Electronic Supplementary Material (ESI) for Natural Product Reports.

Supplementary information (SI)

Marine paralytic shellfish toxins: chemical properties, mode of action, newer analogues, and structure-toxicity relationship

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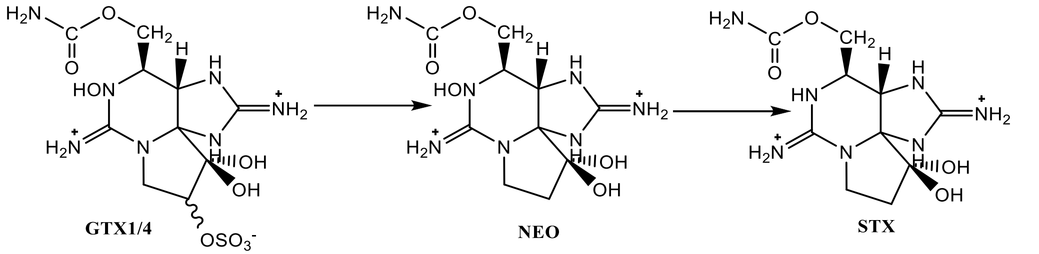
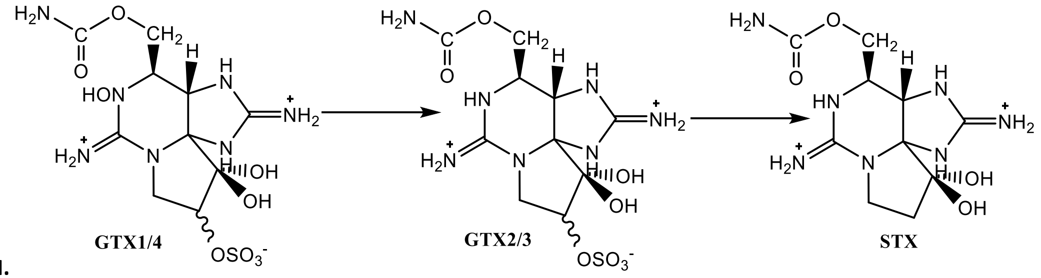
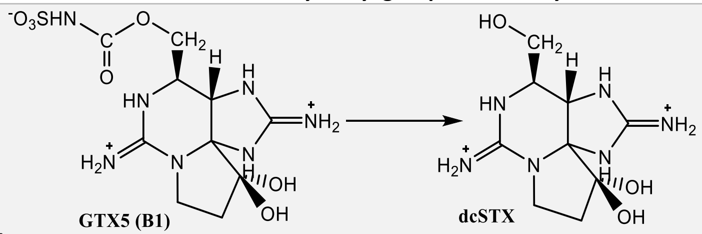
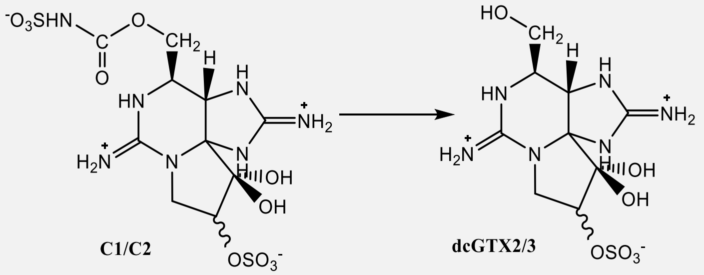
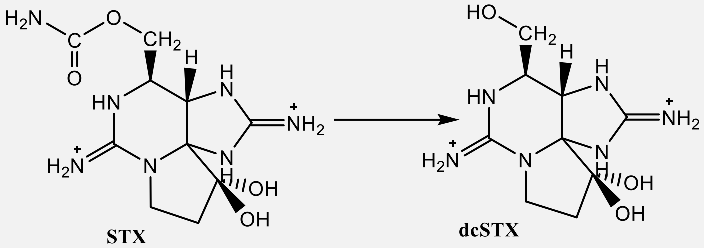
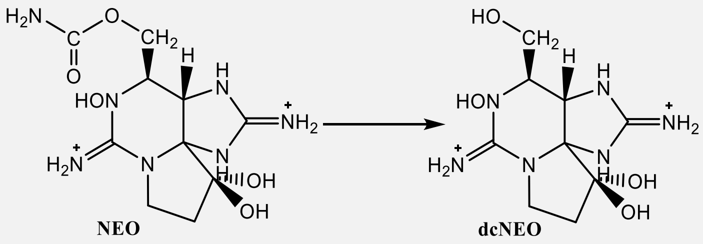
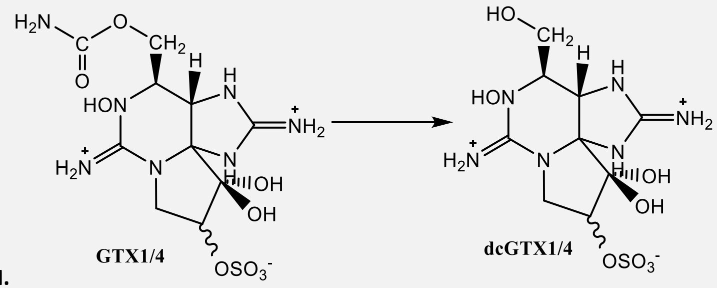
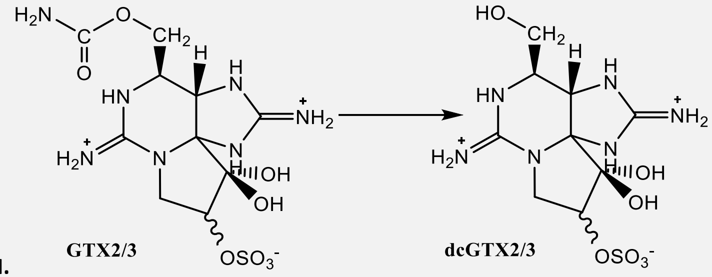
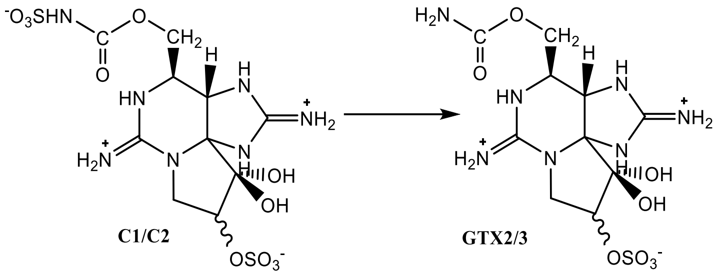
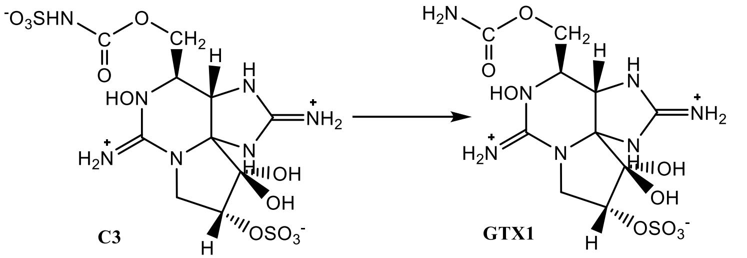
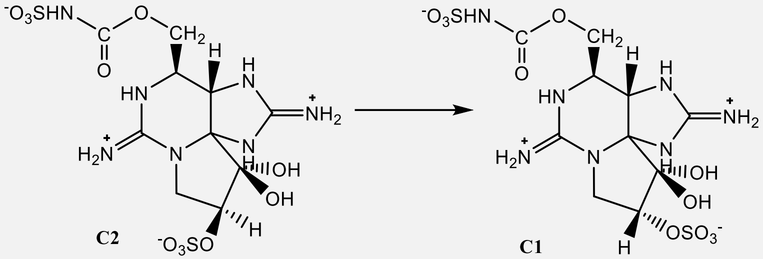
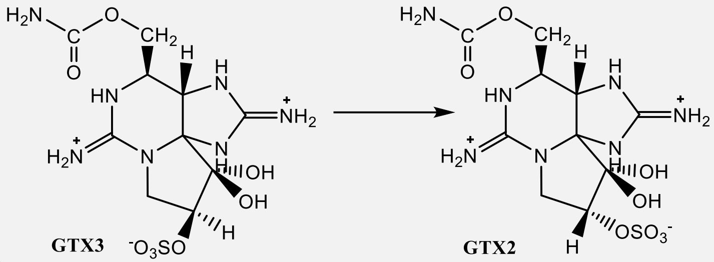
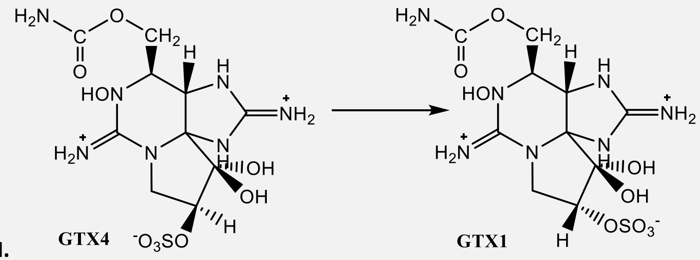
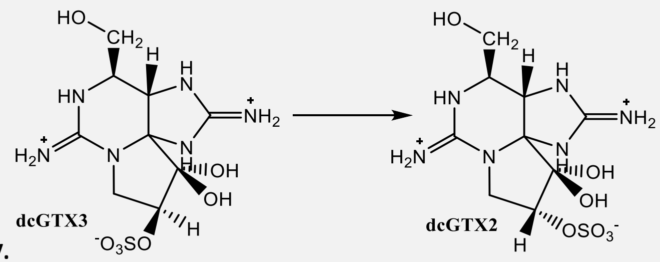
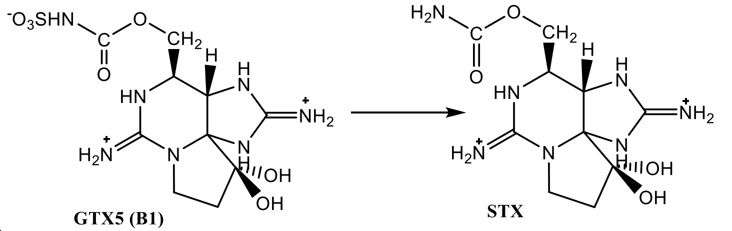
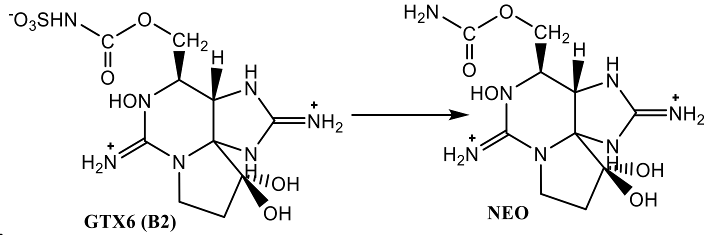
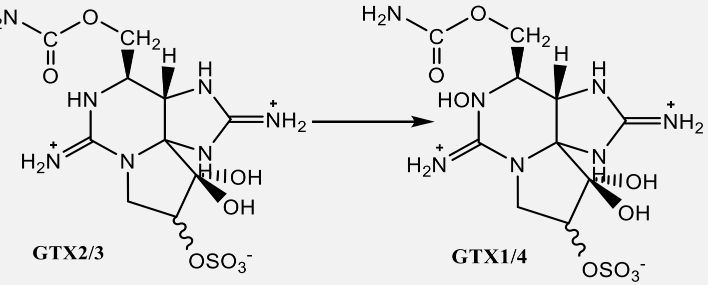
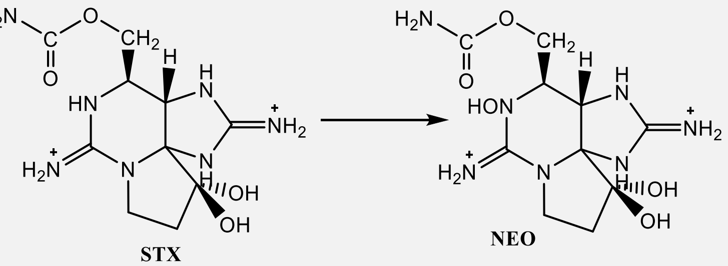
Table S1 *– Bioconversion reactions reported in dinoflagellates, shellfish and/or humans (only STXs toxins produced by marine dinoflagellates were considered).*

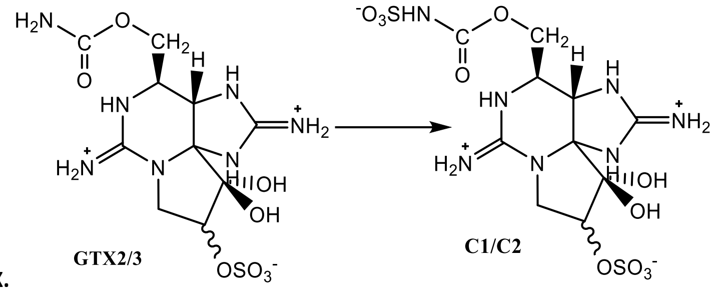
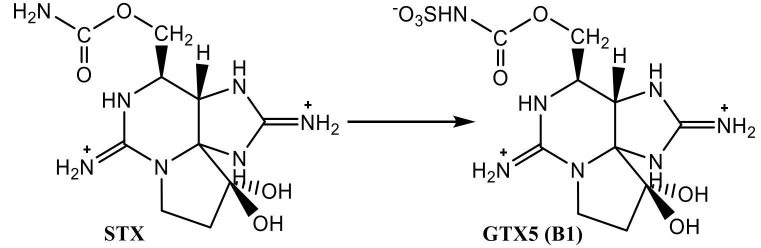
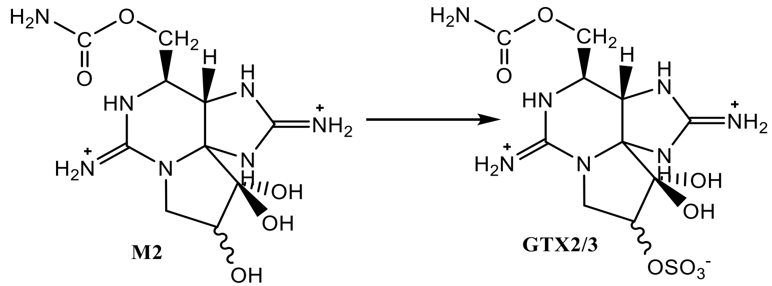
Bioconversion reactions (metabolic transformations)	**Observations**	**Organisms**		
		Dinoflagellates	**Bivalves**	**Humans**
 GTX1/4, NEO, STX **I.** **Reductive cleavage of the *O*-sulphate group followed by reductive elimination of the N-hydroxyl group.**  GTX1/4, GTX2/3, STX **II.** **Reductive elimination of the N-hydroxyl group followed by reductive cleavage of the *O*-sulphate group.**	Described in different parts of the scallops *Placopecten magellanicus*[123]; in digestive glands of scallop *Patinopecten yessoensis* contaminated with *A. tamarense*[124]; in butter clams fed with *A. catenella*[125]; in mussel *Perna viridis* contaminated with *A. minutum* (first part of reaction II)[126]. The conversion GTX2/3 into STX was reported as involving bacteria isolated from intestines of a mussel *Mytilus edulis*[127]. This reaction was first proposed in other marine species (crabs and snails), involving the bacteria *Vibrio* and *Pseudomonas* spp. isolated from their viscera (incubation ≥ 20h)[128]. Reduction of the *O*-suphate group at C11 from GTX1/4 and GTX2/3 to NEO and STX, respectively, is thought to be mediated by glutathione[124]. The conversion GTX2/3 into STX (part of reaction II) is proposed to occur also in humans (gut samples), based on the experiments performed by other authors[134].		X	X
III.  GTX5 (B1), dcSTX	Observed in littleneck clam *Protothaca staminea*, using purified toxins from *Gonyaulax* sp.[71]; from *A. excavatum* and *A. minutum*[130]; incubated with a toxin mixture[131]. Also observed in clam *Spisula solidissima* fed with *Alexandrium* spp.[101]; and (except reaction IV) in digestive glands of clams *Spisula solida* and *Scrobicularia plana* (in less extension) contaminated with *G. Catenatum*[111].		X	

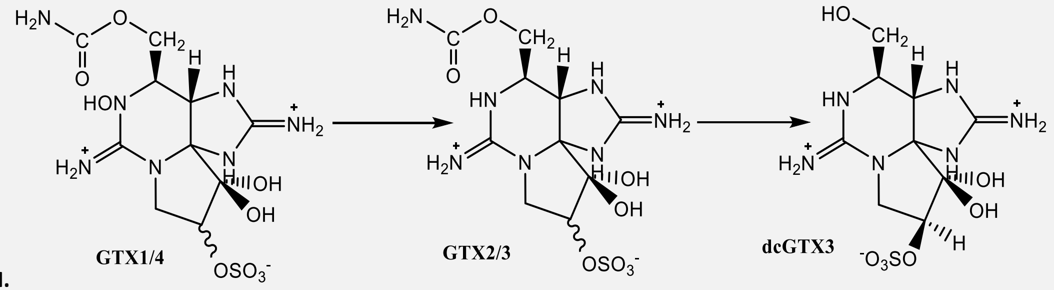
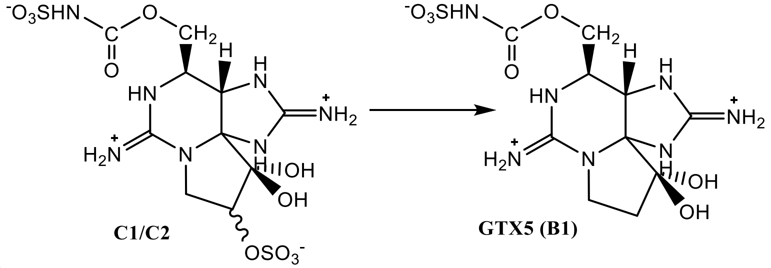
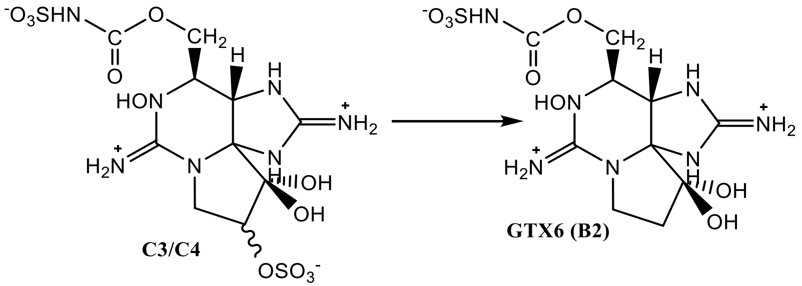
 **IV.**  **V.**  **VI.**  **VII.**	Not detected in butter clams *Saxidomus giganteus* and mussels *M. edulis*, contaminated with *Gonyaulax* sp.[71]; or in clams *M. arenaria* fed with *Alexandrium* spp.[101] and incubated with a toxin mixture[131]. Reaction IV was also described in adductor muscle of scallops *Chlamys nobilis* (higher extension) and in digestive gland of mussels *P. viridis* (lesser extension) fed with toxic *A. tamarense*[110]. Additionally, reaction VIII was observed after 24 h in mussels *M. edulis* (dcGTX3 prevailed over dcGTX2), but not observed in queen scallops *Chlamys opercularis,* after 144 h[129]. These reactions appear to be promoted by the enzymatic activity, namely by hydrolytic enzymes, the carbamoylases[71,111,130]. Reaction V was reported in post-mortem analysis of human tissues (liver, kidney, lung) [18] and human urine [134].			X

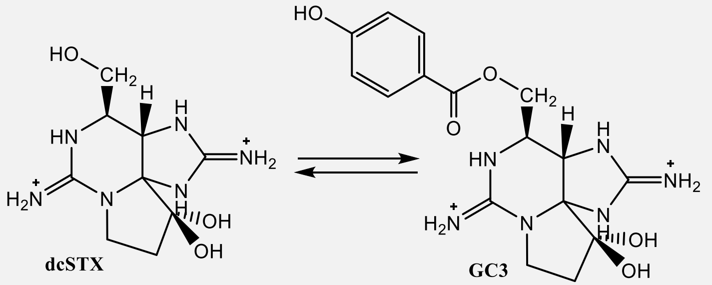
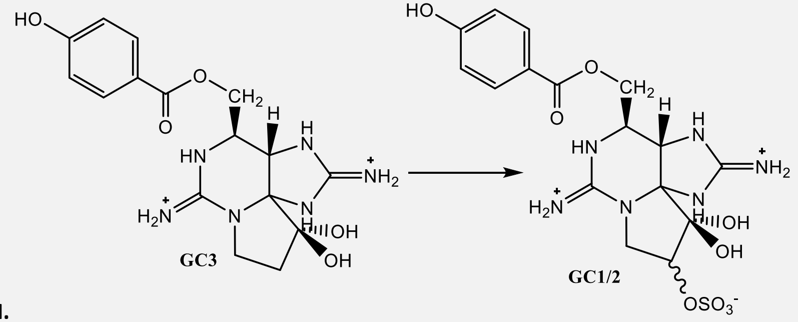
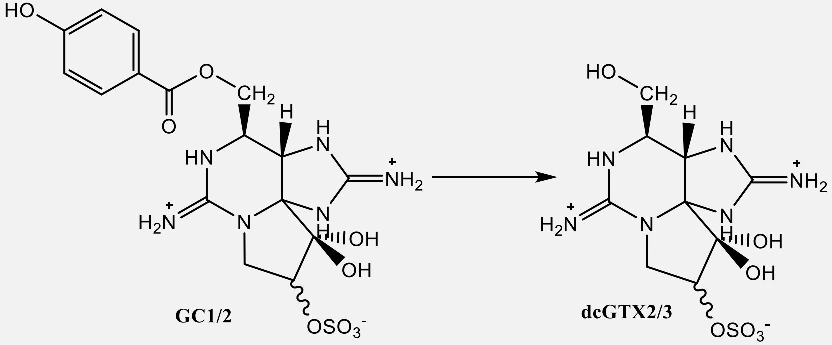
 **VIII.** **Hydrolysis of the carbamate ester from *N*-sulfacarbamoyl toxins (reactions III and IV) and from carbamate toxins (reactions V to VIII), originating some common products (decarbamoyl toxins).**				
 **IX.**  **X.** **Hydrolysis of the *N*-sulfa group (side chain), originating the correspondent carbamate.**	Reported in short-necked clams *Tapes (Amygdala) japonica*, mussels *M. edulis* and oysters *Crassostrea gigas* contaminated with two strains of *A. tamarense* isolated from toxic planktons[109]. Reaction IX reported in the northern quahog (clam) *M. mercenaria* exposed in laboratory to cultured isolates of *Alexandrium* (*A. tamarense* and *A. fundyense*)[105], and in digestive glands of mussels *P. viridis* fed with *A. tamarense*[110].		X	

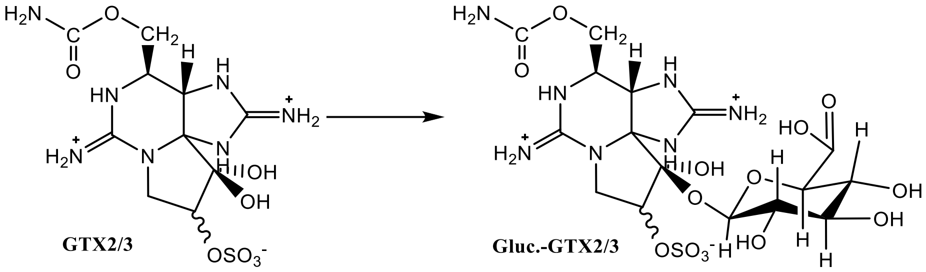
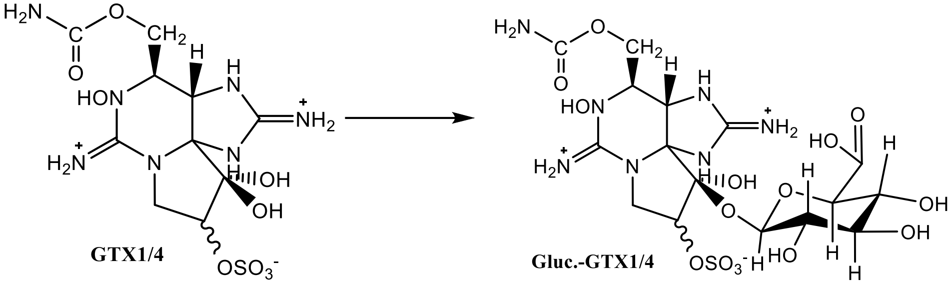
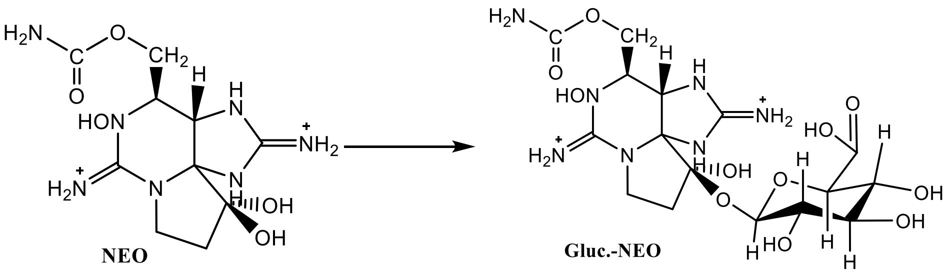
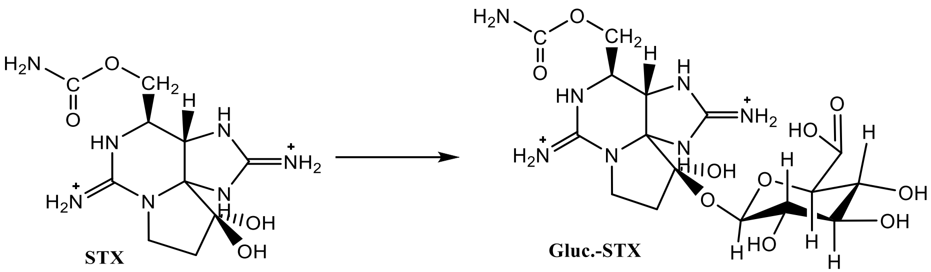
 **XI.**  **XII.**  **XIII.**  **XIV.** **Epimerization β (C2, GTX3, GTX4, dcGTX3) → α (C1, GTX2, GTX1, dcGTX2)**	Suggested (reactions XI-XIII) in mussels *M. edulis* and *Modiolus modiolus*, oysters *C. gigas*, cockles *Cardium edule*, clams *Arctica islandica* and *Mactra stultorum*, sword razor *Ensis ensis*, scallops *Pecten maximus* contaminated with *A. fundyense*[30,107]; and in oyster *C. gigas* (except reaction XIII) fed with *A. minutum*[113]. Bioconversions of C4 and dcGTX4 (β epimers) into C3 and dcGTX1 (α epimers), respectively, are also expected[106].		X	

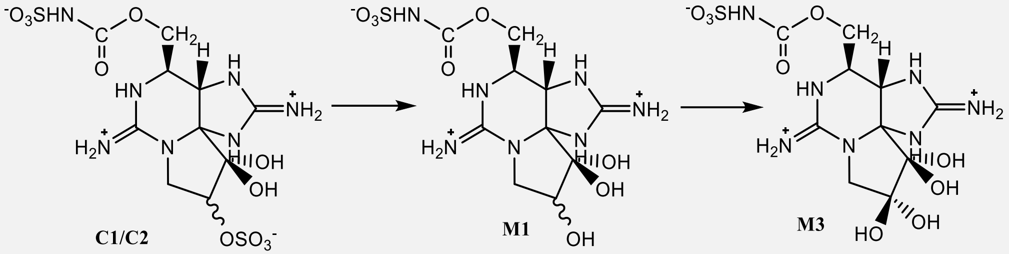
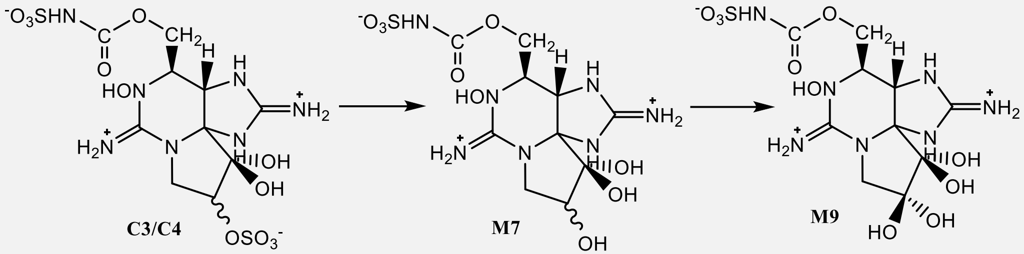
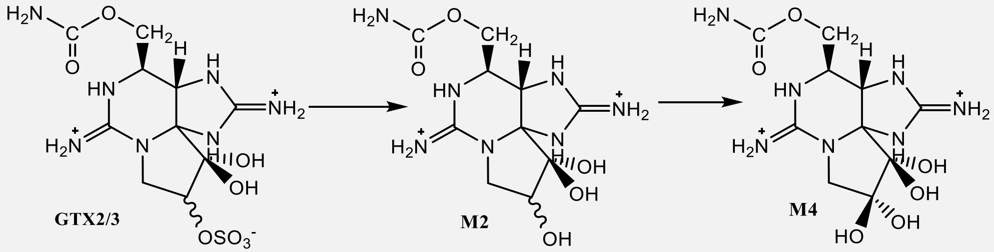
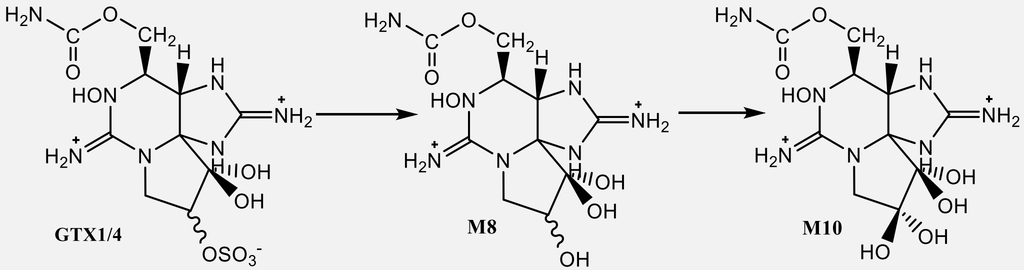
 **XV.**  **XVI.** **Hydrolysis of the *N*-sulfa group (side chain), originating the correspondent carbamate.**	Suggested in clam *Tapes japonica* fed with *A. catenella*[116]. Reaction XV documented in blue mussels [132]. Hydrolysis of the *N*-sulfa group is proposed based on the above reactions (IX – X), in agreement with Oshima[106], who also proposed reaction XVI.		X	
 **XVII.**  **XVIII.** **Oxidation at N1**	Reaction XVII proposed in *A. tamarense* extracts[106], in *G. catenatum*[120]. Both reactions also suggested in human samples[18,134-136]. These reactions in humans occurred in presence of nicotinamide adenine dinucleotide phosphate oxidase (NADPH)[135,136].	X		X

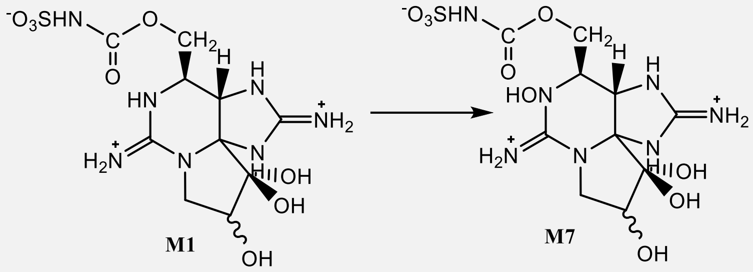
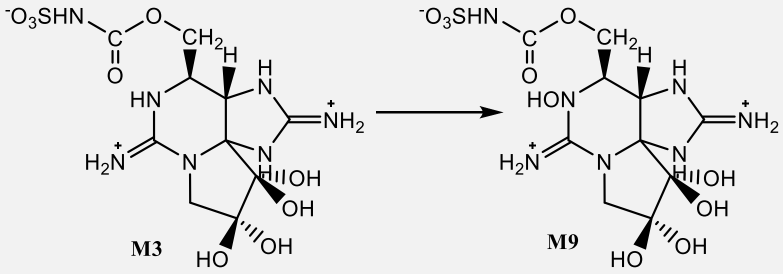
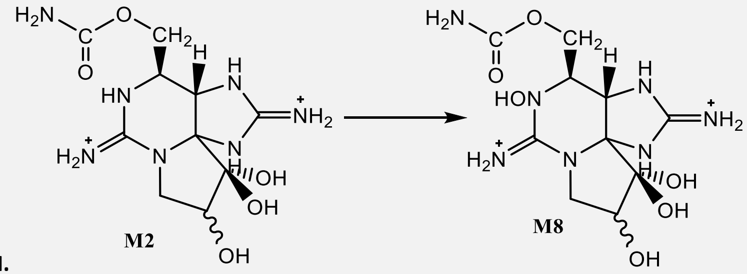
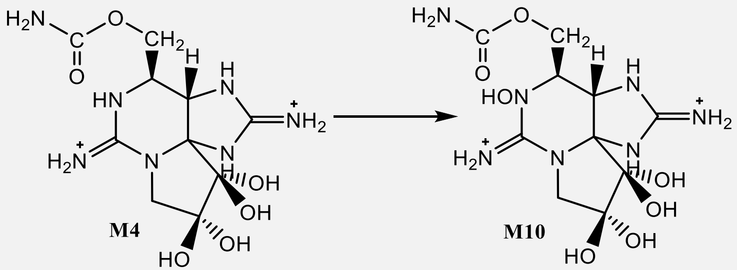
 **XIX.**  **XX.** **Introduction of a sulphate group on carbamoyl group (side chain sulfonylation, at N-21)**  **XXI.** **Introduction of a sulphate group (sulfonylation) at O-22**	Reactions proposed in cultures of *G. catenatum*[120,121]. Reaction XIX also suggested in cultures of *A. tamarense*[122] and in human samples[17]. The reaction XX did not occur when an extract of *A. tamarense* was tested, while the occurrence of reaction XXI was not reported[122]. Specific sulfotransferase (ST) enzymes have been reported to be responsible for reactions XIX and XX (N-ST) and for reaction XXI (O-ST), both using the sulphate group from 3'-phosphoadenosine 5'-phophosulfate (PAPS) as source[120,121].	X		X

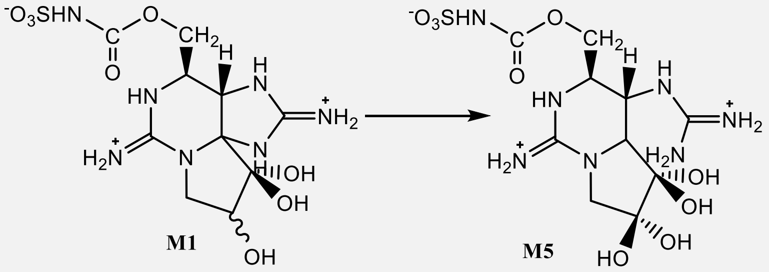
 **XXII.** **Reductive elimination of the *N*-hydroxyl group followed by hydrolysis of the carbamate ester.**	Described in mussels *M. edulis* (after 24 h)[129]. The increase of GTX2/3 was also observed in lesser extension in cockles *Cerastoderma edule* and razorfish *Ensis arcuatus*, but only after 144 h. Not observed in queen scallops *C. opercularis* (after 144 h).		X	
 **XXIII.**  **XXIV.** **Reductive cleavage of the *O*-sulphate group at C11.**	Reaction XXIII reported in digestive gland of mussels *P. viridis* fed with A. *tamarense*, but not in scallops *C. nobilis*[110]. Like other reactions (e.g., second step of reaction II), this may be mediated by natural reductants, like glutathione. Reaction XXIV is also expected[106].		X	

 **XXV.**  **XXVI.**  **XXVII.**	Reactions XXV (direct sense) and XXVI found in a *G. catenatum* strain[27]. Reactions XXV (inverse sense) and XXVII described in blue mussels (*M. galloprovincialis*), common cockles (*C. edule*), clams (*R. decussata*, *V. pullastra*, *S. plana*), offshore clams (*D. trunculus*, *S. solida*, *C. gallina*) and oysters (*Crassostrea japonica*) contaminated with *G. catenatum*[117]. The carbamoylase activity exclusively targeted at benzoate STXs is proposed to be involved in these hydrolysis reactions of the carbamate ester.	X	X	

 **XXVIII.**  **XXIX.**  **XXX.**  **XXXI.** **Glucuronidation at hydroxyl C12**	Enzymatic transformations proposed in human liver microsomes after incubation with purified STXs[135,136]. The reactions occurred in the presence of uridine 5'-diphospho-glucuronic acid (UDPGA), which transferred the glucuronosyl group to the toxin. Glucuronidation products were searched in scallops *Chlamys farreri* and mussels *M. galloprovincialis* exposed to *A. pacificum*, but they were not found[114].			X

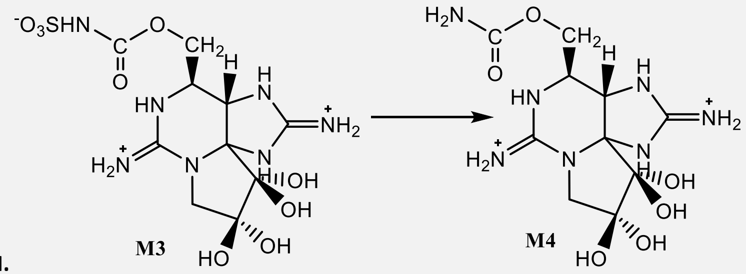
 **XXXII.**  **XXXIII.**  **XXXIV.**  **XXXV.** ***O*-desulfonylation at C11 followed by the addition of an oxyhydroxide (hydroxylation)**	Bioconversions XXXII to XXXVII proposed in mussels *M. galloprovincialis* contaminated from *A. tamarense* strains[29]. Reactions XXXII to XXXV were also suggested in scallops *C. farreri* and mussels *M. galloprovincialis* exposed to *A. pacificum*[114], but the authors did not detect M4 or M10 toxins. Reactions XXXII, XXXV, XXXVIII, XXXIX and XLII suggested based on STX analogues identified in clams *Saxidomus purpuratus* and scallops *P. yessoensis*[133]. M10 formation was also proposed to occur from NEO in mussels *M. galloprovincialis* (an intermediate product may be formed)[50].		X X	

 **XXXVI.**  **XXXVII.**  **XXXVIII.**  **XXXIX.** **Oxidation at N1**	Reactions XXXII, XXXIII, XXXVI, XXXVII described in mussels *M. edulis* fed with toxic *A. fundyense* culture[30]. Additionally, reactions XL to XLIV were suggested. The second step of reaction XXXII (hydroxylation of M1 into M3) was suggested in several bivalve species (mussels *M. galloprovincialis*, cookles *C. edule*, clams *Ruditapes decussatus* and *Donax trunculus*, and razor clams *Ensis* spp.) contaminated with *G. catenatum*[112]. However, this author proposed another precursor to M1 (reaction XLV).		X	

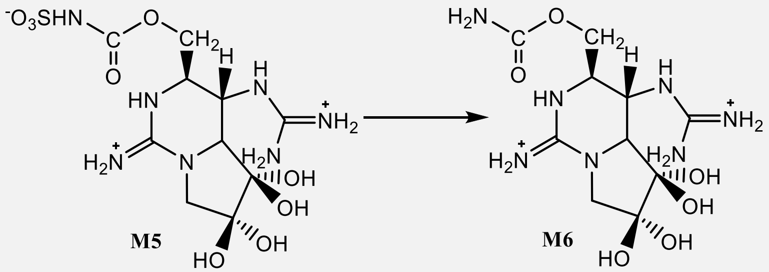


XL.

Loss of H_2O followed by the attack of hydroxyl group at C11 and consequent opening of ring at C4.

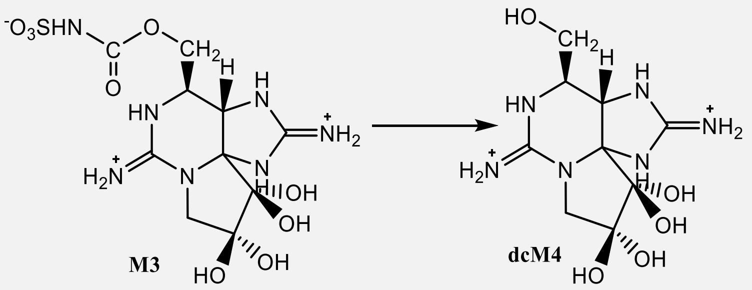


XLI.

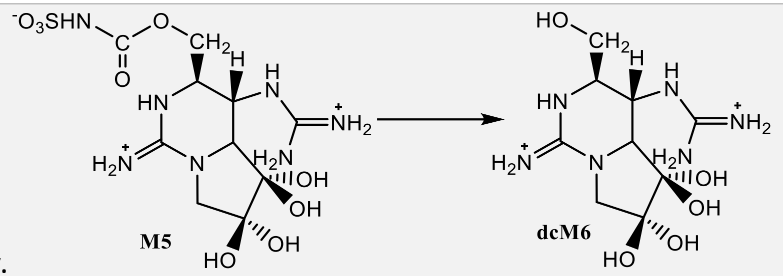
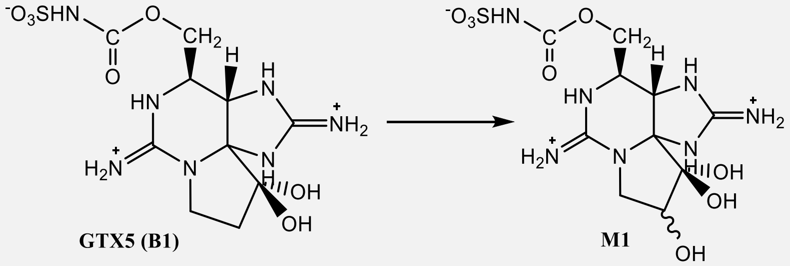


XLII.

Hydrolysis of the *N*-sulfa group (side chain), originating the corresponding carbamate.



XLIII.

 **XLIV.** **Hydrolysis of the carbamate ester from *N*-sulfacarbamoyl toxins, originating the corresponding decarbamoyl toxins.**  **XLV.** **Hydroxylation at C11**				