IN THE HIGH COURT OF JUSTICE **HP-2014-000037**
CHANCERY DIVISION
PATENTS COURT

BETWEEN:

HOSPIRA UK LIMITED

Claimant

-v-

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

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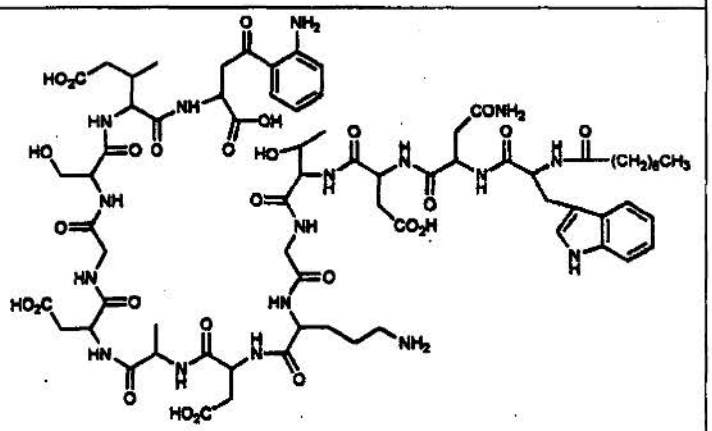
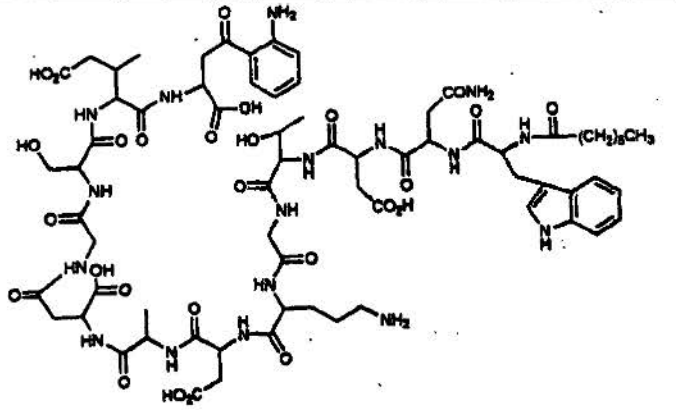
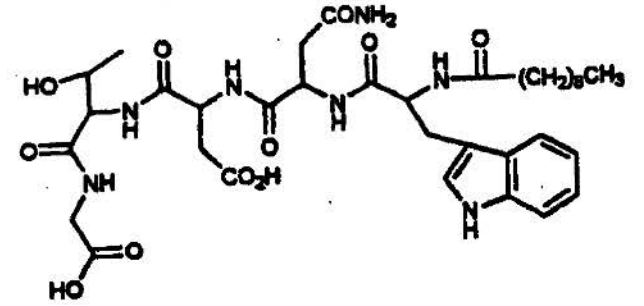
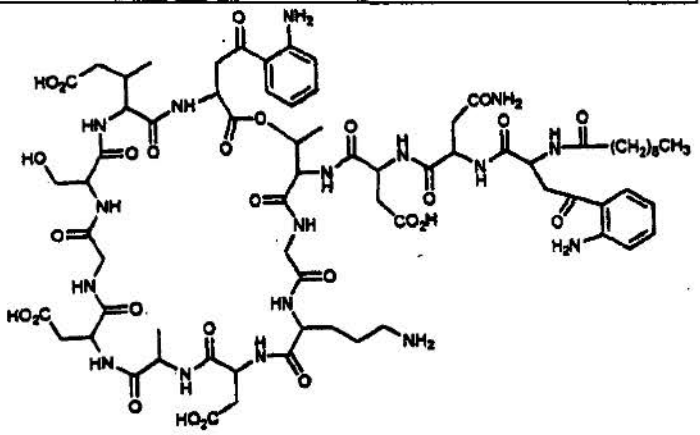
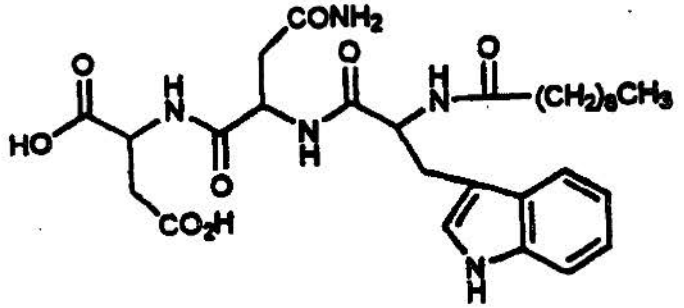
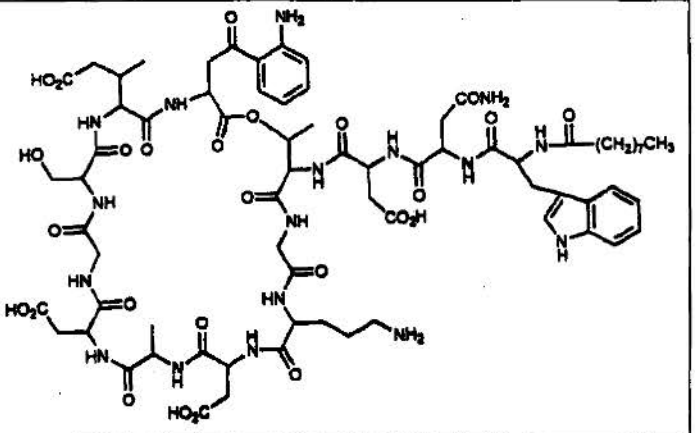
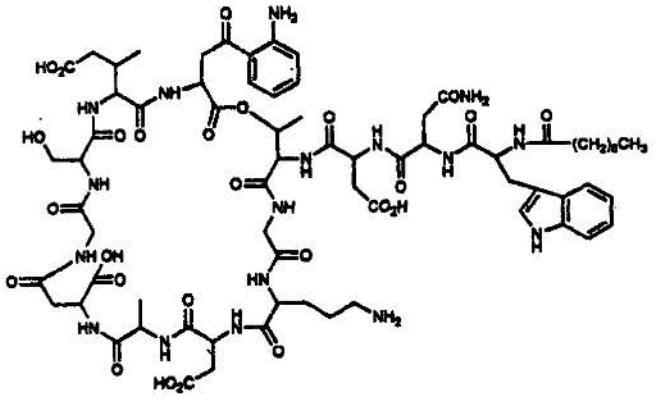
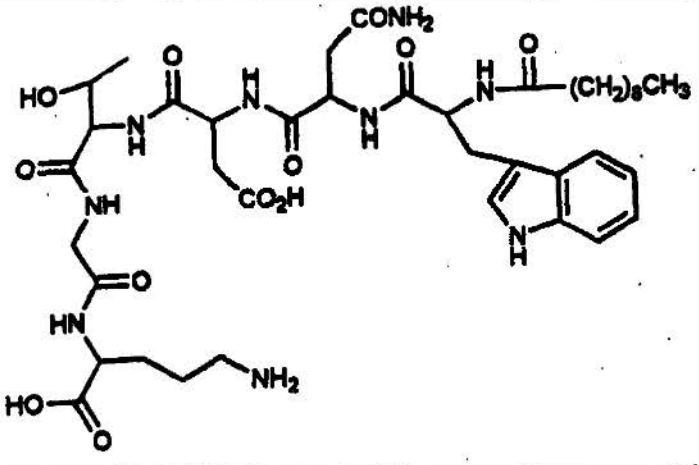
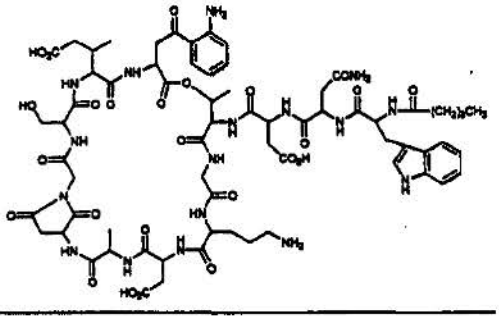
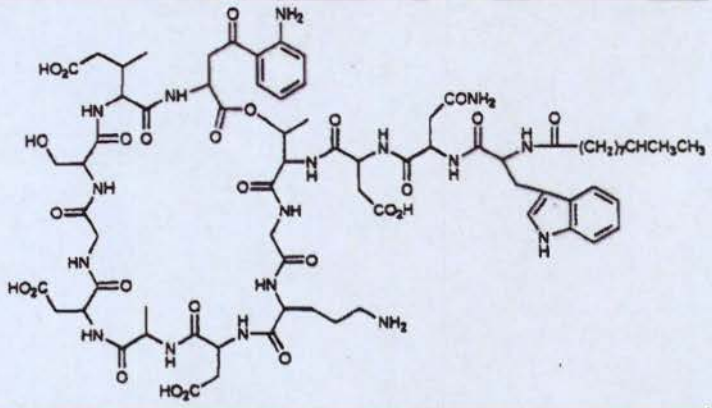
1. A method to purify daptomycin, wherein daptomycin is selected from the group consisting of essentially pure daptomycin, daptomycin that is at least 98% pure, daptomycin that is substantially free of anhydro-daptomycin and substantially free of beta-isomer of daptomycin, daptomycin that is essentially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

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

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

Impurity	Retention time (minutes)	Molecular weight	Structure
14	24.53	1634	

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;

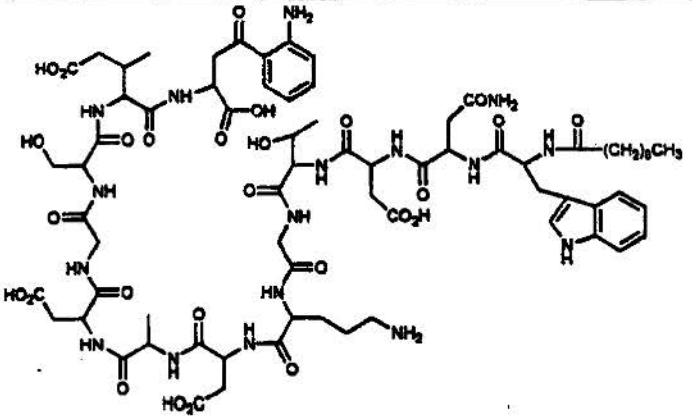
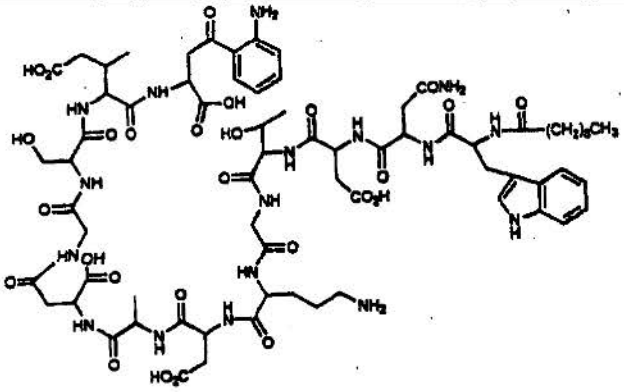
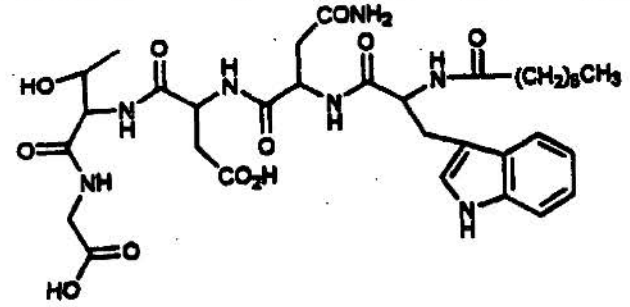
d) eluting daptomycin in the presence of the modified buffer under conditions that separate the purified daptomycin from the β-isomer of daptomycin; and

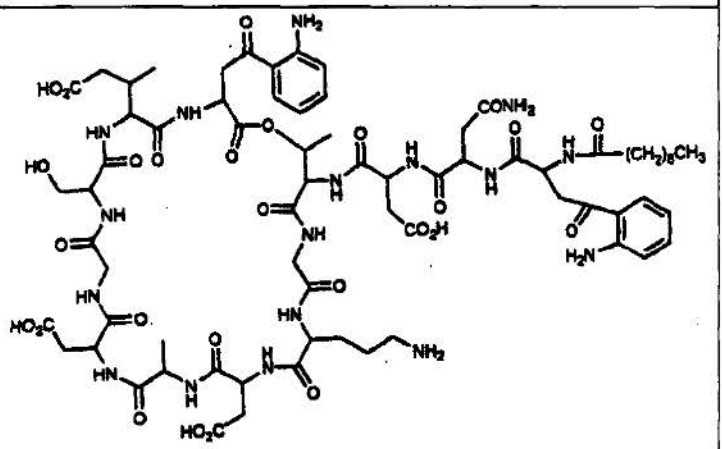
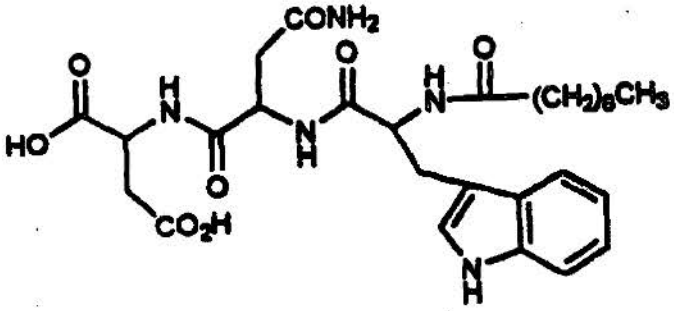
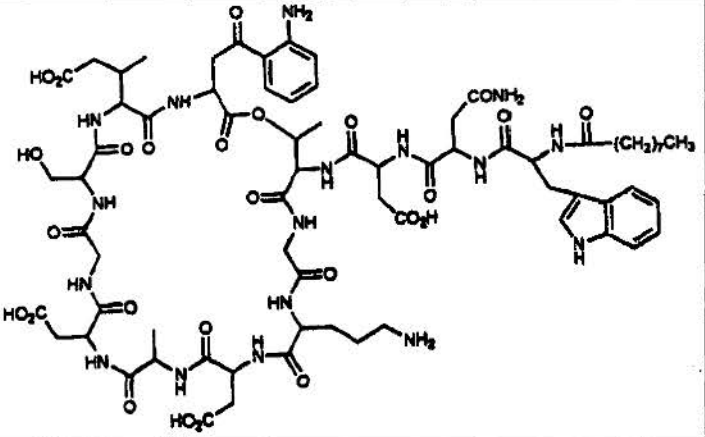
e) obtaining purified daptomycin.

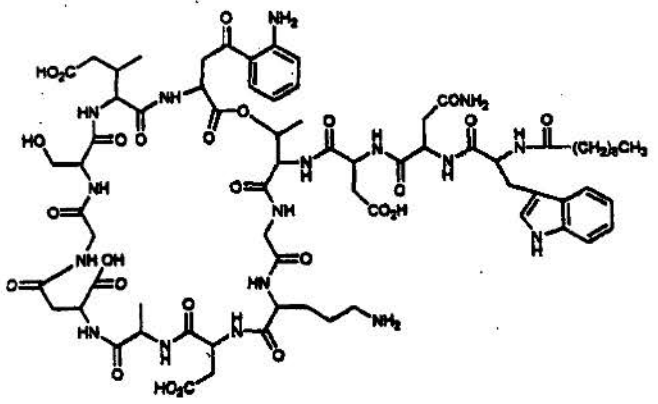
2. The method according to claim 1, further comprising the step of filtering and concentrating the eluted daptomycin.

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:

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

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

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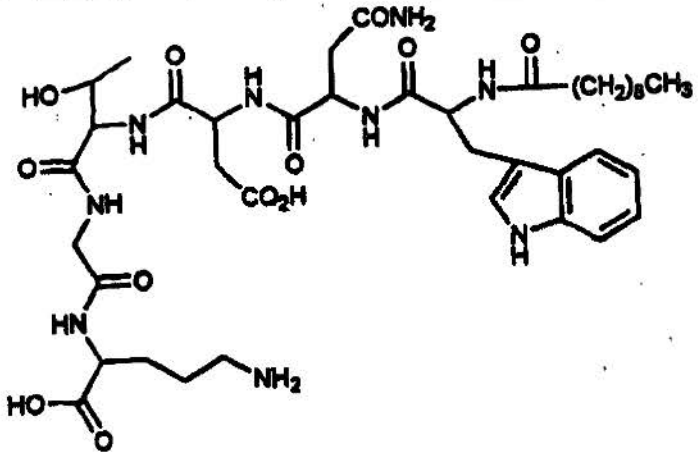
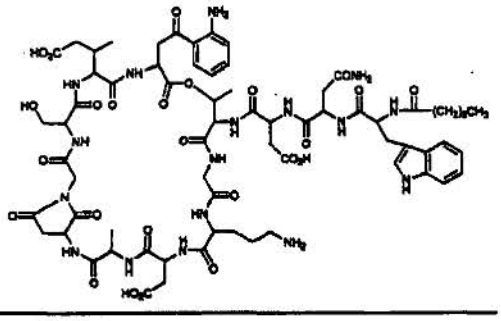
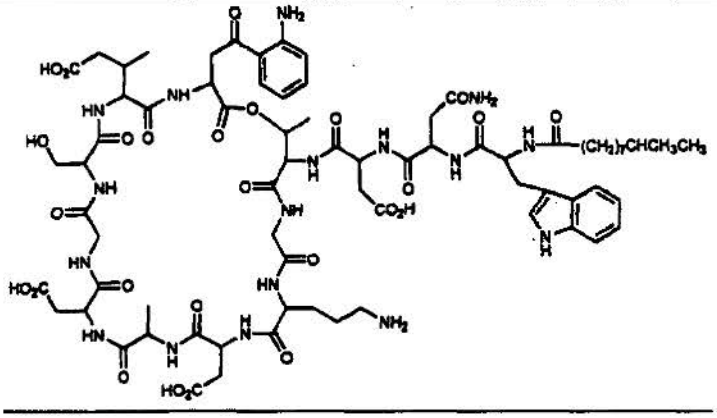
11	19.57	1635	=
12	20.93	859	
13	23.11	1602	

Impurity	Retention time (minutes)	Molecular weight	Structure
14	24.53	1634	

comprising the step of:

a) fermenting Streptomyces reseosporus 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 form 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.