Bioorganic & Medicinal Chemistry 16 (2008) 10295–10300

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Polyethylene glycol-based homologated ligands for nicotinic acetylcholine receptors ☆

Bradley A. Scates, Bethany L. Lashbrook, Benjamin C. Chastain †, Kaoru Tominaga †, Brandon T. Elliott †, Nicholas J. Theising †, Thomas A. Baker †, Richard W. Fitch *

Department of Chemistry, Indiana State University, 600 Chestnut Street, Science S35E, Terre Haute, IN 47809, USA

ARTICLE INFO

Article history:
Received 16 August 2008
Revised 16 October 2008
Accepted 18 October 2008
Available online 1 November 2008

Keywords:
Pyridyl ethers
A-84543
SAR
ADMP
DMPP

ABSTRACT

A homologous series of polyethylene glycol (PEG) monomethyl ethers were conjugated with three ligand series for nicotinic acetylcholine receptors. Conjugates of acetylaminocholine, the cyclic analog 1-acetyl-4,4-dimethylpiperazinium, and pyridyl ether A-84543 were prepared. Each series was found to retain significant affinity at nicotinic receptors in rat cerebral cortex with tethers of up to six PEG units. Such compounds are hydrophilic ligands which may serve as models for fluorescent/affinity probes and multivalent ligands for nAChR.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

Nicotinic acetylcholine receptors (nAChR) are a subclass of receptors activated by the neurotransmitter acetylcholine (ACh). These ligand-gated ion channels are activated by the tobacco alkaloid nicotine, as opposed to muscarinic acetylcholine receptors (mAChR), which are G-protein coupled receptors (GPCR) and are activated by the mushroom muscarine.[1]

Nicotinic receptors are important targets in the development of therapeutics for a variety of neurological disorders.[1b,2,3] They are involved in cellular signaling in both the peripheral (PNS) and central (CNS) nervous systems. Nicotinic receptors exist as a number of different subtypes based on subunit constitution of this pentameric protein. At present there are approximately 20 different characterized subtypes and differential activation/inhibition of these are considered to be responsible for nicotine's myriad pharmacological effects. In the neuromuscular $\alpha1\beta1\gamma(\varepsilon)\delta$ nAChR mediate muscle contraction by the triggering of action potentials (in concert with voltage-gated sodium channels) at the muscle endplate.[1b] In the CNS, nAChR mediate much of the fast synaptic transmission in several areas of the brain. Nicotine's effects on ganglionic receptors are thought to mediate some of its toxic effects, while central neuronal receptors, especially $\alpha4\beta2$ are considered to mediate the cognitive enhancing and addictive properties of nicotine.[4] The $\alpha7$ homopentamer has been implicated in inflammation and schizophrenia and it has been observed that a high percentage (>80%) of schizophrenics smoke heavily, possibly as a form of self-medication.[5] Some of the major foci for the development of drugs for nAChR include analgesia, cognitive enhancement, and smoking cessation.[2,3]

While the analgesic activity of nicotine has been known for many decades, its clinical use is precluded by its toxicity and addictive liability.[3] However, since discovery of epibatidine, a highly potent nicotinic receptor agonist from a poison frog,[6] the field of nAChR-mediated analgesia has become quite active. Epibatidine and several derived analogs are sensitive nAChR probes many with significant subtype selectivity (Fig. 1). Notably, the pyridyl ethers developed by Abbott have been shown to have useful analgesic and cognitive enhancing effects,[6] though groups at several companies have entered the market with nAChR based therapeutics, notably Pfizer, with their antismoking drug varenicline (Chantix®).[8]

We are interested in the development of nicotinic ligands with external tethers, with potential applications to fluorescent and/or affinity tags,[9] as well as multivalent ligands.[10] As a prerequisite to such development, it is necessary to demonstrate the ability to homologate a particular ligand of interest such that it retains affinity for the receptor of interest. Such a ligand serves as the recognition element for receptor binding, but appropriate point(s) of attachment must first be selected such that the tether is placed

☆ Presented at the 233rd National Meeting of the American Chemical Society, Chicago, IL, March 24–29, **2007** (Poster MEDI 0291).

* Corresponding author. Tel.: +1 812 237 2244; fax: +1 812 237 2232.
E-mail address: rfitch@indstate.edu (R.W. Fitch).
† Undergraduate student.

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.10.045

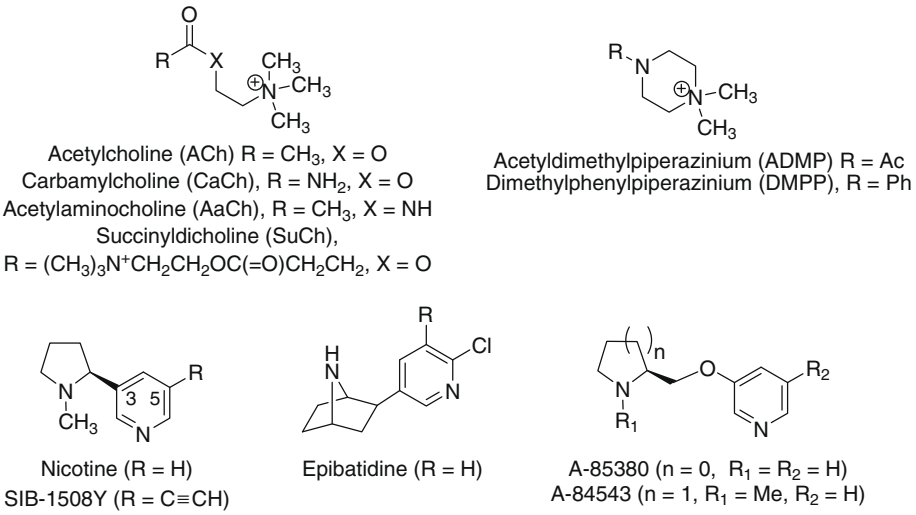


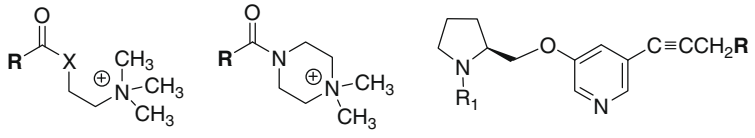
Figure 1.

in a non-congested location when the ligand binds to the receptor, such that affinity is retained. Further the tether composition must be compatible with receptor, biological system, and solvent for both reactivity and physical property considerations (solubility, lipophilicity).[11] We thus examined potential sites of homologation for several nicotinic ligands. Our first interest was in quaternary ammonium compounds analogous to ACh or its bioisosteric counterparts carbamylcholine (CaCh) or acetylaminocholine (AaCh, Fig. 2). We initially considered that these ligands would be amenable to homologation based on the observation that succinyldicholine (SuCh), a paralytic agent employed during surgery, and other bivalent ACh homologs demonstrate the ability to substitute ACh at the α-carbon of the acetyl group.[2] It has also been shown that the ester linkage may be replaced by amide.[12] Along this line, rigid bioisosteric quaternary analogs of acetyldimethypiperazinium (ADMP), a well-known nAChR-selective quaternary agonist were of interest to us as they showed increased potency over the acyclic amide or acetylcholine itself.[13] Given the structural similarities to ACh, we anticipated these would tolerate substitution at the α-carbon of the acetyl group as well. Finally, a series of homologated versions of both epibatidine and A-84543, a pyridyl ether from Abbott, were recently described.[14] These derivatives were appended with ω-alcohol and hydrocarbon moieties up to ten carbons. Indeed, Abbott had described 5-substituted derivatives of their pyridyl ether ligands previously.[7b] However, we anticipated the need for very long tethers (>30 atoms) for our purposes and postulated that hydrocarbon backbones would be undesirable due to increasing hydrophobicity and the anticipated potential for undesired interactions with membranes and solubility problems. Polyethylene glycols (PEGs), on the other hand, have been shown to be a hydrophilic, biocompatible polymer scaffold with a number of applications.[11b] We describe herein a series of highly water soluble homologated ligands (Fig. 2) based on AaCh (**1a**–**g**), ADMP (**2a**–**g**), and A-84543 (**3a**–**g**), and their activity at nicotinic receptors. We anticipate these ligands to have significant utility as models for application to other receptor systems.

2. Results and discussion

2.1. Chemistry

We initially approached the synthesis of the quaternary AaCh and ADMP homologs. For our purposes, we felt that PEG derivatives of this type should be accessible in straightforward fashion by reaction of *N*,*N*-dimethylethylenediamine, or 1-methylpiperazine with an appropriate PEG-based acylating agent. For examination of the effect of tether length, we chose to use polyethylene glycol monomethyl ethers $(mPEG)_nOH$ where $n = 0$–6 (**4a**–**g**) as the homologous series. Monomethyl ethers **4b**–**d** are available[15] commercially while the higher oligomers **4e**–**g** with 4–6 PEG units were synthesized by alkylation of tetraethylene glycol with methyl



R	X	Aminocholines	Piperazines	Pyridines
CH_3O	NH	**1a**	**2a**	**3a**
$CH_3(OCH_2CH_2)O$	NH	**1b**	**2b**	**3b**
$CH_3(OCH_2CH_2)_2O$	NH	**1c**	**2c**	**3c**
$CH_3(OCH_2CH_2)_3O$	NH	**1d**	**2d**	**3d**
$CH_3(OCH_2CH_2)_4O$	NH	**1e**	**2e**	**3e**
$CH_3(OCH_2CH_2)_5O$	NH	**1f**	**2f**	**3f**
$CH_3(OCH_2CH_2)_6O$	NH	**1g**	**2g**	**3g**

Figure 2.

iodide (for **4e**) or triethylene glycol with mPEG mesylates **5c**,**d** (for **4f**,**g**).[15–18]

With the alcohols in hand, we set about synthesizing the amides corresponding to the two quaternary series (Scheme 1).[12,13,19] There are two two possible ways to approach this. The literature describes formation of 4-methylpiperazine-1-carbonyl chloride from methylpiperazine using phosgene or the more user-friendly di- or triphosgene compounds,[20] which we envisioned using in situ to acylate the mPEG alcohols **4** to produce the intermediate urethanes **6** and **7**. However, in our hands this was complicated significantly by formation of the symmetrical ureas, leading to reduced yields. Fortunately, formation of the mPEG chloroformates by reaction of mPEG alcohols **4** with phosgene in toluene proceeded smoothly overnight and in situ acylation of *N*,*N*-dimethylethylenediamine afforded a convenient one-pot synthesis of the mPEG-acyldimethylethylenediamines **6b**–**g** in good yield.[12] Acylation of *N*,*N*-dimethylethylenediamine proceeded likewise, affording the acylmethylpiperazines **7b**–**g**.[13] Predictably, aqueous workups of these highly water soluble compounds gave poor yields, affording only 7–15% recoveries even after saturating with NaCl, while direct flash chromatography of the reaction mixtures gave 60–70% yields. The amines were subsequently quaternized using methyl iodide in acetone to produce **1b**–**g** and **2b**–**g** smoothly, though in several cases oils were obtained rather than the expected crystalline compounds.[21] The parent compounds AaCh, ADMP, and the corresponding methoxycarbonyl analogs **1a** and **2a** were prepared in analogous fashion by acylation of *N*,*N*-dimethylethylenediamine or 1-methylpiperazine respectively with acetyl chloride or methyl chloroformate, to produce compounds **8**, **9**, **6a**, and **7a**, which were quaternized as above to provide crystalline methiodides.[12,13]

A variety of pyridyl ethers were developed by Abbott laboratories in the mid 1990s and several have advanced to the clinic with useful cognitive enhancing and/or analgesic properties.[2a,3a] These compounds can be looked at as homologs of nicotine and/or bioisosteric variants of epibatidine (Fig. 3). Several of these compounds have been shown to have significant selectivity for β2-containing subtypes, especially α4β2 receptors, which are the major subtype present in cortex and thought to be responsible for antinociception and cognitive enhancement.[4] Two of these have gone into clinical trials (ABT-089 and ABT-594). It has also been shown that in this series, the 5-position of the pyridine ring is amenable to substitution.[7] Indeed, another compound, SIB-1508Y shows substitution tolerance in nicotine at the 5- position.[22] Recently, it was demonstrated that similar substitution of both A-84543 and epibatidine can be achieved and enhances selectivity for α4β2 receptors.[14]

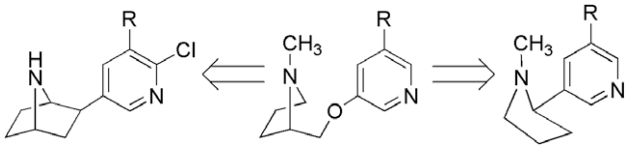
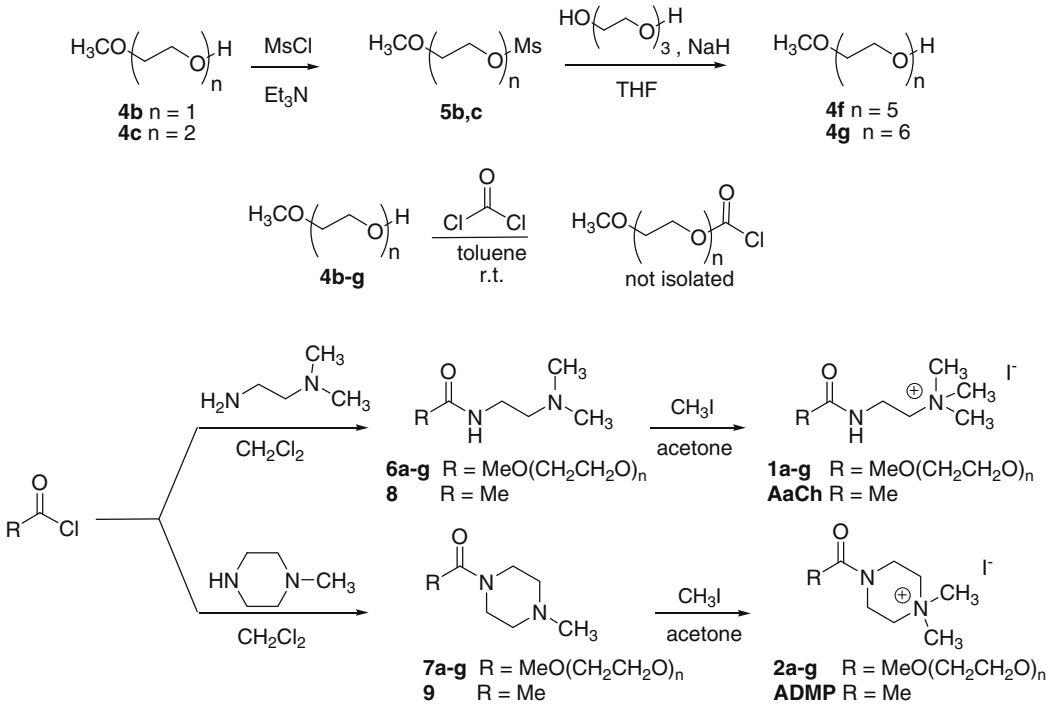
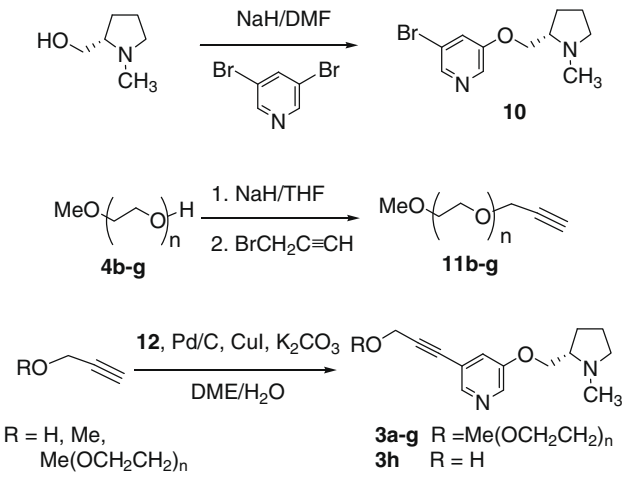


Figure 3.

We prepared a series of homologated ligands analogous to this series with oligomeric mPEG side chains (**3a**–**g**) based on similar chemistry (Scheme 2). We chose the A-84543 homologs as the chemistry is simplified relative to the azetidine clinical candidates noted above. We prepared 5-bromo-A-84543 (**10**) using an S_NAr reaction essentially as described from commercially available 3,5-dibromopyridine and (*S*)-*N*-methylprolinol using NaH in DMF.[7a,14] Initial attempts to conjugate mPEG alcohols **4a**–**f** with **10** in the same manner were unsuccessful due to the increasing deactivation of the pyridine ring to a second S_NAr substitution by the presence of the electron-donating ether installed from the first displacement. We observed no reaction at room temperature in DMF for extended reaction times and elevated temperature led only to decomposition products. We then examined the Sonogashira reaction as described for the installation of the hydrocarbon side chains.[7,14] Alkylation of mPEG alcohols with propargyl bromide afforded the alkyne coupling partners (**11b**–**g**).[23] Sonogashira coupling proceeded in moderate yield with an excess of alkyne to



Scheme 1. Synthesis of quaternary ammonium compounds.



Scheme 2. Synthesis A-84543 homologs.

afford the corresponding ethynylpyridines (**3b**–**g**).[24] As reference compounds, we prepared propargylic alcohol (**3h**) and corresponding methyl ether (**3a**) in analogous fashion using commercially available propargyl alcohol and methoxypropyne, respectively, as described.[14]

2.2. Pharmacology

With the compounds in hand, we examined their affinity for nicotinic receptors in rat cerebral cortex (Table 1).[25] The acylaminocholine analogs (**1a**–**g**) were found to be poor ligands, having affinities 15–200 μM. The piperazinium series fared better, with affinities 1 to 40 μM. In each series, exchange of the acetyl for methoxycarbonyl (**1a**, **2a**) led to an improvement in potency. The A-84543 analogs (**3a**–**g**) were very potent, with K_i values in the 2–15 nM range. In all three series, increasing chain length produced a regular but modest decrease in potency (a factor of 1.7–2.2 per PEG unit), losing just over an order of magnitude over the series. However, the retention of affinity, especially the high affinity for the A-84543 homologs suggests that very long tethers may be appended to these molecules thus making them useful for tagging nAChR. We are currently evaluating this behavior and results will be forthcoming.

The retention of affinity for these series is in keeping with the idea that these tethers extend out of the binding pocket of the receptor into the extracellular space. The currently used models for nicotinic receptors account for this as the binding site extends directly into the extracellular space without little protein interaction, save for the recognition motif.[14,26b] Of course, one must always be cautious as to such interpretation, as the majority of these models are based on the acetylcholine binding protein[27] and may not accurately represent the full receptor, in spite of the homology of the native protein to the extracellular portion of the α7 receptor.[22] These models are typically produced by virtual mutation of residues with the homologous counterparts from the receptor subtype of interest. We had originally designed the length of the tethers to reach the channel lumen through the channels proposed by Unwin based on electron diffraction data obtained on the Torpedo receptor.[26a] However, the models based on AChBP and newer data from the Unwin group now show the binding site to be accessed from the opposite side of the protein extending directly into the extracellular space to the side of the receptor.[26b] Nonetheless, we find that the tethers are easily accommodated and the uniform decrease in affinity suggests a nonselective effect and no sharp transition in affinity to indicate emergence from a tunnel into the extracellular space, and is thus consistent with the current model. However, this issue highlights the need for high-resolution X-ray data for this receptor and it is hoped that newer techniques in the crystallization of membrane proteins may resolve this problem.

3. Conclusions

We have successfully prepared and evaluated homologated nicotinic receptor ligands based on three binding motifs. Of these, the pyridyl ether series showed the strongest binding in the 1–10 nM range. Functionalized variants of these ligands should have significant utility as models for the design of multivalent ligands and/or affinity probes for nicotinic receptors.

4. Experimental

4.1. General

Commercially available reagents were used as received unless otherwise noted. All moisture sensitive reactions were carried out under nitrogen or argon in oven dried glassware. Tetrahyrofuran (THF) was freshly distilled from sodium/benzophenone under an argon atmosphere. Dichloromethane and acetonitrile were distilled from CaH_2 under an argon atmosphere. Dimethoxyethane (DME) was dried and freed of peroxides by filtration through Al_2O_3. Celite® filtrations utlilized Johns-Manville 545 grade. Flash column chromatography utilized 230–400 mesh silica gel EM Science 9385, while silica filtrations utilized EM Science 7734 (70–230 mesh) material. Thin layer chromatography was performed using silica gel plates (EM Science 5715), visualized with UV light, and stained with $KMnO_4$, iodine, or ninhydrin as appropriate. NMR spectra were obtained on Bruker AC-250 or Bruker Avance II 400 spectrometers. Chemical shifts are reported in ppm relative to TMS as calibrated by internal TMS for $CDCl_3$ and by the residual proton signals for other solvents. ^{13}C spectra in D_2O are referenced to the deuterium signal and are uncorrected. Coupling constants

Table 1
Pharmacologic data.

Compound	K_i (nM)	Compound	K_i (nM)	Compound	K_i (nM)
AaCh	63,000 ± 16,000	ADMP	3600 ± 1500	**3h**	3.1 ± 1.2
1a	14,000 ± 5000	**2a**	670 ± 130	**3a**	4.7 ± 1.5
1b	54,000 ± 12,000	**2b**	3000 ± 1700	**3b**	15 ± 7
1c	32,000 ± 8000	**2c**	8200 ± 3900	**3c**	7.5 ± 4.7
1d	70,000 ± 15,000	**2d**	48,000 ± 23,000	**3d**	15 ± 1
1e	15,0000 ± 37,000	**2e**	26,000 ± 10,000	**3e**	30 ± 1
1f	190,000 ± 50,000	**2f**	38,000 ± 5000	**3f**	40 ± 4
1g	30,000 ± 2000	**2g**	19,000 ± 5000	**3g**	63 ± 3

Data represent mean SEM for three experiments conducted in triplicate. K_i values were determined in rat cerebral cortex against 200 pm [^{3}H](±)-epibatidine hydrochloride with K_d taken as 40 pM (observed IC_{50} for cold (±)-epibatidine hydrochloride was determined to be 230–310 pM).

are reported in Hz. Carbon signals marked with an asterisk represent methyl and methine carbons, and quaternary carbons are designated with a (q) as determined on the basis of DEPT-edited experiments. FTIR spectra were obtained with a Midac M200 instrument and are expressed in cm^{-1}. Optical rotations were obtained with a Perkin-Elmer 241 Polarimeter operating at 589 nm at ambient room temperature. Boiling points were obtained by fractional or kugelrohr distillation as noted and are uncorrected. Melting points were obtained with an Electrothermal Mel-Temp apparatus and are uncorrected. Unit mass resolution electron impact mass spectra (EIMS) were taken with a Thermo-Finnigan GCQ instrument consisting of a programmable capillary GC interfaced to an ion trap autotuned against standard perfluorotributylamine. Where applicable, unit resolution chemical ionization mass spectra (CIMS) were obtained using ammonia as the reagent gas. High resolution mass spectrometry (HRMS) was performed by the Washington University Center for Biomedical and Bioorganic Mass Spectrometry (NIH Research Resource, Grant No. P41RR0954). Mass spectral formulas are calculated according to the most abundant isotopic formula and m/z determined relative to exact ^{12}C.

4.2. General procedure for the preparation of mPEG propargyl ethers.[23]

To suspension of sodium hydride (1.2 equiv, 0.7 M) in THF (cooled in an ice bath) was added a solution of a oligoethylene glycol monomethyl ether (1.0 equiv, 0.5 M) in THF. The reaction was stirred for fifteen minutes and propargyl bromide (80% in toluene, 1.1 equiv) was added. The ice bath was removed and the reaction allowed to warm to room temperature. When the reaction was judged to be complete by TLC, the reaction was carefully quenched with water (2 equiv) and the product isolated. For the lower oligomers (n = 1–3), the reaction was performed on 40 mmol scale and the product was isolated by aqueous-organic extraction and distillation. For the higher oligomers (n = 4–6) the reaction was performed on 1–2 mmol scale and the workup consisted of filtration concentration and the product was purified by flash column chromatography (EtOAc/MeOH).

4.3. General procedure for Sonogashira coupling reactions

The reactions were done in parallel analogous to the literature procedure for related compounds.[14] To a dry reaction vial was added in the following sequence, **6** (70 mg, 0.25 mmol), K_2CO_3 (90 mg, 0.65 mmol), triphenylphosphine (10 mg, 0.04 mmol), alkyne (1.5–4 mmol),[23] CuI (10 mg, 0.05 mmol), and 10% Pd/C (10 mg, 0.009 mmol). The compounds were suspended in a 1:1 mixture of dimethoxyethane/water the suspensions were sparged with Ar, then sealed and heated to 80 °C with stirring on a heating block for 6 days. The reactions were then cooled, filtered through Celite®, and the products isolated by flash column chromatography with an EtOAc/MeOH gradient.

4.4. General procedure for the preparation of mPEG urethanes

To neat **1a**–**f** (1.0 mmol) at 0 °C under N_2 was added a solution of phosgene (0.50 mL, 1.9 M in toluene, 0.95 mmol). The reaction was allowed to reach room temperature and stirred overnight. The reaction was sparged with dry N_2 briefly to expel unreacted phosgene, cooled in an ice bath, dry CH_2Cl_2 (1 mL) was added and the corresponding amine (2.5 mmol) was added dropwise. The reaction was warmed to room temperature and the reaction stirred at room temperature for 3–12 h. The product was isolated from the solution by flash column chromatography with a $CHCl_3$/MeOH gradient to yield **3b**–**g**, **5b**–**g** as colorless to pale yellow oils.

4.5. General procedure for the synthesis of mPEG quaternary salts

To a dry culture tube was added 30–50 mg of amine (**3b**–**g**, **5b**–**g**). The compounds were suspended in 1 mL of acetone (dried over 4 Å molecular sieves). Iodomethane (3–5 equiv) was added to the solutions and the reactions were allowed to stand in the dark overnight. Anhydrous diethyl ether was added dropwise as needed to promote precipitation, and the reactions were then returned to the dark for 48 h. The supernatant was removed and the salts were washed with ether and dried under high vacuum. While the lighter amines gave crystalline salts, many of the higher quaternary salts failed to crystallize, thus precluding a precise yield determination. Melting points are noted where crystalline materials were obtained.

4.6. Pharmacology

Binding assays in rat cerebral cortex were performed essentially as described using [^{3}H]epibatidine as the radioligand.[25] Functional fluorescence assays for the measurement of intracellular calcium dynamics were performed essentially as described[28] in HEK cells expressing rat α3 and β4 subunits for neuronal nicotinic acetylcholine receptors.[29] Detailed procedures are provided in the Supplementary material.

Acknowledgments

Mr. Ying Chau (Allen) Liu, Ms. Kiersten Yake, Mr. Fernand M. Bedi, and Ms. Amanda Clay are acknowledged for performing exploratory experiments related to this work. A generous gift of HEK cells expressing various subunit combinations for nicotinic receptors by Drs. Kenneth J. Kellar and Yingxian Xiao of Georgetown University Medical School is gratefully acknowledged.[29] Dr. John W. Daly is acknowledged for many helpful discussions, and his passing is deeply mourned. This work was performed with startup funds from the Department of Chemistry and College of Arts and Sciences at Indiana State University, as well as the National Institute for General Medical Sciences (1R21GM072780), and the National Science Foundation (CHE0521075 for a 400 MHz NMR spectrometer at Indiana State University).

Supplementary data

Detailed experimental procedures and NMR spectra for compounds and intermediates are provided. This material accompanies the online version of this article. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.10.045.

References and notes

1. (a) Nicotinic see: Brown, J. H.; Taylor, P. In *Goodman and Gilman's Pharmacological Basis of Therapeutics*, 10th ed.; Hardman, J. G.; Limbird, L. E., Eds., New York: McGraw-Hill, Chapter 9, pp. 193–213.; (b) Muscarinic, see: Brown, J. H.; Taylor, P. In *Goodman and Gilman's Pharmacological Basis of Therapeutics*, 10th ed.; Hardman, J. G.; Limbird, L. E., Eds., New York: McGraw-Hill, Chapter, Chapter 7 pp. 154–173.
2. (a) Arneric, S. P.; Holladay, M.; Williams, M. *Biochem. Pharmacol.* **2007**, *74*, 1092; (b) Gundisch, D. *Expert Opin. Ther. Pat.* **2005**, *15*, 1221.
3. Nicotinic receptors and pain (a) Bunnelle, William H.; Decker, Michael W. *Exp. Opin. Therap. Pat* **2003**, *13*, 1003–1021; (b) Vincler *Expert Opin. Investig. Drugs* **2005**, *14*, 1191.
4. Tapper, A. R.; McKinney, S. L.; Nashmi, R.; Schwarz, J.; Desphande, P.; Labarca, C.; Whiteaker, P.; Marks, M. J.; Collins, A. C.; Lester, H. A. *Science* **2004**, *306*, 1029.
5. (a) Voineskos, S.; De Luca, V.; Mensah, A.; Vincent, J. B.; Potapova, N.; Kennedy, J. L. *J. Psychiatry Neurosci.* **2007**, *32*, 412; (b) DeLeon, J.; Dadvand, M.; Canuso, C.; White, A. O.; Stanilla, J. K.; Simpson, G. M. *Am. J. Psychiatry* **1995**, *152*, 453.

6. Spande, T. F.; Garraffo, H. M.; Edwards, M. W.; Yeh, H. J. C.; Pannell, L.; Daly, J. W. *J. Am. Chem. Soc.* **1992**, *114*, 3475.
7. (a) Lin, N. H.; He, Y.; Holladay, M. W.; Ryther, K.; Li, Y. 3-Pyridyloxymethyl Heterocyclic Ether Compounds Useful in Controlling Chemical Synaptic Transmission. Patent US 5,629,325, 1997.; (b) Lin, N-H.; Li, Y.; He, Y.; Holladay, M. W.; Kuntzweiler, T.; Anderson, D. J.; Campbell, J. E.; Arneric, S. P. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 631.
8. (a) Gonzales, D.; Rennard, S. I.; Nides, M.; Oncken, C.; Azoulay, S.; Billing, C. B.; Watsky, E. J.; Gong, J.; Williams, K. E.; Reeves, K. R. *J. Am. Med. Assoc.* **2006**, *296*, 47; (b) Jorenby, D. E.; Hays, J. T.; Rigotti, N. A.; Azoulay, S.; Watsky, E. J.; Williams, K. E.; Billing, C. B.; Gong, J.; Reeves, K. R. *J. Am. Med. Assoc.* **2006**, *296*, 56.
9. Moaddel, R.; Oliveira, R. V.; Kimura, T.; Hyppolite, P.; Juhaszova, M.; Xiao, Y.; Kellar, K. J.; Bernier, M.; Wainer, I. W. *Anal. Chem.* **2008**, *80*, 48.
10. (a) Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 2755; (b) Gordon, E. J.; Gestwicki, J. E.; Strong, L. E.; Kiessling, L. L.; Gordon, E. J.; Gestwicki, J. E.; Strong, L. E.; Kiessling, L. L. *Chem. Biol.* **2000**, *1*, 9; (c) Fan, E.; Zhang, Z.; Minke, W. E.; Hou, Z.; Verlinde, C. L. M. J.; Hol, W. G. J. *J. Am. Chem. Soc.* **2000**, *122*, 2663.
11. (a) Haag, R.; Kratz, F. *Angew. Chem. Int. Ed.* **2006**, *45*, 1198–1215; (b) Bentley, M.D.; Bossard, M.J.; Burton, K.W.; Viegas, Tacey X. Poly(ethylene) Glycol Conjugates of Biopharmaceuticals, In *Drug Delivery in Modern Biopharmaceuticals*. Berlin: Wiley-VCH, 2005; Vol. 4, pp. 1193–1418.
12. (a) Barlow, R. B.; Bremner, J. B.; Soh, K. S. *Br. J. Pharmacol.* **1978**, *62*, 39; (b) Price, C. C.; Kabas, G.; Nataka, I. *J. Med. Chem.* **1965**, *8*, 650.
13. (a) Spivak, C. E.; Gund, T. M.; Liang, R. F.; Waters, J. A. *Eur. J. Pharmacol.* **1986**, *120*, 127; (b) Manetti, D.; Bartolini, A.; Borea, P. A.; Bellucci, C.; Dei, S.; Ghelardini, C.; Gualtieri, C.; Romanelli, M. N.; Scapecci, S.; Teodori, E.; Variani, K. *Bioorg. Med. Chem.* **1999**, *7*, 457.
14. (a) Wei, Z. L.; Xiao, Y.; Yuan, H.; Baydyuk, M.; Petukhov, P. A.; Musachio, J. L.; Kellar, K. J.; Kozikowski, A. P. *J. Med. Chem.* **2005**, *48*, 1720; (b) Kozikowski, A. P.; Chellappan, S. K.; Henderson, D.; Fulton, R.; Giboureau, N.; Xiao, Y.; Wei, Z. L.; Guilloteau, D.; Emond, P.; Dolle, F.; Kellar, K. J.; Kassiou, M. *ChemMedChem* **2007**, *2*, 54.
15. For consistency the letter system for each series is such that **a**–**g** represent 0–6 ethylene oxide units. Thus, compound **4a** is simply methanol and mesylate **5a** does not appear.
16. Bertozzi, C. R.; Bednarski, M. D. *J. Org. Chem.* **1991**, *56*, 4326.
17. (a) Reddy, V. V. S.; Whitten, J. E.; Redmill, K. A.; Varshney, A.; Gray, G. M. *J. Organomet. Chem.* **1989**, *372*, 207; (b) Schmidt, M.; Amstutz, R.; Crass, G.; Seebach, D. *Chem. Ber.* **1980**, *113*, 1691; (c) Hurd, C. D.; Fowler, G. W. *J. Am. Chem. Soc.* **1939**, *61*, 249.
18. (a) Sokolowski, A.; Burczyk, B.; Acetals, et al *Pol. J. Chem.* **1979**, *53*, 905; b Matter, M.; Rossi, A.; von Sprecher, H. Polyglycol Ether Acid Anilides. US Patent 2,769,838, 1956.
19. Belaby, R.; Hazard, R.; Cheymol, J.; Chabrier, P.; Najer, H. "Diuréthans et Leur Préparation" Fr. 1,173,901, 1959, *Chem. Abstr.* **1962**, *56*, 1354f.
20. (a) Jorand-Lebrun, C.; Valognes, D.; Halazy, S. *Synth. Commun.* **1998**, *28*, 1189; (b) Sharma, S.; Agarwas, V. K.; Dubey, S. K.; Iyer, R. N.; Anand, N.; Chatterjee, R. K.; Chandra, S.; Sen, A. B. *Ind. J. Chem. B.* **1987**, *26B*, 748.
21. This is a fairly standard method for quaternization of amines and worked well for the preparation of ADMP, AaCh and lower mPEG oligomers ($n = 0–2$), affording crystalline compounds. However, crystallization of the higher oligomers proved more difficult and in many cases, we were unable to obtain precipitates from acetone or acetonitrile and addition of ether or hydrocarbon solvents produced only oils. While we do not have an explanation for this, the compounds gave satisfactory HRMS and NMR data (see Section 4).
22. Cosford, N. D. P.; Bleicher, L.; Herbaut, A.; McCallum, J. S.; Vernier, J-M.; Dawson, H.; Whitten, J. P.; Adams, P.; Chavez-Noriega, L.; Correa, L. D.; Crona, J. H.; Mahaffy, L. S.; Menzaghi, F.; Rao, T. S.; Reid, R.; Sacaan, A. I.; Santori, E.; Stauderman, K. A.; Whelan, K.; Lloyd, G. K.; McDonald, I. A. *J. Med. Chem.* **1996**, *39*, 3235.
23. (a) De Halleux, V.; Mamdouh, W.; De Feyter, S.; De Schryver, F.; Levin, J.; Geerts, Y. H. *J. Photochem. Photobiol. A* **2006**, *178*, 251; (b) Li, Q. X.; Casida, J. E. *Bioorg. Med. Chem.* **1995**, *3*, 1667.
24. An excess of the alkyne is required as the alkynylpyridine product is virtually inseparable from the starting bromopyridine and thus the reaction must be forced to completion.
25. Houghtling, R. A.; Davila-Garcia, M. I.; Kellar, K. J. *Mol. Pharmacol.* **1995**, *48*, 280.
26. (a) Miyazawa, A.; Fujiyoshi, Y.; Stowell, M.; Unwin, N. *J. Mol. Biol* **1999**, *288*, 765; (b) Unwin, N. *J. Mol. Biol.* **2005**, *346*, 967.
27. (a) Smit, A. B.; Syed, N. I.; Schapp, D.; van Minnen, J.; Klumperman, J.; Kits, K. S.; Lodder, H.; van der Schors, R. C.; van Elk, R.; Sorgedrager, B.; Brejc, K.; Sixma, T. K.; Geraerts, W. P. M. *Nature* **2001**, *411*, 261; (b) Brejc, K.; van Dijk, W. J.; Klassen, R. V.; Schuurmans, M.; van der Oost, J.; Smit, A. B.; Sixma, T. K. *Nature* **2001**, *411*, 269.
28. (a) Veliçelebi, G.; Stauderman, K. A.; Varney, M. A.; Akong, M.; Hess, S. D.; Johnson, E. C. *Meth. Enzymol.* **1999**, *279*, 20; (b) Fitch, R. W.; Pei, X. F.; Kaneko, Y.; Gupta, T.; Shi, D.; Federova, I.; Daly, J. W. *Bioorg. Med. Chem. Lett.* **2004**, *12*, 179.
29. (a) Xiao, Y.; Kellar, K. J. *J. Pharmacol. Exp. Ther.* **2004**, *310*, 98; (b) Xiao, Y. l.; Meyer, E. L.; Thompson, J. M.; Surin, A.; Wroblewski, J.; Kellar, K. J. *Mol. Pharmacol.* **1996**, *54*, 322.