

THE ultra-short-acting nondepolarizing neuromuscular blocking agent (NMBA) gantacurium (AV430A or GW 280430A) rapidly combines with L-cysteine *in vitro* to form a presumably less-active degradation product (adduct). Gantacurium itself and its adduct may undergo further breakdown by alkaline hydrolysis. All the above reactions are nonenzymatic.^{1,2}

Gantacurium (fig. 1A) is an asymmetrical isoquinolinium diester of chlorofumaric acid in which one of the central double-bonded (olefinic) carbons is activated (given increased electrophilic character) by a strongly electronegative

chlorine substitution designed to accelerate the adduction reaction. We hypothesized that nonhalogenated olefinic isoquinolinium compounds analogous to gantacurium, such as symmetrical or asymmetrical maleates or fumarates, should also undergo the adduction reaction with L-cysteine but at slower rates. The absence of chlorine in such compounds should result in less activation of the olefinic carbons, which are then influenced only by the two relatively weakly electronegative adjacent α -carboxyl (ester) groups located on both sides of the central olefinic double bond. We tested this hypothesis in two new nonhalogenated olefinic diester ana-

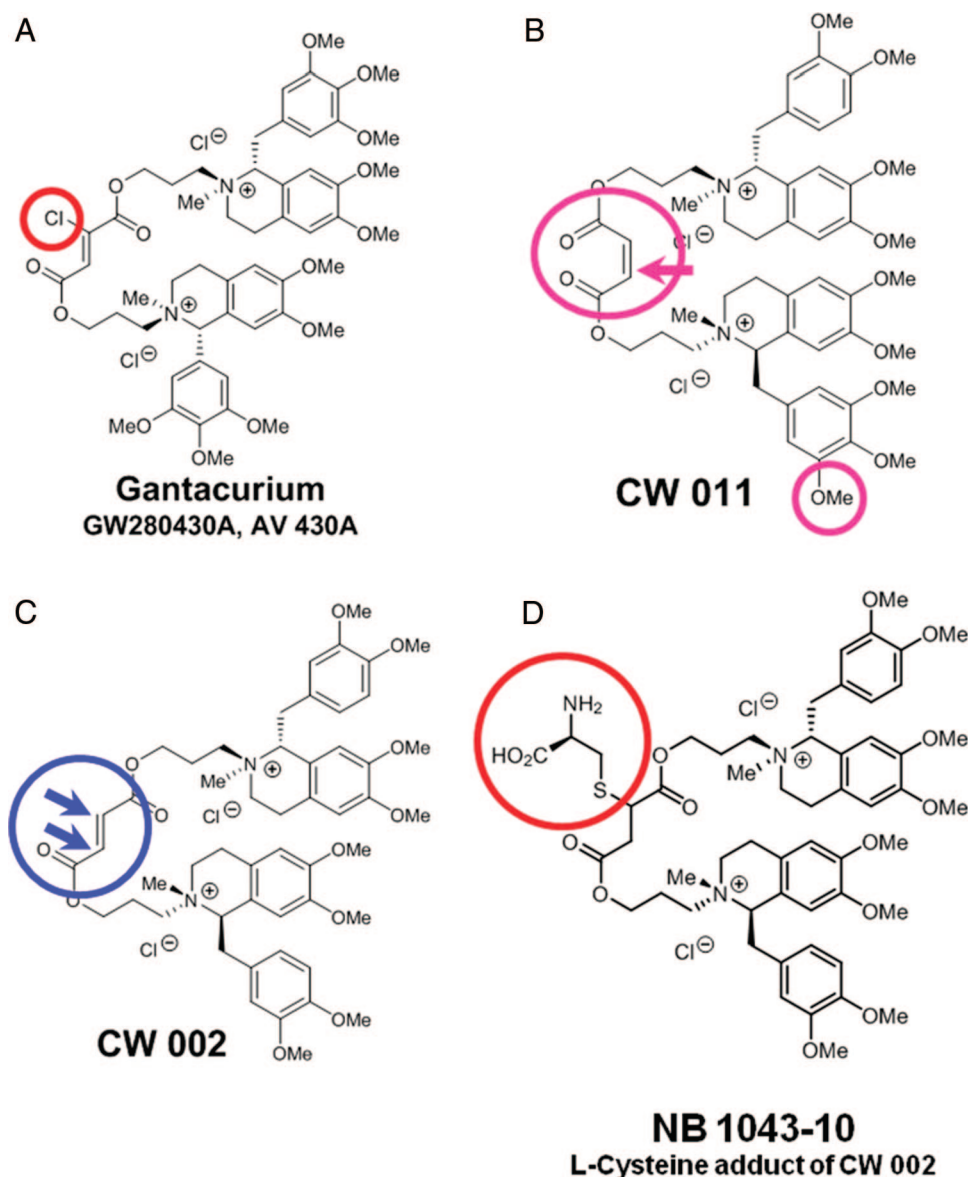


Fig. 1. The chemical formulae of gantacurium (A), CW 011 (B), and CW 002 (C). Chemical features are as follows: chlorine substitution (red circle) on the olefinic double bond of gantacurium, a chlorofumarate, is designed to accelerate the L-cysteine adduction reaction (see Supplemental Digital Content 1, appendix 3, <http://links.lww.com/ALN/A590>). The fumarate CW 002 is symmetrical with no halogen (chlorine) substitutions and undergoes L-cysteine adduction more slowly than gantacurium, at either olefinic carbon (blue arrows), enabled by the adjacent α -carboxyl (ester) groups. The maleate CW 011 is asymmetrical in that one isoquinolinium group contains an extra methoxy substitution (magenta circle). This may reduce access of L-cysteine to the olefin (magenta arrow) and may decrease the rate of the adduction reaction (see table 3). The chemical formula of NB 1043-10, the L-cysteine adduct of CW 002, is also shown (D). The L-cysteine adduction is highlighted by the red circle.

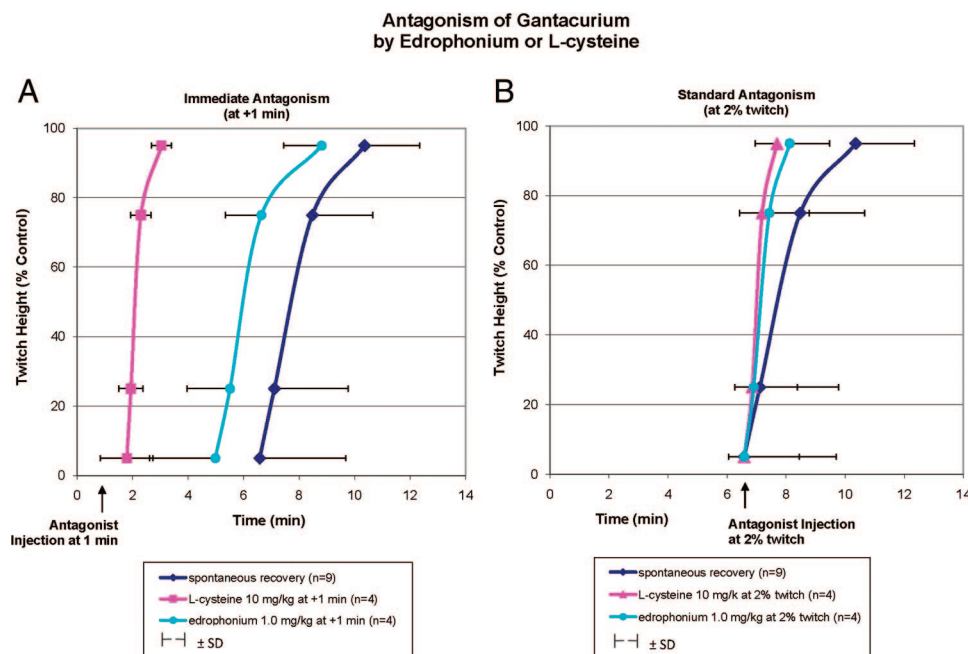


Fig. 3. Comparative antagonism of gantacurium (0.5 mg/kg or $\sim 5 \times \text{ED}_{95}$) by edrophonium (1.0 mg/kg with atropine 0.05 mg/kg) or L-cysteine (10 mg/kg). Gantacurium was injected at $t = 0$. Antagonist was given at the beginning of recovery at 2% twitch height (standard antagonism, B); or at 1 min after gantacurium administration (immediate antagonism, A). Antagonism by L-cysteine is faster than edrophonium at 2% twitch and is equally rapid at either 2% twitch or at 1 min after gantacurium ($P < 0.05$). Antagonism by L-cysteine at 1 min is significantly faster ($P < 0.001$) than edrophonium, which shifts the dose–duration curve to the left by only ~ 2 min. Curves show summarized data from experiments in anesthetized rhesus monkeys in which twitch of the extensor digitorum was elicited at 0.15 Hz (see also fig. 4).

6). The 5–95% interval after L-cysteine antagonism at 1 min after 0.15 mg/kg CW 002 was similar to the same interval after L-cysteine antagonism during the beginning of recovery at 2% twitch height: 1.7 ± 1.1 min (SD) versus 2.1 ± 0.6 min, respectively ($P > 0.05$).

L-Cysteine Does Not Antagonize Cisatracurium-induced Block. L-Cysteine (50 mg/kg) given at 2% twitch height at initiation of spontaneous recovery had no effect on recovery from cisatracurium; there was no change in the total duration or the 5–95% recovery interval ($P > 0.05$, see table 3).

Immediate L-Cysteine Antagonism at +1 min of Escalating Dosage of CW 011 ($4\text{--}64 \times \text{ED}_{95}$). L-Cysteine (50 mg/kg) caused highly significant acceleration ($P < 0.001$

in all cases) of recovery from CW 011 at all CW 011 doses tested from 0.1 to 1.6 mg/kg ($4 \times$ to $64 \times \text{ED}_{95}$). Both total duration and 5–95% recovery interval were shortened significantly (fig. 7).

Discussion

Relation of L-Cysteine Degradation in Vitro to Duration in Vivo

Because the durations of action of gantacurium, CW 002, and CW 011 were inversely related to the respective rates of degradation of the compounds by L-cysteine adduction *in vitro* (fig. 2), the adduction reaction is most likely the rate-

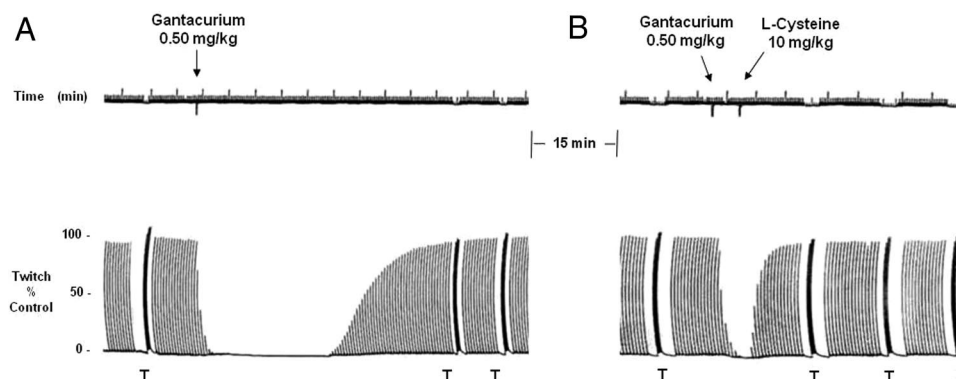


Fig. 4. An example of immediate antagonism of gantacurium by L-cysteine. Twitch was elicited at 0.15 Hz in an anesthetized rhesus monkey. Train-of-four (TOF) stimulation was interposed at T. (A) A control dose of gantacurium, 0.5 mg/kg ($\sim 5 \times \text{ED}_{95}$), was injected at marker. (B) One minute after a second dose, L-cysteine (10 mg/kg) restored twitch and TOF to normal within 2 min.

**Immediate Antagonism of $\sim 4 \times \text{ED}_{95}$ CW 002 (0.15 mg/kg)
by L-cysteine or Neostigmine at +1 min**

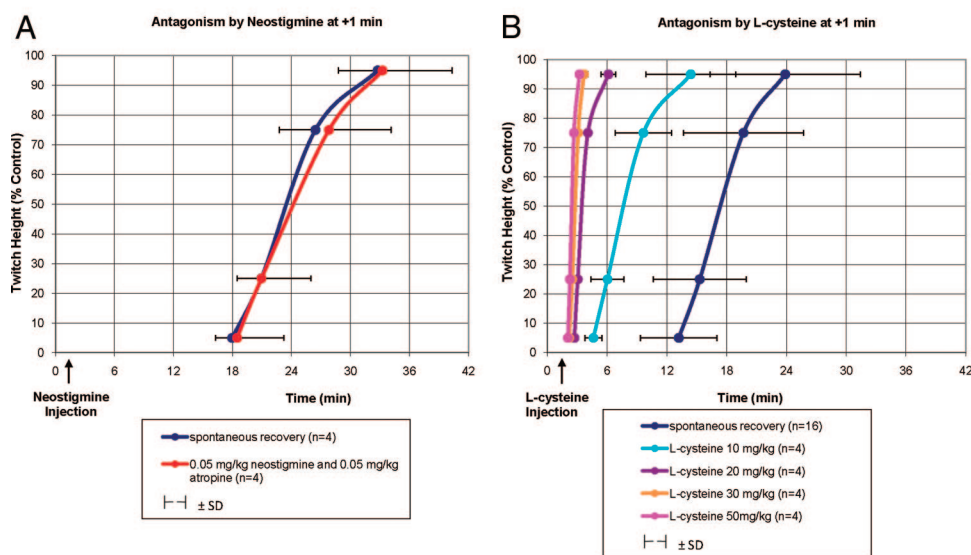


Fig. 5. Immediate antagonism of CW 002 blockade 1 min after CW 002 dosage of 0.15 mg/kg, or $\sim 4 \times \text{ED}_{95}$, injected at $t = 0$. Neostigmine (0.05 mg/kg + atropine 0.05 mg/kg) or L-cysteine (10, 20, 30, or 50 mg/kg) was given at +1 min. Neostigmine did not shorten recovery (A), whereas L-cysteine produced a dose-related acceleration of recovery (B), peaking at 50 mg/kg. Data were taken from anesthetized rhesus monkeys. Twitch of the extensor digitorum was elicited at 0.15 Hz (see also fig. 6).

limiting step governing the kinetics of neuromuscular block *in vivo*. The adduction products seem far less potent than the parent compounds (compare CW 002 and NB 1043–10); the adducts subsequently undergo alkaline hydrolysis to less potent fragments within a few hours (the potency of these fragments is in the range of 15–25 mg/kg for neuromuscular

blockade in monkeys; unpublished laboratory records, John J. Savarese, M.D., Weill Medical College of Cornell University, New York, New York, 2009). All fragments are positively charged monoquaternary substances (see figs. 8–10 in the appendix) and quite soluble in water; therefore, they are likely excretable in urine and possibly bile (not as yet proven

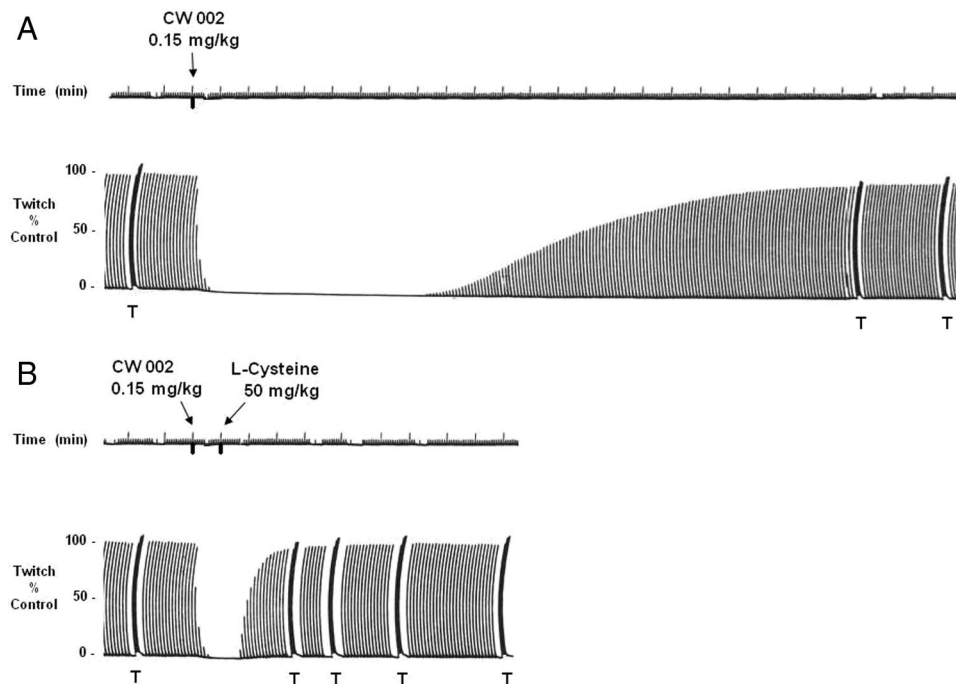


Fig. 6. An example of immediate antagonism of CW 002 by L-cysteine. Twitch of the extensor digitorum was elicited at 0.15 Hz in a rhesus monkey under isoflurane. (A) A control dose of CW 002 (0.15 mg/kg, $\sim 4 \times \text{ED}_{95}$) was injected at marker and allowed to recover spontaneously. (B) Sixty minutes later, the same dose was given at marker, followed 1 min later by L-cysteine (50 mg/kg). Twitch and train-of-four (TOF) (T) were restored to baseline within 2 and 3 min, respectively.

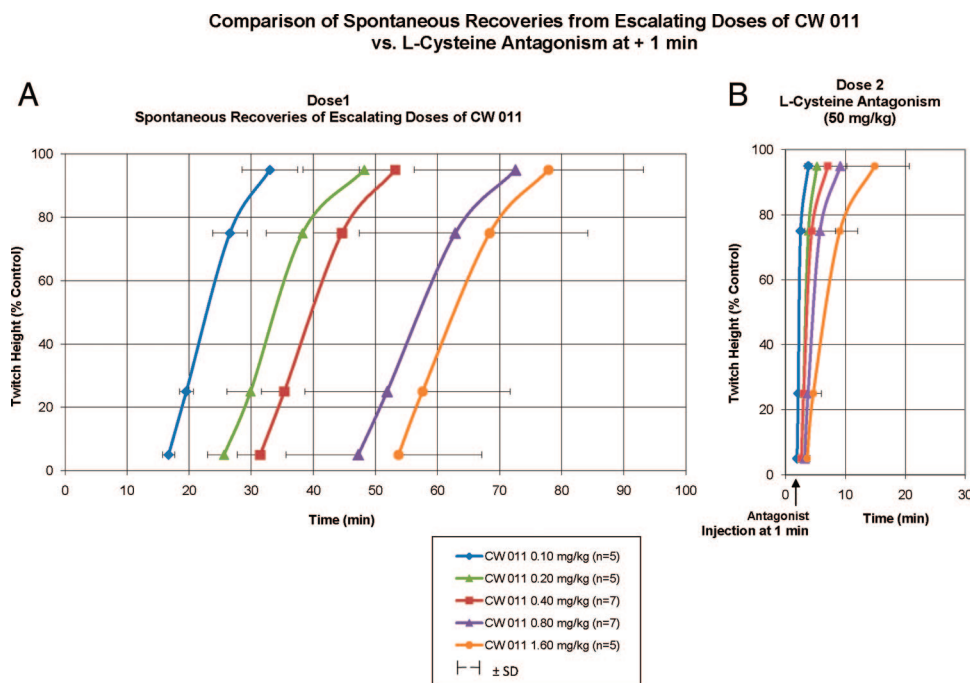


Fig. 7. Immediate L-cysteine (50 mg/kg) antagonism of escalating dosage of CW 011 ($4\text{--}64 \times \text{ED}_{95}$): comparison of spontaneous recoveries (A) vs. recovery accelerated by L-cysteine given at +1 min after CW 011 (B). CW 011 was injected at $t = 0$. All paired comparisons $P < 0.001$. Data were from groups of anesthetized monkeys; twitch of extensor digitorum was elicited at 0.15 Hz. Dosage pairs are color coded.

experimentally). Facile antagonism of blockade by administration of exogenous L-cysteine at +1 min, or at 2% twitch height, after approximately $4\text{--}5 \times \text{ED}_{95}$ doses indicates that the adduction reaction can be accelerated *in vivo* at any time according to laws of mass action.

L-Cysteine Antagonism: Comparisons versus Anticholinesterases

L-Cysteine antagonism was significantly faster than reversal by anticholinesterases. Evaluations at 1 min after approximately $4\text{--}5 \times \text{ED}_{95}$ doses of the new compounds especially showed a marked superiority of chemical antagonism or inactivation by L-cysteine over the competitive effect of anticholinesterases in terms of speed and completeness of antagonism.

Comparisons of L-cysteine antagonism of gantacurium with reversal by edrophonium are appropriate. The rapidly acting edrophonium would theoretically be considered a better antagonist of the ultra-short-acting gantacurium than neostigmine in view of the more slowly developing antagonistic effect of neostigmine.^{5,6}

The requirement for higher dosage of L-cysteine to induce rapid antagonism of the intermediate-duration compounds CW 002 and CW 011 is consistent with the slower rate of adduction *in vitro* by L-cysteine to these compounds compared with gantacurium and the longer duration in monkeys. CW 002 and CW 011 are about two and four times more potent than gantacurium, respectively. This means that concentrations *in vivo* needed for neuromuscular blockade will be approximately two and four times lower than ganta-

curium concentration. Plasma L-cysteine levels remain more or less constant throughout life in humans.⁷ Therefore, according to mass action mechanisms, the adduction reaction will be slower in the presence of the same L-cysteine concentrations in plasma in the case of potent NMBA in which plasma levels of the NMBA will be lower. This necessitates higher concentrations of L-cysteine (higher dosage of exogenous L-cysteine) during chemical antagonism *in vivo* to bring the reaction kinetics up to rates comparable with the reaction with the less-potent gantacurium, thereby inducing antagonism in a similar time-frame of about 2–3 min. Higher concentrations (dosage) of L-cysteine must also be needed for similarly rapid antagonism of the nonhalogenated compounds to increase the kinetics of the adduction reaction to compensate for the lack of the accelerating effect of the chlorine substitution.

Comparative Antagonism of Gantacurium, CW 002, and CW 011 by L-Cysteine

Comparative data in tables 1, 2, and 3, and figures 3, 5, and 7 show that L-cysteine dosage for rapid ($\sim 2\text{--}3$ min) antagonism of $4\text{--}5 \times \text{ED}_{95}$ doses of gantacurium at +1 min after injection is much lower (10 mg/kg) than the dosage requirement for similarly rapid antagonism of CW 002 or CW 011 (50 mg/kg). This difference can be explained by the differences in molecular design of the compounds in which chlorine substitution in gantacurium is a powerful accelerator of L-cysteine adduction to the olefin, resulting in a short (0.2 min) $t_{1/2}$ for the adduction reaction (fig. 2 and table 2). This is the likely mechanism explaining both the ultra-short du-

Appendix: Chemical Pathways of Degradation of CW 002, CW 011, and Gantacurium (Figs. 8–10)

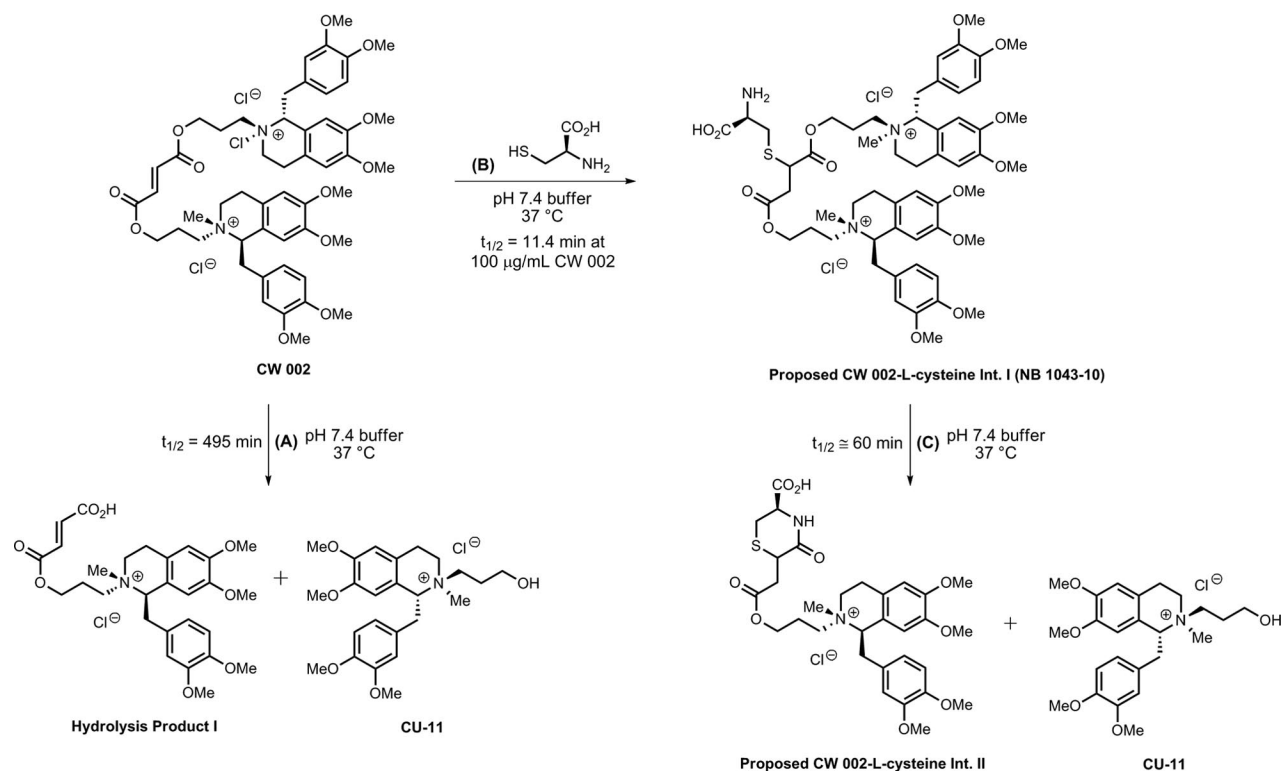


Fig. 8. Degradation of CW 002. The molecule is relatively stable at pH 7.4 and 37°C (A) undergoing relatively slow alkaline hydrolysis in the absence of L-cysteine. Addition of L-cysteine (B) results in rapid conversion to the adduct (Int. I) with reaction half-time ($t_{1/2}$) = 11.4 min. The adduct itself then undergoes alkaline hydrolysis with $t_{1/2} \sim 60$ min (C) to the fragments Intermediate (Int.) II and CU-11.

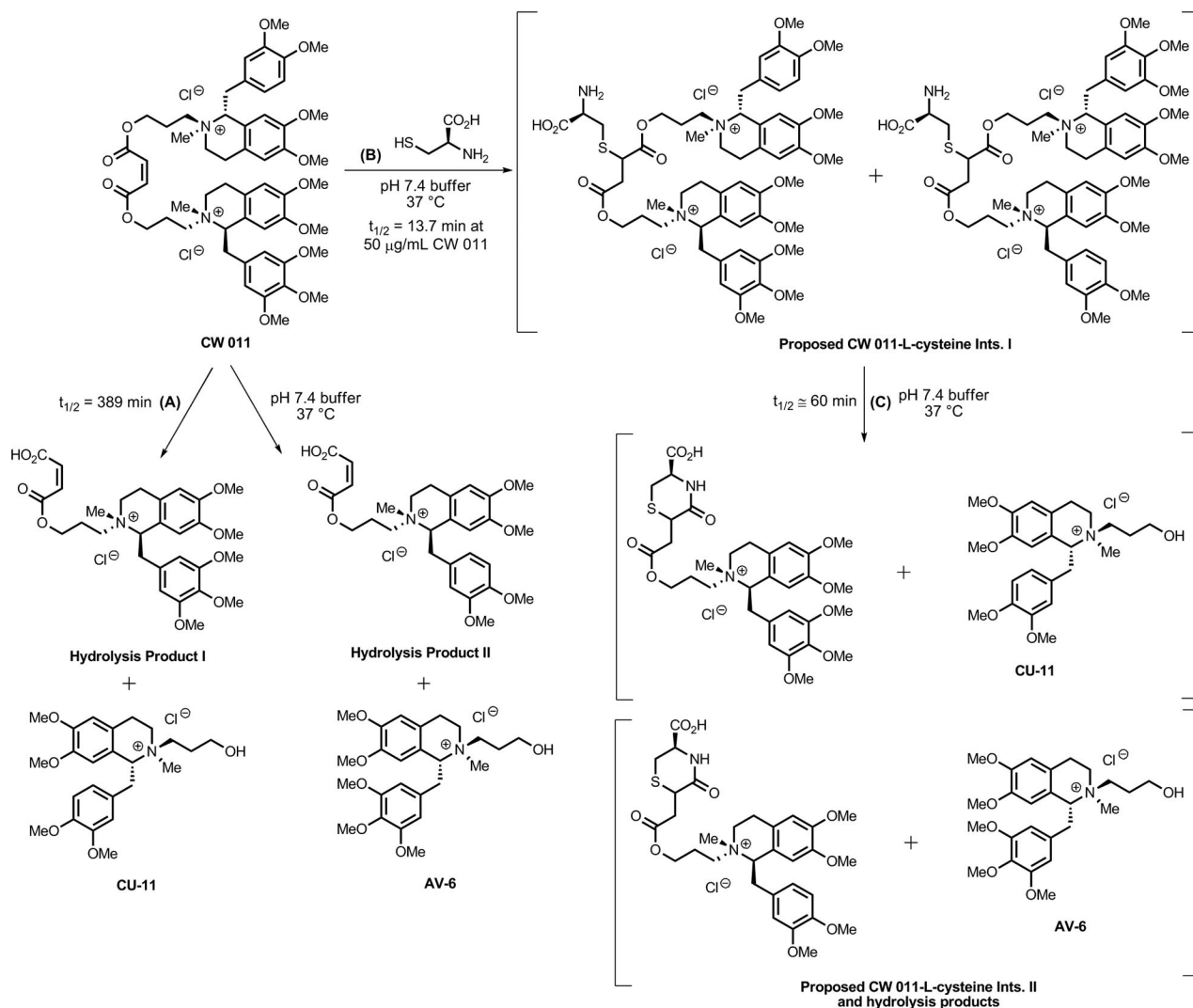
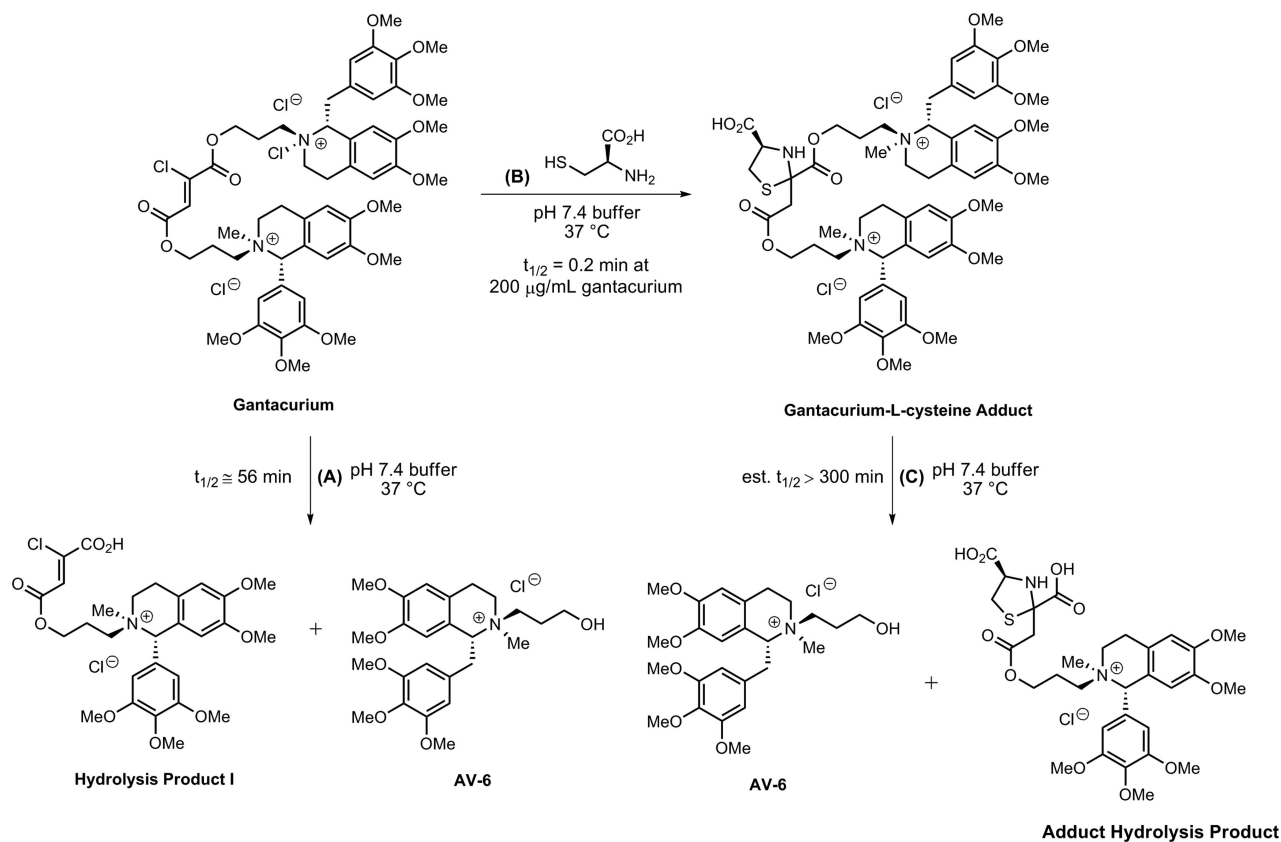


Fig. 9. Degradation of CW 011. The molecule is relatively stable at pH 7.4 and 37°C (A). Because the molecule is asymmetrical, conversion to two adducts results on addition of L-cysteine (B), and both adducts then undergo alkaline hydrolysis with reaction half-time ($t_{1/2}$) ~ 60 min (C).



Hydrolysis Products

Fig. 10. Degradation of gantacurium. The molecule is relatively unstable at pH 7.4 and 37°C and undergoes relatively rapid alkaline hydrolysis with reaction half-time ($t_{1/2}$) = 56 min (A). With addition of L-cysteine, there is rapid conversion to the adduct (B), followed by much slower alkaline hydrolysis of the adduct itself (C).