Bis- and Mixed-Tetrahydroisoquinolinium Chlorofumarates: New Ultra-Short-Acting Nondepolarizing Neuromuscular Blockers

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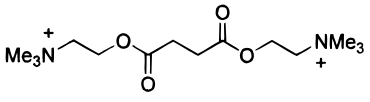
Introduction. Neuromuscular blocking agents (NMBs)[1] are used as adjuncts to anesthesia to provide skeletal muscle relaxation during surgery and to facilitate tracheal intubation. Emergency tracheal intubation maneuvers are routinely accomplished with the ultra-short-acting[2] depolarizing NMB succinylcholine (suxamethonium).[3] Although still widely used, succinylcholine is a nicotinic acetylcholine receptor agonist and can produce a number of potentially dangerous side effects[1,3] secondary to its mechanism of action. These include hyperkalemia, malignant hyperthermia, and cardiac arrhythmias. Common side effects include fasciculations, severe muscle pain, increased intraocular pressure, and increased intragastric tension.

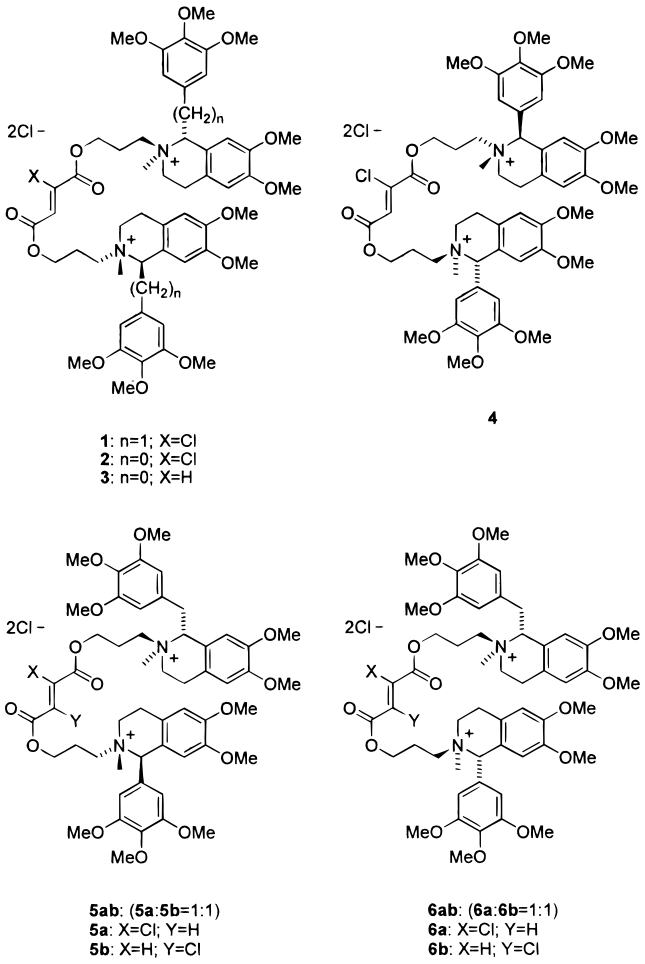


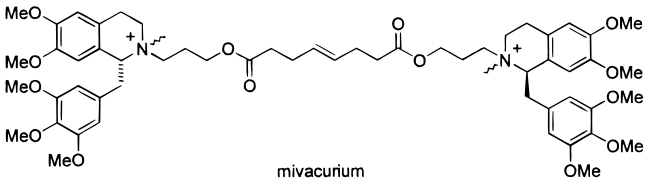
succinylcholine

Nondepolarizing NMBs are nicotinic acetylcholine receptor antagonists and are devoid of the side effects typically associated with depolarizing relaxants. A number of short, intermediate, and long-acting nondepolarizing NMBs are available in the clinic; however, no ultra-short-acting nondepolarizing NMB is currently available to anesthesiologists who have long recognized the need for a nondepolarizing succinylcholine replacement.[3,4]

We recently launched a medicinal chemistry program to discover[5] an ultra-short-acting nondepolarizing NMB, and in the course of this work we synthesized the bis- and mixed-tetrahydroisoquinolinium fumarates **1**−**6**. The structures and absolute stereochemistries of these new NMBs are provided in Chart 1. Mixed-onium chlorofumarate **6a** is a potent, ultra-short-acting, nondepolarizing NMB with a mean ED_{95} value of 0.063 mg/kg in the rhesus monkey. The duration of action of **6a** in this species is very similar to that of succinylcholine and about one-half that of the short-acting nondepolarizing NMB mivacurium (Mivacron).

Chart 1





mivacurium

The structural features of the mixed-onium derivatives **5** and **6** are particularly noteworthy. In addition to four stereogenic centers, two of which are quaternary ammonium, these molecules display disparate headgroup functionality and, unlike mivacurium, are not bis-1-benzylisoquinolines. As a result of their mixed-onium structure, compounds **5** and **6** also manifest defined chlorofumarate regiochemistry. Although these structural features render mixed-onium chlorofumarates challenging targets for practical synthesis, we found that incorporating these stereochemical elements was necessary to obtain an NMB with the desired preclinical pharmacological profile.[6] In this Communication we describe a general method for the preparation of bis- and mixed-onium fumarate derivatives **1**−**6** and com-

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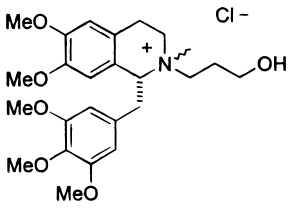
pare their neuromuscular pharmacodynamics in the rhesus monkey with those of succinylcholine and mivacurium.

Chemistry. Tetrahydroisoquinolinium headgroups **7**−**9** were prepared as mixtures of *cis* and *trans* stereoisomers (with respect to the trimethoxybenzyl or trimethoxyphenyl substituent at C-1 and the 3-hydroxypropyl substituent at N-2) from the corresponding enantiomerically pure 1-phenyl[7]- and 1-benzyl[5a]- 1,2,3,4-tetrahydroisoquinolines employing methods previously described[5a] (*trans*:*cis* ratio ca. 3:1). The (1*R*)-benzylisoquinolinium headgroup **7** was enriched in its *trans*-(1*R*,2*S*)-isomer (ca. 95% *trans*) by flash chromatography on silica gel. The (1*R*)-phenylisoquinolinium headgroup **8** and the (1*S*)-isomer **9** were similarly enriched in their *trans*-(1*R*,2*S*)- and *trans*-(1*S*,2*R*)-isomers, respectively, or were used as *trans*/*cis* mixtures without further purification. Relative stereochemistries of the *trans*-onium headgroups were supported by nuclear Overhauser and exchange NMR spectroscopy (NOESY).



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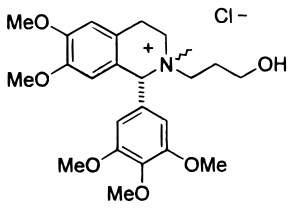

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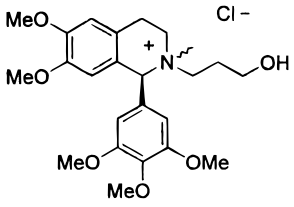

9

Chlorofumaryl chloride (**10**) was prepared from *dl*-tartaric acid and phosphorus pentachloride.[8] Fumaryl chloride (**11**) was obtained commercially. Bis-onium chlorofumarates **1**−**4** were prepared by mixing diacid chloride **10** or **11** with 2 mol equiv of headgroup **7**, **8**, or **9** in dichloroethane at ambient temperature. Mixed-onium chlorofumarates **5ab** and **6ab** were prepared by dropwise addition of an equimolar solution of **7** and **8** (or **7** and **9**) to chlorofumaryl chloride (**10**) in dichloroethane. This method produced complex product mixtures, and the desired bis- and mixed-onium diesters were isolated by preparative HPLC (Scheme 1).[9] Mixed-onium regioisomers **6a** and **6b** were prepared selectively[10] from **7**, **9**, and *trans*-2,3-dichlorosuccinic anhydride.[11]

Neuromuscular Pharmacology. Rhesus monkeys (adult males, 8−15 kg) were anesthetized with ketamine (5 mg/kg, im) or sodium pentobarbital (2−5 mg/kg, iv). Anesthesia was maintained with a mixture of nitrous oxide (60%), oxygen (40%), and halothane (0.5−1%). Ventilation was accomplished mechanically via an endotracheal tube while monitoring esophageal temperature and electrocardiograms. The common peroneal nerve was stimulated supramaximally with square wave pulses of 0.2-ms duration at a rate of 0.15 Hz. Twitch responses of the *extensor digitorum* were recorded via a force transducer. A femoral vein and artery were cannulated for drug administration and for recording arterial blood pressure, respectively. All compounds were tested as their dichloride salts and were injected as bolus doses (saline solutions) intravenously over 1−2 s. Potencies were determined as ED_{95} values, i.e., the dose required to produce 95% suppression of the evoked twitch response. Mivacurium[12,13] was administered to the same animals as a positive control.

Biological Results and Discussion. Potency values (ED_{95}), onset times, and duration of NMB effect for compounds **1**−**6**, mivacurium, and succinylcholine in the rhesus monkey are provided in Table 1.[14] In this species, succinylcholine has an ED_{95} of 1.3 mg/kg, an onset at the ED_{95} dose of 50 s, and a duration of action of ca. 5 min. We felt that an ultra-short-acting nondepolarizing NMB should have an onset in the rhesus monkey of less than 60 s at low multiples of the ED_{95} dose and a duration of action similar to that of succinylcholine.[15] We were also interested in progressing compounds with ED_{95} values less than 0.1 mg/kg.

Although a number of clinically used nondepolarizing NMBs can induce rapid onsets at high doses,[1] none combine the three qualities of good potency, rapid onset, and ultra-short duration characteristic of succinylcholine. The chlorofumarate inter-onium linker was recently discovered in our laboratories to elicit ultra-short duration of action in the bis-isoquinolinium series of NMBs.[16] Reacting onium headgroup **7** with chlorofumaryl chloride (**10**) provided bis-(1*R*,2*S*)-benzylisoquinolinium chlorofumarate **1** which had an ED_{95} of 0.109 mg/kg, an onset of 73 s, and a duration of 8.7 min. In contrast, the corresponding bis-(1*R*,2*S*)-phenylisoquinolinium chlorofumarate **2** was more than 1 order of magnitude less potent (ED_{95} = 1.79 mg/kg), suggesting

Scheme 1

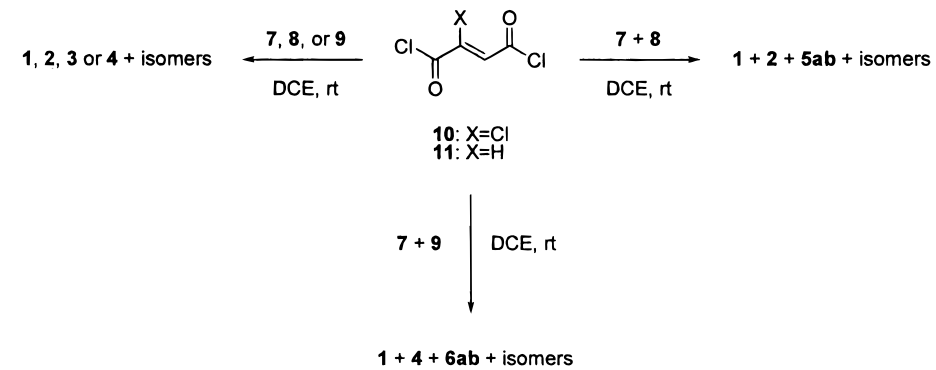

Table 1. Potency Values (ED_{95}), Onset Times, and Durations of NMB Effect at the ED_{95} Dose for Compounds **1**−**6**, Mivacurium, and Succinylcholine in Male Rhesus Monkeys[a]

compd	N[b]	ED_{95} (mg/kg)[c]	onset (s)[d]	duration (min)[e]
1	2	0.109 ± 0.008	73 ± 15	8.7 ± 2.9
2	2	1.79 ± 0.13	46 ± 3	5.1 ± 0.4
3	1	1.55	114	25.5
4	1	8.50	55	11.2
5ab	2	0.31 ± 0.10	37 ± 2	3.7 ± 1.3
6ab	6	0.163 ± 0.030	51 ± 4	5.6 ± 0.6
6a	8	0.063 ± 0.002	88 ± 5	5.6 ± 0.4
6b	2	0.25 ± 0.02	52 ± 2	3.4 ± 0.3
mivacurium (dichloride)	3	0.062 ± 0.009	107 ± 13	12.0 ± 1.9
succinylcholine (dichloride)	3	1.29 ± 0.34	50 ± 7	5.0 ± 0.6

[a] Data are expressed as means ± SE ($N > 1$).[14] [b] N = number of animals. [c] Dose producing 95% suppression of the twitch response. [d] Time from injection to peak inhibition of the twitch response at the ED_{95} dose. [e] Time from injection to recovery of the twitch to 95% of baseline at the ED_{95} dose.

that the (1*R*,2*S*)-benzylisoquinolinium headgroup in **1** has a greater affinity for the nicotinic acetylcholine receptor.

Fumarate **3**, the deschloro analogue of **2**, was prepared from **8** and fumaryl chloride (**11**) in order to assess the importance of the linker halogen on duration of action. This NMB was 5-fold longer acting than the corresponding chlorofumarate congener **2** but had similar potency. Bis-(1*S*,2*R*)-phenylisoquinolinium chlorofumarate **4**, prepared from **9** and chlorofumaryl chloride (**10**), was ca. 5-fold less potent than its enantiomer (**2**). Both **2** and **4** induced noticeably more rapid onsets of action compared with **1**. This structure−activity relationship is consistent with the reported observation that less potent compounds produce more rapid onsets of action (at their ED_{95}) within a given structural class of NMBs.[17]

Following the evaluation of bis-onium chlorofumarates **1**−**4**, we embarked on the synthesis of structurally distinct NMBs that incorporated 1-phenyl- and 1-benzyltetrahydroisoquinolinium headgroups in the same molecule. Accordingly, the mixed-onium chlorofumarates **5** and **6** were prepared by the same nonselective technique outlined in Scheme 1. The α- and *β*-chlorofumarates of **5** and **6** were found to coelute under the preparative HPLC conditions employed and were isolated as 1:1 mixtures of chlorofumarate regioisomers (**a** and **b**). Interestingly, the **6ab** mixture was about 2-fold more potent than the **5ab** mixture, illustrating an unusual mixed-onium synergy; namely, the *trans*-(1*S*,2*R*)-isomer of **9** yielded the least potent bis-onium chlorofumarate (**4**) and the most potent mixed-onium pair (**6ab**).

To assess the neuromuscular blocking properties of **6a** and **6b**, the individual regioisomers were prepared by selective synthesis.[10] Interestingly, α-chlorofumarate **6a** was 4-fold more potent than **6b** and maintained an ultra-short duration of action (5−6 min) at its ED_{95} dose. Onset and recovery times for NMBs **1**, **6ab**, and **6a** at 0.2 and 0.4 mg/kg are shown in Table 2. No untoward cardiovascular effects were noted at these doses. Blood pressure, heart rate, and twitch (*extensor digitorum*) recordings from a rhesus monkey following a 0.2 mg/kg bolus dose of **6a** are shown in Figure 1. The neuromuscular pharmacodynamics of **6a** in the rhesus monkey closely resemble succinylcholine and are exceptional for a nondepolarizing relaxant, particularly at the higher doses shown in Table 2 (3−6 × ED_{95}).

Table 2. Onset Times and Durations of NMB Effect from 0.2 and 0.4 mg/kg Bolus Doses of **1**, **6ab**, and **6a** in Male Rhesus Monkeys[a]

		0.2 mg/kg		0.4 mg/kg	
compd	N[b]	onset (s)[c]	duration (min)[d]	onset (s)[c]	duration (min)[d]
1	5	36 ± 7	9.78 ± 0.94	23 ± 2	13.4 ± 2.4
6ab	6	44 ± 4	7.10 ± 1.02	27 ± 2	8.7 ± 1.5
6a	8	38 ± 5	8.46 ± 0.45	25 ± 3	10.0 ± 0.5

[a] Data are expressed as means ± SE. All compounds produced 100% suppression of the twitch response at the doses indicated. [b] N = number of animals. [c] Time from injection to 95% suppression of the twitch response at the dose indicated. [d] Time from injection to 95% recovery of the twitch response at the dose indicated.

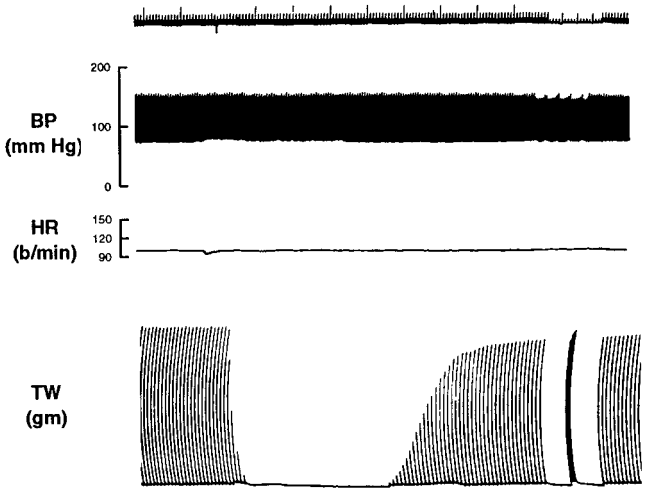

Figure 1. Blood pressure, heart rate, and twitch (*extensor digitorum*) recordings from a male rhesus monkey following a 0.2 mg/kg bolus dose of **6a**. The time from injection (inverted hash mark) to 95% recovery is ca. 9 min.

The ultra-short duration of the title compounds appears to result from the chlorofumarate linker as illustrated by the comparative pharmacodynamics of **2** and **3** (see Table 1). Furthermore, this activated inter-onium linker is susceptible to pH-dependent hydrolysis. For example, compound **6a** has excellent stability in pH 3 saline but undergoes more rapid hydrolysis at physiologic pH.[18] Chemodegradation of drug substance in vivo[19] could provide a clinical advantage by eliminating the problems associated with prolonged neuromuscular blockade in patients with low plasma cholinesterase activity.[20] The chlorofumarates listed in Table 2 are significant additions to the NMB family as they combine good potency (ED_{95} < 0.2 mg/kg), rapid onset, and ultra-short duration—three qualities not typically associated with a single nondepolarizing relaxant.[4]

The NMB properties of **6a** recently led to its clinical evaluation as an ultra-short-acting nondepolarizing succinylcholine replacement. A full account of the chemistry and structure−activity relationships leading to the discovery of this potentially useful adjunct to surgical anesthesia will be reported in due course.

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ceased) for preliminary synthetic work. The analytical support provided by Andrea M. Sefler, Randy D. Rutkowski, Lisa St. John-Williams, and William R. Hall is gratefully acknowledged. We also thank William B. Wastila for helpful discussion, Matthew R. Belmont, M.D., for assistance with the rhesus monkey studies, and Mark J. Sacchetti, Paul R. Johnson, Alan R. Brown, and Michael J. McNulty for the in vitro hydrolysis experiments with **6a**.

Supporting Information Available: Synthetic procedures, analytical data, and copies of ^{1}H NMR spectra for compounds **1**−**6** including chemical shift assignments, NOESY spectra, and dynamic NMR of **6a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) Compounds **1**−**6** produced no changes in heart rate or mean arterial blood pressure at the bolus doses described herein. The cardiovascular effects of some of the title compounds in the high dose range (10−100 × ED_{95}) will be reported in due course.

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(13) At 2 × ED_{95} in humans (0.15 mg/kg), mivacurium provides complete suppression of the twitch response in 2−4 min and 95% recovery in 20−30 min following injection.[1a]

(14) Data in Table 1 are expressed as means ± SE (N > 1). For each animal, the dose producing a 95% block of the *extensor digitorum* (ED_{95}) was computed from the linear regression slopes of log−probit plots of dose versus percent block. The onset at each dose was determined from the time of injection to the time of peak inhibition of the twitch response. The duration at each dose was determined from the time of injection to the time of recovery of the twitch to 95% of baseline.

(15) The intubating dose for succinylcholine in humans (1 mg/kg) produces ablation of the twitch response in 60−90 s and 95% recovery in 12−15 min after injection.[1b]

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(18) Compound **6a** has a half-life of 178 days in pH 3 saline at 23 °C. The half-life of **6a** at 37 °C in pH 7.4 phosphate buffer is 37 min.

(19) Accumulated data indicate that plasma esterases are not involved in the metabolism of **6a**; this will be the focus of a future publication.

(20) This rare but well-known problem has been observed in the clinical application of both mivacurium and succinylcholine; see: Frampton, J. E.; McTavish, D. Mivacurium: A Review of its Pharmacology and Therapeutic Potential in General Anaesthesia. *Drugs* **1993**, *45*, 1066−1089.

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