(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 March 2010 (25.03.2010)

PCT

(10) International Publication Number
WO 2010/032219 A1

(51) International Patent Classification:
*C07C 231/02* (2006.01) *C07C 231/24* (2006.01)
*C07C 231/12* (2006.01) *C07C 237/26* (2006.01)

(21) International Application Number:
PCT/IB2009/054110

(22) International Filing Date:
18 September 2009 (18.09.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2206/DEL/2008 18 September 2008 (18.09.2008) IN

(71) Applicant *(for all designated States except US)*: **RANBAXY LABORATORIES LIMITED** [IN/IN]; Plot No. 90, Sector - 32, Gurgaon, Haryana 122001 (IN).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **SINGH, Ashwani, Kumar** [IN/IN]; Opposite Post Office, Main Road, Khemganj, Sirsaganj, Firozabad 205151, Uttar Pradesh (IN).

(74) Common Representative: **RANBAXY LABORATORIES LIMITED**; c/o DEYONKER, James J., Intellectual Property, 600 College Road East, Suite 2100, Princeton, NJ 08540 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published**:

— *with international search report (Art. 21(3))*

WO 2010/032219 A1

(54) Title: PROCESS FOR THE PREPARATION OF TIGECYCLINE

(57) Abstract: The present invention relates to an improved process for the preparation of tigecycline. The process comprises treating minocycline hydrochloride with nitrating agent at low temperature followed by reduction in presence of catalyst to produce 9-aminominocycline disulphate in granular form. It is then reacted with N-t-butylglycyl chloride hydrochloride at pH below 3 under nitrogen atmosphere to obtain tigecycline.

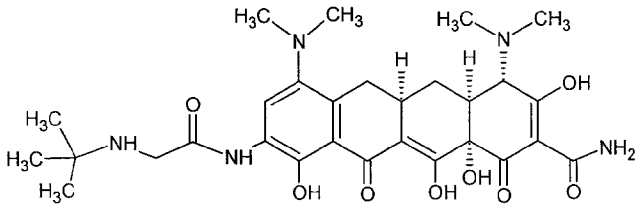
# PROCESS FOR THE PREPARATION OF TIGECYCLINE

## Field of the Invention

The present invention relates to improved process for the preparation of Tigecycline.

## Background of the Invention

Tigecycline chemically is (4S,4aS,5aR,12aS)-9-[2-(tert-butylamino)acetamido]-4,7-bis(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide represented by Formula-I.



**Formula-I**

Tigecycline is reported to be active against methicillin-resistant Staphylococcus aureus, penicillin-resistant Streptococcus pneumoniae, vancomycin-resistant enterococci and against organisms carrying either of the two major forms of tetracycline resistance: efflux and ribosomal protection (U.S. Patent Application No. 2007/0049562).

Tigecycline and its use are disclosed in U.S. Patent Nos. 5,494,903 and 5,529,990, respectively. U.S. Patent Nos. 5,281,628; 5,401,863; 5,284,963; 5,675,030; U.S. Patent Application 2007/0049562 and PCT patent application WO 05/056538 disclose either process for preparation of tigecycline or intermediate compounds thereof. Two synthetic methods for preparing tigecycline are disclosed by *Drugs of the Future 2001, 26(9), 851-858.* The preparation of intermediate compounds is also disclosed by *J. Med. Chem. 1994, 37(1), 184-188.*

The methods disclosed in the cited literature teach three important steps i.e. nitration, reduction and acylation for the preparation of tigecycline starting from minocycline.

One of the known methods involves nitration of minocycline to obtain 9-nitrominocycline which is subsequently reduced to 9-aminominocycline. Finally acylation of

9-aminominocycline with N-t-butylglycyl chloride provides tigecycline of formula I (*Drugs of the Future 2001, 26(9), 851-858).*

Another method describes reaction of 9-aminominocycline with chloroacetyl chloride to produce an acylated intermediate which on reacting with t-butylamine provides tigecycline of formula I (U.S. Patent No. 5,284,963; *Drugs of the future 2001, 26(9), 851-858).*

## Summary of the Invention

The present invention provides a process for preparing highly pure tigecycline which has lower content of degradation products and /or impurities. The reduction step is performed in the presence of organic solvent and water. The use of water is safe to handle catalyst operations. The quantity of catalyst used during reduction step is also less. The 9-aminominocycline disulphate produced is granular in form and thus helps during filtration and reduces filtration time. The pH of the solution at the acylation step is adjusted so that it effectively produces tigecycline in good yields.

## Detailed Description of the Invention

The Following terminology is used in the present application, when describing aspects of the present invention. These are not intended in any way to limit the scope of the present invention. Several variants of these terminology would be evident to persons ordinarily skilled in the art.

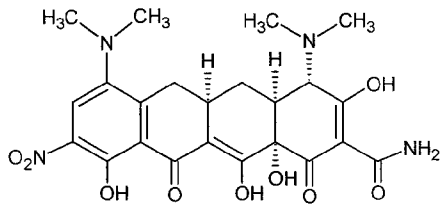
The term 'nitrating agent' as used herein refers to the reagent that can add a $-NO_2$ substituent to minocycline. Exemplary nitrating agents include nitric acid and nitrate salts, such as alkali metal salts, $KNO_3$.

The terms 'minocycline', '9-nitrominocycline' or '9-aminominocycline' as used herein include the free base as well as pharmaceutically acceptable salt forms thereof. For example, the term minocycline wherever mentioned includes minocycline as free base or its pharmaceutically acceptable salt. If the term minocycline hydrochloride is used, it specifically means hydrochloride salt form of minocycline.

The term 'pharmaceutically acceptable salt' as used herein refers to acid addition salts. Examples include, but are not limited to, hydrochloride, hydrobromide, hydroiodide, phosphoric, nitric, sulfuric, acetic, benzoic, citric, fumaric, glycolic, maleic, succinic, tartaric, sulfate, alkyl ($C_1$-$C_6$) or aryl ($C_6$-$C_{10}$) sulfonates and chlorobenzenesulfonate salt. Preferred pharmaceutically acceptable salt is hydrochloride or sulfate salt.

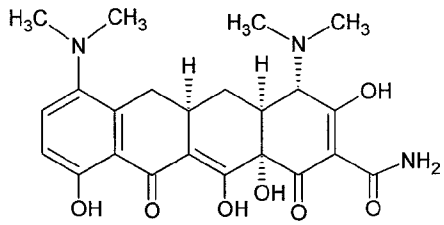
The term 'organic solvent' as used herein refers to a group consisting of alcohols (for example, $C_1$-$C_6$ alcohols like methanol, ethanol, isopropanol, butanol etc.); ethers (for example, diethyl ether, diisopropyl ether, tetrahydrofuran, etc); hydrocarbon solvents (for example, hexane, heptane, etc.); chlorinated solvents like dichloromethane, chloroform, etc.; acetone; acetonitrile; 1,4-dioxane and mixture(s) thereof.

A first aspect of the present invention provides a process for preparation of 9-nitrominocycline of formula III



**Formula-III**

comprising reacting minocycline of formula II



**Formula-II**

with nitrating agent at low temperature.

In an embodiment of this aspect the nitrating agent is added to the reaction in 30-45 minutes under nitrogen atmosphere.

The term 'low temperature' refers to temperature or temperature range below 0°C. Preferably, the range -10° to -1°C can be used for the nitration of minocycline.

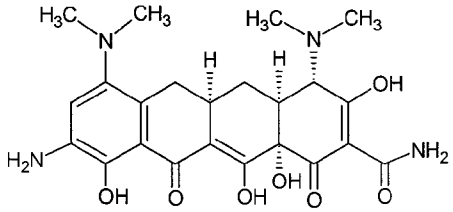
In another embodiment of this aspect, the minocycline used can be in the form of its hydrochloride salt.

In another embodiment of this aspect, the 9-nitrominocycline produced can be in the form of its disulphate salt.

Accordingly, potassium nitrate is added to a solution of minocycline hydrochloride in concentrated sulphuric acid under nitrogen atmosphere in 30-45 minutes at -1° to -10°C. The reaction mixture is then stirred for 1-2 hours at -5°C to produce 9-nitrominocycline disulphate. The product 9-nitrominocycline disulphate can either be isolated from the reaction mixture or used as such (*in situ*) for preparation of tigecycline.

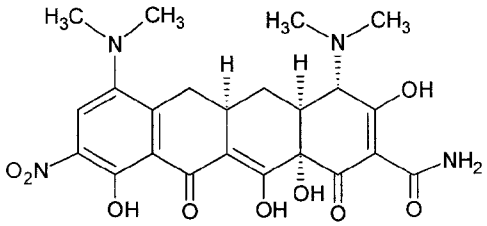
In another embodiment of this aspect, the 9-nitrominocycline disulphate can be used for the preparation of tigecycline.

A second aspect of the present invention provides a process for preparation of 9-aminominocycline of formula IV



**Formula-IV**

comprising reducing 9-nitrominocycline of formula III



**Formula-III**

or reaction mixture thereof with reducing agent in presence of catalyst.

In an embodiment of this aspect, the reduction can be carried out in presence of organic solvent and deionized water.

In another embodiment of this aspect, the reducing agent is hydrogen gas.

The reducing agent is provided in the presence of at least one catalyst. Exemplary catalysts include, but are not limited to, rare earth metal oxides, Group VIII metal-containing catalysts, and salts of Group VIII metal-containing catalyst. An example of a Group VIII metal-containing catalyst is palladium, such as palladium on carbon.

The catalyst is present in an amount ranging from 0.01 to 0.02 parts relative to the amount of minocycline or its pharmaceutically acceptable salt which is used for the preparation of 9-nitrominocycline.

In another embodiment of this aspect, the 9-nitrominocycline used is in the form of its disulphate salt.

In another embodiment of this aspect, the 9-aminominocycline produced is in the form of its disulphate salt.

Accordingly, 9-nitrominocycline or reaction mixture thereof is added to an ice-cooled mixture of organic solvent and deionized water while controlling temperature below 20°C. The organic solvent used in this aspect can be water miscible (for example, methanol, ethanol etc.), immiscible (for example, diisopropyl ether) or mixtures thereof. The resultant mixture is stirred below 20°C and the formed aqueous layer comprising 9-nitrominocycline and water miscible organic solvent is separated. The separated aqueous layer is reduced at hydrogen pressure ranging from 1 to 75 psi in presence of catalyst to produce 9-aminominocycline. The produced 9-aminominocycline can either be isolated from the aqueous layer or used as such (*in situ*) for preparation of tigecycline.

In a preferred embodiment of this aspect, the water miscible solvent is methanol and the water immiscible solvent is diisopropyl ether.

The 9-nitrominocycline or the pharmaceutically acceptable salt thereof can either be isolated from the reaction mixture or *in situ* used for the reduction reaction of this aspect.

A third aspect of the present invention provides a process for the preparation of granules of 9-aminominocycline or its pharmaceutically acceptable salt by controlled addition

of aqueous solution comprising 9-aminominocycline or its pharmaceutically acceptable salt in organic solvent.

In an embodiment of this aspect, the pharmaceutically acceptable salt of 9-aminominocycline is disulphate salt.

In another embodiment of this aspect, preferably the organic solvent is selected from group comprising isopropyl alcohol, acetone, acetonitrile, 1,4-dioxane and mixture thereof.

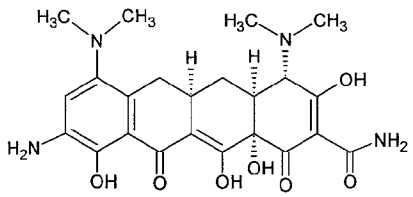
In another embodiment of this aspect, the controlled addition comprises addition of the aqueous solution in its 2 to 6 times of the organic solvent at 25 ±2°C in 30-60 minutes.

The term granules as used herein refer to non-fluffy, easily filterable form of 9-aminominocycline or its pharmaceutically acceptable salt.

The aqueous solution used herein refers to the solution comprising water and 9-aminominocycline or its pharmaceutically acceptable salt. The medium in which 9-nitrominocycline or its pharmaceutically acceptable salt is reduced to 9-aminominocycline or its pharmaceutically acceptable salt can also be referred to as aqueous solution.

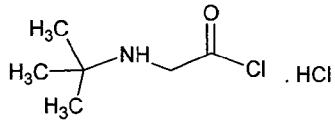
Accordingly, the aqueous solution comprising 9-aminominocycline disulphate is added to isopropyl alcohol at 25 ±2°C in 30-60 minutes. As the granules are formed, addition of isopropyl alcohol produces the granular 9-aminominocycline disulphate. The granular 9-aminominocycline disulphate can be isolated or *in-situ* used for the preparation of tigecycline.

A fourth aspect of the present invention provides a process for the preparation of tigecycline comprising reacting 9-aminominocycline of formula IV




**Formula IV**

with N-t-butylglycyl chloride of formula V



**Formula V**

at pH below 3.

In an embodiment of this aspect, the reaction is performed at temperature below 0°C.

In another embodiment of this aspect, 9-aminominocycline is used as its disulphate salt and having chromatographic purity of more than 90%.

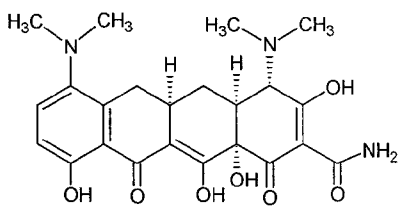
Accordingly, the 9-aminominocycline disulphate is mixed with de-ionized water having dissolved oxygen content below 10 ppm under nitrogen atmosphere and pH of the solution is adjusted in between 2 to 3 with the help of sodium carbonate. N-t-butylglycyl chloride hydrochloride is added to the solution under continuous stirring at below 0°C. The Tigecycline formed is extracted from the reaction mass with the help of organic solvents like dichloromethane, acetone, chloroform, etc.

In another embodiment of this aspect, the extraction of tigecycline from the reaction mass is performed at pH 6.7±0.2.

The 9-aminominocycline or its pharmaceutically acceptable salt (such as disulphate salt) used in this aspect is prepared by following the methods described hereinabove in second and third aspects. The 9-aminominocycline or its pharmaceutically acceptable salt can either be isolated or *in situ* used for the preparation of tigecycline.

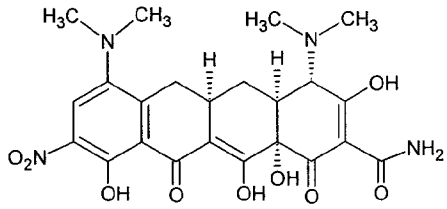
A fifth aspect of the present invention provides a process for the preparation of tigecycline comprising

a) reacting minocycline of formula-II



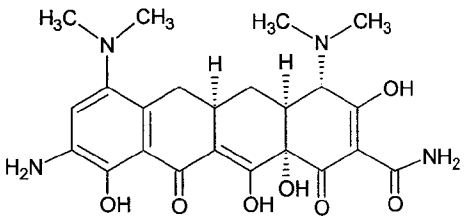
**Formula-II**

with a nitrating agent at low temperature to form 9-nitrominocycline of formula-III



**Formula-III**

b) reducing 9-nitrominocycline of formula-III to obtain 9-aminominocycline of formula-IV



**Formula-IV**

c) converting 9-aminominocycline of formula-IV to tigecycline.

Accordingly, potassium nitrate is added to a solution of minocycline hydrochloride in concentrated sulphuric acid under nitrogen atmosphere in 30-45 minutes at -1° to -10°C. The reaction mixture is then stirred for 1-2 hours at -5°C to produce 9-nitrominocycline disulphate. The produced 9-nitrominocycline disulphate can either be isolated from the reaction mixture or used as such (*in situ*) for preparation of tigecycline.

The reduction can be carried out in the same manner as described hereinabove in second aspect of the invention. The reduction method, embodiments, reagents, solvents, reaction conditions (e.g. temperature, pressure, catalyst, etc.) and description mentioned in the second aspect can also be incorporated herein in order to explain the step-b.

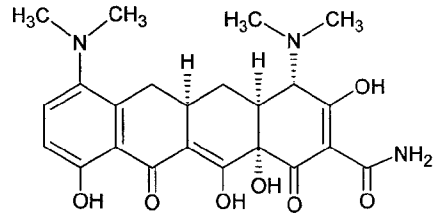
In further embodiment of this aspect, the reduction can be performed using hydrogen in presence of catalyst such as palladium on carbon.

The 9-aminominocycline can be converted to tigecycline by following the procedure and embodiments described hereinabove in fourth aspect of the present invention. The

process, embodiments, reagents, solvents, reaction conditions (e.g. temperature, pH, reaction time, etc.) and description mentioned in the fourth aspect can also be incorporated herein in order to describe how step-c can be carried out.

A sixth aspect of the present invention provides a process for the preparation of tigecycline comprising-

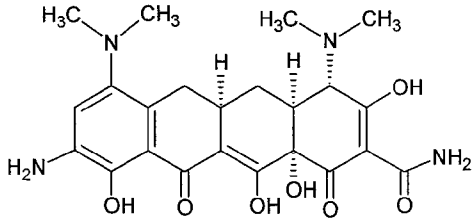
a) nitrating minocycline of formula-II



**Formula-II**

with nitrating agent at low temperature followed by reduction with reducing agent in presence of catalyst

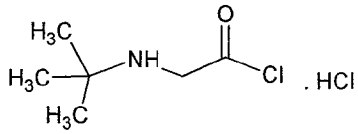
b) isolating 9-aminominocycline of formula-IV



**Formula-IV**

from the reaction mass obtained in step-a

c) reacting 9-aminominocycline of formula-IV isolated in step-b with N-t-butylglycyl chloride hydrochloride of formula-V



**Formula-V**

at pH below 3.

In an embodiment to this aspect, the process for the preparation of tigecycline is carried out under nitrogen atmosphere.

Step a:

In an embodiment of this aspect, nitrating agent is added to the reaction in 30-45 minutes under nitrogen atmosphere.

The term 'low temperature' has the same meaning as defined hereinbefore.

In another embodiment of this aspect, the minocycline used herein in this aspect is in the form of its hydrochloride salt.

In another embodiment of this aspect, minocycline hydrochloride is *in situ* converted into 9-aminominocycline.

In another embodiment of this aspect, the reduction can be carried out in presence of organic solvent and deionized water.

In another embodiment of this aspect, the reducing agent is hydrogen gas.

The reducing agent is used in the presence of at least one catalyst. Exemplary catalysts include, but are not limited to, rare earth metal oxides, Group VIII metal-containing catalysts, and salts of Group VIII metal-containing catalyst. An example of a Group VIII metal-containing catalyst is palladium, such as palladium on carbon.

The catalyst is present in an amount ranging from 0.01 to 0.02 parts relative to the amount of minocycline or its pharmaceutically acceptable salt which is used for the preparation of 9-nitrominocycline.

Accordingly, potassium nitrate is added to a solution of minocycline in concentrated sulphuric acid under nitrogen atmosphere in 30-45 minutes at -1° to -10°C. The reaction mixture is then stirred for 1-2 hours at -5°C. The reaction mixture is added to ice-cooled mixture of organic solvent and deionized water while controlling temperature below 20°C. The organic solvent at this stage can be selected from water miscible (for example, methanol, ethanol, etc.), immiscible (for example, diisopropyl ether) or mixtures thereof. The resultant mixture is stirred below 20°C and the formed aqueous layer containing 9-nitrominocycline

and water miscible organic solvent is separated. The separated aqueous layer is reduced at hydrogen pressure ranging from 1 to 75 psi in presence of the catalyst to produce 9-aminominocycline. The produced 9-aminominocycline can either be isolated from the aqueous layer or used as such (*in situ*) for preparation of tigecycline.

In a preferred embodiment of this aspect, the water miscible solvent is methanol and the water immiscible solvent is diisopropyl ether.

In another embodiment of this aspect, the 9-aminominocycline prepared is in the form of disulphate salt.

The produced 9-aminominocycline disulphate can either be isolated from the aqueous solution or used as such (*in situ*) for preparation of tigecycline.

Step b:

In an embodiment of this aspect, the isolation of 9-aminominocycline comprises granules formation and crystallization steps.

In another embodiment of this aspect, the granules of 9-aminominocycline disulphate salt are prepared.

In another embodiment of this aspect, the granules of 9-aminominocycline disulphate are prepared by a process comprising controlled addition of aqueous solution comprising 9-aminominocycline disulphate in organic solvent.

In another embodiment to this aspect, 2 to 6 times of organic solvent relative to the aqueous solution is used for obtaining the said granules.

In another embodiment of this aspect, the controlled addition comprises addition of the aqueous solution in its 2 to 6 times of the organic solvent at 25 ±2°C in 30-60 minutes.

The term granules as used herein refers to non-fluffy, easily filterable form of 9-aminominocycline disulphate.

The aqueous solution used herein refers to the solution comprising water and 9-aminominocycline disulphate. The reaction medium in which 9-nitrominocycline is reduced to 9-aminominocycline can also be referred as aqueous solution.

In another embodiment of this aspect, preferably the organic solvent is selected from group comprising of isopropyl alcohol, acetone, acetonitrile, 1,4-dioxane and mixture thereof.

Accordingly, the aqueous solution comprising 9-aminominocycline disulphate is added to isopropyl alcohol at 25 ±2°C in 30-60 minutes. As granules are formed, more of isopropyl alcohol is added. This is stirred for 1 hour to crystallize and filtered under nitrogen to get cake of 9-aminominocycline disulphate. This is optionally washed with acetone and dried.

Step c:

The obtained 9-aminominocycline disulphate is reacted with N-t-butylglycyl chloride of formula-V at pH below 3 under nitrogen atmosphere.

In an embodiment of this aspect, the reaction is performed at temperature below 0°C.

In another embodiment of this aspect, 9-aminominocycline disulphate having chromatographic purity more than 90% is used for tigecycline preparation.

Accordingly, the 9-aminominocycline disulphate is mixed with de-ionized water having dissolved oxygen content below 10 ppm under nitrogen atmosphere and pH of the solution is adjusted to or in between 2 to 3 with the help of sodium carbonate. N-t-butylglycyl chloride hydrochloride is added to the solution under continuous stirring at below 0°C. The tigecycline formed is extracted from the reaction mass with the help of organic solvents like dichloromethane, acetone, chloroform, etc.

In an embodiment of this aspect, the extraction of tigecycline from the reaction mass is performed at pH 6.7±0.2.

The N-t-butylglycyl chloride hydrochloride used hereinabove for the preparation of tigecycline is prepared by reacting chloroacetyl chloride with benzyl alcohol to obtain benzyl chloroacetate which upon treatment with t-butylamine produces benzyl N-t-butyl glycinate. Benzyl N-t-butyl glycinate is hydrolyzed to obtain corresponding acid. The acid is then chlorinated to obtain N-t-butylglycyl chloride hydrochloride.

If seeding of tigecycline is required during the preparation of tigecycline, then the seeds may be prepared by following any one of the methods known in the prior-art such as by following the process described in U.S. Patent No. 5,284,963.

While the present invention has been described in terms of its specific aspects, certain modifications and equivalents will be apparent to those skilled in the art and are intended to be included within the scope of the present invention.

In the following section, aspects of the present invention are described by way of examples to illustrate the processes of the invention. However, this is not intended in any way to limit the scope of the present invention. Several variants of the example would be evident to persons ordinarily skilled in the art.

## EXAMPLE

### Preparation of Tigecycline

a) Preparation of 9-aminominocycline disulphate from Minocycline hydrochloride

Dry nitrogen gas was purged through concentrated sulphuric acid (450 ml) at 28±2°C, and it was cooled to -5°C to 0°C. Minocycline hydrochloride (150 g) was added portion-wise to the sulphuric acid while controlling temperature below -1°C under stirring (exothermic reaction observed). After complete addition of minocycline hydrochloride, the solution was stirred for 15-20 minutes at below -1°C. Potassium nitrate (38.4 g) was added portion-wise to the solution while controlling temperature below -1°C under stirring (Exothermic reaction was observed). The reaction mixture obtained was stirred for 1-2 hours at -5°C.

In a flask, de-ionized water (420 ml) having dissolved oxygen content below 10 ppm, methanol (200 ml) and diisopropyl ether (750 ml) were added and cooled to -30°C to -25°C. The reaction mixture obtained above was added to the flask by controlling temperature below 15°C (exothermic reaction was observed). The resultant mixture in the flask was washed with de-ionized water (105 ml). The mixture was stirred for 10-15 minutes below 15°C and then it was allowed to stand for 20-30 minutes. The product enriched aqueous layer was separated. To the separated aqueous layer, activated carbon (20 gm) was added followed by stirring for 20-30 minutes. The aqueous layer was filtered through hyflo bed followed by washing of the

bed with methanol (200 ml) at 20-30°C under nitrogen. The filtrate obtained was transferred to parr apparatus bottle for hydrogenation. To the filtrate, 10% Pd/C (3.0 g; 50% wet) was added and it was flushed twice with nitrogen, once with hydrogen and finally hydrogen pressure of around 50 psi was applied to it. It was maintained for 2-5 hours at 20-25°C. The resultant mixture was filtered through hyflo bed followed by washing with methanol (200 ml) at 20-30°C under nitrogen.

b) Preparation of granular 9-aminominocycline disulphate and crystallization thereof

In another flask containing isopropyl alcohol (5.25 L) at 20-30°C, the filtrate obtained (from step a) was dispersed for precipitation at 25±2°C in 30-60 minutes. When granules formation was observed, isopropyl alcohol (4.5 L) was added to it simultaneously. This was stirred for 1 hour and simultaneously cooled to 0-5°C. It was filtered under nitrogen pressure using sintered funnel at 20-30°C to obtain cake of 9-aminominocycline disulphate.

c) Preparation of Tigecycline from 9-aminominocycline disulphate

The cake obtained (from step b) was washed with acetone (250 x 3 ml) to obtain slurry under nitrogen at 20-30°C. This was flushed with dry nitrogen. The wet cake (370 g) obtained was added at 0°C to de-ionized water (750 ml) purged with nitrogen gas. It was stirred to dissolve at 0°C. The pH of the solution was adjusted to 2-3 slowly with sodium carbonate solution (approx. 0.6-1.0 L) maintaining temperature below 0°C and then N-t-butylglycyl chloride hydrochloride (260 g) was added to it in various lots under stirring and maintaining temperature below 0°C.

After completion of the reaction, pH of the reaction mass was adjusted to 6.7±0.2 with sodium carbonate solution at below 0°C and dichloromethane (9.0 L) was added to it at 20-30°C. It was stirred for 30 minutes at 20-25°C under nitrogen and pH of the resultant solution was then adjusted, if required, to 6.7±0.2 with sodium carbonate solution. The solution was allowed to stand for 30 minutes and then product enriched organic layer (dichloromethane layer) was separated and preserved in another flask. To this layer, 20% activated carbon (30 g) and anhydrous sodium sulphate (700 g) were added at 20-25°C.

To the remaining aqueous layer, sodium carbonate solution was added, if required, the pH was adjusted to 6.7±0.2 at below 15°C. To this layer, dichloromethane (6.0 L) was added followed by stirring at below 25°C for 30 minutes. The solution was allowed to stand for 30 minutes and then product enriched organic layer was separated and added to the flask comprising previously separated organic layer for carbon treatment. This process was repeated again starting from further remaining aqueous layer.

To the flask comprising the organic layer, 20% activated carbon (30 g) was added and it was stirred for 20-30 minutes. The organic layer was then filtered through hyflo having anhydrous sodium sulphate bed and after filtration organic layer was transferred to another flask for dichloromethane recovery below 30°C under vacuum. The dichloromethane was recovered completely at below 20°C. Methanol (600 ml) was added to the resultant mass followed by stirring to dissolve the material. If required, it was heated to 40-45°C to dissolve. This methanolic solution was then cooled to 0°C gradually under stirring in 30-45 minutes. This solution was seeded with tigecycline at 0°C and stirred for 60-90 minutes after crystallization at 0°C. This was filtered under nitrogen pressure using sintered funnel. The obtained cake was washed with ice-cooled methanol (600 ml) at 0°C to obtain slurry which was continue flushed with nitrogen till the removal of maximum solvent for approx. 30-60 minutes at 20-30°C. The wet cake was unloaded and dried under vacuum at 60-65°C for 12-16 hours. The material was unloaded and packed under nitrogen and placed at 5±3°C in a closed airtight container protected from light.

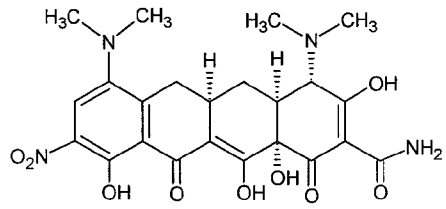
Dry weight of solid: 90-97.5 g

Yield (% w/w): 0.60-0.65

Chromatographic purity: NLT 99%

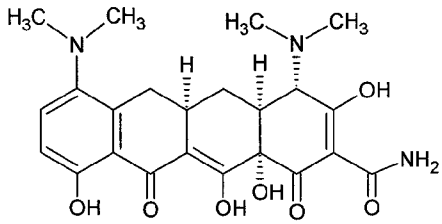
We Claim:

1. A process for the preparation of 9-nitrominocycline of formula III



**Formula-III**

comprising reacting minocycline of formula II



**Formula-II**

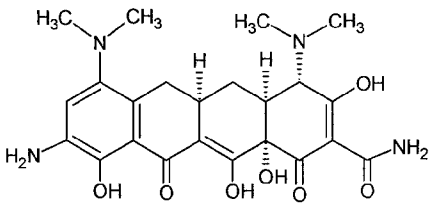
with a nitrating agent at low temperature.

2. The process of claim 1 wherein the nitrating agent is added to the reaction in 30-45 minutes under nitrogen atmosphere.

3. The process of claim 1 wherein the low temperature is the temperature or temperature range below 0°C.

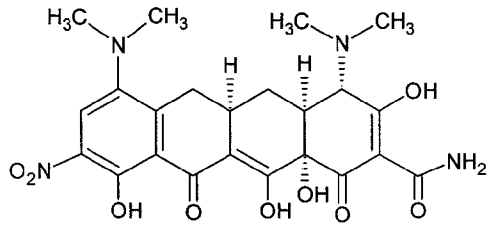
4. The process of claim 1 wherein the 9-nitrominocycline of formula III is used for the preparation of tigecycline.

5. A process for the preparation of 9-aminominocycline of formula IV



**Formula-IV**

comprising reducing 9-nitrominocycline of formula III



**Formula-III**

or reaction mixture thereof with reducing agent in presence of catalyst.

6. The process of claim 5 wherein the reduction is performed in presence of organic solvent and deionized water.

7. The process of claim 5 wherein the reducing agent is hydrogen gas.

8. The process of claim 5 wherein the catalyst is palladium on carbon.

9. The process of claim 5 wherein the 9-aminominocycline of formula IV is used for the preparation of tigecycline.

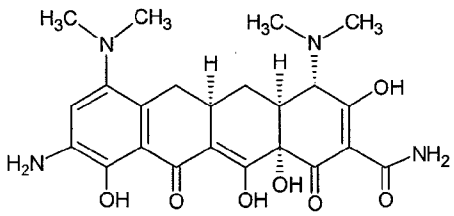
10. A process for the preparation of granules of 9-aminominocycline or its pharmaceutically acceptable salt by controlled addition of aqueous solution comprising 9-aminominocycline or its pharmaceutically acceptable salt in organic solvent.

11. The process of claim 10 wherein the controlled addition comprises addition of the aqueous solution in its 2 to 6 times of the organic solvent at 25 ±2°C in 30-60 minutes.

12. The process of claim 10 wherein the pharmaceutically acceptable salt of 9-aminominocycline is disulphate salt.

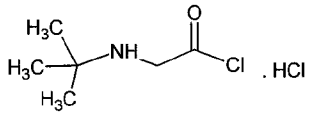
13. The process of claim 10 wherein organic solvent is selected from the group comprising of methanol, ethanol, isopropyl alcohol, butanol, diethyl ether, diisopropyl ether, tetrahydrofuran, hexane, heptane, acetone, acetonitrile and 1,4-dioxane.

14. A process for the preparation of tigecycline comprising reacting 9-aminominocycline of formula IV



**Formula IV**

with N-t-butylglycyl chloride of formula V



**Formula V**

at pH below 3.

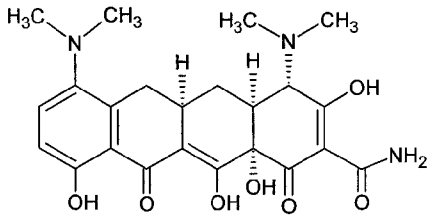
15. The process of claim 14 wherein the reaction is performed at temperature below 0°C.

16. The process of claim 14 wherein 9-aminominocycline is used as its disulphate salt and having chromatographic purity of more than 90%.

17. The process of claim 14 wherein tigecycline is obtained by extracting it from the reaction mass at pH 6.7±0.2.

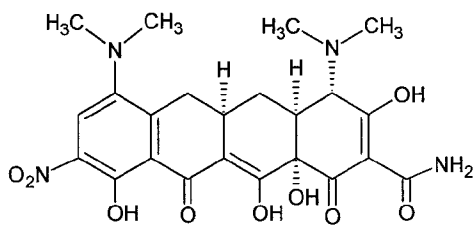
18. A process for the preparation of tigecycline comprising

a) reacting minocycline of formula-II



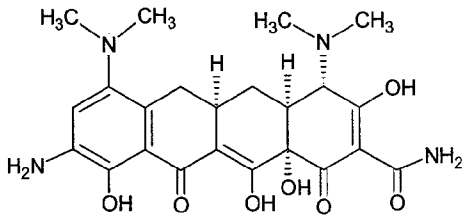
**Formula-II**

with nitrating agent at low temperature to form 9-nitrominocycline of formula-III



**Formula-III**

b) reducing 9-nitrominocycline of formula-III to obtain 9-aminominocycline of formula-IV



**Formula-IV**

c) converting 9-aminominocycline of formula-IV to tigecycline.

19. The process of claim 18 wherein nitrating agent is added to the reaction in 30-45 minutes under nitrogen atmosphere.

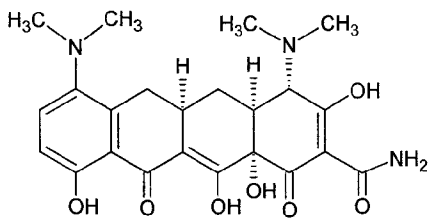
20. The process of claim 18 wherein the low temperature is the temperature or temperature range below 0°C.

21. The process of claim 18 wherein reduction of 9-nitrominocycline of formula-III is performed using hydrogen gas in presence of palladium on carbon.

22. The process of claim 18 wherein 9-aminominocycline of formula IV is reacted with N-t-butylglycyl chloride at pH below 3 to obtain tigecycline.

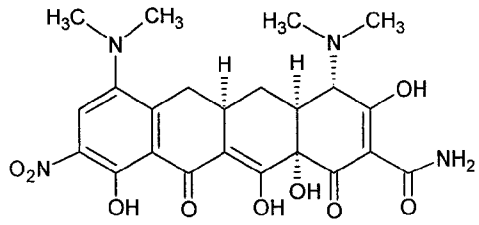
23. A process for the preparation of tigecycline comprising

a) nitrating minocycline of formula-II



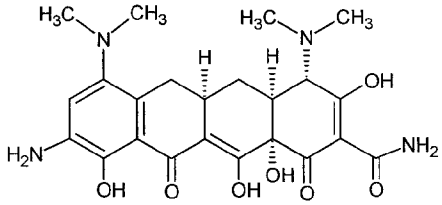
**Formula-II**

to form 9-nitrominocycline of formula-III



**Formula-III**

b) reducing 9-nitrominocycline of formula-III or reaction mixture thereof with reducing agent in presence of catalyst to obtain 9-aminominocycline of formula-IV



**Formula-IV**

c) converting 9-aminominocycline of formula-IV to tigecycline.

24. The process of claim 23 wherein nitration of minocycline is performed at temperature or temperature range below 0°C.

25. The process of claim 23 wherein the reduction is performed in presence of organic solvent and deionized water.

26. The process of claim 23 wherein the reducing agent is hydrogen gas.

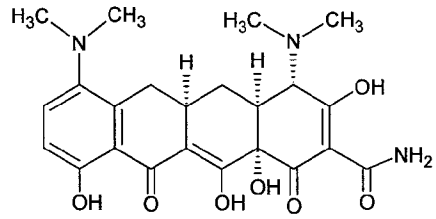
27. The process of claim 23 wherein the catalyst is palladium on carbon.

28. The process of claim 23 wherein the catalyst is present in an amount ranging from 0.01 to 0.02 parts relative to the amount of minocycline.

29. The process of claim 23 wherein 9-aminominocycline of formula IV is converted to tigecycline by reacting it with N-t-butylglycyl chloride at pH below 3.

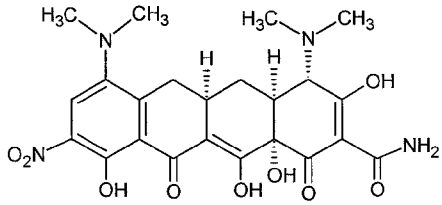
30. A process for the preparation of tigecycline comprising

a) nitrating minocycline of formula-II



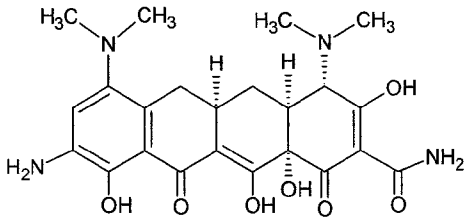
**Formula-II**

to form 9-nitrominocycline of formula-III



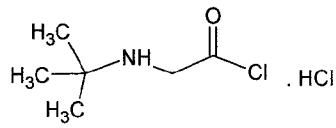
**Formula-III**

b) reducing 9-nitrominocycline of formula-III to obtain 9-aminominocycline of formula-IV



**Formula-IV**

c) reacting 9-aminominocycline of formula-IV with N-t-butylglycyl chloride of formula-V



**Formula-V**

at pH below 3.

31. The process of claim 30 wherein nitration of minocycline is performed at temperature or temperature range below 0°C.

32. The process of claim 30 wherein reduction of 9-nitrominocycline of formula-III is performed using hydrogen gas in presence of palladium on carbon.

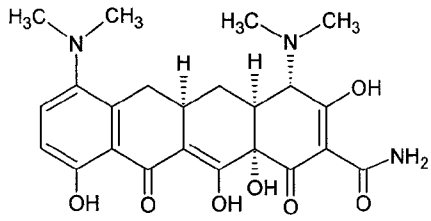
33. The process of claim 30 wherein the step c) reaction is performed at temperature below 0°C.

34. The process of claim 30 wherein 9-aminominocycline is used as its disulphate salt and having chromatographic purity of more than 90%.

35. The process of claim 30 wherein tigecycline is obtained by extracting it from the reaction mass at pH 6.7±0.2.

36. A process for the preparation of tigecycline comprising

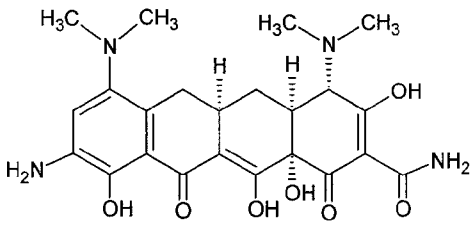
a) nitrating minocycline of formula-II



**Formula-II**

with nitrating agent at low temperature followed by reduction with reducing agent in presence of catalyst

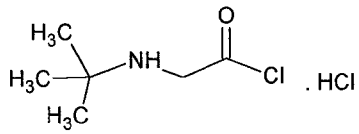
b) isolating 9-aminominocycline of formula-IV



**Formula-IV**

from the reaction mass obtained in step-a

c) reacting 9-aminominocycline of formula-IV isolated in step-b with N-t-butylglycyl chloride hydrochloride of formula-V



**Formula-V**

at pH below 3.

37. The process of claim 36 wherein nitrating agent is added to minocycline in 30-45 minutes under nitrogen atmosphere.

38. The process of claim 36 wherein the low temperature is the temperature or temperature range below 0°C.

39. The process of claim 36 wherein the reduction is performed in presence of organic solvent and deionized water.

40. The process of claim 36 wherein the reducing agent is hydrogen gas.

41. The process of claim 36 wherein the catalyst is palladium on carbon.

42. The process of claim 36 wherein the catalyst is present in an amount ranging from 0.01 to 0.02 parts relative to the amount of minocycline.

43. The process of claim 36 wherein 9-aminominocycline of formula-IV is isolated as its disulphate salt and in the form of granules.

44. The process of claim 36 wherein the step c) reaction is performed at temperature below 0°C.

45. The process of claim 36 wherein 9-aminominocycline is isolated as its disulphate salt and having chromatographic purity of more than 90%.

46. The process of claim 36 wherein tigecycline is obtained by extracting it from the reaction mass at pH 6.7±0.2.

# INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2009/054110

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07C231/02 C07C231/12 C07C231/24 C07C237/26

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 2006/130418 A (WYETH CORP [US]; KRISHNAN LALITHA [US]; SUM PHAIK-ENG [US]; HORNE JEFF) 7 December 2006 (2006-12-07)<br>claim 68<br>paragraphs [0060], [0061], [0067], [0087], [0097], [0098], [0188]<br>page 46, table 2, entries 1-6,10-17<br>page 47, table 3, last two entries<br>pages 65,66; examples 22,23 | 1-5,7-9, 14, 17-24, 26-32, 35-38, 40-42, 45,46 |
| Y | pages 49,50; example 2 | 1-46 |

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 19 November 2009 | 30/11/2009 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL – 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016 | Fitz, Wolfgang |

# INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2009/054110

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | EP 0 535 346 A (AMERICAN CYANAMID CO [US]) 7 April 1993 (1993-04-07) claim 9 page 16, line 58 page 13, line 33 | 1,3,5,7, 8 |
| Y | page 18; examples 2,3 | 1-46 |
| X | SUM P-E ET AL: "GLYCYLCYCLINES. 1. A NEW GENERATION OF POTENT ANTIBACTERIAL AGENTS THROUGH MODIFICATION OF 9-AMINOTETRACYCLINES" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY, WASHINGTON, US, vol. 37, no. 1, 1 January 1994 (1994-01-01), pages 184-188, XP002323590 ISSN: 0022-2623 cited in the application page 185, scheme 1, compound 17 page 186, column 1, paragraph 4 - column 2, paragraph 1 | 1,4,5, 7-9,18, 21,23, 26-28 |
| Y | page 188, column 1, line 7: disulfate | 1-46 |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2009/054110

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 2006130418 A | 07-12-2006 | AR 057032 A1 | 14-11-2007 |
| | | AU 2006252783 A1 | 07-12-2006 |
| | | CA 2609306 A1 | 07-12-2006 |
| | | CN 101228112 A | 23-07-2008 |
| | | EC SP078059 A | 23-01-2008 |
| | | EP 1888507 A1 | 20-02-2008 |
| | | JP 2008545701 T | 18-12-2008 |
| | | KR 20080015470 A | 19-02-2008 |
| | | NI 200700301 A | 24-07-2008 |
| | | ZA 200710153 A | 26-08-2009 |
| EP 0535346 A | 07-04-1993 | AT 129697 T | 15-11-1995 |
| | | AU 651734 B2 | 28-07-1994 |
| | | CA 2079703 A1 | 05-04-1993 |
| | | CN 1071415 A | 28-04-1993 |
| | | CZ 9202972 A3 | 14-04-1993 |
| | | DE 69205792 D1 | 07-12-1995 |
| | | DE 69205792 T2 | 20-06-1996 |
| | | DK 0535346 T3 | 04-12-1995 |
| | | ES 2081005 T3 | 16-02-1996 |
| | | FI 924453 A | 05-04-1993 |
| | | GR 3017978 T3 | 29-02-1996 |
| | | HK 1000319 A1 | 27-02-1998 |
| | | HU 64298 A2 | 28-12-1993 |
| | | HU 9500602 A3 | 29-01-1996 |
| | | IL 103319 A | 23-07-1996 |
| | | JP 3183729 B2 | 09-07-2001 |
| | | JP 5239006 A | 17-09-1993 |
| | | MX 9205573 A1 | 01-05-1993 |
| | | NO 923856 A | 05-04-1993 |
| | | NZ 244556 A | 27-06-1994 |
| | | PH 30343 A | 02-04-1997 |
| | | PL 296140 A1 | 02-11-1993 |
| | | PL 170939 B1 | 28-02-1997 |
| | | SK 297292 A3 | 07-12-1994 |
| | | US 5326759 A | 05-07-1994 |
| | | US 5281628 A | 25-01-1994 |
| | | ZA 9207608 A | 13-04-1993 |