Structural Modification of Anatoxin-a. Synthesis of Model Affinity Ligands for the Nicotinic Acetylcholine Receptor

Nicholas J. S. Huby,[a] Philip Thompson,[a] Susan Wonnacott[b] and Timothy Gallagher*[a]

[a] *School of Chemistry, and* [b] *School of Biological Sciences, University of Bath, Bath BA2 7AY, UK*

The functionalization of anatoxin-a **1** at C-11 has been examined and the synthesis of ester **10** and amide **11**, models for a polymer-bound affinity ligand system, is described.

Anatoxin-a **1** is currently recognized as the most potent agonist known for the nicotinic acetylcholine receptor (nAChR).[1] As a consequence this molecule has emerged as an important pharmacological probe and is, together with synthetic analogues, providing valuable information about the agonist binding site of this receptor protein.[2] Anatoxin-a can also make significant contributions to this area in other ways since the high affinity binding of this molecule to the nAChR makes it a promising candidate for generating a polymer-bound ligand system for use in affinity chromatography.[3] Such a ligand system (Fig. 1) would be of value in the purification and characterization of nAChR and in this communication we describe preliminary synthetic work that is directed towards this end.[4] Our primary goals, which have now been achieved, were, first, to develop methods that would allow the incorporation of a functionalized spacer arm into anatoxin-a and, secondly, to utilize these derivatives to generate affinity ligand models retaining a high level of nicotinic potency.

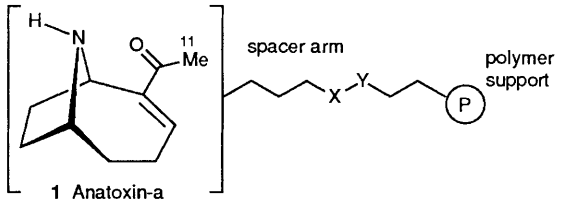
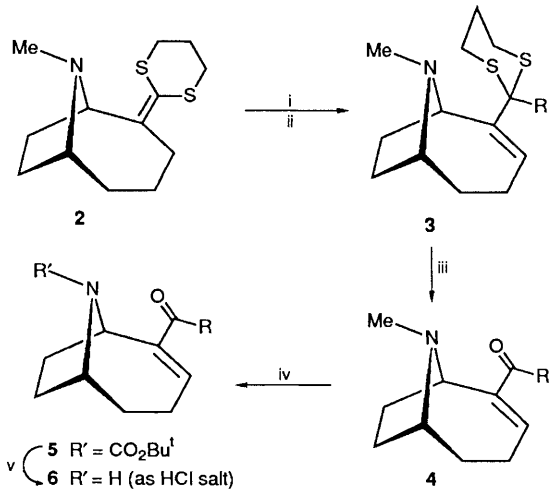
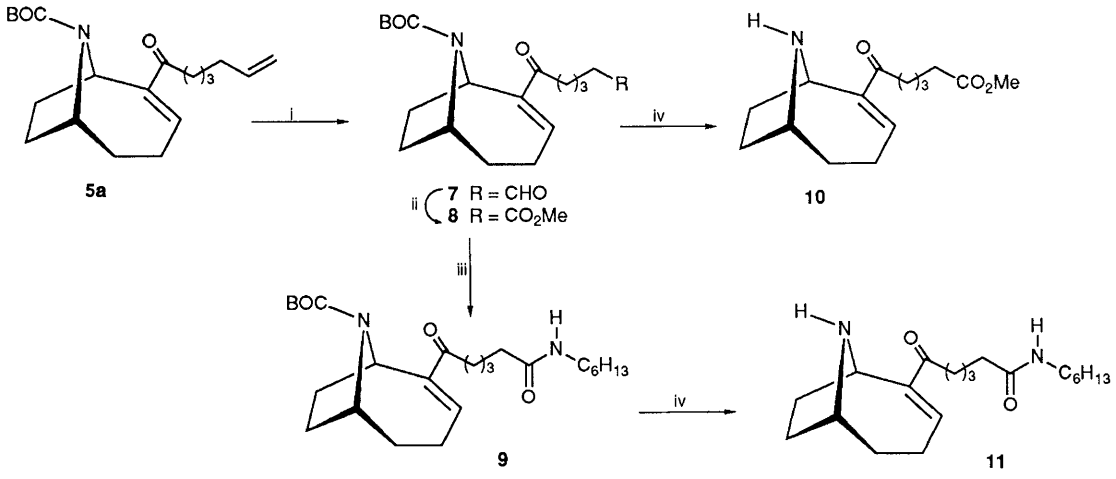


Fig. 1 Affinity chromatography ligand system based on anatoxin-a **1**



Scheme 1 *Reagents and conditions*: i, Bu^{n}Li, tetrahydrofuran (THF); ii, R–Y(Y = Br, I) (see Table 1); iii, HCl, dioxane or $(CF_3CO_2)IPh$;[7] iv, CH_2=CHOCOCl followed by $(Bu^tCO)_2O$; v, CF_3CO_2H, then HCl



Scheme 2 *Reagents and conditions*: i, O_3, CH_2Cl_2, −78 °C, then PPh_3, −78 °C to room temp.; 51%; ii, Br_2, MeOH, $NaHCO_3$; 24%; iii, LiOH, H_2O, THF, then dicyclohexylcarbodiimide, n-hexylamine; 64%; iv, CF_3CO_2H; 100%

Several factors must be considered when designing an affinity ligand system if a viable level of biological activity is to be maintained.[3] These include, for example, the nature of the spacer arm (length, hydrophobic characteristics) and its position and means of connection to the ligand skeleton. In addition there must be a mechanism available to enable the spacer arm to be anchored to a polymeric support, *i.e.* the X–Y link. There is well established methodology available for this latter task but decisions relating to the structure of the spacer and the optimal point of its attachment to the ligand skeleton are more difficult to assess.

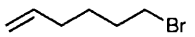
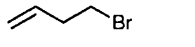
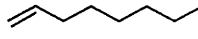
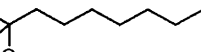
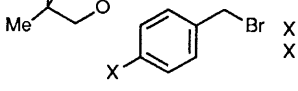
In this study we have focused on the use of C-11 of anatoxin-a as the point of attachment of the spacer arm. Although the synthetic accessibility of this site was an important consideration, we also felt that substitution at this centre was unlikely to lead to any adverse changes to the conformational characteristics of either the bicyclic framework or the enone function as compared to anatoxin-a itself.†

A variety of functionalized spacer arms were examined and were introduced into the skeleton of anatoxin-a using a modification of the procedure described by Lindgren *et al.*[6] (Scheme 1). The ketene dithioacetal **2** underwent allylic deprotonation (Bu^nLi, tetrahydrofuran) and regioselective α-alkylation with a series of alkyl and benzyl halides to give the masked enones **3**. Previous work[6] in this area had suggested the use of hexamethylphosphoramide (HMPA) as a cosolvent in the alkylation of the intermediate allyllithium but we found this to be unnecessary in the examples shown in Table 1. Thioacetal hydrolysis and *N*-demethylation were then carried out using established methods to give, after reprotection of the secondary amine function, the *N*-(t-butoxycarbonyl) (Boc) protected C-11-substituted anatoxin-a derivatives **5**. The use of a sequence involving *N*-demethylation followed by thioacetal cleavage, which was examined in the case of adducts **3b** and **3c**, proved to be less efficient.

Preliminary biological evaluation of the *N*-methyl enones **4** was carried out and this suggested that the benzyl derivatives **4e** and **4f**, which did not show a significant level of binding to nAChR, were unlikely to be of further use in our studies. Activity within the *N*-methyl series was taken as an indicator of nicotinic potency for the corresponding N–H derivatives.

† The bioactive conformation (s-*cis vs.* s-*trans*) of the enone moiety is the subject of some debate.[5] Our own molecular mechanics studies (MMX force field in PCMODEL) suggest that, relative to anatoxin-a, substitution at C-11 (*i*) does not result in significant ligand distortion and (*ii*) has a negligible effect on the relative energies of the s-*trans* and s-*cis* enone conformations, with the s-*trans* being consistently more favoured (by 2–3 kcal mol^{-1}; 8–12 kJ mol^{-1}).

Table 1 Preparation of the affinity ligand candidates

R—Y	Yield (%) 3	4	5
	**3a**, 92	**4a**, 76	**5a**, 60
	**3b**, 78		**5b**, 13[b]
	**3c**, 72		**5c**, 12[b]
	**3d**, 71[a]		
 X = Br	**3e**, 78	**4e**, 66	*c*
X = I	**3f**, 79	**4f**, 77	*c*

[a] All attempts to hydrolyse **3d** resulted in decomposition. [b] Overall yield from **2** based on *N*-demethylation followed by thioacetal cleavage (see text). [c] *N*-Demethylation of **4e** and **4f** was not examined.

This was based on the earlier observation that *N*-methylanatoxin-a, while not as potent as anatoxin-a itself, does display appreciable binding to the nAChR.[8] In addition, we were unsuccessful in our attempts to carry out a selective hydrolysis of orthoester **4d** and further work on this derivative was also abandoned.

The ω-alkenyl derivatives **5a–c** were more promising as affinity ligand candidates and the transformation of the alkenyl side chain into a carboxy function suitable for coupling *via* an ester or amide link (XY = CO·O or CO·NH in Fig. 1) to a Sepharose-based support is illustrated in Scheme 2 for the 6-hexenyl analogue **5a**. The terminal alkene of **5a** underwent selective oxidative cleavage with ozone to give, after workup with PPh_3, aldehyde **7**. This unstable intermediate was immediately oxidized under Williams' conditions[9] to give ester **8**, albeit in low overall yield (24%). Hydrolysis of ester **8** followed by coupling of the resulting carboxylic acid with n-hexylamine (a model for the amine residue of AH-Sepharose 4B) gave the corresponding amide **9**. Acid-induced cleavage of the Boc-protecting group from both **8** and **9** was achieved in essentially quantitative yield to give the fully deprotected model affinity ligands **10** and **11** respectively.

These synthetic models incorporate many of the structural features that would be required in a fully functional polymer-bound affinity chromatography system although the full potential of these ligands will only be realized when incorporation onto a polymer support has been achieved. Nevertheless,

we have established the viability of using C-11 of anatoxin-a as a point of attachment for a spacer arm and with **5b** and **5c** available an opportunity also exists for further structural optimization.‡ Full biological data concerning the ligands described in this communication will be published elsewhere, but our results indicate that monosubstitution at C-11 of anatoxin-a does not result in a significant loss of nicotinic binding.

Received, 9th October 1990; Com. 0/04547K

References

1 C. E. Spivak, B. Witkop and E. X. Albuquerque, *Mol. Pharmacol.*, 1980, **18**, 384; K. L. Swanson, C. N. Allen, R. S. Aronstam, H. Rapoport and E. X. Albuquerque, *Mol. Pharmacol.*, 1986, **29**, 250; X. Zhang, P. Stjernlöf, A. Adem and A. Nordberg, *Eur. J. Pharmacol.*, 1987, **135**, 457; D. MacAllen, G. G. Lunt, S. Wonnacott, K. L. Swanson, H. Rapoport and E. X. Albuquerque, *FEBS Lett.*, 1988, **226**, 357.

‡ Direct alkylation of *N*-Boc-protected anatoxin-a at C-11 using Rapoport's[2] conditions for kinetic enolate formation has also been achieved (N. J. S. Huby, unpublished results).

2 K. L. Swanson, H. Rapoport, R. S. Aronstam and E. X. Albuquerque; *A.C.S. Symposium Series*, 1990, **418**, 107. For synthetic studies relating to side-chain modified anatoxin-a analogues, see: M. H. Howard, F. J. Sardina and H. Rapoport, *J. Org. Chem.*, 1990, **55**, 2829 and references therein.
3 For comprehensive reviews of this technique, see: *Affinity Chromatography; Principles and Methods*, Pharamacia LKB Biotechnology, Uppsala, 1988; I. Parikh and P. Cuatrecasas, *Chem. Eng. News*, August 26, 1985, p. 17.
4 For previous studies directed towards the isolation of the nicotinic binding protein complex, see: L. G. Abood, W. Latham and S. Grassi, *Proc. Natl. Acad. Sci. USA*, 1983, **80**, 3536; H. Nakayama, M. Shirase, T. Nakashima, Y. Kurogochi and J. M. Lindstrom. *Mol. Brain Res.*, 1990, **7**, 221; P. G. Waser, D. M. Bodmer and W. H. Hopff, *Eur. J. Pharmacol.*, 1989, **172**, 231.
5 A. M. P. Koskinen and H. Rapoport, *J. Med. Chem.*, 1985, **28**, 1301; B. Witkop and A. Brossi, in *Natural Products and Drug Development*, Alfred Benzon Symp. 20, ed. P. Krogsgaard-Larsen, S. B. Christensen and H. Kofod, Munksgaard, Copenhagen, 1984, p. 282; U. Hacksell and C. Mellin, *Prog. Brain Res.*, 1989, **79**, 95.
6 B. Lindgren, P. Stjernlöf and L. Trogen, *Acta Chem. Scand., Ser. B*, 1987, **41**, 180.
7 G. Stork and K. Zhao, *Tetrahedron Lett.*, 1989, **30**, 287.
8 P. Kofuji, Y. Aracava, K. L. Swanson, R. S. Aronstam, H. Rapoport and E. X. Albuquerque, *J. Pharmacol. Exp. Ther.*, 1990, **252**, 517.
9 D. R. Williams, F. D. Klingler, E. E. Allen and F. W. Lichtenthaler, *Tetrahedron Lett.*, 1988, **29**, 5087.