Neuromuscular Blocking Activity and Therapeutic Potential of Mixed-Tetrahydroisoquinolinium Halofumarates and Halosuccinates in Rhesus Monkeys

Eric E. Boros,* Vicente Samano, John A. Ray, James B. Thompson, David K. Jung, Istvan Kaldor, Cecilia S. Koble, Michael T. Martin, Virgil L. Styles, Robert A. Mook, Jr., Paul L. Feldman, John J. Savarese,† Matthew R. Belmont,† Eric C. Bigham, G. Evan Boswell,‡ Mir A. Hashim, Sanjay S. Patel,⊥ James C. Wisowaty, Gary D. Bowers, Caroline L. Moseley, John S. Walsh, Mindy J. Reese, Randy D. Rutkowske, Andrea M. Sefler, and Timothy D. Spitzer

GlaxoSmithKline Research & Development, Five Moore Drive, Research Triangle Park, North Carolina 27709 and Department of Anesthesiology, New York-Presbyterian Hospital, The University Hospitals of Columbia and Cornell, 525 East 68th Street, New York, New York 10021

Received December 19, 2002

Structure−activity relationships in rhesus monkeys for a novel mixed-onium class of ultra-short-acting nondepolarizing tetrahydroisoquinolinium neuromuscular blockers (NMBs) are described. Bis-onium chlorofumarate **20a** with (1*R*,2*S*)-benzyltetrahydroisoquinolinium groups was a potent lead compound (ED_{95} = 0.079 mg/kg) with an ultra-short duration of NMB effect (7.1 min) and a selectivity index (SI: defined as a ratio of the cardiovascular threshold dose to the ED_{95}) similar to that of mivacurium (**3**). The mean threshold dose for cardiovascular effects with **20a** was ca. 20 times its ED_{95} value (SI = 20). A novel mixed-onium analogue of **20a** was prepared by replacing the benzyltetrahydroisoquinolinium group distal to the fumarate chlorine atom with a (1*S*,2*R*)-phenyltetrahydroisoquinolinium moiety. The resulting mixed-onium chlorofumarate **24a** displayed good NMB potency (ED_{95} = 0.063 mg/kg), ultra-short duration of action (5.6 min) and an improved selectivity index (SI = 57). Several other mixed-onium derivatives containing octanedioate (**25a**; ED_{95} = 0.103 mg/kg), difluorosuccinate (**27c**; ED_{95} = 0.056 mg/kg), and fluorofumarate (**28a**; ED_{95} = 0.137 mg/kg) linkers were also potent, ultra-short-acting NMBs with good to excellent selectivity index values (SI = 37−96). Octanedioate **25a** was longer acting at higher doses compared to difluorosuccinate **27c** and chlorofumarate **24a**. Durations of NMB effect following a 0.4 mg/kg bolus dose (100% block) of **25a**, **27c**, and **24a** were 16.9, 13.0, and 10.0 min, respectively. Recovery time for mixed-onium chlorofumarate **24a** following a 1 h continuous infusion at 10−20 μg/kg/min (95−100% block) was ca. 5 min which is similar to that observed following a 0.2 mg/kg bolus dose of this compound and indicates a lack of cummulative effects. Preliminary studies with chlorofumarate **24a** in whole human blood revealed that mixed-onium thiazolidine **29** was the major metabolite and that plasma cholinesterases do not play the primary role in duration of NMB effect. The NMB properties of **24a** in rhesus monkeys led to its clinical evaluation as a possible alternative to succinylcholine.

Introduction

Administration of the long-acting neuromuscular blocker (NMB) *d*-tubocurarine (curare) (**1a**) to induce skeletal muscle relaxation during surgery and facilitate tracheal intubation maneuvers transformed the practice of anesthesia.[1] Since that time a variety of semisynthetic and synthetic NMBs with varying durations of NMB (curare-like) activity became available in the clinic.[1,2] Neuromuscular blockers are categorized both by their mechanism of action (nondepolarizing or depolarizing) and by their duration of action (ultra-short, short-, intermediate-, and long-acting).[3] Examples of these adjuncts to anesthesia include the long-acting agent metocurine (**1b**), the ultra-short-acting NMB succinylcholine (**2**), the short-acting relaxant mivacurium (**3**), and the long-acting agent doxacurium (**4**) (Chart 1).

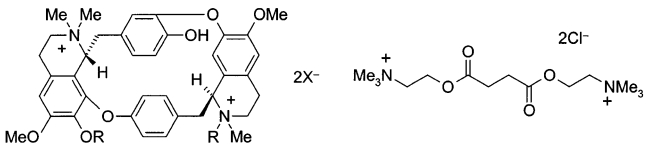
The benzyltetrahydroisoquinoline-based relaxants are nondepolarizing NMBs, and succinylcholine (**2**) is a depolarizing agent. Depolarizing NMBs are nicotinic acetylcholine receptor agonists and produce a number of unwanted side-effects associated with their mechanism of action.[4] These untoward effects can in rare instances include anaphylaxis, hyperkalemia, malignant hyperthermia, and cardiac arrhythmias. More common side-effects of depolarizing NMBs include fasciculations, severe muscle pain, increased intraocular pressure, and increased intragastric tension. Nondepolarizing NMBs are nicotinic acetylcholine receptor antagonists and are typically devoid of the side-effects associated with depolarizing relaxants.

Although a variety of long-, intermediate-, and short-acting nondepolarizing NMBs exist in the clinic, no ultra-short-acting nondepolarizing NMB is currently

* Corresponding author. GlaxoSmithKline Research & Development, Five Moore Drive, Research Triangle Park, NC 27709. E-mail: eric.e.boros@gsk.com. Phone: (919) 483-8116. Fax: (919) 315-0430.
† New York-Presbyterian Hospital, New York, NY.
‡ Present address: Pfizer, Kalamazoo, MI.
⊥ Present address: Avera Pharmaceuticals, San Diego, CA.

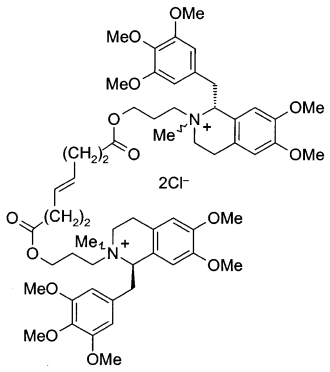
10.1021/jm020574+ CCC: $25.00
Published on Web 05/08/2003

Chart 1

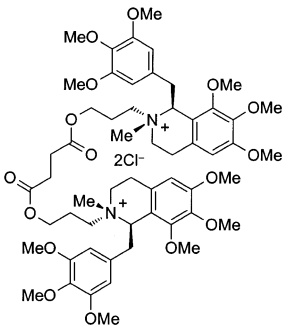


1a: R = H (*d*-tubocurarine)
1b: R = Me (metocurine)

2 (succinylcholine)



3 (mivacurium)



4 (doxacurium = meso + *dl*-forms)

available. Consequently, when anesthesiologists require an ultra-short-acting NMB, they must choose succinylcholine which can produce a number of unwanted side-effects, some of which (in rare instances) are life-threatening.[4] Despite considerable research effort,[5,6] there still exists no reliable substitute for succinylcholine in rapid sequence emergency intubations and treatment of laryngospasm. Succinylcholine's rapid onset and offset times and ultra-short duration of action allow stabilization of a patient's airway within 60 s and will generally ensure spontaneous recovery within a few minutes.

The ultra-short duration of succinylcholine results from its rapid metabolism by plasma cholinesterases. Individuals with low plasma cholinesterase activity will experience prolonged neuromuscular blockade lasting up to several hours.[7] Thus, the ideal succinylcholine replacement should be a nondepolarizing NMB with a duration of action comparable to succinylcholine coupled with a chemical (nonenzymatic) mechanism of deactivation in the blood stream.[8]

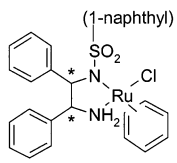
In preliminary communications,[9] we described an unusual class of nondepolarizing mixed-onium NMBs that combined 1-benzyl- and 1-phenyltetrahydroisoquinolinium groups in a single molecule. These compounds showed NMB profiles similar to those of succinylcholine in rhesus monkeys and were devoid of cardiovascular side-effects at doses producing onset times of 30−50 s and durations of 7−10 min in this species. Because the rhesus monkey is an excellent model for neuoromuscular blocking activity in humans, we were encouraged by these results and subsequently selected compound **24a** for study in healthy human volunteers. Results from these preliminary human trials were described by Belmont, Lien, and Savarese.[10] We now wish to elaborate on the structure−activity relationships of mixed-onium chlorofumarates in the rhesus monkey and their improved selectivity index values (SI: defined as a ratio of the cardiovascular threshold dose to the ED_{95}) in this species compared to mivacurium (**3**). In addition, a mechanism for the deactivation of **24a** in the blood stream is described.

Chemistry

Tetrahydroisoquinolinium propanol trans/cis mixtures **10a**−**h** and trans isomers **13a**−**i** (trans and cis refer to the relative orientation of the 1-aryl (or 1-benzyl) substituent and the 2-(3-hydroxylpropyl) side chain) were prepared by methods outlined in Scheme 1. Structures of synthetic intermediates **5**−**12** (Scheme 1) may be inferred from structural formulas **10a**−**h** and **13a**−**i** (Figure 1). Dihydroisoquinolines **5** were prepared by the Bischler−Napieralski[11] synthesis, and racemic tetrahydroisoquinolines **6** were obtained by reduction of **5** with $NaBH_4$. Tetrahydroisoquinoline enantiomers **8** were obtained by classical resolution of (±)-**6**, asymmetric reduction of **5** or other asymmetric synthesis methods (see below). Racemic **7** and enantiomerically pure *N*-methyl derivatives **9** were obtained by *N*-methylation of **6** and **8**, respectively, with formaldehyde and $NaBH_4$ (R′ = CH_2Ar) or under Eschweiler−Clarke[12] conditions (R′ = Ar).

The *R*-enantiomers of 5′-methoxylaudanosine ((*R*)-**9a**)[13] and (*R*)-5′,8-dimethoxylaudanosine ((*R*)-**9c**) were obtained by classical resolution of their (L)-dibenzoyltartrate salts. Synthesis of (*R*)-**9c** was also accomplished by asymmetric transfer hydrogenation[14] of **5c** (see below) followed by *N*-methylation of (*R*)-**8c** as described above. (*R*)-Norcryptostyline-III ((*R*)-**8d**) was prepared by classical resolution of its (*S*)-(+)-mandelic acid salt. (*R*)-Norcryptostyline-II ((*R*)-**8f**) and (*S*)-norcryptostyline-III ((*S*)-**8g**) were resolved as their 2,3:4,6-di-*O*-isopropylidine-2-keto-L-gulonic acid salts according to published procedures.[15] Compound (*S*)-**8g** was also synthesized by asymmetric transfer hydrogenation[14] of **5g** (see below).

Tetrahydroisoquinolines (*R*)-**8b** and (*R*)-**8c** were prepared by asymmetric transfer hydrogenation of dihydroisoquinolines **5b** and **5c** in the presence of Noyori's[14] chiral ruthenium catalyst (*S*,*S*)-**14**. Compounds (*S*)-**8g** and (*S*)-**8i** were prepared in a similar manner from **5g** and **5i** by employing chiral catalyst (*R*,*R*)-**14**.[14] Hexamethoxy tetrahydroisoquinoline (*R*)-**8e** and trimethoxy derivative (*S*)-**8h** were obtained by Kibayashi's[16] nucleophilic addition of aryl Grignard reagents or metal hydrides to chiral hydrazonium ions followed by removal of the chiral auxiliary.

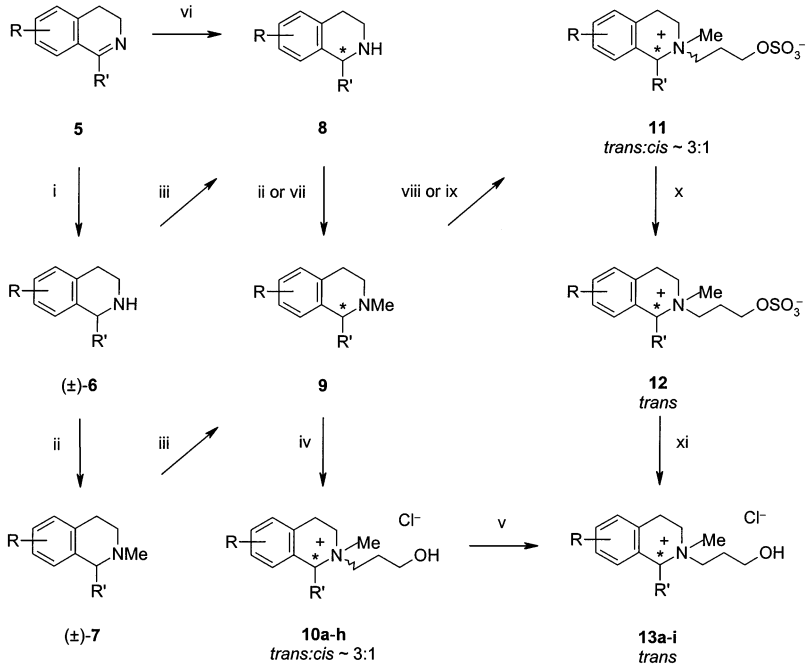


(*S*,*S*)-**14**
(*R*,*R*)-**14**

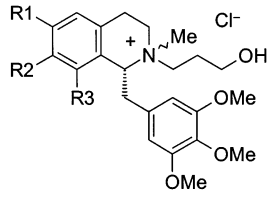
15

Tetrahydroisoquinolinium propanol trans/cis mixtures **10a**−**h** and trans isomers **13a**−**i** (Figure 1) were prepared from the corresponding *N*-methyl tetrahydroisoquinolines **9** using two alternative procedures (Scheme 1). One method involved quaternization of **9**

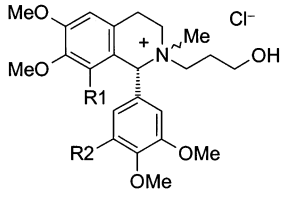
Scheme 1. Synthesis of Tetrahydroisoquinolinium Propanol Trans/Cis Mixtures **10a**−**h** (trans:cis ~ 3:1) and Trans Isomers **13a**−**i** from the Corresponding Dihydro (**5**) and Tetrahydro (**6**−**9**) Isoquinolines[a]



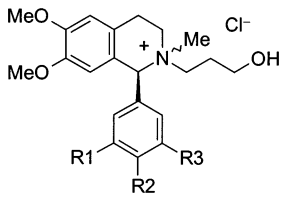
[a] *Denotes (1*R*)- or (1*S*)-configuration; (i) $NaBH_4$, MeOH; (ii) HCO_2H, HCHO, 100 °C (R′ = Ar); (iii) classical resolution or preparative chiral HPLC; (iv) 3-chloro-1-propanol, NaI, MEK, reflux; (v) prep-HPLC; (vi) (*R*,*R*)-**14** or (*S*,*S*)-**14**, TEA, HCO_2H, CH_2Cl_2 or CH_3CN; (vii) HCHO, $NaBH_4$, 0 °C (R′ = CH_2Ar); (viii) **15**, CH_3CN (R = Ar); (ix) **15**, acetone (R = CH_2Ar); (x) trans-selective crystallization from reaction mixture; (xi) AcCl, MeOH.



10a or **13a**: R1 = R2 = OMe; R3 = H
13b: R1-R2 = -OCH_2O-; R3 = H
10c or **13c**: R1 = R2 = R3 = OMe



10d or **13d**: R1 = H; R2 = OMe
10e R1 = R2 = OMe
10f: R1 = R2 = H



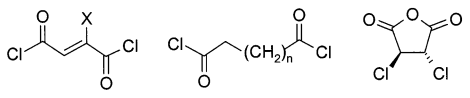
10g or **13g**: R1 = R2 = R3 = OMe
10h: R1 = R3 = H; R2 = OMe
13i: R1 = H; R2 = R3 = F

Figure 1. Structures of tetrahydroisoquinolinium propanol trans/cis mixtures **10a**−**h** (trans:cis ~ 3:1) and trans isomers **13a**−**i**.

with 3-chloro-1-propanol in the presence of sodium iodide. This process produced mixtures of tetrahydroisoquinolinium stereoisomers **10** favoring the trans product (trans:cis ratio ca. 3:1) as evidenced by ^{1}H NMR.[17] These trans/cis mixtures **10** were further enriched in the trans isomer **13** by preparative HPLC or were used without further purification. The second method for preparation of **13a**−**i** involved quaternization of **9** with 1,3,2-dioxathiane 2,2-dioxide (**15**).[9b] This method produced a mixture of tetrahydroisoquinolinium betaines **11** also favoring the trans product (trans:cis ratio ca. 3:1); however, the *trans*-betaines **12** crystallized selectively from the reaction mixture. Methanolysis of **12** provided the *trans*-tetrahydroisoquinolinium propanols **13**.

Structures of tetrahydroisoquinoline propanol **13j** and conformationally constrained tetrahydroisoquinolinium propanols **13k** and **13l** are shown in Figure 2. Compound **13j** was prepared by Michael addition of (*S*)-**6j** to methyl acrylate followed by reduction of the ester with $LiBH_4$. Syntheses of **13k** and **13l** have been described in detail.[18]

Chlorofumaryl chloride (**16a**),[19] dichlorosuccinic anhydride (±)-**18**,[20] and fluorinated cyclic anhydrides **19a**[21] and **19c**[22] were prepared by established procedures. Difluoroglutaric anhydride **19b** was obtained by



16a: X = Cl
16b: X = H

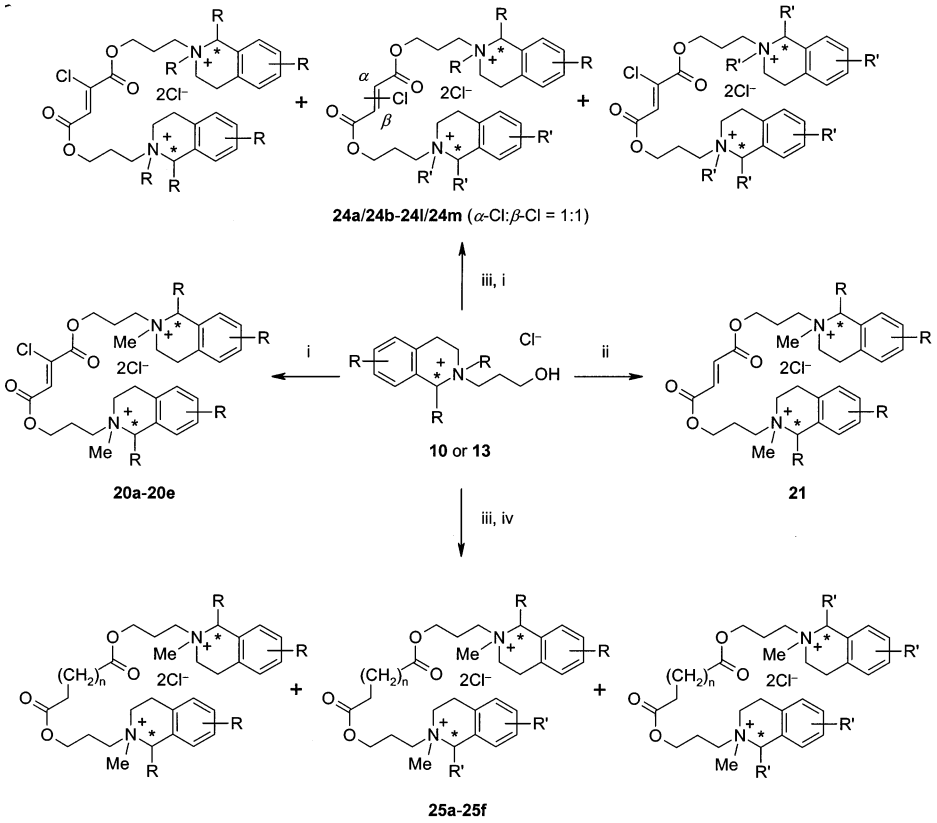
17a: n = 3
17b: n = 4
17c: n = 5
17d: n = 6
17e: n = 7

(±)-**18**

19a: X = H; n = 1
19b: X = F; n = 2
19c: X = F; n = 1

reduction of diethyl 4,4-difluoro-2-pentenedioate[23] followed by hydrolysis and anhydride formation,[24] respectively. Fumaryl chloride **16b** and acid chlorides **17a**−**e** were obtained commercially. Preparation of bis-onium chlorofumarates and fumarates and the nonselective synthesis of mixed-onium chlorofumarates and alkanedioates are illustrated in Scheme 2. Bis-onium chlorofumarates **20a**−**e** and fumarate **21** were synthesized by reacting 2 equiv of the corresponding tetrahydroisoquinolinium propanol **10** or **13** with 1 equiv of chlorofumaryl chloride (**16a**) or fumaryl chloride (**16b**). Mixed-onium chlorofumarate mixtures **24a**/**24b**−**24l**/**24m** were

Scheme 2. Synthesis of Bis-Onium Chlorofumarates and Fumarates (Chart 2), Mixed-Onium α- and *β*-Chlorofumarate Mixtures (Charts 2−4), and Mixed-Onium Alkanedioates (Chart 4)[a]



[a] *Denotes (1*R*)- or (1*S*)-configuration; (i) **16a**, CH_2Cl_2 or DCE; (ii) **16b**, CH_2Cl_2 or DCE; (iii) **10′** or **13′**, CH_2Cl_2 or DCE; (iv) **17a**−**e**.

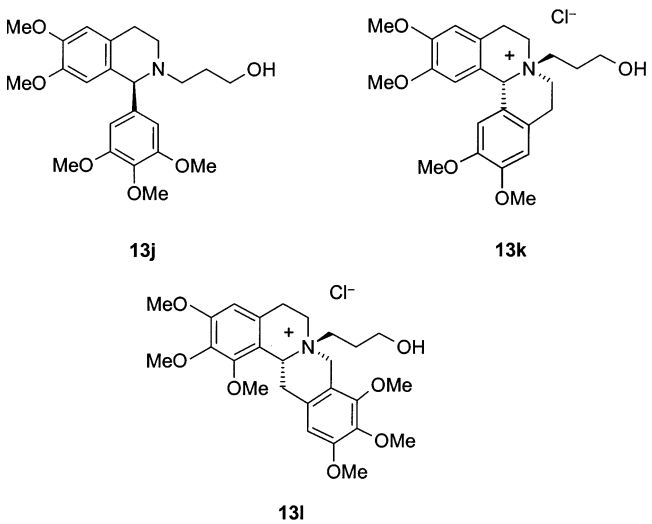
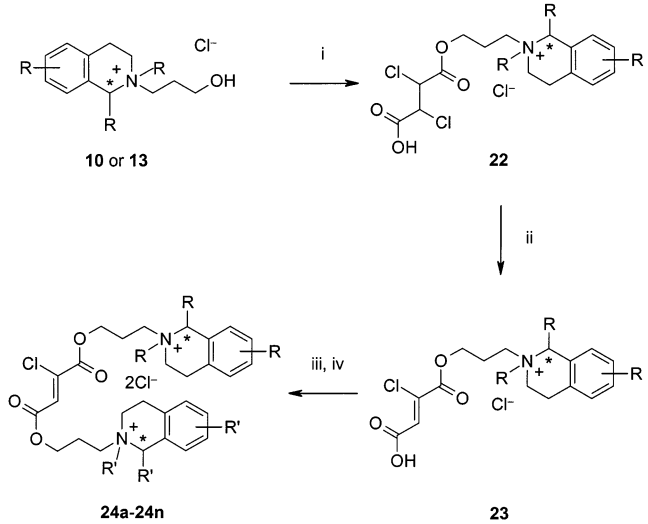


Figure 2. Structures of tetrahydroisoquinoline propanol **13j** and conformationally constrained tetrahydroisoquinolinium propanols **13k** and **13l**.

prepared nonselectively by reacting an equal molar ratio of two *disparate* tetrahydroisoquinolinium propanols (e.g., **13** and **13′**) with one equivalent of **16a** (e.g., **13a** and **13g** to make the **24a**/**24b** mixture). The desired products were isolated by preparative HPLC as 1:1 mixtures of inseparable α- and *β*-chlorofumarates. Mixed-onium alkanedioates **25a**−**25f** were prepared in a similar nonselective fashion using the appropriate alkanedioyl chlorides **17a**−**e**.

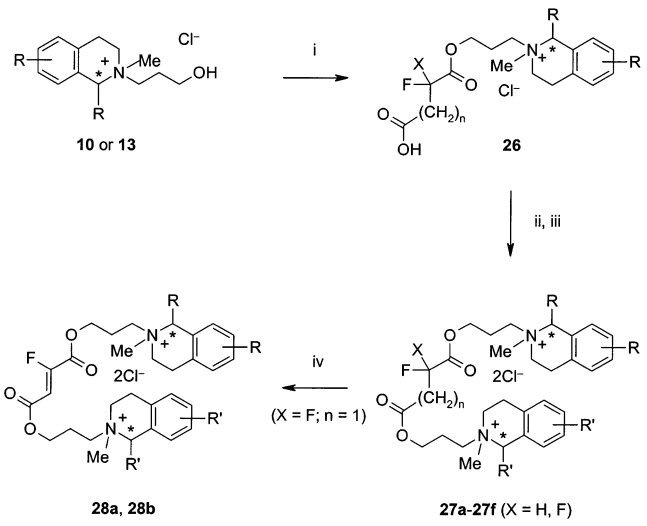
Regioselective synthesis of mixed-onium α- and *β*-chlorofumarates **24a**−**24n** entailed ring opening of dichlorosuccinic anhydride (±)-**18** with the necessary tetrahydroisoquinolinium alcohol **10** or **13** followed by regio- and stereoselective elimination[9b,18] of HCl from the intermediate dichlorosuccinate monoester **22** (Scheme 3).[25] Reaction of the resulting chlorofumarate monoester **23** with oxalyl chloride and coupling to the second tetrahydroisoquinolinium alcohol **10′** or **13′** gave the desired product as a single chlorofumarate regioisomer.

Scheme 3. Regioselective Synthesis of Mixed-Onium α- and *β*-Chlorofumarates (Charts 2−4)[a]



[a] *Denotes (1*R*)- or (1*S*)-configuration; (i) (±)-**18**, CH_2Cl_2; (ii) TEA, CH_2Cl_2 or DBU, CH_3CN; (iii) $(COCl)_2$, CH_2Cl_2; (iv) **10′** or **13′**.

Scheme 4. Regioselective Synthesis of Mixed-Onium α- and *β*-Monofluorosuccinates, -Difluorosuccinates, and -Fluorofumarates (Chart 5)[a]



[a] *Denotes (1*R*)- or (1*S*)-configuration; (i) **19a**−**c**, CH_2Cl_2 or DCE; (ii) $(COCl)_2$, CH_2Cl_2; (iii) **10′** or **13′**; (iv) K_2CO_3, DMF.

Mixed-onium fluoroalkanedioates **27a**−**f** and fluorofumarates **28a** and **28b** were prepared regioselectively through ring-opening[21b,22] of fluorinated cyclic anhydrides **19** with the requisite tetrahydroisoquinolinium alcohol **10** or **13** (Scheme 4). Treatment of the ring-opened products **26** with oxalyl chloride, and reaction of the resulting acid chloride with the second tetrahydroisoquinolinium alcohol **10′** or **13′**, gave the fluorinated succinate and glutarate mixed-onium products **27**. Fluorofumarates **28a** and **28b** were prepared from **27c** and **27d**, respectively, by treatment with K_2CO_3 in DMF.

Neuromuscular Pharmacology

The following procedures are typical of those used for the in vivo evaluation of NMBs in Charts 1−5. Adult male rhesus monkeys (8−15 kg) were anesthetized with ketamine (5 mg/kg, i.m.) and sodium pentobarbital (2−5 mg/kg, iv). The trachea was sprayed with 1−1.5 mL of 2% lidocaine (2 mg/kg) and intubation was performed without a muscle relaxant. Anesthesia was maintained with a mixture of nitrous oxide (60%), oxygen (40%), and halothane (0.5−1%). Ventilation was controlled at 200−250 mL/kg/min maintaining end-tidal CO_2 in the range 25 ± 5 mmHg. Esophageal and peripheral skin temperatures were monitored and kept in the range 37−38 °C and 34−35 °C, respectively, using thermal blankets and infrared heating lamps. EKG (lead II) was monitored continuously. Peripheral oxygen saturation was kept at 98−100%, as indicated by pulse oximetry.

A 20-gauge peripheral intravenous cannula was placed percutaneously and Ringer's lactate was infused at a rate of 15−20 mL/kg/h. During longer experiments (>4 h), the bladder was catheterized. A 20-gauge catheter was also placed percutaneously in the superficial tibial artery between the ankle and the knee of either leg. Arterial pressure was recorded via a Statham *P*23D transducer. Heart rate was monitored by a Grass 7P44A tachometer triggered by the arterial pulse wave.

Under sterile conditions, a surgical cut-down was performed through a 1-cm incision on a tendon of the *extensor digitorum* on the dorsum of the uncatheterized foot. The tendon was split longitudinally and a small slip was then tied to a Grass FT10 transducer at baseline tension of 50 gm for recording of twitch and train-of-four (TOF) responses. Sterile conditions were maintained for the duration of the experiment. The common peroneal nerve was stimulated at the knee at a rate of 0.15 Hz with supramaximal square-wave pulses 0.2 ms in duration via 23 gauge steel needle electrodes placed percutaneously. The stimuli were generated by a Grass S-88 laboratory stimulator and SIU5 isolation unit. Stimulation by TOF was interposed during the experiment whenever appropriate. Recordings of neuromuscular and cardiovascular responses were made simultaneously on a Grass model 7B polygraph.

Stable baseline recordings of twitch, TOF, blood pressure, and heart rate were obtained for at least 20 min. Animals could receive sequential NMB doses of 0.03, 0.05, 0.08, 0.20, 0.40, 0.80, 1.60, 3.20, and 6.4 mg/kg depending upon the NMB effects of the compound and the desire to establish a threshold dose for cardiovascular effects. Dose−response data for neuromuscular blockade, blood pressure, and heart rate were generated from these experiments. In a separate series of experiments to evaluate lack of cumulation and ease of maintenance of blockade, some animals received continuous infusions of NMB, while maintaining twitch at ca. 95% block. Slope and speed of recovery following discontinuation of infusion was compared with recovery slopes of individual bolus doses observed during spontaneous recovery.

At the end of each experiment, all surgical wounds were closed in sterile fashion. Animals were awakened from anesthesia, placed back in their cages, and attended until sitting, walking, and climbing. All animals maintained normal body weight, remained clinically healthy, and maintained normal clinical laboratory values (CBC, chemistries, urinalysis) throughout the study period. A comparative series of experiments with mivacurium was performed in the same animals on different occasions during the same study period and under the identical experimental protocol.

Biological Results and Discussion

Data in Tables 1−6 are expressed as means ± SE ($N > 1$). For each animal, the dose producing 95% block of the *extensor digitorum* (ED_{95}) was computed from linear regression slopes of log-probability plots of dose vs percent block. The onset at each dose was determined from the time of injection to the time of peak inhibition of the twitch response. Duration at each dose was determined from the time of injection to the time of recovery of the twitch to 95% of baseline. Onsets and durations at ED_{95} values were determined from plots of onset and duration, respectively, vs dose. A dose producing ≥10% change in heart rate or mean arterial pressure was defined as the cardiovascular threshold dose (CV dose). The selectivity index (SI) was defined as the mean cardiovascular threshold dose divided by the mean ED_{95} value.

We sought a nondepolarizing NMB with an onset of less than 60 s and a duration of action less than 10 min at 2−3 times the ED_{95} dose. We also wished to develop

Table 1. Neuromuscular Blocking Effects of Succinylcholine (**2**) and Mivacurium (**3**) (Chart 1) in Male Rhesus Monkeys[a]

compd	N[b]	ED_{95} (mg/kg)[c]	onset (s)[d]	duration (min)[e]	CV dose (mg/kg)[f]	SI (CV dose/ED_{95})
2	3	1.29 ± 0.34	50 ± 7	5.0 ± 0.6	nt	nt
3	3	0.062 ± 0.009	107 ± 13	12.0 ± 1.9	1.5 ± 0.4 (N = 6)	24 ± 7

[a] Data are expressed as means ± SE (N > 1). [b] N = number of animals. [c] Dose producing 95% supression of the twitch response at the *extensor digitorum*. [d] Time from injection to peak inhibition of the twitch response at the ED_{95} dose. [e] Time from injection to 95% recovery of the twitch response at the ED_{95} dose. [f] Dose producing ≥10% changes in heart rate or mean arterial pressure. nt = not tested.

Table 2. Neuromuscular Blocking Effects and Cardiovascular Selectivity of Bis- and Mixed-Onium Fumarates and Chlorofumarates (Chart 2) in Male Rhesus Monkeys[a]

compd	N[b]	ED_{95} or dose (mg/kg)[c]	onset (s)[d]	duration (min)[e]	CV dose (mg/kg)[f]	SI (CV dose/ED_{95})
20a	6	0.079 ± 0.025	96 ± 25	7.1 ± 2.4	1.6 ± 0 (N = 4)	20 ± 6
20b	1	0.62	27	2.4	0.8	1.3
20c	1	8.50	55	11.2	12.8	1.5
20d	2	1.79 ± 0.13	46 ± 3	5.1 ± 0.4	6.4 (N = 1)	3.6 ± 0.3
20e	1	2.0 (93%)[g]	45	3.6	2	1
21	1	1.55	114	25.5	1	0.65
24a/24b	6	0.163 ± 0.030	51 ± 4	5.6 ± 0.6	4.4 ± 1.2 (N = 4)	27 ± 7
24a	8	0.063 ± 0.002	88 ± 5	5.6 ± 0.4	3.6 ± 0.8 (N = 8)	57 ± 13
24b	2	0.25 ± 0.02	52 ± 2	3.4 ± 0.3	nt	nt

[a] Data are expressed as means ± SE (N > 1). [b] N = number of animals. [c] Dose producing 95% supression of the twitch response at the *extensor digitorum*. [d] Time from injection to peak inhibition of the twitch response at the ED_{95} dose. [e] Time from injection to 95% recovery of the twitch response at the ED_{95} dose. [f] Dose producing ≥10% changes in heart rate or mean arterial pressure. [g] Dose (% block). nt = not tested.

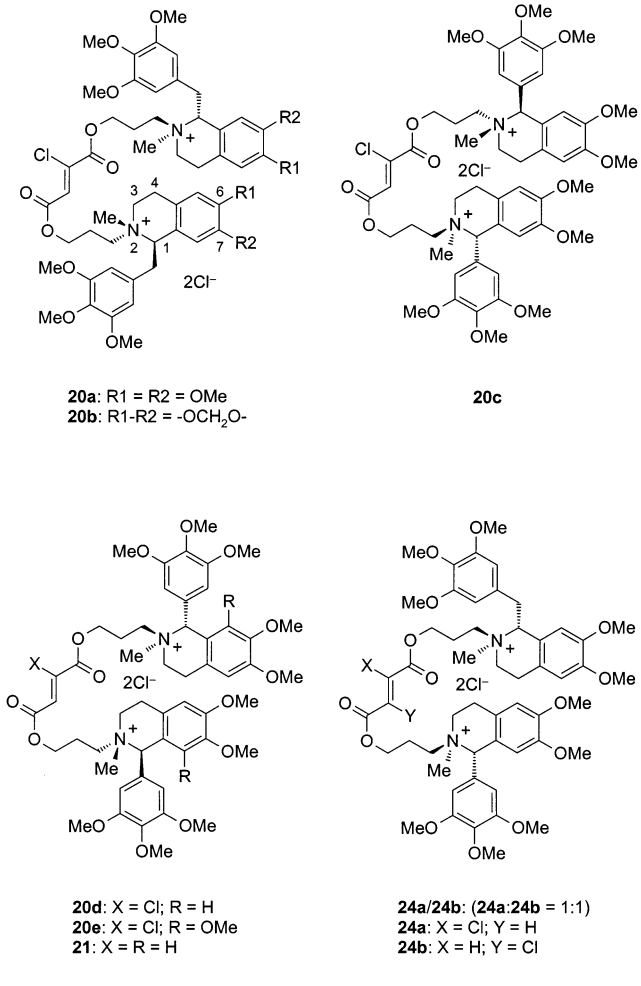
an ultra-short acting NMB with an ED_{95} value less than 0.1 mg/kg and a selectivity index at least 2-fold higher than that of mivacurium (i.e., ca. 50). Potency values (ED_{95}), onset times, durations of NMB effect, cardiovascular threshold doses (CV dose), and selectivity index values (SI) for the study compounds in male rhesus monkeys are provided in Tables 1−5. Structural formulas for the NMBs in these tables are provided in the corresponding Charts 1−5.

Succinylcholine and Mivacurium (Chart 1/Table 1). Structural formulas of succinylcholine (**2**) and mivacurium (**3**) and are shown in Chart 1 and their NMB effects in rhesus monkeys are provided in Table 1. Succinylcholine (**2**) has a mean ED_{95} value of 1.29 mg/kg in this species with an onset of 50 s and a duration of action of 5 min at this dose.[26] Mivacurium (**3**) has a mean ED_{95} value of 0.062 mg/kg in the rhesus monkey with an onset of 107 s, a duration of action of 12 min,[27] and a selectivity index of 24.[28]

Bis- and Mixed-Onium Chlorofumarates and Fumarates (Chart 2/Table 2). Structures and NMB effects of bis- and mixed-onium chlorofumarates and fumarates are provided in Chart 2 and Table 2. Chlorofumarate **20a** which contains two mivacurium-like onium groups was the initial lead molecule for this study. This compound showed an ED_{95} value of 0.079 mg/kg, a 7.1 min duration of action and a selectivity index similar to that of mivacurium (SI = 20). To assess the importance of the 6,7-methoxy subtituents in **20a** for NMB potency, the corresponding 6,7-methylenedioxy analogue (**20b**) was prepared. This substitution reduced NMB potency, onset time, duration of action, and selectivity index compared to **20a**. The rapid onset time of **20b** is typical of NMBs with high ED_{95} values[29] and is ascribed to the higher plasma concentrations required with less potent compounds and a rapid equilibration between plasma and the effect site.[10b]

The enantiotopic bis-1-phenyltetrahydroisoquinolinium NMBs **20c** and **20d** were ca. 100- and 20-fold less potent than their bis-1-benzyltetrahydroisoquinoline congener **20a**, respectively. Methoxy substitution at the

Chart 2



8-position of the 1-phenyltetrahydroisoquinoline ring system (e.g., **20e**) had minimal effects on potency, onset time, and duration of action compared to **20d**. Interestingly, the importance of a linker chlorine atom in determining ultra-short duration of action was confirmed when the deschloro analogue **21** was found to be 5-fold longer acting than the corresponding chloro-

Table 3. Neuromuscular Blocking Effects and Cardiovascular Selectivity of Mixed-Onium Chlorofumarate Analogues (Chart 3) in Male Rhesus Monkeys[a]

compd	N[b]	ED_{95} or dose (mg/kg)[c]	onset (s)[d]	duration (min)[e]	CV dose (mg/kg)[f]	SI (CV dose/ED_{95})
24c/24d	2	0.31 ± 0.10	37 ± 2	3.7 ± 1.3	nt	nt
24e/24f	1	0.067	57	10	1.6	24
24e	5	0.053 ± 0.005	113 ± 7	9.9 ± 1.2	0.8 ± 0 (N = 3)	15 ± 1
24g/24h	1	0.05 (41%)[g]	145	6.5	1.2	nt
24g	1	0.05 (91%)[g]	160	13.3	nt	nt
24i	1	0.87	38	5.4	nt	nt
24j	1	0.136	69	7.4	0.8	6
24k	3	0.09 ± 0.02	72 ± 5	4.7 ± 0.6	1.6 (N = 1)	18 ± 4

[a] Data are expressed as means ± SE (N > 1). [b] N = number of animals. [c] Dose producing 95% supression of the twitch response at the *extensor digitorum*. [d] Time from injection to peak inhibition of the twitch response at the ED_{95} dose. [e] Time from injection to 95% recovery of the twitch response at the ED_{95} dose. [f] Dose producing ≥10% changes in heart rate or mean arterial pressure. [g] Dose (% block). nt = not tested.

fumarate **20d**. Perhaps more significantly, bis-1-phenyltetrahydroisoquinoline chlorofumarate **20c** produced no cardiovascular effects at doses up to 6.4 mg/kg and produced only a 15% decrease in mean arterial blood pressure at 12.8 mg/kg.

The remarkably high cardiovascular threshold dose for **20c** (12.8 mg/kg) prompted us to replace one of the benzyltetrahydroisoquinolinium groups in **20a** with phenyltetrahydroisoquinolinium propanol **13g** in the hopes of enhancing the selectivity index. To this end, we embarked on the synthesis of mixed-onium chlorofumarates **24a** and **24b**, novel NMBs containing a 1-phenyltetrahydroisoquinoline moiety for improved selectivity and a 1-benzyltetrahydroisoquinoline headgroup for optimum NMB potency. A 1:1 mixture of **24a** and **24b** was prepared from chlorofumaryl chloride (**16a**) and tetrahydroisoquinolinium propanols **13a** and **10g** using the nonselective methodology outlined in Scheme 2.[9] The **24a**/**24b** mixture had a mean ED_{95} value of 0.163 mg/kg, an ultra-short duration of NMB effect and showed a 35% improvement in selectivity index over **20a**.

To establish NMB properties of individual chlorofumarate isomers **24a** and **24b**, each regioisomer was synthesized independently by the selective methodology outlined in Scheme 3.[9b,18] We were gratified to discover that α-chlorofumarate **24a** was ca.4-fold more potent than its β-chloro isomer **24b** and exhibited a mean selectivity index of 57.

Mixed-Onium Chlorofumarates (Chart 3/Table 3). Substituent and stereochemical modifications to the mixed-onium chlorofumarate motif and the resulting NMB effects are illustrated in Chart 3 and Table 3. Methoxy substitution at the 8-position of the 1-benzyltetrahydroisoquinoline group (e.g., **24e**, **24g**, and the **24e**/**24f** and **24g**/**24h** mixtures) typically produced potent NMBs (ED_{95} = 0.1−0.05 mg/kg); however, this modification also increased duration of action (duration = 6.5−13.3 min) beyond those of **24a** and the **24a**/**24b** mixture shown in Table 2. In contrast, replacing the *trans*-(1*S*,2*R*)-phenyltetrahydroisoquinoline stereochemistry with the enantiotopic (1*R*,2*S*)-configuration often decreased duration of action and NMB potency. For example, the **24c**/**24d** mixture (Table 3) was shorter acting (duration = 3.7 min) and less potent (ED_{95} = 0.31 mg/kg) than the **24a**/**24b** mixture (Table 2).

Removal of the *N*-methyl substituent from the 1-phenyltetrahydroisoquinoline group (e.g., **24i**) significantly reduced NMB potency (ED_{95} = 0.87 mg/kg) and had little effect on duration of action (duration = 5.4 min) compared to **24a**. This result is comparable to the structure−activity relationships observed with tubocurarine (**1a**) and its more potent dimethyl derivative metocurine[1] (**1b**), both of which have similar durations of action. Replacing methoxy groups on the exocyclic phenyl ring of **24a** with hydrogen (**24j**) or fluorine (**24k**) provided relatively potent NMBs (ED_{95} = 0.09−0.136 mg/kg) of ultra-short duration (duration = 4.7−7.4 min), but reduced the selectivity ratios (SI = 6−18).

Dibenzoquinolizidine Chlorofumarates and Mixed-Onium Alkanedioates (Chart 4/Table 4). Effects of conformational restriction and alkanedioate linkers on the NMB properties of mixed-onium NMBs are illustrated in Chart 4 and Table 4. Restricting the 1-phenyltetrahydroisoquinolinium group in the form of

Chart 3

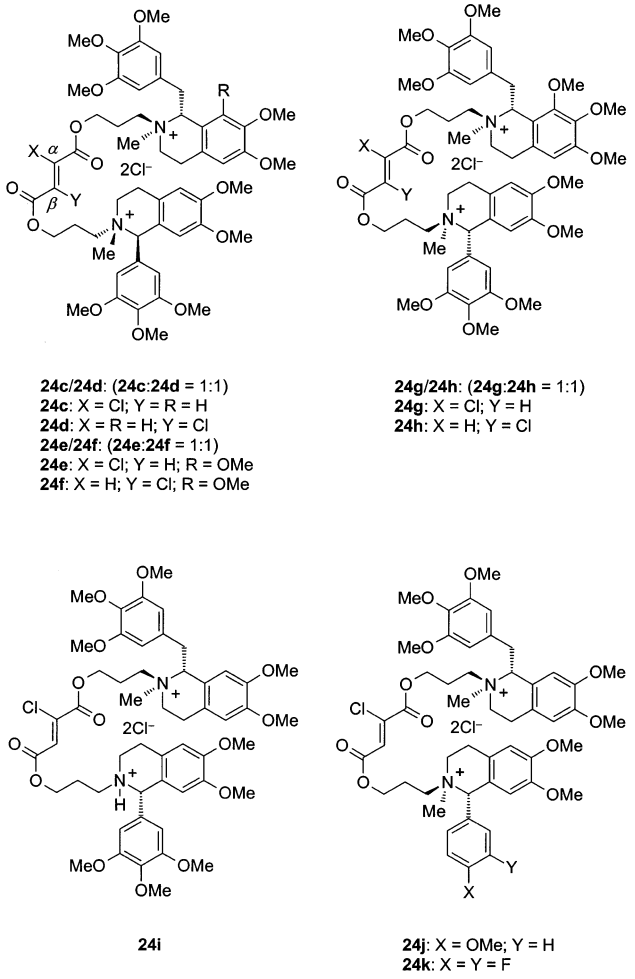
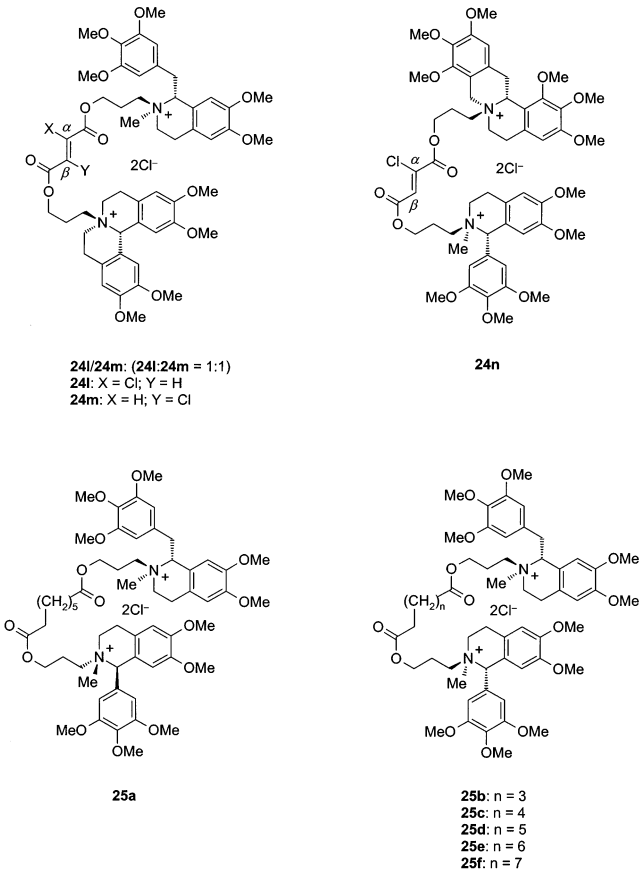


Table 4. Neuromuscular Blocking Effects and Cardiovascular Selectivity of Conformationally Constrained Mixed-Onium Chlorofumarates and Mixed-Onium Alkanedioates (Chart 4) in Male Rhesus Monkeys[a]

compd	N^b	ED_{95} or dose (mg/kg)[c]	onset (s)[d]	duration (min)[e]	CV dose (mg/kg)[f]	SI (CV dose/ED_{95})
24l/24m	1	0.078	94	5.2	0.4	5
24n	1	0.8	28	4.5	nt	nt
25a	4	0.103 ± 0.014	75 ± 6	8.6 ± 0.5	4.8 ± 1.6 (N = 2)	47 ± 17
25b	1	0.136	75	13	nt	nt
25c	1	0.2 (80%)[g]	40	7.1	nt	nt
25d	3	0.067 ± 0.017	117 ± 2	10.9 ± 0.7	0.8 (N = 1)	12 ± 3
25e	2	0.17 ± 0.05	79 ± 6	9.7 ± 1.3	3.2 (N = 1)	19 ± 5
25f	1	0.2	110	13.5	nt	nt

[a] Data are expressed as means ± SE (N > 1). [b] N = number of animals. [c] Dose producing 95% supression of the twitch response at the *extensor digitorum*. [d] Time from injection to peak inhibition of the twitch response at the ED_{95} dose. [e] Time from injection to 95% recovery of the twitch response at the ED_{95} dose. [f] Dose producing ≥10% changes in heart rate or mean arterial pressure. [g] Dose (% block). nt = not tested.

Chart 4



a dibenzo[*a*,*h*]quinolizidine[18] produced a potent, ultra-short-acting NMB mixture (**24l/24m**; ED_{95} = 0.078 mg/kg) but significantly lowered the selectivity index (CV dose = 0.4 mg/kg; SI = 5). Conversely, constraining the 1-benzyltetrahydroisoquinolinium moiety as a dibenzo-[*a*,*g*]quinolizidine[18] afforded compound **24n** which showed very low NMB potency (ED_{95} = 0.8 mg/kg). These results clearly demonstrate that SI and ED_{95} values can be strongly influenced by structural modification to the 1-phenyl- and 1-benzyltetrahydroisoquinolinium groups, respectively.

Alkanedioate mixed-onium derivatives **25a**−**25f** typically showed longer durations of action compared to the corresponding chlorofumarates. For example, one of the shortest acting alkanedioates **25a** (duration = 8.6 min) was 54% longer acting than mixed-onium chlorofumarate **24a** (duration = 5.6 min). Substituting the (1*R*,2*S*)-phenyltetrahydroisoquinolinium group in octanedioate **25a** (ED_{95} = 0.103 mg/kg) with the corresponding (1*S*,2*R*)-onium moiety increased potency and duration of NMB effect (e.g., **25d**; ED_{95} = 0.067 mg/kg; duration = 10.9 min) as observed in the chlorofumarate series. However, this substitution also decreased the selectivity index (SI = 12) which is opposite to that observed with mixed-onium chlorofumarates. Octanedioate **25d** was a potent NMB with a mean ED_{95} value of 0.067 mg/kg. Increasing the linker-length to nonanedioate and decanedioate (**25e** and **25f**) or decreasing the length to heptanedioate and hexanedioate (**25b** and **25c**) produced less potent NMBs.

Mixed-Onium Fluoroalkanedioates and Fluorofumarates (Chart 5/Table 5). The impact of fluorinated linkers on the pharmacology of mixed-onium NMBs are illustrated in Chart 5 and Table 5. Monofluorosuccinate **27a** was longer acting (duration = 35 min) than the corresponding difluorosuccinate **27c** (duration = 7.3 min) indicating that the number of linker fluorine atoms controls the duration of NMB effect. Difluorosuccinate **27c** was a particularly potent NMB (ED_{95} = 0.056 mg/kg) with an exceptionally high selectivity index value (SI = 96) compared to mivacurium (SI = 24, Table 1); however, its extreme hydrolytic instability posed a considerable challenge to pharmaceutical development.[30] The corresponding difluoroglutarate **27b** was also ultra-short acting (duration = 5.7 min) but significantly less potent (ED_{95} = 0.22 mg/kg).

The enhanced potency and slightly longer duration of NMB effect observed for α-halo isomers in the chlorofumarate series was also evident with mixed-onium difluorosuccinates. Thus, α-difluorosuccinate **27c** (ED_{95} = 0.056 mg/kg; duration = 7.3 min) was ca. 7-fold more potent and 33% longer acting than its *β*-difluorosuccinate isomer **27f** (ED_{95} = 0.39 mg/kg; duration = 4.9 min). Replacing the (1*S*,2*R*)-phenyltetrahydroisoquinolinium moiety in **27c** with a (1*R*,2*S*)-dimethoxyphenyltetrahydroisoquinolinium group (e.g., **27e**) reduced onset time (onset = 55 s) and duration of action (duration = 3.9 min), but maintained similar NMB potency (ED_{95} = 0.06 mg/kg).

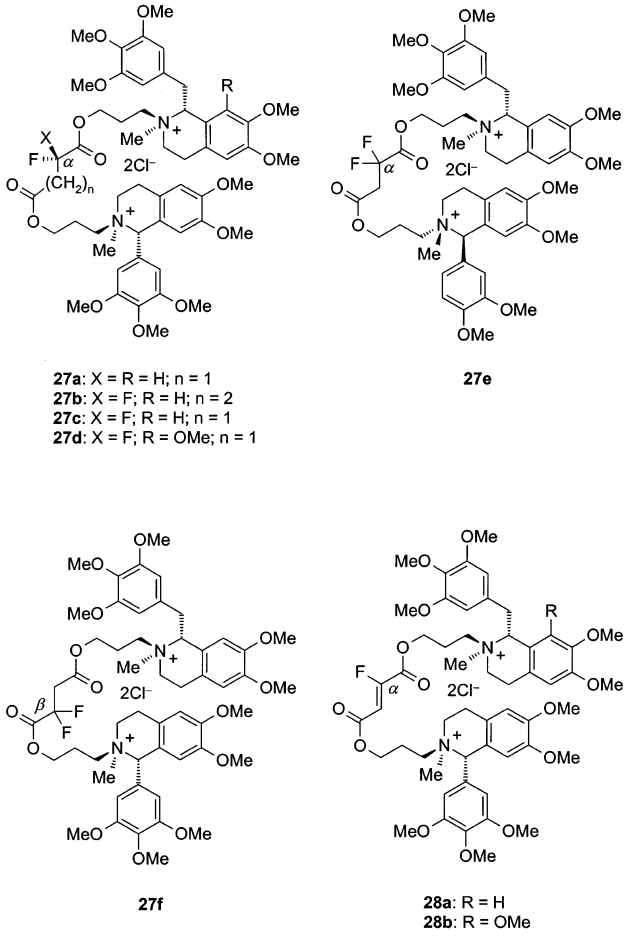
Fluorofumarate **28a** had a mean ED_{95} value of 0.137 mg/kg and duration of action of 4.4 min which is ca. 20% shorter acting and 2-fold less potent than the corresponding chlorofumarate **24a** (Table 2). Methoxy substitution at the 8-position of the 1-benzyltetrahydroisoquinoline ring system in mixed-onium difluorosuccinates and fluorofumarates (e.g., **27d** and **28b**) provided potent NMBs (ED_{95} = 0.04−0.077 mg/kg) and increased dura-

Table 5. Neuromuscular Blocking Effects and Cardiovascular Selectivity of Mixed-Onium Fluoroalkanedioates and Fluorofumarates (Chart 5) in Male Rhesus Monkeys[a]

compd	N^b	ED_{95} or dose (mg/kg)[c]	onset (s)[d]	duration (min)[e]	CV dose (mg/kg)[f]	SI (CV dose/ED_{95})
27a	1	0.05 (100%)[g]	80	35	nt	nt
27b	1	0.22	48	5.7	nt	nt
27c	6	0.056[h]	78[h]	7.3[h]	5.4 ± 0.6	96 ± 11
27d	4	0.040 ± 0.004	217 ± 19	14 ± 2	2.4 ± 0.5	60 ± 14
27e	1	0.06	55	3.9	nt	nt
27f	1	0.39	26	4.9	nt	nt
28a	5	0.137 ± 0.016	66 ± 5	4.4 ± 0.7	5.1 ± 0.8	37 ± 7
28b	3	0.077 ± 0.016	85 ± 9	10 ± 1	1.6 ± 0	21 ± 4

[a] Data are expressed as means ± SE ($N > 1$). [b] N = number of animals. [c] Dose producing 95% supression of the twitch response at the *extensor digitorum*. [d] Time from injection to peak inhibition of the twitch response at the ED_{95} dose. [e] Time from injection to 95% recovery of the twitch response at the ED_{95} dose. [f] Dose producing ≥10% changes in heart rate or mean arterial pressure. [g] Dose (% block). [h] Computed from individual doses of six separate animals. nt = not tested.

Chart 5



tion of action (duration = 10−14 min) as observed in the mixed-onium chlorofumarate and alkanedioate series.

Comparative NMB Effects of 24a, 25a and 27c at 0.2 and 0.4 mg/kg (Table 6). Chlorofumarate **24a**, octanedioate **25a**, and difluorosuccinate **27c** met our preliminary criteria for potency, ultra-short duration of action, and selectivity index. Onset and recovery times for these NMBs following a 0.2 and 0.4 mg/kg bolus dose are shown in Table 6. All three compounds produced onset times below 60 s at 0.2 mg/kg and below 30 s at 0.4 mg/kg. Chlorofumarate **24a** had the shortest NMB action of these compounds followed by difluorosuccinate **27c**. Octanedioate **25a** was 69−73% longer acting than chlorofumarate **24a** and 30−42% longer acting than difluorosuccinate **27c** at these doses.

Table 6. Neuromuscular Blocking Effects of 0.2 and 0.4 mg/kg Bolus Doses of Mixed-Onium Chlorofumarate **24a**, Octanedioate **25a**, and Difluorosuccinate **27c** 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]
24a	8	38 ± 5	8.46 ± 0.45	25 ± 3	10.0 ± 0.5
25a	3	50 ± 5	14.6 ± 1.8	25 ± 8	16.9 ± 1.2
27c	3	37 ± 2	10.3 ± 0.6	22 ± 4	13.0 ± 0.9

[a] Data are expressed as means ± SE ($N > 1$). All compounds produced 100% supression of the twitch response at the *extensor digitorum* following the bolus dose indicated. [b] N = number of animals. [c] Time from injection to peak inhibition of the twitch response at the dose indicated. [d] Time from injection to 95% recovery of the twitch response at the dose indicated.

Blood pressure, heart rate, and twitch (*extensor digitorum*) recordings from a male rhesus monkey following a 0.08 mg/kg bolus dose of **24a** and subsequent continuous infusion at 10−20 μg/kg/min are illustrated in Figure 3. Time of recovery from 0 to 95% of baseline is ca. 5 min which is similar to the recovery slope for a 0.2 mg/kg bolus dose of **24a** in this species.[9a] Maintenance of neuromuscular blockade was readily achieved at this infusion rate, and no cumulative NMB effects were observed with the compound.

Deactivation of 24a In Whole Blood. The ultra-short duration of the title compounds appears to be controlled by the activated inter-onium linker as shown by the comparative durations of chlorofumarate **20d** (duration = 5.1 min, Table 2), fumarate **21** (duration = 25.5 min, Table 2), monofluorosuccinate **27a** (duration = 35 min, Table 5), and difluorosuccinate **27c** (duration = 7.3 min, Table 5). Indeed, these halogenated linkers are sensitive to pH-dependent hydrolysis. For example, chlorofumarate **24a** has excellent stability in pH 3 saline but hydrolyzes rapidly at physiologic pH[31] and the slightly longer acting difluorosuccinate **27c** is even more susceptible to hydrolysis in vitro.[30,31] This latter result suggests that the ultra-short duration of **24a** is not controlled by chemical hydrolysis.

The major metabolite of **24a** isolated from whole human blood was shown by LC/MS to have a molecular weight consistent with addition of cysteine and loss of HCl. Treatment of **24a** with cysteine in pH 7.4 buffer generated a compound that was indistinguishable by LC/MS/MS from the whole blood-derived material. The chemical structure of the metabolite was shown by a combination of 2D COSY, 2D NOESY, HMBC, and HMQC NMR techniques to be the mixed-onium thiazolidine **29** (Scheme 5).[32] Two pathways for deactivation of **24a** are illustrated in Scheme 5. Hydrolysis of **24a**

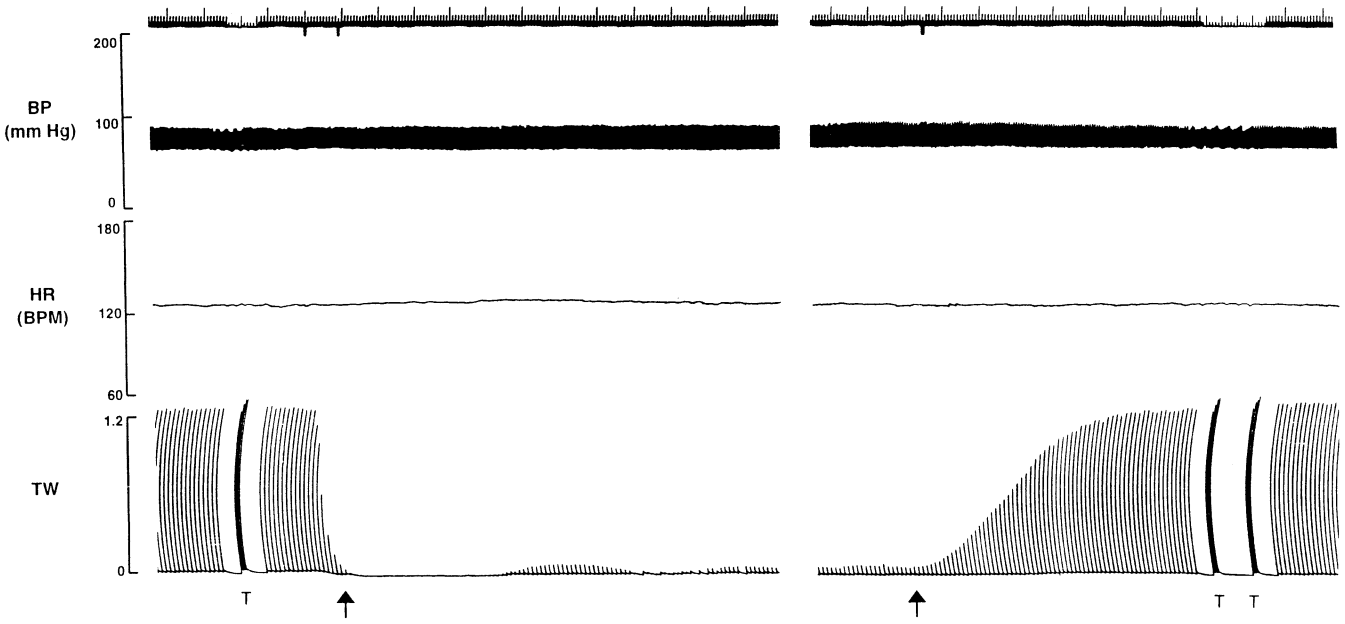
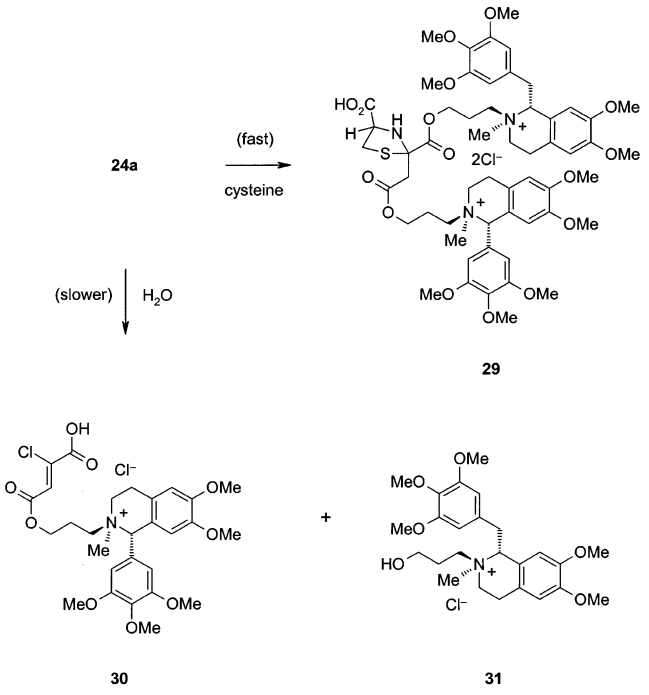


Figure 3. Blood pressure, heart rate, and twitch (*extensor digitorum*) recordings from a male rhesus monkey following a 0.08 mg/kg bolus dose of **24a** followed by continuous infusion from 10 to 20 μg/kg/min. Time scale is divided into 1-min increments by large hash marks. Total infusion time was 1 h (13−57 min time period not shown). Time from the end of infusion (last inverted hash mark) to 95% recovery is ca.5 min. T = train-of-four stimulation. Arrows indicate start and stop of infusion.

Scheme 5. Deactivation Pathways of Mixed-Onium Chlorofurmarate **24a** in Whole Human Blood



occurs to a lesser extent and preferentially at the ester bond proximal to the linker chlorine atom affording chlorofumarate monoester **30** and alcohol **31**. A recovery mechanism of this type could provide a clinical advantage by eliminating problems of prolonged neuromuscular blockade in patients with reduced plasma cholinesterase activity.

Conclusions. Mixed-onium chlorofumarates, difluorosuccinates, and fluorofumarates provide excellent NMB potency, rapid onset, and ultra-short duration of NMB effect in male rhesus monkeys. The structure−activity relationships of these NMBs illustrate the importance of three key structural features-namely, a halogenated linker for ultra-short duration, a 1-phenyltetrahydroisoquinolinium group for reduced cardiovascular effects, and a 1-benzyltetrahydroisoquinolinium moiety for improved NMB potency. These qualities, coupled with the potential for a plasma cholinesterase-independent recovery mechanism represent a significant contribution to NMB pharmacology. The NMB properties and therapeutic potential of mixed-onium chlorofumarate **24a** in rhesus monkeys[9] led to its clinical evaluation as a possible nondepolarizing alternative to succinylcholine.[10]

Experimental Section

General. Experimental details for the synthesis of **10a**,[9a] **10g**,[9a] **13a**,[9b] **13g**,[9b] **20c**,[9a] **20d**,[9a] **20e**,[9a] **21**,[9a] **24a**,[9b] **24b**,[9b] **24n**,[18] the **24a**/**24b**,[9a] the **24c**/**24d**,[9a] and **24l**/**24m** mixtures[18] may be found in the Supporting Information of refs 9a and 9b and the Experimental Section of ref 18. Analytical HPLC analyses were performed on 4 × 250 mm 5μ Si60 LiChrosorb columns (E. Merck, Darmstadt, Germany) at a flow rate of 1.6 mL/min. Preparative HPLC separations were performed on twin Porasil (15−20 μ) cartridges (Waters/Millipore, Milford, MA) at a flow rate of 60 mL/min. The mobil phase for analytical and preparative HPLC separations consisted of 0−25% MeOH/CH_2Cl_2 mixtures containing 0.25 mL methanesulfonic acid/L. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA. Proton NMR spectra were recorded at 300, 400, or 500 MHz and coupling constants are in Hz. Chemical shifts are reported in ppm relative to the residual protonated solvent resonance of DMSO-d_6 (δ 2.50) or D_2O (δ 4.75). Positive ion flow injection electrospray mass spectra (MS) are reported in the form *m*/*z* (charged ion, relative intensity).

(*Z*)-2-Chloro-bis{3-{(1*R*,2*S*)-2-methyl-6,7-methylenedioxy-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl}-2-butenedioate Dichloride (20b). Obtained from a mixture of **16a**, **13a** and **13b** as described for synthesis of the **24a**/**24b** mixture:[9] white powder (6% yield); ^{1}H NMR 400 MHz (DMSO-d_6) δ 7.03 (s, 1H), 6.82 (s, 2H), 6.37 (s, 4H), 5.89 (br s, 2H), 5.84 (d, 4H, J = 9.3), 4.71 (m, 2H), 4.17 (m, 4H), 3.82 (m, 2H), 3.67 (m, 2H), 3.66 (s, 12H),

3.60 (s, 6H), 3.51 (dd, 2H, J = 13, 4), 3.38 (m 4H), 3.31 (s, 6H), 3.05 (m 4H), 2.90 (m 2H), 2.17 (m, 4H); MS m/z 487 (M^{2+}, 100); Anal. ($C_{52}H_{63}N_2O_{14}Cl_3 \cdot 4H_2O$) C, H, N, Cl.

(*Z*)-2-Chloro-bis{3-[(1*R*,2*S*)-2-methyl-6,7,8-trimethoxy-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-isoquinolinio]propyl}-2-butenedioate Dichloride (20e). Prepared from a mixture of **16a** and 2 equiv of **10e** as described for synthesis of the **24a**/**24b** mixture:[9] white powder (9% yield); ^{1}H NMR 300 MHz (DMSO-d_6) δ 7.37 (br, 2H), 7.26 (s, 1H), 6.83 (s, 2H), 6.02 (br, 2H), 5.80 (s, 1H), 5.79 (s, 1H), 4.28 (m, 4H), 4.00−3.20 (br m, 24H), 3.82 (s, 6H), 3.68 (s, 3H), 3.67 (s, 3H), 3.92 (s, 6H), 3.22 (s, 6H), 2.86 (s, 6H), 2.28 (m, 4H); MS m/z 519 (M^{2+}, 100); Anal. ($C_{54}H_{71}N_2O_{16}Cl_3 \cdot 5H_2O$) C, H, N, Cl.

(*Z*)-2-Chloro-1-{3-[(1*R*,2*S*)-6,7-dimethoxy-2-methyl-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-isoquinolinio]propyl}-4-{3-{(1*R*,2*S*)-2-methyl-6,7,8-trimethoxy-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl}-2-butenedioate Dichloride and (*Z*)-2-Chloro-4-{3-[(1*R*,2*S*)-6,7-dimethoxy-2-methyl-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-isoquinolinio-]propyl}-1-{3-{(1*R*,2*S*)-2-methyl-6,7,8-trimethoxy-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl}-2-butenedioate Dichloride (1:1) (24e/24f). Synthesized from **16a**, **13c**, and **10d** as described for synthesis of the **24a**/**24b** mixture:[9] white powder (13% yield); ^{1}H NMR 300 MHz (DMSO-d_6) δ 7.29 (br, 2H), 7.19 (s, 1H), 7.15 (s, 1H), 6.95 (s, 2H), 6.74 (s, 2H), 6.41 (s, 4H), 6.38 (s, 1H), 6.37 (s, 1H), 6.11 (br, 2H), 5.90 (s, 1H), 5.86 (s, 1H), 4.99 (m, 2H), 4.3−4.1 (m, 8H), 3.91−2.92 (m, 28H), 3.76 (s, 6H), 3.69 (s, 12H), 3.68 (s, 12H), 3.62 (s, 6H), 3.61 (s, 6H), 3.58 (s, 6H), 3.56 (s, 6H), 3.54 (s, 6H), 3.31 (s, 6H), 3.22 (s, 6H), 2.84 (s, 6H), 2.34−1.99 (m, 8H); MS m/z 511 (M^{2+}, 22); Anal. ($C_{54}H_{71}N_2O_{15}Cl_3 \cdot 4.5H_2O$) C, H, N.

(*Z*)-2-Chloro-4-{3-[(1*R*,2*S*)-6,7-dimethoxy-2-methyl-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-isoquinolinio]propyl}-1-{3-{(1*R*,2*S*)-2-methyl-6,7,8-trimethoxy-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl}-2-butenedioate Dichloride (24e). Prepared from (±)-**18**, **10c**, and **13d** as described for the synthesis of **24a**:[9] white powder (23% yield); ^{1}H NMR 400 MHz (DMSO-d_6): δ 7.23 (br, 1H), 7.13 (s, 1H), 6.93 (s, 1H), 6.72 (s, 1H), 6.39 (s, 2H), 6.34 (s, 1H), 6.1 (br, 1H), 5.83 (s, 1H), 4.97 (t, 1H, J = 6), 4.24 (t, 2H, J = 6), 4.2 (t, 2H, J = 6), 3.8−3.0 (m, 16H), 3.77 (s, 3H), 3.76 (s, 3H), 3.68 (s, 3H), 3.66 (s, 6H), 3.62 (s, 3H), 3.59 (s, 3H), 3.58 (s, 3H), 3.55 (s, 3H), 3.31 (s, 3H), 3.20 (s, 3H), 2.97 (m, 1H), 2.85 (s, 3H), 2.3−2.0 (m, 4H); MS m/z 511 (M^{2+}, 100); Anal. ($C_{54}H_{71}N_2O_{15}Cl_3 \cdot 4H_2O$) C, H, N.

(*Z*)-2-Chloro-1-{3-[(1*S*,2*R*)-6,7-dimethoxy-2-methyl-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-isoquinolinio]propyl}-4-{3-{(1*R*,2*S*)-2-methyl-6,7,8-trimethoxy-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl}-2-butenedioate Dichloride and (*Z*)-2-Chloro-4-{3-[(1*S*,2*R*)-6,7-dimethoxy-2-methyl-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-isoquinolinio]propyl}-1-{3-{(1*R*,2*S*)-2-methyl-6,7,8-trimethoxy-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl}-2-butenedioate Dichloride (1:1) (24g/24h). Synthesized from **16a**, **13c**, and **13g** as described for synthesis of the **24a**/**24b** mixture:[9] white powder (3% yield); ^{1}H NMR 300 MHz (DMSO-d_6) δ 7.29 (br 2H), 7.13 (s, 1H), 7.08 (s, 1H), 6.94 (s, 2H), 6.73 (s, 2H), 6.41 (s, 4H), 6.38 (s, 1H), 6.37 (s, 1H), 6.11 (br, 2H), 5.82 (s, 1H), 5.80 (s, 1H), 4.97 (m, 2H), 4.34−4.14 (m, 8H), 3.91−2.92 (m, 28H), 3.76 (s, 6H), 3.69 (s, 12H), 3.68 (s, 12H), 3.62 (s, 6H), 3.61 (s, 6H), 3.57 (s, 6H), 3.55 (s, 6H), 3.53 (s, 6H), 3.31 (s, 6H), 3.22 (s, 6H), 2.84 (s, 6H), 2.34−1.99 (m, 8H); MS m/z 511 (M^{2+}, 100); Anal. ($C_{54}H_{71}N_2O_{15}Cl_3 \cdot 8H_2O$) C, H, N.

(*Z*)-2-Chloro-4-{3-[(1*S*,2*R*)-6,7-dimethoxy-2-methyl-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-isoquinolinio]propyl}-1-{3-{(1*R*,2*S*)-2-methyl-6,7,8-trimethoxy-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl}-2-butenedioate Dichloride (24g). Synthesized from (±)-**18**, **10c**, and **13g** as described for the synthesis of **24a**:[9] white powder (23% yield); ^{1}H NMR 300 MHz (DMSO-d_6) δ 7.3 (br, 1H), 7.21 (s, 1H), 7.0 (s, 1H), 6.79 (s, 1H), 6.47 (s, 2H), 6.43 (s, 1H), 6.2 (br, 1H), 5.91 (s, 1H), 5.06 (t, 1H, J = 6), 4.3−4.2 (m, 4H), 3.9−3.0 (m, 20H), 3.84(s, 6H), 3.75 (s, 3H), 3.74 (s, 6H), 3.71 (s, 3H), 3.67 (s, 3H), 3.64 (s, 3H), 3.63 (s, 3H), 3.28 (s, 3H), 2.93 (s, 3H), 2.4−2.1 (m, 4H); MS m/z 511 (M^{2+}, 22); Anal. ($C_{54}H_{71}N_2O_{15}Cl_3 \cdot 3H_2O$) C, H, N.

(*Z*)-2-Chloro-1-{3-{(1*R*,2*S*)-6,7-dimethoxy-2-methyl-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl}-4-{3-[(1*S*)-6,7-dimethoxy-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-isoquinolinyl]-propyl}-2-butenedioate Chloride Monohydrochloride (24i). Synthesized from (±)-**18**, **10a**, and **13j** as described for the synthesis of **24a**:[9] white powder (10% yield); ^{1}H NMR 400 MHz (D_2O) δ 6.91 (s, 1H), 6.81 (s, 1H), 6.62 (s, 2H), 6.49 (s, 1H), 6.21 (s, 1H), 6.18 (s, 2H), 5.52 (br, 1H), 5.51 (s, 1H), 4.51 (m, 2H), 4.25 (m, 2H), 4.13 (m, 1H), 4.04 (m, 1H), 3.81 (s, 3H), 3.71 (s, 3H), 3.70−2.90 (m, 12H), 3.69 (s, 6H), 3.68 (s, 3H), 3.65 (s, 3H), 3.60 (s, 6H), 3.54 (s, 3H), 3.39 (s, 3H), 3.21 (s, 3H), 2.94 (br t, 1H, J = 11), 2.27 (m, 3H), 2.11 (m, 1H); MS m/z 489 (M^{2+}, 100); Anal. ($C_{52}H_{67}N_2O_{14}Cl_3 \cdot 3.5H_2O$) C, H, N, Cl.

(*Z*)-2-Chloro-4-{3-[(1*S*,2*R*)-6,7-dimethoxy-1-(4-methoxyphenyl)-2-methyl-1,2,3,4-tetrahydro-2-isoquinolinio]propyl}-1-{3-{(1*R*,2*S*)-6,7-dimethoxy-2-methyl-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl}-2-butenedioate Dichloride (24j). Synthesized from (±)-**18**, **13a**, and **10h** as described for the synthesis of **24a**:[9] white powder (19% yield); ^{1}H NMR 300 MHz (DMSO-d_6) δ 7.33 (br, 2H), 7.11 (s, 1H), 7.00 (d, 2H, J = 9), 6.93 (s, 1H), 6.84 (s, 1H), 6.39 (s, 2H), 6.31 (s, 1H), 5.88 (s, 1H), 5.70 (s, 1H), 4.79 (m, 1H), 4.24 (m, 4H), 3.89 (m, 1H), 3.78 (s, 3H), 3.77 (s, 3H), 3.74 (m, 1H), 3.70 (s, 3H), 3.64 (s, 6H), 3.64−3.24 (m, 8H), 3.61 (s, 3H), 3.53 (s, 3H), 3.38 (s, 3H), 3.27 (s, 3H), 3.19 (m, 1H), 3.09 (m, 2H), 2.89 (br t, 1H, J = 11), 2.79 (s, 3H), 2.50 (m, 4H); MS m/z 466 (M^{2+}, 62); Anal. ($C_{51}H_{65}N_2O_{12}Cl_3 \cdot 4H_2O$) C, H, N,Cl.

(*Z*)-2-Chloro-4-{3-[(1*S*,2*R*)-1-(3,4-difluorophenyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydro-2-isoquinolinio]-propyl}-1-{3-{(1*R*,2*S*)-6,7-dimethoxy-2-methyl-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl}-2-butenedioate Dichloride (24k). Prepared from (±)-**18**, **13a**, and **13i** as described for the synthesis of **24a**:[9] white powder (35% yield); ^{1}H NMR 300 MHz (DMSO-d_6) δ 7.65 (br, 1H), 7.55 (m, 1H), 7.25 (br, 1H), 7.14 (s, 1H), 6.95 (s, 1H), 6.84 (s, 1H), 6.40 (s, 2H), 6.35 (s, 1H), 6.01 (s, 1H), 5.71 (s, 1H), 4.80 (dd, 1H, J = 9, 4), 4.23 (m, 4H), 3.90 (m, 1H), 3.80−3.30 (m, 6H), 3.77 (s, 3H), 3.70 (s, 3H), 3.63 (s, 6H), 3.60 (s, 3H), 3.55 (s, 3H), 3.47 (br t, 2H, J = 8), 3.39 (s, 3H), 3.27 (s, 3H), 3.23 (m 1H), 3.15 (m, 1H), 3.09 (m, 2H), 2.89 (m, 1H), 2.86 (s, 3H), 2.26 (m, 4H); MS m/z 469 (M^{2+}, 100); Anal. ($C_{50}H_{61}N_2O_{11}Cl_3F_2 \cdot 3.5H_2O$) C, H, N.

{3-[(1*R*,2*S*)-6,7-Dimethoxy-2-methyl-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-isoquinolinio]propyl} {3-{(1*R*,2*S*)-6,7-Dimethoxy-2-methyl-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl} Octanedioate Dichloride (25a). Synthesized from **17c**, **13a**, and **10d** as described for synthesis of the **24a**/**24b** mixture:[9] white powder (9% yield); ^{1}H NMR 400 MHz (DMSO-d_6) δ 7.17 (br, 1H), 6.96 (s, 1H), 6.87 (s, 1H), 6.39 (s, 1H), 6.38 (s, 2H), 6.12 (br, 1H), 5.79 (s, 1H), 5.71 (s, 1H); 4.71 (dd, 1H, J = 9, 3), 4.05 (m, 2H), 4.00 (m, 2H), 3.87 (m, 2H), 3.78 (s, 3H), 3.72 (s, 3H), 3.70 (s, 3H), 3.65 (s, 6H), 3.61 (s, 3H), 3.80−3.20 (br m, 12H), 3.57 (s, 3H), 3.42 (m, 2H), 3.32 (s, 3H), 3.29 (s, 3H), 3.16 (m, 1H), 3.10 (m, 2H), 2.89 (dd, 1H, J = 12, 10), 2.83 (s, 3H), 2.19 (t, 2H, J = 7), 2.16 (m, 4H), 2.12 (t, 2H, J = 7), 1.36 (m, 4H), 1.14 (m, 4H); MS m/z 508 (M^{2+}, 100); Anal. ($C_{57}H_{80}N_2O_{14}Cl_2 \cdot 3.5H_2O$) C, H, N, Cl.

{3-[(1*S*,2*R*)-6,7-Dimethoxy-2-methyl-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-isoquinolinio]propyl} {3-{(1*R*,2*S*)-6,7-Dimethoxy-2-methyl-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl} Hexanedioate Dichloride (25b). Synthesized from **17a**, **13a**, and **10g** as described for synthesis of the **24a**/**24b** mixture:[9] white powder (13% yield); ^{1}H NMR 400 MHz (DMSO-d_6) δ 7.17 (br, 1H), 6.96 (s, 1H), 6.86 (s, 1H), 6.38 (s, 2H), 6.12 (br, 1H),

5.80 (s, 1H), 5.71 (s, 1H), 4.71 (dd, 1H, J = 8, 3), 4.05 (m, 2H), 3.99 (m, 2H), 3.92−3.22 (br m, 13H), 3.87 (m, 2H), 3.78 (s, 3H), 3.71 (s, 3H), 3.70 (s, 3H), 3.65 (s, 6H), 3.61 (s, 3H), 3.57 (s, 3H), 3.42 (m, 2H), 3.36 (br, 3H), 3.29 (s, 3H), 3.15 (m, 1H), 3.09 (m, 2H), 2.88 (dd, 1H, J = 10, 9), 2.84 (s, 3H), 2.19 (m, 4H), 2.18 (t, 2H, J = 7), 2.13 (t, 2H, J = 7), 1.36 (m, 4H); MS m/z 494 (M^{2+}, 100); Anal. ($C_{55}H_{76}N_2O_{14}Cl_2 \cdot 4H_2O$) C, H, N, Cl.

{3-[(1*S*,2*R*)-6,7-Dimethoxy-2-methyl-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-isoquinolinio]propyl} {3-{(1*R*,2*S*)-6,7-Dimethoxy-2-methyl-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl} Heptanedioate Dichloride (25c). Synthesized from **17b**, **13a**, and **10g** as described for synthesis of the **24a**/**24b** mixture:[9] white powder (13% yield); ^{1}H NMR 400 MHz (DMSO-d_6) δ 7.17 (br, 1H), 6.96 (s, 1H), 6.87 (s, 1H), 6.38 (s, 2H), 6.12 (br, 1H), 5.80 (s, 1H), 5.71 (s, 1H), 4.73 (dd, 1H, J = 11, 4), 4.06 (m, 2H, $J \sim 6$), 4.01 (m, 2H, $J \sim 6$), 3.82 (m, 2H), 3.80−3.20 (br m, 13H), 3.78 (s, 3H), 3.72 (s, 3H), 3.70 (s, 3H), 3.65 (s, 6H), 3.62 (s, 3H), 3.58 (s, 3H), 3.41 (m, 2H), 3.33 (br, 3H), 3.27 (s, 3H), 3.15 (m, 1H), 3.10 (m, 2H), 2.88 (dd, 1H, J = 11, 10), 2.82 (s, 3H), 2.19 (m, 4H), 2.18 (t, 2H, J = 7), 2.12 (t, 2H, J = 7), 1.36 (m, 4H, $J \sim 8$), 1.14 (m, 2H); MS m/z 501 (M^{2+}, 100); Anal. ($C_{56}H_{78}N_2O_{14}Cl_2 \cdot 4H_2O$) C, H, N, Cl.

{3-[(1*S*,2*R*)-6,7-Dimethoxy-2-methyl-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-isoquinolinio]propyl} {3-{(1*R*,2*S*)-6,7-Dimethoxy-2-methyl-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl} Octanedioate Dichloride (25d). Synthesized from **17c**, **13a**, and **10g** as described for synthesis of the **24a**/**24b** mixture:[9] white powder (12% yield); ^{1}H NMR 400 MHz (DMSO-d_6) δ 7.17 (br, 1H), 6.96 (s, 1H), 6.87 (s, 1H), 6.38 (s, 4H), 5.80 (s, 1H), 5.71 (s, 1H), 4.73 (dd, 1H, J = 10, 6), 4.06 (m, 2H), 3.99 (m, 2H), 3.85 (m, 2H), 3.80−3.20 (br m, 13H), 3.79 (s, 3H), 3.72 (s, 3H), 3.70 (s, 3H), 3.65 (s, 6H), 3.62 (s, 3H), 3.58 (s, 3H), 3.43 (m, 2H), 3.35 (br, 3H), 3.27 (s, 3H), 3.24 (m, 1H), 3.16 (m, 1H), 3.10 (m, 2H), 2.90 (dd, 1H, J = 11, 10), 2.82 (s, 3H), 2.20 (t, 2H, J = 7), 2.17 (m, 4H), 2.12 (t, 2H, J = 7), 1.36 (m, 4H), 1.13 (m, 2H); MS m/z 508 (M^{2+}, 100); Anal. ($C_{57}H_{80}N_2O_{14}Cl_2 \cdot 5H_2O$) C, H, N, Cl.

{3-[(1*S*,2*R*)-6,7-Dimethoxy-2-methyl-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-isoquinolinio]propyl} {3-{(1*R*,2*S*)-6,7-Dimethoxy-2-methyl-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl} Nonanedioate Dichloride (25e). Synthesized from **17d**, **13a**, and **10g** as described for synthesis of the **24a**/**24b** mixture:[9] white powder (17% yield); ^{1}H NMR 400 MHz (DMSO-d_6) δ 7.20 (br, 1H), 6.96 (s, 1H), 6.86 (s, 1H), 6.37 (s, 2H), 6.16 (br, 1H), 5.79 (s, 1H), 5.70 (s, 1H), 4.71 (dd, 1H, J = 10, 5), 4.03 (m, 2H), 3.97 (m, 2H), 3.85 (m, 2H), 3.80−3.20 (br m, 13H), 3.78 (s, 3H), 3.71 (s, 3H), 3.69 (s, 3H), 3.64 (s, 6H), 3.61 (s, 3H), 3.57 (s, 3H), 3.42 (m, 2H), 3.34 (br, 3H), 3.29 (s, 3H), 3.14 (m, 1H), 3.07 (m, 2H), 2.87 (dd, 1H, J = 12, 11), 2.82 (s, 3H), 2.19 (t, 2H, J = 7), 2.18 (m, 4H), 2.11 (t, 2H, J = 7), 1.37 (m, 4H), 1.14 (m, 6H); MS m/z 515 (M^{2+}, 100); Anal. ($C_{58}H_{82}N_2O_{14}Cl_2 \cdot 4.5H_2O$) C, H, N, Cl.

{3-[(1*S*,2*R*)-6,7-Dimethoxy-2-methyl-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-isoquinolinio]propyl} {3-{(1*R*,2*S*)-6,7-Dimethoxy-2-methyl-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl} Decanedioate Dichloride (25f). Synthesized from **17e**, **13a**, and **10g** as described for synthesis of the **24a**/**24b** mixture:[9] white powder (10% yield); ^{1}H NMR 300 MHz (DMSO-d_6) δ 7.17 (br, 1H), 6.96 (s, 1H), 6.86 (s, 1H), 6.38 (s, 2H), 6.16 (br, 1H), 5.80 (s, 1H), 5.71 (s, 1H), 4.73 (m, 1H), 4.05 (m, 2H), 3.99 (m, 2H), 3.80−3.20 (br m, 17H), 3.78 (s, 3H), 3.71 (s, 3H), 3.70 (s, 3H), 3.65 (s, 6H), 3.61 (s, 3H), 3.58 (s, 3H), 3.32 (s, 3H), 3.28 (s, 3H), 3.08 (m, 3H), 2.87 (m, 1H), 2.84 (s, 3H); 2.19 (t, 2H, J = 7), 2.17 (m, 4H), 2.12 (t, 2H, J = 7), 1.37 (m, 4H), 1.16 (m, 8H); MS m/z 522 (M^{2+}, 100); Anal. ($C_{59}H_{84}N_2O_{14}Cl_2 \cdot 4H_2O$) C, H, N, Cl.

2,2-Difluoro-1-{3-{(1*R*,2*S*)-6,7-dimethoxy-2-methyl-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl} Hydrogen Butanedioate Monochloride (26c). A solution of **13a** (3.0 g, 6.22 mmol) and **19c** (0.94 g, 6.91 mmol) in 1,2-dichloroethane (23 mL) was stirred for 15 min at room temperature. The solvent was evaporated, and the remaining material was triturated with EtOAc and dried under high vacuum to afford **26c** as a rigid foam: (3.21 g, 83% yield); ^{1}H NMR 400 MHz (DMSO-d_6) δ 6.84 (s, 1H), 6.36 (s, 2H), 5.72 (s, 1H), 4.70 (dd, 1H, J = 9, 3), 4.02 (q, 2H, J = 7), 3.89 (m, 1H), 3.80−3.20 (br m, 5H), 3,72 (s, 3H), 3.65 (s, 6H), 3.61 (s, 3H), 3.32 (s, 3H), 3.30 (s, 3H), 3.14−3.00 (m, 3H), 2.88 (br t, 1H, J = 12), 2.24 (m, 2H), carboxyl proton not observed; MS m/z 582 (M^+, 70); HPLC: one major peak (95%) (see general Experimental Section for analytical HPLC conditions).

(2*S*)-4-{3-[(1*S*,2*R*)-6,7-Dimethoxy-2-methyl-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-isoquinolinio]propyl}-1-{3-{(1*R*,2*S*)-6,7-dimethoxy-2-methyl-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl}-2-fluorobutanedioate Dichloride (27a). Synthesized from **19a**,[21] **13a**, and **10g** as described below for the synthesis of **27c**: white powder (26% yield); ^{1}H NMR 400 MHz (DMSO-d_6) δ 7.26 (br, 1H), 6.94 (s, 1H), 6.82 (s, 1H), 6.38 (s, 2H), 6.36 (s, 1H), 6.08 (br, 1H), 5.85 (s, 1H), 5.70 (s, 1H), 5.31 (ddd, 1H, J = 46.6, 7.2, 3.6), 4.78 (dd, 1H, J = 8, 3), 4.10 (br m, 4H), 3.90−2.70 (br m, 22H), 3.77 (s, 3H), 3.70 (s, 3H), 3.68 (s, 3H), 3.63 (s, 6H), 3.60 (s, 3H), 3.56 (s, 3H), 3.34 (s, 3H), 3.26 (s, 3H), 2.84 (s, 3H), 2.18 (m, 4H); MS m/z 489 (M^{2+}, 55); Anal. ($C_{53}H_{71}N_2O_{14}Cl_2F \cdot 5H_2O$) C, H, N.

2,2-Difluoro-5-{3-[(1*S*,2*R*)-6,7-dimethoxy-2-methyl-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-isoquinolinio]propyl}-1-{3-{(1*R*,2*S*)-6,7-dimethoxy-2-methyl-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl}pentanedioate Dichloride (27b). Synthesized from **19b**,[23,24] **13a**, and **13g** as described below for the synthesis of **27c**: white powder (32% yield); ^{1}H NMR 400 MHz (DMSO-d_6) δ 7.31 (br, 1H), 6.93 (s, 1H), 6.82 (s, 1H), 6.39 (s, 2H), 6.35 (s, 1H), 6.08 (br, 1H), 5.88 (s, 1H), 5.68 (s, 1H), 4.80 (dd, 1H, J = 10, 4), 4.21 (br t, 2H, J = 6), 4.07 (t, 2H, J = 6), 4.00−3.20 (m, 19H), 3.76 (s, 3H), 3.69 (s, 3H), 3.68 (s, 3H), 3.62 (s, 6H), 3.59 (s, 3H), 3.55 (s, 3H), 3.32 (s, 3H), 3.20−3.00 (m, 3H), 2.84 (s, 3H), 2.85 (m, 1H), 2.40 (t, 2H, J = 7), 2.22 (m, 6H); MS m/z 505 (M^{2+}, 100); Anal. ($C_{54}H_{72}N_2O_{14}Cl_2F_2 \cdot 3H_2O$) C, H, N.

2,2-Difluoro-4-{3-[(1*S*,2*R*)-6,7-dimethoxy-2-methyl-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-isoquinolinio]propyl}-1-{3-{(1*R*,2*S*)-6,7-dimethoxy-2-methyl-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl}butanedioate Dichloride (27c). Neat oxalyl chloride (25 mL, 0.28 mol) was added dropwise to a solution of **26c** (7.0 g, 11.0 mmol) in DCE (150 mL). The solution was stirred at room temperature for 3.5 h. The solvent and excess oxalyl chloride were removed at reduced pressure, and the remaining foam was reconstituted in DCE (35 mL). A solution of **13g** (4.7 g, 10.0 mmol) in DCE (35 mL) was added, and the reaction mixture was stirred overnight at room temperature. The solvent was evaporated, and the product was purified by preparative HPLC. Chromatography fractions were assayed by analytical HPLC (see general Experimental Section for analytical and preparative HPLC conditions), and those fractions containing pure **27c** were combined and coevaporated with 1−2 volumes of $CHCl_3$ to a final volume of ca.0.5 L. This solution was washed with 1:1 brine/H_2O, dried over Na_2SO_4, filtered, and concentrated. The resulting material was lyophilized from water to afford **27c** as a white powder (5.63 g, 53% yield): ^{1}H NMR 400 MHz (DMSO-d_6) δ 7.2 (br, 1H), 6.94 (s, 1H), 6.82 (s, 1H), 6.37 (s, 2H), 6.1 (br, 1H), 5.80 (s, 1H), 5.69 (s, 1H), 4.76 (dd, 1H, J = 10, 4), 4.23 (t, 2H, J = 6), 4.07 (t, 2H, J = 6), 3.95−2.95 (br m, 22H), 3.77 (s, 3H), 3.69 (s, 3H), 3.68 (s, 3H), 3.63 (s, 6H), 3.60 (s, 3H), 3.56 (s, 3H), 3.33 (s, 3H), 3.26 (s, 3H), 2.87 (br t, 1H, $J \sim 11$), 2.83 (s, 3H), 2.21 (m, 4H); MS m/z 498 (M^{2+}, 100); Anal. ($C_{53}H_{70}N_2O_{14}Cl_2F_2 \cdot 4H_2O$) C, H, N.

2,2-Difluoro-4-{3-[(1*S*,2*R*)-6,7-dimethoxy-2-methyl-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-isoquinolinio]propyl}-1-{3-{(1*R*,2*S*)-2-methyl-6,7,8-trimethoxy-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl}butanedioate Dichloride (27d).

Prepared from **19c**, **13c**, and **13g** as described above for the synthesis of **27c**: white powder (73% yield); ^{1}H NMR 400 MHz (DMSO-d_6) δ 7.20 (br s, 1H), 6.94 (s, 1H), 6.71 (s, 1H), 6.38 (s, 2H), 6.36 (s, 1H), 6.10 (br s, 1H), 5.74 (s, 1H), 4.95 (t, 1H, J = 6), 4.21 (t, 2H, J = 6), 4.07 (t, 2H, J = 6), 3.9−2.9 (br m, 22H), 3.77 (s, 6H), 3.68 (s, 3H), 3.66 (s, 6H), 3.64 (s, 3H), 3.60 (s, 3H), 3.58 (s, 3H), 3.56 (s, 3H), 3.16 (s, 3H), 2.81 (s, 3H), 2.30−2.00 (br m, 4H); MS m/z 513 (M^{2+}, 100); Anal. ($C_{54}H_{72}N_2O_{15}$-Cl_2F_2·$4H_2O$) C, H, N.

2,2-Difluoro-4-{3-[(1*R*,2*S*)-6,7-dimethoxy-1-(3,4-dimethoxyphenyl)-2-methyl-1,2,3,4-tetrahydro-2-isoquinolinio]propyl}-1-{3-{(1*R*,2*S*)-6,7-dimethoxy-2-methyl-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl}butanedioate Dichloride (27e). Prepared from **19c**, **13a**, and **10f** as described above for the synthesis of **27c:** white powder (17% yield); ^{1}H NMR 400 MHz (DMSO-d_6) δ (aromatic region, 2H, too broad for observation), 6.99 (br, 1H), 6.95 (s, 1H), 6.84 (s, 1H), 6.40 (s, 2H), 6.34 (br s, 1H), 5.85 (br s, 1H), 5.71 (s, 1H), 4.81 (dd, 1H, $J \sim$ 10, 4), 4.25 (t, 2H, J = 6), 4.09 (t, 2H, J = 7), 3.85 (m, 2H), 3.80−3.30 (br m, 12H), 3.78 (br s, 6H), 3.71 (s, 3H), 3.65 (s, 6H), 3.61 (s, 3H), 3.55 (s, 3H), 3.36 (s, 3H), 3.28 (s, 3H), 3.20−3.01 (m, 4H), 2.88 (dd, 1H, $J \sim$ 12, 10), 2.82 (s, 3H), 2.25 (m, 4H); MS m/z 483 (M^{2+}, 100); Anal. ($C_{52}H_{68}N_2O_{13}Cl_2F_2$·$5H_2O$) C, H, N, Cl.

2,2-Difluoro-1-{3-[(1*S*,2*R*)-6,7-dimethoxy-2-methyl-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-isoquinolinio]propyl}-4-{3-{(1*R*,2*S*)-6,7-dimethoxy-2-methyl-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl}butanedioate Dichloride (27f). Prepared from **19c**, **13g**, and **13a** as described above for the synthesis of **27c**: white powder (20% yield); ^{1}H NMR 400 MHz (DMSO-d_6) δ 7.30 (br, 1H), 6.94 (s, 1H), 6.86 (s, 1H), 6.40 (s, 2H), 6.36 (s, 1H), 6.10 (br, 1H), 5.88 (s, 1H), 5.72 (s, 1H), 4.78 (dd, 1H, $J \sim$ 10, 4), 4.30 (t, 2H, J = 6), 4.04 (m, 3H), 3.85 (m, 2H), 3.80−3.20 (br m, 13H), 3.78 (s, 3H), 3.72 (s, 3H), 3.69 (s, 3H), 3.65 (s, 6H), 3.61 (s, 3H), 3.56 (s, 3H), 3.50 (m, 2H), 3.36 (s, 3H), 3.27 (s, 3H), 3.16 (m, 1H), 3.10 (m, 2H), 2.88 (m, 1H), 2.85 (s, 3H), 2.30 (m, 2H), 2.16 (m, 2H); MS m/z 498 (M^{2+}, 100); Anal. ($C_{53}H_{70}N_2O_{14}Cl_2F_2$·$4H_2O$) C, H, N.

(*Z*)-4-{3-[(1*S*,2*R*)-6,7-Dimethoxy-2-methyl-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-isoquinolinio]propyl}-1-{3-{(1*R*,2*S*)-6,7-dimethoxy-2-methyl-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl}-2-fluoro-2-butenedioate Dichloride (28a). Solid K_2CO_3 (97 mg, 0.702 mmol) was added to a solution of **27c** (750 mg, 0.702 mmol) in DMF (5 mL), and the mixture was stirred at room temperature for 1 h and then filtered. The filtrate was diluted with $CHCl_3$ (50 mL) and washed with 1:1 brine/H_2O (adjusted to pH $\sim$ 1 with HCl). The organic layer was dried and concentrated, and the residue was triturated with Et_2O and purified by preparative HPLC (see general Experimental Section). Workup of chromatography fractions and lyophilization as described for **27c** gave **28a** as a white powder (404 mg, 52% yield): ^{1}H NMR 400 MHz (DMSO-d_6) δ 7.15 (br, 1H), 6.92 (s, 1H), 6.83 (s, 1H), 6.38 (s, 2H), 6.35 (s, 1H), 6.27 (d, 1H, J = 31), 6.1 (br, 1H), 5.82 (s, 1H), 5.69 (s, 1H), 4.77 (dd, 1H, J = 8, 3), 4.21 (m, 4H), 3.95−3.05 (m, 22H), 3.75 (s, 3H), 3.69 (s, 3H), 3.68 (s, 3H), 3.62 (s, 6H), 3.59 (s, 3H), 3.55 (s, 3H), 3.36 (s, 3H), 2.87 (br t, 1H, $J \sim$ 13), 2.85 (s, 3H), 2.24 (m, 4H); MS m/z 488 (M^{2+}, 80); Anal. ($C_{53}H_{69}N_2O_{14}$-Cl_2F·$4H_2O$) C, H, N.

(*Z*)-4-{3-[(1*S*,2*R*)-6,7-Dimethoxy-2-methyl-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydro-2-isoquinolinio]propyl}-1-{3-{(1*R*,2*S*)-2-methyl-6,7,8-trimethoxy-1-[(3,4,5-trimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-2-isoquinolinio}propyl}-2-fluoro-2-butenedioate Dichloride (28b). Synthesized from **27d** as described above for the synthesis of **28a**: white powder (44% yield); ^{1}H NMR 400 MHz (DMSO-d_6) δ 7.20 (br s, 1H), 6.92 (s, 1H), 6.71 (s, 1H), 6.40 (s, 2H), 6.25 (d, 1H, J = 30.8), 6.35 (s, 1H), 6.10 (br s, 1H), 5.79 (s, 1H), 4.95 (t, 1H, J = 5.6), 4.21 (m, 4H), 3.8−2.9 (br m, 23H), 3.77 (s, 3H), 3.75 (s, 3H), 3.68 (s, 3H), 3.66 (s, 3H), 3.64 (s, 3H), 3.60 (s, 3H), 3.57 (s, 3H), 3.54 (s, 3H), 3.19 (s, 3H), 2.84 (s, 3H), 2.30−2.00 (br m, 4H); MS m/z 503 (M^{2+}, 80); Anal. ($C_{54}H_{71}N_2O_{15}Cl_2F$·$5H_2O$) C, H, N, Cl.

References

(1) Savarese, J. J.; Miller, R. D.; Lien, C. A.; Caldwell, J. E. Pharmacology of Muscle Relaxants and Their Antagonists. In *Anesthesia*, 4th ed.; Miller, R. D., Ed.; Churchill Livingstone: New York, 1994; pp 417−488.

(2) (a) Lee, C. Structure, Conformation and Action of Neuromuscular Blocking Drugs. *Br. J. Anaesth.* **2001**, *87*, 755−769. (b) Rees, D. C.; Hill, D. R. Drugs in Anesthetic Practice. *Annu. Rep. Med. Chem.* **1996**, *31*, 41−50. (c) Bevan, D. R. Newer Neuromuscular Blocking Agents. *Pharmacol. Toxicol.* **1994**, *74*, 3−9.

(3) Maximum clinical duration (time for return to *25% of control* twitch response) of NMBs after a 2 × ED_{95} dose are defined by the FDA as ultrashort (8 min), short (20 min), intermediate (50 min), and long acting (≫50 min), see: Bedford, R. F. From the FDA. *Anesthesiology* **1995**, *82*, 33A.

(4) (a) Naguib, M.; Flood, P.; McArdle, J. J.; Brenner, H. R. Advances in Neurobiology of the Neuromuscular Junction. Implications for the Anesthesiologist. *Anesthesiology* **2002**, *96*, 202−231. (b) Mahajan, R. P. Is Suxamethonium Now Obsolete? *Curr. Anaesth. Crit. Care* **1996**, *7*, 289−294. (c) Belmont, M. R. Succinylcholine/Suxamethonium. *Curr. Opin. Anesthesiol.* **1995**, *8*, 362−366. (c) Durant, N. N.; Katz, R. L. Suxamethonium. *Br. J. Anaesth.* **1982**, *54*, 195−208.

(5) (a) Sparr, H. J.; Beaufort, T. M.; Fuchs-Buder, T. Newer Neuromuscular Blocking Agents. How Do They Compare with Established Agents? *Drugs* **2001**, *61*, 919−942. (b) White, P. F. Rapacuronium: Why Did It Fail as a Replacement for Succinylcholine? *Br. J. Anaesth.* **2002**, *88*, 163−165. (c) Adam, J. M.; Bennett, D. J.; Bom, A.; Clark, J. K.; Feilden, H.; Hutchinson, E. J.; Palin, R.; Prosser, A.; Rees, D. C.; Rosair, G. M.; Stevenson, D.; Tarver, G. J.; Zhang, M.-Q. Cyclodextrin-Derived Host Molecules as Reversal Agents for the Neuromuscular Blocker Rocuronium Bromide: Synthesis and Structure−Activity Relationships. *J. Med. Chem.* **2002**, *45*, 1806−1816. (d) Bom, A.; Bradley, M.; Cameron, K.; Clark, J. K.; Van Egmond, J.; Feilden, H.; MacLean, E. J.; Muir, A. W.; Palin, R.; Rees, D. C.; Zhang, M.-Q. A Novel Concept of Reversing Neuromuscular Block: Chemical Encapsulation of Rocuronium Bromide by a Cyclodextrin-Based Synthetic Host. *Angew. Chem., Int. Ed.* **2002**, *41*, 265−270.

(6) (a) Dewar, G. H.; Marshall, I. G.; Patel, S. S.; Waigh, R. D. Chemodegradable Neuromuscular Blocking Agents-I: *Bis*-3,4-Dihydroisoquinolinium Salts. *Pharm. Sci. Commun.* **1993**, *4*, 3−14. (b) Stenlake, J. B.; Williams, W. D.; Dhar, N. C.; Marshall, I. G. Neuromuscular Blocking Agents: Stereochemical Studies on 1-Benzyltetrahydroisoquinolinium Salts. *Eur. J. Med. Chem.* **1974**, *9*, 233−238 and references therein. (c) Stenlake, J. B.; Dhar, N. C.; Haddow, J.; McDonald, I. M.; Maehr, R. B.; Wastila, W. B. Neuromuscular Blocking Agents. Some Approaches to Short Acting Compounds. *Eur. J. Med. Chem.* **1992**, *27*, 463−477. (d) Dhar, N. C.; Maehr, R. B.; Masterson, L. A.; Midgley, J. M.; Stenlake, J. B.; Wastila, W. B. Approaches to Short-Acting Neuromuscular Blocking Agents: Nonsymmetrical Bis-tetrahydroisoquinolinium Mono- and Diesters. *J. Med. Chem.* **1996**, *39*, 556−561. (e) Gyermek, L.; Lee, C.; Nguyen, N. Pharmacology of G-1−64, a New Nondepolarizing Neuromuscular Blocking Agent with Rapid Onset and Short Duration of Action. *Acta Anaesthesiol. Scand.* **1999**, *43*, 651−657.

(7) This rare but well-known problem has been observed in the clinical application of both mivacurium and succinylcholine (see ref 1).

(8) For examples of the inactive metabolite (soft drug) approach in drug design, see: (a) Hwang, S.-K.; Juhasz, A.; Yoon, S.-H.; Bodor, N. Soft Drugs. 12. Design, Synthesis, and Evaluation of Soft Bufuralol Analogues. *J. Med. Chem.* **2000**, *43*, 1525−1532. (b) Feldman, P. L.; James, M. K.; Brackeen, M. F.; Bilotta, J. M.; Schuster, S. V.; Lahey, A. P.; Lutz, M. W.; Johnson, M. R.; Leighton, H. J. Design, Synthesis, and Pharmacological Evaluation of Ultra-Short- to Long-Acting Opioid Analgetics. *J. Med. Chem.* **1991**, *34*, 2202−2208 and references therein.

(9) (a) Boros, E. E.; Bigham, E. C.; Mook, R. A., Jr.; Patel, S. S.; Savarese, J. J.; Ray, J. A.; Thompson, J. B.; Hashim, M. A.; Wisowaty, J. C.; Feldman, P. L.; Samano, V. Bis- and Mixed-Tetrahydroisoquinolinium Chlorofumarates: New Ultra-Short-Acting Nondepolarizing Neuromuscular Blockers. *J. Med. Chem.* **1999**, *42*, 206−209 and Supporting Information. (b) Samano, V.; Ray, J. A.; Thompson, J. B.; Mook, R. A., Jr.; Jung, D. K.; Koble, C. S.; Martin, M. T.; Bigham, E. C.; Regitz, C. S.; Feldman, P. L.; Boros, E. E. Synthesis of Ultra-Short-Acting Neuromuscular Blocker GW 0430: A Remarkably Stereo- and Regioselective Synthesis of Mixed-Tetrahydroisoquinolinium Chlorofumarates. *Org. Lett.* **1999**, *1*, 1993−1996 and Supporting Information.

(10) (a) Belmont, M. R.; Lien C. A.; Savarese, J. J.; Patel, S.; Fischer, G.; Mook, R. Neuromuscular Blocking Effects of GW280430A at the Adductor Pollicis and Larynx in Human Volunteers. *Br. J. Anaesth.* **1999**, *82*, A419. (b) Moore, E. W.; Hunter, J. M. The New Neuromuscular Blocking Agents: Do They Offer Any Advantages? *Br. J. Anaesth.* **2001**, *87*, 912−925 and references therein.

(11) Whaley, K. W.; Govindachari, T. R. The Preparation of 3,4-Dihydroisoquinolines and Related Compounds by the Bischler-Napieralski Reaction. *Org. React.* **1951**, *6*, 74−150.

(12) Kaluszyner, A.; Galun, A. B. *N*-Methylation of Amino Alcohols and Amino Mercaptans. *J. Org. Chem.* **1961**, *26*, 3536−3537.

(13) Swaringen, R. A., Jr.; El-Sayad, H. A.; Yeowell, D. A.; Savarese, J. J. Novel Compounds. US Patent 4,761,418, 1988.

(14) Uematsu, N.; Fujii, A.; Hashiguchi, S.; Ikariya, T.; Noyori, R. Asymmetric Transfer Hydrogenation of Imines. *J. Am. Chem. Soc.* **1996**, *118*, 4916−4917.

(15) Brossi, A.; Teitel, S. Synthesis and Absolute Configuration of Cryptostylines I, II, and III. *Helv. Chim. Acta* **1971**, *54*, 1564−1571.

(16) Suzuki, H.; Aoyagi, S.; Kibayashi, C. Asymmetric Synthesis of 1-Substituted Tetrahydroisoquinolines by Nucleophilic Addition to Hydrazonium Ions. Application to the Enantioselective Syntheses of (+)- and (−)-Salsolidines and (−)-Cryptostyline II. *Tetrahedron Lett.* **1995**, *36*, 6709−6712.

(17) (a) Bernath, G.; Kobor, J.; Koczka, K.; Radics, L.; Kajtar, M. Quaternization of 1,2-Disubstituted 6,7-Dimethoxy-1,2,3,4-Tetrahydroisoquinolines. *Tetrahedron Lett.* **1968**, 225−229. (b) Ribar, B.; Lazar, D.; Kalman, A.; Kobor, J.; Bernath, G. Stereochemical Studies. Part 29. Crystal Structure of 2-Benzyl-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-phenylisoquinolinium Iodide. *J. Chem. Soc., Perkin Trans. 2* **1977**, 1141−1144. (c) El-Sayad, H. A.; Swaringen, R. A.; Yeowell, D. A.; Crouch, R. C.; Hurlbert, S.; Miller, R. W.; McPhail, A. T. Nuclear Magnetic Resonance and X-ray Stereochemical Studies on 1-Benzyltetrahydroisoquinolinium Salts. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2067−2077.

(18) Kaldor, I.; Feldman, P. L.; Mook, R. A., Jr.; Ray, J. A.; Samano, V.; Sefler, A. M.; Thompson, J. B.; Travis, B. R.; Boros, E. E. Stereocontrolled Synthesis of *cis*-Dibenzoquinolizine Chlorofumarates: Curare-Like Agents of Ultrashort Duration. *J. Org. Chem.* **2001**, *66*, 3495−3501.

(19) Akhtar, M.; Botting, N. P.; Cohen, M. A.; Gani, D. Enantiospecific Synthesis of 3-Substituted Aspartic Acids via Enzymic Amination of Substituted Fumaric Acids. *Tetrahedron* **1987**, *43*, 5899−5908.

(20) (a) Erickson, L. E. Proton Nuclear Magnetic Resonance Spectra of Substituted Succinic Anhydrides, Acids, Salts and Esters. *J. Am. Chem. Soc.* **1965**, *87*, 1867−1875. (b) Feuer, H.; Rubinstein, H. Preparation of 1-(3′,1′H,6′-Pyridazinone)-3,6-pyridazinedione. Attempts to Prepare Bicyclic Dimaleic Hydrazide. *J. Org. Chem.* **1959**, *24*, 811−813.

(21) (a) Lowe, G.; Potter, B. V. L. Synthesis, Absolute Configuration, and Circular Dichroism of the Enantiomers of Fluorosuccinic Acid. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2029−2032. (b) Leeper, F. J.; Rock, M. The Synthesis of a Fluorinated Analogue of 5-Aminolaevulinic Acid, A Potential Inhibitor of Porphyrin Biosynthesis. *J. Fluorine Chem.* **1991**, *51*, 381−396.

(22) Partridge, J. J.; Shiuey, S.-J.; Uskokovic, M. R. Process for the Preparation of 24,24-Difluoro-1α,25-Dihydroxy Vitamin D_3 and Intermediates Obtained Therefrom. US Patent 4,421,690, 1988.

(23) (a) Tsukamoto, T.; Kitazume, T. Preparation and Reaction of Difluorinated Malonaldehydic Acid Derivatives: A New Route to Functionalized α,α-Difluorinated Esters and Amides. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1177−1181. (b) Tsukamoto, T.; Kitazume, T. Difluorinated Malonaldehyde Derivatives as Useful Difluoromethylene-Containing Building Blocks. *J. Chem. Soc., Chem. Commun.* **1992**, 540−541.

(24) Fried, J. Certain Fluorine Substituted PGI_2 Compounds. US Patent 4,324,730, 1982.

(25) For similar examples of regio- and stereoselective elimination of HCl from 2,3-dichloropropanoates, see: Mercedes, C. C.; Garay, R. O. Elimination from Diastereomeric Methyl 3-Acetoxy-2-halogeno- and 2,3-Dihalogeno-3-phenylpropanoates. *J. Chem. Soc., Perkin Trans. 2* **1987**, 1473−1476.

(26) 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 (see ref 1).

(27) 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 (see ref 2c).

(28) The threshold dose for cardiovascular effects with mivacurium in humans is ca. 2.7 × ED_{95} (0.2 mg/kg): see (a) Frampton, J. E.; McTavish, D. Mivacurium: A Review of its Pharmacology and Therapeutic Potential in General Anaesthesia. *Drugs* **1993**, *45*, 1066−1089. (b) Savarese. J. J. Mivacurium: A Comparison with Other Benzylisoquinolinium Nondepolarizing Muscle Relaxants. *J. Drug Dev.* **1993**, *5*, 1−5.

(29) (a) Bowman, W. C.; Rodger, I. W.; Houston, J.; Marshall, R. J.; McIndewar, I. Structure: Action Relationships among Some Desacetoxy Analogues of Pancuronium and Vecuronium in the Anesthetized Cat. *Anesthesiology* **1988**, *69*, 57−62. (b) Kopman, A. F. Onset of Action of Neuromuscular Blockade: Contributing Factors. *Curr. Opin. Anaesth.* **1992**, *5*, 561−567.

(30) Compound **27c** was stabilized in pH 2−3 saline solution at ice-bath temperature for in vivo studies. Its half-life in pH ∼ 2 DCl/D_2O at 25 °C is ca. 24 h based on ^1H NMR analysis.

(31) Compound **24a** has a half-life of ca. 178 days in pH 3 saline at 23 °C. Its half-life in pH 7.4 phosphate buffer at 37 °C is ca. 37 min.

(32) (a) Reese, M. J.; Walsh, J. S.; Spitzer, T.; Bower, G.; Stafford, C. Comparative Metabolic Profiles of the Neuromuscular Blocker GW280430 in Human, Monkey, and Dog, and Characterization of a Major Metabolite as an Unusual Cyclized Cysteine Conjugate. Presented at the 9th North American ISSX Meeting (*issx.org*); Nashville, TN.; October, 1999. (b) Spitzer, T.; Walsh, J.; Reese, M.; Stafford, C.; Facchine, K.; Burinsky, D.; Sides, S. Structure Elucidation of the Primary Metabolite of the Neuromuscular Blocker GW280430A. Presented at the SMASH NMR Conference (*smashnmr.org*); Argonne, IL.; July, 1999.

JM020574+