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Anionic Cyclophanes as Potential Reversal Agents of Muscle Relaxants by Chemical Chelation

Kenneth S. Cameron,[c] Lee Fielding,[c] Rona Mason,[b] Alan W. Muir,[b] David C. Rees,[a,*] Simon Thorn[a] and Ming-Qiang Zhang[a]

[a]*Department of Medicinal Chemistry, Organon Laboratories Ltd., Newhouse, Lanarkshire ML1 5SH, Scotland, UK*
[b]*Department of Pharmacology, Organon Laboratories Ltd., Newhouse, Lanarkshire ML1 5SH, Scotland, UK*
[c]*Department of Analytical Chemistry, Organon Laboratories Ltd., Newhouse, Lanarkshire ML1 5SH, Scotland, UK*

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Abstract—A series of carboxyl-containing cyclophanes have been designed and synthesised as chemical chelators (or *host* molecules) of cationic muscle relaxant drugs (or *guest* molecules). Three of these cyclophane derivatives, **1**–**3**, have been shown by NMR to form 1:1 complexes with the muscle relaxants pancuronium, **4** and gallamine, **5** in D_2O, with association constants up to 10^4 M^{-1}. When tested in an in vitro chick biventer muscle preparation, the cyclophanes reversed the neuromuscular block induced by pancuronium and gallamine, with **3** having the most effective reversal against pancuronium (EC_{50} 40 μM).

Inspired by the fascinating developments in the area of supramolecular chemistry,[1] the aim of this study was to investigate whether chemical chelation (or *complexation*, *encapsulation*, *sequestration*) of muscle relaxant drugs could be used as a mechanism for reversing their pharmacological actions.

Skeletal muscle relaxants (neuromuscular blocking agents, NMBA's) are widely used surgically during general anesthesia particularly to paralyse laryngeal muscles prior to airway intubation and also to paralyse other skeletal muscles before general surgery.[2] Most currently used muscle relaxants in surgery today achieve their effect by blocking the physiological effects of acetylcholine (ACh) at nicotinic acetylcholine receptors (nAChR) on skeletal muscle. To reverse the blockade in patients, for example to assist recovery of muscle function after surgery, it is most common to administer acetylcholinesterase (AChE) inhibitors (e.g., neostigmine, pyridostigmine or edrophonium). However, this mechanism of action of the reversal agent increases ACh levels induced by AChE inhibition and leads to non-selective activation of muscarinic acetylcholine receptors (mAChR) causing many side effects, for example, bradycardia, hypotension, increased salivation and so on. Therefore, in practice, these reversal agents are usually used in combination with a mAChR antagonist such as atropine which itself has also a number of side effects, for example dry mouth, blurred vision, tachycardia, and so on.

We hypothesised that chemical chelation of NMBAs by an exogenous host molecule would reverse neuromuscular block. Since this mechanism of action does not involve direct activation of cholinergic systems it would be expected to circumvent the undesired clinical side effects of the currently used AChE inhibitors.

Complexation of quaternary ammonium guests by negatively charged cyclophanes is known in the literature.[3] Also, complexation of steroidal guests (neutral or negatively charged) with cyclophanes is known.[4] We sought to synthesise negatively charged cyclophanes **1**–**3** and to investigate their chelation of quaternary ammonium NMBAs such as the aminosteroid pancuronium **4** and gallamine **5** (Fig. 1).

The carboxyls on the periphery of the cyclophanes **1**–**3** were designed to provide potential for electrostatic interaction with the quaternary ammoniums as well as water-solubility (required for intravenous administration). The rigid cyclophane backbones provide a

*Corresponding author at present address: Medical Chemistry Dept. AstraZeneca, R&D Mölndal, S-431 83, Sweden. Fax: +46-31-776-3868; e-mail: david.c.rees@astrazeneca.com

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hydrophobic cavity for NMB (guest) chelation in an aqueous environment. The cyclophanes **1**–**3** were synthesised as outlined in Scheme 1. The commercially available 5,5′-methylenedisalicylic acid **6** was protected as the methyl ester **7**. Bis alkylation of the phenol to give **8** was followed by macrocyclisation with the phenol **7** using syringe pump techniques to give the cyclophane **9**. With the increasing hydrophobicity of the macrocycles, the final hydrolysis step became more difficult, with longer reaction times and use of DMSO as co-solvent in order to obtain **1**–**3**.[5]

The binding affinity of the cyclophanes **1**–**3** with the muscle relaxants pancuronium, **4** and gallamine, **5** was determined by ^{1}H NMR (400 MHz). Titration of phosphate buffered (pH 7.6) D_2O solutions of **4** or **5** with the cyclophanes **1**–**3** (0–4 mM) resulted in concentration dependent changes of chemical shifts of protons of the muscle relaxants. Job plot analysis by the method of continuous variation[6] indicates that all three cyclophanes form 1:1 complexes with **4** and **5**. The association constants (K_a's, Table 1) were calculated by an in-house curve fitting method that closely resembles those reported in literature.[7] The values in Table 1 are the average from independent observations of several protons of the muscle relaxants. For pancuronium, **4** the chemical shift changes of both A-ring and D-ring N–CH_3 were observed. In addition, the changes of D-ring acetate CH_3 were also clearly visible in the titration with **2** and **3** and changes of 19-CH_3 were observed during titration with **1** and **3**. In the case of gallamine binding, all three cyclophanes formed symmetrical complexes as seen from the retention of the 2:1 ratio of signals in the ^{1}H NMR spectra. This implies that complexation takes place with the central ammonium ion to give an axis of symmetry.

The NMR binding affinity of cyclophanes **1**–**3** with the NMBs (Table 1) correlates with the size and the aromatic area of the cyclophane ring, that is **3** > **2** > **1**. This is in agreement with the previous observation that host molecules with a high aromatic content tend to have high affinity for quaternary ammonium compounds,[3a–c] probably due to an ion-dipole attraction between the positive charge of the ammonium and the polarisable π bonds of the aromatic parts of the host. In addition, the increased aromatic area in the cyclophane ring increases the lipophilicity of the cavity, which favours interaction with the lipophilic part of the guest molecules, for

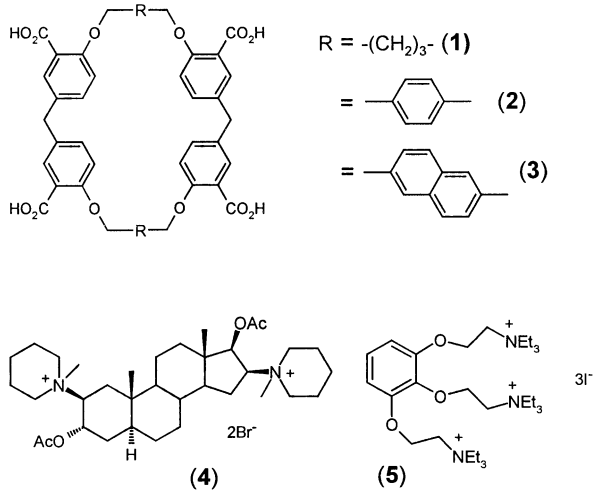
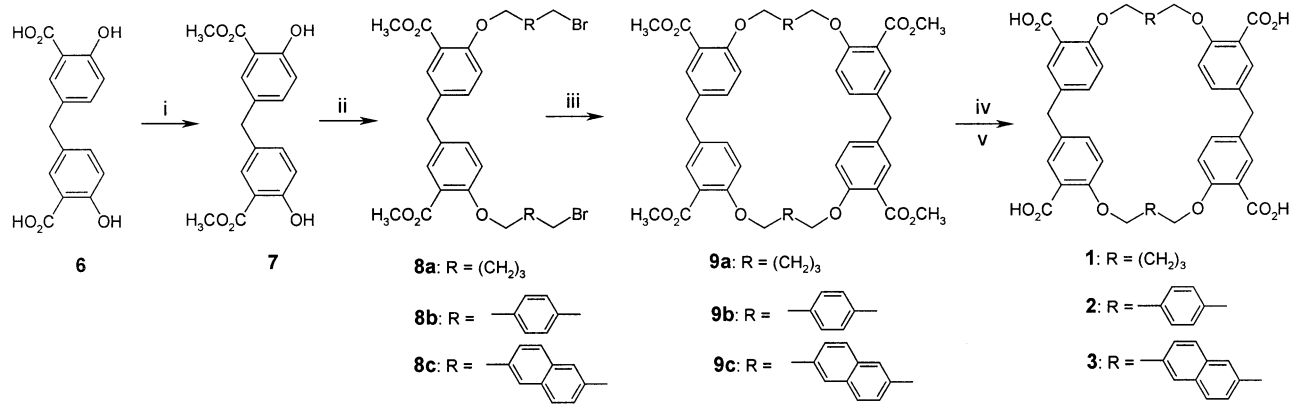


Figure 1. Structures of target cyclophanes (**1**–**3**) and muscle relaxants pancuronium (**4**) and gallamine (**5**).

Table 1. Association constants of cyclophanes **1**–**3** to the muscle relaxants **4** and **5**, and the reversal of 4- and 5-induced muscle relaxation by **1**–**3**

Compd	Pancuronium (**4**)		Gallamine (**5**)	
	Assoc. constant[a] K_a M^{-1}	Reversal EC_{50}, μM[b]	Assoc. constant[a] K_a, M^{-1}	Reversal EC_{50}, μM[b]
1	167	142	608	1030
2	2500	292	3275	563
3	10,450	40	13,200	104

[a]Means of at least three independent calculations based on chemical shift changes of different protons of **4** or **5**.
[b]Concentrations that produced 50% reversal of pancuronium- or gallamine-induced neuromuscular block in the chick biventer assay.[10] Data are means of two independent determinations.



Scheme 1. Synthesis of anionic cyclophanes **1**–**3**. Reagents and conditions: (i) MeOH, HCl, reflux, 11 h, (22%); (ii) K_2CO_3, $Br(CH_2)_5Br$, DMF, 1 h (60%) or NaH, $BrCH_2ArCH_2Br$, 50 °C; (iii) **7** (over 3 h), K_2CO_3 10 equiv (for **8a**), DMF, 80 °C, 4.5 h (10%) or NaH (for **8b** and **8c**), DMF, 120 °C (10–25%); (iv) for **1**: NaOH 20 equiv, MeOH–H_2O (3:1), reflux, 3 h (45%); for **2**: NaOH, MeOH–H_2O–DMSO (1:1:1), 60 °C, 12 h; for **3**: NaOH, THF–DMSO–H_2O (1:1:1), 60 °C, 48 h; (v) HCl (40%).

example, the steroid backbone of pancuronium **4**. Previous work in the area of crown ethers[8] and work by Koga[9] has shown that preorganisation favours good binding. The increased rigidity and therefore better defined cavity from **1** to **3** may also contribute to the increased affinity from **1** to **3** for the guest molecules.

Encouraged by these results we then investigated the ability of these guest molecules to reverse neuromuscular block in vitro as determined in the isolated chick biventer cervicis muscle preparation.[10] In these experiments, reversal activity was measured against an ~90% block of muscle twitch induced by cumulative additions of pancuronium, **4** or gallamine, **5**. The concentrations of **1**–**3**, which produced 50% reversal (EC_{50}), are listed in Table 1. For comparison, neostigmine, which is commonly used surgically as a NMB reversal agent via AChE inhibition, reverses vecuronium-induced block in chick biventer with an EC_{50} of 0.028 μM.

Although much less potent than the standard AChE inhibitor neostigmine, these cyclophane-based chelators do produce significant reversal of pancuronium, **4** or gallamine, **5** induced neuromuscular block in vitro. When cyclophane **3** was tested at the higher concentration of 2.3 mM it produced almost full reversal (>90%) of both **4**- and **5**-induced block in this chick biventer preparation.[11]

The cyclophanes **1**–**3** have also been examined for their intrinsic activities on isolated muscle by cumulative addition (1 μM–1 mM) to the tissue bath of unblocked chick biventer muscles. None of the compounds modified the height of the electrically-induced twitch response or affected the resting baseline tension of the biventer preparation.[12]

Taken together, these results suggest that the reversal activity of these cyclophanes seems to stem from their ability to form complexes with the muscle relaxants. For example the cyclophane **3** with the highest affinity to the blockers has the highest reversal potency, although there are some discrepancies of **1** and **2** in this respect.

In summary, this study aims at reversing the pharmacological effects of NMBAs by a mechanism involving chemical chelation (or sequestration). The cyclophane **3** binds to pancuronium **4** in an aqueous environment with an NMR binding constant of 10^4 and it reverses the biological effect of **4** and **5** in an in vitro muscle preparation. These results came from the first attempts in our laboratories to design and synthesise host molecules acting as reversal agents for NMBAs. Subsequent studies, which further develop the concept, are reported elsewhere.[13]

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5. Compound **1**: MS (EI) *m/z* 711 (M−H); ^{1}H NMR (DMSO) δ 1.51 (m, 4H), 1.68 (m, 8H), 3.81 (s, 4H), 3.99 (t, J=5.6 Hz, 8H), 6.96 (m, 4H), 7.20 (m, 4H), 7.43 (m, 4H), 12.25 (br s, 4H); ^{13}C NMR (DMSO) δ 21.57, 27.81, 38.72, 68.47, 113.99, 121.77, 130.28, 132.60, 133.21, 155.61, 167.27. Compound **2**: MS (EI) *m/z* 779 (M−H); ^{1}H NMR (DMSO) δ 3.75 (s, 4H), 5.15 (s, 8H), 6.91 (d, J=8.58 Hz, 4H), 7.20 (m, 4H), 7.32 (m, 8H), 7.49 (m, 4H), 12.40 (br s, 4H); ^{13}C NMR (DMSO) δ 68.69, 84.57, 114.52, 126.54, 129.98, 132.32, 133.81, 136.28, 154.60, 167.50. Compound **3**: MS (EI) *m/z* 879 (M−H); ^{1}H NMR (DMSO) δ 3.76 (s, 4H), 5.30 (s, 8H), 6.90 (m, 4H), 7.19 (m, 4H), 7.49 (m, 8H), 7.74 (m, 4H), 7.83 (m, 4H), 12.60 (br s, 4H); ^{13}C NMR (DMSO) δ 69.21, 83.84, 114.73, 121.88, 125.25, 125.37, 127.96, 130.34, 132.04, 132.67, 133.97, 135.03, 154.91, 167.52.
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11. An attempt to determine the in vivo reversal activity of compound **3** in an anesthetised guinea-pig failed due to insufficient potency.
12. An anti-AChE component to the reversal activity of compounds **1**–**3** is ruled out because they show no AChE inhibition when tested in concentrations up to 50 μM (cf. neostigmine IC_{50} 0.6 μM).
13. Note added in proof: see Bom, A.; Bradley, M.; Cameron, K; Clark, J. K.; van Egmond, J.; Fielden, H.; MacLean, E. J.; Muir, A. W.; Palin, R.; Rees, D. C.; Zhang, M.-Q. *Angew. Chem. Int. Ed. Engl.* **2002**, in press.