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Graphical Abstract

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The preparation of novel histrionicotoxin analogues and their activity towards the α4β2 and α7 nicotinic acetylcholine receptors

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Cecily Eldridge[a], Gracia Quek[b], Michael Sako[b], John H. Ryan[a,*], Simon Saubern[a], Mary Chebib[b,*], and James M. Macdonald[a,*]

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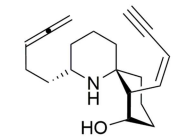
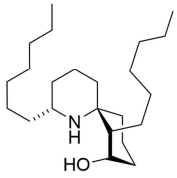
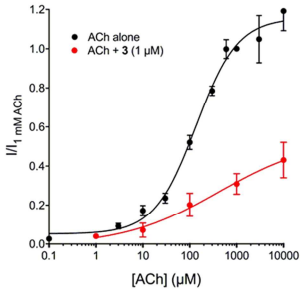


(–)-histrionicotoxin 285A



3
(+/–)-histrionicotoxin analogue



α4β2 antagonist activity

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The preparation of novel histrionicotoxin analogues and their activity towards the α4β2 and α7 nicotinic acetylcholine receptors

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ABSTRACT

Four histrionicotoxin analogues were prepared in an efficient manner utilizing a nitrone dipolar cycloaddition reaction as the key step in forming tricyclic intermediate **13**. The nitrile in intermediate **13** was reduced with DIBAL to an aldehyde which then underwent *Z*-selective Wittig reactions to produce intermediates containing the *Z*-alkene side-chain. Hydrogenation of the *Z*-alkenes produced saturated histrionicotoxin analogues whereas reduction with SmI_2 afforded the unsaturated histrionicotoxin analogues. The histrionicotoxin analogues were shown to be potent non-competitive antagonists of the α4β2 and α7 nAChR's with the most potent analogue **3** displaying IC_{50}'s of 0.10 μM and 0.45μM against the α4β2 and α7 nAChR's, respectively. The unsaturated analogues **15** and **18** displayed Hill slope (n_H) of approximately 1 whilst the saturated analogues **16** and **3** had a n_H of approximately 0.5, which may indicate that the saturated analogues are binding to more than one binding site.

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1. Introduction

The nicotinic acetylcholine receptor (nAChR) is a member of the ligand-gated ion channel family that mediate fast synaptic transmission of cations between cells both in the central (neuronal type) and peripheral (muscle) nervous systems.[1] These receptors are a pentameric protein assembly that can be formed from various subunit proteins which mix and match to form either at neuromuscular junction or in mammal brain.[1] In this study, we focused on the neuronal α4β2 and α7 nAChR's as these subtypes are most widely expressed and are implicated in processes as diverse as cognition, consciousness, mood, nicotine addiction, and nociception among others.[2]

Since their discovery,[3] the histrionicotoxins such as 285A **1** isolated from the poison arrow frog *Dendrobates histrionicus* as well as the unnatural perhydrohistrionicotoxin **2** have inspired a wealth of synthetic approaches and total syntheses.[4] In addition to these challenging synthetic targets possessing an intriguing molecular architecture, the histrionicotoxins are also potent inhibitors of nAChR.[5] Over the years, there have been some biological and toxicity studies of histrionicotoxins but these were limited to animal studies,[3] although some pharmacological studies were performed on nAChRs with frog nerve preparations[6] and on *Torpedo* electroplax membranes.[7] To date, there have not been any functional studies of histrionicotoxins on any recombinant nAChRs. This paper seeks to prepare novel unnatural histrionicotoxin variants such as compound **3** and test their activity toward the neuronal α4β2 and α7 nAChR's.

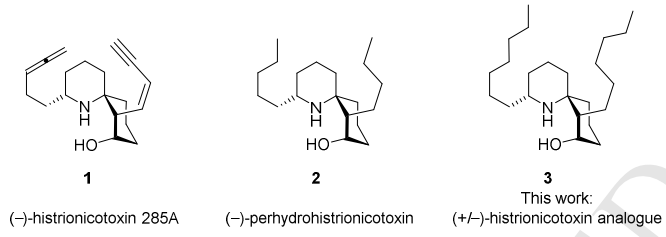
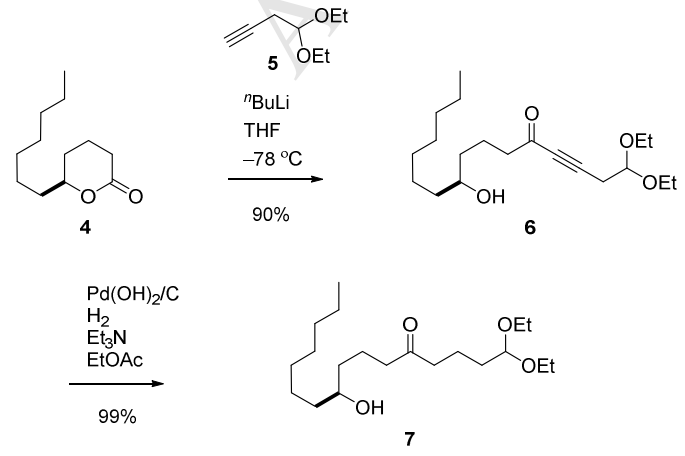


Figure 1. Structures of histrionicotoxins.

2. Results and discussion

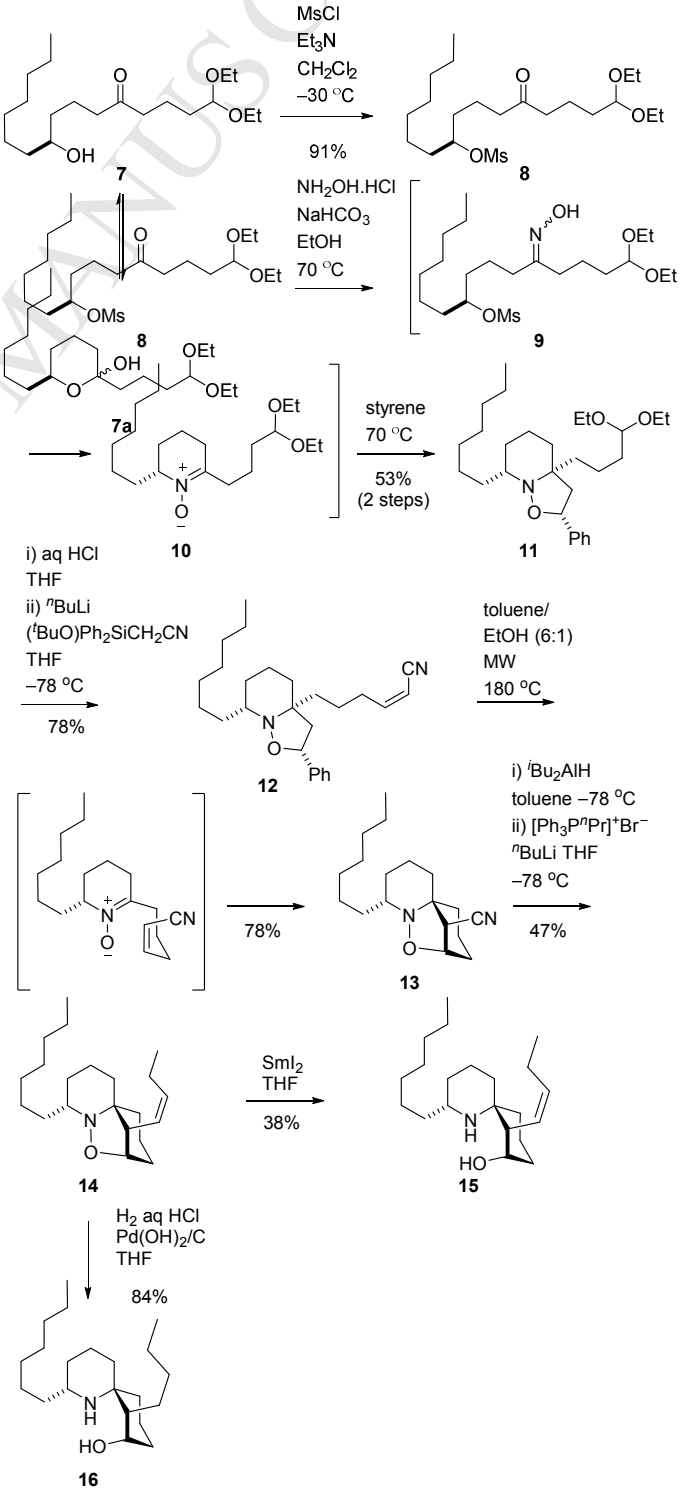
One synthetic strategy to generate histrionicotoxins involved a nitrone dipolar cycloaddition reaction as the key step[8] and was also utilized to successfully synthesize histrionicotoxins **1**[9] and **2**.[9,10] The efficiency and potential for late-stage divergency of these chemistries formed the inspiration to prepare novel analogues such as **3**.

Racemic lactone **4** was chosen as the starting material, being readily available and already containing the 7-carbon alkyl chain that would ultimately be reflected in the final product. The lithium acetylide of alkyne **5** efficiently opened lactone **4** to furnish the alkynone **6** in excellent yield. Next, catalytic hydrogenation gave the saturated derivative **7** (Scheme 1. Relative stereochemistry is depicted throughout).



Scheme 1. Preparation of intermediate 7.

The next step was to activate the alcohol of compound **7** as a good leaving group. ^{1}H NMR spectra obtained for compound **7** revealed the four protons adjacent to the carbonyl significantly under-integrated to what would be expected for the product **7** and consistent with compound **7** being in equilibrium with its hemi-ketal isomer **7a** in an approximate ratio of (**7**/**7a**) 4:1. Indeed, an attempted mesylation (0 °C) of this isomeric mixture gave the desired, somewhat unstable mesylate in 74% yield with another unstable product being isolated but not characterized (presumably the mesylate product of isomer **7a**). We reasoned that the reaction rate of mesylation of a secondary alcohol relative to the hydroxyl of a hemi-ketal should be faster. Gratifyingly, repeating this mesylation procedure at –30 °C resulted in just a single product;



the desired mesylate isomer **8** in excellent yield (Scheme 2). 4).

Scheme 2. Preparation of intermediate **8**.

Treatment of mesylate **8** with hydroxylamine accomplished the formation of intermediate oxime **9** and then *N*-nucleophilic displacement of the mesylate to form the cyclic nitrone **10**. The nitrone was isolated and immediately treated with styrene which underwent a 1,3-dipolar cycloaddition reaction to effectively protect the nitrone functionality as its styrene adduct; isoxazolidine **11**. The next step involved elaboration of the diethyl acetal of **11** to a *Z*-α,β-unsaturated nitrile functionality. This was achieved by acid catalyzed hydrolysis of the diethyl acetal to liberate the aldehyde followed by olefination employing the modified Peterson reagent as described by Kojima *et. al.*[11] It is noteworthy that a simple modification to the preparation of this important reagent (using two equivalents of lithium diisopropyl amide instead of one) resulted in a superior, and more predictable, yield of *tert*-butoxydiphenylsilylacetonitrile (80% vs previously reported 62%; see experimental section). Careful chromatography separated the desired *Z*-isomer from trace amounts of the *E*-isomer. In this manner, *Z*-α,β-unsaturated nitrile **12** was prepared in 78% yield from diethylacetal **11**. As described previously,[8-10] simply heating compound **12** resulted in the following reaction cascade; a 1,3-dipolar cycloreversion (extrusion of styrene) followed by an intramolecular 1,3-dipolar cycloaddition reaction between the liberated nitrone and α,β-unsaturated nitrile functionalities (under thermodynamic control) to generate tricycle **13**. The nitrile functionality of tricycle **13** was reduced to the aldehyde and then Wittig olefination gave exclusively the *Z*-alkene **14**. The isoxazolidine moiety of **14** could be selectively reduced under Brandi (SmI_2) conditions[12] to give rise to unnatural histrionicotoxin derivative **15**. Alternatively, catalytic hydrogenation gave the fully reduced variant; histrionicotoxin analogue **16** (Scheme 3).

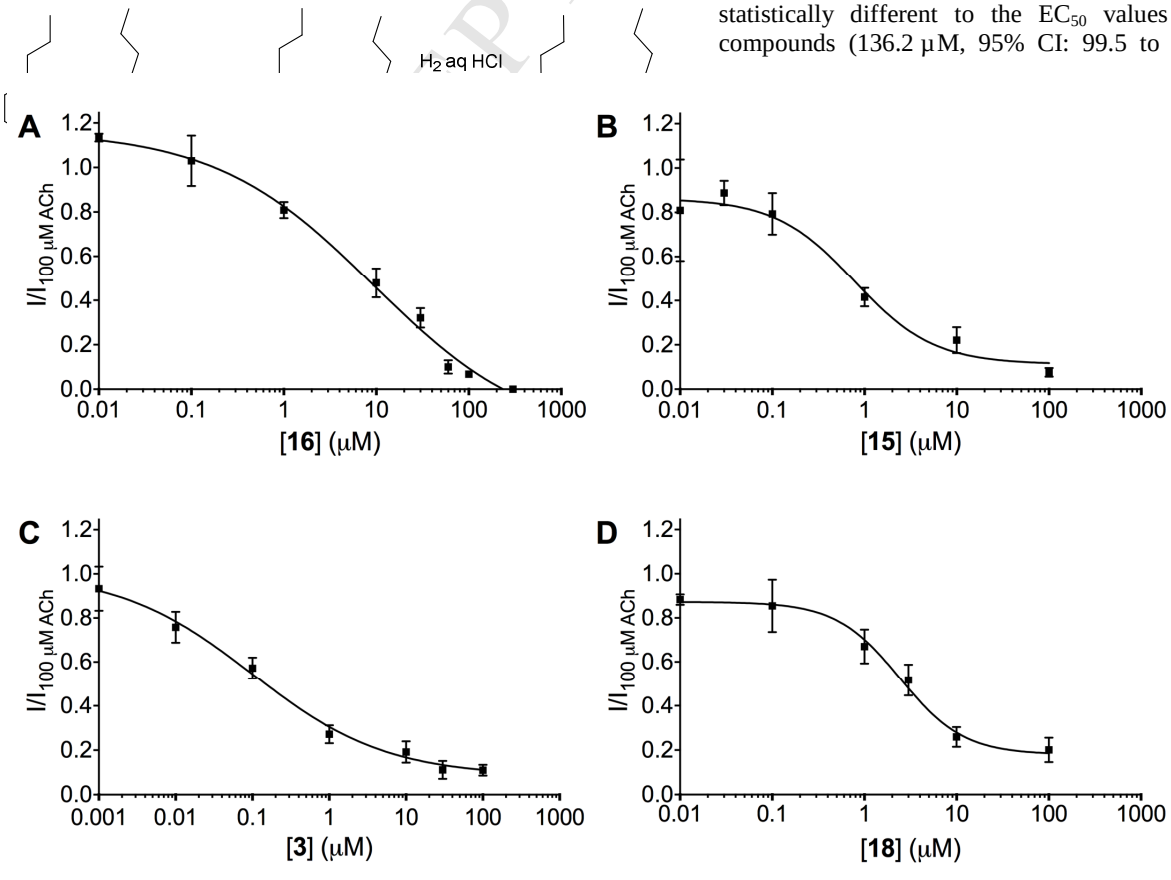
Scheme 3. Preparation of histrionicotoxin analogues **15** and **16**.

Additionally, two further unnatural histrionicotoxin analogues **18** and **3** were prepared from tricycle **13**. Relative to the majority of histrionicotoxins found in Nature,[3] these latter analogues contain “arms” elongated with two carbon atoms each (Scheme

Scheme 4. Preparation of histrionicotoxin analogues **18** and **3**.

The effects of **16**, **15**, **3** and **18** were evaluated on rat α4β2 nAChRs recombinantly expressed in *Xenopus* oocytes using 2-electrode voltage clamp electrophysiology. Neither **16**, **15**, **3** or **18** activated the α4β2 nAChRs on their own indicating that they are neither agonists nor partial agonists (data not shown). We subsequently evaluated the compounds for antagonist effect. In order to evaluate antagonist effects, the compounds were pre-incubated for 3 mins before being co-applied with ACh in order to give them time to access their binding site. In the presence of 100 µM ACh, the ACh induced currents were reduced or blocked, indicating that **16**, **15**, **3** and **18** were antagonists. Inhibitory concentration response curves were constructed with increasing concentrations of **16**, **15, 3** and **18** in the presence of 100 µM ACh and the resulting currents normalised to 100 µM ACh alone (Figure 1). **15** was more potent than **16** (IC_{50} = 9.38 µM; 95% CI: 3.30 to 26.75 µM) while **3** (IC_{50} = 0.10 µM; 95% CI: 0.03 to 0.30 µM) was more potent than **18.** The order of potency is as follows: **3** > **15** > **18** > **16.** Table 1 summarises the data. Interestingly the Hill slope (n_H) differed for saturated vs unsaturated histrionicotoxins. Unsaturated **15** and **18** displayed a n_H of approximately 1 while the saturated analogues **16** and **3** had a n_H of approximately 0.5 (Table 2). A shallow n_H i.e a slope < 1 may indicate that histrionicotoxins are binding to more than one site. Indeed different histrionicotoxin analogues have been reported to bind to more than one site.[7]

We then individually evaluated the effect of a fixed concentration of the histrionicotoxins in the presence of varying concentrations of ACh. Histrionicotoxins **16** (30 µM), **15** (1 µM), **3** (1 µM) and **18** (1 µM) all significantly reduced the maximal current elicited by a saturating concentration of ACh such that the maximal current was reduced from 116% (95% CI: 106 to 126) in the absence of histrionicotoxin to approximately 45% in the presence of histrionicotoxin (F-test on n_H; $p<0.05$) at the α4β2 nAChR (Example shown in Figure 2 with **3**). The reduction in the maximal current indicates that high concentrations of ACh cannot compensate for the antagonist effect. In addition, the EC_{50} values of ACh in the presence of **16**, **15**, **3** and **18** ranged from 62.15 µM to 207.9 µM (Table 2), but this change was not statistically different to the EC_{50} values in the absence of compounds (136.2 µM, 95% CI: 99.5 to 186.4 µM; F-test on



logEC$_{50}$, p>0.05). This pattern is indicative of a non-competitive antagonist that exerts its effects on saturating concentrations of an agonist, inferring that binding of the histrionicotoxins is to an allosteric site, rather than to the ACh or the orthosteric binding site at α4β2 nAChRs. In summary, histrionicotoxins **16**, **15**, **3** and **18** are potent non-competitive antagonists of recombinant α4β2 nAChRs.

Figure 1: Inhibitory concentration response curves of increasing concentrations of **A 16**, **B 15**, **C 3** and **D 18** in the presence of 100 µM ACh at rat α4β2 nAChRs recombinantly expressed in *Xenopus* oocytes. Histrionicotoxins were pre-incubated for 3 mins before co-addition with 100µM ACh. Data are normalised to 100 µM ACh and presented as mean ± SEM (n= 3-8 oocytes; >2 batches of oocytes).

Table 1: Pharmacological data of the inhibitory effects of histrionicotoxins on rat α4β2 nAChRs recombinantly expressed in Xenopus oocytes.

	16	**15**	**3**	**18**
IC$_{50}$ (µM)[a]	9.39	0.78	0.10	2.72
95% CI	3.30 to 26.75	0.13 to 4.75	0.03 to 0.30	0.87 to 8.52
n_H	0.51	1.02	0.49	0.96
95% CI	0.78 to 0.23	3.02 to 0.98	0.79 to 0.19	1.98 to 0.06

[a]IC$_{50}$ is the effective concentration that inhibits 50% of the current response produced by 100 µM ACh. n_H is the Hill slope. Errors are expressed as 95% confidence interval (CI).

Table 2: Pharmacological data of increasing concentrations of ACh alone and in the presence histrionicotoxis on α4β2 nAChRs recombinantly expressed in Xenopus oocytes.

	ACh	**16**	**15**	**3**	**18**
EC$_{50}$ (µM)[a]	136.2	182.6	207.9	94.94	62.15
95% CI	99.5 to 186.4	37.7 to 884.5	55.9 to 773.5	17.7 to 509.0	17.6 to 219.0
I_{Max} (%)	116	46	52	41	51
95% CI	106 to 126	36 to 55	40 to 63	31 to 52	40 to 61

[a]EC$_{50}$ is the effective concentration that activates 50% of the receptors. I_{Max} is the maximum normalised current produced by ACh alone (1 mM). Errors are expressed as 95% confidence interval (CI).

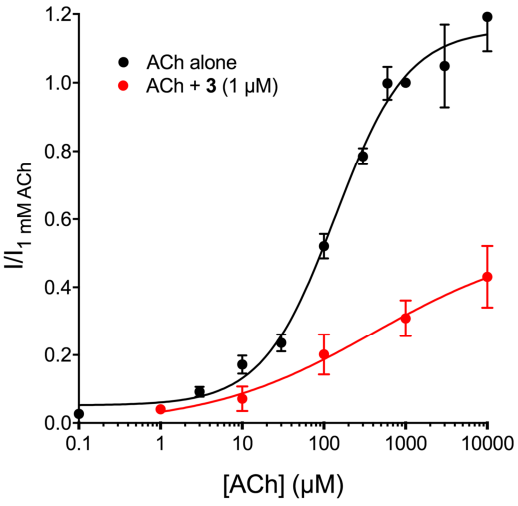


Figure 2. Concentration response curves of increasing concentrations of ACh alone (●) and in the presence of 1 µM **3**(●) on rat α4β2 nAChRs recombinantly expressed in *Xenopus* oocytes. Oocytes were pre-incubated for 3 mins with 1 µM **3** before the the co-application of ACh. Data are normalised to 1mM ACh and presented as mean ± SEM (n= 3-6 oocytes; >2 batches of oocytes).

We then evaluated the most potent histrionicotoxin series, **3** and **18**, on homomeric rat α7 nAChRs. Both **3** and **18** inhibited the current exhibited by an EC$_{50}$ concentration of ACh in a concentration dependent manner. Histrionicotoxin **3** had an IC$_{50}$ of 0.45 µM (95% CI 0.23-0.90 µM; Figure 3A) while **18** had an IC$_{50}$ of 0.62 µM (95% CI 0.10-3.7 µM; Figure 3B). Given that both **3** and **18** had similar potencies on both α4β2 and α7 nAChRs indicates the histrionicotoxins are not very selective and that there is a common binding at both receptor subtypes. In summary, histrionicotoxins are potent antagonists of nAChRs and may act at sites distinct to the ACh binding site.

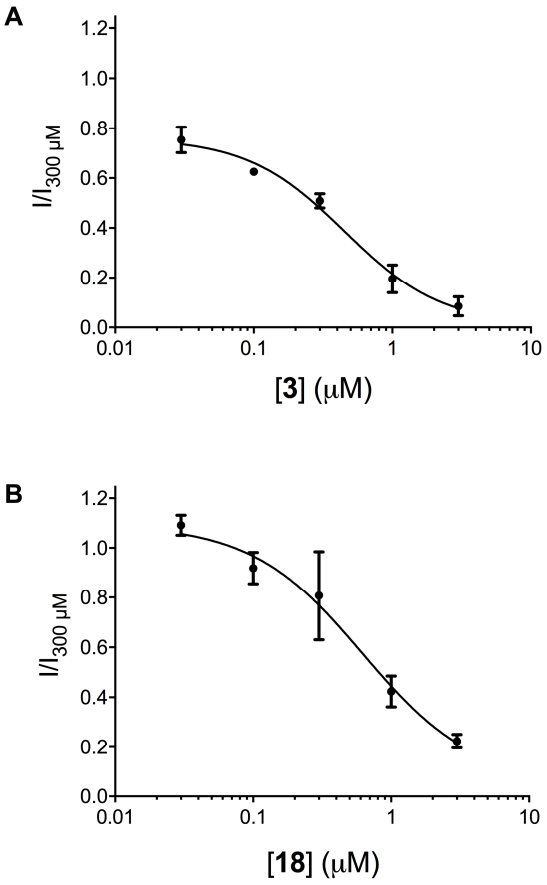


Figure 3. Inhibitory concentration response curves of increasing concentrations of **A 3** and **B 18** in the presence of 300 µM ACh at rat α7 nAChRs recombinantly expressed in *Xenopus* oocytes. Histrionicotoxins were preincubated for 3 mins before co-addition with 300µM ACh. Data are normalised to 300 µM ACh and presented as mean ± SEM (n= 3-8 oocytes; >2 batches of oocytes).

3. Conclusion

Four closely related histinicotoxin analogues were prepared in an efficient manner and shown to be potent non-competitive antagonists of nAChR's. While the unsaturated analogues appear to have one binding site, the saturated analogues would appear to have more than one binding site. Further work is required to elucidate the binding sites and develop structure-activity relationships.

4. Chemistry Experimental Section

4.1. General Experimental

Hexanes refer to the fraction with a boiling point of 40-60 °C. Dry tetrahydrofuran (THF), dichloromethane, and toluene were obtained by passing these solvents through activated alumina columns. Unless otherwise specified, all ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Av400 spectrometer at 400 MHz and 100.6 MHz respectively, or, a Bruker DRX508 spectrometer at 500 MHz and 125.8 MHz respectively, using $CDCl_3$ solutions. Chemical shifts (δ) are measured in ppm. Positive ion EI mass spectra were run on a ThermoQuest MAT95XL mass spectrometer using an ionization energy of 70eV. Accurate mass measurements were obtained with a resolution of 5000-10000 using perfluorokerosene as the reference compound. High resolution positive ion electrospray mass spectra were acquired with a Micromass Q-TOF II mass spectrometer using a cone voltage of 50V and a capillary voltage of 3.0kV. The sample was introduced by direct infusion at a rate of 5µl/min using PEG400 as an internal calibrant. Flash chromatography[13] was carried out using Merck Kieselgel 60 (230-400 mesh; particle size 0.04-0.63 mm) silica gel. Analytical thin layer chromatography (TLC) was conducted on Sigma-Aldrich silica gel coated aluminium sheets and visualised with UV and/or by dipping in a phosphomolybdic S2 acid/EtOH solution and heating at 400 °C. IR analysis was carried out using a Perkin Elmer 2000 FT-IR spectrophotometer, in absorbance mode, using NaCl disks as the background reference. Microwave reactions were carried out in sealed reaction vessels using a Biotage Initiator 2.0 (400W). Melting points were recorded on an Electrothermal IA9300 digital melting point apparatus, and are uncorrected.

*4.2. 4,4-Diethoxybut-1-yne (**5**)*

Alkyne **5** was prepared from propargyl bromide (17.8 g, 0.150 mol) according to the literature procedure.[14] ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.18 (t, J = 7.1 Hz, 6H), 1.98 (t, J = 2.6 Hz, 1H), 2.48 – 2.50 (m, 2H), 3.48 – 3.56 (m, 2H), 3.64 – 3.69 (m, 2H), 4.63 (t, J = 5.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 15.07, 24.69, 61.75, 69.80, 79.58, 100.57.

*4.3. 1,1-diethoxy-9-hydroxyhexadec-3-yn-5-one (**6**)*

4,4-Diethoxybut-1-yne **5** (6.66 g, 44.1 mmol) in dry THF (120 mL) was cooled to −78 °C. ^{n}BuLi (27.7 mL of a 1.59 M solution in hexanes, 44.0 mmol) was added dropwise (2 min), which gave a pale yellow solution, that was stirred (10 min). A solution of δ-dodecalactone **4** (6.99 g, 35.2 mmol) in dry THF (30 mL) was added dropwise (5 min) and the solution was stirred (1 h). The reaction was quenched with saturated aqueous NH_4Cl and allowed to warm to room temperature. This mixture was diluted with EtOAc and H_2O and the organic layer was separated. The aqueous phase was re-extracted (2 times) with EtOAc. The combined organics were washed sequentially with saturated aqueous $NaHCO_3$, saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes, gradient; 10:90, 15:85; 25:75 then 30:70) to yield alkyne **6** (10.8 g 90%) as a clear yellow oil: R_f 0.34 (30:70 EtOAc/hexanes); IR (neat) 3448, 2937, 2858, 2217, 1674, 1457, 1373, 1346, 1228, 1162, 1120, 1063 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 – 0.90 (m, 3H, CH3), 1.20 – 1.25 (m, 6H, 2 X O-CH2-C*H*3), 1.25 – 1.53 (m, 15H), 1.61 (b s, 1H, OH), 1.66 – 1.89 (m, 2H), 2.58 (t, J = 7.2 Hz, 2H,), 2.70 (d, J = 5.6 Hz, 2H), 3.51 – 3.61 (m, 2H) 3.64 – 3.73 (m, 2H), 4.70 (t, J =5.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.02, 15.12, 20.00, 22.58, 25.39, 25.59, 29.22, 29.57, 31.76, 36.47, 37.45, 45.25, 62.08, 71.36, 81.73, 89.25, 100.96, 187.95; HRMS (EI) *m/z* 339.2516. $C_{20}H_{35}NO_4$ [M-1]$^{+\bullet}$ requires 339.2530.

*4.4. 1,1-Diethoxy-9-hydroxyhexadecan-5-one (**7**)*

A mixture of the alkyne **6** (5.25 g, 16.0 mmol) and $Pd(OH)_2$ on carbon (520 mg of 20% Pd catalyst) in EtOAc (200 mL) was shaken vigorously under an atmosphere of H_2 (30 psi, 2 h). The mixture was filtered through Celite™, washing with EtOAc. The combined filtrate and washings were concentrated. The residue was purified by flash chromatography (EtOAc/hexanes, gradient; 15:85 then 40:60) to yield the alkane **7** (5.3 g, 99%) as white waxy solid: R_f 0.19 (30:70 EtOAc/hexanes); IR (neat) 3459, 3010, 2930, 2872, 2858, 1710, 1458,1376, 1216, 1128, 1059, 757, 667 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) *Data for the major isomer* δ 0.83 – 0.88 (m, 3H), 1.18 (t, J = 7.1 Hz, 6H), 1.22 – 1.31 (m, 9H), 1.22 – 1.47 (m, 5H), 1.54 – 1.73 (m, 7H), 2.39 – 2.45 (m, 4H), 3.41 – 3.51 (m, 2H), 3.51 – 3.57 (m, 1H), 3.57 –

3.67 (m, 2H) 4.43 – 4.50 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) *Data for the major isomer* δ14.03, 15.28, 19.03, 19.65, 22.60, 25.61, 29.23, 29.60, 31.77, 33.00, 36.79, 37.44, 42.31, 42.53, 61.05, 71.40, 102.66, 210.99; HRMS (EI) *m/z* 326.2812. $C_{20}H_{38}O_3$ $[M-H_2O]^{+\bullet}$ requires 326.2815.

*4.5. 1,1-Diethoxy-9-methanesulfonylhexa-decan-5-one (**8**)*

To a solution of alcohol **7** (5.00 g, 14.5 mmol) and Et_3N (8.10 mL, 58.1 mmol) in dry CH_2Cl_2 (150 mL) at –30 ºC, was added MsCl (2.25 mL, 29.3 mmol) in dry CH_2Cl_2 (50 mL) dropwise (25 min) and stirred (30 min). The mixture was quenched with aqueous saturated $NaHCO_3$, the organic layer was separated, washed with saturated aqueous NaCl solution, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes, gradient; 10:90 then 30:70) to yield mesylate **8** (5.1 g, 91%) as an unstable, clear, colourless oil: R_f 0.29 (30:70 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.83 – 0.93 (m, 3H, CH_3), 1.19 (t, J =7.1 Hz, 6H, 2 x OCH_2CH_3), 1.23 – 1.43 (m, 10H), 1.55 – 1.72 (m, 10H), 1.98 – 2.46 (m, 4H,CH_2COCH_2), 3.00 (s, 3H, SO_3CH_3), 3.41 – 3.53 (m, 2H, 2 x OCH*H*CH$_3$), 3.57 – 3.66 (m, 2H, 2 x OC*H*HC*H*$_3$) 4.45 – 4.50 (m, 1H, C*H*$(OEt)_2$), 4.64 – 4.73 (m, 1H, C*H*SO_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.03, 15.30, 18.83, 19.00, 22.57, 24.97, 29.06, 29.28, 31.70, 32.99, 33.74, 34.28, 38.66, 41.89, 42.41, 61.09, 83.50, 102.65, 210.10

4.6. (2S,6R*,8R*)-2-(Heptyl)-6-(4',4'-diethoxy-1'-butyl)-8-phenyl-1-aza-9-oxabicyclo[4,3,0]nonane (**11**)*

To the mesylate **8** (5.92 g, 14.0 mmol) in EtOH (250 mL) was added $NaHCO_3$ (8.47 g, 100 mmol) and $NH_2OH{\cdot}HCl$ (3.58 g, 98.1 mmol). This mixture was stirred at room temperature (15 min) and then at 70 ºC (20 h). H_2O was added (250 mL); and then the mixture was concentrated to approximately 250 mL. The mixture was diluted with CH_2Cl_2 and the organic layer was separated. The aqueous phase was re-extracted (4 times) with CH_2Cl_2. The combined organics were washed with aqueous saturated NaCl solution, dried ($MgSO_4$), filtered and concentrated to give, presumably, the somewhat unstable crude nitrone **10** (crude 4.9 g) as an oil that was used immediately: [R_f 0.27 (8:92 EtOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) *data from crude nitrone* δ0.82 – 0.88 (m, 3H, CH_3), 1.18 (t, J = 7.1 Hz, 6H), 1.21 – 1.35 (m, 10H), 1.53 –1.97 (m, 8H), 2.06 – 2.35 (m, 2H), 2.39 (t, J = 6.3 Hz, 2H), 2.44 – 2.59 (m, 2H), 3.43 – 3.52 (m, 2H, 2 x OCH*H*CH_3), 3.57 – 3.65 (m, 2H, 2 x OC*H*HCH_3), 3.65 – 3.73 (m, 1H, C*H*N^+), 4.45 – 4.50 (m, 1H, C*H*$(OEt)_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.03, 15.30, 15.59, 19.78, 22.58, 26.11, 26.44, 28.85, 29.13, 29.41, 31.63, 31.75, 32.36, 33.62, 61.20, 61.34, 67.01, 102.66, 148.26. This oil (4.94 g) was taken up in styrene (100 mL), and hydroquinone (~ 2 mg) was added. The mixture was heated (70 °C) for 4 days and then concentrated. The residue was purified by flash chromatography (EtOAc/hexanes, gradient; 4:96, 5:95, 6:94 then 7:93) to give isoxazolidine **11** (2.9 g, 53% over 2 steps) as a clear pale yellow oil: R_f 0.21 (10:90 EtOAc/hexanes); IR (neat) 3081, 3061, 2928, 2864, 1603, 1494, 1453, 1374, 1127, 1063, 754, 699 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.83 – 0.95 (m, 3H, CH_3), 1.10 – 1.19 (m, 6H, 2 x OCH_2CH_3), 1.20 – 1.32 (m, 10H), 1.34 – 1.72 (m, 11H), 1.78 – 1.89 (m, 2H), 1.89 – 1.97 (m, 1H), 1.98 – 2.03 (dd, J = 12.4, 5.2 Hz, 1H), 2.58 – 2.67 (m, 1H), 2.68 – 2.76 (m, 1H), 3.32 – 3.47 (m, 2H, 2 x OCH*H*CH_3), 3.49 – 3.61 (m, 2H, OC*H*HCH_3), 4.39 (t, J = 5.6, 1H, CH$(OEt)_2$), 5.39 (dd, J = 10.0, 5.2 Hz, 1H, C*H*Ph), 7.20 – 7.40 (m, 5H, Ar); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.07, 15.32, 18.97, 19.99, 22.64, 25.81, 29.31, 29.48, 29.93, 31.28, 31.87, 33.96, 34.69, 41.58, 42.25, 59.37, 60.74, 61.04, 67.86, 77.20, 102.66, 126.00, 127.00, 128.29, 142.03 ; HRMS (EI) *m/z* 445.3552. $C_{28}H_{47}NO_3$ requires 445.3550.

4.7. ^{t}Butoxychlorodiphenylsilane Kojima et. al.[11]

(^{t}BuO)Ph_2SiCl was prepared according to the literature procedure.[11] Ph_2SiCl_2 (15.0 g, 59.2 mmol) was converted to (^{t}BuO)Ph_2SiCl and distilled to a clear colourless oil (12.2 g, 76%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.39 (s, 9H), 7.37 – 7.48 (m, 6H), 7.70 – 7.74 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 31.70, 31.99, 76.37, 127.31, 127.87, 130.53, 134.30, 134.86, 135.17.

4.8. (^{t}Butoxydiphenylsilyl)acetonitrile.[11]

To a solution of ^{i}Pr$_2$NH (9.12 mL, 64.5 mmol) in dry THF (180 mL) at 0 °C, ^{n}BuLi (38.7 mL of a 1.59 M solution in hexanes, 61.5 mmol) was added dropwise (2 min) which gave a clear yellow solution that was stirred (5 min). MeCN (1.70 mL, 32.3 mmol) was added dropwise (1 min) giving a pale purple solution that was stirred (30 min). (^{t}BuO)Ph_2SiCl (8.94 g, 30.7 mmol) in THF (70 mL) was added dropwise (2 min), which gave a bright yellow clear solution. The solution was stirred (7 min) at 0 °C and then allowed to warm to room temperature and stirred (2.5 h). The reaction was then quenched with saturated aqueous NH_4Cl. The organic layer was separated and the aqueous phase was re-extracted with Et_2O. The combined organics were dried ($MgSO_4$), filtered and concentrated. The residue was purified by silica gel funnel chromatography (CH_2Cl_2/hexanes, gradient; 10:90 then 40:60), to give (^{t}BuO)Ph_2SiCH_2CN (7.3 g, 80%) as white solid: mp 54 °C (lit.[11] 56°C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.30 (s, 9H), 2.15 (s, 2H), 7.36 – 7.51 (m, 6H), 7.51 – 7.52 (m,4H) ; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 5.66, 31.92, 75.12, 118.40, 128.11, 130.67, 133.65, 134.60.

4.9. (2S,6S*,8R*)-2-(Heptyl)-6-(5'-cyanopent-4'-en-1'-yl)-8-phenyl-1-aza-9-oxabicyclo[4,3,0]nonane (**12**)*

To diethylacetal **11** (1.71 mg, 3.84 mmol) in THF (50 mL) was added aqueous HCl (5.00 mL of 2.00 M solution) and the solution was stirred (1.5 h). The reaction was quenched with saturated aqueous $NaHCO_3$ and diluted with EtOAc. The organic layer was separated, the aqueous phase was re-extracted (2 times) with EtOAc. The combined organics were washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated. The residue was purified by silica gel funnel chromatography (EtOAc:hexanes, gradient; 25:75) to give a clear yellow oil, presumably the aldehyde, that was used immediately. Meanwhile, to a solution of (^{t}BuO)Ph_2SiCH_2CN (1.39 mg, 4.71 mmol) in dry THF (80 mL) at –78 °C was added ^{n}BuLi (2.96 mL of 1.59 M solution in hexanes, 4.71 mmol) dropwise (~1 min). This mixture was allowed to warm to 0 °C and kept at this temperature for 10 min, and then cooled to –78 °C. To this mixture was added the crude aldehyde in dry THF (30 mL) dropwise (8 min). The reaction was stirred (1 h) and quenched with saturated aqueous NH_4Cl and allowed to warm to rt. The organic layer was separated and the aqueous phase was re-extracted with EtOAc (2 times). The combined organics were washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography (EtOAc:hexanes, gradient; 1:99, 2:98, 3:97, 4:96 then 7:93) to yield nitrile **12** (1.2 g, 78%) as a clear yellow oil: R_f 0.21 (10:90 EtOAc/hexanes); IR (neat) 3063, 3029, 2927, 2857, 2219, 1685, 1494, 1456, 1375, 1074, 1028, 753, 700m cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ]0.84 – 0.90 (m, 3H, CH_3), 1.06 – 1.45 (m, 14H), 1.52 – 1.70 (m, 5H), 1.77 – 1.85 (m, 2H), 1.96 (dd and m, J = 12.5, 5.0 Hz, 2H, CH*H*CHPh and C*H*H), 2.30 – 2.36 (m, 2H, CH_2CH=CHCN), 2.65 – 2.75 (m, 2H, C*H*HCHPh and C*H*N),

5.19 (d, *J* = 10.9 Hz, 1H, CH=C*H*CN), 5.37 – 5.43 (dd, *J* = 10.0, 5.1 Hz, 1H, C*H*Ph), 6.31 (dt, *J* = 10.9, 7.6 Hz, 1H, C*H*=CHCN) 7.22 – 7.39 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.09, 19.94, 22.65, 25.75, 29.32, 29.49, 29.93, 31.04, 31.87, 31.97, 34.61, 41.31, 42.08, 59.41, 67.58, 76.80, 99.43, 115.98, 125.90, 127.06, 128.35, 141.98, 155.03; HRMS (EI) *m/z* 394.2978. $C_{26}H_{38}N_2O$ $[M]^{+\bullet}$ requires 394.2979.

4.10. (1R,5R*,8S*,12R*)-12-Cyano-5-(heptyl)-6-aza-7-oxatricyclo-[6.3.1.0^{1,6}] dodecane (**13**)*

A solution of the α,β-unsaturated nitrile **12** (2.38 g, 6.03 mmol) in toluene (72 mL) and EtOH (12 mL) was divided equally into 6 vials. These vials were each sealed and irradiated in a microwave reactor for 50 min at 180 °C (pressure was ~ 11 bar). The contents of the vials were combined, concentrated and the residue was purified by flash chromatography (EtOAc/hexanes, gradient; 2:98, 3:97, 3.5:96.5 then 4:96) to yield the tricycle **13** (1.4 g, 78%) as a clear yellow oil: R_f 0.24 (10:90 EtOAc/hexanes); IR (neat), 2926, 2855, 2240, 1457, 1376, 1360, 1115, 1084, 945, 931 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.83 – 0.89 (m, 3H, C*H*$_3$), 1.05 – 1.37 (m, 8H), 1.50 – 2.04 (m, 15H), 2.12 – 2.20 (m, 1H) 2.33 – 2.42 (m, 1H, C*H*N), 3.43 (d, *J* = 5.8 Hz, 1H, C*H*CN), 4.66 – 4.74 (m, 1H, C*H*O); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.06, 17.52, 19.19, 22.62, 25.62, 27.14, 29.23, 29.59, 29.82, 31.81, 32.24, 34.23, 36.02, 38.11, 65.42, 65.51, 75.55, 117.91; HRMS (EI) *m/z* 290.2351. $C_{18}H_{30}N_2O$ $[M]^{+\bullet}$ requires 290.2353.

4.11. (1R,5R*,8S*,12S*)-1'-(Z)-12-(But-1'-enyl)-5-(heptyl)-6-aza-7-oxatricyclo[6.3.1.0^{1,6}] dodecane (**14**)*

To a solution of the nitrile **13** (769 mg, 2.65 mmol) in toluene (50 mL) at −78 °C was added ^{i}Bu$_2$AlH in toluene (1.5 M, 2.20 mL, 3.30 mmol). After 2 h, MeOH (0.5 mL) was added and the mixture was allowed to warm to room temperature. This mixture was diluted with EtOAc (40 mL) and aqueous potassium sodium tartrate (1.4 M, 70 mL) and stirred vigorously (16 h). The organic layer was separated and the aqueous layer was re-extracted (2 times) with EtOAc and the combined organics were washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated to give, presumably the crude aldehyde (550 mg, 71% crude) as an unstable colourless oil: [R_f 0.48 (30:70 EtOAc/hexanes] that was used immediately. Meanwhile, to a mixture of 1-propyltriphenylphosphonium bromide (1.53 g, 3.98 mmol) in dry THF (60 mL) at −78 °C was added ^{n}BuLi (2.50 mL of a 1.59 M solution in hexanes, 3.98 mmol) dropwise (3 min). The bright yellow mixture was allowed to warm to 0 °C and stirred for 30 min during which the mixture had turned orange. The mixture was cooled (−78 °C) and the crude aldehyde (550 mg) in THF (30 mL) was added dropwise (10 min). This mixture was allowed to slowly warm to room temperature (over ~ 4 h) and then stirred overnight. Saturated aqueous NH_4Cl was added, and the mixture was diluted with H_2O and EtOAc. The organic layer was separated, and the aqueous layer was re-extracted (2 times) with EtOAc. The combined organics were washed sequentially with saturated aqueous $NaHCO_3$, saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes, gradient; 2:98, 2.5:97.5 then 3:97) to yield the alkene **14** (402 mg, 47% over two steps) as a clear, yellow oil: R_f 0.33 (10:90 EtOAc/hexanes); IR (neat) 3024, 2928, 2858, 1650, 1460, 1375, 1113, 1075, 925, 721 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ0.83 – 0.91 (m, 3H, C*H*$_3$), 0.96 – 1.04 (m, 3H, C*H*$_3$), 1.06 – 1.65 (m, 20H), 1.73 – 1.82 (m, 2H), 1.90 – 2.04 (m, 2H), 2.07 – 2.18 (m, 2H, CH=CHC*H*$_2$), 2.60 – 2.70 (m, 1H, C*H*N), 3.39 – 3.48 (m, 1H, C*H*CH=CH), 4.35 – 4.43 (m, 1H, C*H*O), 5.34 – 5.48 (m, 1H, CHC*H*=CH), 5.59 – 5.70 (m, 1H, CH=C*H*CH$_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.08, 14.39, 17.87, 19.65, 21.22, 22.65, 24.97, 26.02, 29.30, 29.81, 29.97, 31.86, 32.30, 34.14, 34.73, 42.56, 64.86, 75.56, 77.99, 123.32, 136.31; HRMS (EI) *m/z* 319.2864 $C_{21}H_{37}NO$ $[M]^{+\bullet}$ requires 319.2870.

4.12. (2S,6R*,7S*,8S*)-(1'Z)-7-(But-1'-enyl)-2-(heptyl)-1-azaspiro[5.5]undecan-8-ol (**15**)*

To alkene **14** (318 mg, 0.995 mmol) was added SmI_2 in THF (0.1 M, 14 mL, 1.4 mmol) in 1 mL portions over a week. Fresh SmI_2 in THF was added whenever the solution turned yellow (~ every morning and evening). MeOH (0.5 mL) and aqueous NH_3 (14 M, 2 mL) were added and the mixture was stirred (30 min).The pH was adjusted to ~10 (2 mL 14 M NH_3), then saturated aqueous $Na_2S_2O_3$ was added, and the mixture was diluted with H_2O and Et_2O, and the organic layer was separated. The aqueous layer was re-extracted (3 times) with Et_2O and then the combined organics were washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes, gradient; 10:90, 20:80, 25:75, 30:70 then MeOH/CH_2Cl_2, 2:98; then 14 M aq NH_3/MeOH/CH_2Cl_2, gradient; 1:2:87 and then 1:3:96) to give bicycle **15** (124 mg, 38%, with 162 mg starting material recovered) as an oil: R_f 0.48 (1:5:94 16 M NH_3/MeOH/CH_2Cl_2); IR (neat) 3257, 3011, 2929, 2856, 1457, 1375, 1088, 970m, 727w cm^{-1};^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.71 - 0.90 (m, 5H, C*H*$_3$, C*H*, C*H*), 0.98 (t, *J* = 7.5 Hz, 3H, CH_3), 1.17 – 1.47 (m, 16H), 1.47 – 1.65 (m, 6H), 1.97 – 2.20 (m, 3H, CHCH=CHC*H*$_2$, C*H*$_2$), 2.88 – 2.90 (m, 1H, C*H*NH), 3.22 – 3.29 (m, 1H, C*H*CH=CHCH$_2$, 3.67 – 3.75 (m, 1H, C*H*OH), 5.16 – 5.25 (m, 1H, CHC*H*=CHCH$_2$), 5.39 – 5.47 (m, 1H, CHCH=C*H*CH$_2$), 7.30 – 8.20 (v br s, 1H, O*H*); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.05, 14.43, 15.29, 19.47, 20.82, 22.63, 25.93, 28.59, 29.17, 29.91, 31.81, 32.79, 36.55, 37.42, 37.74, 38.03, 50.32, 54.59, 72.43, 126.63, 133.86; HRMS (EI) *m/z* 321.3022 $C_{21}H_{39}NO$ $[M]^{+\bullet}$ requires 321.3026.

4.13. (2S,6R*,7S*,8S*)-7-(Butyl)-2-(heptyl)-1-azaspiro[5.5]undecan-8-ol (**16**)*

Alkene **14** (132 mg, 0.413 mmol), was added to a mixture of $Pd(OH)_2$ on carbon (100 mg of 20% Pd catalyst) and aqueous HCl (1.03 mL, 2 M solution, 2.06 mmol) in dry THF (50 mL). This mixture was shaken vigorously under an atmosphere of H_2 (25 psi, 16 h). The mixture was filtered through Celite washing with THF/H_2O (4:1, 100 mL). The mixture was then neutralised ($NaHCO_3$) and concentrated until all the THF was removed and then extracted (4 times) with CH_2Cl_2. The organic phase was washed with aqueous saturated NaCl, dried (MgSO4), filtered and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes, gradient; 10:90, 20:80, 25:75, 30:70; MeOH/CH_2Cl_2, gradient; 2:98 then 14 M aq NH_3/MeOH/CH_2Cl_2, gradient; 1:2:87 and then 1:3:96) to give bicycle **16** (130 mg, 84%) as waxy solid. R_f 0.38 (1:10:89 16 M NH_3/MeOH/CH_2Cl_2); IR (thin film) 3253, 2929, 2857, 1715, 1537, 1377, 1067, 970 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.79 – 0.94 (m, 8H, 2 x C*H*$_3$, C*H*, C*H*), 1.00 – 1.15 (m, 2H), 1.18 – 1.44 (m, 18H), 1.46 – 1.85 (m, 8H), 1.95 – 2.90 (m, 1H, C*H*), 2.16 – 2.23 (m, 1H), 2.89 – 3.00 (m, 1H, C*H*NH), 3.90 – 3.98 (m, 1H, C*H*OH), 7.31 – 8.35 (v br s, 1H, OH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.01, 14.05, 15.18, 19.48, 22.63, 23.02, 25.89, 27.54, 27.64, 29.16, 29.88, 30.26, 31.81, 33.00, 36.78, 36.86, 37.68, 38.05, 50.07, 55.34, 69.76; HRMS (EI) *m/z* 323.3174 $C_{21}H_{41}NO$ $[M]^{+\bullet}$ requires 323.3183.

4.14. (1R,5R*,8S*,12S*)-1'-(Z)-5-(heptyl)-12-(hex-1'-enyl)-6-aza-7-oxatricyclo[6.3.1.0^{1,6}] dodecane (**17**)*

To a solution of the nitrile **13** (699 mg, 2.41 mmol) in toluene (50 mL) at −78 °C was added $^{i}Bu_2AlH$ in toluene (1.50 M, 1.93 mL, 2.89 mmol). After 2 h, MeOH (0.5 mL) was added and the mixture was allowed to warm to room temperature. This mixture was diluted with EtOAc (40 mL) and aqueous potassium sodium tartrate (1.40 M, 70.0 mL) and stirred vigorously (16 h). The organic layer was separated, the aqueous layer was re-extracted (2 times) with EtOAc and the combined organics were washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated to give, presumably the crude aldehyde as an unstable colourless oil that was used immediately. Meanwhile, to a mixture of 1-pentyltriphenylphosphonium bromide (1.49 g, 3.61 mmol) in dry THF (60 mL) at −78 °C was added ^{n}BuLi (2.27 mL of a 1.59 M solution in hexanes, 3.61 mmol) dropwise (3 min). The bright yellow mixture was allowed to warm to 0 °C and stirred for 30 min after which the mixture had turned orange. The mixture was cooled (−78 °C) and the crude aldehyde (751 mg) in THF (30 mL) was added dropwise (10 min). This mixture was allowed to slowly warm to room temperature (over ~ 4 h) and then stirred overnight. Saturated aqueous NH_4Cl was added and the mixture was diluted with H_2O and EtOAc. The organic layer was separated and the aqueous layer was re-extracted (2 times) with EtOAc. The combined organics were washed sequentially with saturated aqueous $NaHCO_3$, saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes, gradient; 4:96, 5:95, 6:94, 7:93 then 8:92) to yield the alkene **17** (179 mg, 64%) as a clear yellow oil: R_f 0.27 (10:90 EtOAc/hexanes); IR (neat) 3024, 2928, 2858, 1651, 1460, 1377, 1109w, 1080w, 944, 924, 699 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.82 – 0.95 (m, 6H, 2 x C*H*$_3$), 1.20 – 2.05 (m, 28H), 2.06 – 2.16 (m, 2H, CH=CHC*H*$_2$), 2.60 – 2.69 (m, 1H, C*H*N), 3.39 – 3.47 (m, 1H, C*H*=CHCH$_2$), 4.34 – 4.40 (m, 1H, C*H*O), 5.42 – 5.50 (m,1H, CHC*H*=CH), 5.59 – 5.68 (m, 1H, CH=C*H*CH$_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.98, 14.08, 17.89, 19.73, 22.35, 22.65, 25.00, 26.00, 27.62, 29.31, 29.83, 29.98, 31.86, 31.94, 32.34, 34.20, 34.78, 42.62, 64.78, 64.84, 77.98, 123.89, 134.74; HRMS (EI) *m/z* 347.3180. $C_{23}H_{41}NO$ $[M]^{+\bullet}$ requires 347.3183.

4.15. (2S,6R*,7S*,8S*)-2-(Heptyl)-7-(hexyl)-1-azaspiro[5.5]undecan-8-ol (**3**)*

Alkene **17** (167 mg, 0.48 mmol), was added to a mixture of $Pd(OH)_2$ on carbon (100 mg of 20% Pd catalyst) and aqueous HCl (0.96 mL, 2 M solution, 1.92 mmol) in dry THF (50 mL). This mixture was shaken vigorously under an atmosphere of H_2 (25 psi, 16 h). The mixture was filtered through Celite washing with THF/H_2O (4:1, 100 mL). The mixture was then neutralised ($NaHCO_3$) and concentrated until all the THF was removed. It was then diluted with CH_2Cl_2 and the organic layer was separated. The aqueous phase was re-extracted (3 times) with CH_2Cl_2. The combined organics were washed with aqueous saturated NaCl, dried (MgSO4), filtered and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes, gradient; 40:60, then MeOH/CH_2Cl_2, 5:95 and then 14 M aq NH_3/MeOH/CH_2Cl_2, 1:10:89) to give bicycle **3** (136 mg, 81%) as waxy crystals. R_f 0.39 (1:10:89 16 M NH_3/MeOH/CH_2Cl_2); IR (neat) 3172, 2932, 2860, 2649, 1777, 1678, 1556, 1459, 1154, 1061 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.68 – 0.94 (m, 8H, 2 x CH_3, CH, CH), 1.00- 1.05 (m, 2H), 1.16 – 1.44 (m, 23H), 1.44 – 1.67 (m, 5H), 1.67 – 1.84 (m, 2H), 1.95 – 2.10 (m, 1), 2.17 – 2.25 (m, 1H) 2.83 – 2.97 (v br s, 1H, C*H*NH), 3.85 – 3.96 (br s, 1H, C*H*OH) 7.30 – 7.90 (v br, OH exchange); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.05, 14.08, 15.20, 19.56, 22.63, 25.91, 27.75, 27.94, 28.05, 29.16, 29.68, 29.91, 31.81, 31.81, 33.28, 37.02, 37.07, 37.90, 38.14, 49.98, 55.10, 69.81; HRMS (EI) *m/z* 351.3497. $C_{23}H_{45}NO$ $[M]^{+\bullet}$ requires 351.3496.

4.16. (2S,6R*,7S*,8S*)-(1'Z)-2-(Heptyl)-7-(hex-1'-enyl)-1-azaspiro[5.5]undecan-8-ol (**18**)*

To alkene **17** (57 mg, 0.144 mmol) was added SmI_2 in THF (0.1 M, 14.0 mL, 1.4 mmol) in 1 mL portions over a week. MeOH (0.5 mL) and aqueous NH_3 (14 M, 2 mL) were added and the mixture was stirred (30 min). The pH was adjusted to ~10 (2 mL 14 M NH_3), then saturated aqueous $Na_2S_2O_3$ was added, the mixture was diluted with H_2O and Et_2O, and the organic layer was separated. The aqueous layer was re-extracted (3 times) with Et_2O. The combined organics were washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography (EtOAc/hexanes, gradient; 40:60, then MeOH/CH_2Cl_2, 5:95 and then 14 M aq NH_3/MeOH/CH_2Cl_2, 1:10:89) to afford olefin **18** (30 mg, 52%, 15 mg starting material recovered) as an oil: R_f 0.46 (1:5:94 16 M NH_3/MeOH/CH_2Cl_2); IR (neat) 3258, 3011, 2927, 2856, 1457, 1377, 1089, 971, 724 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.78 – 0.97 (m, 8H, 2 x CH_3, CH, CH), 1.20 – 1.48 (m, 19H), 1.50 – 1.77 (m, 7H), 1.98 – 2.09 (m, 1H), 2.10 – 2.19 (m, 2H), 2.92 – 3.04 (m, 1H, C*H*NH), 3.26 (d, *J* = 10.2 Hz, 1H, C*H*CH=CH), 3.70 – 3.80 (m, 1H, C*H*OH), 5.18 – 5.27 (m, 1H, CHC*H*=CHCH$_2$), 5.40 – 5.50 (m, 1H, CHCH=C*H*CH$_2$), 7.40 – 7.80 (v br s, 1H, OH exchange); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.02, 14.05, 15.27, 19.41, 22.46, 22.63, 25.91, 27.30, 28.49, 29.17, 29.89, 31.81, 32.02, 32.41, 36.24, 37.11, 37.99, 50.55, 55.00, 72.33, 126.83, 132.57; HRMS (EI) *m/z* 349.3333 $C_{23}H_{43}NO$ $[M]^{+\bullet}$ requires 349.3339.

5. Pharmacology Experimental Section

The cDNAs encoding rat nAChR for α4 subunit was subcloned in pSP64, for α7 subunit subcloned in pBS SK (+), and β2 subunit subcloned in the pSP65 vectors and were generous gifts from Professor Jim Boulter (University of California, Los Angeles, CA). Preparation of cRNAs for α4 and β2 subunits and two-electrode voltage-clamp recordings were undertaken as previously described.[15] In brief, oocytes were surgically removed from *Xenopus* laevis while under general anaesthesia (tricaine, 850 mg/500 mL) in accordance with the National Health and Medical Research Council of Australia's ethical guidelines and approved by the University of Sydney Animal Ethics Committee. Harvested lobes were treated with collagenase A (2 mg mL-1; Roche Diagnostics, Australia) in oocyte releasing buffer 2 (OR-2; 82.5mM NaCl, 2 mM KCl, 1 mM MgCl2, 5 mM HEPES, pH 7.5) for 1.5 h. cRNAs (1-10 ng) were mixed in a 1:1 ratio of α4:β2 in a total volume of 50.6 nL and injected into stage V-VI oocytes. Injected oocytes were kept in frog Ringer solution (96mM NaCl, 2mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$ and 5 mM HEPES; pH 7.4) containing 2.5 mM sodium pyruvate, 0.5μM theophylline, and 4μg/mL of kanamycin, and stored for 2-5 days at 18°C in an orbital shaker before being used for recording. Oocytes expressing α4β2 nAChRs were clamped at -60 mV and continually perfused with Ca^{2+}-free solution (115 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, , 1.8 mM $BaCl_2$, 10 mM HEPES; pH 7.4). Glass electrodes used for recording had a resistance of 0.2-2MΩ and were filled with 3 M KCl. Compounds were stored at -20°C and made up to the required concentrations in Ca^{2+}-free solution before applying to the oocyte by gravity flow. Whole-cell currents were measured using a GeneClamp 500 amplifier (Axon Instruments, Foster City, CA, USA), a MacLab 2e recorder (AD Instruments, Sydney, NSW, Australia) and Chart Version 4.0.1 program.

Inhibitory concentration response curves were determined in the presence of 100 μM ACh for α4β2 or 300 μM for α7 nAChRs by first incubating the histrionicotoxins 3 mins before

co-addition with 100 µM or 300 µM ACh, respectively. Concentration response curves were determined over an ACh concentration range of 0.1-10000 µM either alone or in the presence of **16** (30 µM), **15** (1 µM), **3** (1 µM) and **18** (1 µM) Currents were normalised to the currents elicited by 100 µM ACh and presented as mean ± SEM and fitted using sigmoidal fit (variable slope) equation from GraphPad Prism 4.0:

$$I=I_{MAX}([A]^{nH}/([A]^{nH} + EC_{50}{}^{nH}))$$

where I is the current, I_{MAX} is the current produced by ACh at a given concentration (100 µM, 300 µM or 1 mM as specified), [A] is the ligand concentration and n_H is the Hill slope. From this equation, the concentration of the agonist that activates 50% of expressed receptors (EC_{50}) or in the case of inhibitory concentration response curves the concentration of the antagonist that inhibits 50% of the evoked ACh current (IC_{50}) were calculated. Data are presented as mean (95% confidence intervals) from a minimum of 3 oocytes over a minimum of 2 batches. Unless otherwise stated statistical differences between groups were calculated using Student's t-test.

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7. References and notes

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Supplementary Material

Copies of ^{1}H and ^{13}C NMR spectra for Compounds **6**–**8**, **10**–**17**, **3** and **18**.