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Synthesis of UB-165: A Novel Nicotinic Ligand and Anatoxin-a/Epibatidine Hybrid

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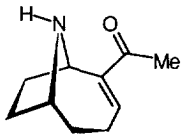
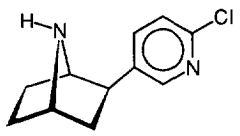
Abstract. UB-165 (**5**), a hybrid corresponding to natural anatoxin-a and epibatidine, has been synthesised and shows significant potency at the high affinity nicotine binding site in rat brain. *Ent*-(**5**) shows a much lower level of activity which parallels the sense of enantiospecificity associated with anatoxin-a. © 1997 Elsevier Science Ltd.

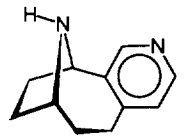
Anatoxin-a (**1**)[1] and epibatidine (**2**)[2] are two of the most potent agonists known for the nicotinic acetylcholine receptor (nAChR), itself now recognised as a therapeutically important drug target. These two ligands do, however, differ in a number of respects and in particular show a marked contrast in terms of the degree of enantiospecificity associated with the ligand-receptor interaction. While naturally occurring anatoxin-a (**1**) is a potent agonist and *ent*-(**1**) is inactive, both enantiomers of epibatidine display similar (and high) levels of activity at the nAChR.[3] Probing this issue of enantiospecificity is important for refining our concept of the nicotinic pharmacophore[4] and this goal could be achieved using ligands such as PHT (**3**)[5] and epiboxidine (**4**).[6] These molecules represent hybrids of anatoxin-a and nicotine, and epibatidine and ABT-418 respectively but, to date, have only been reported as racemates.



1 Anatoxin-a



2 Epibatidine



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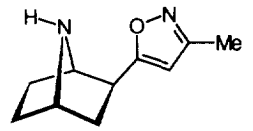

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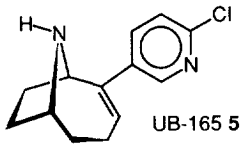
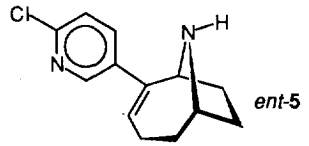


UB-165 5



ent-5

We now describe the synthesis and preliminary biological profile of UB-165 (**5**) as well as *ent*-(**5**) which represent the two enantiomers of a novel anatoxin-a/epibatidine hybrid. This hybrid retains the bulky azabicyclo[4.2.1]nonane moiety of anatoxin-a together with the pyridyl unit (hydrogen bond acceptor component in the general pharmacophore model[4]) of epibatidine. Additionally, UB-165 (**5**) has an absolute configuration corresponding to that of natural anatoxin-a.

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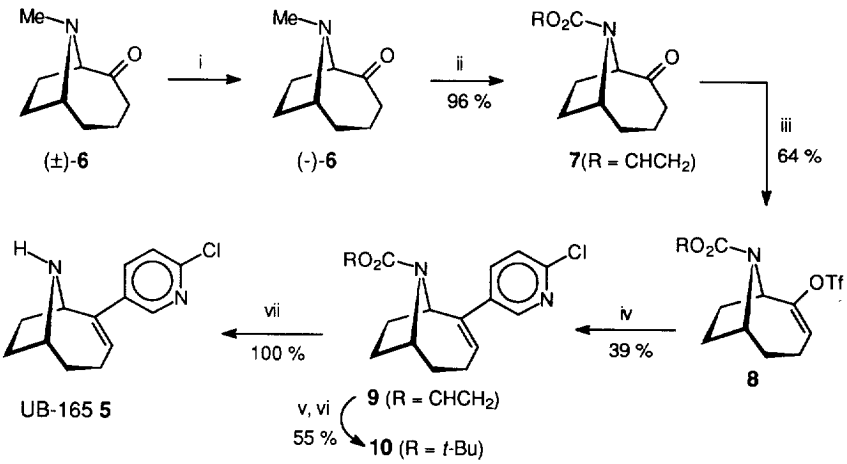
The synthesis of UB-165 (**5**) is shown in Scheme 1 and is based on resolution of the azabicyclic ketone (**6**), available in 3 steps from *cis*-1,5-cyclooctanediol.[7] Resolution[8] of (±)-(**6**) using (-)-dibenzoyl tartrate gave ketone (**6**) {$[\alpha]_D^{26}$ -51.6 (c=0.92, MeOH); lit.,[8] $[\alpha]_D^{20}$ -52.5 (c 1.0, MeOH)} and *N*-demethylation (with vinyl chloroformate) gave carbamate (**7**) in 96 % yield. Conversion of (**7**) to vinyl triflate (**8**) was carried out using the Comins reagent[9] and this synthetically versatile intermediate was then coupled, using Pd(0)-catalysis, to the organozinc species generated from 2-chloro-5-lithiopyridine and $ZnCl_2$ to give adduct (**9**) in 39 % yield.[10] Deprotection of (**9**) was carried out under aqueous acidic conditions and the crude amine product [i.e. (**5**)] was then reprotected as the *N*-Boc derivative (**10**) in 55 % yield from (**9**). This was done because the *N*-Boc intermediate (**10**) was not only readily purified but was also more amenable to storage. Finally, deprotection of purified (**10**) was carried out using aqueous acid and UB-165 (**5**) was then isolated (as the corresponding HCl salt) in essentially quantitative yield.[11]



SCHEME 1. *Reagents and conditions:* i, (-)-dibenzoyl tartrate, EtOH, reflux; ii, vinyl chloroformate, K_2CO_3, CH_2Cl_2; iii, KHMDS, 2-N(Tf_2) -5-chloropyridine, THF, -78 °C; iv, 2-chloro-5-iodopyridine, *n*-BuLi, THF, then $ZnCl_2$, THF followed by (**8**), $Pd(PPh_3)_4$; v, conc. HCl, aq. dioxane, reflux; vi, Boc_2O, Et_3N, aq. THF; vii, 2M HCl, dioxane.

Using exactly the same chemistry, *ent*-(**5**) (which corresponds to the absolute configuration of *unnatural* anatoxin-a) was obtained from (+)-(**6**) {$[\alpha]_D^{29}$ +47.8 (c=0.96, MeOH); lit.,[8] $[\alpha]_D^{20}$ +55.0 (c 1.0, MeOH)}. However, the enantiomeric purity of (+)-(**6**), based on optical rotation, was not as high as that observed for (-)-(**6**) (as used in Scheme 1) and the likelihood that *ent*-(**5**) is contaminated by a small amount of UB-165 must not be ignored (*see below*).

Both UB-165 (**5**) and *ent*-(**5**) were evaluated against the high affinity [^{3}H]nicotine binding sites in rat brain and our preliminary biological data are shown below (Figure 1 and Table 1).[12] Both anatoxin-a and epibatidine used in the assay experiments were racemic and the racemic hybrid [(±)-(**5**)] was also prepared.

Most significantly, UB-165 (**5**) was identified as a potent nicotinic ligand and showed a potency that was intermediate between anatoxin-a and epibatidine. *Ent*-(**5**) was approximately 20 times less potent than UB-165 which indicates a significant degree of enantiospecificity is associated with this hybrid arrangement. It is, however, important to appreciate that this may only represent a conservative assessment since the activity associated with *ent*-(**5**) could be accounted for by contamination (*ca.* 5 %) of this less active enantiomer by the more potent UB-165.

FIGURE 1

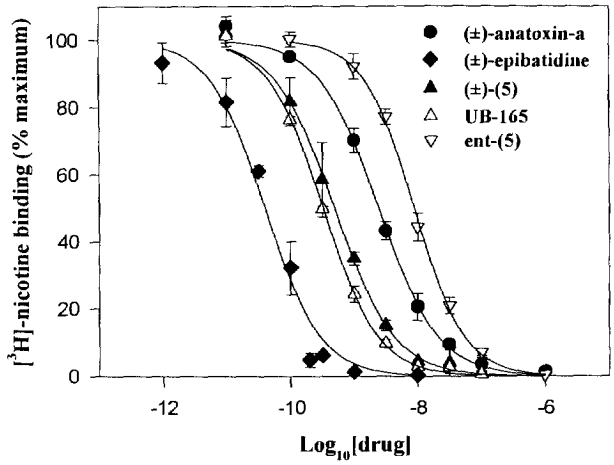


TABLE 1. Inhibition constants for interaction with [^{3}H]nicotine binding site in rat P2 brain membranes[12]

Ligand	IC_{50} (nM)	K_i (nM)
(±)-anatoxin-a	2.49 ±0.41	1.25
(±)-epibatidine	0.041 ±0.01	0.021
(±)-(**5**)	0.53 ±0.1	0.27
UB-165 (**5**)	0.34 ±0.02	0.17
ent-(**5**)	8.79 ±1.06	4.40

The enantiospecificity observed with UB-165 (**5**) and *ent*-(**5**) is associated with the bulk of the azabicyclo[4.2.1]nonane framework which serves to support those elements defined by the nicotinic pharmacophore. The bulk of this framework may, as a consequence, play a critical role in determining the fit of the ligand within the receptor although pharmacophore models have not, in general, characterised this aspect of ligand structure. Interestingly, the N_{amine}-$N_{pyridine}$ distance[13] of (**5**) ranges from 5.33 Å to 6.22 Å, with the higher value being significantly greater than that encountered with either anatoxin-a or epibatidine.[4]

Further studies aimed at exploiting the scope and potential offered by this new category of nicotinic ligand, as well as extending the characterisation of the associated biological profile are underway.[14]

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11. *N*-Boc Derivative (**10**): $[\alpha]_D^{28}$ -31.4 (c=1.13, MeOH), R_f 0.49 (1:4 EtOAc:petrol); ν_{max} (Film/cm^{-1}) 1690 (s); δ_H (400 MHz, $CDCl_3$, shows amide resonance) 1.34 (4.5H, s, C(CH_3)$_3$), 1.48 (4.5H, s, C(CH_3)$_3$), 1.60-1.70 (2H, m, CH_2), 1.71-2.15 (2H, m, CH_2), 2.16-2.25 (2H, m, CH_2), 2.26-2.44 (2H, m, CH_2), 4.37-4.40 (0.5H, m, C*H*), 4.46-4.50 (0.5H, m, C*H*), 4.70-4.73 (0.5H, m, C*H*), 4.76-4.78 (0.5H, m, C*H*), 5.82-5.85 (0.5H, m, C=C*H*), 5.87-5.90 (0.5H, m, C=C*H*), 7.27 (1H, dd, *J* 8, 3, Ar*H*), 7.63 (0.5H, dd, *J* 8, 3, Ar*H*), 8.10 (0.5H, dd, *J* 8, 3, Ar*H*), 8.33 (0.5H, d, *J* 2.5, Ar*H*), 8.37 (0.5H, d, *J* 2.5, Ar*H*); *m/z* (EI) 336/334 (M^+, 3 %); *m/z* (CI) 337/335 (M+1, 100 %); HRMS (CI) Found: 335.1526. $C_{18}H_{23}{}^{35}ClN_2O_2$ + H requires 335.1540 (3.9 ppm).

    UB-165 (**5**) (as free base): R_f 0.11 (1:19 MeOH:$CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.75-2.54 (8H, m, CH_2), 3.95-4.08 (1H, m, C*H*-6), 4.28-4.35 (1H, m, C*H*-1), 5.87-6.00 (1H, m, C=C*H*), 7.25 (1H, d, *J* 8, Ar*H*), 7.59 (1H, dd, *J* 8, 2.5, Ar*H*), 8.31 (1H, d, *J* 2.5 Ar*H*); δ_C (75.5 MHz, $CDCl_3$) 23.54 (CH_2), 27.61 (CH_2), 28.34 (CH_2), 39.96 (CH_2), 58.88 (*C*H-6), 59.01 (*C*H-1), 124.02 (Ar*C*H), 134.90 (*C*H), 136.19 (*C*), 137.02 (Ar*C*H), 139.17 (*C*), 147.41 (Ar*C*H), 150.50 (*C*); *m/z* (EI) 236/234 (M^+, 43 %); *m/z* (CI) 237/235 (M+1, 100 %); HRMS (CI) Found: 235.1006. $C_{13}H_{15}{}^{35}ClN_2$ +H requires 235.1002 (1.8 ppm).
12. Rat brain P2 membranes (10 mg protein/ml, 250 µl) were incubated with [^{3}H]-(-)-nicotine (10 nM, 64.4 Ci/mmol) in the absence or presence of 1 mM nicotine, to define total and non-specific binding respectively, or in the presence of serial dilutions of drug. Binding in the presence of drug was calculated as a percentage of specific binding. Data points were fitted to the Hill equation and IC_{50} values were determined from the curves, with values shown in Table 1 being the mean ± sem of three independent assays and (±)-(**5**), UB-165 (**5**) and *ent*-(**5**) were all used as the corresponding HCl salts.
13. This range of values for the variation of the N_{amine}-$N_{pyridine}$ distance against torsion angle was determined with an energy minimised structure using Discover 2.95 (cvff) from MSI/Biosym.
14. Very recently, a tropane variant of epibatidine (derived from (-)-cocaine) has been reported which also uses a Pd(0)-mediated coupling of an organozinc nucleophile (as in Scheme 1) to a tropane-based vinyl triflate: Zhang, C.; Gyermek, L.; Trudell, M. L. *Tetrahedron Lett.* 1997, **38**, 5619.

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