Cyclodextrin-Derived Host Molecules as Reversal Agents for the Neuromuscular Blocker Rocuronium Bromide: Synthesis and Structure−Activity Relationships

Julia M. Adam,[†] D. Jonathan Bennett,[†] Anton Bom,[†] John K. Clark,[†] Helen Feilden,[†] Edward J. Hutchinson,[†] Ronald Palin,[†] Alan Prosser,[†] David C. Rees,[†,‡] Georgina M. Rosair,[§] Donald Stevenson,[†] Gary J. Tarver,[†] and Ming-Qiang Zhang*[,†]

Departments of Medicinal Chemistry and Pharmacology, Organon Laboratories Ltd., Newhouse ML1 5SH, Scotland, U.K., and Department of Chemistry, Heriot-Watt University, Edinburgh EH14 4AS, Scotland, U.K.

Received November 21, 2001

A series of mono- and per-6-substituted cyclodextrin derivatives were synthesized as synthetic receptors (or host molecules) of rocuronium bromide, the most widely used neuromuscular blocker in anaesthesia. By forming host−guest complexes with rocuronium, these cyclodextrin derivatives reverse the muscle relaxation induced by rocuronium in vitro and in vivo and therefore can be used as reversal agents of the neuromuscular blocker to assist rapid recovery of patients after surgery. Because this supramolecular mechanism of action does not involve direct interaction with the cholinergic system, the reversal by these compounds, e.g., compound **14** (Org 25969), is not accompanied by cardiovascular side effects usually attendant with acetylcholinesterase inhibitors such as neostigmine. The structure−activity relationships are consistent with this supramolecular mechanism of action and are discussed herein. These include the effects of binding cavity size and hydrophobic and electrostatic interaction on the reversal activities of these compounds.

Introduction

Neuromuscular blockers (NMBs, also known as skeletal muscle relaxants), together with hypnotics and analgesics, constitute the three major classes of drugs routinely used in modern anaesthesia.[1] Intravenous administration of NMBs causes skeletal muscle relaxation, thus facilitating endotracheal intubation and allowing surgical access to body cavities, in particular the abdomen and thorax, without hindrance from voluntary or reflex muscle movement.[2] NMBs are also used in the care of critically ill patients undergoing intensive therapy, to facilitate compliance with mechanical ventilation when sedation and analgesia alone have proved to be inadequate.[3]

On the basis of their mechanisms of action, NMBs are divided into two categories: depolarizing and nondepolarizing. Most of the clinically used NMBs are nondepolarizing. These include atracurium, mivacurium, pancuronium, vecuronium, and rocuronium. They act as competitive antagonists of the nicotinic acetylcholine receptor (nAChR) at the neuromuscular junction. By blocking the acetylcholine-induced activation of the ion channel, nondepolarizing NMBs prevent cell membrane depolarization, and as a result, the muscle becomes flaccid (Figure 1A). Depolarizing NMBs act as agonists of the nAChR. They stimulate an initial opening of the ion channel, producing contractions known as fasciculations. However, since these drugs are broken down relatively slowly by cholinesterase enzymes, compared to the very rapid hydrolysis of acetylcholine by acetylcholinesterase (AChE), they bind to the receptor longer than acetylcholine. This causes persistent depolarization and desensitization of the end-plate. Succinylcholine is the only depolarizing NMB that is still in clinical use.

At the end of surgery or a period of intensive care, a reversal agent of NMBs (nondepolarizing only) is often administered to the patient to assist the recovery of muscle function and/or to prevent residual neuromuscular block.[4−6] All clinically used reversal agents are AChE inhibitors, such as neostigmine (**1**; Chart 1) and edrophonium (**2**; Chart 1), which inhibit the breakdown of acetylcholine to increase the level of acetylcholine at the neuromuscular junction and to gain competitive advantage for acetylcholine to bind to the nAChR (Figure 1B).

The use of AChE inhibitors as NMB reversal agents has several drawbacks. First of all, AChE inhibition causes nonselective potentiation of neurotransmission to all synapses (both somatic and autonomic) involving acetylcholine, especially those in the heart, and leads to many side effects including bradycardia, hypotension, etc. Therefore, in practice, these agents are often used in combination with a muscarinic acetylcholine receptor (mAChR) antagonist such as atropine or glycopyrrolate to antagonize the muscarinic effects of acetylcholine in the autonomic parasympathetic neuroeffector junctions (e.g., the heart). Unfortunately mAChR antagonists themselves cause a number of side effects such as tachycardia, dry mouth, blurred vision, etc. and furthermore may affect cardiac conduction. Second, AChE

* To whom correspondence should be addressed. Current address: Shire BioChem Inc., 275 Armand-Frappier Blvd., Laval, Quebec, Canada H7V 4A7. Phone: 1-450-978-7810. Fax: 1-450-978-7777. E-mail: mzhang@ca.shire.com.
[†] Organon Laboratories Ltd.
[‡] Current address: AstraZeneca R&D Mölndal, S-431 83 Mölndal, Sweden.
[§] Heriot-Watt University.

10.1021/jm011107f CCC: $22.00
Published on Web 03/21/2002

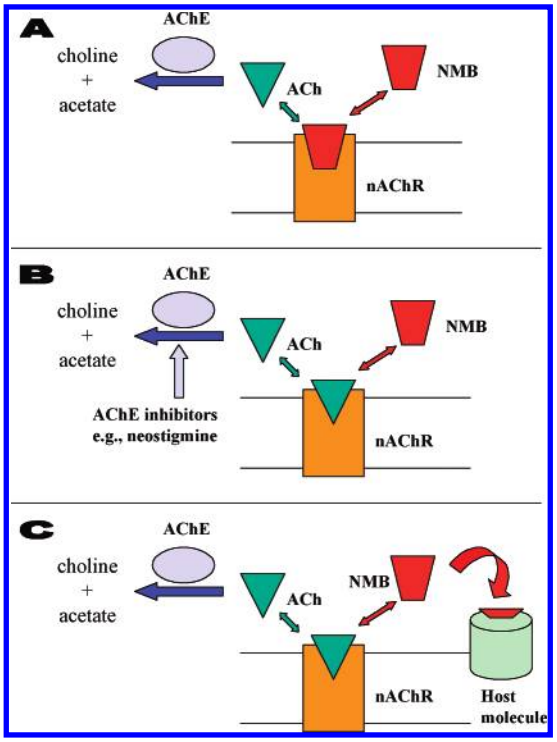
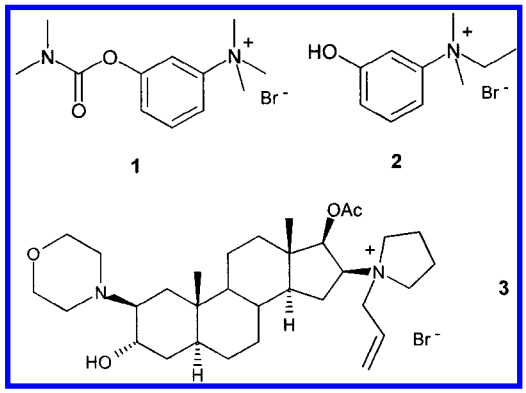


Figure 1. Concept of using a synthetic receptor (or host molecule) as a NMB reversal agent. (A) The neuromuscular blocker (NMB, or muscle relaxant) binds to nicotinic acetylcholine receptor (nAChR) at the neuromuscular junction and blocks neurotransmission induced by acetylcholine. (B) A conventional reversal agent inhibits acetylcholinesterase (AChE) and increases the acetylcholine (ACh) level at the neuromuscular junction, giving ACh the competitive advantage to reactivate the receptor. However this artificially increased ACh level causes cardiovascular side effects. (C) A host molecule encapsulates the NMB and promotes its dissociation from the receptor, leaving the receptor available to ACh. No ACh homeostasis is disturbed, and hence, there are no cardiovascular side effects.

Chart 1. Structures of Most Widely Used NMB Reversal Agents Neostigmine (**1**), Edrophonium (**2**), and the Neuromuscular Blocker Rocuronium (**3**)



inhibitors can only be used when residual neuromuscular activity (>10% twitch activity) is present. Occasionally, because of either hypersensitivity of the patient or accidental overdose, administration of NMBs can cause complete blockade of neuromuscular function (also known as "profound block"). At present, there is no reliable treatment to reverse such a "profound block". Attempts to overcome a "profound block" with high doses of AChE inhibitors has the risk of inducing a life-threatening "cholinergic crisis", resulting in a broad range of symptoms related to overenhanced stimulation of nAChR and mAChR. Third, since depolarizing NMBs (e.g., succinylcholine) are themselves cholinergic agonists, AChE inhibitors are not suitable for the reversal of depolarizing NMBs.

We have therefore been interested in the discovery of a NMB reversal agent that can overcome the above drawbacks and limitations of AChE inhibitors.[7,8]

We hypothesised that chemical encapsulation of a NMB by an exogenous host molecule would promote the dissociation of the NMB from its site of action, leading to the reversal of neuromuscular blockade (Figure 1C).[8] Since this mechanism of action does not involve direct interaction with cholinergic systems, it should circumvent the undesired side effects attendant with AChE inhibitors. Since the host molecule does not compete with the NMB for binding to nAChR and instead acts as a synthetic receptor of the NMB, it could be effective in reversing depolarizing NMBs. Furthermore, this chemical removal of a NMB from its site of action does not need the presence of residual neuromuscular activity before application and could be safely employed for the reversal of "profound block".

This paper describes the design, synthesis, and structure−activity relationship of a series of cyclodextrin-derived host molecules as potential reversal agents for rocuronium bromide (**3**; Chart 1), the most widely used NMB in anaesthesia. Also discussed in this paper is the relationship between structural factors that influence host−guest complex formation and the activities of these compounds to reverse rocuronium-induced neuromuscular blockade.

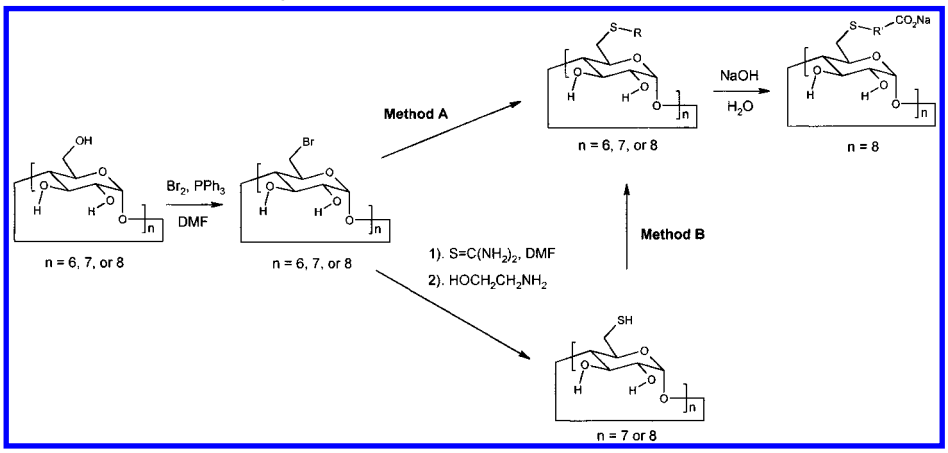
Design and Synthesis

We chose cyclodextrins (CDs) as our target molecules because of three characteristics of this group of cyclic oligosaccharides: (1) a well-defined lipophilic cavity, (2) high water solubility, and (3) good biological tolerance.[10−12] Several CDs have been successfully used as pharmaceutical excipients to increase water solubility, stability, or bioavailability of lipophilic drugs.[11,12]

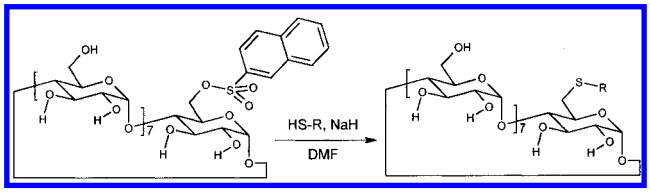
It is known that the most important factors involved in CD−organic guest binding are van der Waals and hydrophobic interactions.[13] The van der Waals interactions are highly dependent on the size and shape complementarity between the guest and the CD cavity, and hydrophobic interactions are related to the total hydrophobic area involved in the interaction. We therefore designed a series of cyclodextrin derivatives based on the architectures of α-, *β*-, and *γ*-CDs to assess the influence of cavity size on reversal activity. We also designed CDs with mono- and per-6 substitutions to examine the influence of total hydrophobic cavity area on the complexation-induced reversal of NMB activity.

Rocuronium has a positively charged quaternary nitrogen in its structure. We therefore decided to place negatively charged functional groups, e.g., a carboxyl, at the rim of the CD cavity in order to increase binding affinity to rocuronium by electrostatic interaction. Because of their salt-forming capability, these negatively charged functional groups would help to maintain the high water solubility of the resulting derivatives. In addition, when a CD is perfacially substituted, electrostatic repulsion between identically charged groups

Scheme 1. Synthesis of Per-6-thiolated Cyclodextrins



Scheme 2. Synthesis of Mono-6-thiolated γ-Cyclodextrins



would also keep the entrance to the cavity open instead of closed because of hydrophobic collapse of side chains.

The biggest challenge in chemical modification of CDs is the presence of a large number of similarly reactive hydroxyl groups.[14,15] The number of possible positional isomers increases rapidly with each further substituent. Isolation and purification of these positional isomers require laborious techniques such as dialysis and chromatography and very often can only be achieved on a small scale. In fact, many of the cyclodextrin derivatives described in the literature are complex mixtures of compounds with varying degrees of substitution.[14] To obtain uniform products, especially with the potential of large-scale manufacturing, it is only feasible to prepare mono- and per-substituted CD derivatives.

It should be mentioned that this synthetic feasibility of uniform CD derivatives had a significant impact on our design of target molecules. The CD derivatives described in this paper are all constructed via a thioether linkage. This is because thiols are much better nucleophiles than hydroxyls, and therefore side products from hydroxyl-participating reactions are minimized.[16,17]

As shown in Scheme 1, the per-6-thiolated CD derivatives were prepared either by reacting an appropriate thiol with the readily prepared per-6-bromo-α-, β-, or γ-CDs[18−20] (method A) or by alkylating the per-6-thiol-CDs with an appropriate alkyl halide (method B). The per-6-thiol-CDs were prepared according to a literature procedure[18] by reacting per-6-bromo-CD with thiourea, followed by ethanolamine. In certain cases where the substituent contains a terminal ester or amide function, the compound was further hydrolyzed under basic conditions to afford carboxylsodium salts. For mono-substituted CD derivatives, a simple nucleophilic substitution of mono-6-naphthalenesulfonyl-γ-CD[21] by an appropriate thiol was used for the preparation (Scheme 2).

Results and Discussion

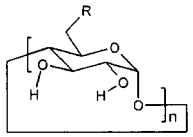
All compounds were screened for their activities to reverse rocuronium-induced neuromuscular block in an isolated mouse hemidiaphragm in vitro and in anaesthetized guinea pigs in vivo (Table 1). It should be mentioned that in separate in vitro experiments these compounds did not modify mouse vas deferens contractions induced by field stimulation, mouse hemidiaphragm contractions induced by phrenic nerve stimulation, or rat aortic ring contractions induced by (−)-noradrenaline, adrenaline, dopamine, and 5-HT. Pretreatment of mouse diaphragm with up to 10-fold the effective reversal concentration of the selected compounds did not show any change in force of contraction but gave 100% protection against 3.6 μM rocuronium, the concentration that produces a 90% block in the untreated mouse diaphragm. These data indicate that the CD derivatives do not possess any intrinsic biological activities that could interfere with their host−guest complexation induced reversal of rocuronium activity.

As shown in Table 1, the reversal potencies of these compounds are related to their cavity sizes. Among the compounds with comparable substituents, all α-CD derivatives (**4**−**7**) are less potent than β-CD derivatives (**8**−**11**), which in turn are less potent than γ-CD derivatives (**12**−**15**). This is most likely due to the smaller cavity sizes (diameter less than 6.5 Å) of α- and β-CDs, which cannot form stable complexes with the bulky steroid guest rocuronium (molecule width of ∼7.5 Å). In contrast, γ-CDs with a cavity diameter of 7.5−8.3 Å have greater structural complementarity with rocuronium and can therefore form much more tightly bound complexes with the aminosteroid.

Among the per-substituted γ-CD derivatives, the chain length of the substituents generally has little effect on the reversal potency, although a greatly extended spacer group between the CD backbone and terminal carboxyl group may decrease reversal potency, e.g., **17** and **23**. Incidentally, these two compounds were obtained in low purity (∼70%). Whether the lower potencies of these two compounds are due to their lower purity or more stable self-inclusion (hydrophobic collapse) of the more flexible side chains remains inconclusive.

Further branching of the linking unit between the CD backbone and terminal carboxyl caused reduction in

Table 1. Structures and Reversal Activities of Per-6-thiolated CDs against Rocuronium-Induced Neuromuscular Block



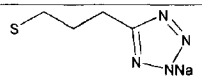
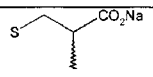
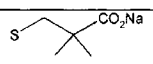
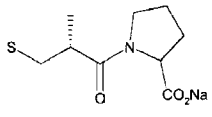
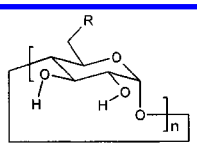
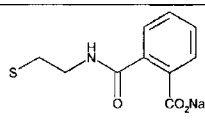
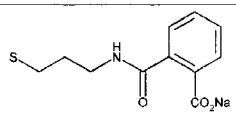
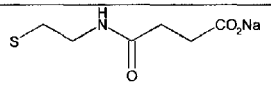
Compounds						In vitro reversal activity vs ~90% block by rocuronium [a] (isolated mouse hemi-diaphragm)		In vivo reversal activity vs ~90% block by rocuronium [b] (i.v., guinea-pigs)	
Compound No.	n	R	Prep. Method	MW	Purity [c]	EC_{50}, μM	max reversal, % (conc., μM)	ED_{50}, μmol/kg	max reversal, % (dose, μmol/kg)
4	6	OH	n.a. [d]	972.9	>98% [e]	> 360.0	9.7 ± 3.0 (360)	1575.0 ± 1025.0	6.4 ± 3.9 (1018)
5	6	SCH_2CO_2Na	A	1549.3	>99%	> 18.0	22.8 ± 13.0 (18)	> 21	3.6 (21)
6	6	$SCH_2CH_2CO_2Na$	A	1633.5	>70%	> 360.0	0.0 ± 0.0 (360)	> 16	3.2 (16)
7	6	$SCH_2CH_2CH_2CO_2Na$	A	1717.7	>92%	> 18.0	5.3 ± 3.4 (18)	> 16	3.4 (16)
8	7	OH	n.a. [d]	1135.1	>98% [e]	> 360.0	29.0 ± 15.4 (360)	20.0 ± 7.0	92.9 ± 10.3 (113)
9	7	SCH_2CO_2Na	A	1807.6	>88% [f]	6.5 ± 1.5	97.3 ± 16.2 (16.2)	0.93 ± 0.26	89.6 ± 12.8 (9.6)
10	7	$SCH_2CH_2CO_2Na$	A	1905.8	>90%	3.3 ± 0.7	100.1 ± 2.8 (9)	0.75 ± 0.35	81.3 ± 9.4 (2.6)
11	7	$SCH_2CH_2CH_2CO_2Na$	A	2004.0	>90%	5.0 ± 0.7	95.3 ± 5.2 (14.4)	0.49 ± 0.10	99.6 ± 0.1 (3.2)
12	8	OH	n.a.	1297.2	>98% [e]	34.6 ± 10.4	94.1 ± 2.0 (144)	4.0 ± 0.0	104.7 ± 8.6 (47)
13	8	SCH_2CO_2Na	A	2065.8	>97%	1.2 ± 0.2	93.8 ± 2.7 (3.6)	0.10 ± 0.05	103.3 ± 4.3 (0.5)
14	8	$SCH_2CH_2CO_2Na$	A	2000.0	>97%	1.2 ± 0.8	95.1 ± 2.3 (3.6)	0.03 ± 0.00	92.5 ± 5.3 (0.3)
15	8	$SCH_2CH_2CH_2CO_2Na$	A	2290.2	>97%	1.4 ± 0.0	98.5 ± 4.5 (3.6)	0.06 ± 0.01	93.4 ± 10.6 (0.3)
16	8	$SCH_2CH_2CH_2CH_2CO_2Na$	A	2402.5	>97%	1.8 ± 0.1	98.9 ± 5.2 (5.4)	0.07 ± 0.00	99.0 ± 3.5 (0.3)
17	8	$SCH_2CH_2CH_2CH_2CH_2CO_2Na$	A	2514.7	>70%	7.0 ± 0.4	81.7 ± 12.6 (12.6)	0.74 ± 0.10	78.4 ± 7.7 (2.5)
18	8	*ortho*-S-Ph-CO_2Na	A	2562.4	>90%	226.7 ± 65.4	42.3 ± 13.8 (216)	n.t. [g]	n.t. [g]
19	8	*meta*-S-Ph-CO_2Na	A	2562.4	>80%	3.3 ± 0.5	95.7 ± 2.3 (7.2)	0.28 ± 0.05	102.0 ± 5.7 (1.3)
20	8	*para*-S-Ph-CO_2Na	A	2562.4	>99%	1.0 ± 0.1	97.0 ± 4.2 (2.2)	0.12 ± 0.01	97.6 ± 6.3 (1.4)
21	8	*meta*-S-Ph-CH_2CO_2Na	A	2674.6	>99%	1.7 ± 0.6	99.1 ± 5.2 (7.2)	0.53 ± 0.10	104.6 ± 0.8 (2)
22	8	*para*-S-Ph-CH_2CO_2Na	A	2674.6	>99%	1.4 ± 0.1	98.9 ± 1.1 (2.9)	0.14 ± 0.04	103.8 ± 2.8 (1.6)
23	8	*meta*-S-Ph-$CH_2CH_2CO_2Na$	A	2786.8	>70%	14.8 ± 3.0	65.2 ± 12.0 (18)	22.77	35.3 (16)
24	8	*para*-S-Ph-$CH_2CH_2CO_2Na$	A	2786.8	>90%	3.0 ± 0.7	95.4 ± 5.2 (7.2)	1.01 ± 0.35	94.3 ± 7.8 (2.8)
25	8	SCH_2SO_3Na	B	2466.4	>87%	0.36 ± 0.00	101.4 ± 1.4 (7.2)	0.06 ± 0.01	106.5 ± 4.8 (1.7)
26	8	$SCH_2CH_2SO_3Na$	B	2578.7	>80%	3.6 ± 3.6	95.6 ± 2.0 (3.6)	0.05 ± 0.01	92.8 ± 8.5 (0.5)
27	8	$SCH_2CH_2CH_2SO_3Na$	B	2690.9	>85%	1.8 ± 1.8	99.3 ± 1.4 (3.6)	0.07 ± 0.01	98.6 ± 3.2 (0.2)
28	8		B	2482.5	>85%	0.6 ± 1.7	89.9 ± 2.1 (3.6)	0.22 ± 0.10	109.0 ± 1.2 (1.2)
29	8		A	2004.0	>90%	9.5 ± 1.9	77.5 ± 6.0 (18)	0.73	97.8 (8)
30	8		A	2402.5	>90%	20.3 ± 6.6	47.6 ± 15.3 (18)	1.32 ± 0.70	98.8 ± 1.2 (15)
31	8		A	3075.3	>99%	33.9 ± 15.5	35.7 ± 9.1 (18)	1.49	103.6 (10)

Table 1 (Continued)



Compounds						In vitro reversal activity vs ~90% block by rocuronium [a] (isolated mouse hemi-diaphragm)		In vivo reversal activity vs ~90% block by rocuronium [b] (i.v., guinea-pigs)	
Compound No.	n	R	Prep. Method	MW	Purity [c]	EC_{50}, μM	max reversal, % (conc., μM)	ED_{50}, μmol/kg	max reversal, % (dose, μmol/kg)
32	8		B	3131.0	>90%	0.1 ± 1.6	88.3 ± 2.9 (5.4)	0.09 ± 0.04	94.5 ± 10.3 (0.5)
33	8		B	3243.2	>75%	0.1 ± 3.0	90.7 ± 6.7 (7.2)	0.27 ± 0.08	83.0 ± 5.6 (1.6)
34	8		B	2744.6	>70%	1.08 ± 1.08	99.4 ± 1.4 (5.4)	0.07 ± 0.02	107.1 ± 8.9 (0.6)
35	8	SCH_2CH_2OH	A	1778.1	97.3% [h]	4.0 ± 0.5	86.4 ± 3.5 (7.2)	0.21 ± 0.06	95.8 ± 6.6 (2)
36	8	$SCH_2CH_2CH_2OH$	A	1890.3	>97%	4.1 ± 0.4	96.5 ± 3.4 (9)	0.52	98.3 (6.4)
37	8	$SCH_2CH_2CH_2CH_2OH$	A	2002.5	>98%	6.5 ± 0.9	78.9 ± 2.5 (18)	3.94 ± 1.47	52.8 ± 15.3 (3.9)
38	8	$SCH_2CH_2OCH_2CH_2OH$	A	2130.5	>96%	9.1 ± 4.5	66.1 ± 13.9 (16.2)	0.47 ± 0.03	92.2 ± 7.8 (2.1)
39	8	*para*-S-Ph-OH	A	2162.4	90%	4.9 ± 2.2	93.2 ± 6.3 (14.4)	4.1	79.3 (25.6)
40	8	$SCH_2C(O)NHCH_3$	A	1994.3	>95%	6.4 ± 0.8	79.6 ± 2.9 (14.4)	1.54 ± 0.97	102.2 ± 6.5 (7.3)
41	8	$SCH_2CH_2CH_2C(O)NH_2$	A	2105.5	>85%	5.0 ± 0.6	85.4 ± 4.9 (10.8)	0.58 ± 0.01	94.2 ± 11.6 (33.1)
Neostigmine bromide	--	--	--	--	--	0.9 ± 0.1	74.4 ± 9.5 [i]	0.04 ± 0.00	65.3 ± 5.3 (0.08)

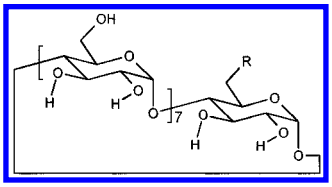
[a] Data are presented as means ± SEM of at least four independent experiments. The concentration of rocuronium bromide in the organ bath was 3.6 μM, which produced ~90% reduction of the twitch height. EC_{50} is the concentration that produces 50% recovery of muscle twitch compared with prereversal twitch height. Max reversal (%) is the maximum twitch recovery achieved, with highest concentration tested in parentheses. [b] Data are presented as means ± SEM of at least two independent experiments or a single experiment when the potency was of low interest. An average of 90% (80−97%) neuromuscular block was achieved by continuous iv infusion (~10 nmol kg^{-1} min^{-1}) of rocuronium bromide and applying cumulative doses of CD. ED_{50} is the dose (iv) that produces 50% recovery of muscle twitch compared with preblock twitch height. Max reversal (%) is the maximum twitch recovery achieved, with highest dose tested in parentheses. [c] Data are represented as the minimum purity determined by two diverse chromatography systems: HPLC-ELSD with a Phenomenex Aqua C18 (25 cm × 0.46 cm) column eluted with CH_3CN/H_2O 25/75 + 0.1% HCOOH and a time-of-flight (TOF) LC−MS with a Jupiter C18 (150 mm × 4.6 mm) eluted with CH_3CN/H_2O gradient + 0.1% HCOOH. Results from 400 MHz ^1H NMR spectroscopy were also used. [d] Not applicable. [e] Data were provided by the commercial supplier Wacker-Chemie GmbH and were not checked by the authors. [f] Reported previously in the literature.[19] [g] Not tested. [h] Reported previously in the literature.[20] [i] Cumulative administration of neostigmine bromide caused variable results. In some preparations neostigmine caused complete reversal, whereas in other preparations only limited reversal occurred.

reversal activity (**29**−**31**) probably due to increased steric hindrance preventing the entry of rocuronium into the cavity.

The importance of hydrophobic cavity area to complex formation is supported by the fact that all monosubstituted γ-CD derivatives (Table 2) are less potent than their corresponding per-substituted analogues (**42**−**44** vs **13**−**15** and **45**−**51** vs **18**−**24**).

The most interesting structure−activity relationship within this series concerns the roles of negatively charged carboxyls at the rim of the CD cavity. First of all, they contribute to the reversal activity by forming electrostatic interaction with the quaternary nitrogen of rocuronium. This is evidenced by the fact that the negatively charged γ-CD derivatives **13**−**15** are all more potent than their corresponding neutral hydroxyl analogues **35**−**37**. Isothermal titration calorimetry (ITC) studies of **14** and **36**, both of which have a cavity depth extended by three carbon atoms from the primary face, showed that **14** ($K_a = 1.8 \times 10^7$ M^{-1}, $\Delta H = -28.9$ kJ mol^{-1}, $\Delta S = 42.8$ J mol^{-1} K^{-1}) forms a more stable complex with rocuronium than **36** ($K_a = 2.0 \times 10^5$ M^{-1}, $\Delta H = -16.2$ kJ mol^{-1}, $\Delta S = 47.6$ J mol^{-1} K^{-1}). Comparison of these thermodynamic data seems to suggest that the carboxyls of **14** contribute approximately −12.7 kJ mol^{-1} toward the enthalpy gain. Second, the sodium salts of these carboxylic derivatives are more water-soluble than their neutral hydroxyl

Table 2. Structures and Reversal Activities of Mono-6-thiolated CDs against Rocuronium-Induced Neuromuscular Block



	compounds			in vitro reversal activity vs ~90% block by rocuronium[a] (isolated mouse hemidiaphragm)		in vivo reversal activity vs ~90% block by rocuronium[b] (iv, guinea pigs)	
ORG code	R	MW	purity,[c] %	EC_{50}, μM	max reversal, % (concentrated, μM)	ED_{50}, μmol/kg	max reversal, % (dose, μmol/kg)
42	SCH_2CO_2Na	1393.2	>70	60.8 ± 27.1	28.9 ± 9.2 (18)	5.79 ± 0.73	96.5 ± 5.7 (16)
43	$SCH_2CH_2CO_2Na$	1407.3	95−97	17.2 ± 28.5	94.5 ± 4.8 (144)	1.30 ± 0.04	100.6 ± 0.6 (12)
44	$SCH_2CH_2CH_2CO_2Na$	1421.3	75	>18.0	14.4 ± 10.2 (18)	*d*	*d*
45	ortho-S−Ph−CO_2Na	1455.3	85	97.6 ± 14.1	86.3 ± 2.0 (252)	1.3 ± 0.8	93.0 ± 10.6 (11)
46	meta-S−Ph−CO_2Na	1455.3	>88	>18.0	36.1 ± 12.9 (18)	2.41	102.7 (16)
47	para-S−Ph−CO_2Na	1455.3	70−80	50.3 ± 6.6	94.7 ± 3.8 (180)	0.94	102.4 (8)
48	meta-S−Ph−CH_2CO_2Na	1469.3	>85	>18.0	30.4 ± 18.6 (18)	1.67 ± 0.58	92.8 ± 4.1 (16)
49	para-S−Ph−CH_2CO_2Na	1469.3	94	34.7 ± 14.6	45.0 ± 17.7 (18)	4.55 ± 0.60	97.1 ± 8.8 (30.7)
50	meta-S−Ph−$CH_2CH_2CO_2Na$	1483.4	>85	>18.0	3.9 ± 2.6 (18)	12.64	55.8 (15)
51	para-S−Ph−$CH_2CH_2CO_2Na$	1483.4	93	32.8 ± 12.2	36.2 ± 15.1 (18)	3.33 ± 0.50	93.8 ± 3.3 (31.8)

[a] Data are presented as means ± SEM of at least four independent experiments. The concentration of rocuronium bromide in the organ bath was 3.6 μM, which produced ~90% reduction of the twitch height. EC_{50} is the concentration that produces 50% recovery of muscle twitch compared with prereversal twitch height. Max reversal (%) is the maximum twitch recovery achieved, with highest concentration tested in parentheses. [b] Data are presented as means ± SEM of at least two independent experiments or a single experiment when the potency was of low interest. An average of 90% (80−97%) neuromuscular block was achieved by continuous iv infusion (~10 nmol kg^{-1} min^{-1}) of rocuronium bromide and applying cumulative doses of CD. ED_{50} is the dose (iv) that produces 50% recovery of muscle twitch compared with preblock twitch height. Max reversal (%) is the maximum twitch recovery achieved, with highest dose tested in parentheses. [c] Data are represented as the minimum purity determined by two diverse chromatography systems: HPLC-ELSD with a Phenomenex Aqua C18 (25 cm × 0.46 cm) column eluted with CH_3CN/H_2O 25/75 + 0.1% HCOOH and a time-of-flight (TOF) LC−MS with a Jupiter C18 (150 mm × 4.6 mm) eluted with CH_3CN/H_2O gradient + 0.1% HCOOH. Results from 400 MHz ^1H NMR spectroscopy were also used. [d] Not tested.

analogues (data not shown). Third, the electrostatic repulsion between these negatively charged groups keeps the entrance to the cavity open. This is most convincingly demonstrated by the X-ray crystal structures of **14** and **35** (Figure 2). In the X-ray crystal structure of **14**, there is only one side chain that is partially self-included in the cavity and the entrance to the cavity is largely open because of electrostatic repulsion between the negatively charged carboxyls. In the X-ray crystal structure of **35**, because of the absence of electrostatic repulsion between hydroxyls, there are at least three side chains that are self-included in the cavity, blocking completely the entrance to the cavity. The shape of molecule **35** looks more like a bowl rather than a torus. This may at least partially explain why the chain length between the CD backbone and the terminal carboxyl had little effect on the reversal activities of **13**−**16** (see above), because the identical charges of the carboxyls keep these groups away from each other and thus do not set an extra energy barrier to adopt a conformation (open cavity entrance) for rocuronium encapsulation. Theoretically there should be a limit to the length of such a flexible and hydrophobic side chain before self-inclusion restricts cavity entrance significantly. This is visible among the neutral compounds **35**−**38**; i.e., side chains longer than three methylene groups caused reduction in reversal activity. This is in agreement with the depth of the hydrophobic cavity in the X-ray crystal structure of **14**, i.e., ~11 Å, similar to the approximate distance between rocuronium the A-ring C-3 and the D-ring C-16. In other words, an extended γ-CD cavity by three carbon atoms would allow full encapsulation of all four hydrophobic steroidal rings of rocuronium.

Not surprisingly other acidic functional groups, e.g., sulfonic acid (**25**−**27**), tetrazole (**28**), etc., have similar effects as carboxyls.

The in vivo efficacy of these CD derivatives is mostly in agreement with their in vitro potency; i.e., the potency rank order stays the same in vivo as in vitro among the series (Tables 1 and 2). However, most of the compounds appeared more potent in the in vivo assay than in the in vitro assay (i.e., $ED_{50} < EC_{50}$). This is most likely due to the increased renal clearance of rocuronium by these CD derivatives. For example, the urine level of rocuronium in guinea pigs within the first hour of intravenous administration of **14** increased by several-hundred-fold compared with that of saline control. The diuretic effects of cyclodextrins have been reported in the literature.[22]

To investigate the cardiovascular effects of these host molecules in comparison with the standard AChE inhibitor neostigmine, we tested one of the most potent compounds in this series, compound **14** (coded Org 25969), in anaesthetized cats. As shown in Figure 3, administration of **14** (1.0 mg/kg or 0.46 μmol/kg, iv) rapidly restored the muscle contraction, taking only about 1.5 min to achieve full recovery while the spontaneous recovery required about 7 min.

Cumulative dosing of **14** up to 1.8 μmol/kg (almost 4 times the effective reversal dose) did not produce significant changes in all hemodynamic parameters measured, e.g., mean arterial blood pressure (BP), heart rate (HR), left ventricular pressure (LVP), left ventricular contractility (LVdP/dt), bradycardia induced by vagal stimulation, and nictitating membrane contractions induced by sympathetic stimulation (Table 3). In con-

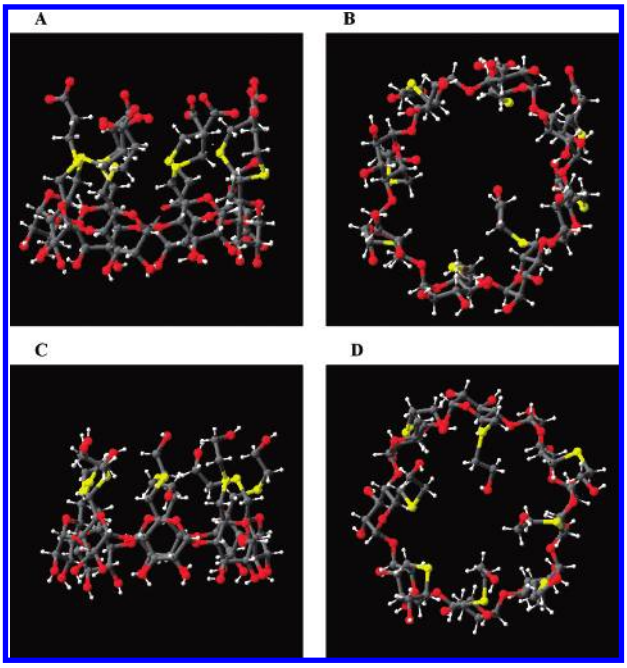


Figure 2. X-ray crystallography of **14** (A, B) and **35** (C, D). (A) Side view of the crystal structure of **14**. The per-6-mercaptopropionic acid side chains form an extension of the γ-CD cavity. The depth of the cavity is ~11 Å as estimated from the average distance between the carboxyl O atoms and the hydroxyl O atoms at the secondary face (9.8−11.4 Å). (B) Perspective view from the carboxyl side of **14**. One side chain is self-included in the cavity, rendering the ring somewhat puckered. The width of the cavity thus ranges from 5.6 Å (carboxyl face) to 11 Å (second OH face). (C) Side view of the crystal structure of **35**. The per-6-mercaptoethanol side chains form the edges of the upside down "bowl". The depth of the cavity is about 9.5 Å. (D) Perspective view from the primary OH face (bottom of the "bowl") of **35**. Three side chains are self-included in the cavity, restricting the entrance of the cavity.

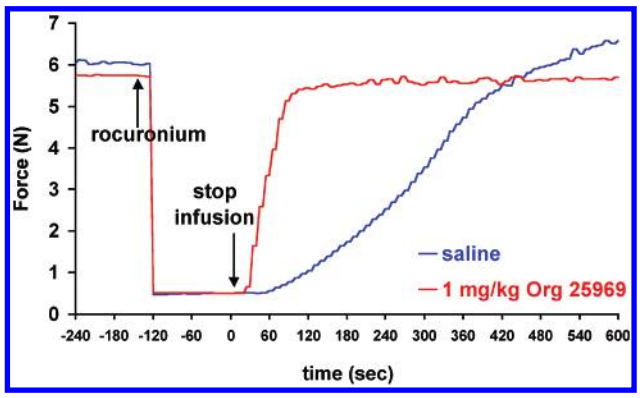


Figure 3. Reversal of rocuronium-induced neuromuscular block in anaesthetized cats by **14**. Continuous infusion of rocuronium bromide (10.24 ± 1.31 nmol kg^{-1} min^{-1}) resulted in ~95% block of *M. tibialis* contractions. Immediately after switching off the infusion, a single dose of 1.0 mg/kg (or 0.46 μmol/kg) **14** or saline was injected intravenously. The administration of **14** caused rapid restoration of the contraction, reaching the near-complete recovery (>90%) within 1.5 min. The control saline administration needed 7−8 min to reach the same level of recovery. The graph represents the average of three independent experiments.

trast, the clinical standard combination of neostigmine and atropine exerted a profound effect on vagal stimulation.

Conclusions

In conclusion we have designed and synthesized a series of cyclodextrin derivatives that reverse the neuromuscular blockade induced by rocuronium bromide in vitro and in vivo. To achieve high potency, the CD host needs to have a large enough lipophilic cavity capable of full encapsulation of all four steroidal rings of rocuronium. We achieved this by perfacial substitution on the primary face of γ-CD and demonstrated that an extension of the cavity depth by at least 2−3 carbon atoms increased the reversal potency by 30 (in vitro) to >100-fold (in vivo). The negatively charged groups at the rim of the cavity are important for high reversal potency because they contribute to the complexation with rocuronium by electrostatic interaction with its positively charged nitrogen, maintain the preorganization of the cavity by mutual electrostatic repulsion, and provide salt-forming capability for high water solubility. The full characterization of the rocuronium complex with one of the CDs within the current series, compound **14**, has been reported elsewhere.[23]

Because their supramolecular mechanism of action does not involve direct interaction with the cholinergic system, these host molecules appear to be superior to currently clinically used reversal agents. The reversal produced is not only highly efficacious but also without significant cardiovascular side effects, as demonstrated herein with the example of **14** in anaesthetized cats. This cyclodextrin derivative **14** (coded Org 25969) is now under clinical development and in our opinion serves as a good example of how host molecules can be designed as medicinal agents.

Experimental Section

All cyclodextrin starting materials, α-, β-, and γ-CDs, were purchased from Wacker-Chemie GmbH (Munich, Germany), and they were of the pharmaceutical grade with >98% purity. All other chemical reagents were obtained from commercial suppliers and used without further purification. All NMR spectra were recorded at 200 or 400 MHz on Bruker AM200 or 400 spectrometers, and chemical shifts are reported in ppm relative to TMS or sodium 3-trimethylsilylpropionate. The purity of all compounds was determined by at least two diverse chromatography systems: HPLC-ELSD with a Phenomenex Aqua C18 (25 cm × 0.46 cm) column eluted with CH_3CN/H_2O (25/75) + 0.1% HCOOH; a time of flight (TOF) LC−MS using a PerSeptive Biosystems Mariner TOF instrument with a Jupiter C18 (150 cm × 4.6 mm) eluted with CH_3CN/H_2O gradient + 0.1% HCOOH.

Method A. Example 1. 6-Perdeoxy-6-per(2-carboxyethyl)thio-γ-cyclodextrin Sodium Salt (14). Anhydrous cesium carbonate (16.3 g, 50 mmol) was added to a stirred solution of 6-perdeoxy-6-perbromo-γ-cyclodextrin[18] (9.0 g, 5 mmol) and methyl 3-mercaptopropionate (6.65 mL, 60 mmol) in DMF at room temperature under nitrogen. The temperature was raised to 50 °C, and the system was left stirring under nitrogen for 25 h. After this time the slurry was added directly to water (800 mL). The precipitate was collected by filtration and washed with water. This gave 6-perdeoxy-6-per(2-methoxycarbonylethyl)thio-γ-cyclodextrin as a white powder (5.78 g, 60%).

Sodium hydroxide solution (10 mL, 1 M) was added to the methyl ester intermediate (2.11 g, 1.08 mmol), and the system was stirred at room temperature (with flask stoppered under nitrogen) for 20 h. The solution was placed directly into dialysis tubing and dialyzed for 6 h (the external water being changed every 2 h). The contents of the dialysis tubing was then poured into a flask, and the water evaporated under reduced pressure. This gave the title compound as a glassy solid (1.87 g, 86%).

Table 3. Cardiovascular Effects of **14** and the Standard Combination of Neostigmine Plus Atropine in Anaesthetized Cats

	maximum changes[a] (%)			
drug dose	BP (mmHg)	HR (bpm)	increase in contractions nictitating membrane	decrease in HR (bpm) by vagal stimulation
saline	+1.9	−5.9	0	+3.8[b]
14 (0.2 μmol/kg)	−7.3	−4.1	0	−2.9
14 (0.4 μmol/kg)	−6.5	−5.5	0	−6.7
14 (0.6 μmol/kg)	+3.5	−7.5	0	−6.7
14 (0.8 μmol/kg)	−3.3	−6.3	+3.7	+2.2
14 (1.0 μmol/kg)	−3.2	−3.4	+8.7	0
14 (1.2 μmol/kg)	−3.3	−4.0	+3.7	0
14 (1.4 μmol/kg)	−3.3	−3.7	+3.7	+0.7
14 (1.6 μmol/kg)	−5.0	−6.9	+3.7	0
14 (1.8 μmol/kg)	−5.0	−14.3	+12.5	+11.0
neostigmin + atropine (24.3 + 15.0 μg/kg)	?[c]	−7.1	−7.2	−64.8[d]

[a] Data are means of three independent experiments. Increasing doses of **14** were administered by bolus injections. BP = mean arterial blood pressure; HR = heart rate. [b] Data vary from −25.7 to +37. [c] Data vary from −17.7 to +17.7. [d] Data vary from −51.4 to −78.8.

^{1}H NMR (D_2O) δ 5.19 (8H, d, J = 3.7 Hz), 4.07 (8H, m), 3.97 (8H, t, J = 9.5 Hz), 3.73−3.61 (16H, m), 3.15 (8H, d, J = 12.8 Hz), 3.01 (8H, dd, J = 12.3 and 5.0 Hz), 2.88 (16H, t, J = 7.2), 2.58−2.43 (16H, m); LC−MS m/z 2000.5 $(M - 8Na + 7H)^-$.

Example 2. 6-Perdeoxy-6-per(4-carboxyphenyl)thio-γ-cyclodextrin Sodium Salt (20). To a solution of 4-mercaptobenzoic acid (0.86 g) in DMF (30 mL) was added 60% sodium hydride dispersed in oil (0.37 g) portionwise over 30 min. The mixture was cooled, and 6-perdeoxy-6-perbromo-γ-cyclodextrin[18] (1.0 g) was added in one portion. The mixture was then heated to 70 °C and stirred at this temperature for 24 h. After cooling to room temperature, the mixture was quenched with the addition of water (20 mL) before evaporating to a low volume. The solution was poured into ethanol (250 mL), and the precipitate was collected by filtration, dissolved in water (20 mL), and dialyzed (MWCO 1000) by changing the external water four times. The internal solution was evaporated to low volume and poured into acetone (250 mL). The solid precipitate was collected by filtration and dried under vacuum at 70 °C to afford the title compound (1.2 g) as a white solid. ^{1}H NMR at 353 K (D_2O) δ 8.23 (16H, d, J = 8.3 Hz), 7.77 (16H, d, J = 8.1 Hz), 5.65 (8H, s), 4.55−4.47 (16H, m), 4.06−4.01 (24H, m), 3.72−3.70 (8H, m); LC−MS m/z 2385 $(M - 8Na + 7H)^-$.

The following compounds were synthesized in a similar way as described above for the two examples.

5: ^{1}H NMR (D_2O) δ 5.11 (6H, d, J = 3.2 Hz), 3.96−3.92 (12H, m), 3.65−3.56 (12H, m), 3.13 (6H, d, J = 12.1 Hz), 2.96−2.94 (6H, m), 2.81 (12H, t, J = 7.4 Hz), 2.45 (12H, t, J = 7.4 Hz); LC−MS m/z 1584 $(M - 6Na + 5H)^-$.

6: ^{1}H NMR (D_2O) δ 5.07 (6H, d, J = 3.2 Hz), 3.98−3.91 (12H, m), 3.67 (6H, t, J = 9.2 Hz), 3.58 (6H, dd, J = 10.4 and 3.6 Hz), 3.32 (12H, d, J = 8.0 Hz), 3.13 (6H, d, J = 12.4 Hz), 2.95−2.89 (6H, m); LC−MS m/z 1498.5 $(M - 6Na + 3H)^-$.

7: ^{1}H NMR (D_2O) δ 5.11 (6H, br s), 4.18−4.08 (6H, m), 3.97 (6H, t, J = 9.3 Hz), 3.60 (12H, dd, J = 10.0 and 3.6 Hz), 3.17 (6H, d, J = 13.3 Hz), 3.00−2.90 (6H, m), 2.69−2.65 (12H, m), 2.28−2.25 (12H, m), 1.89−1.84 (12 H, m); LC−MS m/z 1584 $(M - 6Na + 5H)^-$.

9: ^{1}H NMR (D_2O) δ 4.92 (7H, s), 3.88−3.85 (7H, m), 3.70 (7H, t, J = 9.4 Hz), 3.49 (7H, t, J = 9.4 Hz), 3.42 (7H, dd, J = 9.8 and 4.0 Hz), 3.16 (14H, d, J = 9.0 Hz), 2.95 (7H, d, J = 12 Hz), 2.79−2.72 (7H, m); LC−MS m/z 1650.4 $(M - 7Na + 4H)^-$.

10: ^{1}H NMR (D_2O) δ 5.05 (7H, d, J = 3.6 Hz), 3.91−3.82 (14H, m), 3.55−3.48 (14H, m), 3.02 (7H, d, J = 12.0 Hz), 2.89−2.87 (7H, m), 2.73 (14H, t, J = 7.2 Hz), 2.36 (14H, t, J = 7.2 Hz); LC−MS m/z 1750 $(M - 7Na + 6H)^-$.

11: ^{1}H NMR (D_2O) δ 5.06 (7H, d, J = 3.6 Hz), 3.88−3.81 (14H, m), 3.52−3.46 (14H, m), 3.05 (7H, d, J = 11.7 Hz), 2.89−2.87 (7H, m), 2.60−2.56 (14H, m), 2.17 (14H, t, J = 7.4 Hz), 1.77−1.73 (14H, m); LC−MS m/z 1848.7 $(M - 7Na + 6H)^-$.

15: ^{1}H NMR (D_2O) δ 5.17 (8H, d, J = 3.7 Hz), 3.98−3.89 (16H, m), 3.58−3.54 (16H, m), 3.10 (8H, d, J = 11.5 Hz), 2.96−2.93 (8H, m), 2.65 (16H, t, J = 7.4 Hz), 2.23 (16H, t, J = 7.9 Hz), 1.84−1.80 (16H, m); LC−MS m/z 2113 $(M - 8Na + 7H)^-$.

16: ^{1}H NMR (D_2O) δ 5.22 (8H, d, J = 3.6 Hz), 3.98−3.91 (16H, m), 3.62−3.57 (16H, m), 3.15 (8H, d, J = 12.0 Hz), 2.99−2.92 (8H, m), 2.70 (16H, br s), 2.20 (16H, br s), 1.63 (32H, br s); LC−MS m/z 2225 $(M - 8Na + 7H)^-$.

17: ^{1}H NMR (D_2O) δ 5.17 (8H, s), 3.93−3.87 (16H, m), 3.62−3.52 (16H, m), 3.20 (8H, d), 2.99−2.84 (8H, m), 2.70−2.66 (16 H, m), 2.21−2.17 (16H, m), 1.64−1.57 (32H, m), 1.40 (16H, m); LC−MS m/z 2362 $(M - 7Na + 8H)^-$.

18: ^{1}H NMR (D_2O) δ 7.52 (8H, d, J = 7.2 Hz), 7.14−7.10 (25 H, m), 5.13 (8H, s), 4.14−4.12 (8H, m), 4.03 (8H, t, J = 9.5 Hz), 3.70 (8H, t, J = 9.3 Hz), 3.54 (8H, dd, J = 3.3 and 9.8 Hz), 3.10 (16 H, s); LC−MS m/z 2383.8 $(M - 8Na + 5H)^-$.

19: ^{1}H NMR (D_2O) δ 7.76 (8H, s), 7.55 (8 H, d, J = 6.0 Hz), 7.11−7.09 (16 H, m), 5.16 (8H, s), 4.00−3.93 (16H, m), 3.58−3.53 (16H, m), 3.42 (8H, d, J = 12 Hz), 3.24−3.20 (8H, m); LC−MS m/z 2382.3 $(M - 8Na + 7H)^-$.

21: ^{1}H NMR at 323 K (D_2O) δ 7.15−6.97 (32H, m), 5.13 (8H, d, J = 3.0 Hz), 3.96−3.88 (16H, m), 3.61−3.54 (16H, m), 3.40−3.22 (32H, m); LC−MS m/z 2497.7 $(M - 8Na + 6H)^-$.

22: ^{1}H NMR at 343 K (D_2O) δ 7.16 (16H, d, J = 7.9 Hz), 7.01 (16H, d, J = 7.9 Hz), 5.00 (8H, d, J = 3.5 Hz), 3.86−3.78 (16H, m), 3.52−3.48 (16H, m), 3.32−3.29 (24H, m), 3.19−12 (8H, m); LC−MS m/z 1249 $(M - 8Na + 5H)^{2-}$.

23: ^{1}H NMR at 353 K (D_2O) δ 7.11−6.97 (32H, m), 5.21−5.02 (8H, m), 3.97−3.89 (16H, m), 3.64−3.55 (24H, m), 3.35−3.12 (8H, m), 2.81−2.69 (16H, m), 2.45−2.30 (16H, m); LC−MS m/z 2609 $(M - 8Na + 7H)^-$.

24: ^{1}H NMR at 343 K (D_2O) δ 7.21 (16H, d, J = 7.4 Hz), 7.07 (16H, d, J = 7.6 Hz), 5.07 (8H, s), 3.98−3.82 (16H, m), 3.62−3.56 (16H, m), 3.39−3.18 (16H, m), 2.73 (16H, t, J = 8.0 Hz), 2.35 (16H, t, J = 7.7 Hz); LC−MS m/z 2607 $(M - 8Na + 4H)^-$.

29: ^{1}H NMR (D_2O) δ 5.10−5.05 (7H, m), 3.92−3.80 (14H, m), 3.57−3.46 (14H, m), 3.03−2.96 (7H, m), 2.90−2.72 (14H, m), 2.52−2.38 (14H, m), 1.09−1.05 (21H, m); LC−MS m/z 1848 $(M - 5H - 7Na)^-$.

30: ^{1}H NMR (D_2O) δ 5.29 (8H, s), 3.99 (16H, t, J = 12 Hz), 3.64 (8H, t, J = 8 Hz), 3.58 (8H, dd, J = 8.0 and 3.0 Hz), 3.12 (8H, d, J = 16 Hz), 3.01−2.95 (8H, m), 2.90−2.80 (16H, m), 1.19 (48H, s); LC−MS m/z 1472 $(M - 8Na)^-$.

31: ^{1}H NMR at 353 K (D_2O) δ 5.65 (8H, br s), 4.79−4.73 (8H, m), 4.38−4.38 (16H, m), 4.21−4.09 (40H, m), 3.69−3.62 (8H, m), 3.46−3.44 (16H, m), 3.19−3.12 (8H, m), 2.76−2.62 (8H, m), 2.50−2.46 (24H, m), 1.72−1.69 (24H, m); LC−MS m/z 2890.8 $(M - H - 8Na)^-$.

35: ^{1}H NMR at 303 K (D_2O/DMSO-d_6) δ 5.09 (8H, d, J = 3.8 Hz), 3.90 (8H, t, J = 8.0 Hz), 3.82 (8H, t, J = 8.0 Hz), 3.72 (16H, t, J = 6.5 Hz), 3.61 (8H, dd, J = 12.0 and 4.0 Hz), 3.51 (8H, t, J = 8.0 Hz), 3.22 (8H, d, J = 12.5 Hz), 2.97−2.89 (8H, m), 2.81 (16H, t, J = 6.5 Hz); LC−MS m/z 1775.4 $(M - H)^-$.

36: ^{1}H NMR (DMSO-d_6) δ 4.90 (8H, s), 3.79−3.63 (8H, m), 3.58 (8H, t, J = 8.2 Hz), 3.45 (16H, t, J = 6.3 Hz), 3.34−3.21 (16H, m), 3.03 (8H, d, J = 12.2 Hz), 2.85−2.78 (8H, m), 2.60 (16H, t, J = 7.2), 1.68−1.64 (16H,m); LC−MS m/z 1889 $(M - H)^-$.

37: ^{1}H NMR (DMSO-d_6) δ 6.00 (16H, br s), 4.97 (8H, d, J = 3.48 Hz), 4.41 (8H, br s), 3.82−3.81 (8H, m), 3.61 (8H, t, J = 9.3 Hz), 3.45−3.33 (24H, m), 3.07 (8H, d, J = 12.1 Hz), 2.90−

2.80 (8H, m), 2.63 (16H, t, J = 13.8 Hz), 1.60−1.52 (40H, m); LC−MS m/z 2000 $(M - H)^-$.

38: ^{1}H NMR (D_2O) δ 5.32 (8H, s), 4.11−4.01 (16H, m), 3.93−3.88 (40H, m), 3.85−3.79 (8H, m), 3.72 (8H, m), 3.45 (8H, d), 3.12−3.07 (32H, m); LC−MS m/z 2175 $(M - H)^-$.

39: ^{1}H NMR at 333 K (D_2O) δ 7.01 (16H, d, J = 8.5 Hz), 6.48 (16H, d, J = 8.5 Hz), 4.86 (8H, s), 3.79−3.72 (8H, m), 3.57 (8H, t, J = 7.5 Hz), 3.34 (8H, t, J = 7.6 Hz), 3.25 (8H, d, J = 8 Hz), 3.07 (16H, s); LC−MS m/z 2159.9 $(M - H)^-$, 1018 $(M - 2H)^-$.

40: ^{1}H NMR (D_2O) δ 5.12 (8H, s), 3.98−3.82 (16H, m), 3.69−3.52 (16H, m), 3.42−3.32 (16H, m), 3.20 (8H, d, J = 12.2 Hz), 2.95−2.78 (8H, m), 2.78 (24H, s); LC−MS m/z 1991.7 $(M - H)^-$.

41: ^{1}H NMR (D_2O/DMSO) δ 4.92 (8H, m), 3.80−3.71 (8H, m), 3.61 (8H, t, J = 10.0 Hz), 3.41−3.31 (16H, m), 3.03 (8H, d, J = 12.1 Hz), 2.78−2.88 (8H, m), 2.57 (16H, t, J = 8.0 Hz), 2.18 (16H, t, J = 8.2 Hz), 1.79−1.71 (16H, m); LC−MS m/z 2105 $(M - H)^-$.

Method B. 6-Perdeoxy-6-per(2-sulfoethyl)thio-γ-cyclodextrin Sodium Salt (26). A mixture of 6-perdeoxy-6-perbromo-γ-cyclodextrin[18] (20.0 g) and thiourea (13.5 g) in DMF (100 mL) was stirred at 65 °C for 3 days, and then ethanolamine (20 mL) was added. After addition, the stirring was continued for 2 h. The mixture was cooled to room temperature and diluted with ice−water. The product was separated by centrifugation. The solid was washed twice with water and dried in a vacuum at 65 °C, giving the 6-perdeoxy-6-perthiol-γ-cyclodextrin (7.3 g). ^{1}H NMR (DMSO-d_6) δ 2.82 (m, 8H), 3.20 (d, 8H), 3.35 (m, 16H), 6.65 (t, 8H), 7.75 (t, 8H), 5.0 (s, 8H). LC−MS m/z 1424 (M − H).

A mixture of γ-CD-per-6-thiol (1.0 g), 2-bromoethane sulfonic acid sodium salt (1.4 g), and cesium carbonate (2.2 g) in DMF (10 mL) was stirred at 64 °C overnight. Most of the solvent was evaporated under vacuum, and the residue was dissolved in water. Sodium bicarbonate solution (5% w/w, 5 mL) was added, and the solution was dialyzed three times with water. The internal solution was evaporated to dryness, and the residue was dissolved in sodium bicarbonate solution (10 mL), dialyzed, and evaporated as before. This process was repeated once, and the resulting solid was dissolved in a small volume of water and precipitated with methanol. This solid precipitate was collected to give the title compound as a white solid (1.2 g). ^{1}H NMR (D_2O) δ 5.19 (8H, d, J = 4.0 Hz), 4.02−3.92 (16H, m), 3.64−3.56 (16H, m), 3.18 (8H, d, J = 12.0 Hz), 3.03−2.98 (24H, m), 2.82 (16H, t, J = 8.0 Hz), 2.08−2.05 (16H, m) ppm.

The following compounds were synthesized in a similar way as described above.

25: ^{1}H NMR (D_2O) δ 5.16 (8H, d, J = 3.8 Hz), 4.08−4.02 (8H, m), 3.89 (8H, t, J = 9.5 Hz), 3.63−3.59 (16H, m), 3.21−3.15 (24H, m), 3.00−2.94 (24H, m).

27: ^{1}H NMR (D_2O) δ 5.19 (8H, d, J = 3.7 Hz), 3.97−3.90 (16H, m), 3.62−3.55 (16H, m), 3.16 (8H, d, J = 11.7 Hz), 2.94−2.89 (24H, m), 2.74−2.69 (16H, m), 1.86−1.74 (32H, m); LC−MS m/z 1255 $(M - 8Na + 6H)^{2-}$.

28: ^{1}H NMR (D_2O) δ 5.06 (8H, d, J = 3.6 Hz), 3.92−3.85 (16H, m), 3.58 (8H, dd, J = 10.0 and 3.6 Hz), 3.51 (8H, t, J = 5.6 Hz), 3.04 (8H, d, J = 12.0 Hz), 2.89−2.84 (24H, m), 2.58 (16H, t, J = 6.8 Hz), 1.96 (16H, t, J = 7.2 Hz); LC−MS m/z 1153 $(M - 4H - 8Na)^{2-}$.

Method B, Including an Extra Step of Hydrolysis. 6-Perdeoxy-6-per[[2-(2-carboxybenzoyl)amino]ethyl]thio-γ-cyclodextrin Sodium Salt (32). 6-Perdeoxy-6-perthiol-γ-cyclodextrin[18] (1.0 g, 0.7 mmol) (see above) was dissolved in DMF (10 mL), and the solution was stirred at room temperature under a nitrogen atmosphere. To this solution was added *N*-(2-bromoethyl)phthalimide (1.6 g, 6.2 mmol) and cesium carbonate (2.0 g, 6.2 mmol). The resultant suspension was stirred at 60 °C overnight under a nitrogen atmosphere. After the mixture was cooled to room temperature, the DMF was removed under reduced pressure and to the residue was added water (100 mL) with vigorous stirring. The precipitate was collected by filtration, washed with water (3 times), and dried in a vacuum to yield 1.7 g of a cream-colored solid. This phthalimide intermediate (0.6 g) was then dissolved in a sodium hydroxide solution (1 M, 20 mL) and stirred at room temperature overnight under a nitrogen atmosphere. The solution was then dialyzed with deionized water until a constant pH was attained, and it was evaporated to dryness under reduced pressure to yield the desired product as a glassy solid (0.5 g). ^{1}H NMR (D_2O) δ 7.59 (8H, d, J = 8.0 Hz), 7.50−7.39 (24H, m), 5.02 (8H, d, J = 3.6 Hz), 3.88 (16H, t, J = 9.3 Hz), 3.60−3.42 (32H, m), 3.21 (8H, d, J = 12.1 Hz), 2.90−2.82 (24H, m); LC−MS m/z 1472 $(M - 5H - 8Na)^{2-}$.

The following compounds were synthesized in a similar way as described above.

33: ^{1}H NMR (D_2O) δ 7.59 (8H, d, J = 8.0 Hz), 7.51−7.35 (24H, m), 5.04 (8H, s), 3.98−3.83 (16H, m), 3.56−3.38 (32H, m), 3.18 (8H, d, J = 12.0 Hz), 2.92−2.84 (8H, m), 2.78−2.69 (16H, m), 1.91−1.82 (16H, m); LC−MS m/z 1532 $(M - 4H - 8Na)^{2-}$.

34: ^{1}H NMR (D_2O) δ 5.16 (8H, d, J = 4.0 Hz), 4.98−3.87 (16H, m), 3.63 (8H, dd, J = 8.0 and 3.5 Hz), 3.55 (8H, t, J = 8.0 Hz), 3.46−3.38 (16H, m), 3.21 (8H, d, J = 12.2 Hz), 3.97−3.91 (8H, m), 2.82−2.80 (16H, m), 2.48 (32H,br s); LC−MS m/z 1285 $(M + 5H - 8Na)^2$.

6-Monosubstituted γ-CDs. 6-Monodeoxy-6-mono(4-carboxyphenyl)thio-γ-cyclodextrin Sodium Salt (47). γ-Cyclodextrin (2.0 g, 1.5 mmol, dried at 110 °C overnight) was added to pyridine (120 mL) under nitrogen. After dissolution, 2-naphthalenesulfonyl chloride (1.1 g, 4.6 mmol) in pyridine (20 mL) was added and the mixture was stirred at room temperature for 24 h. After the reaction was quenched with water (50 mL), the solution was evaporated to dryness to give crude 6-mono-*O*-(2′-naphthalenesulfonyl)-γ-cyclodextrin.[21]

To a suspension of sodium hydride (0.38 g, 15.8 mmol) in dry DMF (20 mL) was added 4-mercaptobenzoic acid (0.7 g, 4.6 mmol), and the resulting mixture was stirred for 20 min. 6-Mono-*O*-(2′-naphthalenesulfonyl)-γ-cyclodextrin (3.2 g, 2.12 mmol) was then added to the mixture, and the reaction mixture was heated to 100 °C and stirred at this temperature for 90 min. After the mixture was cooled to room temperature, acetone was added to the solution and the precipitate was collected by filtration. The solid was reprecipitated from water/acetone. This was then redissolved in water (20 mL). The pH of the solution was adjusted to 7.0 by adding 2 N hydrochloric acid before chromatography on a Sephadex DEAE A-25 column. Appropriate fractions were collected, combined, and dialyzed. The title compound (0.4 g) was obtained by precipitation twice from water/acetone as a white solid. ^{1}H NMR (D_2O) δ 7.82 (2H, d, J = 7.6 Hz), 7.45 (2H, d, J = 8.0 Hz), 5.12 (1H, d, J = 4 Hz), 5.05−5.04 (7H, m), 4.13 (1H, t, J = 8.1 Hz), 3.94−3.87 (29H, m), 3.70−3.48 (17 H, m), 3.30−3.25 (1H, m); LC−MS m/z 1477.7 $(M + Na)^+$.

The following compounds were synthesized in a similar way as described above.

42: ^{1}H NMR (D_2O) δ 5.42−5.38 (1H, m), 5.16−5.08 (7H, m), 4.05−3.83 (30H, m), 3.68−3.61 (16H, m), 3.28 (2H, d, J = 4.3 Hz), 3.17−3.12 (1H, m), 2.87−2.82 (1H, m); LC−MS m/z 1368.8 $(M - Na)^-$.

43: ^{1}H NMR at 323 K (D_2O) δ 5.00 (1H, s), 4.97−4.89 (7H, m), 3.86−3.67 (30H, m), 3.50−3.35 (16H, m), 2.96 (1H, d, J = 9.5 Hz), 2.72−2.64 (3H, m), 2.35 (2H, t, J = 8.0 Hz); LC−MS m/z 1382.8 $(M - Na)^-$.

44: ^{1}H NMR at 323 K (D_2O) δ 5.05 (1H, d, J = 4.0 Hz), 4.97−4.95 (7H, m), 3.79−3.68 (29H, m), 3.52−3.43 (16H, m), 3.35 (1H, t, J = 7.5 Hz), 2.95 (1H, d, J = 12.0 Hz), 2.65 (1H, dd, J = 12.6 and 7.0 Hz), 2.50 (2H, t, J = 8.0 Hz), 2.32 (2H, t, J = 7.9 Hz), 1.78−1.70 (2H, m); LC−MS m/z 1398 $(M - Na)^-$.

46: ^{1}H NMR (D_2O) δ 7.79 (1H, s), 7.63 (1H, d, J = 8.0 Hz), 7.56 (1H, d, J = 7.9 Hz), 7.35 (1H, t, J = 7.6 Hz), 5.29 (1H, d, J = 3.6 Hz), 5.16−5.09 (7H, m), 4.30−4.22 (1H, m), 4.05−3.59 (46H, m), 3.30−3.22 (1H, m); LC−MS m/z 1432.0 $(M - Na)^-$.

48: ^{1}H NMR (D_2O) δ 7.33 (2H, d, J = 7.7 Hz), 7.25 (1H, t, J = 7.9 Hz), 7.10 (1H, d, J = 8.0 Hz), 5.28 (1H, d, J = 3.7 Hz),

Table 4. Crystal Data and Structure Refinement for **14** and **35**

	14	**35**
cryst system	orthorhombic	orthorhombic
space group	$P2_12_12_1$	$P2_12_12_1$
a, Å	16.839(5)	14.559(5)
b, Å	21.026(5)	17.521(5)
c, Å	35.345(5)	36.519(5)
α, deg	90.000(5)	90.000(5)
β, deg	90.000(5)	90.000(5)
γ, deg	90.000(5)	90.000(5)
Z	4	4
density (calcd), Mg/m^3	1.446	1.422
abs coeff, mm^{-1}	0.275	0.291
cryst size, mm^3	0.08 × 0.62 × 0.42	0.12 × 0.62 × 0.38
θ range for data collection, deg	1.93−25.01	1.82−24.99
reflns collected	13 817	10 631
independent rflns	13 503 [*R*(int) = 0.0281]	10 325 [*R*(int) = 0.0534]
completeness to *θ* = 24.99°, %	99.1	99.5
final *R* indices [*I* > 2 *σ*(*I*)]	R1 = 0.0792, wR2 = 0.2190	R1 = 0.0825, wR2 = 0.1852
R indices (all data)	R1 = 0.0942, wR2 = 0.2312	R1 = 0.1696, wR2 = 0.2197
absolute structure parameter	0.04(12)	0.08(15)
largest diff peak and hole, e Å^{-3}	1.124 and −0.967	0.746 and −0.537

5.15−5.10 (7H, m), 3.99−3.54 (49H, m), 3.15−3.08 (1H, m); LC−MS *m*/*z* 1446.0 (M − Na)$^-$.

49: ^{1}H NMR (D_2O) *δ* 7.32 (2H, d, *J* = 8.1 Hz), 7.17 (2H, d, *J* = 8.1), 5.17 (1H, d, *J* = 3.8 Hz), 5.08−5.04 (7H, m), 3.91−3.51 (49H, m), 3.02−2.97 (1H, m); LC−MS *m*/*z* 1445.9 (M − Na)$^-$.

50: ^{1}H NMR (D_2O) *δ* 7.35−7.25 (3H, m), 7.15 (1H, d, *J* = 7.8 Hz), 5.35 (1H, d, *J* = 3.6 Hz), 5.20−5.11 (7H, m), 4.09−3.60 (45H, m), 3.60−3.50 (2H, m), 3.10−3.05 (1H, m), 2.93 (2H, t, *J* = 8.0 Hz), 2.63 (2 H, t, *J* = 7.8 Hz); LC−MS *m*/*z* 1460.1 (M − Na)$^-$.

51: ^{1}H NMR (D_2O) *δ* 7.29 (2H, d, *J* = 8.1 Hz), 7.13 (2H, d, *J* = 8.1 Hz), 5.16 (1H, d, *J* = 3.9 Hz), 5.09−5.04 (7H, m), 4.02−3.50 (45H, m), 3.45−3.35 (2H, m), 2.95−2.89 (1H, m), 2.84 (2H, t, *J* = 6.8 Hz), 2.55 (2 H, t, *J* = 6.7 Hz); LC−MS *m*/*z* 1459.9 (M − Na)$^-$.

X-ray Crystallography Determination of 14 and 35. Crystallization of Compound 14. A suspension of compound **14** (200 mg) in DMF (5 mL) was heated at 80−90 °C and to this was added water dropwise till a clear solution was obtained. A further two drops of water were then added, and the solution was allowed to cool slowly, giving crystals over 2 days. A 20 mg sample was recrystallized by heating in a 2 mL mixture of DMF/H_2O (2.5:1) and allowing the mixture to cool slowly over 2 days. The resulting crystals were a mixture of prisms and fine needles. A needle-shaped single crystal was used for X-ray crystallography determination.

Crystallization of Compound 35. Approximately 100 mg of **35** was dissolved in water (7 mL) with heating and then allowed to cool slowly (warm oven, cotton wool insulation). After 3 days, crystals were obtained as large, dense needles.

The colorless crystals of **14** and **35** were coated in Nujol and with vaccum grease mounted on glass fibers. Diffraction data were measured at 160(2) K on a Bruker AXS P4 four-circle diffractometer fitted with graphite-monochromated Mo Kα radiation, *λ* = 0.710 73 Å.

Semiempirical absorption corrections by *ψ*-scans were applied. Structure solution and refinements (full-matrix least-squares on F^2) were performed using the SHELXL 5.1 suite of programs.[24] All non-aqua hydrogen atoms were included in the model, constrained to idealized positions, and refined using a riding model with riding isotropic displacement parameters. Hydrogen atoms were not located for solvent water molecules. In **35**, sulfur atom S3 was disordered and modeled as two positions S3 and S3A with 75% and 25% occupancy, respectively.

Crystallographic data for **14** (sodium salt) and **35** (CCDC 174659 and 174660, respectively) have been deposited with the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 IEZ, U.K. (e-mail: deposit@ccdc.cam.ac.uk). Table 4 lists some of the more essential information.

Reversal of Rocuronium-Induced Neuromuscular Block in Isolated Mouse Hemidiaphragm in Vitro. The hemidiaphragm with its phrenic nerve from male mice (20−30 g) was mounted on a tissue holder in a 20 mL tissue bath filled with a modified Krebs−Henseleit buffer (pH 7.4) at 37 °C, bubbled with 95% oxygen and 5% carbon dioxide. The buffer contains the following composition in mM: NaCl 118, KCl 5, KH_2PO_4 1, $MgSO_4$ 1, $NaHCO_3$ 30, $CaCl_2$ 2.5, and glucose 20. The phrenic nerve was stimulated continuously using a Grass S88E stimulator (rectangular pulses of 0.2 ms every 20 s at a supramaximal voltage of 2.5 V), and the isometric force was recorded using Grass FT03 transducers and a Grass 79D recorder. After a stimulation period of at least 30 min, rocuronium bromide was added to the bath (final concentration of rocuronium, 3.60 *μ*M) to produce ∼90% twitch block. After 20 min, increasing concentrations of reversal agents were added in a cumulative fashion at intervals of 10 min. Concentrations of compounds producing 50% and maximum recovery of twitch height were determined.

Reversal of Rocuronium-Induced Neuromuscular Block in Anaesthetized Guinea Pigs in Vivo. Male Dunkin−Hartley guinea pigs (body weight: 600−900 g) were anaesthetized by ip administration of 10 mg/kg pentobarbitone and 1000 mg/kg urethane. After tracheotomy, the animals were artificially ventilated using a Harvard small animal ventilator. A catheter was placed into the carotid artery for continuous monitoring of arterial blood pressure and the taking of blood samples for blood gas analysis. Heart rate was derived from the blood pressure signal. The sciatic nerve was stimulated (rectangular pulses of 0.5 ms duration at 10 s (0.1 Hz) intervals at a supramaximal voltage, using a Grass S88 Stimulator), and the force of *M. gastrocnemius* contractions was measured using a Grass FT03 force-displacement transducer. Contractions, blood pressure, and heart rate were recorded on a multichannel Grass 7D recorder. Catheters were placed in both jugular veins. One catheter was used for the continuous infusion of a neuromuscular blocking agent. The infusion rate of the neuromuscular blocking agent was increased until a steady-state block of 85−90% was obtained. The other catheter was used for administration of increasing doses of the reversal agent. During continuous infusion of the neuromuscular blocking agent, single doses of increasing concentration of reversal agent were given. At the end of the experiment, the measured force of muscle contractions was plotted against the concentration of reversal agent, and by use of regression analysis techniques, the 50% reversal concentration was calculated.

Reversal of Rocuronium-Induced Neuromuscular Block in Anaesthetized Cats in Vivo. Female cats (3.3−3.7 kg) were anaesthetized with a mixture of medetomidine

(80 μg/kg) and ketamine (2.5 mg/kg, iv), followed by 90 mg/kg α-chloralose, ip.

A tracheotomy was performed, and animals were ventilated with room air via a dual-phase respirator pump. Heart rate and arterial and left ventricular blood pressure were recorded. A catheter was placed in a femoral vein for the infusion of rocuronium bromide. Bradycardia was induced by stimulating the right vagus nerve using 10 s trains of rectangular 0.25 ms pulses every 120 s. The left vagus and cervical sympathetic nerves were tied, and the cervical sympathetic nerve was stimulated preganglionically. A suture was tied to the nictitating membrane and attached to a force-displacement transducer. The left tibialis anterior muscle was dissected free from surrounding tissue, and the tendon was attached to a force-displacement transducer. The muscle was electrically stimulated via the sciatic nerve at supramaximal voltage using rectangular pulses of 0.25 ms duration at 10 s intervals (0.1 Hz). After surgery animals were allowed to stabilize for 1 h. Rocuronium was dissolved in a phosphate buffer (pH 4). Org 25969 was dissolved in saline.

Continuous infusion of rocuronium (10.24 ± 1.31 nmol kg^{-1} min^{-1}) resulted in a 93 ± 2% block of *M. tibialis* contractions. This was followed by bolus injections of increasing doses of **14**.

Acknowledgment. We thank our colleagues in the Department of Analytical Chemistry for generating spectroscopic data and purity determination. We thank Frank Hope, Rona Mason, and Susan Miller for their expert technical assistance in performing pharmacology experiments. We acknowledge that Niall Hamilton was involved in the project as a line manager of D.J.B., H.F., E.J.H., and D.S. between April and December 1999.

Note Added after ASAP Posting. This article was released ASAP on 03/21/2002 with data missing from Table 1. The correct version was posted on 03/27/2002.

References

(1) Rees, D. C.; Hill, D. R. Drugs in Anesthetic Practice. In *Annual Reports in Medicinal Chemistry*; Bristol, J., Ed.; Academic Press: New York, 1996; Vol. 31, Chapter 5, pp 41−50.

(2) Hunter, J. M. New neuromuscular blocking drugs. *N. Engl. J. Med*. **1995**, *332*, 1691−1699.

(3) Hanson, C. W. Pharmacology of neuromuscular blocking agents in the intensive care unit. *Crit. Care Clin*. **1994**, *10*, 779−97.

(4) Bevan, D. R.; Donati, F.; Kopman A. F. Reversal of neuromuscular blockade. *Anesthesiology* **1992**, *77*, 785−805.

(5) Berg, H.; Roed, J.; Viby-Mogensen, J.; Mogensen, C. R.; Engbaek, J.; Skovgaard, L. T.; Krintel, J. J. Residual neuromuscular block is a risk factor for postoperative pulmonary complications. A prospective, randomised, and blinded study of postoperative pulmonary complications after atracurium, vecuronium and pancuronium. *Acta Anaesthesiol. Scand*. **1997**, *41*, 1095−1103.

(6) Eriksson, L. I. The effects of residual neuromuscular blockade and volatile anesthetics on the control of ventilation. *Anesth. Analg.* **1999**, *89*, 243−251.

(7) Grove, S. J. A.; Kaur, J.; Muir, A. W.; Pow, E.; Tarver, G. J.; Zhang, M.-Q. Oxyaniliniums as acetylcholinesterase inhibitors for the reversal of neuromuscular block. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 193−196.

(8) (a) Bom, A.; Muir, A.; Rees, D. C. Use of chemical chelators as reversal agents for drug-induced neuromuscular block. PCT Int. Appl. WO 0112202 A2, 2001; *Chem. Abstr*. **2001**, *134*, 193457. (b) Zhang, M.-Q.; Palin, R.; Bennet, D. J. 6-Mercapto-cyclodextrin derivatives: Reversal agents for drug-induced neuromuscular block. PCT Int. Appl. WO 0140316 A1, 2001; *Chem. Abstr*. **2001**, *135*, 29151.

(9) Wallimann, P.; Marti, T.; Fürer, A.; Diederich, F. Steroids in molecular recognition. *Chem. Rev*. **1997**, *97*, 1567−1608.

(10) D'Souza, V. T., Lipkowitz, K. B., Eds. *Cyclodextrins* (thematic issue); Chemical Reviews, Vol. 98; American Chemical Society: Washington, DC, 1998; pp 1741−2076.

(11) Szente, L.; Szejtli, J. Highly soluble cyclodextrin derivatives: chemistry, properties, and trends in development. *Adv. Drug Delivery Rev*. **1999**, *36*, 17−28.

(12) Zhang, M.-Q.; Rees, D. C. A review of recent applications of cyclodextrins for drug discovery. *Expert Opin. Ther. Pat.* **1999**, *9*, 1697−1717.

(13) Rekharsky, M. V.; Inoue, Y. Complexation thermodynamics of cyclodextrins. *Chem. Rev*. **1998**, 98, 1875−1917.

(14) Wenz, G. Cyclodextrins as building blocks for supramolecular structures and functional units. *Angew. Chem., Int. Ed. Engl*. **1994**, *33*, 803−822.

(15) Khan, A. R.; Forgo, P.; Stine, K. J.; D'Souza, V. T. Methods for selective modifications of cyclodextrins. *Chem. Rev*. **1998**, *98*, 1977−1996.

(16) Chmurski, K.; Coleman, A. W.; Jurczak, J. Direct synthesis of amphiphilic α-, β- and γ-cyclodextrins. *J. Carbohyd. Chem*. **1996**, *15*, 787−796.

(17) De Robertis, L.; Lancelon-Pin, C.; Driguez, H. Synthesis of new oligosaccharidyl-thio-β-cyclodextrins (CDs): A novel family of potent drug-targeting vectors. *Bioorg. Med. Chem. Lett*. **1994**, *4*, 1127−1130.

(18) Gorin, B. I.; Riopelle, R. J.; Thatcher, G. R. J. Efficient perfacial derivatization of cyclodextrins at the primary face. *Tetrahedron Lett*. **1996**, *37*, 4647−4650.

(19) Guillo, F.; Hamelin, B.; Jullien, L.; Canceill, J.; Lehn, J.-M.; De Robertis, L.; Driguez, H. Synthesis of symmetrical cyclodextrin derivatives bearing multiple charges. *Bull. Soc. Chim. Fr*. **1995**, *132*, 857−866.

(20) Ling, C.-C.; Darcy, R. 6-S-Hydroxyethylated 6-thiocyclodextrins: expandable host molecules. *J. Chem. Soc., Chem.Commun*. **1993**, *2*, 203−205.

(21) Uekama, K.; Minami, K.; Hirayama, F. 6^A-O-[(4-Biphenylyl)-acetyl]-α-, -β-, and -γ-cyclodextrins and 6^A-deoxy-6^A-[[(4-biphenylyl)acetyl]amino]-α-, -β-, and -γ-cyclodextrins: potential prodrugs for colon-specific delivery. *J. Med. Chem*. **1997**, *40*, 2755−2761.

(22) Szejtli, J. Medicinal applications of cyclodextrins. *Med. Res. Rev*. **1994**, *14*, 353−386.

(23) 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*, 256−270.

(24) (a) *SHELXTL*, version 5.1; Bruker AXS, Inc.: Madison, WI, 1998. (b) Sheldrick, G. M. *SHELXL97 and SHELXS97*; University of Göttingen: Göttingen, Germany, 1997.

JM011107F