

PREPARATIVE SCALE CONVERSION OF GONYAUTOXINS TO NEOSAXITOXIN

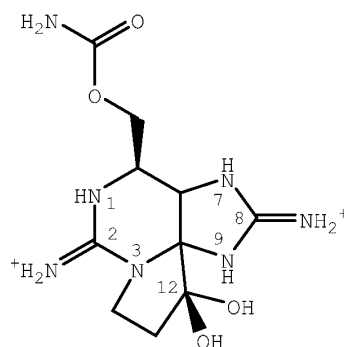
TECHNICAL FIELD

The invention relates to the conversion of a mixture of gonyautoxin 1 (GTX1) and gonyautoxin 4 (GTX4) to neosaxitoxin (neoSTX) on a preparative scale and the purification of the neoSTX for use as an active pharmaceutical ingredient (API).

BACKGROUND ART

As stated in the publication of Garcia-Altare (2017), marine microalgal toxins constitute one of the most diverse and sophisticated groups of natural products. Examples are paralytic shellfish toxins (PSTs) such as saxitoxin (STX), its analogues and derivatives. Gonyautoxins (GTXs) are sulphated analogues of STX and marine bacteria can transform GTXs into STX through reductive eliminations. In marine environments the main producers of STX are eukaryotic dinoflagellates.

STX is a monoterpene indole alkaloid containing a tricyclic 3,4-propinoperhydropurine system with 2 guanidinium moieties formed by the NH_2 groups in the positions C2 and C8 of the reduced purine:



STX blocks voltage-gated sodium channels (VGSCs), but also binds to calcium and potassium channels. The nature of the substituents greatly influences the overall toxicity of saxitoxin analogues. The hydroxylation of N1, e.g. as in neosaxitoxin (neoSTX) does not play a major role in binding affinity, but seems to increase potency.

The prior art is replete with disclosures of the cosmetic and therapeutic applications of PSTs, including their use as local anaesthetics and analgesics. The publication of Mezher (2018) discloses that the US Food and Drug Administration (FDA) plans to develop guidance documents to encourage the development of extended-release local anaesthetics which could replace the need for systemic oral opioids in certain situations. The expectations of

the US FDA are for the development of new non-opioid drugs to treat chronic pain that could provide a safer alternative for patients who require long-term use of analgesic drugs. The publications of Kohane *et al* (2000), Rodriguez-Navarro *et al* (2011), Templin *et al* (2015) and Wylie *et al* (2012) disclose the use of neoSTX in these applications. A limitation on the exploitation and widespread adoption of these applications is the availability of the PSTs in sufficient quantity and of sufficient purity to render their use in the manufacture of pharmaceutical preparations commercially viable.

The following publications disclose the preparation of gonyautoxin 1 (GTX1), gonyautoxin 4 (GTX4) or neosaxitoxin (neoSTX). Often the preparation is on an analytical scale, or does not provide the quantity and purity required for use of the preparation as an active pharmaceutical ingredient (API).

The publication of Hall *et al* (1984) discloses the confirmation by x-ray crystallography of the position and identity of the three substituents which, with the parent compound, form the array of twelve saxitoxins found in *protogonyaulax*.

The publication of Daigo *et al* (1985) discloses the extraction and isolation of neosaxitoxin (neoSTX) from specimens of crab. The dose-death time curve obtained for the isolated neoSTX was clearly distinguishable from the curve for saxitoxin (STX).

The publication of Laycock *et al* (1994) discloses methods for the purification of some of the common paralytic shellfish poisoning (PSP) toxins in quantities sufficient for use as analytical standards. The PSP toxins were purified from the dinoflagellate *Alexandrium excavatum*, the giant sea scallop (*Plagopecten magellanicus*) hepatopancreas and the cyanobacterium *Aphanizomenon flos-aquae*.

The publication of Ravn *et al* (1995) discloses what are asserted to be optimal conditions for extraction of paralytic shellfish toxins from a clone of *Alexandrium tamarense*. The paralytic shellfish toxins are extracted with acetic acid and hydrochloric acid in the concentration range 0.01 to 1.0 N. Concentrations of hydrochloric acid in the range 0.03 to 1.0 N were observed to cause the amount of C1 and C2 toxins to decrease sharply with a concomitant increase in the amount of gonyautoxins 2 (GTX2) and 3 (GTX3).

The publication of Tsai *et al* (1997) discloses the detection of paralytic toxicity by a tetrodotoxin bioassay in specimens of crab. Partial purification and characterisation of the toxins demonstrated the main toxin to be tetrodotoxin with minor amounts of gonyautoxins (GTXs) and neosaxitoxin (neoSTX).

The publication of Siu *et al* (1997) discloses the examination of the effects of environmental and nutritional factors on population dynamics and toxin production in *Alexandrium catenella*. Optimum conditions for the growth of this species of dinoflagellate are disclosed along with the toxin profile for a species grown under these conditions. The toxin profile as detected by HPLC was found to include in descending order GTX4, GTX3, GTX1, B2, neosaxitoxin (neoSTX) and saxitoxin (STX).

The publication of Sato *et al* (2000) discloses the transformation of the O-sulfate group of GTX1 and GTX4 to methylene to form neosaxitoxin. The transformation was achieved using thiols such as glutathione and intermediates of the conversion were isolated.

The publication of Parker *et al* (2002) discloses an investigation of the autotrophic growth of the toxic dinoflagellate, *Alexandrium minutum*, in three different high biomass culture systems, assessing growth, productivity and toxin production. The organism was grown in aerated and non-aerated two litre Erlenmeyer flasks, 0.5 litre glass aerated tubes, and a four litre lab scale alveolar panel photobioreactor. A marked increase in biomass and productivity in response to aeration was observed. A maximum cell concentration of 3.3×10^5 cells/mL, a mean productivity of 0.4×10^4 cells/mL/day and toxin production of approximately 20 µg/L/day with weekly harvesting was reported.

The publication of Baker *et al* (2003) discloses the production by bacterial strains isolated from saxitoxin-producing dinoflagellates of compounds that could easily be mistaken for gonyautoxin 4 (GTX4).

The publication of Miao *et al* (2004) discloses the isolation of gonyautoxins (GTX1, GTX2, GTX3 and GTX4) from two strains of *Alexandrium minutum* Halim. The strain of *Alexandrium minutum* Halim designated Amtk4 is asserted to be suitable for the preparation of gonyautoxins.

The publication of Jiang and Jiang (2008) discloses the establishment of optimal conditions for the extraction of paralytic shellfish poisoning toxins from the gonad of *Chlamys nobilis*. The extraction uses acetic acid and hydrochloric acid in the concentration range of 0.04 to 1.0 mol/L. The use of hydrochloric acid in the concentration range of 0.25 to 1.0 mol/L was shown to cause a significant decrease of the toxins C1, C2 and GTX5 and the concomitant increase in the toxins GTX2,3. The amount of the three unstable toxins did not show any change when acetic acid was used in the extraction.

The publication of Liu *et al* (2010) discloses the culture of toxin producing *Alexandrium catenella* in the laboratory. A maximum cell density of 0.4×10^4 cells/mL was obtained within eight days of culture. Analysis by high performance liquid chromatograph (HPLC) of a crude extraction showed the

major toxic components to be C1/2, GTX4, GTX5 and neoSTX at concentrations of about 0.04550, 0.2526, 0.3392, 0.8275 and 0.1266 $\mu\text{mol/L}$, respectively.

The publication of Foss *et al* (2012) discloses a comparison of extraction methods for paralytic shellfish toxins (PSTs) from the filamentous cyanobacterium *Lyngbya wollei*. In the absence of commercially available standards for the unique toxins produced by this cyanobacterium it was not possibly to quantify the toxins extracted.

The publication of Li *et al* (2013) discloses a method for the rapid screening and identification of paralytic shellfish poisoning (PSP) toxins in red tide algae. The method utilises hydrophilic interaction chromatography-high resolution mass spectrometry (HILIC-HR-MS) combined with an accurate mass database. Limits of detection (LOD) of ten common PSP compounds were in the range of 10 to 80 nmol/L. The developed method was asserted to be a useful tool for the rapid screening and qualitative identification of common PSP toxins in harmful algae.

The publication of Bernardi Bif *et al* (2013) discloses the sensitivity of sea urchins to toxic cell extracts containing saxitoxins.

The publication of Poyer *et al* (2015) discloses the development of an analytical method to characterise and differentiate saxitoxin analogs, including sulfated (gonyautoxins) and non-sulfated analogs. Hydrophilic interaction liquid chromatography (HILIC) was used to separate sulfated analogs. Ion mobility mass spectrometry (IM-MS) was used as a new dimension of separation based on ion gas phase confirmation to differentiate the saxitoxin analogs. Positive and negative ionisation modes were used for gonyautoxins. Positive ionisation mode was used for non-sulfated analogs. The coupling of three complementary techniques, HILIC-IM-MS, permitted the separation and identification of saxitoxin analogs, isomer differentiation being achieved in the HILIC dimension with non-sulfated analogs separated in the IM-MS dimension.

The publication of Rubio *et al* (2015) discloses a method to purify saxitoxin using a liquid chromatography methodology based on ionic pairs. The saxitoxin is extracted using hydrochloric acid and treated with ammonium sulfate following a treatment with trichloroacetic acid and hexane/diethyl ether (97/3). Samples were analysed by a semi-preparative HPLC in order to collect pure fractions of saxitoxin and these fractions were eluted in solid-phase cationic interchange STX extraction columns. The purified saxitoxin was reported to be stable and homogenous and its identity confirmed by LC-MS-MS. Analogs such as neosaxitoxin of a decarbamoyl saxitoxin were reported to be absent from the purified saxitoxin.

The publication of Chen et al (2016) discloses the application of serial coupling of reverse-phase liquid chromatography (RPLC) and hydrophilic interaction chromatography (HILIC) combined with high resolution mass spectrometry (HR-MS) to the simultaneous screening and identification of known lipophilic and hydrophilic toxins in the algae of harmful algal blooms (HABs). Lipophilic and hydrophilic toxins were extracted simultaneously by the use of ultrasound-assisted extraction (UAE). The publication demonstrated that HPLC/HILIC-HR-MS combined with an accurate mass list of known marine algal toxins may be used as a powerful tool for screening of different classes of known toxins in marine harmful algae.

The publication of Cho et al (2016) discloses the analysis of crude extracts of toxin-producing dinoflagellates by column switching and two-step gradient elution using hydrophilic-interaction chromatograph (HILIC) combined with mass spectrometry. The publication states that the data obtained supports the hypothesis that the early stages of the saxitoxin biosynthesis and shunt pathways are the same in dinoflagellates and cyanobacteria.

The publication of Beach et al (2018) discloses the sensitive multiclass analysis of paralytic shellfish toxins, tetrodotoxins and domoic acid in seafood using a capillary electrophoresis (CE)-tandem mass spectrometry (MS/MS) method. A novel, highly acidic background electrolyte comprising 5 M formic acid was used to maximise protonation of analytes and is asserted to be generally applicable to simultaneous analysis of other classes of small, polar molecules with differing pKa values.

The publication of Kellmann and Neilan (2007) discloses the fermentative production of neosaxitoxin and its analogs in recombinant *Escherichia coli* strains.

The publications of Lagos Gonzáles (2010, 2015a, 2015b and 2016) disclose the purification of phycotoxins from cyanobacteria produced in a continuous culture. The phycotoxins are isolated primarily from the bacteria, but can also be isolated from the culture medium. In one embodiment of the process disclosed only neosaxitoxin (neoSTX) and saxitoxin (STX) are produced. In another embodiment of the process disclosed only gonyautoxin 2 (GTX2) and gonyautoxin 3 (GTX3) are produced.

The publication of Wang et al (2010) discloses the preparation of a paralytic shellfish poison (PSP) standard solution. The standard solution is prepared by removing impurities from shellfish material, collecting shellfish meat, adding distilled water and 0.1-0.3 mol/L hydrochloric acid solution, regulating pH to 1.5 to 5.0, and homogenising to obtain homogenate, precooling at -20°C for 30 minutes to 24 hours, and lyophilising to obtain a core sample, grinding, and sieving, precooling at -20°C for 10 minutes to six

hours and lyophilising to obtain the standard sample. The method of preparation is asserted to have the advantages of low raw material cost and a simple preparation process.

The publication of Xiong and Qiu (2009) discloses the application of biguanido purine derivatives and their salts and esters for improving the therapeutic effect and reducing the side effects of antitumor agents. The biguanido purine derivatives are saxitoxin analogs.

It is an object of the invention to provide a method of preparing neosaxitoxin in sufficient quantity and of sufficient purity to enable its use in the manufacture of pharmaceutical preparations. This object is to be read in the alternative with the object to at least provide a useful choice.

SUMMARY OF INVENTION

In a **first** aspect the invention provides a method of preparing a quantity of neoSTX comprising the steps of:

1. contacting in solution in a reaction solvent a quantity of GTX1,4 and a quantity of dithiol for a period of time and at a temperature sufficient to provide a reaction product in which greater than 97.5% (w/w) of the GTX1,4 has been converted to neoSTX; and then
2. applying the reaction product to a silica based weak cation exchange sorbent and eluting with an aqueous weak organic acid to separate the neoSTX from the dithiol and provide the quantity of neoSTX,

where the pH of the solution is in the range 7.4 to 7.6.

Preferably, the reaction solvent is buffered aqueous acetic acid.

Preferably, the dithiol is selected from the group consisting of dithiothreitol (DTT) and dithiobutylamine (DTBA). More preferably, the dithiol is dithiothreitol (DTT).

Preferably, the quantity of neoSTX is greater than 100 mg with a purity greater than 99.5% (w/w).

Preferably, the quantity of GTX1,4 is of a purity of at least 97.5% (w/w). More preferably, the quantity of GTX1,4 is of a purity of at least 98.75% (w/w). More preferably, the quantity of GTX1,4 is of a purity of at least 99% (w/w).

Preferably, the method of preparing a quantity of neoSTX is a near quantitative method.

The method of the invention provides for the batch preparation of neoSTX in a quantity and of a purity not previously obtainable (*cf.* Lagos Gonzáles (2010, 2015a, 2015b and 2016)).

In the description and claims of this specification the following abbreviations, acronyms, phrases and terms have the meaning provided: "batch preparation" means prepared discontinuously; "biosynthetic" means prepared within living organisms or cells; "CAS RN" means Chemical Abstracts Service (CAS, Columbus, Ohio) Registry Number; "comprising" means "including", "containing" or "characterized by" and does not exclude any additional element, ingredient or step; "consisting of" means excluding any element, ingredient or step not specified except for impurities and other incidentals; "consisting essentially of" means excluding any element, ingredient or step that is a material limitation; "GTX" means gonyautoxin; "GTX1" mean gonyautoxin 1 [CAS RN 60748-39-2]; "GTX4" means gonyautoxin 4 [CAS RN 64296-26-0]; "GTX1,4" means an unresolved mixture (as solid or in solution) comprising gonyautoxin 1 and gonyautoxin 4; "GTX2,3" means an unresolved mixture (as solid or in solution) comprising gonyautoxin 2 and gonyautoxin 3; "near quantitative" means greater than 97.5 % (w/w) of substrate, e.g. GTX1,4, is converted to product, e.g. neoSTX; "neoSTX" means (3aS,4R,10aS)-2-amino-4-[[[(aminocarbonyl)oxy]methyl]-3a,4,5,6,8,9-hexahydro-5-hydroxy-6-imino-1H,10H-pyrrolo[1,2-c]purine-10,10-diol [CAS RN 64296-20-4]; "preparative scale" means prepared in batches of greater than 100 mg; and "semi-synthetic" means prepared by chemical conversion of an at least partially purified biosynthetic precursor. A paronym of any of the defined terms has a corresponding meaning.

The terms "first", "second", "third", etc. used with reference to elements, features or integers of the matter defined in the Summary of Invention and Claims, or with reference to alternative embodiments of the invention, are not intended to imply an order of preference. Where concentrations or ratios of reagents are specified the concentration or ratio specified is the initial concentration or ratio of the reagents. Where values are expressed to one or more decimal places standard rounding applies. For example, 1.7 encompasses the range 1.650 recurring to 1.749 recurring. Purity of the isolated neoSTX is determined according to Method 3 [F. Analysis].

The invention will now be described with reference to the following example and figure of the accompanying drawings page.

BRIEF DESCRIPTION OF FIGURES

Figure 1. Plot of concentration of gonyautoxins (GTX 1 (♦) and GTX 4 (■)) versus time.

DESCRIPTION

The publication of Laycock *et al* (1994) discloses the extraction and purification of GTX1 and GTX4 from hepatopancreas of scallops (*Placopecten magallanicus*):

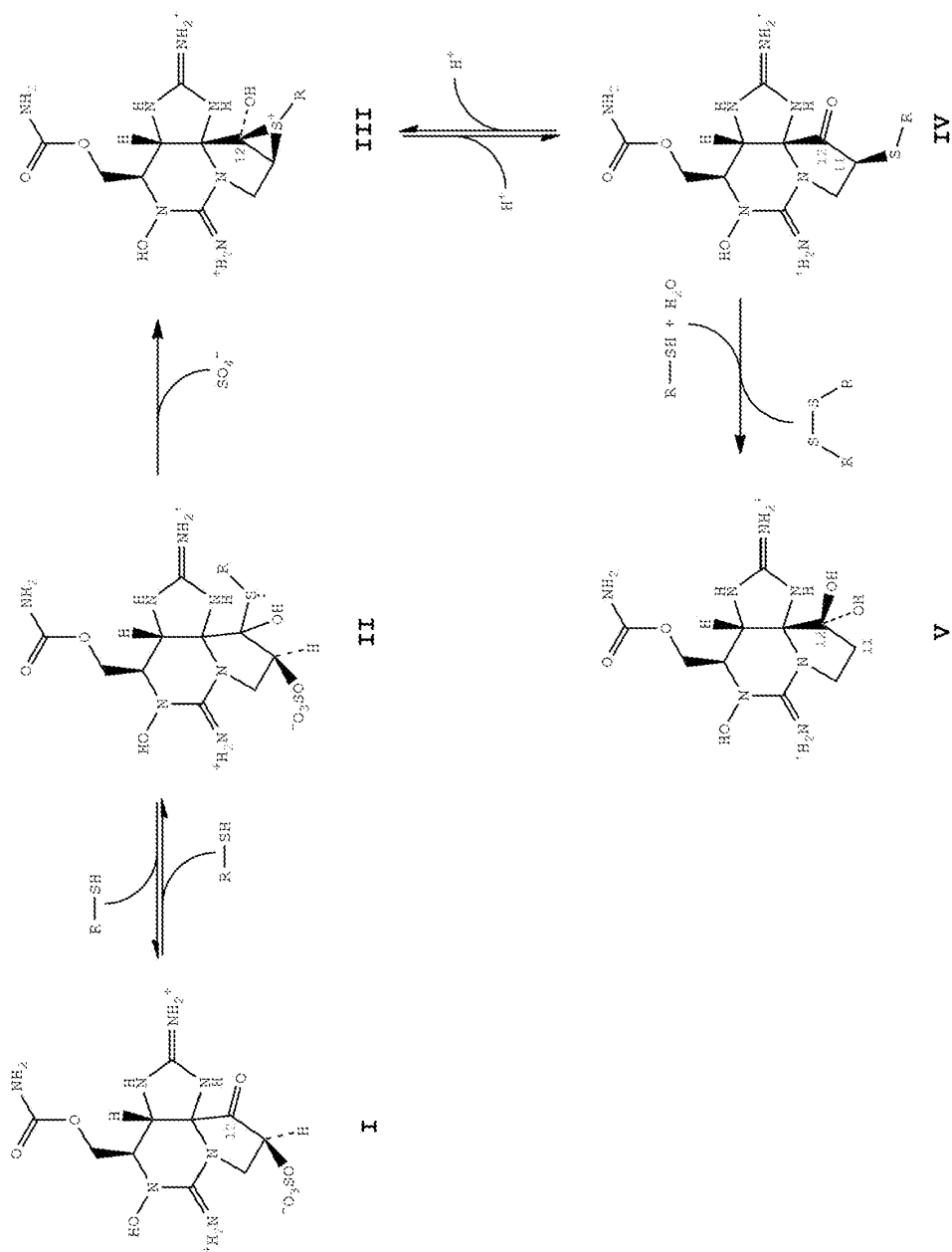
5 *Tissues (1 kg) were homogenized in 1 L of 0.1 M HCl using a Polytron tissue homogenizer, (Model PT10/35, Brinkman Instruments Canada Ltd, Rexdale, ON). The slurry was heated to 80°C for 30 min, then cooled and centrifuged (5,000g, 20 min) to remove precipitated protein. The supernatant fluid was extracted twice with dichloromethane (500 ml*
10 *each). The aqueous layer was concentrated by rotary evaporation to 200 ml then poured onto a column (10 cm ID x 15 cm) of a mixture of activated charcoal (Norite, A, 500 g, BDH Ltd.) and Celite (500 g, Johns-Manville). The column was washed with a solution of 20% ethanol and 1% acetic acid. Several one liter fractions were*
15 *collected and toxin concentrations monitored by HPLC-FD. Toxin-containing fractions were concentrated by rotary evaporation and lyophilized.*

The publication further discloses separation of Bio-Rex-70 was not complete for any of the gonyautoxins. However, by repeatedly removing GTX2 and re-equilibrating the mixture, the proportion of GTX2 and GTX3 contaminating the GTX1 and GTX4 fractions was gradually reduced.

The publication of Laycock *et al* (1995) discloses that dithiothreitol at a concentration of 100 mM in aqueous solution at pH 8.5 rapidly converted GTX1,4 to NeOSTX and a small (less than 10%) amount of neosaxitoxinol (as
25 determined by capillary electrophoresis). By contrast, it has now been determined that when performing the conversion at a preparative - as opposed to analytical - scale the optimum pH is lower and in the range 7.2 to 7.8, more specifically 7.4 to 7.6, when dithiothreitol (DTT) is used as the reducing agent.

30 In solution, GTX1 and GTX4 are believed to exist as a pair of epimers of which GTX1 is the thermodynamically most favoured. Epimerisation is believed to occur under most conditions via keto-enol equilibration at C-12. In the first step of the postulated 2-step reaction mechanism according to SCHEME I a thiol group of the dithiol (R-SH) attacks the electrophilic C-12 of the
35 keto form (I) to form a thiohemiketal (II). Conversion to a thioether (IV) occurs via an episulfonium ion intermediate (III) when the leaving group (O-sulfate) is oriented anti to the sulphur atom (as in the reactive epimer GTX1). In the second step of the proposed reaction mechanism the thiol group

SCHEME I



of the dithiol reacts with the sulphur of the thioether (IV) to form a disulfide thereby yielding an enolate that readily hydrates to neoSTX (V).

The optimal pH for the conversion of GTX1,4 to neoSTX described in the following example has been determined to be around 7.5, and without wishing to be bound by theory it is believed that this pH ensures both (i) an optimal rate of epimerisation between the gonyautoxin epimers and (ii) optimal degrees of electrophilicity at C-12 and deprotonation of the dithiol used as the reducing agent. The use of dithiols such as dithiothreitol (DTT) and dithiobutylamine (DTBA) is preferred over the use of monothiols such as glutathione (GSH) and mercaptoethanol (ME) (*cf.* Sakamoto *et al* (2000) and Sato *et al* (2000)). Higher rates of conversion are obtained when using the dithiols, rendering them more suitable for use in the production of neoSTX on a preparative scale

The excess dithiol, sodium phosphate buffer and unreacted GTX1,4 has been found to be most conveniently removed from the neoSTX containing conversion product by the use of cation exchange chromatography. The silica based weak cation exchange sorbent Septra™ WCX has been determined to be a suitable sorbent as it has been determined not to retain DTT. Trials of the polymeric based weak cation exchange sorbent Strata-X™ CW (Phenomenex) determined this sorbent to be unsuitable for purification of neoSTX from the conversion product on a preparative scale. The excess dithiol is retained by both an ion exchange and a reverse phase mechanism when using this sorbent. Although a portion of the excess DTT is eluted with organic solvents such as acetonitrile/water a further portion is eluted with 1 M acetic acid frustrating the purification of the neoSTX when using this sorbent.

A. Analysis

Samples of GTX1,4 or neoSTX are diluted to a concentration of 200 µg/mL in 10 mM acetic acid. The diluted sample is then further diluted 100-fold in 8% acetonitrile/0.25% acetic acid to provide a solution of product at a concentration of 20 mg/mL for quantitative analysis. A mixed standard containing a number of reference paralytic shellfish toxins (PSTs) is also prepared in the same solvent. A solution of 2 µL of the diluted product (20 ng/mL) is injected by means of an autosampler maintained at a temperature of 40°C onto a column (2.1 x 100 mm) of 1.7 µm Waters Acquity UPLC BEH amide eluted at a flow rate of 0.6 mL/min while being maintained at a temperature of 60°C. The column is eluted stepwise with 80% Mobile Phase B/20% Mobile Phase C for a period of time of 6 minutes following injection, followed by 55% Mobile Phase B/45% Mobile Phase C for a period of time of 0.50 minutes before reverting to 80% Mobile Phase B/20% Mobile Phase C. The eluate is monitored by mass spectrometry monitoring in ESI- and ESI+ ionisation modes.

B. Conversion

A quantity of 183 mg (as the free base) of GTX1,4 is dissolved in a total volume of 5 mL of dilute acetic acid and mixed with a volume of 45 mL of 0.2 M phosphate buffer at a pH of 7.5 in a 100 mL round bottom flask. The mixture is placed on ice and the pH adjusted from 6.8 to 7.5 with the addition whilst stirring of solid sodium carbonate. A quantity of 1.5 g of dithiothreitol (DTT) is added to the pH adjusted mixture and its dissolution promoted by placing the reaction mixture containing round bottom flask in an ultrasonic bath before transferring to a water bath maintained at a temperature of 50°C. Aliquots of a volume of 10 µL are removed from the reaction mixture and transferred to the water bath (T=0) and periodically (every 15 minutes) thereafter. Aliquots are diluted 50-fold by the addition of a volume of 490 µL 80% acetonitrile 0.25% acetic acid immediately following removal from the reaction mixture and analysed by LC-MS as described (A. Analysis) to monitor the progress of the reaction in near real time (Figure 1). After incubation for 45 minutes at 50°C the reaction mixture is chilled by transferring the round bottom flask to an ice slurry. Under these conditions near quantitative conversion of GTX1,4 to neoSTX is observed with close to 100% yield.

C. Isolation

The conversion product is loaded onto a quantity of 39 g Septra™ WCX packed in an empty flash cartridge (Grace) and preconditioned with a volume of 250 mL of 50% (w/w) acetonitrile followed by a volume of 250 mL of deionised water. The conversion product is loaded onto the packed cartridge with rinses of deionised water with collection of the effluent (about 200 mL). The dissolution of any crystals formed during storage of the conversion product at 4°C is achieved by the addition of a minimal amount of deionised water. The loaded packed cartridge is then eluted at a rate of 50 mL/min with a total volume of 1.5 L, followed by elution with a continuous gradient to 1 M acetic acid over 20 minutes and the collection of sequential volumes of 10 mL of eluate as fractions while monitoring UV absorbance at 205 nm and 254 nm.

A volume of 5 µL of fractions demonstrating UV absorbance at 205 nm is diluted 100,000-fold in 80% acetonitrile 0.25% acetic acid and analysed by LC-MS. Fractions confirmed to comprise neoSTX are combined, frozen at -70°C and lyophilised. The dried neoSTX is dissolved in a small volume of 10 mM and transferred to a pre-weighed 10 mL glass vial and a volume of 10 µL analysed. The purity and quantity of a batch (CNC00063) of neoSTX prepared according to the foregoing method is provided in Table 1.

Component	Quantity (mg)	% (w/w)
neoSTX	118	99.58
L-arginine	0.444	0.37
STX	0.0546	0.05
DTT	<0.005	<0.004
TOTAL	119	100

Table 1. Specification for a batch (CNC00063) of neoSTX prepared according to the semisynthetic method described.

Although the invention has been described with reference to embodiments or examples it should be appreciated that variations and modifications may be made to these embodiments or examples without departing from the scope of the invention. Where known equivalents exist to specific elements, features or integers, such equivalents are incorporated as if specifically referred to in this specification. Variations and modifications to the embodiments or examples that include elements, features or integers disclosed in and selected from the referenced publications are within the scope of the invention unless specifically disclaimed. The advantages provided by the invention and discussed in the description may be provided in the alternative or in combination in these different embodiments of the invention.

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CLAIMS

- 1) A **method** of preparing a quantity of neoSTX comprising the steps of:
 - (a) contacting in solution in a reaction solvent a quantity of purified GTX1,4 and a quantity of dithiol for a period of time and at a temperature sufficient to provide a reaction product in which greater than 97.5% (w/w) of the GTX1,4 has been converted to neoSTX; and then
 - (b) applying the reaction product to a silica based weak cation exchange sorbent and eluting with an aqueous weak organic acid to separate the neoSTX from the dithiol and provide the quantity of neoSTX, where the pH of the solution is in the range 7.4 to 7.6.
- 2) The method of claim 1 where the reaction solvent is buffered aqueous acetic acid.
- 3) The method of claim 1 or 2 where the dithiol is selected from the group consisting of dithiothreitol (DTT) and dithiobutylamine (DTBA).
- 4) The method of claim 1 where the dithiol is dithiothreitol (DTT).