

IN THE HIGH COURT OF JUSTICE
CHANCERY DIVISION
PATENTS COURT

HP-2014-000037

BETWEEN:

HOSPIRA UK LIMITED

Claimant

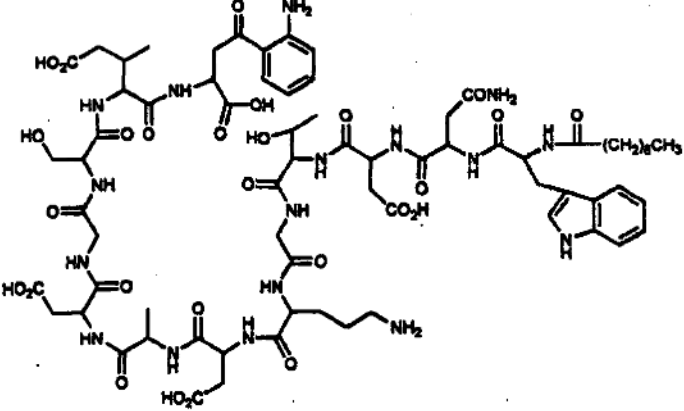
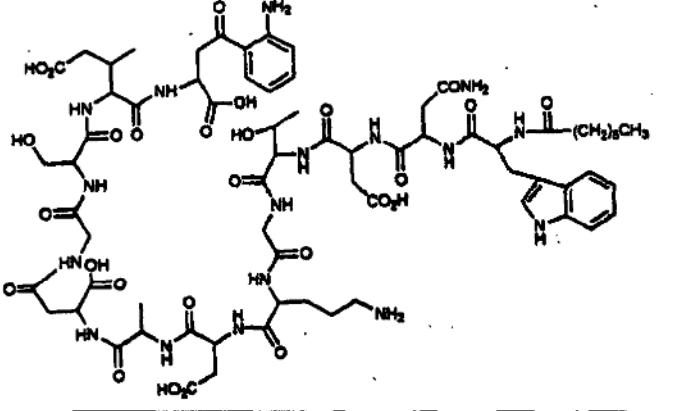
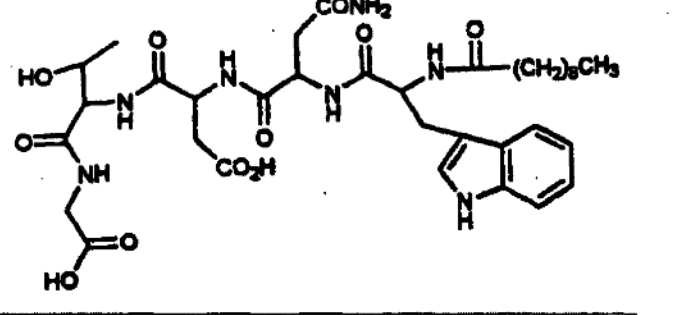
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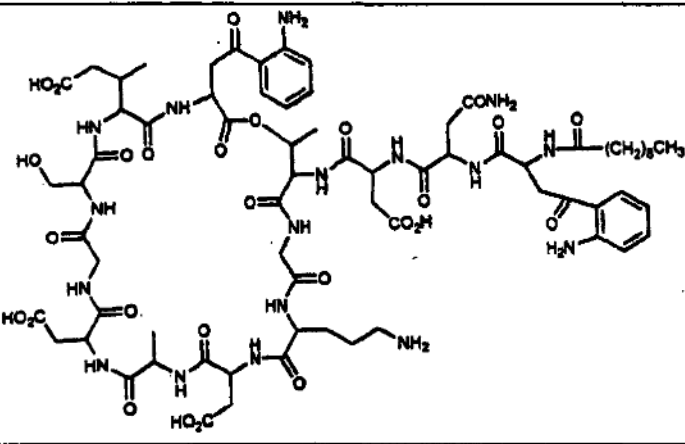
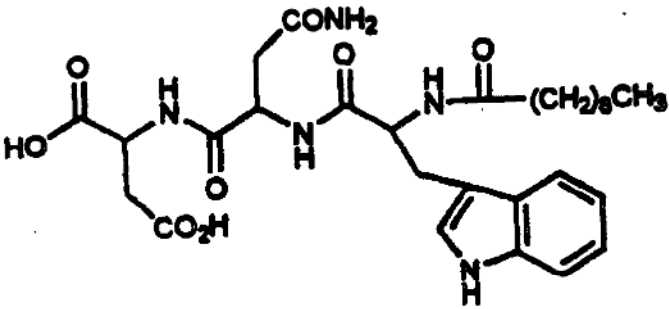
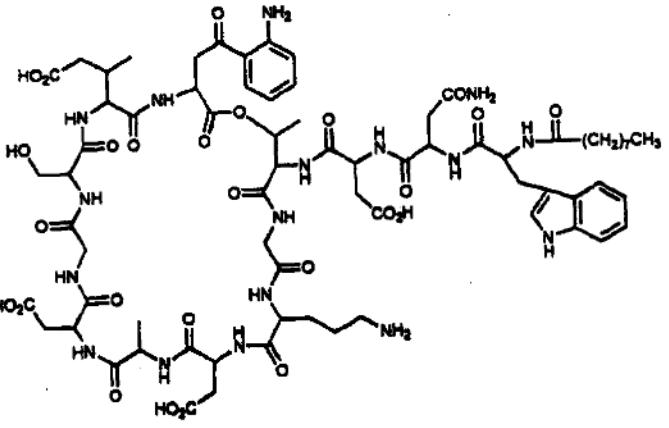
CUBIST PHARMACEUTICALS INC

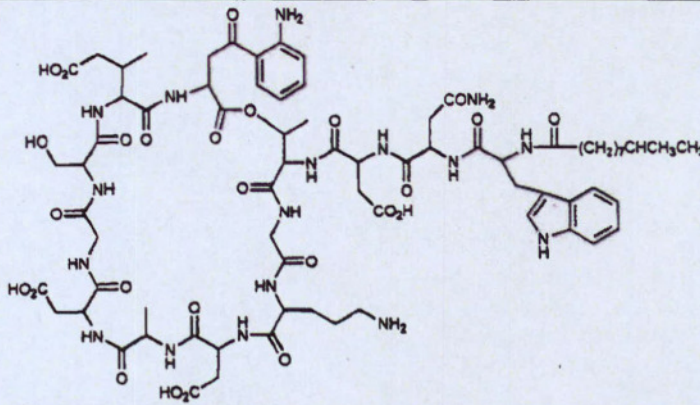
Defendant

AMENDED CLAIMS AS REFERRED TO IN
STATEMENT OF REASONS IN SUPPORT OF
APPLICATION TO CONDITIONALLY AMEND
EUROPEAN PATENT (UK) NO. 1 252 179

1. A method to purify daptomycin, wherein daptomycin is selected from the group consisting of essentially pure daptomycin, daptomycin that is at least 98% pure, daptomycin that is substantially free of anhydro-daptomycin and substantially free of beta-isomer of daptomycin, daptomycin that is essentially free of anhydro-daptomycin and substantially free of beta-isomer of daptomycin, daptomycin that is free of anhydro-daptomycin and substantially free of beta-isomer of daptomycin, daptomycin that is substantially free of impurities 1 to 14 and daptomycin that is essentially free of impurities 1 to 14, wherein impurities 1 to 14 are as follows:

Impurity	Retention time (minutes)	Molecular weight	Structure
1	7.96	1638	
2	9.11	1638	
3	11.54	745	

<u>Impurity</u>	<u>Retention time (minutes)</u>	<u>Molecular weight</u>	<u>Structure</u>
<u>4</u>	<u>12.28</u>	<u>1624</u>	
<u>5</u>	<u>13.10</u>	<u>1618</u>	=
<u>6</u>	<u>14.43</u>	<u>587</u>	
<u>7</u>	<u>14.43</u>	<u>1606</u>	

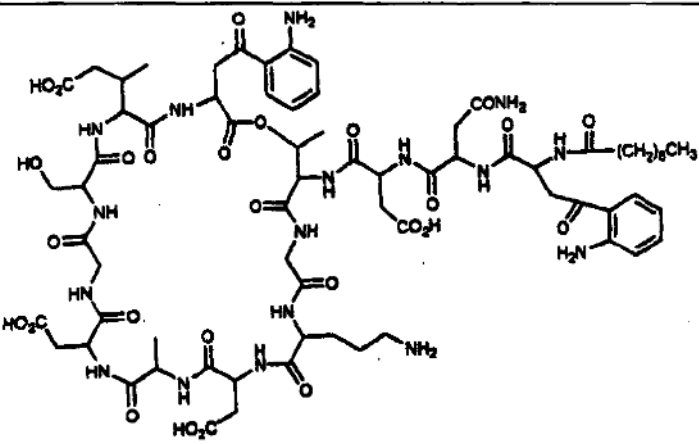
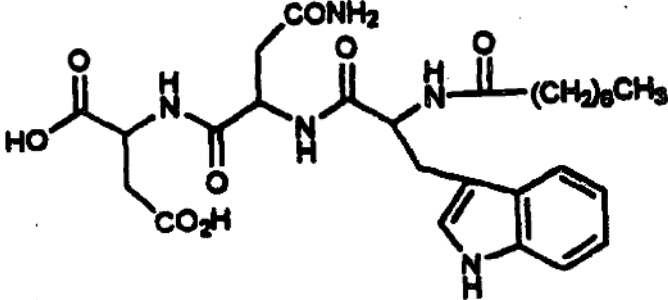
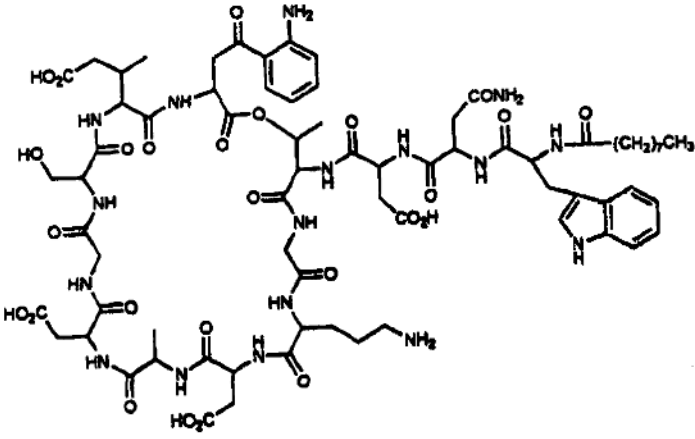
<u>Impurity</u>	<u>Retention time (minutes)</u>	<u>Molecular weight</u>	<u>Structure</u>
<u>14</u>	<u>24.53</u>	<u>1634</u>	

comprising the steps of:

- a) supplying a daptomycin preparation that contains at least 2.5% of a combined amount of anhydro-daptomycin and β -isomer of daptomycin;
 - b) binding the daptomycin preparation to an anion exchange resin in the presence of a modified buffer under conditions in which daptomycin binds to the anion exchange resin in a monomeric and non-micellar state, wherein the modified buffer comprises a buffering agent selected from acetate, phosphate, citrate and Tris-HCl and one or more chaotropic agents selected from ammonia, urea, benzoate and ascorbate;
 - c) washing the anion exchange resin in the presence of the modified buffer under conditions that elutes anhydro-daptomycin but retains daptomycin;
 - d) eluting daptomycin in the presence of the modified buffer under conditions that separate the purified daptomycin from the β -isomer of daptomycin; and
 - e) obtaining purified daptomycin.
2. The method according to claim 1, further comprising the step of filtering and concentrating the eluted daptomycin.
3. A method to purify daptomycin, wherein daptomycin is selected from the group consisting of essentially pure daptomycin, daptomycin that is at least 98% pure, daptomycin that is substantially free of anhydro-daptomycin and substantially free of beta-isomer of daptomycin, daptomycin that is essentially free of anhydro-daptomycin and substantially free of beta-isomer of daptomycin, daptomycin that is free of anhydro-daptomycin and substantially free of beta-isomer of daptomycin, daptomycin that is

substantially free of impurities 1 to 14 and aptomycin that is essentially free of impurities 1 to 14, wherein impurities 1 to 14 are as follows:

[illegible]

<u>Impurity</u>	<u>Retention time (minutes)</u>	<u>Molecular weight</u>	<u>Structure</u>
4	12.28	1624	
5	13.10	1618	=
6	14.43	587	
7	14.43	1606	

<u>Impurity</u>	<u>Retention time (minutes)</u>	<u>Molecular weight</u>	<u>Structure</u>
<u>8</u>	<u>15.10</u>	<u>1620</u>	
<u>9</u>	<u>17.92</u>	<u>874</u>	=
<u>10</u>	<u>19.57</u>	<u>1810</u>	=
<u>11</u>	<u>19.57</u>	<u>1635</u>	=
<u>12</u>	<u>20.93</u>	<u>859</u>	
<u>13</u>	<u>23.11</u>	<u>1602</u>	

[illegible]

comprising the step of:

- a) fermenting *Streptomyces reeseus* with a feed of n-decanoic acid to produce daptomycin in a fermentation broth;
 - b) clarifying the fermentation broth;
 - c) subjecting the fermentation broth to anion exchange chromatography to obtain an enriched daptomycin preparation;
 - d) subjecting the enriched daptomycin preparation to hydrophobic interaction chromatography to obtain a semi-purified daptomycin preparation; and
 - e) subjecting the semi-purified daptomycin preparation to modified buffer enhanced anion chromatography, wherein the modified buffer comprises a buffering agent selected from acetate, phosphate, citrate and Tris-HCl and one or more chaotropic agents selected from ammonia urea, benzoate and ascorbate to obtain purified daptomycin.
4. The method according to claim 3, wherein the feed of n-decanoic acid in step a) is regulated to achieve a residual concentration of n-decanoic acid of no more than 50 parts per million (ppm) during fermentation; said clarifying in step b) comprises extracting the fermentation broth with a buffer comprising butanol; the anion exchange chromatography in step c) is performed on FP-DA 13 resin; or either or both steps c) or e) comprises the use of a continuous salt gradient or step salt gradient.
5. The method according to claim 3, wherein the modified buffer enhanced anion exchange chromatography in step e) comprises the step of:
- i. supplying the semi-purified daptomycin preparation from step d) in a buffer appropriate for modified buffer enhanced anion exchange chromatography;
 - ii. binding the daptomycin preparation to an anion exchange resin in the presence of the modified buffer under conditions in which daptomycin binds to the anion exchange resin in a monomeric and non-micellar state;
 - iii. washing the anion exchange resin in the presence of the modified buffer under conditions that elutes anhydro-daptomycin but retains daptomycin; and
 - iv. eluting daptomycin in the presence of the modified buffer under conditions that permit the separation of daptomycin from β -isomer.
6. The method according to claim 3, further comprising:
the step of anion exchange chromatography prior to step e); or
the step of filtering and/or concentrating daptomycin.

7. The method according to claim 3, further comprising the step of depyrogenating daptomycin.

8. The method according to claim 7, further comprising the step of lyophilizing daptomycin.