbon variant has not been examined. The current study started with the concept of increasing the steric bulk around the amine and also the examination of the importance of amine relative stereochemistry. Previous syntheses of MA, although concise, have relied on camphene derivatives, which meant exploring options other than methyl groups adjacent to the amine would be synthetically challenging.[26,27] In order to explore these options an alternative new synthetic route would be required.

As a model study, the preparation of MA from norboranone (**4**) was attempted – if this methodology were successful it should allow alternative groups to be installed in the place of methyl units around the amine. Treatment of norboranone (**4**) with LDA at low temperature gave the expected enolate which was methylated with methyl iodide forming **5** in 84% yield. Note that the addition of the electrophile occurred from the *exo*-face due to the 'picket fence' effect.[28] Attempts to add a second methyl group by treatment with LDA and methyl iodide led to incomplete reaction. However, the use of NaHMDS, in place of LDA, followed by treatment with methyl iodide generated the dimethylated ketone **6**, and exposure of this ketone to methyl lithium formed tertiary alcohol **7**. Again the 'picket-fence' effect directed the methyl lithium to the *exo*-face with no evidence of alternative stereoisomers by NMR spectroscopy.



Scheme 1 – Formation of the tertiary alcohol

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The procedure for the conversion of alcohol **7** to azide **8** was based on the methodology developed by Arcus[29] but required a degree of optimisation. Alcohol **7** was exposed to sodium azide in a biphasic system consisting of sulfuric acid and chloroform and in this case, the reaction was highly dependent on the concentration of sulfuric acid employed (Table 1). Employing 40% sulfuric acid no reaction was observed, 50% gave the desired tertiary azide **8** in 67% yield while 75% sulfuric acid gave the rearranged product **9**. Presumably, during the formation of the desired azide **8** the reaction proceeds via a S_N1 process with the azide anion adding selectively from the *exo*-face. Azide **8** is relatively volatile and care must be taken when removing the solvent at reduced pressure.

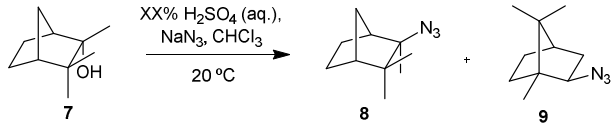
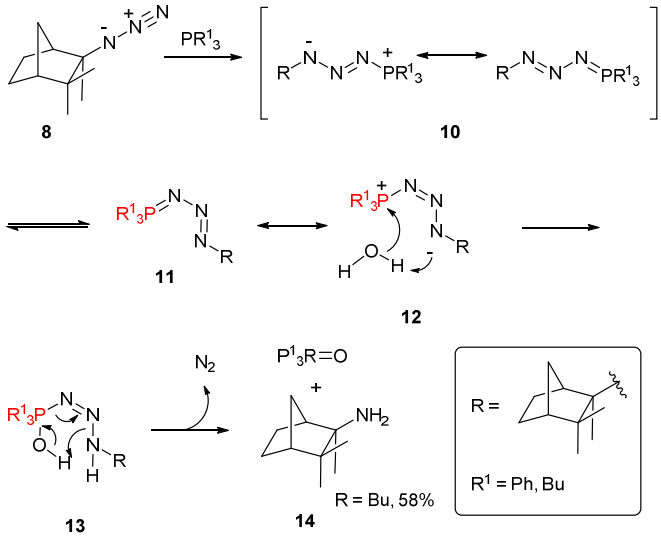


Table 1: Formation of azides 8 and 9

Sulfuric Acid Conc. % (M)	Time (h)	Product Distribution (%) 7	8	9
75 (13.8)	3	0	0	100
50 (9.2)	4	0	100	0
40 (7.4)	24	100	0	0

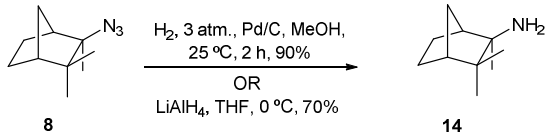
Reduction of the azide **8** also proved interesting. Initially the reduction was attempted using typical Staudinger Reaction conditions,[30] with triphenylphosphine and water in THF at room temperature however, this was unsuccessful. Repeating the reaction in toluene at reflux was also failed to provide any of the desired amine. Vaultier[31] has shown that, in the case of hindered azides, it can be beneficial to complete the formation of the intermediate iminophosphorane prior to the addition of water. Treatment of the azide **8** with triphenylphosphine in toluene at reflux formed a new synthetic intermediate (R^1 = Ph, indicated by ^{31}P NMR) but the expected amine was not produced upon addition of water, instead the intermediate slowly reverted back to the starting azide **8**. This indicated that the intermediate observed was not the expected iminophosphorane as diatomic nitrogen gas would be lost, irreversibly, in the process of its formation. It is known that tributylphosphine is more reactive than triphenylphosphine in the Staudinger Reaction[32] and it was employed in this case. Treatment of azide **8** with tributylphosphine in THF generated a new product (presumably phophoazide **10**, R^1 = Bu, indicated by ^{31}P NMR) after 4 hours at room temperature. Addition of water followed by treatment with 2M HCl in diethyl ether provided the expected amine salt **14.HCl**. Formation of the HCl salt allowed simple removal of tributylphosphine oxide by washing with butanone. Treatment with aqueous NaOH gave the expected amine **14** in 58% yield.



Scheme 2 – Azide reduction

During the Staudinger Reaction with tributylphosphine it was noted that only upon addition of water was a gas (presumably nitrogen) evolved. It was therefore speculated that the highly congested bicyclic system in combination with the bulky phosphines formed intermediates that were unable to undergo the standard Staudinger-type mechanism and that the reaction therefore stalled at the phosphoazide intermediate **10**. In the case of the tributylphosphine we suggest that the added water is able to bridge the *cis*-phosphoazide **12** and effect the loss of nitrogen without the requirement for a formal formation of a 4-membered ring. In the case of the triphenyl derivative, it is suspected that the intermediate is too hindered and/or electronically unreactive for water to have the same effect providing a means for the phosphoazide (**10**, R^1 = Ph) to revert back to the azide **8**. Attempts at aza-Wittig reactions from either of the initial phosphine addition products (prior to the addition of water, usually expected to be the iminophosphorane) were unsuccessful.

Although the Staudinger Reaction was attractive from the perspective of functional group compatibility, allowing access to more elaborate analogues for future SAR work, other reduction methodologies were also explored (Scheme 3). Treatment of azide **8** with 10% Pd/C and hydrogen produced the amine **14** in 90% yield and treatment with lithium aluminium hydride provided the expected compound **14** in 70% yield.



Scheme 3 – Alternative azide reduction.

Reductive amination allowed the successful addition of a methyl group to **14** after problems associated with dimethylation were solved by a slightly modified procedure. The reaction of amine **14**

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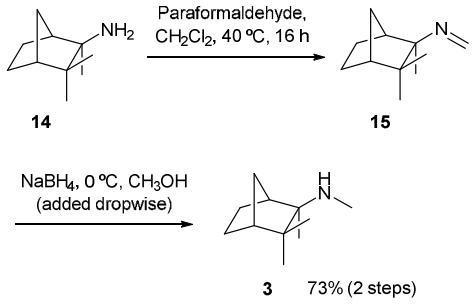
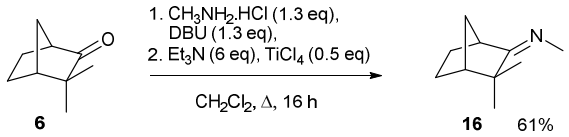
with paraformaldehyde in dichloromethane was monitored by proton NMR spectroscopy until complete formation of the imine had occurred at which point sodium borohydride was added followed by the dropwise addition of methanol which gave MA (**3**) in 72% yield (Scheme 4). It was important to add a limited quantity of methanol, dropwise, to prevent decomposition of the imine. Alternatively, deprotonation of amine **14** with butyl lithium before the addition of methyl iodide gave MA in 49% yield.



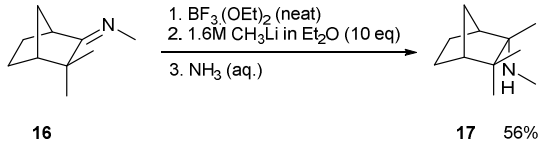
Scheme 4 – Methylation to form mecamylamine

Having prepared MA itself attention turned to the synthesis of *endo*-MA (**17**) in which the amine is positioned on the same face as the two-carbon bridge (Scheme 6). The synthetic approach involved attack of a nucleophile on the appropriate imine **16**, hopefully controlled by the picket fence effect; the first task was to prepare the hindered imine. Although the synthesis of *endo*-MA **(17)** has been reported by Suchocki[25] it was formed in trace quantities (<1%) that only permitted characterisation by mass spectral analysis. Rather than employ the same method of imine formation (which required bubbling methyl amine through a reaction mixture in toluene at reflux for 18 hours) we employed the methodology developed by Moss (Scheme 5).[33] The reaction was performed in a sealed system, and the order of reagent addition proved critical. DBU was added to a suspension of methylamine hydrochloride and stirred until the mixture became homogeneous. Titanium tetrachloride and trimethylamine were added sequentially and the mixture heated at reflux for 20 minutes before the ketone **6** was added and heating continued for 16 hours. After work-up the hindered imine **16** was recovered in 61% yield.



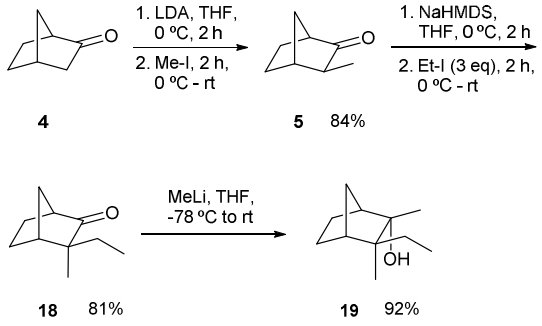
Scheme 5 – Formation of a hindered imine

Treatment of the imine **16** with methyl lithium, methylmagnesium bromide or methylcerium trichloride gave none of the desired amine. However, precomplexation with borontrifluoride etherate to form a solid prior to addition of 10 equivalents of methyl lithium in diethyl ether gave *endo*-MA (**17**) in 56% yield after work-up with concentrated ammonia (Scheme 6).



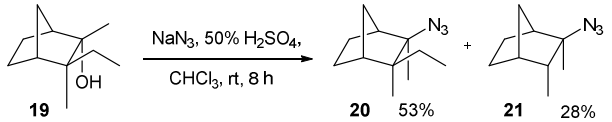
Scheme 6 – Preparation of endo-mecamylamine

Having developed a new synthetic route to MA and having prepared *endo*-MA (**17**) in sufficient quantities for analysis and biological evaluation, the versatility of the new synthetic approach was investigated further. Previous SAR work had shown that the replacement of hydrogen with methyl groups around the amine gave improved activity. It was decided to examine the effect of adding further steric bulk with the exchange of one of the methyl groups for an ethyl group. It was hoped that the "picket-fence" effect[28] could be used to control the addition of nucleophiles and electrophiles to the *exo*-face, at least during the early stages of the synthetic sequence. Fortunately this was indeed the case and the two deprotonation/alkylation steps with the appropriate electrophiles gave ketone **18** in 68% yield (Scheme 7). Subsequent nucleophilic attack of methyllithium gave alcohol **19** in 92% yield. All reactions occurred diastereoselectively from the *exo*-face, with no indication (by NMR spectroscopy) of the formation of alternative stereoisomers.



Scheme 7 – Replacing a methyl group with ethyl

The formation of azide **20** was achieved by treatment of alcohol **19** with sodium azide in a biphasic solvent system with sulfuric acid and chloroform (Scheme 8). Once again, use of 9.2M (50%) sulfuric acid facilitated the formation of the desired azide in 53% yield without the concomitant formation of the azide formed by the Wagner-Meerwein-type rearrangement. However, in this reaction a second azide was formed in 28% yield that appeared to be lacking an ethyl group and was tentatively assigned as **21**.



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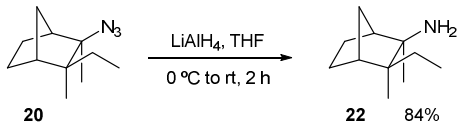
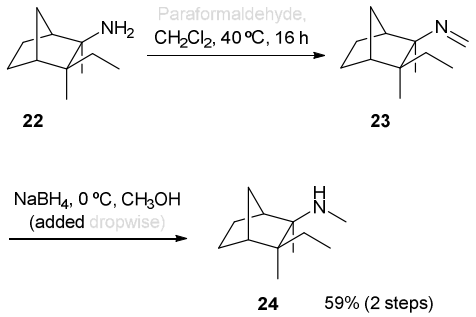
Scheme 8 – Azide formation

Attempts to reduce the azide by catalytic hydrogenation led to decomposition however, fortunately, treatment with $LiAlH_4$ in THF at 0 ºC gave the expected amine **22** in 84% yield (Scheme 9).



Scheme 9 – Azide reduction

The final step was the methylation of the amine and the two-step reductive amination methodology was employed once again (Scheme 10). The reaction of amine **22** with paraformaldehyde in dichloromethane was monitored by proton NMR spectroscopy until complete formation of the imine **23** had occurred at which point sodium borohydride was added followed by the dropwise addition of methanol which gave the MA derivative **24** in 59% yield.



Scheme 10 – Formation of a mecamylamine analogue

Having prepared mecamylamine and two novel analogues all three compounds were evaluated by assessing their ability to reduce the action of agonists at two nAChR subtypes. In order to gain preliminary insight into the effects of the structural alterations the compounds were tested at fixed concentrations against the $\alpha_4\beta_2$ subtype (expressed in oocytes, 3 µM vs ACh 100 µM) and the $\alpha_3\beta_4$ subtype (SH-SY5Y cells, 100 µM vs nicotine 100 µM). Interestingly, *endo*-MA (**17**, entry 3 of table 2) was more active than MA (**3**, entry 2 of Table 2) at $\alpha_4\beta_2$ but less active than MA (**3**) at $\alpha_3\beta_4$. Compound **24** (entry 4 of Table 2) had a similar profile and was more active than MA (**3**) at $\alpha_4\beta_2$ but less active then MA (**3**) at $\alpha_3\beta_4$. Pleasingly, the preliminary pharmacological data indicate that manipulation of the structure will allow the enhancement of both activity and selectivity, at least in the case of the $\alpha_4\beta_2$receptor, and work will continue to develop compounds that are more selective for the $\alpha_3\beta_4$ receptor.

Table 2 – Biological activity of the parent compound *exo*-mecamylamine and two new analogues

Entry	Compound	$\alpha_4\beta_2$ response (%)	$\alpha_3\beta_4$ response (%)
1	--	100	100
2	**3.HCl**	33 ± 8%	6.3 ± 1%
3	**17.HCl**	18 ± 7%	13.8 ± 7%
4	**24.HCl**	18 ± 5%	12.0 ± 2%

Conclusions

A new route to mecamylamine and mecamylamine analogues which allowed the facile synthesis of MA itself and two analogues is described. Preliminary pharmacological evaluation of the simple MA derivatives indicates that minor structural changes e.g. methyl to ethyl have interesting effects on activity at nAChR subtypes. Of particular note is the fact that these small structural changes show enhanced selectivity and activity at the two subtypes assessed. The versatile route should allow installation of a variety of functional groups which is significant in light of the biological effect of the simple structural modifications described. Clearly more detailed pharmacology is required but the work reported herein indicates that the formation of analogues related to MA may be fruitful in the pursuit of effective treatments and/or tool compounds for addiction and other psychiatric disorders associated with nAChRs.

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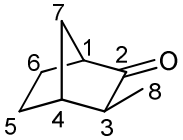
Experimental procedure

General Experimental section

Melting points were determined using a standard melting point apparatus and are uncorrected. Infrared spectra were obtained on a Perkin Elmer Spectrum 100 FT-IR spectrometer equipped with a universal ATR sampling accessory. Proton nuclear magnetic resonance (NMR) spectra were recorded on: Bruker Avance III 400 MHz, Bruker DPX400 400 MHz and Bruker Avance II 600 MHz spectrometers (^{1}H NMR spectra were recorded at 400.23 MHz, 400.13 MHz and 600.13 MHz respectively). Chemical shifts are reported in ppm relative to tetramethylsilane and coupling constants (*J*) are quoted in Hertz. Carbon NMR spectra were recorded on the previously mentioned instruments (100.64 MHz, 100.61 MHz & 150.9 MHz, respectively) with total proton decoupling. HSQC, HMBC, TOCSY and nOe NMR experiments were used to aid assignment of NMR peaks when required. A Waters micromass LCT-tof mass spectrometer was used in ES positive and ES negative modes for electrospray mass spectrometry. Electron impact mass spectra were determined on a Quatro-II mass spectrometer in the EI mode. Mass spectra were recorded in CSCB Trinity College Dublin, CSCB University College Dublin. Flash chromatography was performed using Merk Kiesegel 60 (art. 9385). Merck precoated Kiesegel foils 60F_{254} were used for thin-layer chromatography and slides were visualised by UV irradiation, $KMnO_4$, or anisaldehyde staining. Toluene, CH_2Cl_2, and triethylamine were distilled from calcium hydride.

3-*exo*-Methylbicyclo[2.2.1]heptan-2-one (5)



A solution of freshly distilled diisopropylamine (1.70 mL, 1.72 g, 11.84 mmol) in anhydrous THF (11 mL) was cooled to -78 °C, a solution of 2.5 M *n*-butyllithium in hexanes (4.60 mL, 11.36 mmol) was added. The reaction mixture was allowed to warm to 0 °C and a solution of bicyclo[2.2.1]heptan-2-one (1.00 g, 9.08 mmol) in anhydrous THF (2 mL) was then added dropwise. After stirring for 2 hours at 0 °C, iodomethane (1.70 mL, 3.87 g, 27.30 mmol) was added dropwise. After stirring for 2 hours at room temperature, a solution of 1M HCl (8 mL) was added. The product was extracted using diethyl ether (2 x 10 mL), washed with brine (10 mL) and dried over $MgSO_4$. The solvent was evaporated at reduced pressure to yield the title compound as a yellow oil. (0.95g, 84%)

IR υ_{max} (cm^{-1}): 2956, 2874, 1736, 1459, 1082, 937, 850.

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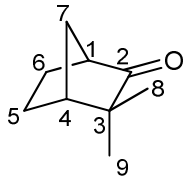
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 1.06 (d, *J* = 7.5 Hz, 3H, H8), 1.43-1.56 (m, 3H, H5a, H6a, H7a), 1.77-1.91 (m, 4H, H5b, H6b, H3, H7b), 2.30-2.35 (m, 1H, H4), 2.53-2.58 (m, 1H, H1).
^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 13.7 (CH_3, C8), 23.3 (CH_2, C6), 27.5 (CH_2, C5), 34.0 (CH_2, C7), 41.0 (CH, C3), 47.5 (CH, C4), 49.2 (CH, C1), 220.7 (q, C2).
HRMS: (*m/z* - CI) calcd. for $C_8H_{13}O$ $(M+H)^+$ 125.0966, found 125.0974.

3,3-Dimethylbicyclo[2.2.1]heptan-2-one (6)

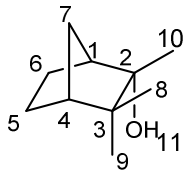


General procedure A

A solution of 3-*exo*-methylbicyclo[2.2.1]heptan-2-one (1.24 g, 10 mmol) in anhydrous THF (12 mL) was added to a cooled (-78 ºC) 1M solution of sodium bis(trimethylsilyl)amide in THF (15.00 mL, 15.00 mmol) and the reaction mixture allowed to warm to 0 ºC. After stirring for 2 hours at 0 ºC, iodomethane (1.93 mL, 4.40 g, 30.98 mmol) was added dropwise and the reaction mixture allowed to warm to room temperature. After stirring for 2 hours at room temperature, a solution of 1M HCl (8 mL) was added. The product was extracted using diethylether (2 x 10 mL), washed with brine (10 mL) and the combined organic extracts dried over $MgSO_4$. The solvent was evaporated at reduced pressure to provide a pale yellow oil which was purified by flash chromatography on silica gel eluting with 97:3 hexane:ethylacetate to yield the title compound as a pale yellow oil. (1.15g, 83%)

IR υ_{max} (cm^{-1}): 2967, 2874, 1739, 1464, 1290, 1153, 949, 748.
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 1.04 (s, 3H, H9), 1.08 (s, 3H, H8), 1.43-1.53 (m, 2H, H6a, H7a), 1.58-1.72 (m, 1H, H5a), 1.76-1.93 (m, 2H, H5b, H6b), 1.97-2.03 (m, 1H, H7b), 2.30-2.35 (m, 1H, H4), 2.53-2.58 (m, 1H, H1).
^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 21.5 (CH_3, C9), 23.26 (CH_3, C8), 23.31 (CH_2, C5), 24.6 (CH_2, C6), 35.1 (CH_2, C7), 46.2 (CH, C4), 47.2 (q, C3), 50.2 (CH, C1), 223.3 (q, C2).
HRMS: (*m/z*-EI) calcd. for $C_9H_{14}O$ $(M)^+$ 138.1045, found 138.1045.

2-*exo*-,3,3-Trimethylbicyclo[2.2.1]heptan-2-ol (7)

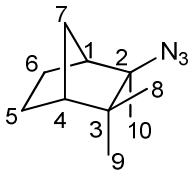


General procedure B

A solution of 3,3-dimethylbicyclo[2.2.1]heptan-2-one (0.897 g, 6.50 mmol) in anhydrous THF (20 mL) was added dropwise to a cooled (-78 ºC) solution of methyllithium (1.6 M in diethylether, 8.13 mL, 13.00 mmol). The reaction mixture was allowed to warm to room temperature and after 3 hours, 10% aqueous NH_4Cl (20 mL) was added. The product was extracted using diethylether (2 x 20 mL), washed with brine (20 mL) and the combined organic extracts dried over $MgSO_4$. The solvent was evaporated at reduced pressure to yield a pale yellow oil which was purified by flash chromatography on silica gel eluting with 98:2 hexane:ethyl acetate to give the title compound as a white solid (0.89g, 89%). M.p. 118-120 °C (lit.,[34] 113-115°C)

IR υ_{max} (cm^{-1}): 3411, 2929, 2871, 1473, 1371, 1295, 1087, 927
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 0.94 (s, 3H, H9), 0.97 (s, 3H, H8), 1.19-1.15 (m, 1H, H7a), 1.25 (s, 3H, H10), 1.27-1.36 (m, 3H, H5a, H6a, H11), 1.71-1.78 (m, 3H, H7s, H4, H6b), 1.85-1.90 (m, 1H, H5b), 1.97-2.01 (m, 1H, H1)
^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 21.1 (CH_2, C5), 21.8 (CH_3, C9), 24.0 (CH_2, C6), 26.3 (CH_3, C10), 27.0 (CH_3, C8), 34.6 (CH_2, C7), 42.0 (q, C3), 49.7 (CH, C4), 50.9 (CH, C1), 78.8 (q,C2).
HRMS: (*m/z* - EI) calcd. for $C_{10}H_6O_4N_2S$ $(M)^+$ 154.1358, found 154.1353.

2-Azido-2-*endo*-,3,3,-trimethyl-bicyclo[2.2.1]heptane (8)

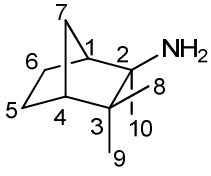


General Procedure C

A solution of HN_3 was prepared by carefully adding a solution of 50% aqueous H_2SO_4 (10 mL) to NaN_3 (2.00 g, 30.77 mmol) in $CHCl_3$ (50 mL) at 0 ºC. To this was added 2,3,3-trimethylbicyclo[2.2.1]heptan-2-ol (1.00 g, 6.49 mmol). After stirring for 4 hours at room temperature, ice-cold water (30 mL) was added. The product was extracted with CH_2Cl_2 (3 x 30 mL). The combined organic extracts were washed with 5% $NaHCO_3$ solution (30 mL), dried over $MgSO_4$, and evaporated at reduced pressure to give a yellow oil which was purified by flash chromatography on silica gel using 100% hexane to yield the title compound as pale yellow oil. (0.78 g, 67% yield)

IR υ_{max} (cm^{-1}): 2934, 2876, 2083, 1454, 1253, 1071, 801
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 0.94 (s, 3H, H9), 1.06 (s, 3H, H8), 1.12-1.16 (m, 1H, H7a), 1.25-1.35 (m, 4H, H10, H5a), 1.41-1.51 (m, 2H, H6), 1.55-1.63 (m, 1H, H5b), 1.75-1.80 (m, 1H, H4), 1.99-2.05 (m, 1H, H7b), 2.11-2.15(m, 1H, H1).
^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 17.3 (CH_3, C10), 23.1 (CH_2, C6), 23.5 (CH_3, C9), 23.7 (CH_2, C5), 26.8 (CH_3, C8), 34.8 (CH_2, C7), 43.9 (q, C3), 48.8 (CH, C1), 49.8 (CH, C4), 72.6 (q, C2).
HRMS: (*m/z* - ES) calcd. for $C_{10}H_{18}N$ $(M+H-N_2)^+$ 152.1439, found 152.1443.

2-*endo*-,3,3-Trimethyl-bicyclo[2.2.1]heptan-2-amine (14)



Reduction using lithium aluminium hydride:

General procedure D

To a solution of 2-azido-2-*endo*-,3,3-trimethylbicyclo[2.2.1]heptane (179 mg, 1 mmol) in anhydrous THF (10 mL) under an argon atmosphere cooled to 0 ºC, was added a solution of lithium aluminium hydride

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(2M in THF, 400 µL, 0.80 mmol) dropwise. The reaction mixture was allowed to warm to room temperature over 2 hours. After this time, TLC analysis showed that no starting material remained. The reaction mixture was cooled to 0 ºC and 2 M NaOH (10 mL) was added slowly. After stirring for 30 minutes at room temperature, the product was extracted using diethyl ether (2 x 10 mL), washed with brine (10 mL), the combined organic extracts dried over $MgSO_4$ and concentrated to give a clear viscous oil. The crude oil was further purified by flash chromatography on silica gel eluting with 50:50 hexane:ethyl acetate to provide the title compound (107 mg, 70%) M.p. 110 ^{o}C -112 ^{o}C (Sublimes)

Reduction via Staudinger protocol:

To a solution of 2-azido-2-*endo*-,3,3-trimethylbicyclo[2.2.1]heptane (179mg, 1 mmol) in anhydrous THF (10 mL) under an argon atmosphere, tributylphosphine (375 µL, 304 mg, 1.5 mmol) was added. The reaction mixture was stirred for 4 hours at room temperature. H_2O (180 µL, 10 mmol) was added and effervescence was observed. The reaction mixture was stirred for a further 16 hours at room temperature. The organic solvent was evaporated to yield a pale yellow oil. This oil was redissolved in DCM (20 mL) and dried over $MgSO_4$. A 2 M solution of hydrogen chloride in diethyl ether (1 mL, 2 mmol) was added to form the hydrochloride salt of the amine that was triturated to give a pale yellow oil that was purified by flash chromatography on silica gel using a eluent gradient from 100% ethyl acetate to 50:50 ethyl acetate:methanol. After removing the solvent under vacuum, the amine was extracted from 1M NaOH (15 mL) using CH_2Cl_2 (2 x 15 mL), dried over $MgSO_4$, filtered and concentrated to yield the title compound (88 mg, 58%) M.p. 110 ^{o}C -112 ^{o}C (Sublimes)

Reduction by hydrogenation:

To a solution of 2-azido-2-*endo*-3,3-trimethylbicyclo[2.2.1]heptane (0.30 g, 1.72 mmol), in methanol (20 mL) palladium on charcoal (0.03 g, 10% w/w) was added. The reaction vessel was evacuated and filled with hydrogen. The reaction mixture was stirred vigorously under a blanket of hydrogen at atmospheric pressure overnight. The reaction mixture was filtered through celite, dried over magnesium sulfate and filtered before the volatiles were removed at reduced pressure. The free amine was isolated by the addition of a 1M solution of NaOH and extraction with ethyl acetate (2 x 20 mL). The combined organic extracts were dried over magnesium sulfate and filtered before the volatiles were removed at reduced pressure to yield the title compound as a white solid (0.24 g, 1.56 mmol, 90%). M.p. 110 ^{o}C -112 ^{o}C (Sublimes).

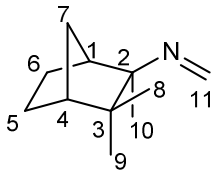
IR υ_{max} (cm^{-1}): 3387, 2953, 2870, 1464, 1386, 1258, 1127, 806, 743.

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 0.93 (3H, s, H8), 1.00 (3H, s, H9), 1.07-1.13 (4H, m, H10, H7a), 1.23-1.33 (1H, m, H5a), 1.33-1.43 (1H, m, H6a), 1.50-1.66 (2H, m, H5b, H6b), 1.71-1.76 (1H, m, H1), 1.80-1.85 (1H, m, H4), 1.91-1.97 (1H, m, H7b).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 22.8 (C10), 23.0 (C6), 23.1 (C8), 23.6 (C5), 25.9 (C9), 34.0 (C7), 42.5 (q, C3), 49.9 (C1), 52.1 (C4), 59.5 (q, C2).

HRMS: (*m/z* - EI) Calculated for $C_{10}H_{19}N$ $(M)^+$ 153.1517, found 153.1510.

2-*endo*-3,3-Trimethyl-*N*-methylenebicyclo[2.2.1]heptan-2-amine (15)



General Procedure E

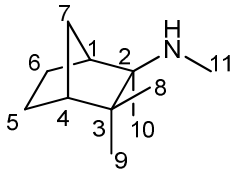
To a solution of 2-*endo*-,3,3-trimethyl-bicyclo[2.2.1]heptan-2-amine (1.00 g, 6.54 mmol) in anhydrous DCM (20 mL), under an argon atmosphere, was added paraformaldehyde (1.18 g, 39.25 mmol) and 4Å molecular sieve pellets (1 g). The reaction mixture was heated at reflux under an argon atmosphere until NMR indicated complete formation of the imine (*ca*. 12 h). After this time, the molecular sieves and any unreacted paraformaldehyde were removed by filtration. The residue was washed with DCM (40 mL). The washings were combined and the solvent evaporated at reduced pressure to yield the title compound as a clear oil (0.98g, 91%) that was used in the next step without further purification.

IR υ_{max} (cm^{-1}): 2934, 2867, 1647, 1460, 1095, 937, 782, 1386, 1290, 1075, 971, 878

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 0.88 (s, 3H, H9), 0.94 (s, 3H, H8), 1.06 (s, 3H, H10), 1.09-1.13 (m, 1H, H7a), 1.32-1.40 (m, 1H, H5a), 1.42-1.50 (m, 1H, H6a), 1.53-1.63 (m, 1H, H6b), 1.65-1.74 (m, 1H, H5b), 1.77-1.82 (m, 1H, H4), 2.02-2.12 (m, 2H, H1, H7b), 7.32-7.45 (m, 2H, H11).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 18.3 (CH_3, C10), 23.1 (CH_3, C8), 23.3 (CH_2, C6) 23.57 (CH_3, C9), 23.62 (CH_2, C5), 27.6 (CH_3, C9), 33.9 (CH_2, C7), 43.2 (q, C3), 48.7 (CH, C4), 49.7 (CH, C1), 70.1 (q, C2), 146.5 (CH_2, C11).

***N*,2,3,3-tetramethylbicyclo[2.2.1]heptan-2-amine (3)**



General Procedure E

2-*endo*-3,3-trimethyl-*N*-methylenebicyclo[2.2.1]heptan-2-amine (1.00 g, 6.06 mmol) was added to $NaBH_4$ (345 mg, 9.09 mmol) in anhydrous DCM (20 mL), under an argon atmosphere, at -78 ºC. Anhydrous methanol (0.97 mL, 30.6 mmol) was added dropwise and the reaction mixture allowed to warm to room temperature. After 1 hour, water (20 mL) was added and the product was extracted using DCM (2 x 20 mL). The organic extracts were combined and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield the title compound as a clear oil (0.82 g, 81%). The product was dissolved in anhydrous diethyl ether (8 mL) and a solution of hydrogen chloride (2 M in diethyl ether, 4.0 mL, 8.0 mmol) was added, the resultant solid was isolated by filtration and dried under vacuum to yield the expected hydrochloride salt compound as a white solid in quantitative yield. M.p. 239 ºC -241 ºC (decomposes), litt[22] 245 ºC -246 ºC.

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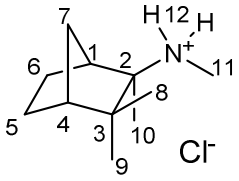
Alkylation via quantitative deprotonation and subsequent alkylation:

A solution of *n*-butyllithium (2.5M in hexanes, 222 µL, 0.56 mmol) in anhydrous THF (2 mL) was prepared and cooled to 0 ºC. To this was added a solution of 2-*endo*-,3,3-trimethylbicyclo[2.2.1]heptan-2-amine (0.081 g, 0.53 mmol) in anhydrous THF (1 mL) dropwise. The reaction mixture was cooled to -78 ºC before freshly distilled iodomethane (34 µL, 0.57 mmol) was added dropwise and the reaction mixture allowed to warm to room temperature over 30 minutes. Water (10 mL) was added and the product was extracted with DCM (2 x 20 mL). The organic extracts were combined and dried over $MgSO_4$. The solvent was evaporated at reduced pressure to yield the title compound as a clear oil (0.44 g, 49 %).

N,2-*endo*-,3,3-Tetramethylbicyclo[2.2.1]heptan-2-amine (3)

IR υ_{max} (cm^{-1}): 2930, 2830, 1450, 1410, 1255, 1110
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 0.95 (s, 3H, H9), 1.01-1.05 (m, 4H, H8, H7a), 1.06 (s, 3H, H10), 1.23-1.32 (m, 1H, H5a), 1.33-1.47 (m, 2H, H6), 1.52-1.65 (m, 1H, H5b), 1.65-1.70 (m, 1H, H1), 1.82-1.90 (m, 1H, H7b), 2.17-2.22 (m, 1H, H4), 2.31 (s, 3H, H11).
^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 17.5 (CH_3, C10), 22.9 (CH_2, C6), 23.1 (CH_3, C9), 23.7 (CH_2, C5), 25.1 (CH_3, C8), 29.7 (CH_3, C11), 33.9 (CH_2, C7), 43.4 (q, C3), 44.5 (CH, C4), 50.0 (CH, C1), 63.3 (q, C2).
HRMS: (*m/z* - ES) calcd. for $C_{11}H_{22}N$ $(M+H)^+$ 168.1747, found 168.1752.

N,2,3,3-tetramethylbicyclo[2.2.1]heptan-2-aminium chloride (3.HCl)

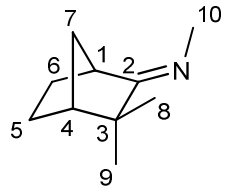


IR υ_{max} (cm^{-1}): 2933, 1594, 1462, 1387, 1112, 1071.
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 1.00 (s, 3H, H9), 1.19-1.25 (d, *J* = 12.3 Hz, 1H, H7a), 1.28 (s, 3H, H8), 1.30-1.37 (m, 1H, H5a), 1.38-1.50 (m, 5H, H10, H6), 1.51-1.61 (m, 1H, H5b), 1.82-1.85 (m, 1H, H4), 2.34-2.43 (m, 2H, H1, H7b), 2.59-2.69 (m, 3H, H11), 8.40 (bs, 1H, H12), 9.05 (bs, 1H, H12).
^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 16.1 (CH_3, C10), 22.9 (CH_2, C6), 23.3 (CH_3, C9), 23.4 (CH_2, C5), 26.2 (CH_3, C8), 28.9 (CH_3, C11), 34.5 (CH_2, C7), 44.6 (q, C3), 44.9 (CH, C4), 50.5 (CH, C1), 70.1 (q, C2).
HRMS: (*m/z* - ES) calcd. for $C_{11}H_{22}N$ M^+ 168.1747, found 168.1741.

70

N-(3,3-Dimethylbicyclo[2.2.1]heptan-2-ylidene) methanamine (16).

71



72

73

Methylamine hydrochloride (640 mg, 9.48 mmol) was ground into a fine powder and heated to 100 ºC under high vacuum to remove all traces of water. A reflux condenser was fitted to the flask and the system flushed with argon and a thick septum added. 1,8-Diazabicyclo[5.4.0]undec-7-ene (19.24 mmol, 1.406 mL, 1.434 g) was added and the reaction mixture stirred for 5 minutes. Triethylamine (4.545 mL, 3.30 g, 32.61 mmol) was added. Titanium tetrachloride solution (1 M in DCM, 3.60 mL, 3.60 mmol) was added and the reaction was stirred for 20 minutes at 40 ºC. 3,3-dimethylbicyclo[2.2.1] heptan-2-one (1.00 g, 7.25 mmol) was added slowly and the reaction mixture turned from bright red to dark brown. After stirring at 40 ºC for 16 hours, the reaction mixture was poured into diethyl ether (200 mL) and filtered thorough celite to remove triethylamine hydrochloride. The organic fraction was dried over $MgSO_4$ and evaporated at reduced pressure to yield the title compound as a clear oil (663 mg, 61%) which was employed in the next step without further purification.

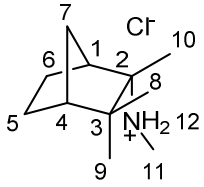
74

IR υ_{max} (cm^{-1}): 2965, 2869, 1686, 1463, 1379, 1105, 1066.
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 1.08 (s, 3H, H9), 1.09 (s, 3H, H8), 1.23-1.33 (m, 1H, H6a), 1.41-1.46 (m, 1H, H7a), 1.53-1.63 (m, 1H, H5a), 1.74-1.88 (m, 3H, H5b, H6b, H7b), 2.08-2.11 (m, 1H, H4), 3.11 (s, 3H, H10), 3.19-3.24 (m, 1H, H1).
^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 22.6 (CH_3, C9), 22.9 (CH_2, C5), 24.6 (CH_2, C6), 24.7 (CH_3, C8), 35.4 (CH_2, C7), 38.3 (CH_3, C10), 40.1 (CH, C1), 44.3 (q, C3), 46.8 (CH, C4), 189.8 (q, C2).
HRMS: (*m/z* - ES) calcd. for $C_{10}H_{18}N$ $(M+H)^+$ 152.1439, found 152.1444.

75

N,2,3,3-tetramethylbicyclo[2.2.1]heptan-2-aminium chloride (17.HCl).

76



77

78

N-(3,3-Dimethylbicyclo[2.2.1]heptan-2-ylidene) methanamine (250 mg, 1.66 mmol) was placed in a RBF with a stirring bar in the absence of any solvent under an argon atmosphere. To this was added boron trifluoride diethyl etherate (316 µL, 354 mg, 2.49 mmol) dropwise and a crystalline solid formed. The reaction mixture was cooled to 0 ºC and methyllithium solution (1.6 M in diethyl ether, 10.00 mL, 16.00 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 1 hour. Ammonium hydroxide solution (30% in water, 10 mL was added and the product extracted with diethyl ether (2 x 10 mL), washed with brine (10 mL) and the combined organic extracts dried over $MgSO_4$. The volatiles were removed at reduced pressure to yield the desired amine as a viscous oil. This product was redissolved in anhydrous diethyl ether (2 mL) and a solution of hydrogen chloride (2 M in diethyl ether, 1.0 mL, 2.0 mmol) was added. The hydrochloride salt was isolated by filtration and dried under vacuum to yield the title compound as a white solid (191 mg, 56%). M.p. 140 ºC -145 ºC (decomposes).

79

IR υ_{max} (cm^{-1}): 3358, 2965, 1457, 1381, 1341, 1081, 912.
^{1}H NMR (d_6-DMSO, 400 MHz): δ (ppm) 1.00 (s, 3H, H8), 1.11-1.23 (m, 4H, H7a, H9), 1.24 (s, 3H, H10), 1.30-1.47 (m,

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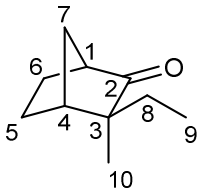
DOI: 10.1039/C6OB01974A

2H, H5a, H6a), 1.50-1.68 (m, 2H, H5b, H6b), 1.73-1.77 (m, 1H, H4), 1.82-1.89 (m, 1H, H7b), 2.14-2.18 (m, 1H, H1), 2.47 (t, *J* = 5.34 Hz, 3H, H11), 7.91 (br s, 1H, H12a), 9.25 (br s, 1H, H12b).
^{13}C NMR (d_6-DMSO, 100 MHz): δ (ppm) 19.1 (CH_3, C10), 21.8 (CH_3, C9), 21.8 (CH_2, C6), 23.4 (CH_2, C5), 27.5 (CH_3, C8), 28.9 (CH_3, C11), 34.7 (CH_2, C7), 42.9 (q, C3), 46.4 (CH, C1), 49.7 (CH, C4), 68.4 (q, C2).
HRMS: (*m/z* - ES) calcd. for $C_{11}H_{22}N$ (M^+) 168.1752, found 168.1751.

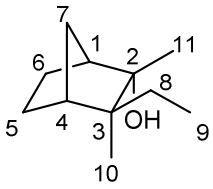
3-*exo*-Ethyl,3-methylbicyclo[2.2.1]heptan-2-one (18)



Prepared as per general Procedure A using 3-*exo*-methylbicyclo[2.2.1]heptan-2-one (1.76 g, 14.20 mmol), 1 M sodium bis(trimethylsilyl)amide in THF (21.3 mL, 21.3 mmol), THF 25 mL and iodoethane (3.41 mL, 6.65 g, 42.62 mmol) to yield the title compound as a clear oil. (1.75 g, 81%).

IR υmax (cm^{-1}): 2960, 2876, 1739, 1460, 1083, 938, 850.
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 0.89 (t, *J* = 7.2 Hz, 3H, H9), 0.97 (s, 3H, H10), 1.33-1.53 (m, 4H, H6a, H7a, H8), 1.56-1.66 (m, 1H, H5a), 1.68-1.77 (m, 1H, H5b), 1.79-1.90 (m, 1H, H6b), 1.93-2.01 (m, 1H, H7b), 2.36 (br s, 1H, H4), 2.52-2.57 (m, 1H, H1).
^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 8.4 (CH_3, C10), 17.9 (CH_3, C9), 23.2 (CH_2, C5), 25.2 (CH_2, C6), 34.8 (CH_2, C7), 42.8 (CH, C4), 50.1 (q, C3), 50.2 (CH, C1), 223.3 (q, C2).
HRMS: (*m/z* - ES) calcd. for $C_{10}H_{17}O$ (M^+) 153.1279, found 153.1279.

3-*exo*-Ethyl-2-*exo*-,3-dimethylbicyclo[2.2.1]heptan-2-ol (19)

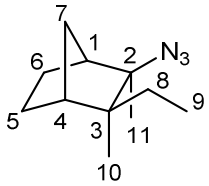


Prepared as per general procedure B using 3-*exo*-ethyl,3-methylbicyclo[2.2.1]heptan-2-one (4.25g, 27.92 mmol), methyllithium (1.6M in diethylether, 34.9 mL, 55.84 mmol) and THF (80 mL) to yield the title compound as a clear oil. (4.32 g, 92%).

IR υmax (cm-1): 3455, 2953, 2875, 1454, 1370, 1292, 1199, 1148, 1003, 899, 876
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 0.86 (t, *J* = 7.50Hz, 3H, H9), 0.89 (s, 3H, H10), 1.11-1.16 (m, 1H, H7a), 1.25 (s, 3H, H11), 1.26-1.36 (m, 2H, H5a, H6a), 1.38 (q, *J* = 7.5 Hz, 2H, H8), 1.60-1.69 (m, 2H, H7b, H6b), 1.81-1.90 (m, 1H, H5b), 1.94-1.98 (m, 1H, H4), 2.02-2.06 (m, 1H, H1).
^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 9.7 (CH_3, C9), 17.0 (CH_3, C10), 20.6 (CH_2, C5), 23.7 (CH_2, C6), 25.3 (CH_3, C11), 29.5 (CH_2, C8), 34.1 (CH_2, C7), 43.7 (CH, C4), 44.4 (q, C3), 50.7 (CH, C1), 79.2 (q, C2).

HRMS: (*m/z* - CI) calcd. for $C_{11}H_{21}O$ $(M+H)^+$ 169.1592, found 169.1592.

2-Azido-3-*exo*-ethyl-2-endo-,3-dimethylbicyclo[2.2.1] heptane (20)

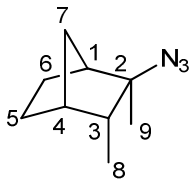


Prepared as per general procedure C using 3-*exo*-ethyl-2-*exo*-,3-dimethylbicyclo[2.2.1]heptan-2-ol (500 mg, 2.98 mmol), 50% H_2SO_4 (10 mL), NaN_3 (2.40 g, 36.9 mmol) and CHCl3 (50 mL) with a reaction time of 8 hours to yield 2-azido-3-*exo*-ethyl-2,3-dimethylbicyclo[2.2.1]heptane as a clear oil (304 mg, 53%) and a related azide tentatively assigned as 2-azido-2-endo-,3-dimethylbicyclo[2.2.1]heptane as a clear oil (140 mg, 28%).

2-azido-3-*exo*-ethyl-2-endo-,3-dimethylbicyclo[2.2.1]heptane (**20**):

IR υmax (cm^{-1}): 2917, 2849, 2087, 1463, 1378, 1131.
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 0.87 (s, 3H, H10), 0.88 (t, *J* = 7.38 Hz, 3H, H9), 1.11-1.14 (m, 1H, H7a), 1.28-1.35 (m, 4H, H5a, H11), 1.39-1.60 (m, 5H, H8, H6, H5b), 1.94-1.97 (m, 1H, H4), 1.98-2.02 (m, 1H, H7b), 2.15-2.18 (m, 1H, H1).
^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 10.1 (CH_3, C9), 17.7 (CH_3, C11), 19.3 (CH_3, C10), 23.0 (CH_2, C6) 24.2 (CH_2, C5), 30.7 (CH_2, C8), 34.9 (CH_2, C7), 46.1 (CH, C4), 46.5 (q, C3), 48.7 (CH, C1), 73.7 (q, C2).
HRMS: (*m/z* - ES) calcd. for $C_{11}H_{20}N$ $(M+H-N_2)^+$ 166.1596, found 166.1596.

2-Azido-2-*endo*-,3-dimethylbicyclo[2.2.1]heptane (**21**):



IR υ_{max} (cm^{-1}): 2965, 2879, 2082, 1458, 1261, 1111, 1079, 741.
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 0.92 (d, *J* = 7.41 Hz, 3H, H8), 1.25 (s, 3H, H9), 1.26-1.48 (m, 5H, H7a, H5, H6), 1.77-1.83 (m, 1H H7b), 1.91-2.00 (m, 1H, H3), 2.11-2.17 (m, 2H, H4, H1).
^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 12.13 (CH_3, C8), 17.6 (CH_3, C9), 20.1 (CH_2, C5), 24.0 (CH_2, C6), 37.2 (CH_2, C7), 42.7 (CH, C4), 44.6 (CH, C3), 48.0 (CH, C1), 69.6 (q, C2).

3-*exo*-Ethyl-2-*endo*-,3-dimethylbicyclo[2.2.1]heptan-2-amine (22)

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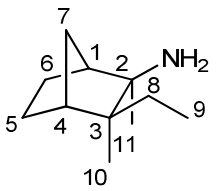


100
101

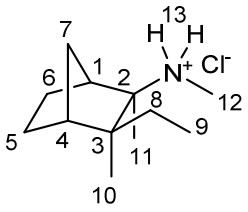
Prepared as per general procedure D using 2-azido-3-*exo*-ethyl-2-*endo*-,3-dimethylbicyclo[2.2.1]heptane (150 mg, 0.78 mmol), lithium aluminium hydride solution (2 M in THF, 500 µL, 1 mmol) and anhydrous THF (5 mL) to yield the title compound as a clear oil (110 mg, 84%).

102

IR υ_{max} (cm^{-1}): 2960, 2937, 2874, 1463, 1379, 805.
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 0.83 (s, 3H, H10), 0.86 (t, *J* = 7.31 Hz, 3H, H9), 1.02-1.10 (m, 4H, H11, H7a), 1.21-1.58 (m, 6H, H5, H6, H8), 1.73-1.79 (m, 1H, H1), 1.82-1.90 (m, 1H, H7b), 1.92 (br s, 1H, H4).
^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 10.6 (CH_3, C9), 19.3 (CH_3, C10), 23.6 (CH_3, C11), 23.7 (CH_2, C6), 23.9 (CH_2, C5), 29.7 (CH_3, C8), 34.4 (CH_2, C7), 45.2 (q, C3), 45.5 (CH, C4), 52.7 (CH, C1), 60.6 (q, C2).
HRMS: (*m/z* - ES) calcd. for $C_{11}H_{22}N$ $(M+H)^+$ 168.1752, found 168.1751.

3-ethyl-*N*,2,3-trimethylbicyclo[2.2.1]heptan-2-aminium chloride (24.HCl)

103



104
105

Prepared as per general procedures E and F using 3-*exo*-ethyl-2-*endo*-,3-dimethylbicyclo[2.2.1]heptan-2-amine (120 mg, 0.62 mmol), paraformaldehyde (70 mg, 2.33 mmol), molecular sieves (200 mg), CH_2Cl_2 (5 mL) and sodium borohydride (110 mg, 2.91 mmol) to yield the desired amine as a viscous oil. This oil was dissolved in anhydrous diethyl ether (1.0 mL) and hydrogen chloride solution (2 M in diethylether, 800 µL, 1.6 mmol) was added. The desired HCl salt was obtained by filtration and dried under vacuum to yield a white solid (80 mg, 59%).

106

IR υ_{max} (cm^{-1}): 3359, 2953, 1457, 1370, 1150, 1112, 914, 892.
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 0.94 (t, *J* = 7.0 Hz, 3H, H9), 0.98 (s, 3H, H10), 1.5 (d, *J* = 11.4 Hz, 1H, H7a), 1.34-1.60 (m, 7H, H5, H6, H11), 1.78-1.90 (m, 1H, H8a), 2.03-2.14 (m, 1H, H8b), 2.20-2.23 (m, 1H, H4), 2.35 (d, *J* = 11.4 Hz, 1H, H7b), 2.39-2.43 (m, 1H, H1), 2.69 (br s, 3H, H12), 8.40 (br s, 1H, H13a), 9.00 (br s, 1H, H13b).
^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 10.0 (CH_3, C_9), 16.0 (CH_3, C11), 19.1 (CH_3, C10), 23.1 (CH_2, C5), 23.4 (CH_3, C6), 27.9 (CH_2, C8), 29.0 (CH_3, C12), 34.5 (CH_2, C7), 44.1 (CH, C4), 44.7 (CH, C1), 47.6 (q, C3), 71.5 (q, C2)
HRMS: (*m/z* - ES) calcd. for $C_{12}H_{24}N$ (M^+) 182.1909, found 182.1911.

Biological testing

Xenopus oocyte expression and electrophysiological recordings

107

Stage V and VI *Xenopus* oocytes were prepared as previously described.[35,36] Wild-type α4 or β2 subunit cDNAs, ligated into the pCI (Promega) expression vector, were dissolved in distilled water at a concentration of 1 µg/µL (spectrophotometric and agarose gel electrophoresis determinations). Mixtures of wild-type α4 and β2 cDNA at 1:1 ratios were injected into the nuclei of oocytes in a volume of 18.4 nL/oocyte, using a Nanoject Automatic Oocyte Injector (Drummond, Broomall, PA, U.S.A.). The total amount of cDNA injected per oocyte was kept constant at 2 ng. After injection oocytes were incubated at 18 °C for 2-5 days in a modified Barth's solution containing 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.3 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 15 mM Hepes and 5 mg/L neomycin (pH 7.6) Recordings were performed 3 - 5 days post-injection.

108 Oocytes were placed in a 0.1 mL recording chamber and perfused with modified Ringer solution (in mM: NaCl 150, KCl 2.8, Hepes 10, $BaCl_2$ 1.8; pH 7.2, adjusted with NaOH) at a rate of 10 mL/min. We chose a nominally Ca^{2+} free solution in order to minimise the contribution to the response of Ca^{2+}-gated chloride channels which are endogenous to the *Xenopus* oocyte and may be activated by Ca^{2+} entry through the nAChRs, as previously reported.[35]
Oocytes were impaled by two agarose-cushioned microelectrodes filled with 3 M KCl (0.5-2.0 MΩ) and voltage-clamped at –60 mV using a Geneclamp 500B amplifier (Axon Instruments, CA, U.S.A.). All experiments were carried out at room temperature. A minimum interval of 4 minutes was allowed between acetylcholine applications as this was found to be sufficient to ensure reproducible recordings. The sensitivity of the receptors to inhibition by the novel nAChR antagonists (3 µM) was tested by first superfusing the antagonist for 2 min and then coapplying it with an EC_{50} of ACh ($\alpha_4\beta_2$ nAChR EC_{50}: 100 µM)

109

SH-SY5Y cells (passages 19 to 28) were cultured in an incubator at 37°C and 5% CO_2.[37] Cells were maintained in Advanced DMEM/F-12 medium supplemented with 2% FBS, 2mM L-glutamine, 190 U/mL penicillin and 0.2mg/mL streptomycin. For assays, cells were plated in 96 well plates (Falcon) and grown under the same culture conditions until confluent before being used.
Ca^{2+} response assay Cells in 96 well plates were washed twice with TSS (137mM NaCl, 2.7mM KCl, 1mM $MgCl_2$, 1.8mM $CaCl_2$, 0.2mM NaH_2PO_4, 12mM $NaHCO_3$, 5.5mM glucose; pH 7.4). Cells were then incubated in TSS with 10µM fluo-3 AM and 0.02% pluronic acid for 1 hour, at room temperature in the dark. Cells were washed twice more with TSS and left in 80µL TSS. For inhibitor pre-incubations, the 80µL TSS was replaced with 80µL TSS containing appropriate drug concentrations. All drugs were pre-incubated at room temperature in the dark 10 for 10 minutes, except for $CdCl_2$ which was pre-incubated for 15 minutes. Four replicate wells were used per condition in each assay. Stimulation drugs, either 100µM (-)-nicotine hydrogen tartrate or 100mM KCl, were added in 20µL TSS and fluorescence was measured (485nm excitation, 538nm emission) using a Fluoroskan Ascent plate reader and Ascent software (Labsystems). Maximum (F_{max}) and minimum (F_{min}) fluorescence values for each well were obtained by taking readings after adding 1% Triton X-100 and 350mM $MnCl_2$ respectively (both in TSS). These measurements were used to calculate F_{max}-F_{min}, and assay results were normalised as a percentage of F_{max}-F_{min}.