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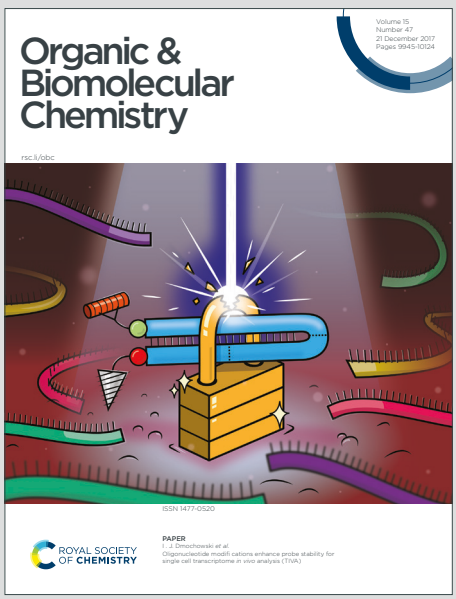

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ARTICLE

Supramolecular neuromuscular blocker inhibition by a pillar[5]arene through aqueous inclusion of rocuronium bromide

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A water-soluble pillar[5]arene, decafunctionalized with thioether and carboxylate fragments, was synthesized as a structural analogue of Sugammadex. Its ability to rescue contraction of the diaphragm muscle by encapsulating the muscle relaxant rocuronium bromide was demonstrated. Using UV-vis, NMR and fluorescence spectroscopy, it was shown that the muscle relaxant is associated with the pillar[5]arene with association constant of 4500 M^{-1} and a stoichiometry of 1:1. The structure of the inclusion complex of the pillar[5]arene with rocuronium bromide was additionally investigated by quantum chemical methods.

Introduction

In 1987 Jean-Marie Lehn, Charles J. Pedersen and Donald J. Cram were awarded the Nobel Prize for fundamental research involving the creation of artificial molecules that can imitate vital chemical reactions occurring in living organisms.[1] It took almost thirty years for these basic research to find application in the field of pharmacology and medical chemistry.[2a] One of the clearest examples of the application of the fundamentals of supramolecular chemistry in medical practice is the use of derivatives of γ-cyclodextrin. The most prominent representative among them is Sugammadex (tradename Bridion®) used to reverse neuromuscular blockade by drugs administered during surgery.[2b]

For most surgical interventions it is necessary to relax the skeletal muscles.[2c] Neuromuscular blockers (NMBs, also known as skeletal muscle relaxants) are used for this purpose. They block synaptic transmission at the neuromuscular junction by binding to the postsynaptic nicotinic acetylcholine receptors.[2] Rapid recovery of neuromuscular blockade (NMB) at the end of anesthesia is essential for the return of adequate respiration and upper airway muscle function.[3,4]

Currently, two concepts of NMB reversal are exist. An acetylcholinesterase inhibitor, such as neostigmine, can reverse NMB by increasing the lifetime of the neurotransmitter acetylcholine at the neuromuscular junctions by inhibiting its hydrolysis by acetylcholinesterase. The increased levels of acetylcholine compete with the NMBs molecules to binding to the postsynaptic nicotine receptors and tip the balance towards recovery of muscle contractions. This mechanism does not depend on the type of muscle relaxant, thus neostigmine can be used with all NMBs. Compounds that can selectively encapsulate free plasma NMB molecules represent an alternative recovery strategy.[4c,5] Sugammadex is able to encapsulate lipophilic guest molecules such as rocuronium bromide and vecuronium bromide (the two most widely used NMBs).[6] It is worth noting that the use of Sugammadex for NMB reversion revealed several advantages over neostigmine.[3] Thus, recovery of self-breathing in patients reversed with Sugammadex was two times faster than in those who received neostigmine. This leads to a significant reduction in mortality during the postoperative period.[6] In addition, Sugammadex is better tolerated by patients and is devoid of neostigmine side effects caused by inhibition of acetylcholinesterase in heart and smooth muscles.

Although the introduction of Sugammadex represents a great improvement in the reversal of NMB, there are some important aspects that deserve consideration. Firstly, only NMB induced by rocuronium bromide and vecuronium bromide can be reversed with Sugammadex,[7] leaving acetylcholinesterase inhibitors the only choice for reversal of

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the other NMBs, such as Cisatracurium. In the future, new broad-spectrum encapsulating agents may become available for all NMBs but they do not yet exist. Secondly, the cost of Sugammadex remains very high but it is unclear whether Sugammadex reversal leads to an improved postoperative outcome that justifies its cost.[8]

In this regard, over the last ten years, research has been conducted on the study on non-toxic macrocyclic drugs to facilitate recovery from NMBs. Macrocyclic systems based on cucurbiturils,[9a,b] calixarenes[9c] and Calabadion[9d] have been synthesized and studied for this purpose. However, the poor aqueous solubility of these macrocycles and the complexity of functionalization makes their production costly. We propose to replace semisynthetic γ-cyclodextrins used in NMB treatment with fully synthetic, water-soluble decasubstituted derivatives of pillar[5]arene. The benefits of this water-soluble pillar[5]arenes[10] over other macrocyclic platforms are their ease of production and functionalization and low toxicity. Previously, a water-soluble pillar[6]arene has been shown to bind Succinylcholine (Sch) to form host-guest complexes, [10e] however, the yield of the pillar[6]arene is significantly lower than its pillar[5]arene analogue. In this paper, we report the first example of using a water-soluble derivative of pillar[5]arene containing thioether and carboxylate fragments as recovery agent for NMB.

Results and discussion

The similarity of such macrocyclic platforms as cyclodextrins, already used for NMB reversal, and pillar[n]arenes [10d,11] has been noted. Both platforms have a cavity of similar diameter and free hydroxyl groups that can be functionalized. The advantages of using a pillar[5]arene as a starting platform[10, 11] compared with a cyclodextrin are the availability of initial reagents, ease of preparation, high yields of targeted macrocycles and the ability to control the size of the interaction region by changing the length of substituents.

Currently, there are two main ways of forming the macrocyclic platform of pillar[n]arenes:[12] 1) cyclization of a previously functionalized 1,4-hydroquinone unit[13] and 2) functionalization of the macrocyclic platform itself.[14]

The first path has a number of significant advantages, such as good yields of target macrocycles and the possibility of introducing highly reactive functional groups. In the second approach, the macrocyclic platform is used directly, which is obtained with a high yield, but its subsequent functionalization can be carried out only with a limited number of reagents. We chose the first approach, which is currently more versatile for pillar[5]arenes.[12]

It has been previously shown in the literature[15] that cyclization of 1,4-bis(2-bromoethoxy)benzene into a macrocyclic platform proceeds in good yield, while the bromoethoxy-fragment can react with thiols under mild conditions and high yields.[13b, 16] In this regard, we obtained compound **1** from commercially available reagents according to literature methods[15b,c] (Fig. 1, ESI,† S3).

In order to produce a water-soluble decasubstituted derivative of pillar[5]arene capable of removing NMB, macrocycle **1** was reacted with methyl 3-mercaptopropionate, in the presence of potassium carbonate in DMF at room temperature (Fig. 1, ESI,† S3). The reaction time was 48 hours, the yield of the target macrocycle was 84%. Then, using alkaline hydrolysis in isopropyl alcohol, macrocycle **2** was transformed into water-soluble pillar[5]arene **3** in 96% yield (Fig. 1, ESI,† S4). The structures of **2** and **3** were fully confirmed by a range of physical methods and their compositions confirmed by elemental analysis (ESI,† S5).

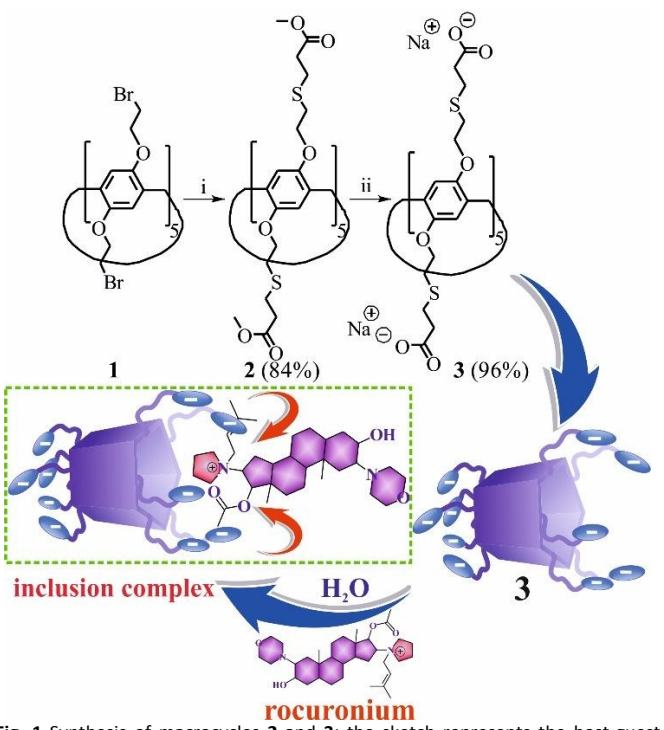

Fig. 1 Synthesis of macrocycles **2** and **3**: the sketch represents the host-guest system of the **3** / rocuronium bromide. Reagents and conditions: i - methyl 3-mercaptopropionate, K_2CO_3, DMF, r.t., 48h; ii – NaOH / H_2O / $CH_3CH(OH)CH_3$, r.t., 4h.

The ability of macrocycle **3** to interact with rocuronium bromide was studied by UV-vis, fluorescence and 2D ^{1}H NMR spectroscopy. Rocuronium bromide is a frequently used commercial muscle relaxants and produced under the trade name Esmeron®.[17] According to UV-vis spectroscopy, pillar[5]arene **3** has one absorption maximum with λ_{max} at 292 nm in water. The rocuronium bromide solution does not absorb in the wavelength range studied (200 - 600 nm). The interaction of macrocycle **3** (10^{-5} M) with rocuronium bromide shows a hyperchromic effect and the absorption band undergoes a red shift (Fig. 2). The study of complexation by the method of isomolar series allowed us to establish a 1:1 stoichiometry for **3**:rocuronium bromide. The association constant was determined on the basis of a spectrophotometric titration in which the concentration of rocuronium bromide varied and the concentration of **3** (10^{-5} M) remained constant. The results were processed using BindFit [18,19] and fitted to a 1:1 binding model (ESI,† S13). The association constant of pillararene **3** with rocuronium bromide was determined as

4500 M^{-1}. In addition, the stoichiometry of the complex was confirmed by titration data processed using host : guest ratios of 1:2 and 2:1. However, in this case the constant was determined with great uncertainty. To assess the role of the macrocyclic platform in the formation of a complex with rocuronium bromide, we additionally synthesized model compound **4** (ESI,† S4).

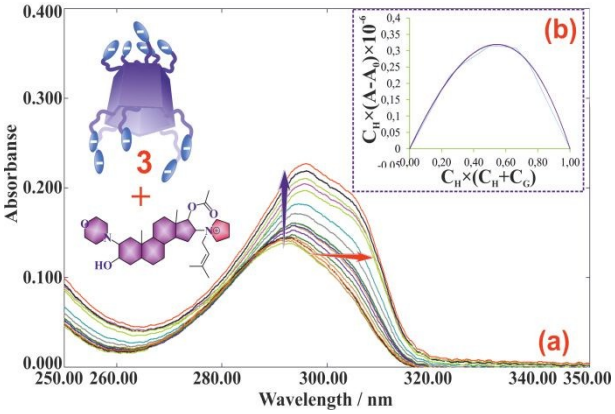

Fig. 2 a) UV-vis spectra of mixtures of pillar[5]arene **3** (10^{-5} M) with different concentrations of rocuronium bromide; b) Job's plot for the complex of rocuronium bromide with **3**.

The absorption spectra of pillar[5]arene **3** and model compound **4** have a different appearance (ESI,† S14). In the spectrum of a model compound, there are absorption bands with maxima at 222 and 283 nm, corresponding to the absorption bands of the benzene fragment associated with π-electron transitions.[20a] The introduction of substituents into the benzene ring causes a shift in the absorption maxima of benzene to the long-wavelength region. The presence of carboxylate groups in both compounds **3** and **4** leads to the appearance in their spectra of the absorption band corresponding to the n → π* transitions, which is superimposed on the absorption band at 222 nm. In the absorption spectrum of pillararene **3**, there is a single long-wavelength absorption band (π → π *) with a maximum at 292 nm, which is bathochromic offset compared to the band in the model compound. Such a shift can be explained by an increase in the polarity of the environment of the aromatic fragments due to the large number of ionized carboxylate groups. The absorption band at 220 nm seen for **4** is absent in the spectrum of the macrocycle. Absorption in this region, corresponding to n → π* transitions, due to the presence of carboxylate groups, undergoes a hypsochromic shift, while the band corresponding to π → π* transitions in aromatic fragments, shifts to the region of long waves. As a result, a broadening of the absorption band is observed, which is manifested by the presence in the spectrum of a shoulder in the region of 200-260 nm (ESI,† S14). To confirm the selectivity of macrocycle **3** for rocuronium bromide, its interaction with another widely used muscle relaxant, Succinylcholine (Sch), was studied. An analysis of the UV spectra of macrocycle **3** (10^{-5} M) in the presence of various concentrations of Sch (10^{-3}-10^{-5}M) in water did not reveal the formation of complexes of macrocycle **3** with Sch, as evidenced by slight changes in the absorption band of pillar[5]arene **3** at 292 nm (ESI, † S16). The selectivity of rocuronium bromide binding remains in the series of compounds of identical structure (gonan structure). For this, complexation **3** with Hydrocortisone acetate was studied. Using UV-vis spectroscopy, it was found that pillar[5]arene **3** (10^{-5} M) does not interact with Hydrocortisone acetate (10^{-4} M) (ESI, † S16). This is evidenced by the absence of changes in the absorption band of aromatic fragments of pillar[5]arene **3** (292 nm) in the UV-visible region of the spectrum of the mixture of pillar[5]arene **3** (10^{-5} M) / hydrocortisone acetate (10^{-4} M) (ESI , † S16)

An interesting and unexpected feature of pillararene **3** was its ability to fluorescence. As a rule, the fluorescent properties of these macrocycles are imparted by the introduction of known fluorophores such as porphyrins or pyrenes.[20b–d] It should be noted that the ability of the pillararenes to Aggregation-Induced Emission was recently discovered.[20e] Interest in compounds of this kind has remained high since they were first discovered in 2001.[20f] It is assumed that emission occurs in molecular aggregates as a result of limiting the intramolecular mobility of the compounds that make them up. However, the study of solutions of pillararene **3** at concentrations (10^{-3}-10^{-5}M) and the corresponding systems with the muscle relaxant by the DLS method showed the absence of the formation of any aggregates in both cases (ESI, † S14). Additionally, the absence of aggregation is evidenced by our analysis of UV-vis spectra, in which there is no noticeable change in the baseline in the red region of the spectrum (Fig. 2),[20f] and the absence of signal broadening in the 1H NMR spectrum of pillar[5]arene **3** in deuterated water (ESI, † S6).

Macrocycle 3 has significant fluorescence in water already at a concentration of 10^{-5}M with an emission maximum at 324 nm (Fig. 3). A small Stokes shift (32 nm) in a polar solvent indicates that in non-polar solvents fluorescence may not be visible against the background of scattered light. Indeed, it is known that toluene has a fluorescence, however, the Stokes shift in this case reaches only 23 nm, and the maximum emission is observed in the range inconvenient for measurements (285 nm).

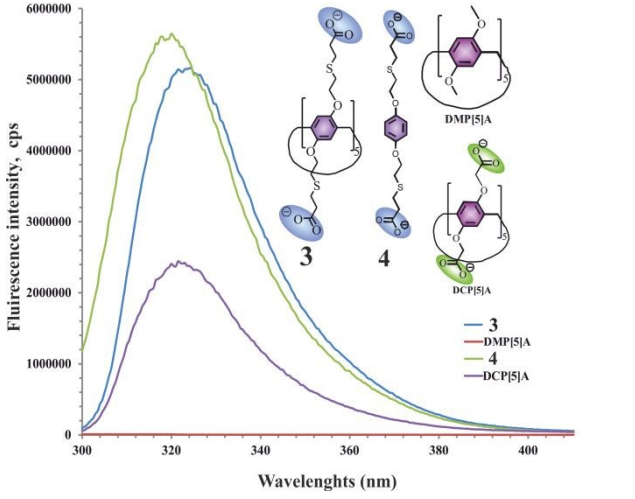

Fig. 3 Fluorescence spectra of **3** (H_2O, 10^{-5}M), **4** (H_2O, 5×10^{-5}M), decamethoxypillar[5]arene (DMP[5]A) ($CHCl_3$, 10^{-5}M) and pillar[5]arene containing 10 carboxylate groups (DCP[5]A) (H_2O, 10^{-5}M).

The introduction of substituents into the benzene ring increases the Stokes shift and shifts the emission maximum to the longwave region.[20g] Based on the assumption that the pillararene platform can impart fluorophore properties, we studied the spectrum of decamethoxypillararene, but no emission was detected for it (Fig. 3). As a result, we hypothesized that the available carboxylate substituents of macrocycle **3** affect the energy levels of the compound, thereby reducing the energy gap between HOMO and LUMO of the functionalized macrocycle **3**. Indirectly, this assumption is confirmed by the data of 2D ^{1}H-^{1}H NOESY NMR spectroscopy: cross-peaks between the protons of $SCH_2CH_2C(O)$ and protons of aromatic fragments **3** indicate the spatial proximity of carboxylate groups with a macrocyclic platform (ESI,† S9). So, we assume that closely spaced carboxylate anionic fragments of pillar[5]arene **3** increase the polarity of the medium around the aromatic rings, thereby lowering the energy of the HOMO and shifting the emission maximum into long waves compared with decamethoxypillararene and toluene. [20h] The hypothesis was confirmed by quantum chemical calculations which showed that the HOMO-LUMO energy gap is smaller for charged pillar[5]arene **3** than for uncharged pillar[5]arene **3** in acid form or model compound **4** (ESI,† S17). In addition, we recorded the emission spectrum of the model compound, which also turned out to be fluorescently active, with a maximum emission at 319 nm (Fig. 3). It is worth noting that pillar[5]arene containing 10 carboxylate groups (DCP[5]A) was also capable of emission (Fig. 3). Thus, it can be assumed that the presence of polar, preferably charged, groups near the macrocyclic platform plays a key role in the appearance of fluorescent properties in pillararenes.

The ability to fluoresce makes it possible to study the interaction of the compounds obtained with various substrates. In the spectrum of model compound **4** in the presence of rocuronium bromide, no changes were observed, however, when rocuronium bromide is added to macrocycle **3**, significant changes were observed in the emission of pillar[5]arene (Fig. 4). The nature of the change in the spectra was unusual as the position of the maximum emission does not change but at low concentrations of the substrate (up to 70 μM), an increase in fluorescence was observed, while a further increase in its content leads to the suppression of emission (Fig. 4). Similar behavior was shown in the interaction of cyclodextrin with rocuronium bromide, but an explanation for the result obtained was not given.[21] In our opinion, it may be due to a change in the mechanism of the interaction between rocuronium bromide and the pillararene. In order to confirm or refute this assumption, we recorded the emission spectra of pillararene with rocuronium bromide at different temperatures (5 and 35 °C) (ESI,† S15). On the basis of the data obtained, Stern-Volmer curves were constructed. [20h] Two areas can be distinguished in which the extinction dependence on the substrate concentration is linear, which indicates the presence of a single interaction mechanism (ESI,† S15) over these concentration ranges. At high concentrations (70-800 μM) of rocuronium bromide (ESI,† S15) quenching is static, indicating the formation of a complex between the macrocycle **3** and rocuronium bromide. Interestingly, at low concentrations of rocuronium bromide (0-60 μM), the relationship is different; with increasing temperature, the emission intensity increases linearly, which indicates the dynamic nature of the flowing process (ESI,† S15). We believe that at low concentrations of the substrate, electrostatic interactions between the cation (rocuronium bromide) and anion (pillar[5]arene **3**) dominate, the rate of which is controlled by diffusion while the substrate is bound by the anionic centers of pillararene **3**. This process is probably accompanied by photoinduced electron transfer (PET) with a change in the arrangement of the molecular orbitals of the donor (carboxylate) groups of the fluorophore, resulting in an increase in fluorescence. [20h] After analyzing the results, we concluded that at low concentrations of muscle relaxant there are no significant changes in the absorption spectra of the macrocycle, which is consistent with the proposed dynamic nature of the process. Subsequent increase in the concentration of the substrate leads to a change in the structure of the complex, with rocuronium bromide included in the macrocyclic cavity. This assumption is favored by the lack of interaction between rocuronium bromide and the model compound **4**.

In order to clarify the structure of the resulting complex, we additionally studied **3** / rocuronium bromide mixtures in a 1:1 ratio (10^{-2} M) using NMR spectroscopy. In the ^{1}H NMR spectra, broadening of the spectral lines of macrocycle **3** and rocuronium bromide is observed, which indirectly confirms the formation of the complex due to the overlap of the signals in a mixture of **3** and rocuronium bromide. This complicates the interpretation of the spectral pattern, however, the displacement of the proton signals of the guest molecule in a strong field, characteristic of the formation of an inclusion complex[22] is not observed in the ^{1}H NMR spectrum. The formation of a complex between macrocycle **3** and rocuronium bromide and their interactions were also confirmed by 2D ^{1}H-^{1}H NOESY and 2D DOSY NMR spectroscopy.

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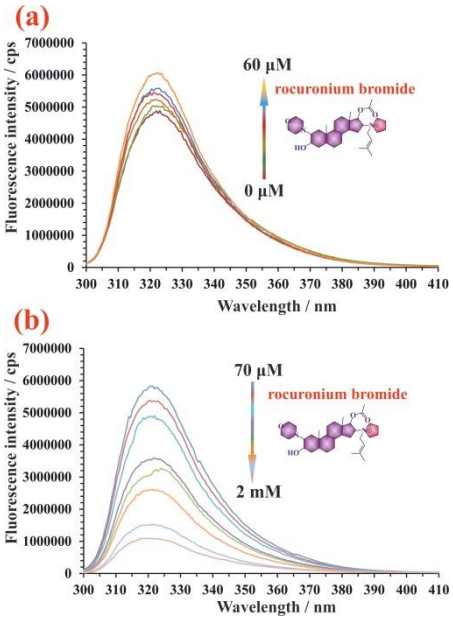

Fig. 4 Fluorescence spectra of **3** (10^{-5}M) at different rocuronium bromide concentrations (0-2×10^{-3}M).

Thus, in the 2D ^{1}H-^{1}H NOESY NMR spectrum of the **3** / rocuronium bromide mixture in a 1:1 (10^{-2} M) ratio (Fig. 5), cross-peaks between the protons **H¹** (aromatic fragments) of pillar[5]arene **3** and **H^{c}** and **H^{d}** protons of methyl groups of the gonan fragment of rocuronium bromide are observed. Cross-peaks between **H²** protons (methylene bridges) of pillar[5]arene **3** with protons **H^{j}** of methyl groups of the allyl fragment of muscle relaxant were also recorded. The formation of complex **3** / rocuronium bromide was additionally confirmed by two-dimensional DOSY spectroscopy (ESI,† S18). The diffusion coefficients of **3**, rocuronium bromide and **3** / rocuronium bromide at 298 K (10^{-2} M) were determined (ESI,† S18). The DOSY spectrum of mixture **3** / rocuronium bromide in a 1: 1 ratio (10^{-2} M) shows the presence of only one type of complex (ESI,† S18). An important criterion confirming the formation of a complex between **3** and rocuronium bromide was a significant decrease in the rate of diffusion of particles with the simultaneous presence in the system **3** / rocuronium bromide (ESI,† S18).

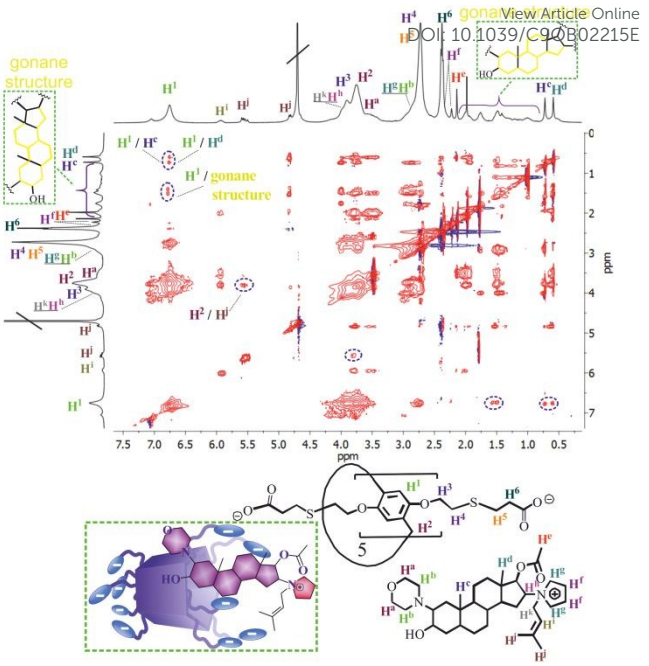

Fig. 5 The 2D ^{1}H-^{1}H NOESY NMR spectrum of the **3** / rocuronium bromide complex (1:1, 1×10^{-2} M) in D_2O at 25 °C and the proposed structure of the complex.

The orientation of the rocuronium bromide molecule relative to pillar[5]arene **3** was refined using PM6 and DFT quantum chemical calculations (B3LYP/6-311G*). As a result, two possible structures of the **3** / rocuronium complex bromide were proposed (Fig. 6a and 6b). Structure 6a represents a "perching complex",[1c] in which the rocuronium bromide molecule is parallel to the pillar[5]arene **3** cavity and is linked by anionic centers of the macrocycle. Structure 6b represents a "nesting complex",[1c] in which the rocuronium bromide molecule is perpendicular to the pillar[5]arene **3** cavity, and it is linked by the ion-ion interaction and the hydrophobic effect of the pseudo-cavity, formed by the substituents of macrocycle. Both the proposed structures are in good agreement with the experimental data obtained with structure (a) (Fig. 6a) in agreement with the hypothesis that electrostatic interactions predominate between the rocuronium cation and pillar[5]arene anion **3** at the initiation of fluorescence. From Fig. 6a it appears that the substrate is bound by the anionic centers of pillararene **3**. Structure (b) (Fig. 6b) agrees with the 2D NMR data when the complex is formed at high concentration (10^{-2} M). Fig. 6b is characterized by the close proximity of the "gonan frame" of rocuronium bromide in relation to the macrocyclic cavity of pillar[5]arene **3**, in agreement with 2D ^{1}H-^{1}H NOESY NMR spectroscopy. The inability of rocuronium bromide fragments to fit within the macrocyclic cavity of pillar[5]arene **3** explains the absence of significant chemical shifts of protons of the guest molecule into a strong field, which is typical for host-guest complexes of pillar[5]arenes.[22a] Thus, quantum chemical calculations support the experimental data obtained, which show that an increase in the concentration of the substrate leads to a change in the structure of the complex. These findings are in good agreement with previous studies[22b] including those of

García-Río[22c] who showed the possibility of forming an external complex with water-soluble decasubstituted derivatives of pillararenes. However, it is worth noting that planar aromatic compounds were used as guest molecules in the latter case.

Pillar[5]arenes are known to interact with alkali and transition metal ions[23] so, to assess the possible interfering effect of metal ions present in the body (Li^+, K^+, Na^+, Zn^{2+}, Fe^{2+}, Mg^{2+}), we studied their possible competition with rocuronium bromide for pillar[5]arene **3**. However, analysis by UV-vis, NMR spectroscopy did not detect the formation of complexes of the macrocycle with these cations, which indicates the absence of their interfering effect.

Having shown that pillar[5]arene **3** is capable of forming stable complexes with rocuronium bromide we wished to compare its efficacy to that of Sugammadex *in vitro* and *ex vivo*.

Thus, changes in rocuronium bromide cytotoxicity following coincubation with Sugammadex or **3** were analyzed. Initially we studied *in vitro* cytotoxicity of rocuronium bromide, Sugammadex and pillar[5]arene **3** against two normal human cell lines (embryonic lung and Chang liver cells). It was shown that both Sugammadex and pillar[5]arene **3** have no influence on cell viability up to 2000 μg/ml. A further increase in concentration was limited by the solubilities of Sugammadex and pillar[5]arene **3** in water. For rocuronium bromide 50% inhibitory concentrations (IC50) against lung and liver cells were determined as 1020±81 μg/ml and 1563±125 μg/ml, respectively (ESI,† Table 1, S19). In a second step, the cytotoxicity of rocuronium bromide in combination with an equal concentration of Sugammadex or pillar[5]arene **3** was studied against lung and liver cells (ESI,† Table 2, S19). It was shown that after treatment with 2000 μg/ml of rocuronium bromide in combination with 2000 μg/ml pillar[5]arene **3** that the cell viability was 100% for both cell lines whereas for the combination of rocuronium and Sugammadex the liver cell viability was only 75% and for lung cells the viability was only 80% (ESI,† Table 2, S19). Thus, pillar[5]arene **3** was able to decrease rocuronium-induced cytotoxicity to a greater extent than Sugammadex.

To study the effect of pillar[5]arene **3** on the restoration of muscle contractility after NMB, *ex vivo* twitch tension measurements was used. Four consecutive contractions (train-of-four, TOF) of mouse hemidiaphragm muscles were evoked by stimulating the phrenic nerve at 2 Hz.[24] The ability of Sugammadex or pillar[5]arene **3** to restore the muscle contractility was expressed as percentage of contraction amplitude for the each diaphragm muscle after drug application, i.e. muscle contractions before rocuronium bromide treatment were taken as 100%. (ESI,† S3).

During the TOFs, the ratio of the amplitude of fourth twitch (T4) to the first twitch (T1) in the control was 91.8 ± 0.8% (n=16 muscles). Several minutes after bathing in a solution of rocuronium bromide (10 μM) contractions were completely blocked (Fig. 7, ESI,† S20). Once contractions were absent, solutions of Sugammadex or pillar[5]arene **3** were applied.

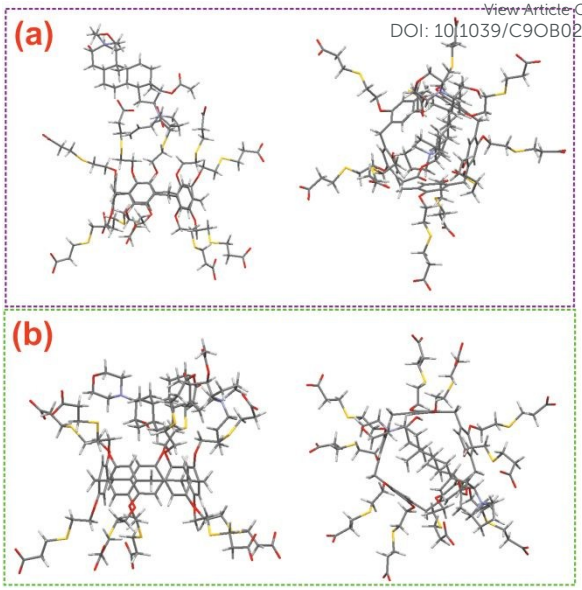

Fig. 6 Two calculated variants of orientation of the complex: with either (a) the pyrrolidine group or (b) the morpholine group oriented towards the macrocyclic annulus.

Application of 10 μM Sugammadex resulted in the restoration of the force of contractions (for T1) to 77.9± 5.6% (n = 8 muscles) from the initial values prior to the blockade by rocuronium bromide. Thus, the recovery of contractions after Sugammadex was incomplete, the amplitude of T1 was significantly less than before the NMB (Fig. 7, ESI,† S20). The mean T4/T1 ratio after treatment of diaphragm muscles with Sugammadex was 89.9 ± 1.36%, this mean T4/T1 ratio does not significantly differ from T4/T1 ratio in intact preparations (paired Wilcoxon test, p = 0.22, n = 8)

Application of 10 μM of **3** also caused partial recovery of the muscle contraction force. The mean recovery of T1 in presence of pillar[5]arene **3** was 87.1 ± 5.0% of the initial values prior to rocuronium blockade (n = 8 muscles). There were no significant differences in mean amplitude of contraction between Sugammadex and pillar[5]arene **3** (Fig.7 ESI,† S20). Thus, pillar[5]arene **3** showed the similar ability to recover NMB as Sugammadex. The mean T4/T1 ratio in the presence of pillar[5]arene **3** was 87.35±3.4%, which does not differ significantly from the mean T4/T1 ratio in the control (paired Wilcoxon test, p = 0.31, n = 8 muscles).

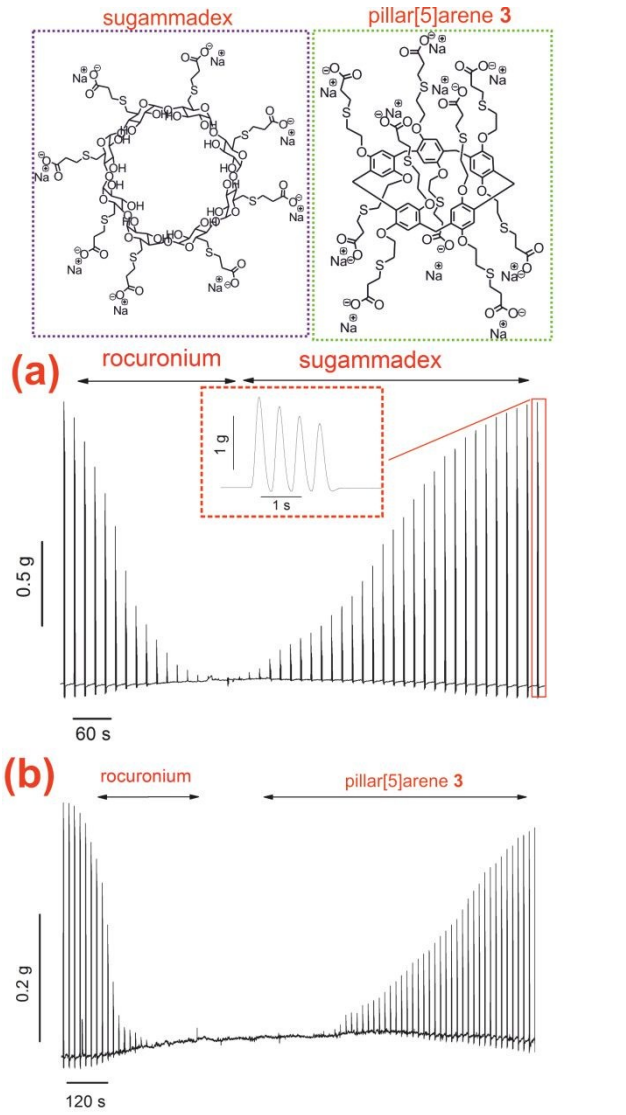

Fig. 7 Representative traces of muscle contractions with rocuronium bromide and subsequent recovery after application of either Sugammadex (a) or pillar[5]arene **3** (b). Typical TOF is shown in Insert.

It should be noted, that the time period necessary for maximal recovery of contractions for pillar[5]arene **3** was 735 ± 37 s, which is significantly longer than in Sugammadex (255 ± 20 s, Fig. 7a, ESI,† S20).

Because both drugs are reach the target locations through passive mechanisms, it can be assumed, that this difference in the rate of recovery of muscle contraction between pillar[5]arene **3** and Sugammadex could be explained by lipophilicity of compounds. The calculated log P for pillar[5]arene **3** (8.60)[25] is significantly higher than log P for Sugammadex (-6.14).[25]

Experimental Materials and methods

Materials and Instrumentation. All the chemical reagents were commercially available and used as supplied without further purification. ^{1}H NMR, ^{13}C and 2D NOESY NMR spectra were obtained on a Bruker Avance-400 spectrometer (^{13}C{^{1}H} - 100 MHz and ^{1}H and 2D NOESY, DOSY - 400 MHz). Mass spectra (MALDI-TOF) were recorded on an Ultraflex III mass spectrometer in a 4-nitroaniline matrix. Electrospray ionization mass spectra (ESI,†) were obtained on an AmazonX mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). Fluorescence spectra were recorded on a Fluorolog 3 luminescent spectrometer (Horiba Jobin Yvon). UV-vis spectra were recorded using the Shimadzu UV-3600 spectrometer. Calculations were undertaken using the Spartan '18 Parallel Suite[S2] running on a Mac Pro with 3.5 GHz 6-Core Intel Xenon E5 processors and two threads per core (ESI,†).

Synthesis of compounds 2.

Anhydrous (0.9 g, 6.5 mmol) K_2CO_3 was added to (0.3 g, 0.18 mmol) pillar[5]arene **1** in anhydrous DMF (15 ml). The reaction mixture was stirred at room temperature for 30 minutes. Methyl 3-mercaptopropionate (at a once) (0.4 ml, 3.6 mmol) was then added to the resulting suspension. The reaction mixture was stirred at room temperature under an argon atmosphere for 48 hours. Then the reaction mixture is poured into 20 ml of distilled H_2O. The precipitated amorphous precipitate was centrifuged and washed with methanol (2 × 20 ml). A light yellow viscous oil was obtained. Yield 0.31 g (84%).

Synthesis of compounds 3.

Pillar[5]arene **2** (0.3 g, 0.14 mmol) suspended in isopropanol (20 ml). Then, NaOH (7 ml of 10% aqueous solution) was added to the reaction mixture. The reaction mixture was heated at 60 °C under argon atmosphere until the precipitate dissolved (~12 hours). Then the reaction was cooled. A white precipitated residue was collected by filtration and washed with cold methanol (2 × 15 ml). A white crystalline powder was obtained. Yield 0.30 g (96%).

Synthesis of compounds 4.

Anhydrous K_2CO_3 (1.2 g, 9 mmol) was added to 1,4-bis(2-bromoethoxy)benzene (1 g, 3 mmol) in anhydrous DMF (20 ml). The reaction mixture was stirred at room temperature for 30 minutes. Then methyl 3-mercaptopropionate (at a once) (0.6 ml, 6.1 mmol) was added to the resulting suspension. The reaction mixture was stirred at room temperature under an argon atmosphere for 24 hours. Then the reaction mixture was poured into a mixture (30 ml) of isopropanol / NaOH solution (10% aqueous solution) (2:1). The reaction mixture was stirred for two hours. A white precipitated residue was collected by filtration and washed with cold isopropanol (2 × 15 ml). A white crystalline powder was obtained. Yield 1.12 g (89%).

To study the effect of **3** on the muscle contractility after the neuromuscular blockade caused with non-depolarizing muscle relaxant rocuronium bromide, isolated mouse hemidiaphragm nerve-muscle preparation was used.

Experiments were performed on neuromuscular preparations from diaphragms excised from white laboratory mice of the ICR line (CD-1) of both sexes (weighting 22–25 g) in strict accordance with European Communities Council Directive 86/609 / EEC; protocol No. 9 - 2013, approved by the ethical committee of the Kazan Federal University.

Animals were deeply anesthetized by isoflurane inhalation until the tail-pinch reflex disappeared. Hemidiaphragm muscles with phrenic nerves were isolated from mice killed by dislocation of the cervical vertebrae. Preparations were

mounted in a temperature-controlled chamber filled with oxygenated (O_2 95%, CO_2 5%) Ringer's-Krebs' solution (in mM): 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 23 mM $NaHCO_3$, 1 mM NaH_2PO_4, and 11 mM glucose. pH was adjusted to 7.2-7.4; experiments were conducted at room temperature of 20±2 °C.

For twitch tension measurements, the central tendon of the hemidiaphragm muscle was tied by a stainless steel rod via a linen thread hook to a force sensor TRI201AD (ADInstruments, serial №1762414) and the ribs were immobilized with two long hooks. The responses were evoked by stimulation of the phrenic nerve using Electronic stimulator 1001 (AD Instruments) by supramaximal current pulses 0.1 ms duration. Signals from the force sensor were acquired and analyzed digitally, using PowerLab system and LabChart 8 software (ADInstruments). The force of the contractions was measured in grams. Four contractions (Train-of-four) were evoked by phrenic nerve stimulation at 2 Hz four times, with 15 s intervals between TOFs. The drugs were delivered to diaphragm muscle by a bath application. TOFs were recorded in intact muscle preparation. Then 10 μM rocuronium bromide containing solution was applied until the muscle contractions completely disappeared, and then either **3** or Sugammadex at final concentration 10 μM were added.

Data are presented as mean +/- SEM. Statistical analysis was performed using Origin 8.1. Paired Wilcoxon test was used for statistical analysis of experimental data. The differences were considered significant at $p < 0.05$.

Pillar[5]arenes **1** were synthesized according to the literature procedures. [15b,c]

Rocuronium bromide (Esmeron®), Succinylcholine, Hydrocortisone acetate and Sugammadex (Bridion®) were parched from Merck.

Detailed information (NMR and MS spectra, quantum chemical calculations, biological assays) is given in the ESI.†

Conclusions

A water-soluble pillar[5]arene **3** containing 10 carboxylate fragments was obtained by macrocyclization followed by thiolation and UV-vis, NMR and fluorescence spectroscopy showed binding by **3** to the muscle relaxant rocuronium bromide. The stoichiometry of the complex was 1:1 with an association constant of 4500 M^{-1}. The ability of water-soluble pillar[5]arenes, which do not contain fluorophore fragments, to fluorescence was shown for the first time and using fluorescence spectroscopy, it was demonstrated that an increase in the concentration of rocuronium bromide led to a change in the structure of its complex with the pillar[5]arene. The different complexes were modeled by quantum chemical calculations (DFT B3LYP/6- 311G*). Administration of **3**, at 10 μM, initiated recovery of rocuronium-inhibited muscle contraction as efficaciously as Sugammadex although muscles took longer to resume contractions. The work showcases the potential of water-soluble pillararenes as universal means to reverse the neuromuscular blockade. These results show that it is possible to construct synthetic macrocyclic receptors from the pillar[5]arene platform that are more affordable compared to natural analogues, which have the potential to further reduce the cost of drug delivery and have a positive influence on the development of healthcare.

Conflicts of interest

There are no conflicts to declare.

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